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ITALIAN JOURNAL OF FOOD SCIENCE

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PAPER

CFD SIMULATIONS AS A SUPPORTING TOOL FOR PROCESS AND CONSTRUCTION OPTIMIZATION IN FOOD INDUSTRY PRODUCTION PRACTICE: A CASE STUDY OF A SINGLE TRUCK SMOKING CHAMBER

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ABSTRACT

This paper presents an overall description of one of many numerical modeling methods (Computational Fluid Dynamics) as a tool supporting the optimization of an existing technological process in relation to changes in the parameters of individual operations, as well as the guidelines for constructional changes in the machines and devices used in these operations. The method is presented on the basis of a case study of basic smoking in a single truck smoking chamber. The modeled problem is the flow of an air and smoke mixture in an electric single truck smoking chamber. Results were obtained in the form of spatial distributions of mixture velocity and the movement tracks of smoke particles. These results enabled an analysis of the uniformity of the mixture flow inside the chamber taking into consideration the spaces where "dead zones" occur.

- Keywords: basic smoking, CFD modeling, fluid flow, simulation, smoking chamber -

INTRODUCTION

The application of modeling has recently become more common in engineering practice. Many design systems contain extra modules for modeling and simulation issues; for example, the strength of constructional elements that are designed. Especially popular are additional modules for approximate calculations applying grid methods, such as Finite Element Method (FEM). Furthermore, the significant increase in PC calculation productivity enables faster stimulation analysis and thus produces data applicable for further optimization, e.g. the construction of drive line elements or constructional elements. Additionally, there are also software packages for simulation using calculation algorithms, which are constantly being improved (SEBASTIAN *et al.*, 2005). One such package is ANSYS CFX (ANSYS, Inc., Canonsburg, PA, USA), a CFD analysis calculation package. This computer program, with a complementary (on the post-processor level) program called FLUENT, is the main tool for the modeling and simulation of the fluid flow phenomenon.

One example of CFX applications are the CFD simulation models of an air and smoke mixture flow in a smoking chamber. A single truck smoking chamber was used as a substitute for the geometric model of a chamber. It is the most frequently used device in small production plants (in this case a smoking chamber produced by Pek-Mont LTD, Bielsk k/Płocka, Poland) (JAKUBOWSKI *et al.*, 2010; KUBIAK and JAKUBOWSKI, 2010a,b; KOSTYRA, 2005).

The reason behind investigating the characteristics of air and smoke mixture flow in this chamber was the need to solve the problem of the occurrence of technological defects during meat smoking. There may be two main defects in smoked meat: either the meat is not sufficiently smoked or it is cured too much; both are caused by improper application of parameters in the process of smoking. In many food industry plants, the first problem is eliminated by an additional smoking operation process, whereas the second problem is eliminated by adding air and diluting the air and smoke mixture during the operation of smoking. The second defect ruins the final product in most cases (in terms of sensory values), which causes an additional loss in food industry plants. Both defects contribute to the generation of additional production costs and can negatively affect the quality of the final product (ROBERTSON *et al.*, 2004; KUBIAK and JAKUBOWSKI, 2010 a,b; JAKUBOWSKI *et al.*, 2010).

MATERIALS AND METHODS

The simulation model was a geometric representation of the working space of a KWP-1 single truck smoking chamber type manufactured by Pek-Mont LTD (Fig. 1a). The internal dimensions of the working part of the chamber were: length - 1,440 mm; width - 1,200 mm; height - 2,950 mm. These chambers are designed and manufactured especially for small- and middle-sized meat processing plants. They are the most appropriate solution for processing different types of meat products as the smoking process needs to be carried out with swift technological parameter changes between the different operations of the given process (JAKUBOWSKI *et al.*, 2010; KUBIAK and JAKUBOWSKI, 2010 a,b). For simulation analysis, four geometric variants were used: in the form of an empty chamber and ones filled with batches composed of spherical and cylindrical hams and an elongated shape similar to the Sopot loin – a typical Polish product. The dimensions of a single filled batch were averaged for each product.

The arrangement projection is only an approximation of reality, i.e. hanging the batch in rows and on smoking sticks. It should also be mentioned that batch geometry and its spatial arrangement is only a simplification because of the assumed regular and homogenous dimensions of each processed batch.

The geometry of a computer model and its discretization (Fig. 1b) were conducted with the use of Ansys Mechanical APDL 12.1 (ANSYS, Inc., Canonsburg, PA, USA). Tetragonal element type Fluid 142 (ANSYS Mechanical, 2010) available in the program library was used to create a mesh of finite elements. Four meshes were generated with ca. 1,250,000 elements (empty chamber) and 1,500,000 (chamber filled with batch) (JAKUBOWSKI and KUBIAK, 2013). The model was then introduced into the CFX program preprocessor. Boundary and primary simulation conditions appropriate for the conditions and realization of the smoking process were set. The next phase was to introduce the model to the solver module and start the simulation (ANSYS CFX, 2010).

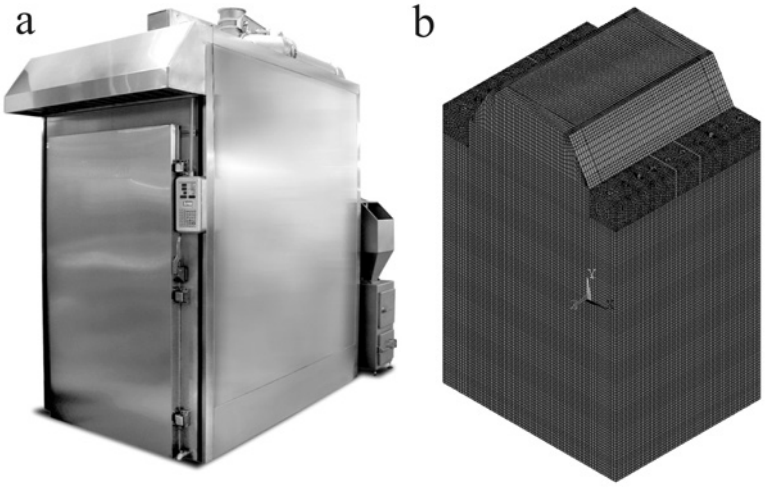

Fig. 1 - Single truck smoking chamber type KWP-1etz by Pek-Mont LTD: a) general view, b) geometric model of workspace with FE grid (www.pekmont.pl).

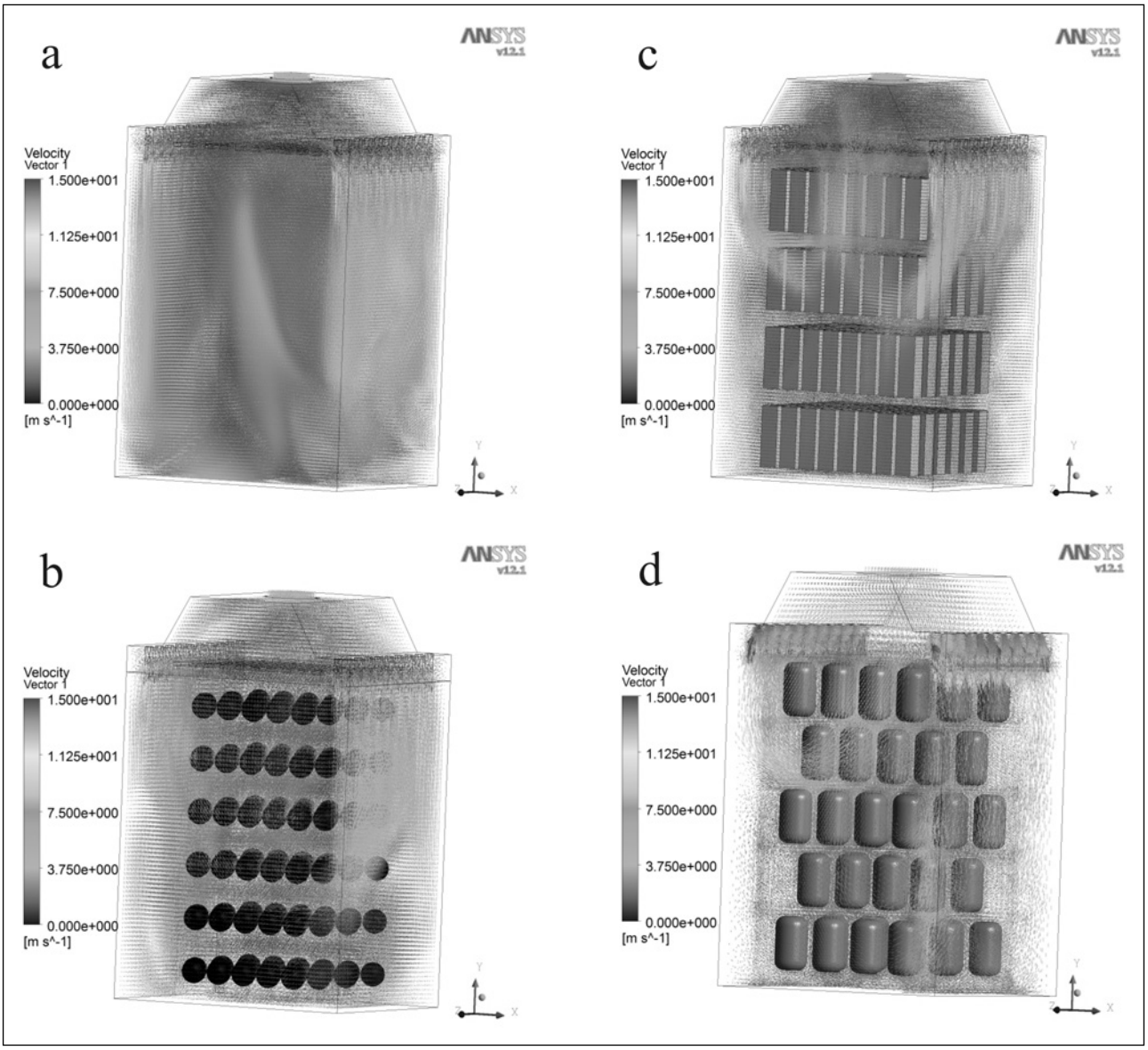

Fig. 2 - Map of the distribution of mixtures flow rates in working space of smoking chamber: a - empty, b - in chamber with charger (spherical shape), c - in chamber with charger (cylindrical shape), d - in chamber with charger (a elongated shape similar to the Sopot loin, typical polish product).

RESULTS AND DISCUSSION

After finishing the calculations, the resulting data were entered into the post processor module in the form of files including information about flows inside the analyzed models of the chamber working space. This part of the processor allows the processing of the resultant values of parameters describing the flow of particles and its visualization, e.g. as three-dimensional velocity distribution maps of the particles present in the mixture inside the chamber. Figure 2 presents the distribution of particle flow velocity in the whole internal space of both an empty chamber (Fig. 2a) and in a chamber filled with batch (Fig. 2b-2d). A simple comparison of the mixture flow velocity distribution in both variants reveals significant differences in distribution for an empty chamber and one filled with batch. For the analyzed cases an existence of asymmetric flow is strongly highlighted, regardless of the regular distribution of the inlet nozzle and batch inside the chamber space. Velocity maps reveal significant flow irregularities inside the chamber, where some obstacles are present in the form of individual parts of batch.

Tracking analysis was performed on the particles present in the dispersed phase of the air and smoke mixture. Figure 3 shows the trajectory of the smoking movement for 5,000 model particles. In the model of the flow in the chamber filled with batch, the assumption has been made that particles that have contact with the product surface have been settled in this model.

The results of the tracking analysis of dispersed phase particles reveal areas (inside the chamber)

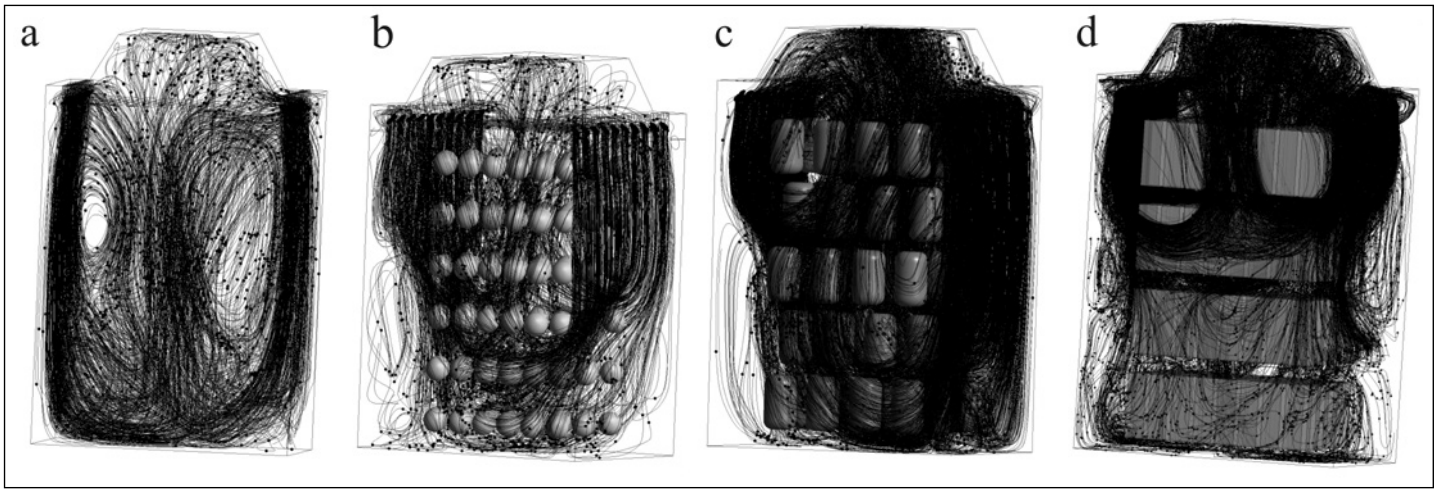

Fig. 3 - Movement path of selected 5,000 smoke particles in working space of smoking chamber: a - empty, b - in chamber with charger (spherical shape), c - in chamber with charger (cylindrical shape), d - in chamber with charger (a elongated shape similar to the Sopot loin, typical polish product)

with higher and lower concentrations of smoking smoke. This allows areas to be indicated where propagation conditions may not be sufficiently favorable, which may in turn determine the efficacy of the realization of the smoking process.

Summarizing the review of selected analysis results it should be noted that the developed model is still being improved. At present, verifying research is being carried out in industrial conditions with the use of a testing chamber manufactured by Pek-Mont LTD. This verification is being conducted at the "Grabowscy" Meat Processing Plant in a small village near Gryfice (West Pomeranian region in Poland). For the verification of results, measurement of smoke flow in selected chamber space areas will be conducted using anemometric sensors (HVAC Miniature Air Velocity Transmitter type: EE575).

CONCLUSIONS

Although the developed model and the consequent calculations made provide only an approximated view of the real situation, they are valuable tools for people responsible for smoking process operations in production settings. This is certainly applies to engineers who are familiar with the conditions and core technological processes of smoking chambers and also their designers and constructors.

The simulation model that was built, tested and analyzed can be used as a tool for the optimization of those criteria which can be defined when searching for new constructional solutions for the inner part of the chamber which will allow for the creation of better (more homogenous) conditions in terms of smoking smoke flow, regardless of the geometrical parameters of the material in question. This will improve the efficiency of the smoking process and also enable the elimination of technological defects of the final smoked product.

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REFERENCES

Ansys CFX 12.1, documentation of program, 2010.

Ansys Mechanical APDL 12.1, documentation of program, 2010.

Jakubowski M., Kubiak M. S. and Diakun J., 2011: Simulation analysis of flow in a single-car curing chamber packed with a charge. Chemical Engineering and Equipment (PL) vol. 50 no 1, 17-18.

Jakubowski M., Kubiak M.S., 2013: Simulation analysis of airflow in a curing chamber with modified distribution of inlets nozzles. Chemical Engineering and Equipment (PL) vol. 52 no 3, 183-184.

Kostyra E., 2005. Wood smoke and smoke flavouring preparation-composition, function, application. Technological Progress in food processing (PL). T 5, no 2, 48-50.

Kubiak M. S. and Jakubowski M., 2010a: Simulation analysis of smoke carrier speed flow distribution in the smoke chamber. Advances of Agricultural Sciences Problem Issues (PL) no 546, 201-206.

Kubiak M. S. and Jakubowski M., 2010b: The simulation model flow condition in the smoke chamber. Technological Progress in food processing (PL) no 1, 55-57.

Robertson A., Tirado C., Lobstein T., Jermini M., Knai C., Jensen J. H., Ferro-Luzzi A. and James W. P. T., 2004. Food and health in Europe: a new basis for action WHO Regional Publications. European Series. Copenhagen. no 96.

Sebastian P., Bruneau D., Collignan A. and Rivier M., 2005. Drying and smoking of meat: heat and mass transfer modeling and experimental analysis, Journal of Food Engineering; vol. 70 (2), 227-243.

www.pekmont.pl - Materials of company Pek-Mont LTD. (PL).

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PAPER

EVALUATION OF DIFFERENT STUNNING METHODS ON ASPECTS OF ANIMAL WELFARE AND MEAT QUALITY OF MATRINXÃ (*BRYCON CEPHALUS*)

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ABSTRACT

Three methods of stunning fish (electronarcosis, CO_2 narcosis, and thermal shock) were compared to study their influence on welfare and meat quality of matrinxã (*Brycon cephalus*). Parameters such as water quality and the time to reach clinical indicators of unconsciousness were observed. *Rigor mortis* index and muscular shrinkage were evaluated 3 and 5 h after stunning and at 1, 4, 7, 12 and 18 days of storage. None of the methods extended shelf-life; however, electronarcosis provided faster clinical indicators of unconsciousness and did not cause loss of meat quality.

- Keywords: CO_2 narcosis, electronarcosis, shelf-life, thermal shock, unconsciousness -

INTRODUCTION

Because fish is the most internationally traded food commodity, quality control and safety, including meat quality and ethical aspects of animal welfare, are fundamental in aquaculture production (FAO, 2009; LAMBOOIJ *et al.*, 2006b). Thus, one of the most critical production stages to maintain fish quality is the stunning process.

Techniques for fish slaughter have been the subject of numerous studies regarding quality control, efficiency and safety procedures (CONTE, 2004). Several studies have also aimed to minimise the time required to establish death and implicitly reduce stress and pain (LAMBOOIJ *et al.*, 2002; ACERETE *et al.*, 2009). Slaughter is usually a two-step process. First, the animal is stunned to induce insensitivity to pain, and death is induced by various methods, including bleeding or oxygen deprivation (OLIVEIRA and GALHARDO, 2007). These two phases may occur together, but when they occur separately, the stunning time should be minimised to avoid recovery of consciousness before death (LINES *et al.*, 2003). When correctly applied, the stunning methods should cause little stress, improve the physical properties of meat, reduce muscular energy exhaustion, generate less lactic acid, maintain muscle pH balance, delay *rigor mortis* and, consequently, produce better fish quality (CONTE, 2004).

A typical stunning method in the fish processing industry is thermal shock (ASHLEY, 2007), in which water temperature is decreased to approximately 1°C. This low temperature decreases metabolic rate and oxygen consumption, causing the fish to become immobilised until death (RIBAS *et al.*, 2007). The effectiveness of this method depends on the difference between culture and stunning water temperature. Thus, thermal shock is more effective when applied to tropical fish species (ACERETE *et al.*, 2004) and is most often used in warm climate regions owing to ease of application and positive results on meat quality (ROBB and KESTIN, 2002b; SCHERER *et al.*, 2005; LAMBOOIJ *et al.*, 2006b; BAGNI *et al.*, 2007).

Some studies demonstrate that electronarcosis can damage fish quality when not conducted properly (ROBB and ROTH, 2003; LINES *et al.*, 2003; POLI *et al.*, 2005b). Nevertheless, it has been demonstrated that species such as Atlantic salmon (*Salmo salar*) (KIESSLING *et al.*, 2004), rainbow trout (*Oncorhynchus mykiss*) (AZAM *et al.*, 1990), eel (*Anguila anguila*) (MORZEL and VAN DE VIS, 2003) and common carp (*Cyprinus carpio)* (LAMBOOIJ *et al.*, 2006a) show good positive results on fish quality because of the low stress level provided by this method.

CO_2 narcosis is mostly used for salmon and trout. This method consists of placing fish inside a box containing water saturated with CO_2, which produces H_2CO_3 in equilibrium with HCO_3 and H^+. This procedure decreases the blood pH and, hence, induces a toxic effect in the animals' brain (POLI *et al.*, 2005b). Sea bass stunned by CO_2 narcosis show high energy reserve in muscle and low plasma cortisol levels (POLI *et al.*, 2005a). This method is efficient and quick for narcotising a large number of fish and is useful for several species of all sizes, but it is highly stimulating to the fish during the first few minutes. The use of a controlled CO_2 delivery system or inert gases to promote anoxia in a more humane way is needed to prevent fish suffering (POLI *et al.*, 2005). Therefore, studies concerning different stunning methods may contribute to achieve greater fish shelf-life and assure animal welfare.

Matrinxã (*Brycon cephalus*) is a freshwater native fish species from the Upper Amazon basin in Peru, Bolivia and Brazil. This species has been considered a promising candidate for Brazilian aquaculture because of its rapid growth rate, good adaptation to captivity and increasing economic importance among farmed fishes in Brazil (ROMAGOSA *et al.*, 1999; GOMES *et al.*, 2000). Although this fish has been the subject of several studies in Brazil, few researchers have studied the biochemical, physical and sensory changes during refrigerated storage (ALMEIDA *et al.*, 2006). Despite the fact that stunning methods are a widely studied subject worldwide, the effects of stunning stress on the physiological responses and meat quality of native freshwater fish species are unknown in Brazil. Thus, we aimed to compare the efficacy of the three stunning methods (electronarcosis, thermal shock and CO_2 narcosis) in evaluating parameters such as behaviour and meat quality of matrinxã (*B. cephalus*) stored in ice.

MATERIAL AND METHODS

Ten month old matrinxã specimens (n = 90; weight, 535.10±121.36 g) were purchased from a local breeding farm. Fish underwent a 24-h fasting period in outdoor tanks. Then, the animals were captured with a net and immediately transferred to plastic boxes (120 L) inside the laboratory. All animals in the treatment (n = 30) were stunned at the same time. As the procedures were the same for all animals, they were subjected to the same stress level. The slaughter methods were performed on the same day to avoid the influence of weather.

For electronarcosis stunning, salinity of the tank water was adjusted to achieve a specific conductance of 700 μS. Two aluminium plates (65×35 cm) were placed parallel to each other inside a box with a distance of 49 cm between them to create an electric field. One of the plates was covered with a polyethylene screen to avoid short circuit by fish contact. An electrode connected to a device capable of discharging 220 V was attached to each plate. Fish were simulta-

neously subjected to an electric current of 7.3 A and 155 V for 3 min.

In CO_2 narcosis, fish (n = 30) were placed in a slaughter box and CO_2 was diffused into the water (23.2°C) for 30 min until apparent death. For thermal shock, fish (n = 30) were immersed in a slaughter box containing water and ice (1:1) for 14 min until apparent death. This is the usual stunning procedure for freshwater farmed fish in the Brazilian aquaculture industry.

Water quality parameters (pH, temperature, salinity, specific conductance and dissolved oxygen) were monitored with a multi-parameter probe (Horiba multi-parameters, template U-10) during all procedures. Fish behaviour was also carefully monitored from the beginning of the stunning procedures until apparent death. All stunning treatments should make fish insensitive to pain; therefore, some behaviours were used as indicators of brain function in the absence of brain activity measuring equipment, as suggested by KESTIN *et al.* (2002). These included no vestibulo-ocular reflex (VOR), opercular (no breathing) or swimming movements, upside down posture and the absence of a reflex when the lateral line was stimulated with a pin. After stunning, fish were kept cold in isothermal boxes containing ice and housed in a cold chamber at 4°C for 18 days. The parameters evaluated were as follows:

a) *Rigor mortis* index (RI), which was measured during each sampling period with three replicates per treatment. This parameter was measured initially every 30 min after stunning to monitor its onset (RI = 100%) and later at 1, 4, 7, 12 and 18 days of storage to verify resolution. RI was determined using the equation described by BITO *et al.*, 1983.

$$RI = \frac{D - D_0 \, X \, 100}{D_0}$$

where RI is the rigor index, D_0 is the initial distance between the table surface and the caudal fin base and D is the final distance between the table surface and the caudal fin base.

b) Fillet length shrinkage, which was measured using three right fish fillets per treatment with the aid of a digital calliper (Starrett, 799A series) at 0, 3 and 5 h after death and at 1, 4, 7, 12 and 18 days of storage. Fish fillets were stored under refrigeration at 4°C at time 0. Fillet shrinkage was measured in the samples at each sampling time. The percentage of variation in fillet length was determined using the following equation:

$$\% \text{ Shrinkage} = 100 - (L \; X \; 100/L_0)$$

where L is the final fillet length and L_0 is the initial fillet length.

c) Volatile base nitrogen (VBN), which was determined in three left fish fillets per treatment immediately after confirming death (time 0) and at 1, 4, 7, 12 and 18 days of storage according to HOWGATE (1976).

d) Instrumental colour analysis, which was performed in three left fish fillets per treatment at 1, 4, 7, 12 and 18 days of storage using a portable colourimeter (Miniscan XE, Hunterlab). The instrument was calibrated with standard black and white before each reading. A D65 light source, 10° viewing angle and 33-mm diameter area were used for the analysis. Colour measurements were expressed according to the CIELab colour scale, where L^* denotes lightness, a^* denotes red to green variation and b^* denotes yellow to blue variation.

e) Instrumental texture, which was measured in three left fish fillets per treatment at 1, 4, 7, 12 and 18 days of storage with a texturometer (TA-XT2i, Stable Micro Systems) previously calibrated with a 5 kg standard weight. Fillets were compressed perpendicularly with the aid of an aluminium probe (SMS P/20) and measured at 40% compression and a 2 mm/s speed with a platform distance of 20 mm in three different locations.

f) Sensory evaluation, which was performed by five trained panellists with three fish per treatment at 1, 4, 7, 12 and 18 days of storage, according to the European Union official scheme (European Union Regulation, EEC 103/76 modified by regulation 2406/96) that determines four levels of fish freshness.

g) Statistical analysis: data were subjected to analysis of variance (ANOVA) at a 5% significance level. Tukey's test was applied to assess differences among stunning methods and regression analyses were performed where appropriate. All analyses were performed with the statistical program SAS version 9.1.3 (SAS/STAT®, 2002).

RESULTS AND DISCUSSION

Water quality parameters

As parameters such as water quality may have varied during stunning causing stress and consequently compromising meat quality, these parameters were monitored and are described in Table 1. As anticipated, in electronarcosis, parameters such as pH, dissolved oxygen and temperature remained stable during application of the electrical current. Salinity increased because NaCl was added to improve specific conductance of the water. pH decreased after adding CO_2 due to the production of carbonic acid; however, dissolved oxygen levels did not decrease. Other parameters such as specific conductance, temperature and salinity remained stable. Water temperature was low during the thermal shock stunning method. Dissolved oxygen was higher in

Table 1 - Water quality parameters: Dissolved oxygen (DO), temperature (TEMP), specific conductance (SC), pH and salinity (SAL) at the beginning (B) and end (E) of matrinxã stunning with electronarcosis, CO_2 narcosis and thermal shock.

Treatment		Water quality parameters				
		DO (mg/L)	TEMP (°C)	SC (µS)	pH (%)	SAL
Electronarcosis	B	5.6	23.5	700	6.5	0.03
	E	5.6	23.5	700	6.5	0.03
CO_2 narcosis	B	5.9	23.2	0.0	6.1	0
	E	5.7	23.4	0.0	4.8	0
Thermal shock	B	19.2	1.4	0.0	6.6	0
	E	19.1	1.7	0.0	5.9	0

Table 2 - Behavior parameters observed during the tested stunning methods in matrinxã fish (*Brycon cephalus*) (n = 30).

Behavior[1]	Electronarcosis	Thermal shock	CO_2 narcosis
	Exposition time to stunning method		
Swimming interruption	2 s	2 min	14 min
Absence of runaway movement	2 s	6 min	14 min
Dorsal inversion	2 s	8 min	32 min
Muscular tonelessness	2 s	8 min	32 min
Absence of operculum movement	2 s	8 min	32 min
Absence of vestíbulo-ocular reflex (vor)	2 s	8 min	32 min
Absence of lateral line reflex	2 s	8 min	32 min

[1](KESTIN *et al*., 2002).

the low temperature water (PROENÇA and BITTENCOURT, 1994). pH, conductance and salinity values also remained within expected ranges.

Time required to reach clinical indicators of unconsciousness

Clinical indicators of unconsciousness were observed when fish remained upside down without opercular or muscular movement or a VOR (KESTIN *et al.*, 2002). Considering that these data were not statistically analysed, as each stunning method was simultaneously performed on thirty fish to simulate the fish industry environment, unconsciousness seemed to differ among treatments (Table 2).

Stunning by electronarcosis resulted in clinical indicators of fish unconsciousness almost instantaneously and apparently faster than those of the other treatments. It is possible to use electronarcosis to instantaneously stun and kill eels by using an electric current for longer periods (ROBB *et al.*, 2002), similar to what we used on matrinxã. Other studies have also reported that stunning fish in water with an electric current is a suitable method (LINES *et al.*, 2003) because it promotes rapid clinical indicators of unconsciousness.

In thermal-shock-stunning gradual loss of consciousness was observed. Although the fish were stunned in groups (n = 30), all fish presented simultaneous clinical signs of unconsciousness. Time to achieve unconsciousness using thermal shock stunning seemed shorter than in electronarcosis, but longer than that in CO_2 narcosis (Table 2), as observed for turbot (*Scophthalmus maximus*) (MORZEL *et al.*, 2003).

In CO_2 narcosis stunning, the time required to reach clinical indicators of unconsciousness was apparently longer than that in the other treatments. Similar studies in other species have reported shorter times to reach loss of consciousness (ROBB and KESTIN, 2002a; ROBB *et al.*, 2002a; KESTIN, 2003; ROTH *et al.*, 2006). These variations may be due to metabolic and physiological differences among species or the manner in which the stunning was executed (if fish are exposed to saturated water or if the gas is diffused into water afterwards). As observed in our experiment, CO_2 narcosis stimulates fish, which is obvious by their quick and violent reactions such as repeated swimming movements, attempts to escape and abnormal activity after stunning (POLI *et al.*, 2005b).

Rigor mortis

The time required for the onset of full *rigor mortis* did not differ ($P > 0.05$) among stunning methods. Two hours were required for the onset of *rigor mortis*, which remained for 96 h (4 days) of storage. These data can vary greatly among species, stunning methods and preservation temperatures after death, as reported by RUFF

et al. (2002) in their study of *S. maximus* (20-36 h) and by PARISI *et al.* (2002) in their study of sea bass stunned by thermal shock and stored for 11 days at 4°C.

The onset of *rigor mortis* occurs within 2 h when tilapia are slaughtered at 0°C and stored in ice (CURRAN *et al.*, 1986), as observed in the present study.

Muscular fillet shrinkage

Similar behaviours were observed within treatments for the muscular fillet shrinkage evaluations, and a significant ($P < 0.05$) increase was observed 12 h after storage. Shrinkage rate tended to stabilise after this time and was higher in fillets from fish stunned by CO_2 narcosis (CO_2: 10.28%; thermal shock: 9.47%; electronarcosis: 8.52%; Fig. 1). In cod (*Gadus morhua*) fillets, (MISIMI *et al.*, 2008), the maximum period for tissue shrinkage (11 h) is similar for fish stressed and not stressed by the stunning method (bleeding); however, the shrinkage rate (11%) is higher in fillets that originated from stressed fish, as observed in our study with matrinxã.

At the time of death, fish muscles are relaxed, soft and have an elastic structure. Gradually, the muscles become harder, changes their geometric form and decrease in size (ROBB *et al.*, 2000a). Fillet shrinkage rate depends on the species (EINEN *et al.*, 2002) and the time of filleting. Fish muscles tend to have greater shrinkage if filleted before *rigor mortis* because the muscles are separated from the bone structure, which normally prevents such retraction (LAMBOOIJ *et al.*, 2010).

VBN

Stunning by electronarcosis resulted in higher levels of VBN during the storage period (19.02 mg 100 g^{-1}) than those of the other methods (CO_2: 17.76 mg 100 g^{-1}; thermal shock: 17.89 mg 100 g^{-1}), probably because of the presence of blood. However, it is important that fish should not be consumed after 18 days of storage regardless of treatment according to sensory evaluation and once they have been categorised as class B (European Union Regulation, EEC 103/76 modified by regulation 2406/96). VBN levels during the 18 day storage period were below the maximum allowed by the Brazilian Control Department for Products of Animal Origin (RIISPOA), which is 30 mg 100 g^{-1}. In another study with matrinxã stored in ice for 16 days after stunning by thermal shock, VBN levels were 19 mg 100 g^{-1} and fillets were considered appropriate for consumption according to the sensory evaluation table proposed by the Torry Research Station, Aberdeen (LESSI *et al.*, 2004).

Total VBN content in fish stored in ice is low during the first storage phase and tends to increase rapidly only when fish quality deteriorates (HUSS, 1997). This was not observed in the present study because VBN levels were stable throughout the entire storage period even when fish were not appropriate for human consumption. HUSS (1997) reported that VBN values should not be used to estimate the first changes in fish but to evaluate the degree of deterioration during the last steps. However, this has been confirmed mainly with regard to sea fish species (LESSI *et al.*, 2004). Therefore, on the ba-

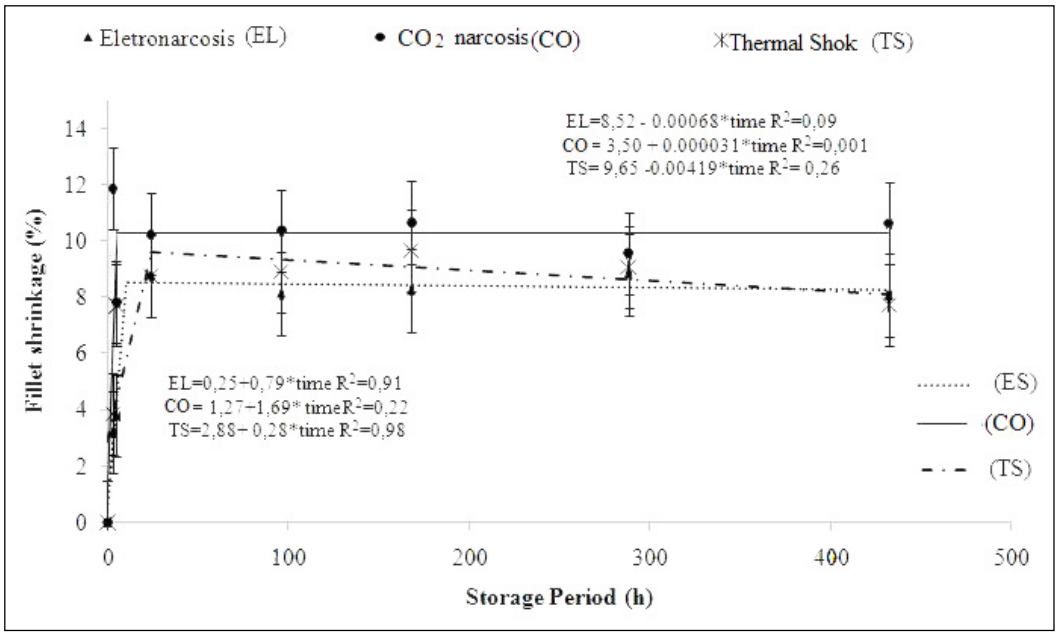

Fig. 1 - Muscular fillet shrinkage of matrinxã fillets stunned by electronarcosis (ES), CO_2 narcosis (CO), and thermal shock (TS) under refrigerated storage for 432 hours.

sis of the three stunning methods used in this study, VBN was not an effective tool to evaluate matrinxã under cold storage.

Sensory analysis

No differences were observed among the tested methods with regard to sensory aspects. The evaluated methods could not extend fish shelf life as the fish stored in ice were classified as 'extra' up to day 4 (96 h), 'fresh' (category A) up to day 18 (432 h) and 'rancid' (category B) after this period (Fig. 2).

A study by LESSI *et al.* (2004) on matrinxã stunned in ice and water and subsequently refrigerated showed that fish maintained good condition until day 26, and fish quality was classified as optimal during the first 13 days. The sensory evaluation was based on the Torry Research Station table, which considers culture conditions, fish habitat and weight at slaughter.

Colour evaluation (L*, a* and b*)

The stunning methods did not alter the intensity of yellow colour (b*) of the fillets. This parameter increased constantly throughout time in the three treatments and could be explained by a single equation, b* = 10.4684 + 0.004209x. The lightness (L*) of the fillets increased ($P < 0.05$) significantly during storage period for CO_2 narcosis and thermal shock treatment. L* values for both methods presented little but constant increase until day 18 of storage. In contrast, fillets from fish stunned by electronarcosis had consistent lightness (L*) throughout storage (average, 53.9 ± 1.58).

Red colour intensity (a*) did not differ during the storage period, but the intensity was higher ($P < 0.05$) in fish stunned by electronarcosis (4.84 ± 0.6) than in those stunned by CO_2 narcosis (0.58 ± 0.6) and thermal shock (0.80 ± 0.6). The highest red intensity was observed due to haemorrhaging caused by the electric current applied during electronarcosis. Higher a* and lower L* values were observed in turbot stunned by electronarcosis (MORZEL *et al.*, 2003). Stunning by electricity is a fast and efficient method to render the fish insensitive and induce unconsciousness (ROBB and ROTH, 2003; LAMBOOIJ *et al.*, 2004). However, injuries, such as vertebra fractures and arterial disruption, caused by this kind of stunning can promote blood stains and haemorrhaging in the meat (ROTH, 2003), compromising product quality. Thus, further studies investigating different electric shock variables (voltage, amperage, etc.) are necessary to improve L* and a* parameters of fillets.

Instrumental texture

Texture was measured with a texturometer to evaluate if the tested methods could cause alterations in muscle texture; however, no differences in fillet resistance were observed when compression strength was applied, regardless of the kind of treatment. In contrast, compression strength diminished during cold storage (Fig. 3). During cold storage, fish suffer degradation in muscle structure owing to protein denaturation and enzyme actions, causing fish muscle to become less rigid, particularly in the first 24 h after slaughter (TOYOHARA and SHIMIZU, 1988; OKA *et al.*, 1990; MOCHIZUKI and SATO, 1996). ROTH *et al.* (2007) did not observe differences in fillet resistance after comparing different stunning methods (brain percussion, bleeding and electronarcosis) and meat texture in turbot.

In summary, the European Union regulations were not effective for this species because some

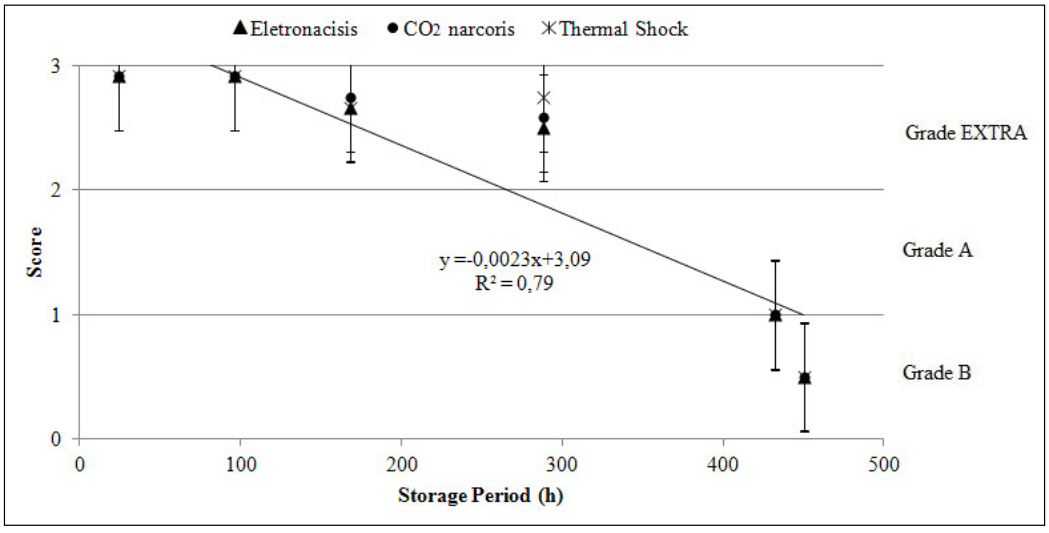

Fig. 2 - Sensory evaluation of matrinxã stunned by electronarcosis, CO_2 narcosis and Thermal Shock stored under refrigeration during 432 hours.

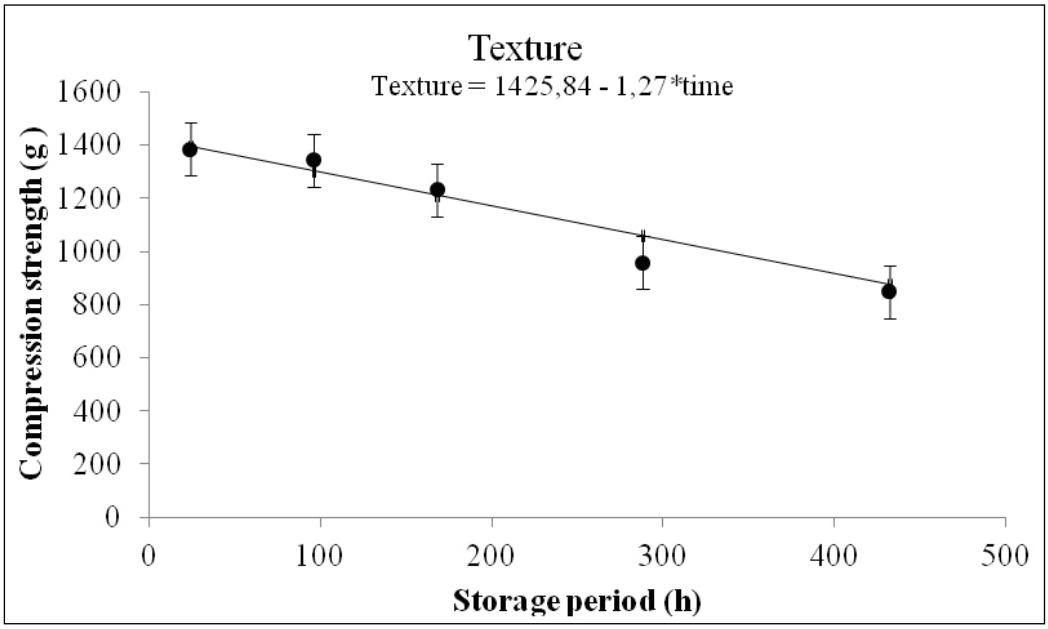

Fig. 3 - Texture Variation of matrinxã fillets stunned by electronarcosis, CO_2 narcosis and thermal shock during refrigeration storage for 432 hours.

evaluation parameters, such as gills and abdominal cavity odour, only occur in typical sea fish. Therefore, developing a specific quality index method (QIM) for matrinxã or utilising an existent scheme for species with similar habitats and feeding behaviour is recommended. Further investigations concerning voltage variations as well as the stunning time and its consequences on fish quality are suggested with the purpose of minimising or eliminating possible damage caused by haemorrhages associated with electric shock.

CONCLUSIONS

The three stunning methods tested were effective in maintaining meat quality when applied to matrinxã. Stunning by electronarcosis seemed slightly deficient when considering colour because of the hemorrhages caused by this method suggests the need for further research. However, the haemorrhaging did not compromise other quality parameters. Electronarcosis is considered to be the most efficient method for matrinxã welfare because it seemed to clinically indicate unconsciousness faster than the other treatments.

REFERENCES

Acerete L., Balasch J.C., Espinosa E., Josa A. and Tort L. 2004. Physiological responses in Eurasian perch (*Perca fluviatilis*, L.) subjected to stress by transport and handling. Aquaculture. 237: 167-178.

Acerete L., Reig L., Alvarez D., Flos R. and Tort L. 2009. Comparison of two stunning/slaughtering methods on stress response and quality indicators of European sea bass (*Dicentrarchus labrax*). Aquaculture. 287: 139-144.

Almeida N.M.De, Batista G.M., Kodaira M. and Lessi E. 2006. Post-mortem alterations in tambaqui (*Colossoma macropomum*) stored in ice. Alterações post-mortem em tambaqui (*Colossoma macropomum*) conservados em gelo. Cienc. Rural 36: 1288-1293.

Ashley P. 2007. Fish welfare: Current issues in aquaculture. Appl. Anim. Behav. Sci. 104: 199-235.

Azam K., Strachan N.J.C., Mackie I.M., Smith J. and Nesvadba P. 1990. Effect of slaughter method on the progress of rigor of rainbow trout (*Salmo gairdneri*) as measured by an image processing system. J. Food Sci. Technol. 25: 477-482.

Bagni M., Civitareale C., Priori A., Ballerini A., Finoia M., Brambilla G. and Marino G. 2007. Pre-slaughter crowding stress and killing procedures affecting quality and welfare in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*). Aquaculture. 263: 52-60.

Bito M., Yamanda K., Mikumo Y. and Amano K. 1983. Studies on rigor mortis of fish. I. Difference in the mode of rigor mortis among some varieties of fish by modified cutting methods. Bulletin Tookai Reg. Fish Res. Lab. 109: 89-96.

Conte F. 2004. Stress and the welfare of cultured fish. Appl. Anim. Behav. Sci. 86: 205-223.

Curran C., Poulten R., Brueton A. and Jones N. 1986. Cold shock reactions in iced tropical fish. J. Food Technol. 21: 289-299.

Einen O., Guerin T., Fjaera S. and Skjervold P. 2002. Freezing of pre-rigor fillets of Atlantic salmon. Aquaculture. 212: 129-140.

Gomes L., Baldisserotto B. and Senhorini J. 2000. Effect of stocking density on water quality, survival, and growth of larvae of the matrinxã, Brycon cephalus (Characidae), in ponds. Aquaculture. 183: 73-81.

Huss H.H. 1997. Control of indigenous pathogenic bacteria in seafood. Food Control. 8: 91-98.

Kestin S., van de Vis J.W. and Robb D. 2002. Protocol for assessing brain function in fish and the effectiveness of methods used to stun and kill them. Vet. Rec. 150: 302-307.

Kiessling A., Espe M., Ruohonen K. and Mørkøre T. 2004. Texture, gaping and colour of fresh and frozen Atlantic salmon flesh as affected by pre-slaughter iso-eugenol or CO_2 anaesthesia. Aquaculture. 236: 645-657.

Lambooij E., Grimsbø E., van de Vis J.W., Reimert H.G.M., Nortvedt R. and Roth B. 2010. Percussion and electrical stunning of Atlantic salmon (*Salmo salar*) after dewatering and subsequent effect on brain and heart activities. Aquaculture. 300: 107-112.

Lambooij E., Kloosterboer R., Gerritzen M. and van de Vis J.W. 2006a. Assessment of electrical stunning in fresh water of African Catfish (*Clarias gariepinus*) and chilling in ice water for loss of consciousness and sensibility. Aquaculture. 254: 388-395.

Lines J.A., Robb D.H., Kestin S.C., Crook S.C. and Benson T. 2003. Electric stunning: A humane slaughter method for trout. Aquacultural Eng. 28: 141-154.

Marx H., Brunner B., Weinzierl W., Hoffmann R. and Stolle A. 1997. Methods of stunning freshwater fish: Impact on meat quality and aspects of animal welfare. Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung a-Food Res. Tech. 204: 282-286.

Misimi E., Erikson U., Digre H., Skavhaug A. and Mathiassen J.R. 2008. Computer vision-based evaluation of pre- and postrigor changes in size and shape of Atlantic cod (*Gadus morhua*) and Atlantic salmon (*Salmo salar*) fillets during rigor mortis and ice storage: Effects of perimortem handling stress. J. Food Sci. 73: E57-E68.

Morzel M. and van de Vis H. 2003. Effect of the slaughter method on the quality of raw and smoked eels (*Anguilla anguilla* L.). Aquaculture Res. 34: 1-11.

Morzel M., Sohier D. and Van de Vis H. 2003. Evaluation of slaughtering methods for turbot with respect to animal welfare and flesh quality. J. Sci. Food Agric. 83: 19-28.

Oliveira R.F. and Galhardo L. 2007. Sobre a aplicação do conceito de bem-estar a peixes teleósteos e implicações para a piscicultura. Revista Brasileira de Zootecni. 36, Suplemento especial: 77-86.

Parisi G., Franci O. and Poli B. 2002. Application of multivariate analysis to sensorial and instrumental parameters of freshness in refrigerated sea bass (*Dicentrarchus labrax*) during shelf life. Aquaculture. 214: 153-167.

Poli B.M., Parisi G., Scappini F. and Zampacavallo G. 2005a. Fish welfare and quality as affected by pre-slaughter and slaughter management. Aquaculture Int. 13: 29-49.

Poli B.M., Parisi G., Scappini F. and Zampacavallo G. 2005b. Fish welfare and quality as affected by pre-slaughter and slaughter management. Aquaculture Int. 13: 29-49.

Proença C.E.M. and Bittencourt P.R.L. 1994. Manual de Piscicultura tropical. Ministério do Meio Ambiente e da Amazônia Legal, Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, Brasília.

Ribas L., Flos R., Reig L., MacKenzie S., Barton B. and Tort L. 2007. Comparison of methods for anaesthetizing Senegal sole (*Solea senegalensis*) before slaughter: Stress responses and final product quality. Aquaculture. 269: 250-258.

Robb D. and Kestin S. 2002a. Methods used to kill fish: Field observations and literature reviewed. Anim. Welf. 11: 269-282.

Robb D., Wotton S., McKinstry J., Sorensen N. and Kestin S. 2000a. Commercial slaughter methods used on Atlantis salmon: determination of the onset of brain failure by electroencephalography. Vet. Rec. 147: 298-303.

Robb D.H.F. and Kestin S. C. 2002b. Methods used to kill fish: Field observations and literature reviewed. Anim. Welf. 11: 269-282.

Robb D.H.F. and Roth B. 2003. Brain activity of Atlantic salmon (*Salmo salar*) following electrical stunning using various field strengths and pulse durations. Aquaculture. 216: 363-369.

Robb D.H.F., O'Callaghan M., Lines J.A. and Kestin S.C. 2002. Electrical stunning of rainbow trout (*Oncorhynchus mykiss*): Factors that affect stun duration. Aquaculture. 205: 359-371.

Robb D.H.F., Phillips A.J. and Kestin S.C. 2003. Evaluation of methods for determining the prevalence of blood spots in smoked Atlantic salmon and the effect of exsanguination method on prevalence of blood spots. Aquaculture. 217: 125-138.

Robb D.H.F., Wotton S.B., McKinstry J.L., Sørensen N.K. and Kestin S.C. 2000b. Commercial slaughter methods used on Atlantic salmon: Determination of the onset of brain failure by electroencephalography. Vet. Rec. 147: 298-303.

Romagosa E., Narahara M., Borella M., Parreira S. and Fenerich-Verani N. 1999. Ultrastructure of the germ cells in the testis of matrinxa, *Brycon cephalus* (Teleostei, Characidae). Tissue Cell. 540-544.

Roth B., Slinde E. and Robb D.H.F. 2006. Field evaluation of live chilling with CO_2 on stunning Atlantic salmon (*Salmo salar*) and the subsequent effect on quality. Aquaculture Res. 37: 799-804.

Ruff N., FitzGerald R., Cross T. and Kerry J. 2002. Fillet shelf-life of Atlantic halibut *Hippoglossus hippoglossus* L. fed elevated levels of α-tocopheryl acetate. Aquaculture Res. 33: 1059-1071.

Scherer R., Augusti P.R., Steffens C., Bochi V.C., Heckthueuer L.H., Lazzari R., Radünz-Neto J., Pomblum S.C.G. and Emanuelli T. 2005. Effect of slaughter method on postmortem changes of grass carp (Ctenopharyngodon idella) stored in ice. J. Food Sci. 70: C348-C353.

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PAPER

EMPLOYING ARTIFICIAL NEURAL NETWORKS AND REGRESSION IN ANALYSIS ON KNOWLEDGE ABOUT SWEET POTATO (*IPOMOEA BATATAS* L.) IN SLOVENIA

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ABSTRACT

This article analyses factors affecting the reputation of sweet potato (*Ipomoea batatas* L.) among people in Slovenia. The inquiry, which included 7 general questions and 19 questions in particular about sweet potato, was completed by 712 respondents. The aim was to find out which factors impact the knowledge about sweet potato, the relations between answers to various questions regarding sweet potato features and willingness of people to know, to buy and to grow it. The methods applied were the Radial basis function neural networks and multiple linear and logistic regressions. It was established that persons with agricultural education are experts and know sweet potato best. Persons from large families are also familiar with it, but to a smaller degree. The answers to 8 questions about sweet potato features were very consistent, since we found out that every answer can be predicted with 98% probability (on the basis of the answers to the other 7 questions). Significant covariates in regression show that the most likely persons to know/buy/grow sweet potato are the people with agricultural education. Older persons are more interested in curative features of sweet potato, while younger and better educated believe in stronger nutritional values. Female respondents are more likely to grow sweet potato than men. Net income also influences willingness to buy sweet potato, because people living with children are more likely to be willing to attend free lectures about sweet potato.

- Keywords: sweet potato, questionnaire, artificial neural networks, regression, Slovenia -

INTRODUCTION

The cultivated sweet potato, *Ipomoea batatas* (L.) Lam., (an autohexaploid species) is a dicotyledonous vegetable plant (AUSTIN and HUAMÁN, 1996). The plant is a perennial but is grown as an annual by vegetative propagation using either storage roots or stem cuttings (ESCALANTE-SÁNCHEZ *et al.*, 2008).

Although sweet potato originated in Central or South America, the world production is centred in Asia. China is the world's leading producer, accounting for 83% of the world supply at 117 million ton produced annually on 5.1 million hectares (JUNG *et al.*, 2011).

In spite of its name, the sweet potato is an entirely different vegetable than potatoes (*Solanum tuberosum* L.). Sweet potatoes belong to the morning glory family (Convolvulaceae), while potatoes are members of the Solanaceae family. Sweet potato roots, leaves, and shoots (stems) are used for table food, starch production, food processing and many other purposes such as livestock feeds and brewing (TAKAHATA *et al.*, 2011). Sweet potatoes are a nutritious food, low in fat and proteins, but rich in carbohydrates (BURRI, 2011). Tubers and leaves are good sources of crude protein, minerals, vitamins and carotenoids (TEOW *et al.*, 2007; FONSECA *et al.*, 2008). Several Slovenian investigators reported that a great deal of interest has been devoted to bioactive food components and phytochemicals found in fruits and vegetables in the past few years, due to the possibility of having nutritional benefits (MIKULIČ PETKOVŠEK *et al.*, 2011; SCHMITZER *et al.*, 2011; SLATNAR *et al.*, 2011; KOCJAN AČKO, 2012).

Sweet potato is one of the most efficient food crops in terms of caloric value per land area, with many agronomic advantages for marginal land (WOOLFE, 1992), because it is relatively easy to grow (van OIRSCHOT *et al.*, 2003). Despite of this the crop is a relatively unknown vegetable in Europe.

The aim of this study is to explore the knowledge and perceptions of people in Slovenia about sweet potato. A questionnaire had been designed and filled by 712 persons of age 18 or older, sampled randomly. It consisted of 7 general questions and 19 questions about sweet potato. The aim was to determine how many people know sweet potato, which factors impacted the knowledge about sweet potato, the relations between answers to various questions regarding sweet potato features and willingness of people to know, to buy and to grow the crop. Besides using basic statistics, correlations and regressions, artificial neural networks – as more advanced data analysis methods – were also applied.

Artificial neural networks (ANNs) are mathematical models, inspired by natural neural networks. An ANN consists of an interconnected group of processing units or neurons, which usually perform a non-linear function. The connections between neurons have their associated weights, which act as free parameters of the model. By proper setting of the weights, some desired relationship between inputs and outputs are set. In most cases it is hard or even impossible to calculate desired weights directly and instead another procedure called learning or training is performed, where the difference between actual and desired outputs, called error, is gradually minimized. ANNs may be viewed as a complex type of regression. They are trained on a training set, where inputs and outputs are known and used later for predicting outputs for patterns or examples with known inputs only (MITTAL *et al.*, 2000; GHAMARI *et al.*, 2010).

ANNs are capable of generalizing; they are tolerant to errors and need no apriority model. They are also capable of modelling non-linear relationships in data. However, there is no general way to determine the optimal number of neurons for a certain problem. It is also possible that the network over-fits the data, which reduces its generalization ability. Besides, the knowledge stored in the network is not easy to interpret (SEYHAN *et al.*, 2005).

In agriculture, the most frequently applied artificial neural network is the Multi-layer perceptron (MLP). CHEN (2005) discussed the advantages and disadvantages of ANNs for use in agriculture. He concluded that ANNs generally outperform econometric methods (meaning standard statistical methods such as regression). Their limitations included a lack of tests for statistical significance. ANNs are perhaps best viewed as supplements to standard methods, not as substitutes.

ANNs were used by several authors for various purposes. According to GHAMARI *et al.* (2010), terminal velocity of agricultural seeds was predicted with artificial neural networks. On the other hand, ŠTASTNY *et al.* (2011) predicted crop yield levels using MLP. ANNs were also applied as classifiers in image analysis of agricultural products (JAYAS *et al.*, 2000). DRUMMOND *et al.* (1998) applied ANN methods to functionally relate soil properties and crop yields. TERZI and ONAL (2012) used ANNs and regression to forecast monthly river flow. ANNs were further used for modeling the impact of climate changes, based on precipitation over the mountains and the basin area coupled with stream flow, on irrigation water supplies (ELGAALI and GARCIA, 2007).

A radial basis function (RBF) neural network is in its predicting capabilities very similar to the MLP, although it works quite differently. RBF neurons use different activation functions and the training procedure usually begins with clustering. We are not aware of any application in the field of agriculture which has applied RBF

networks, which, of course, still does not mean that it does not exist.

Using RBF networks and regression, the study aimed to predict certain variables from a questionnaire on knowledge about sweet potato among citizens of Slovenia, using subsets of other variables. While regression analysis usually gives more explanative results, RBF networks generally yield higher classification accuracy.

Many studies have been published about the willingness to buy or grow food. LAURIE and van HEERDEN (2012) studied the consumer acceptability of four products made from β-carotene-rich sweet potato in South Africa. The acceptability of sweet potato products was high (about 90%). 92% of the consumers liked the colour of the four products, 87% were willing to purchase the products and 88% would produce these products at home. Willingness to buy and to grow these products were mostly indicated in the older respondents.

LEIGHTON *et al.* (2010) reported the consumer taste preference for white-fleshed sweet potato and orange-fleshed sweet potato (OSP). They found a preference for OSP, in terms of both preferring the taste and liking the colour. The majority of respondents indicated a willingness to buy OSP.

WALKER *et al.* (2009) studied the prevalence and reasons for sweet potato use in child nutrition programs in the Southeastern United States. Significantly more white-potato menu items were offered and generated significantly less waste than sweet potato menu items. The most frequently cited reasons for willingness to use potatoes were having more recipes acceptable to children, controlling cost and meeting nutrition guidelines.

CHOWDHURY *et al.* (2011) attempted to assess whether consumers in Uganda will accept the orange sweet potato (OSP), which is high in β-carotene and can reduce the prevalence of vitamin A deficiency. The paper attempts to quantify the magnitude of the premium or discount in consumers' willingness to pay (WTP). The results suggest that the provision of nutrition information leads to substantial premia for the orange varieties (vs. white) and that taste plays an important role in consumer acceptance, indicating that an information campaign may be key to driving market acceptance of the product.

VOON *et al.* (2011) investigated the determinants of willingness to purchase organic food among consumers in a Malaysian city. A major theory on consumer behavior is the Theory of planned behavior by AJZEN (1991). It argues that an individual's intention to perform a behavior is influenced by a combination of behavioral attitudes (beliefs about the desirability of behaviors); subjective norms (perceived relevance and importance of opinions of significant others); and behavioral control (sense of control over behavior). Attitude, subjective norms and affordability (behavioral control) were modeled to impact willingness to pay (WTP) for organic food. Attitude and subjective norms exerted significant positive effects on WTP while the effect of affordability was not significant. Attitude influenced subjective norms and affordability, thus indicating that a promotion of consumption growth should focus on influencing consumer attitudes.

JEKANOWSKI *et al.* (2000) studied demographic and attitudinal factors which are most important in predicting the likelihood of consumers to purchase products that are produced within the local state (Indiana). The results indicated that the willingness to purchase locally produced agricultural products increases with time of residency in the state, and there is a greater tendency for female consumers to purchase such products. They also find that quality perceptions played a critical role in these food purchase decisions.

MATERIALS AND METHODS

Data

A total of 712 persons in Slovenia were interviewed. Each person had to be 18 years or older. The respondents were selected randomly. There were no other inclusion or exclusion criteria.

Each record (a single person's data) consisted of the following variables:

1. Basic data about the person, such as Gender, Age, Status, Education, Type of Education, Net income, Number of family members and questions like "Where do you shop mostly?" These variables will be called "property variables" (Table 1).
2. Answers to the questions about sweet potato, such as "Do you know sweet potato?", "Where have you encountered this plant?", "What is the utility of the plant?" and others are shown in Table 2. These can further be divided into two groups:
   a) Answers to the questions about knowing sweet potato and its features, called **"**knowledge variables**"**.
   b) Answers to the questions commencing with "Would", such as "How would you enlarge the promotion of the plant?", "Would you be willing to attend a free of charge lecture about it?" and similar. These will be called "conditional variables".

Statistical methods

Linear and logistic regression

Multiple linear regression (DRAPER and

Table 1 - Property variables.

Properties	Values					
Gender	Male (25.0%)	Female (74.1%)				
Age	18-25 (25.0%)	26-35 (23.2%)	36-45 (28.6%)	-55 (15.2%)	56-65 (4.5%)	66 or more (3.6%)
Status	Married or with partner (63.4%)	Single (18.8%)	Living with parents (9.8%)	Single parent (3.6%)	Divorced (1.8%)	Widow/er (0.9%)
Education	Primary (1.8%)	Vocational (11.6%)	Secondary (41.1%)	High or university (38.4%)	MSc or PhD (7.1%)	
Type of education	Agricultural (26.8%)	Nutritional (1.8%)	Other (67.0%)			
Net income	< 400 EUR (17.9%)	401-500 EUR (1.8%)	501-750 EUR (14.3%)	751-1000 EUR (15.2%)	1001-1500 EUR (24.1%)	> 1500 EUR (10.7%)
Num. of family members	1 (7.1%)	2 (19.6%)	3 (11.6%)	4 (24.1%)	5 (15.2%)	6 or more (12.5%)
Shopping (where)	Local stores (43.8%)	Shopping centers (51.8%)	Market halls (0.9%)	Ecological markets (0.9%)	Ecological stores (2.7%)	

SMITH, 1998) assumes that the dependent variable is a linear function of independent variables:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p + \varepsilon$$

It is also assumed that the error term ε is normally distributed with E(ε) = 0 and with a constant variance Var(ε) = σ^2. Since the system is usually over-determined, the exact solution does not exist. Model coefficients β_0, β_1, ..., β_p are determined by the method of least squares, which minimizes the sum of squared errors.

Logistic regression (AGRESTI, 2007) applies the logistic function on a linear combination of independent variables:

$$y = \frac{1}{1 + e^{-(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_p x_p)}}$$

The logistic function returns values between 0 and 1 and is useful for modelling binary or dichotomous variables. NAGELKERKE (1992) generalized R^2 to be used as the coefficient of determination:

$$R^2 = 1 - \left(\frac{L(0)}{L(\hat{\theta})}\right)^{2/n}$$

where $L(0)$ is the likelihood of the model with only the intercept, $L(\hat{\theta})$ is the likelihood of the estimated model, and n is the sample size.

The RBF neural network

We also applied a non-linear classification/regression method, the Radial basis function or RBF neural network (MOODY and DARKEN, 1989; POGGIO and GIROSI, 1990; HAYKIN, 1999). It is able to "learn" specified input-output relationships of (usually) previously collected data (training set). By learning is meant adaptation of free parameters of the network, also called weights.

The RBF network has three layers:

The input layer, which merely distributes inputs to the hidden layer.

The hidden layer, which consists of processing units, also called neurons, with radial functions response.

The output layer, whose outputs are linear combinations of hidden layer's basis functions.

Fig. 1 shows a RBF neural network and its typical output. The latter is for a case with only two inputs, otherwise it could not be presented graphically. The RBF network may also have more output neurons.

An output unit performs the following function:

$$\tilde{F}(\mathbf{x_i}) = \sum_{j=1}^{K} w_j \varphi_j(\mathbf{x}) = \sum_{j=1}^{K} w_j \varphi(\|\mathbf{x_i} - \mathbf{t_j}\|)$$

where $\mathbf{x}_i$ is the i-th input vector and φ is the radial basis function, usually Gaussian:

$$\varphi(r) = e^{-\frac{1}{2}\left(\frac{r}{\sigma}\right)^2}$$

with width σ. The center vector of the j-th Gaussian is denoted by $\mathbf{t}_j$.

If Φ is a matrix with elements $\Phi_{ij} = \varphi(||\mathbf{x}_i - \mathbf{t}_j||)$, we have a system of linear equations:

$$\Phi \boldsymbol{w} = \boldsymbol{d}$$

where $\boldsymbol{d}$ is the vector of target or desired outputs and $\boldsymbol{w}$ is the vector of weights. Since the system

is usually over-determined, an exact solution is generally not possible, only approximation. The least squares solution is:

$$w = \Phi^{+} d = (\Phi^{T} \Phi)^{-1} \Phi^{T} d$$

and Φ^{+} is called pseudo-inverse.

The number of hidden units is the only factor that influences the complexity of the RBF function. When this number is too large, the network may adapt itself too much to the training set (over-fitting) and perform badly on other data from the same population (testing set). On the other hand, when the number of hidden units is too small, the network is unable to capture relevant features of the task presented.

RESULTS AND DISCUSSION

Basic statistics and correlations

Tables 1 and 2 show the answers to all questions in percentages. Of all respondents, 24.1% knew sweet potato well, 37.5% knew the crop only superficially, and 38.4% did not know it at all.

From the raw data an additional variable called "Knowledge" was constructed, which was the sum of eight knowledge variables (questions from "What is the utility of the plant?" to "What are its healing effects?" in Table 2), using answers other than "Don't know". All the other possible answers were more or less meaningful, i.e., none of them was false, so it is not sensible to measure how many answers were correct. There were eight strict knowledge variables, so "Knowledge" had integers from 0 to 8 as possible values. It may be considered as a sort of score at a test. Figure 2 shows the distribution of "Knowledge". The mean value was 3.86; thus a little less than 4, which is exactly a half of the maximal value (8).

Twenty persons or 17.9% had the score of 0, although 38.4% of people claimed not to be familiar with sweet potato. It means that half of these people either knew something or simply guessed some of the answers.

The first type of analysis performed was calculation of the Pearson product-moment correlation coefficient between variables. We did not calculate all possible correlations, since some of them were not of interest in terms of sweet potato. For example, mutual correlations between property variables (such as significant positive correlations between "Education level" and "Net income" or between "Age" and "Status: married", or negative correlations between "Age" and "Status: with parents" or between "Education level" and "Status: with parents", etc.).

One of the most significant correlations was between "Education: agricultural" and "Do you know sweet potato?", $r = 0.257$ at significance $p < 0.01$. Then, answers "Don't know" are mostly correlated with each other, which is also logical, since persons who do not know some facts about sweet potato are likely to not know some other facts. Actually, 20 persons knew absolutely nothing about sweet potato, as shown in Fig. 2, which presents the scores that respondents obtained on questions about knowledge of sweet potato.

We also calculated the correlation coefficient between "Knowledge" and "Do you know sweet potato?" and thus verified that persons who claimed to know sweet potato actually knew something about it. The correlation was very significant ($r = 0.662^{**}$ at $p = 0.000$). Further, it was interesting to measure the correlation of "Knowledge" with some of the conditional variables: correlation with "Are you willing to attend a free of charge lecture about it?" was $r = 0.185$ at

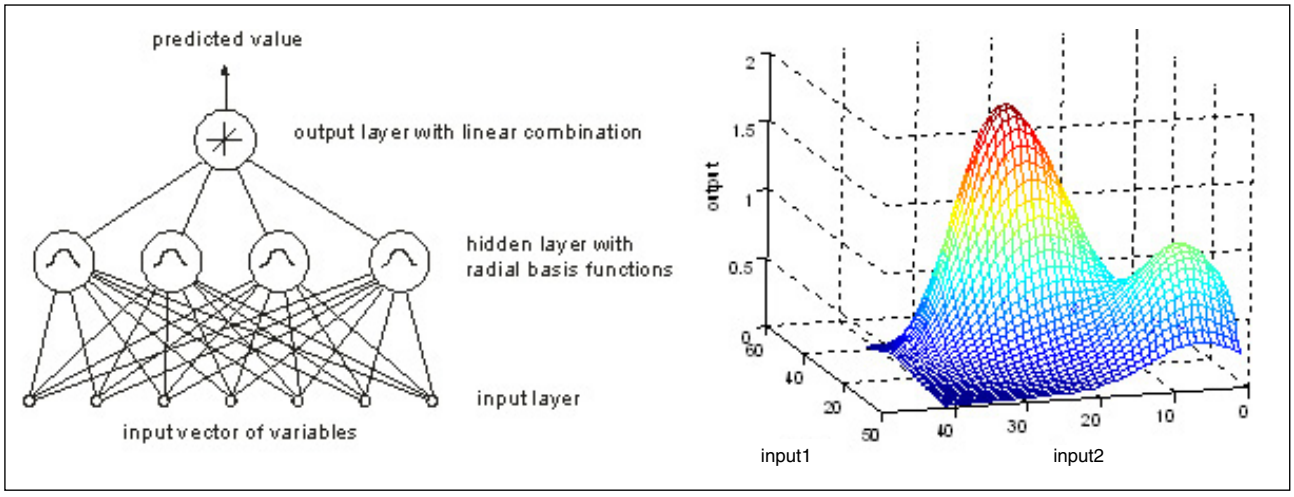

Fig. 1 - RBF neural network and its typical output.

Table 2 - Knowledge and conditional variables.

Questions	Possible answers (frequencies in percent*)						
Do you know sweet potato?	No (38.4%)	I know it merely from pictures/talking (only superficially) (37.5%)	Yes, I know it well (24.1%)				
Where have you encountered this plant?	In a local store center (5.4%)	In a shopping hall (12.5%)	At market store (5.4%)	In garden (9.8%)	At acquaintances (10.7%)	Other (7.1%)	Nowhere yet (46.4%)
What is the utility of the plant?	Decorative (20.5%)	Nutritional (38.4%)	In textile (0.9%)	Don't know (39.3%)			
Does the plant originate from tropical areas?	Yes (15.2%)	No (16.1%)	Perhaps (30.4%)	Don't know (38.4%)			
What are the reasons for its small use?	Too small production in Slovenia (19.6%)	Consumers mainly buy food produced in Slovenia (14.3%)	Insufficient promotion (53.6%)	Other (8.9%)			
What are its temperature demands?	High temperatures (8.0%)	Undemanding (8.0%)	Ordinary temperatures (12.5%)	Don't know (69.6%)			
Which type of propagation it uses?	With tubers (31.2%)	With stem cuts and leaf cuts (4.5%)	With tubers, stem cuts and leaf cuts, micro-propagation (6.2%)	Don't know (57.1%)			
Which are the usable parts of the plant?	Tender leaves (1.8%)	Tubers (52.7%)	Leaves and tubers (4.5%)	Don't know (41.1%)			
Which tuber color is the most frequent?	White (17.0%)	Yellow and orange (18.8%)	Red, rose and purple (18.8%)	Don't know (45.5%)			
What are its healing effects?	Lowering cholesterol, strengthening of immune system (7.1%)	Lowering possibility of stroke, preventing coagulation (5.4%)	For diabetes (15.2%)	Don't know (72.3%)	Other (0.0%)		
Which properties do the ordinary potato and the sweet potato share?	Tuber color (17.0%)	Tuber shape (19.6%)	External structure (leaves, stem, flower) (12.5%)	Don't know (50.9%)			
Do you use it in nutrition?	Yes (15.2%)	No (82.1%)					
How frequently do you use it?	Regularly (7.1%)	Frequently (0.0%)	Seldom (5.4%)	Used only once (3.6%)	Don't use it (83.9%)		

Table 2 - Continued.

Questions	Possible answers (frequencies in percent*)				
For what purpose do you use it in nutrition?	As a dessert (2.7%)	As cooked vegetable side dish (9.8%)	As baked vegetable side dish (1.8%)	Other (1.8%)	
How would you enlarge the promotion of the plant?	With stronger advertising in shopping and garden centers (29.5%)	With stronger promotion in nutritional profession (21.4%)	With stronger promotion as a healing plant (19.6%)	With larger quantities of produced plant (13.4%)	Other (5.4%)
Are you willing to attend a free of charge lecture about it?	Yes (27.7%)	No(11.6%)	Don't know, depends on time (50.9%)	Not interested (6.2%)	
Are you willing to buy it due to its healing effects?	Yes (50.9%)	No (8.0%)	Don't know (37.5%)		
Are you willing to grow (produce) it for your own use?	Yes (30.4%)	No (14.3%)	Maybe, with the help of agricultural advisers (18.8%)	Don't know (32.1%)	

* due to missing values not all frequencies sum to 100.0%

Fig. 2 - Distribution of "Knowledge": number of persons who responded meaningfully to a specified number of questions (knowledge variables).

p = 0.055 and with "Are you willing to grow (produce) it for your own use?" it was r = 0.221* at p = 0.022. Willingness to buy sweet potato is positively correlated with the net income. Besides, persons with agricultural education use sweet potato more frequently than others.

Regression and RBF network

The next type of analysis performed was regression. The purposes of regression are twofold: a) to predict the dependent variables and b) to observe relationships between dependent and independent variables. Linear (multiple) regression was performed on quantitative variables, as well as on ordered categorical variables, which were previously quantified. For non-ordered (or qualitative) categorical variables, it is possible to perform logistic regression on the individual values of the variables. Thus, multiple regressions on any single variable had to be performed.

Since the variable "Do you know sweet potato?" had three ordered values, a linear multiple regression model to predict it could be built. The property variables were placed as the independent ones. The results showed that R was only 0.419 (R^2 = 0.176) and that the only significant (p = 0.004) variable in this model was "Education: agricultural" with B = 0.658 and standardized β = 0.372. Obviously, it was hard to predict the knowledge of sweet potato solely on the basis of property variables, which were probably too general for such a purpose. It was suspected that people's inclination towards plants and towards cooking might play major roles here.

It is also possible to perform the logistic regression when the question "Do you know sweet

Table 3 - Classification table for dependent variable "Do you know sweet potato?" using a RBF neural network.

		Predicted			
	Observed	**Don't know**	**Know only superficially**	**Know**	**Percent Correct**
Training	Don't know	21	3	4	75.0%
	Know only superficially	10	9	1	45.0%
	Know	4	1	6	45.5%
	Overall Percent	59.3%	22.0%	18.6%	61.0%
Testing	Don't know	4	2	1	57.1%
	Know only superficially	4	6	0	60.0%
	Know	4	0	5	55.6%
	Overall Percent	46.2%	30.8%	23.1	57.5%

potato?" has only two answers: "I know it" (combining "I know it merely from pictures/talking" and "I know it well") and "Don't know". In this case Nagelkerke's coefficient of determination R^2 = 0.220, the percentage of correct classification was 61.2%, and the only significant (p = 0.042) variable was again "Education: agricultural" with B = 1.361.

Furthermore a RBF neural network was applied to predict the answers to the question above. The number of hidden units was optimized with respect to the results on a separate validation set. In this case there were 9 hidden units. Table 3 presents the classification table for this case. Ideally, only diagonal entries of the classification table would be nonzero - others are miss-classifications.

It can be stated that persons who do not know sweet potato were mainly classified as such (75%), only seven of them were predicted as to know sweet potato. It is quite obvious that it was hard to determine whether a person knows sweet potato (well) or knows it only superficially. Due to this observation, we tried to predict the modified variable "Do you know sweet potato?" that only has two values: "I know it" (consisting of "Know only superficially" and "I know it well") and "Don't know". That time the obtained results were a bit better (Table 4). However, it is hard to expect the results to be better for this case, since only the property variables were the independent ones.

Table 4 - Classification table for dependent variable "Do you know sweet potato?". This time the variable is dichotomous or binary – it has two values: "Don't know" and "Know", which subsumes "Know only superficially" and "Know".

		Predicted		
	Observed	**Don't know**	**Know**	**Percent Correct**
Training	Don't know	10	11	47.6%
	Know	4	20	83.3%
	Overall Percent	31.1%	68.9%	66.7%
Testing	Don't know	4	8	33.3%
	Know	4	17	81.0%
	Overall Percent	24.2%	75.8%	63.6%

Next, regression analysis was performed on individual knowledge variables, taking the remaining knowledge variables as independent (but of course, not taking into account the remaining values of the dependent variable). For example, when running regression on "Tuber color is white", "Tuber color is yellow or orange" was not taken as an independent variable.

For instance, separate logistic regressions were performed on all values of "Which properties do the ordinary and the sweet potato share?". For "Similarity - Tuber color", Nagelkerke's coefficient was R^2 = 1.000, for "Similarity - Tuber shape" Nagelkerke's R^2 = 0.610, for "Similarity - Structure" Nagelkerke R^2 = 0.963, and for "Similarity - Don't know" Nagelkerke R^2 = 0.961. The classification values for all four cases are presented in Table 5.

In case of "Similarity - Tuber shape", variable "Utility - Nutritional" had B = 8.2 at p = 0.095, so the two variables were considered to be correlated. The results showed that people who believe that the tuber shape of sweet potato is similar to the tuber shape of a potato, also believe that sweet potato has nutritional value.

These results and the results for the other seven knowledge variables are presented in Table 6. A striking result was obtained in this regard: the probability to predict the answer to any of these eight questions (on the basis of the answers to the other seven questions) is very high; 98% on average.

We also tried to predict some of the conditional variables on the basis of property variables and some of the other conditional variables. When predicting "Are you willing to attend a free of charge lecture about it?" the classification accuracy is 73.8% on the training set and 82.4% on the testing set. There were only two target

Table 5 - Classification tables for variables "Similarity - Tuber color", "Similarity - Tuber shape", "Similarity - Structure", and "Similarity - Don't know".

		Predicted Similarity - Tuber color		
Observed		No	Yes	Percent Correct
Similarity - Tuber color	No	52	0	100.0%
	Yes	0	17	100.0%
	Overall Percentage			100.0%
		Predicted Similarity - Tuber shape		
Observed		No	Yes	Percent Correct
Similarity - Tuber shape	No	50	2	96.2%
	Yes	3	14	82.4%
	Overall Percentage			92.8%
		Predicted Similarity - Structure		
Observed		No	Yes	Percent Correct
Similarity - Structure	No	57	0	100.0%
	Yes	1	11	91.7%
	Overall Percentage			98.6%
		Predicted Similarity - Don't know		
Observed		No	Yes	Percent Correct
Similarity - Don't know	No	45	1	97.8%
	Yes	1	22	95.7%
	Overall Percentage			97.1%

Table 6 - Results of logistic regression on knowledge variables. Logistic regression was performed on each answer separately, i.e. 32 times. Each time the independent variables were the answers to the other 7 questions, i.e. 28 variables.

	Values, Nagelkerke R^2, Classification accuracy			
Which properties share the ordinary and sweet potato?	Tuber color 1.000, 100.0%	Tuber shape 0.610, 92.8%	Structure 0.963, 98.6%	Don't know 0.961, 97.1%
Which tuber color is the most frequent?	White 1.000, 100.0%	Yellow or orange 0.980, 98.6%	Red, rose and purple 1.000, 100.0%	Don't know 0.984, 98.6%
Which are the usable parts of the plant?	Tender leaves 1.000, 100.0%	Tubers 1.000, 100.0%	Leaves and tubers 1.000, 100.0%	Don't know 1.000, 100.0%
Which type of propagation it uses?	With tubers 1.000, 100.0%	With stem cuts and leaf cuts 1.000, 100.0%	With tubers, stem cuts and leaf cuts, micro-propagation 1.000, 100.0%	Don't know 1.000, 100.0%
What are its temperature demands?	High temperatures 1.000, 100.0%	Undemanding 1.000, 100.0%	Ordinary temperatures 1.000, 100.0%	Don't know 1.000, 100.0%
Does the plant originate from tropical areas?	Yes 1.000, 100.0%	No 1.000, 100.0%	Perhaps 0.838, 92.8%	Don't know 0.859, 94.2%
What is the utility of the plant?	Decorative 1.000, 100.0%	Nutritional 0.691, 88.4%	In textile	Don't know .956, 98.6%
What are its healing effects?	Cholesterol 1.000, 100.0%	Stroke 1.000, 100.0%	Diabetes 0.578, 85.5%	Don't know 0.681, 88.4%

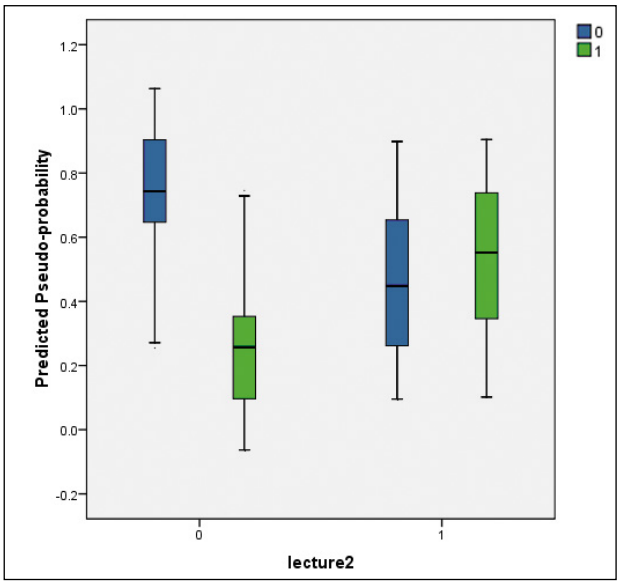

Fig. 3 - Predicted pseudo-probability versus actual outputs for the RBF neural network.

categories: "No or Don't know" and "Yes". Fig. 3 shows the predicted pseudo probability versus actual outputs for the RBF neural network. It is called pseudo probability, since the RBF network's outputs do not sum to 1.

When predicting "Are you willing to buy it due to its healing effects?", the classification accuracies were 78.0 and 68.4%. When predicting "Are you willing to grow (produce) it for your own use?", the classification accuracies are 73.8 and 69.2%.

Table 7 shows statistically significant covariates in regression on different variables. This is important, since it shows factors that best predict the variables. In logistic regression on "Utility: nutritional", there were also significant positive correlations with "Usable tubers" at p = 0.016, with "Usable leaves and tubers" at p = 0.078, and with "Healing stroke" at p = 0.080. "Do you use it in nutrition? "Yes" and "Education: nutritional" are positively correlated, which seems logical. The "Stronger promotion in nutritional profession" is positively correlated with education level and negatively with age, which means that younger and more educated people more firmly believe in the promotion of sweet potato as a vegetable with high nutritional values. Older people and persons from large families believe in stronger promotion as a healing plant. The last three questions "Are you willing to … ?" are positively correlated with "Education: agricultural", with "Status: with children", and with "Gender: female". Willingness to buy sweet potato is also correlated with the net income, which naturally makes sense.

CONCLUSIONS

The present study evaluated knowledge about sweet potato in Slovenia and factors that affect it. 712 respondents answered a questionnaire with 26 questions – 7 general and 19 particular about sweet potato. The main objective was to determine the degree of knowledge about sweet potato, which general factors impact the knowledge about it, relationships between answers, willingness to know/buy/grow sweet potato and factors that affect it.

Table 7 - Statistically significant covariates in regression (logistic or linear, dependent on the variable). Only those covariates are shown that significantly impact the dependent variable (an answer to a question on the left side).

Dependent variables	**Statistically significant covariates, sign of correlation (positive, negative), p**		
What is the utility of the plant? Nutritional	Usable parts: tubers, positive, p = 0.016	Usable parts: leaves and tubers, positive, p = 0.078	Healing stroke, positive, p = 0.080
Do you use it in nutrition? Yes/No	Status: single, negative, p = 0.088	Education nutritional, positive, p = 0.123	
How would you enlarge the promotion of the plant? With stronger promotion in nutritional profession	Age, negative, p = 0.039	Education level, positive, p = 0.060	
How would you enlarge the promotion of the plant? With stronger promotion as a healing plant	Age, positive, p = 0.089	Number of family members, positive, p = 0.057	
Are you willing to attend a free of charge lecture about it?	Status: with children, positive, p = 0.064	Education: agricultural, positive, p = 0.006	
Are you willing to buy it due to its healing effects?	Education: agricultural, positive, p = 0.134	Net income, positive, p = 0.086	
Are you willing to grow (produce) it for your own use?	Gender, positive (female), p = 0.088	Education: agricultural, positive, p = 0.008	

We applied the linear and the logistic multiple regression and artificial neural networks with radial basis functions. Both types of methods are mainly used for prediction, although they can also be applied for extracting the importance of individual variables. Artificial neural networks require no explicit model. They are derived from known examples. They are able to generalize and are tolerant to errors. When compared to the multiple regression, RBF neural networks are generally able to use a more complex model, which can lead to better prediction results. However, it is generally hard to extract knowledge from neural networks – regression is more transparent to the user.

24% of respondents know sweet potato well, while 39% know it only superficially. The most important factor for knowledge about sweet potato is agricultural education. The second (less significant) is the number of family members, which is probably related to the number of children in most cases. However, it is hard to predict the reputation of sweet potato solely on the basis of general properties of people. We suspect that people's inclinations probably play an important role here.

When researching relationships between various answers to 8 specific questions about features of sweet potato, we came upon a very interesting result. Namely, every answer can be very accurately predicted on the basis of answers to the other 7 questions, by accuracy of 98% on average. Of course, the other answers to the same question are not considered. We may therefore conclude that the answers are very self-consistent.

When looking for significant factors in regression on different variables, we found some interesting and logical relationships. It is shown that people with agricultural education are more willing to know/buy/grow SP than others. Older persons are shown to be more interested in healing effects of sweet potato, while younger and better educated believe in stronger nutritional values. Female respondents are more likely to grow it than male. Net income impacts one's willingness to buy sweet potato, because people living with children are willing to attend a free lecture about sweet potato.

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REFERENCES

Agresti A. 2007. Building and applying logistic regression models. In: "An Introduction to Categorical Data Analysis." W.J. Pesce and P.B. Wiley (Ed.), p. 137. Wiley-Interscience, New York.

Ajzen I. 1991. The theory of planned behavior. Organ. Behav. Hum. Dec. 50: 179.

Austin D.F. and Huamán Z. 1996. A synopsis of Ipomoea (Convolvulaceae) in the Americas. Taxon 45: 3.

Burri B.J. 2011. Evaluating sweet potato as an intervention food to prevent vitamin A deficiency. Comp. Rev. Food Sci. Food Safety 10: 118.

Chen J. 2005. Neural Network Applications in Agricultural Economics. Sc. D. Thesis, University of Kentucky.

Chowdhury S., Meenakshi J.V., Tomlins K.I. and Owori C. 2011. Are consumers in developing countries willing to pay more for micronutrient-dense Biofortified foods? Evidence from a field experiment in Uganda. Am. J. Agr. Econ. 93(1): 83.

Draper N.R. and Smith H. 1998. "Applied Regression Analysis" 3rd Ed. John Wiley & Sons, Inc., New York, Usa.

Drummond S., Joshi A. and Sudduth K.A. 1998. Application of neural networks: Precision farming. IEEE Trans. Neural Netw. 9: 211.

Elgaali E. and Garcia L.A. 2007. Using neural networks to model the impacts of climate change on water supplies. J. Water Resour. Plann. Manage. 133: 230.

Escalante-Sánchez E., Rosas-Ramírez D., Linares E., Bye R. and Pereda-Miranda R 2008. Batatinosides II-VI, acylated lipooligosaccharides from the resin glycosides of sweet potato. J. Agric. Food. Chem. 56: 9423.

Fonceca M.J.O., Soares A.G., Freire Junior M., Almeida D.J. and Ascheri J.L.R. 2008. Effect of extrusion-cooking in total carotenoids content in cream and orange flesh sweet potato cultivars. Hort. Brasileira 26: 112.

Ghamari S., Borghei A.M., Rabbani H., Khazaei J. and Basati F. 2010. Modeling the terminal velocity of agricultural seeds with artificial neural networks. Afr. J. Agr. Res. 5: 389.

Haykin S. 1999. "Neural Networks: A Comprehensive Foundation" 2nd Ed. Upper Saddle River, NJ: Prentice Hall.

Jayas D.S., Paliwal J. and Visen N.S. 2000. Multi-layer neural networks for image analysis of agricultural products. J. Agric. Eng. Res. 77: 119.

Jekanowski M.D., Williams II D.R. and Schiek W.A. 2000. Consumers' Willingness to Purchase Locally Produced Agricultural Products: An Analysis of an Indiana Survey. Agr. Resour. Ec. Rev. 29(8): 43.

Jung J.K., Lee S.U., Kozukue N., Levin C.E. and Friedman M. 2011. Distribution of phenolic compounds and antioxidative activities in parts of sweet potato (*Ipomoea batata* L.) plants and in home processed roots. J. Food Comp. Anal. 24: 29.

Kocjan Ačko D. 2012. Importance and possibilities of proso millet (*Panicum miliaceum* L.) production for human nutrition, and animal feed in Slovenia. J. Food. Agric. Environ. 10: 636.

Laurie S.M. and Van Heerden S.M. 2012. Consumer acceptability of four products made from beta-carotene-rich sweet potato. Afr. J. Food Sci. 6(4): 96.

Leighton C.S., Schoenfeldt H.C., Kruger R. Consumer taste preferences for sweet potato. Report, University of Pretoria, 2010.

Mikulič Petkovšek M., Slatnar A., Štampar F. and Veberič R. 2011. Phenolic compounds in apple leaves after infection with apple scab. Biol. Plant. 55: 725.

Mittal G.S. and Zhang J. 2000. Prediction of temperature and moisture content of frankfurters during thermal processing using neural network. Meat Sci. 55: 13.

Moody J. and Darken C.J. 1989. Fast learning in networks of locally tuned processing units. Neural Comput. 1: 281.

Nagelkerke N.J.D. 1992. "Maximum Likelihood Estimation of Functional Relationships." Springer-Verlag, Berlin.

Poggio T. and Girosi F. 1990. "Networks for approximation and learning." In: Procedings of IEEE, 78: 1481.

Schmitzer V., Slatnar A., Mikulič Petkovšek M., Veberič R., Krška B. and Štampar F. 2011. Comparative study of primary and secondary metabolites in apricot (*Prunus armeniaca* L.) cultivars. J. Sci. Food Agric., 91: 860.

Seyhan A.T., Tayfur G., Karakurt M. and Tanoglu M. 2005. Artificial neural network prediction of compressive

strength of VARTM processed polymer composites. Comput. Mater. Sci. 34: 99.

Slatnar A., Klančar U., Štampar F. and Veberič R. 2011. Effect of drying of figs (*Ficus carica* L.) on the content of sugars, organic acids and phenolic compounds. J. Agric. Food Chem. 59: 11696.

Šťastný J., Konečný V. and Trenz O. 2011. Agricultural data prediction by means of neural network. Agric. Econ. 57: 356.

Takahata Y., Kai Y., Tanaka M., Nakayama H. and Yoshinaga M. 2011. Enlargement of the variances in amount and composition of anthocyanin pigments in sweetpotato storage roots and their effect on the differences in DPPH radical-scavenging activity. Scientia Hort. 127: 469.

Teow C.C., Truong V.D., McFeeters R.F., Thompson R.L., Pecota K.V. and Yencho G.C. 2007. Antioxidant activities, phenolic and beta-carotene contents of sweet potato genotypes with varying flesh colours. Food Chem. 103: 829.

Terzi O. and Onal S. 2012. Application of artificial neural networks and multiple linear regression to forecast monthly river flow in Turkey. Afr. J. Agr. Res. 7: 1317.

van Oirschota Q.E.A., Reesa D. and Akedb J. 2003. Sensory characteristics of five sweet potato cultivars and their changes during storage under tropical conditions. Food Qual. Prefer. 14: 673.

Voon J.P., Nguib K.S. and Agrawal A. 2011. Determinants of Willingness to Purchase Organic Food: An Exploratory Study Using Structural Equation Modeling. Int. Food and Agr. Manag. Rev. 14(2): 103.

Walker E.H., Gerald B.L., Hunt A. and Pope J.F. 2009. Factors determining the use of sweet potatoes in school foodservice in the Southeastern United States. J. Foodservice, 20: 100.

Woolfe J.A. 1992. Sweet potato: an untapped food resource. Cambridge Univ. Press., New York, Usa.

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PAPER

HONEY-BASED "ÁGUA-MEL" CHEMICAL CHARACTERIZATION AND MICROBIOLOGICAL QUALITY

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ABSTRACT

In Mediterranean countries such as Italy and Portugal an ancient practice among beekepers is the production of a honey-based product that is called "água-mel" (Portuguese designation) or "abbamele" (Italian designation) that have not only food applications but also medicinal purposes. However, the characterization of such foodstuff is completely absent in Portugal. In our study the main goal was to provide the general chemical characterization and the microbiological quality of samples of "água-mel". The chemical characterization showed a great variability of the ash percentage (0.167-0.474); electrical conductivity (407-1067 μS/cm); free acidity (33.2-91.2 meq/kg); lactone acidity (14.60-20.50 meq/kg); total acidity (53.7-122.72 meq/kg); glucose (185.57-258.52 g/kg); fructose (218.49-315.36 g/kg); total polyphenols (1780.0-4963.8 mg/kg); flavonoids (188.8-1702.4 mg/kg) and 5-(hydroxymethyl)-2-furaldehyde (HMF) (1812.6-8428.9 mg/kg), depending on the beekeeper and production year. The microbiological quality included the counts of aerobic mesophilic bacteria, yeasts and moulds, Enterobacteriaceae, sulphite-reducing *Clostridium* spp. and the presence of *Salmonella* spp. The results showed that from all "água-mel" samples analyzed only one sample was contaminated with *Clostridium* spp. and aerobic mesophilic bacteria. Taken together both chemical and microbiological data indicates a safe consumption of "água-mel".

- Keywords: "água-mel", honey, chemical characterization, microbiological quality -

INTRODUCTION

For a long time beekeepers from Portugal, mainly in the South (Algarve and Alentejo regions), obtain a honey-based product known as "água-mel". Such product is obtained according to that already reported for a traditional honey-based Sardinian product called "abbamele" (SPANO *et al.*, 2008) with little differences. For "abbamele", after the extraction of honey from the honeycombs, the latter are crumbled and dipped into warm water (40°C). The emerging wax separates and the remaining liquid (water, some honey and pollen) is heat-treated (up to 100°C) until a brown, honey-like product. In Portugal, honeycombs are also crumbled and dipped into warm water but at 70°C. The remaining liquid constituted by washing water, some honey, propolis and pollen is, then, heat-treated until a brown, honey-like product with 70°-77° Brix (Fig. 1). This procedure is time-consuming (9-12 hours).

Since ancient times, "água-mel" is used in Portugal as sweetener in cakes, tea, and of great importance as natural medicine on the alleviation of simple symptoms of upper respiratory tract. More recently, the haute cuisine started to use this product in salads and cakes.

In Portugal no information about the physico-chemical characteristics of "água-mel" exists, in contrast to the Italian "abbamele" which has been under research since a few years ago (SPANO *et al.*, 2008; JERKOVIĆ *et al.*, 2011). Thus, the general characterization of the "água-mel" from the South of Portugal is our main goal.

MATERIAL AND METHODS

Samples

Samples of "água-mel" were given by the following producers; through the beekeepers Association "Associação dos Apicultores do Sudoeste Alentejano e Costa Vicentina" (AASACV), Portugal:

- 1A/2008: Producer 1A/year of production 2008;
- 1A/2010: Producer 1A/year of production 2010;
- 1A/2011: Producer 1A/year of production 2011;
- 1B/2010: Producer 1B/year of production 2010;
- 1B/2011: Producer 1B/year of production 2011;
- 1H/2011: Producer 1H/year of production 2011;
- 1I/2011: Producer 1I/year of production 2011;
- 1M/2011: Producer 1M/year of production 2011;
- 2A/2011: Producer 2A/year of production 2011;
- 2B/2011: Producer 2B/year of production 2011.

Samples were kept at room temperature and flasks were opened at aseptic conditions, in a laminar flow chamber (BIOHAZARD, Bio II A, Telstar, Madrid, Spain). For each sample of "água-mel", the producers provided 3 bottles. For each bottle, 3 determinations were done. Thus, data are the mean of 9 determinations (n=9).

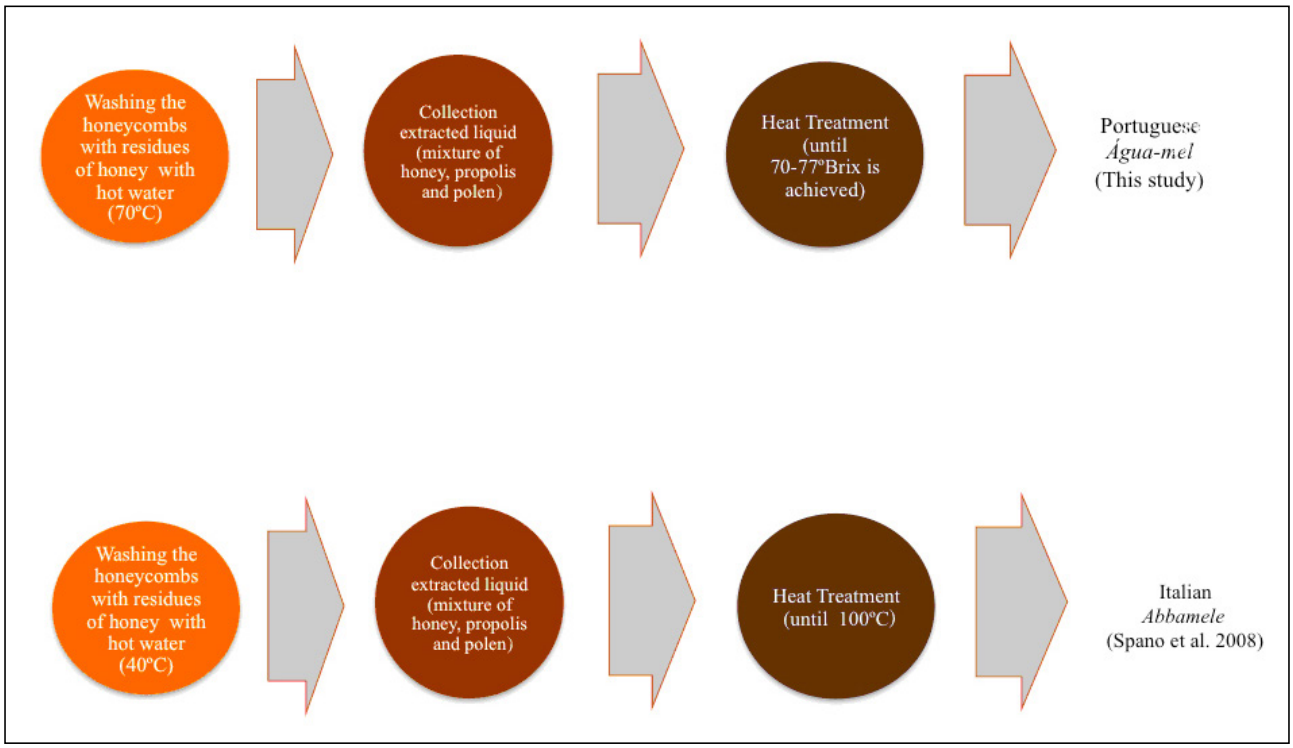

Fig. 1 - Flow chart of "água-mel" and "abbamele" production.

Microbiological analysis

To evaluate the microbiological quality of "água-mel", counts of aerobic mesophilic bacteria (NP-4405:2002), yeasts and moulds (ISO 21527-2:2008), Enterobacteriaceae (ISO 21528-2:2004) and sulphite-reducing *Clostridium* spp. (ISO 15213:2003) were determined. Culture media were purchased from Oxoid (Basingstoke, Hampshire, UK) and Biokar (Paris, France). Ten gram of "água-mel" samples were transferred to 90 mL of peptone water (Oxoid) and homogenized. Decimal dilutions were prepared using the same diluent. The detection procedure for *Salmonella* spp. was done according to the international standard ISO 6579:2002. The microbiological determinations were done in triplicate. Microbial counts were expressed in Log_{10} CFU/g.

Ash

The samples were submitted to 550°C in an electric furnace (Cassel, Portugal) and the residue weighed in an analytical balance (Shimadzu, Aux 220, Philippines), after cooling in a desiccator, according to the harmonized method for honey developed by the International Honey Commission IHC (2002).

Electrical conductivity

Electrical conductivity was measured according to the International Honey Commission IHC (2002) and using a (Thermo Electron corporation, Orion 3 STAR, USA) conductivity meter equipped with a conductivity probe (Orion, 013005MD, USA). The sample solution was prepared using MilliQ water (MQ Integral 5, 2RXP-005TO, Portugal). The cell constant value was checked with 0.1 M aqueous solution of KCl (BHD Prolabo, Leuven, Belgium).

Water content

The water content of the samples was determined by measuring the refractive index at 20°C according to the International Honey Commission IHC (2002) for honey. This determination was conducted using an Abbe Refractometer (HANNA, HI968601, Romania).

pH, free acidity, lactone acidity and total acidity

The measurement of pH and determination of free acidity was performed according to the International Honey Commission IHC (2002), after obtaining a solution of 10 g of sample dissolved in 75 mL of carbon dioxide-free water. The pH was measured using a potentiometer combined with glass electrode (Thermo Electron Corporation, Orion 3 STAR, USA). After the measurement of pH, free acidity was determined by titration with 0.1 M NaOH (Pronalab, Madalena, Portugal) to pH 8.30 (free acidity). Immediately a volume of 10 mL 0.05 M NaOH was added and, without delay, back-titrated with 0.05 M HCl (Fisher Scientific UK Ltd, Loughborough, UK) to pH 8.30 (lactone acidity).

HMF content

The HMF concentration was determined according to the harmonized method for honey. One gram of "água-mel" samples was diluted up to 50 mL with distilled water, filtered on 0.45 μm filter and immediately injected in a HPLC (Hitachi, LaChrom Elite, Japan) equipped with a Diode Array Detector (L-2455), Autosampler (L-2200) and Pump (2100/2130). The HPLC column was a Merck KGaA, Lichrosorb RP-18, 10 μm, Hibar 250-4. The HPLC conditions were the following: isocratic mobile phase, 90% water and 10% methanol HPLC grade (Labscan, Dublin, Ireland); flow rate, 1 mL/min; injection volume, 20 μL. The wavelength was 285 nm. HMF was identified by splitting the peak in "água-mel" with a standard HMF (Acros Organis, New Jersey, USA), and by comparison of the spectra of the HMF standard with that of an "água-mel" sample. The amount of HMF was determined using an external calibration curve (8-500 mg/L). Data were elaborated using EZChrom Elite (VWR International, Carnaxide, Portugal). Each sample was analyzed in triplicate.

Fructose and glucose

Fructose and glucose were determined according to the International Honey Commission IHC (2002). About 0.5 g of "água-mel" was weighed directly into polypropylene tubes and mixed with 10 mL 25% methanol. Afterwards, 1 mL of the solution was filtered through a 0.45 μm filter (VWR International, USA) prior to HPLC analysis. The determination of sugars was performed with the same high-performance liquid chromatograph equipped with a refractive index (RI) detector (Hitachi model L-2490, Japan). The separation was performed by using a Merck NH_2-bonded column for Carbohydrate Analysis (LiChroCART 250-4) with a particle size diameter of 5 μm, equipped with a guard column (Merck LiChroCART 4-4).

The column was kept at 30°C throughout the analysis. The HPLC pumps, autosampler, column oven and RI detector were monitored and controlled using EZChrom Elite system. The mobile phase was composed of 80% acetonitrile HPLC Grade (Panreac, Barcelona, Spain) in water. The injection volumes of the samples were 20 μL, with a flow rate of 1 mL/min.

The HPLC sample peaks were identified by comparing the retention times obtained from standards. The "água-mel" samples were also spiked with standards in order to verify the iden-

tity of the chromatographic peaks. Triplicate injections were performed and average peak areas were used for the peak quantification. The standard of fructose (3-20 g/L) and glucose (4-20 g/L) were from Sigma (S. Louis, MO, USA). The amount of the monosaccharides was determined using an external calibration curve.

Estimation of total polyphenols

The total polyphenol content was determined by a modification of the Folin-Ciocalteu method and the results are expressed as mg gallic acid (Acros Organics, New Jersey, USA)/kg. The method was that followed by some authors for honey (AL *et al.*, 2009). Five grams of "água-mel" were treated with 50 mL of distilled water, mixed and filtered using a qualitative filter. Five hundred microlitres of this solution was mixed with 2.5 mL Folin-Ciocalteu reagent (0.2 N) (Merck KGaA, Darmstadt, Germany) for 5 min and then 2 mL of a Na_2CO_3 (PRONALAB, Lisboa, Portugal) solution were added (75 g/L). All samples were incubated at room temperature in the dark conditions for 2 h, and the absorbance was read at 760 nm.

The blank solution contained water instead of "água-mel". For calibration curve, a stock solution of gallic acid (1 g/L) was prepared for further dilutions (4-500 mg/L).

Estimation of total flavonoids

A method described by ISLA *et al.* (2011) was used for total flavonoids determination. Briefly, 0.5 mL $AlCl_3$ (Carlo ERBA reagents, Val de Reuil, France) (20 g/100 mL) was added to 0.5 mL of "água-mel" samples. After 1 h at room temperature, absorbance was measured. Total flavonoid contents were expressed as mg quercetin (Alfa Aesar GmbH & CoKG, Carsluhe, Germany)/kg of "água-mel" (mg/kg of "água-mel"), using a calibration curve over the range of 15.6-125 mg quercetin/L.

Estimation of proline content

The proline content was determined by using a colour comparison after applying ninhydrin, with a proline standard. The content was expressed as a proportion to the mass of "água-mel" tested. The proline content was determined according to the harmonized method for honey developed by the International Honey Commission IHC (2002). A solution (0.5 mL) of "água-mel" (0.05 g/mL) was mixed with 1 mL of formic acid (80%) (Acros Organis, New Jersey, USA), 1 mL of ninhydrin (Acros Organis, New Jersey, USA) solution [3% in ethylene glycol monomethylether, from (Panreac Quimica, Barcelona, Spain)] and shaken vigorously for 15 min. The mixture was placed in a boiling water bath for 15 min and transferred to a 70°C bath for 10 min. Five mL of 50% 2-propanol (Riedel-de-Haën, Seelze, Germany) in water was then added to the mixture and was left to cool. The absorbance was read at 510 nm, 45 min after removal from the 70°C water bath. Water was used as blank and 0.032 mg/mL solution of proline (Acros Organis, New Jersey, USA) was used as standard solution.

Proline concentration in mg/kg of honey was calculated as follows:

Proline (mg/kg) = (Es/Ea) x (E1/E2) x 80, where Es is the absorbance of the sample solution; Ea is the absorbance of the proline standard solution (average of 3 readings); E1 is the mg of proline used for the standard solution; E2 is the weight of "água-mel" in grams; 80 is the dilution factor. The mean of three readings was used.

Diastase activity

Two different methods are used to determine honey diastase. The traditional Schade method uses starch as a substrate. The diastase activity of "água-mel" was evaluated by the methodology previously reported in the International Honey Commission IHC (2002).

RESULTS AND DISCUSSION

All tested samples were negative for all microbiological indicators, except sample 1M/2011 that was contaminated with 3,41±0,09 Log_{10} CFU/g of aerobic mesophilic bacteria and 4,05±0,11 Log_{10} CFU/g of sulphite-reducing *Clostridium* spp.

The consumption of the tested "água-mel" samples poses no risk to human health, except sample 1M/2011 that evidences a contamination with *Clostridium* spp. The consumption of honey or honey derivatives contaminated with *C. botulinum* poses a particular risk to children, elderly and immunocompromised individuals (EUROPEAN COMMISSION, 2002; ANONYMOUS, 2005). The sources of contamination of honey or its derivatives with *Clostridium* spp. may occur through bee's digestive tract, pollen, soil, dust and from not properly cleaned equipment and processing areas (NEVAS *et al.*, 2006). In Portugal botulism cases are rare: between 2003-2006 the mean of notified cases were 8 cases (Direcção Geral de Saúde, 2007) and the first infant botulism case was just reported (SARAIVA *et al.*, 2012). The case involved a 1-month-old infant that was breastfed but his parents used to give him chamomile tea and occasionally honey (chamomile tea and honey brought from Moldavia). In the case reported *C. botulinum* type B was isolated from infant faeces sample and as well from chamomile tea herbs and honey (SARAIVA *et al.*, 2012). In what concerns "água-mel" sample 1M/2011, the identification of the *Clostridium* spp. contamination source is under investigation and the produc-

er will be instructed with actions to eliminate the source of contamination.

The physicochemical parameters of "água-mel" of beekeepers from Portugal are depicted in Tables 1 and 2. The values found for ash (%) ranged from a minimal 0.167% to a maximal of 0.474%. A great variability was even detected for "água-mel" produced by the same beekeeper but in different years, such as observed for 1A/2010 and 1A/2011, and 1B/2008 and 1B/2011 (Table 1). Ash represents the direct measure of inorganic residues after "água-mel" carbonization. In honey, the ash percentage expresses its richness in mineral content and constitutes a quality parameter, which depends mainly from floral origin of honey (MARCHINI *et al.*, 2007; BOGDANOV *et al.*, 2009; ALOISI, 2010). The diversity of ash percentages found in "água-mel" samples in the same producer but in different years may reveal the utilization of honeys from different floral origins.

Electric conductivity is a quality parameter which is closely related to the concentration of mineral salts, organic acids and proteins and shows a great variability according to the floral origin (ACQUARONE *et al.*, 2007; ZERROUK *et al.*, 2011). The values of electric conductivity found are within the range reported by SPANO *et al.* (2008) for "abbamele" samples from Sardinia. The sample 1M/2011 (1,067 μS/cm) was the sole exception which may be partly explained by the presence of aerobic mesophilic bacteria and *Clostridium* spp.. A linear relationship has been reported between ash and electric conductivity of different types of honey (SANCHO *et al.*, 1991; MALIKA *et al.*, 2005), although some authors consider that such relationship may depend on the floral origin of honeys (THRASYVOULOU and MANIKIS, 1995). In our samples of "água-mel" a direct correlation was found between those two parameters (r=0.980, $P<0.01$). Fig. 2 depicts such correlation.

The water content found in one sample of "água-mel" (1M/2011) exceeded the maximum moisture content of 25% established by European legislation (EU Council, 2002) for water content in honey. The remaining samples had lower levels, nevertheless the majority showed percentages superior to 20%, which is generally found in unifloral honeys from Europe (MATEO and BOSCH-REIG, 1998; PERSANO and PIRO, 2004), and our sample values are within the range found for "abbamele" from Sardinia (SPANO *et al.*, 2008). The only exclusion was samples 1B/2011 and 1H/2011 that showed lower 20% water content. This variability of water content in "água-mel" samples may be attributed to three main factors: the residual moisture

Table 1 - Physico-chemical results obtained from "água-mel" samples of Portugal.

Beekeeper/ Year	Ash (%, w/w)	Electrical conductivity (μS/cm±SD)	Water content (%, ±SD)	pH±SD	Free acidity (meq/kg ±SD)	Lactone acidity (meq/kg ±SD)	Total acidity (meq/kg ±SD)
1A/2010	0.185±0.005	407.89±0.32	22.0±0.1	3.25±0.00	53.6±0.6	19.84±1.14	73.45±1,35
1A/2011	0.425±0.022	715.22±3.05	23.0±0.0	3.35±0.00	83.3±1.6	17.22±1.14	100.51±2.68
1B/2008	0.202±0.019	487.89±3.87	24.2±0.0	3.44±0.01	52.6±0.5	18.53±3.41	71.15±3.08
1B/2010	0.284±0.008	563.33±2.64	25.0±0.0	3.49±0.00	57.3±0.6	18.53±1.97	75.81±2.24
1B/2011	0.425±0.022	751.89±0.27	19.2±0.0	3.35±0.00	102.2±0.5	20.50±0.00	122.72±3.64
1H/2011	0.209±0.013	418.44±0.43	18.3±0.1	3.56±0.01	40.7±0.5	14.60±0.00	55.27±3.67
1I/2011	0.167±0.017	439.44±2.12	22.8±0.0	3.49±0.01	33.2±0.6	20.50±0.00	53.72±0.51
1M/2011	0.474±0.093	1067.00±1.68	>25.0	3.91±0.01	74.8±0.7	15.26±1.14	90.03±1.15
2A/2011	0.442±0.013	765.56±3.20	20.1±0.1	3.35±0.00	91.2±0.2	18.53±3.41	109.76±0.79
2B/2011	0.392±0.013	696.44±3.15	23.4±0.1	3.35±0.00	77.5±0.9	15.91±1.14	93.42±2.07

Table 2 - Monosaccharide, phenols and HMF results obtained from "água-mel"samples of Portugal.

Sample	Glucose (g/kg±SD)	Fructose (g/kg±SD)	Total polyphenols (mg/kg±SD)	Flavonoids (mg/kg±SD)	HMF (mg/kg±SD)
1A/2010	241.16±1.20	306.15±2.03	2718.7±75.45	603.2±6.92	4370.7±71.0
1A/2011	258.52±3.05	266.34±3.79	4508.5±116.36	1316.6±16,14	6620.0±79.8
1B/2008	241.01±3.88	299.19±5,01	2403.1±67.51	582.6±6.57	3327.9±29.9
1B/2010	229.63±6.11	288.02±1.72	2733.9±46,71	698.6±5.04	3938.6±128.2
1B/2011	250.65±0.64	269.22±0.96	4963.8±93.96	1702.4±13.89	6884.2±57.4
1H/2011	252.41±14.76	315.36±20.01	4125.8±96.21	355.6±9.34	1812.6±13.6
1I/2011	243.46±3.24	289.75±3.87	1780.0±40.48	188.8±6.47	1969.5±4.5
1M/2011	185.57±0.33	218.49±0.72	2777.9±23.32	278.3±2.53	4352.9±157.8
2A/2011	246.12±14.53	252.92±0.93	2718.7±81.97	1579.7±11.97	8428.9±42.9
2B/2011	237.94±8.33	271.94±11.45	2611.0±20.42	1155.7±24.98	6506.5±31.1

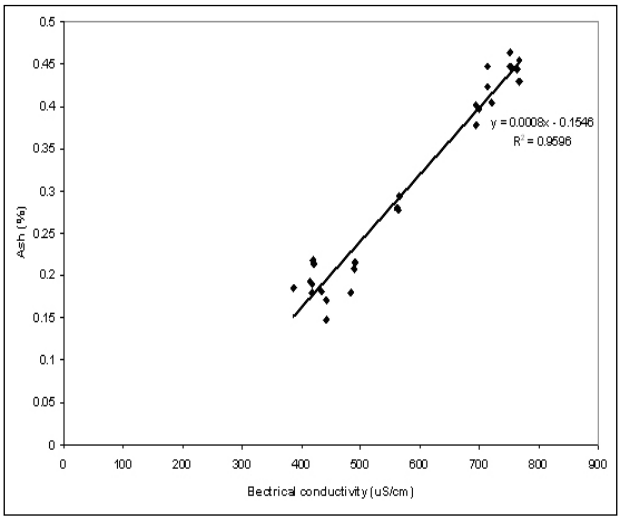

Fig. 2 - Correlation found between ash and electrical conductivity in "água-mel" samples.

in the honeycombs, the amount of water initially added for treating honeycombs, the temperature used and the length of the heating process (SPANO *et al.*, 2008).

The parameter pH is within the ranges reported for honey (3.5-5.5) and "abbamel" from Sardinia (Italy) (3.21-3.92) (SPANO *et al.*, 2008). Nevertheless the free acidity parameter of some samples was superior to that reported for honey (not more than 80 meq/kg for baker's honey) (EU Council, 2002). In our case, a great range of values was found: 33.2 meq/kg for sample 1I/2011 and 102.2 meq/kg for IB/2011 (Table 1).

The acid content of honey is due to the acids added by the bees, being the main one gluconic acid, a product of glucose oxidation by glucose oxidase. This acid is generally present in honey as its internal ester, a lactone, which does not contribute to honey's active acidity (BOGDANOV *et al.*, 2004). Lactone acidity is determined by adding an excess of alkali to a neutralized honey and back-titrating with acid (WHITE *et al.*, 1958). The sum of free acidity and lactone acidity gives the total acidity of honey samples. Lactone acidity in "água-mel" ranged from 14.60 to 20.50 meq/kg. Total acidity exceeding 100 meq/kg were found in samples 1A/2011, 2A/2011 and 1B/2011 (Table 1).

Glucose and fructose were the main sugars present in "água-mel", predominating fructose in all samples. The amounts found in our samples were similar to those already reported for some "abbamele" samples from Sardinia (JERKOVIĆ *et al.*, 2011). The variability found and according to the same authors may be attributed to the preparation procedure, rather than with different types of honey used. In our samples such is not so evident because for the same producer but in different years of production the amounts of the two monosaccharides changed (Table 2). It is our opinion that both factors may be responsible for the observed differences.

The absence of diastase activity in all samples is understandable since "água-mel" is a product obtained by heating the raw material for several hours, inactivating enzymes. The same would be expected for proline as also reported for Italian "abbamele" (SPANO *et al.*, 2008). In fact, only one sample had a significant content of this amino acid (462.4 mg/kg): 1M/2011. This value may be associated to the presence of *Clostridium* ssp. which can synthesize this amino acid by the Stickland reaction (NISMAN, 1954). The absence of proline in all samples, excepting 1M/2011, may be attributed to an increased rate of Maillard reaction, which is initiated by the reaction of a carbohydrate with a compound possessing a free amino group (PAÄT-ZOLD and BRÜCKNER, 2006; CZIPA, 2010). The levels of sugars slightly inferior to those reported for honey samples may also be attributed to their consumption in the Maillard reactions, as reported for OOSTERVELD *et al.* (2003) in roasted *Coffea arabica* beans. These authors reported that during roasting, oligosaccharides hydrolyse to monosaccharides and both are able to react with protein/peptides/amino acids originating Maillard products which are responsible for the formation of volatile compounds and organic acids (OOSTERVELD *et al.*, 2003). The relative high free and total acidity found in "água-mel" samples may be, therefore, endorsed to the liberation of organic acids. Such may explain the inverse correlation obtained between fructose and total acidity ($r=-0.796$; $p<0.01$). Fig. 3 represents this inverse relation between fructose and total acidity, which was not detected for glucose.

At same time, the thermal treatment is also responsible for the degradation of monosaccharides to hydroxymethylfurfural (HMF), and therefore to the decrease of sugars, particularly fructose, because an inverse correlation was obtained between the fructose concentration and

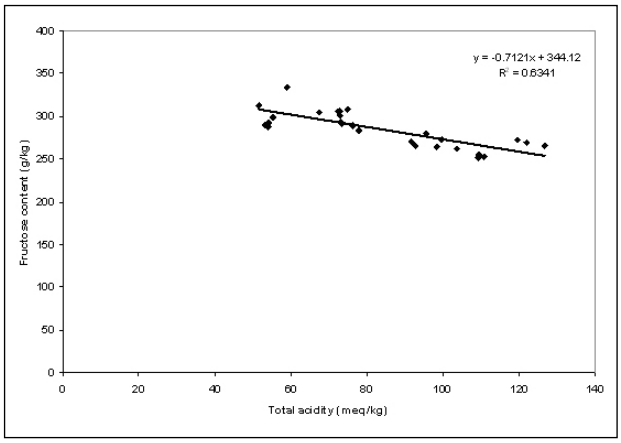

Fig. 3 - Correlation found between total acidity and fructose content in "água-mel" samples.

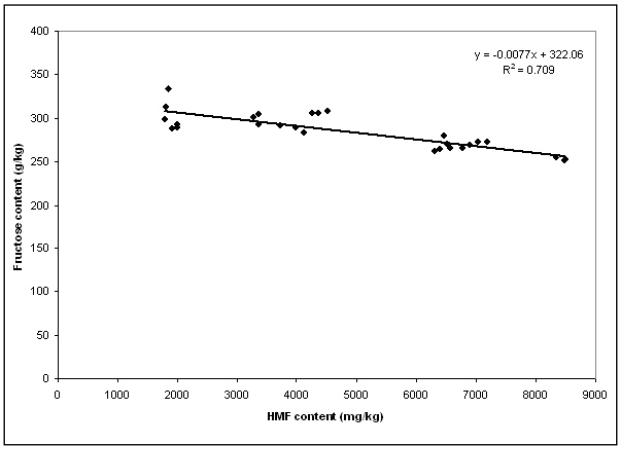

Fig. 4 - Correlation found between HMF content and fructose content in "água-mel" samples.

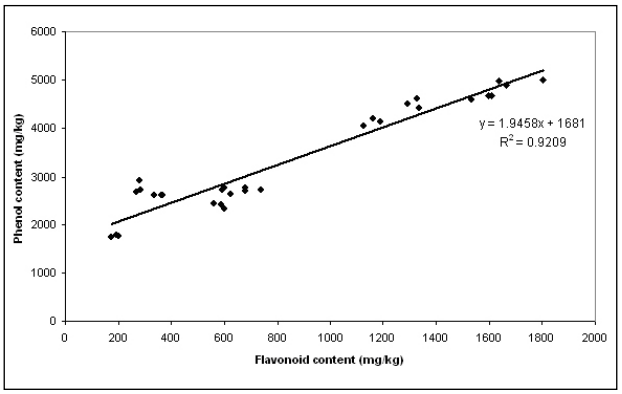

Fig. 5 - Correlation found between flavonoid content and phenol content in "água-mel" samples.

HMF content (r=-0.842, *p<0.01*) as can be seen in the Fig. 4.

As expected, HMF content (Table 2) is largely superior to that of honey samples, nevertheless comparable to those found for "abbamele" (SPANO *et al.*, 2008; JERKOVIĆ *et al.*, 2011). Nevertheless some samples (2A/2011) showed higher concentrations (8428.9 mg/kg) in comparison to the sample with the highest concentration reported by SPANO *et al.* (2008) (4,476 mg/kg), still within the ranges found for liquid caramel as reported by those authors. In order to have reference HMF values from thermally treated foodstuffs the HMF value of liquid caramel, instant coffee powder and coffee substitutes was determined. These foodstuffs showed higher HMF values in comparison to our "água-mel" samples: liquid caramel (9,700 mg/kg), instant coffee powder (11,500 mg/kg), and coffee substitutes (9,700 mg/kg).

Polyphenol concentration ranged from 1780.0 mg/kg in sample 1I/2011 to 4963.8 mg/kg in sample 1B/2011 (Table 2), amounts significantly superior to those reported by SPANO *et al.* (2008), but within the ranges reported by JERKOVIĆ *et al.* (2011) As reported by these authors, such differences may be explained by different sample preparation and data expression.

It is remarkable the great variability of flavonoid content in "água-mel" samples, even in the same producer but in different years of production. For example, for samples 1A (2010 and 2011) and 1B (2008, 2010 and 2011) a large variability in flavonoids content was observed (Table 2). This variability may result from diverse floral origin of honeys used in different years for the production of "água-mel". The levels of flavonoids go along with the amounts of total polypehenols, there is even a significant correlation between these two parameters (r=0.960, *p<0.01*) as can be observed in the Fig. 5.

Our study shows that "água-mel" obtained from honey after thermal treatment continue to possess some of the parameters similar to those of honey, including pH, glucose and fructose as main constituents, but generally relative higher percentages of water content, free acidity and HMF, and low levels or even absence of proline and diastase activity, as expected due to the thermal processing, but the values of HMF in our samples are comparable to other foodstuffs. The presence of phenols and flavonoids in "água-mel" was as predicted due to their invariable presence in honey. Nevertheless higher quantities were detected which must be enlightened because some interference may be responsible for such data. The determination of the microbiological quality of "água-mel" showed the presence of *Clostridium* spp. in one of the samples. This result shows that as for honey the use of "água-mel" is not recommended for infant feed.

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REFERENCES

Acquarone C., Buera P. and Elizalde B. 2007. Pattern of pH and electrical conductivity upon honey dilution as a complementary tool for discriminating geographical origin of honeys. Food Chem. 101: 695.

Al M.L., Daniel D., Moise A., Bobis O., Laslo L. and Bogdanov S. 2009. Physico-chemical and bioactive properties of different floral origin honeys from Romania. Food Chem. 112: 863.

Aloisi P.V. 2010. Determination of quality chemical parameters of honey from Chubut (Argentinean Patagonia). Chil. J. Agric. Res. 70: 640.

Anonymous. 2005. Opinion of the Scientific Panel on Biological Hazards on a request from the Commission related to *Clostridium* spp in foodstuffs. EFSA Journal. 199: 1-65.

Bogdanov S. 2002. Harmonized Methods of the International Honey Commission. (http://www.apiculturacluj.com/ApiculturaCluj/italiano/Documents/IHCmethods_e.pdf) (most recent access date).

Bogdanov S. 2009. Honey Composition (http://fantastic-fla-

vour.com/yahoo_site_admin/assets/docs/Composition-Honey.20105942.pdf) (most recent access date).

Bogdanov S., Ruoff K. and Oddo L.P. 2004. Physico-chemical methods for the characterisation of unifloral honeys: a review. Apidologie 35: S4.

Czipa N. 2010. Comparative study of honeys with different origin, the effect of production-forming on the quality. *PhD thesis*. University of Debrecen, Debrecen, Hungary.

Direcção Geral da Saúde 2007. Doenças de Declaração Obrigatória, 2002-2006, Botulismo, pp 11.Portugal.

EU Council 2002. Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities L10, 47-52.

European Commission, Health and Consumer Protection Directorate-General 2002. Honey and Microbiological Hazards, 1-40.

Isla M.I., Craig A., Ordoñez R., Zampini C., Sayago J., Bedascarrasbure E., Alvarez A., Salomón V. and Maldonado L. 2011. Physico chemical and bioactive properties of honeys from Northwestern Argentina. LWT-Food Sci. Technol. 44: 1922.

ISO 15213:2003 2003. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions. International Standards Organization, Switzerland.

ISO 21527-2:2008 2008. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Enumeration of Yeasts and Moulds – Part 2: Colony Count Technique in Products with Water Activity Less Than or Equal to 0.95. International Standards Organization, Switzerland.

ISO 21528-2:2004 (E) 2004. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the detection and enumeration of Enterobacteriaceae-Part 2: Colony-count method.

ISO 6579:2002 (E) 2002. Microbiology of Food and Animal Feeding Stuffs-Horizontal Method for the Detection of *Salmonella* spp. International Standars Organization, Switzerland.

Jerković I., Kasum A., Marijanović Z. and Tuberoso C.I.G. 2011. Contribution of the characterization of honey-based Sardinian product abbamele: volatile aroma composition, honey marker compounds and antioxidant activity. Food Chem. 124: 401.

Malika N., Mohamed F. and Chakib El A. 2005. Microbiological and physico-chemical properties of Moroccan honey. Int. J. Agric. Biol. 7: 773.

Marchini P.V., Moreti A.C.C.C., Otsuk I.P. and Sodré G.S. 2007. Physicochemical composition of *Apis mellifera* honey samples from São Paulo, Brazil. *Quim. Nova* 30: 1653.

Mateo R. and Bosch-Reig F. 1998. Classification of Spanish unifloral honeys by discriminant analysis of electrical conductivity, color, water content, sugars, and pH. J. Agric. Food Chem. 46: 393.

Nevas M., Lindström M., Hörman A., Keto-Timonen R. and Korkeala H. 2006. Contamination routes of *Clostridium botulinum* in the honey production environment. Environ. Microbiol. 8: 1085.

Nisman B. 1954. The Stickland reaction. Bacteriol. Rev. 18: 16.

NP-4405:2002 2002. Microbiologia Alimentar - Regras gerais para a contagem de microrganismos. Contagem de colónias a 30ºC. Instituto Português da Qualidade, Lisboa, Portugal.

Oosterveld A., Voragen A.G.J. and Schols H.A. 2003. Effect of roasting on the carbohydrate composition of *Coffea arabica* beans. Carbohyd. Polym. 54: 183.

Paätzold R. and Brückner H. 2006. Gas chromatographic detection of D-amino acids in natural and thermally treated bee honeys and studies on the mechanism of their formation as result of the Maillard reaction. Europ. Food Res. Technol. 223: 347.

Persano Oddo L. and Piro R. 2004. Main European unifloral honeys: descriptive sheets. Apidologie 35: S38.

Sancho M.T., Muniategni S., Sánchez M.P., Huidobro J.F. and Simal J. 1991. Relationships between electrical conductivity and total and sulphated ash contents in Basque honeys. Apidologie 22: 487.

Saraiva M., Cunha I.C., Bonito C.C., Pena C., Toscano M.M., Lopes T.T., Sousa I. and Calhau M.A. 2012. First case of infant botulism in Portugal. Food Control 26: 79.

Spano N., Ciulu M., Floris I., Panzanelli A., Pilo M.I., Piu P.C., Scanu R. and Sanna G. 2008. Chemical characterization of a traditional honey-based Sardinian product: Abbamele. Food Chem. 108*:* 81.

Thrasyvoulou A. and Manikis J. 1995. Some physicochemical and microscopic characteristics of Greek unifloral honeys. Apidologie 26: 441.

White J.W., Petty J. and Hager R.B. 1958. The composition of honey. II. Lactone content. JAOAC 41: 194.

Zerrouk S.H., Fallico B.G., Arena E.N., Ballistreri G.F. and Boughediri L.A. 2011. Quality of evaluation of some honey from the Central region of Algeria. Jordan J. Biol. Sci. 4: 243.

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PAPER

SUSCEPTIBILITY OF MAIZE VARIANTS TO *PLODIA INTERPUNCTELLA*

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ABSTRACT

The behavior of the Indian meal moth *Plodia interpunctella* on maize genotypes differing in embryo development, both on whole and longitudinally sectioned kernels, was studied. In the test with whole kernels, damage was very low or absent, and only viviparous mutants were significantly attacked. However, 100% damage was observed in all genotypes on longitudinally sectioned kernels. In this test, mutant seeds lacking embryos were less damaged and showed the lowest mean number of adult insects. These results indicate that larval penetration is influenced by the embryo properties and first shows that the employment of genetic variants is a valuable approach to study insect behavior and an opportunity to highlight maize genotypes with characteristics that can minimize quality reduction caused by insect attacks.

- Keywords: Indian meal moth, maize embryo, maize seed mutants -

INTRODUCTION

Maize, with wheat and rice, is one of the most important crops for the world economy. Several insect pests can infest stored maize and research on the development of pests on different varieties has been carried out in order to find the most tolerant ones (ABDEL-RAHMAN *et al.*, 1968; WISEMAN *et al.*, 1970; DOBIE, 1974, 1977; ADESUYI, 1977; FALOMO, 1981; MORAH and MBATA, 1986; MBATA *et al.*, 1988; SIWALE *et al.*, 2009).

Moths colonize the surface of cereals stored as a mass, causing heavier damage in warehouses than in silos. Moth larvae cause losses by feeding and by contaminating food with silk and frass. Silk net is produced by larvae on the surface of the cereal mass, favoring the development of moulds which can produce mycotoxins. Some authors have studied the development of moths on different varieties of maize (BHATTACHARYA *et al.*, 1976; ROSE and BEHL, 1985; MBATA *et al.*, 1988). *Plodia interpunctella*, the Indian meal moth, is one of the most frequent species that can damage this cereal (ABDEL-RAHMAN *et al.*, 1968; HOCKENSMITH *et al.*, 1986; MBATA, 1987, 1990).

The maize seed comprises two major compartments: the embryo and the endosperm (CONSONNI *et al.*, 2005). The mature embryo consists of a well-differentiated axis, with root and shoot primordia and five or six internodes bearing a leaf at each node, surrounded by a single massive cotyledon, the scutellum. The differentiated endosperm consists of four major cell types or domains: the starchy endosperm, representing the central bulk; the single-cell aleurone layer at the periphery; the embryo-surrounding region, lining the cavity where the embryo develops; and the basal endosperm transfer cells involved in the transport of nutrients from the mother plant (CONSONNI *et al.*, 2005). The endosperm is the main storage site of starch and proteins, whereas the embryo reserves mainly lipids.

The influence of genetic traits related to the maize caryopsis on susceptibility to insect attack has never been evaluated. A better knowledge of maize seed-insect interactions may allow the isolation, and subsequent introduction in commercial hybrids, of genetic variants limiting insect attack.

With this aim we propose in this study the analysis of the effect of maize lines differing in developmental and/or metabolic traits, as a tool to investigate the molecular basis of the interaction between stored product insects and a specific seed compartment (CONSONNI *et al.*, 2005). Particularly in this work we analyze the behavior of *P. interpunctella* on entire and longitudinally sectioned kernels of the RAlex0 line, characterized by high oil content in the embryo, and the B73 reference line. Two embryo-related recessive monogenic mutants were also tested: an *embryo-specific* mutant (*emb*-8908*), that causes an early block in embryo development, and the viviparous mutants (*vp2* and *vp5*), characterized by the precocious germination of the seed while it is still attacked to the ear (GIRAUDAT *et al.*, 1994; HABLE *et al.*, 1998).

MATERIALS AND METHODS

Plant material

Four maize genetic stocks were used in this study. The inbred lines and the viviparous mutants were provided by the Maize Genetics Cooperation Stock Center (http://maizecoop.cropsci.uiuc.edu/), whereas the *emb*-8908* mutant was isolated in our laboratory in an active *Mutator* line.

Inbred lines were propagated via siblings mating. Mutants were backcrossed to the B73 line and segregating ears were obtained by selfing heterozygous plants. Homozygous mutants and related wild-type kernels used in the tests were obtained from F2 segregating ears.

The seeds harvested were dried to about 12-13% moisture content and stored at room temperature.

The B73 line: the inbred line B73, developed at Iowa State University (RUSSELL, 1972), exhibits high yield and is among the most used laboratory accessions and the main source of commercially important germplasm. Its genome sequence was released in 2009 (SCHNABLE *et al.*, 2009).

The RAlex0 genetic stock: also known as Alexander High Oil Synthetic, characterized by the presence of a larger embryo, is the result of a selection made at the University of Illinois for grain with high oil concentration (http://www.maizegdb.org, GERDES *et al.*, 1993).

The *emb*-8908* mutant: the *emb*-8908* recessive mutant was isolated in a genetic line carrying active *Mutator* transposable elements, which act as endogenous mutagens thus allowing single gene specific mutant isolation (WALBOT, 1992). The mutant was subsequently introgressed in the B73 inbred line. It belongs to the class of *embryo specific* (*emb*) mutants, that are characterized by impaired or arrested embryo development but normal endosperm (CLARK and SHERIDAN, 1991). As shown in Fig. 1, emb*-8908 mutants are easily distinguishable from wild type seeds by the absence of the embryo axis and the presence of a scutellum with a reduced size. Seeds used in this work were obtained from ears with a segregation ratio of 3:1 for wild-type: emb phenotype kernels, obtained by selfing heterozygous +/*emb*-8908* plants. The mutant seeds' genotype is indicated as *emb*/*emb*, whereas wild-type seeds' genotype is indicated as *Emb*/-.

The *vp* mutants: maize *viviparous* (*vp*) mutants have been shown to affect either ABA biosynthesis or ABA signalling. *vp2* and *vp5* (GI-

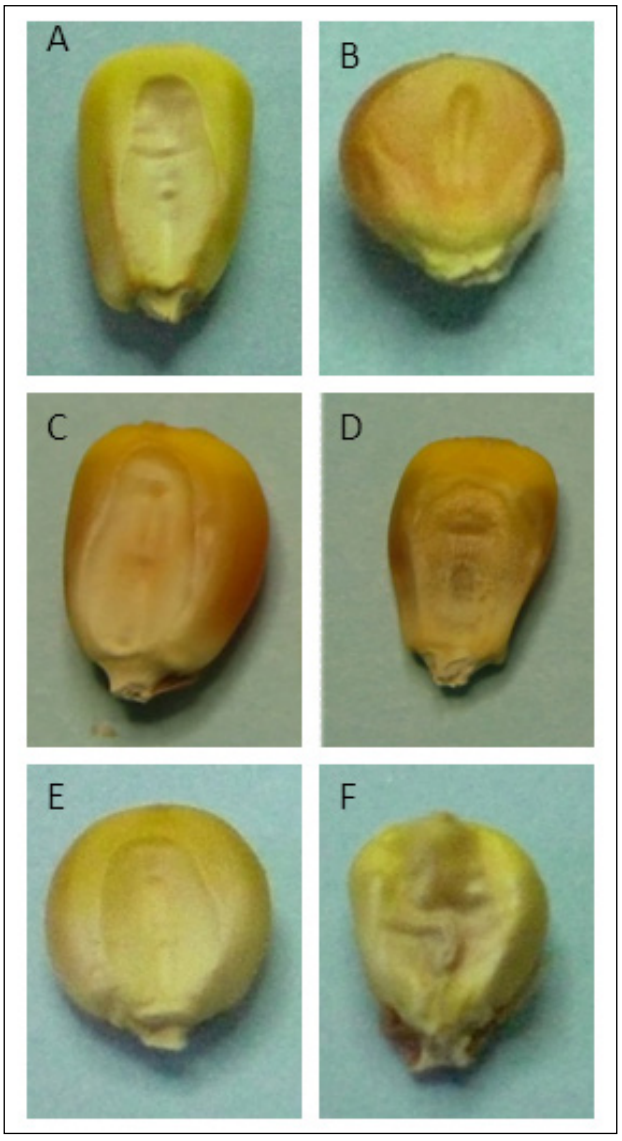

Fig. 1 - Maize seed phenotypes. Representative seeds from: B73 inbred line (A); RAlex0 inbred line (B); Emb*-8908 wild-types (C) and emb*-8908 mutants (D) from a self-pollinated segregating ear; Vp5 wild-types (E) and vp5 mutants (F) from a self-pollinated segregating ear.

RAUDAT *et al.*, 1994; HABLE *et al.*, 1998) are associated with reduced or suppressed carotenoid accumulation in both endosperm and vegetative tissues as a result of a mutational block in the early biosynthetic steps before the branching point separating ABA and carotenoid biosynthesis. Seeds were obtained from ears with a segregation ratio of 3:1 for wild-type: vp phenotype kernels; pools of vp2 and vp5 mutant kernels (*vp/vp*) and of relative wild-type controls (*Vp/-*) were used in each experiment.

Insect rearing and tests

Plodia interpunctella were reared on an artificial diet[1] in a thermostatic chamber at 26°±1°C, 70±5% RH (Relative Humidity), and a photoperiod of 16:8 (light:dark). The tests were carried out by placing maize kernels in glass containers (diameter 35 mm, height 20 mm) with 20 newly emerged larvae. Such containers, closed with a net (120 mesh) to provide ventilation, were placed in an incubator at 26°±1°C, 70±5% RH and 16 h of light alternating with 8 h of darkness. For each of the four maize genotypes, tests were carried out with 20 entire kernels and with 20 longitudinally sectioned kernels. Four replicates were carried out for each test.

The number of emerged adults, the developmental period, and the percentage of damaged seeds were observed and data were analyzed using one-way ANOVA, Duncan's multiple range test and Student's t test (SPSS 19.0 per Windows).

RESULTS

Tests with entire kernels

In Table 1 the results of tests carried out on the development of first instar larvae of *Plodia interpunctella* on entire kernels of the different genetic stocks of maize are reported. In all the genotypes tested no or only one adult developed among the four replicates, with the exception of the homozygous viviparous (*vp/vp*) mutants, which exhibited a mean of 7.5 adults with

[1] Ingredients of the rearing diet: 57 g bran, 61 g corn flour, 55 g wheat flour, 17 g wheat germ, 14 g dried yeast, 85 g glycerine, 67 g honey.

Table 1 - Mean number (±S.D.) of emerged adults[1], mean developmental period (±S.D.) (number of days) of *Plodia interpunctella* (Walker) and damaged seeds percentage observed on whole kernels of different maize genetic stocks.

Maize genetic stocks	Mean number of adults	Min-max	Insect developmental period (mean)	Min-max	Damaged seeds (%)
B73	0.7±0.5b	0-1	51.7±3.05	49-55	15
RAlex0	0.2±0.5b	0-1	48±0	-	7
Emb/-	0±0b	-	0±0	-	0
emb/emb	0.2±0.5b	0-1	51±0	-	2.5
Vp/-	0±0b	-	0±0	-	0
vp/vp	7.5±1.91a	5-9	52.7±7.49	42-68	55

[1]Observations were carried out on twenty newly emerged larvae for each replicate.
One-way Anova: Adults F5,18=48.01 P<0.001; Development period F3,31=0.157 n.s.
Means followed by different letters are significantly different according to Duncan's multiple range test.

55% of seed damage, while the respective control seeds (*Vp*/-) were undamaged.

Even if the number of adults emerged is not significantly different from the other genotype, a higher percentage of damaged seeds was observed in B73, 15%, and in RAlex0, 7%. The mean developmental period of the larvae was between 48 and 53 days. On *Emb*/- and *Vp*/- larvae died without feeding, as seeds were undamaged.

Tests with longitudinally sectioned kernels

In Table 2 the results of tests carried out on 20 first instar larvae of *P. interpunctella* on longitudinally sectioned kernels of the different genetic stocks of maize are reported.

Adults emerged in all the genotypes tested and 100% of damaged seeds were observed in all genotypes, except *emb*/*emb*.

In emb mutants the lowest percentage of damaged seeds, 45%, the lowest mean number of adults, only 1.75, and the longest mean developmental period, 44.3 days, were observed. Even the comparison between emb mutant seeds (*emb*/*emb*) and related wild-types (*Emb*/-) seeds, which exhibited a reduced number of moths in comparison with the other genotypes, showed a significant reduction, according to the Student's t test (t=4.437, df=3.345, P=0.01), in the mean number of moths produced with emb mutant seeds (1.7). The mean developmental period was significantly longer in emb mutants *versus* wild-type seeds (t=3.725, df=6.625, P=0.008). The highest number of emerged adults was observed on RAlex0 (15.2±2.5), followed by B73 (12±3.65), but the two lines were not significantly different for this parameter if compared by Student's t test (t=1.469, df=5.306 P=0.199). Notably, the mean developmental period observed on B73 (34.9±2.07) was the shortest of all genotypes tested, and significantly differs from RAlex0 (t=3.426, df=89.987, P=0.001).

The results obtained in this test with sectioned vp mutants (*vp*/*vp*) did not confirm those obtained in the first test with entire kernels performed in this study. With sectioned kernels the mean number of adults emerged was not significantly different, according to the Student's t test (t=1, df=5.4, P=0.360), from the related controls, *Vp*/-, and the mean developmental period was significantly longer (t=4.172, df=75.58, P=0.0001). The mean number of adults emerged from vp seeds was also lower than that of the two inbred lines.

DISCUSSION

In this study we have examined the behavior of *Plodia interpunctella* on entire and longitudinally sectioned maize kernels with different genotypes affecting embryo development. In most of the maize genotypes tested as entire kernels the mean number of attacked seeds was very low or seeds were not attached (*Emb*/- and *Vp*/-) and few adults of *P. interpunctella* emerged. On the contrary the same genotype tested as longitudinally sectioned kernels showed in most cases 100% of damaged kernels and a higher number of adults.

Several authors observed that the percentage of larval survival increased as the percentage of broken kernels augmented (LOCATELLI and LIMONTA, 1998; KALIYAN *et al.*, 2005) and maize is mainly damaged in the germ area (FRAENKEL and BLEWETT, 1945; ABDEL- RAHMAN *et al.*, 1968; MBATA, 1990). In this study the developmental time required from egg hatching to adult was from 48 to 52 days on entire kernels. However, unlike our results, ARBOGAST (2006) observed a shorter mean developmental period of 34.5 days (from egg to adult) on entire kernels of the maize Pioneer 3320 at 27°C, at 70 or 80% RH, thus indicating that our lines were less favorable to *P. interpunctella* development than the commercial hybrid adopted in the ARBOGAST (2006) study.

Interestingly, in the tests with entire kernels, the only exception was represented by homozygous *vp*/*vp* mutant kernels that were significantly attacked by the insects, with 7.5 the mean number of emerged adults and 55% of damaged

Table 2 - Mean number (±S.D.) of emerged adults[1], mean developmental period (±S.D.) (number of days) of *Plodia interpunctella* (Walker) and damaged seeds percentage observed on longitudinally sectioned kernels of different maize genetic stocks.

Maize genetic stocks	Mean number of adults	Min-max	Insect developmental period (mean)	Min-max	Damaged seeds (%)
B73	12±3.65b	8-16	34.9±2.07d	33-39	100
RAlex0	15.2±2.5a	12-18	36.9±4.1bc	33-53	100
Emb/-	6.5±2.1c	4-9	36.6±2.31bcd	33-40	100
emb/*emb*	1.7±0.5d	1-2	44.3±5.32a	39-53	45
Vp/-	9.5±0.57bc	9-10	35.9±2.63cd	33-42	100
vp/*vp*	10±0.81b	9-11	38.5±2.98b	33-43	100

[1]Observations were carried out on twenty newly emerged larvae for each replicate.
One-way Anova: Adults F5,18=20.59 P<0.001; development period F5,214=14.767 P<0.001
Means followed by different letters are significantly different according to Duncan's multiple range test.

seeds and where 37.5% of larvae reached the adult stage. vp embryo are characterized by deficiency in ABA synthesis, that causes pre-germination of the mutant embryo while still attached to the ear. As consequence the pericarp, the external tegument of the seed, is less hard or fractured in the germ area, thus making the embryo more accessible, and allowing the insect to easily penetrate the kernel surface. The comparison between sectioned vp and wild-type sectioned kernel did not confirm a preference for the mutants, thus proving that the results observed in the entire kernels test were due to physical and not to nutritional properties of the embryos.

We may thus conclude that several factors influence the development of the pest, not only nutrients in the cereal but also the physical structure, such as hardness and the more or less smooth surface. Also SILHACEK and MURPHY (2006) observed that "nutrient availability depends upon the amounts of nutrients actually consumed and upon physical factors that restrict the ingestion of nutrients"

As to the other genotypes in this study, the observation that in longitudinally sectioned emb mutant seeds only few adults with a longer developmental period were observed, provides a proof of the requirement of this seed domain for *P. interpunctella* development. The emb mutant adopted in this study belongs to the class of *embryo specific* (*emb*) mutants, in which only the embryo but not the endosperm is affected (Clark and Sheridan, 1991); mutant seeds lack or exhibit a smaller scutellum and their embryo axis does not differentiate, whereas starchy endosperm seems normal both in volume and texture. As previously shown by FRAENKEL and BLEWETT (1946), *P. interpunctella* can obtain all the nutrients it needs from wheat germ, but it is unable to use the starch in the endosperm. Our work may support the theory that *P. interpunctella* needs nutrients present in the germ, most probably liposoluble vitamins and proteins.

Another interesting result is related to the analysis of the RAlex0 inbred line, which is the result of a selection process aimed at increasing the oil concentration in the scutellum. It is conceivable, on the basis of the number of emerged adults, that RAlex0 nutritional properties have a positive effect on stimulating the speed of insects' development.

A preference for the B73 lines was also observed, as shown in the test with sectioned kernels. The comparison of B73 *versus* RAlex0 with Student's t test indicated that the two lines were not significantly different in terms of number of emerged adults; moreover in the first line the mean developmental period was shorter. B73, which has been selected by breeders for its robustness and suitability in hybrid production, appears indeed not to be the ideal genotype for maize storage since it is a suitable substrate for *P. interpunctella* development. We observed, for instance, in the test with whole kernels that the wild-type controls of the two mutants used in the study, referred to as *Emb*/- and Vp/-, are more resistant to the infestation of *P. interpunctella*. Since both mutations have been introgressed in the B73 line, we may speculate that the introgression has brought some genetic factors that lead to a modification of the kernel properties.

Many mutants affecting embryo and endosperm development and metabolism are available in maize, and have been adopted for the isolation of genes involved in seed formation as well as the characterization of the molecular mechanisms implied. This study, although preliminary, shows that maize genetic variants constitute a valuable tool for the study of insect-seed interactions. Developmental mutants give the opportunity to explore the role of the seed components, i.e. embryo, endosperm and integuments, in determining insects attack. Similarly, mutants showing metabolic defects could be adopted to specifically investigate the role of molecules, such as oils, proteins, carbohydrates and secondary metabolites, in promoting or reducing insect attack.

This type of study will provide useful information for the selection of genotypes resistant to insect pests of stored products. If one takes into account that during its development *P. interpunctella* produces on the surface of the cereal a thick silk net that favors mold proliferation, we can conclude that the adoption of maize seeds more resistant to mechanical damage and insect attacks can also prevent mycotoxin accumulation thus enhancing both quality and safety of stored products.

REFERENCES

Abdel-Rahman H.A., Hodson A.C. and Christensen C. 1968. Development of *Plodia interpunctella* (Hb.) (Lepidoptera: Phycitidae) on different varieties of corn at two levels of moisture. J. Stored Prod. Res. 4, 127-133.

Adesuyi S.A. 1977. Relative resistance of some varieties of maize to attack by *Sitophilus zeamais* (Motsch.). Report of the Nigerian Stored Products Research Institute, 1976/1977, Technical report no. 7, 79-82.

Arbogast R.T. 2006. A wild strain of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) from farm-stored maize in South Carolina: development under different temperature, moisture, and dietary conditions. J. Stored Prod. Res. 43, 160-166.

Bhattacharya A.K., Chaudhary R.R.P. and Rathore R.R.S. 1976. Susceptibility of several varieties of soybean to *Ephestia cautella* (Walker) (Lepidoptera: Phycitidae). J. Stored Prod. Res. 12, 143-148.

Clark J.K. and Sheridan W.F. 1991. Isolation and characterization of 51 embryo-specific mutations of maize. Plant Cell 3, 935-951.

Consonni G., Gavazzi G. and Dolfini S. 2005. Genetic analysis as a tool to investigate the molecular mechanism underlying seed development in maize. Annals of Botany 96, 353-362.

Dobie P. 1974. The laboratory assessment of the inherent susceptibility of maize varieties to post harvest infestation by *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). J. Stored Prod. Res. 10, 183-197.

Dobie P. 1977. The contribution of the tropical stored products centre to the study of insect resistance in stored maize. Tropical Stored Products Information 34, 7-16.

Falomo A.A. 1981. Post-harvest susceptibility of maize varieties to infestation by *Sitophilus zeamais* Motsch. Report of the Nigerian Stored Products Research Institute, 1981, Technical report no. 6, 57-63.

Fraenkel G. and Blewett M. 1945. The dietetics of the caterpillars of three *Ephestia* species, *E. kuehniella*, *E. elutella*, and *E. cautella*, and of a closely related species, *Plodia interpunctella*. J. Exp. Biol. 22, 162-171.

Fraenkel G. and Blewett M. 1946. Linoleic acid, vitamin E and other fat-soluble substances in the nutrition of certain insects, *Ephestia kuehniella*, *E. elutella*, and *E. cautella* and *Plodia interpunctella* (Lep.). J. Exp. Biol. 22, 172-190.

Gerdes J.T., Behr C.F., Coors J.G. and Tracy W.F. 1993. Compilation of North American maize breeding germplasm. Misc. Publ. CSSA, Madison, Wisconsin, USA.

Giraudat J., Parcy F., Bertauche N., Gosti F., Leung J., Morris P.C., Bouvier-Durand M. and Vartanian N. 1994. Current advances in abscisic acid action and signalling. Plant Mol. Biol. 26, 1557-1577.

Hable W.E., Oishi K.K. and Schumaker K.S. 1998. Viviparous-5 encodes phytoenedesaturase, an enzyme essential for abscisic acid (ABA) accumulation and seed development in maize. Mol. Gen. Genet. 257, 167-176.

Hockensmith P.E., Devine T.L., Legg D.E. and Rodriguez J.G. 1986. Energy consumptions and food utilization of the Indian meal moth (Lepidoptera: Pyralidae) on different corn genotypes. J. Kansas Entomol. Soc. 59 (4), 598-603.

Kaliyan N., Carrillo M.A., Morey R.V., Wilcke W.F. and Cannon C.A. 2005. Indian meal moth survivability in stored corn with different levels of broken kernels. Great Lakes Entomologist 38 (3/4), 177-185.

Locatelli D.P. and Limonta L 1998. Development of *Ephestia kuehniella* (Zell.), *Plodia interpunctella* (Hbn.) and *Corcyra cephalonica* (Staint.) (Lepidoptera: Pyralidae) on kernels and wholemeals of *Fagopyrum esculentum* Moench and *Triticum aestivum* L. J. Stored Prod. Res. 34 (4), 269-276.

Mbata G.N. 1987. Studies on the susceptibility of groundnut varieties to infestation by *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae). J. Stored Prod. Res. 23 (1), 57-63.

Mbata G.N. 1990. Suitability of maize varieties for the oviposition and development of *Plodia interpunctella* (Hubner) (Lepidoptera,Pyralidae). Tropical Pest Management 36 (2), 122-127.

Mbata G.N., Osuji F.N.C. and Okere A.N. 1988. Studies on the developmental biology of *Corcyra cephalonica* (Stainton) (Lepidoptera: Galleriidae) on 13 maize varieties. Tropical Science 28 (1), 25-34.

Morah S.C. and Mbata G.N. 1986. An assessment of relative susceptibility of some maize varieties to post harvest infestation by the maize weevil *Sitophilus zeamais* (Motsch.). Report of the Nigerian Stored Products Research Institute, 1982, Technical report no. 5, 63-68.

Rose H.S. and Behl N.K. 1985. Studies on the development of *Corcyra cephalonica* (Stainton) (Gallerinae: Pyralidae: Lepidoptera) on different varieties of maize. Ann. Entomol. 3 (1), 25-28.

Russell W.A. 1972. Registration of B70 and B73 parental lines of maize. Crop Science 12: 721.

Schnable P.S., Ware D., Fulton R., Stein J., Wei F. *et al.* 2009. The B73 maize genome: complexity, diversity and dynamics. Science 326, 1112-1115.

Sedlacek J.D., Komaravalli S.R., Hanley A.M., Price B.D. and Davis P.M. 2001. Life history attributes of Indian meal moth (Lepidoptera: Pyralidae) and Angoumois grain moth (Lepidoptera: Gelechiidae) reared on transgenic corn kernels. J. Econ. Entomol. 94 (2), 586-592.

Silhacek D. and Murphy C. 2006. A simple wheat germ diet for studying the nutrient requirements of the Indian meal moth, *Plodia interpunctella* (Hubner). J. Stored Prod. Res. 42 (4), 427-437.

Siwale J., Mbata K., McRobert J. and Lungu D. 2009. Comparative resistance of improved maize genotypes and landraces to maize weevil. African Crop Science Journal 17 (1), 17-24.

Walbot V. Strategies for mutagenesis and gene cloning using transposon tagging and T-DNA insertional mutagenesis. Annu. Rev. Plant Phys. Plant Mol. Biol. 1992, 43:49-82.

Wiseman R., McMillan R. and Widstrom N.W. 1970. Husk and kernel resistance among maize hybrids to an insect complex. J. Econ. Entomol. 63, 1260-1262.

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PAPER

THE HEDONIC PRICE FOR AN ITALIAN GRAPE VARIETY

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ABSTRACT

Consumers face complex choices when buying wine, a highly differentiated product sold at widely varying prices. This paper aims to provide a monetary valuation of some key credence attributes of wine, such as certifications and quality ratings made by expert tasters. The implicit price of DOC-DOCG certification is of extreme importance. It gives access to a collective reputation and enables a premium price to be captured from consumers. With this in mind, hedonic price estimation was performed on a specific Italian grape variety (Aglianico).

- Keywords: attributes, certification, hedonic, wine -

INTRODUCTION

Wine consumption and production have steadily increased worldwide over the past few decades (ANDERSON and NELGEN, 2009) despite the decrease of consumption in Mediterranean Countries (MARIANI *et al.*, 2012; POMARICI *et al.*, 2012). World wine markets are showing a constant increase in competition, fueled by both enhanced quality and quantity in the supply of new producing Countries, and by an improvement in the quality of wine produced in the traditional Countries (OIV, 2011; WITTWER, 2007). At the same time, consumption patterns and habits have been evolving particularly fast in traditional wine-producing and -consuming Countries (ANDERSON and NELGEN, 2009; CEMBALO *et al.*, 2013): in Italy and France, for example, per capita consumption was about 100 liters per year in the 1970s while the corresponding figure is currently about 45 liters (OIV, 2011). In this context, consumers face complex choices when they buy wine. Most of the complexity is due to the fact that wine is a highly differentiated product sold at prices that vary over a wide range. At the same time, wine embodies a bundle of intrinsic and extrinsic attributes that generate a perception of quality which makes consumers view a wine in different ways (GOLDSTEIN *et al.*, 2008; LECOCQ and VISSER, 2006).

Wine producers also base their product quality strategies on intrinsic and extrinsic attributes. Among the latter, classic wine designations and reputation (sensory quality ratings in magazines) seem to carry the most weight (SCHAMEL and ANDERSON, 2003)[1]. In this setting, the recent EU reform on wine under the Common Market Organization (Reg. EC 479/2008 later included in the Reg. EC 1273/2007) aims to improve the competitiveness of the EU wine sector, encouraging the enhancement of wine quality[2]. Moreover, it obliges EU Member States to adopt a strict system of control on origin certificated wines[3]. This new strategy may have important implications for producers' long-term investment decisions. While a stricter certification system involves observable explicit costs for producers (CARACCIOLO and CEMBALO, 2010; CICIA *et al.*, 2013; CEMBALO *et al.*, 2012), the benefits are mainly unknown and not directly observable in the market. Two possible scenarios may arise: 1. Certification benefits exceed adoption costs, resulting in the success of the collective brand equity; 2. certification and controlling costs exceed their benefits, leading to market failure. In this regard, of paramount importance is the monetary valuation of credence attributes, in particular origin designation labeling. One good argument concerning the benefits of a labeling/certification system is the trust that it generates on consumers. Many experience attributes are not known before purchase. Labeling and certification, as well as reputation, contribute to overcome imperfect information (AKERLOF, 1970). In other words, the expected wine quality is proxied by consumers trough a long term reputation or, more frequently, by a certification system that ensures consumers on quality, turning experience attributes in credence ones. If consumers deem a certification or labeling procedure as trustworthy, then a long term reputation takes place that, very likely, influences market prices (BENFRATELLO *et al.*, 2009).

This paper aims to provide monetary valuation of some relevant credence attributes, such as certifications and quality ratings made by expert tasters. While markets for attributes can be considered implicit, implicit prices of the attributes determine a wine's price (BOMBRUN and SUMMER, 2003). Here, market values for some wine attributes are measured by estimating a hedonic price function, which relates the price of a wine to its various attributes. Hedonic price estimation in the wine sector has been applied worldwide. What is common to all works is that they consider wines in large categories, such as reds, whites, premium or basic wines (ANGULO *et al.*, 2000; COMBRIS *et al.*, 1977; COMBRIS *et al.*, 2000; FOGARTY, 2006; SCHAMEL and ANDERSON, 2003; JONES and STORCHMANN, 2001). In this paper, hedonic price estimation of a specific Italian grape variety (Aglianico) was performed. The reason why our analysis focused on a certain varietal lies in the overall homogeneity of the product. This strategy avoids the possible drawback when a hedonic price estimation is involved in a heterogeneous sample of wines: the relations between price and wine attributes may be biased by unobservable wine characteristics (COSTANIGRO and MCCLUSKEY, 2011). In our empirical approach, this bias is controlled by sample (varietal) homogeneity.

MATERIALS AND METHODS

Data

A set of 1,053 different Aglianico wines was collected from three of the most popular Italian wine guides, (AIS: Ais Duemilavini; GR: Gambero Rosso; VER: Veronelli). Although other guides are available in the literature, those three report systematically the southern Italy production. All the Aglianico wines in the sample were re-

[1] A large part of consumers seem to be influenced by quality labelling even though only a fraction of them knows definition and meaning (VECCHIO and ANNUNZIATA, 2011).

[2] Recital n. 5, in the foreword to the Reg. EC 479/2008, states: "*...achieving the following objectives: increasing the competitiveness of the Community's wine producers; strengthening the reputation of Community quality wine as the best in the world; recovering old markets and winning new ones in the Community and worldwide; creating a wine regime that operates through clear, simple and effective rules that balance supply and demand; ...*"

[3] EU Legislation: Reg. EC 607/2009, Section 6; Italian legislation: D.L.gs 61/2010, Section 4.

Table 1 - Quality ratings made by experts for Aglianico (2001 *vs* 2010).

Aglianico reviewed	AIS		GR		VER	
	2001	2010	2001	2010	2001	2010
Low rating	48	6	60	32	38	15
Average rating	40	86	35	61	53	56
High rating	12	9	5	7	9	29
Total	100	100	100	100	100	100

AIS: "Duemilavini 2010. Il libro guida ai vini d'Italia". GR: "Gambero rosso 2010. Vini d'Italia 2010". VER: "Veronelli, 2010. I vini di Veronelli 2010".

viewed in at least one of the three guides. Aglianico is an important historical variety native to Southern Italy. It occupies approximately 23 and 34% of vine-growing areas in the regions of Campania and Basilicata, respectively. Many denominations of origin are involved: 20 DOC/DOCGs and 15 origin certifications (POMARICI *et al.*, 2004). As shown in Table 1, Aglianico wines improved their overall quality ratings expressed by the experts during the time span from 2001 to 2010. The number of reviewed Aglianico wines, in all three guides, increased almost threefold in the same decade (Table 2).

Prices (in €) are organized into four categories: < 5, 5-10, 10-15, > 15. Most wines in the sample have a price below €10 (73%) (Table 3). Other variables collected were: province of production; certifications (DOC, DOCG, POD, PGI); degree of Aglianico varietal content; number of bottles produced; firm degree of specialization; firm type; and three sources of expert quality ratings. Aglianico wine produced in Campania represents over 75% of the whole sample. 93% is produced by private companies, 13.3% is certified as organic/biodynamic, and more than 54% of the sample falls within origin certification regulations (Table 4). At least half of the wines collected were reviewed in either the Veronelli or Gambero Rosso guide. Only a fourth of all wines were reviewed by the AIS wine guide.

Methodology

The hedonic pricing method assumes that goods consist of a bundle of characteristics and are valued by their utility-generating attributes. In other words, the market price reflects the good composition of the attributes which, on the contrary, have no explicit price. To this extent, it is possible to value the attributes that compose the final good by analyzing the systematic variation in the price (ROSEN, 1974). Hedonic price estimation in the wine sector has been applied worldwide.

In a hedonic price function not only might the objective characteristics appearing, for example, on the label of the bottle be included, but also other wine attributes (COMBRIS *et al.*, 1977; COMBRIS *et al.*, 2000; FOGARTY, 2006; SCHAMEL, 2003; SCHAMEL and ANDERSON, 2003; BENFRATELLO *et al.*, 2009; PANZONE, 2011; CAREW and FLORKOWSKI, 2010; LANDON and SMITH, 1998). Since prices collected were expressed in ranges, the dependent variable consists of four ordinal categories. In this situation, ordered logit estimation is required. The ordered logit method represents a generalization of the Logit model and it is specifically applied to analyze ordinal data (WINKELMANN and BOES, 2006).

Table 2- Aglianico coverage in Italian wine catalogues.

	2010			2001		
	AIS	GR	VER	AIS	GR	VER
No. of wines reviewed	16,000	18,000	16,800	7,000	12,045	6,495
% change (2001-2010)	*129*	*49*	*159*			
Aglianico reviewed	161	231	345	104	91	64
% change (2001-2010)	*55*	*154*	*439*			
Total firms reviewed	1,600	2,253	3,000	1,000	1,681	1,524
% change (2001-2010)	*60*	*34*	*97*			
Aglianico producers reviewed	70	121	155	43	43	33
% Aglianico reviewed wine/total reviewed wine	1	1.3	2.1	1.5	0.8	1
% Aglianico producers reviewed /total firms reviewed	4.4	5.4	5.2	4.3	2.6	2.2
Average rating of Aglianico	2	1.7	2.1	1.6	1.5	1.7

Table 3 - Price classification of sampled wine.

Price range	Dependent variable value	# cases	%
€0-5	1	359	34.09
€5-10	2	414	39.32
€10-15	3	161	15.29
€15	4	119	11.3
Total		1,053	100

The model assumes a latent unobserved continuous process (1):

$$(1)\quad y_w^* = \mathbf{x}_w \beta + e_w, \qquad E[e_w | X_w] = 0,$$
$$e_i \text{ i.i.d. } Logistic\ (0,1) \text{ with } w = 1,\ldots,W$$

It underlies the ordinal observed outcome y_w:

$$(2)\quad y_w = \begin{cases} 1 & if \quad k_0 < y_w^* \le k_1 \\ 2 & if \quad k_1 < y_w^* \le k_2 \\ 3 & if \quad k_2 < y_w^* \le k_3 \\ 4 & if \quad k_3 < y_w^* \le k_4 \end{cases}$$

where $k_0 = -\infty$ and $k_4 = \infty$; k_1 k_2 k_3 are unknown threshold parameters to be estimated in order to indicate the range of the logistic distribution associated with specific values of the stated response variable y_w^*. $\mathbf{x}_w$ is the vector of explanatory variables and β is the vector of unknown parameters. The parameters are obtained by maximizing the log-likelihood: for example, the probability that $y_w = 1$ is:

$$(3)\quad \pi_{w1} = P(y_w = 1 \mid \mathbf{x}_w) = F(k_1 - \mathbf{x}_w \beta) - F(k_0 - \mathbf{x}_w \beta)$$

where F is the cumulative distribution function (cdf) of e_w. In the ordered logit the error term e_w is assumed to be distributed as a standard logistic.

In our study, the hypothesis being tested is that the market price of the selected wine, (in terms of the probability that a wine is being priced in a certain price class) is a function of the wine's extrinsic attributes, specific winery factors influencing the firm's cost curve but also some credence attributes.

As stated previously, hedonic price estimation can be cumbersome when the sample of the analyzed wines is too heterogeneous. Estimation of the attributes' implicit prices can be inconsistent due to the considerable influence of unobservable quality signals (OCZKOWSKI, 2001). The sample of wine under investigation, including only one grape variety, minimizes such drawbacks, excluding the effects of collective reputation related to different product designations (COSTANIGRO and MCCLUSKEY, 2011). Product designation is of paramount importance since it is related to comparative market advantage due to terroir and price differentiation. Market price might thus depend on explicit quality product and specific credence attributes, such as ratings of experts and classic wine designations.

RESULTS AND DISCUSSION

Estimation results

According to the estimation results of the ordered logit described in the previous section (Table 5), most of the explanatory variables included in the model are statistically significant. The following were not significant: organic certification (organic) and degree of vine specialization of producing wineries (specialized). DOCG certification seems to show the greatest impact. As for quality experts' ratings, the AIS guide rating had no impact on the selling price but inclusion in AIS had a positive impact. By contrast, both the wine ratings of Gambero Rosso and Veronelli had a positive influence on price.

On estimating hedonic price modeling the marginal effects can be straightforwardly derived. In the case of an ordered logit model, marginal effects must be interpreted as the impact of a unit increase in an explanatory variable on the probability of shifting from one price class to the next. In order to elicit an implicit price, the change in probability thereby derived was transformed into the average change in the expected value of wines. The procedure consists in summing over the four price classes the predicted change in probability due to the marginal increase in the variables

Table 4 - Explanatory variables included in the empirical model.

	Type	Definition	Mean	St. dev.
Location	Campania	1 if winery is located in Campania	0.756	0.43
	Prov. AV	1 if winery is located in Avellino prov.	0.279	0.449
Organization	Private company	1 if winery is a private company	0.934	0.248
	Specialized	Degree of winery specialization	0.227	0.419
	Q bottled	*ln* no. of bottles produced	11.52	1.561
Certification	Organic	1 if organic	0.133	0.34
	DOCG	1 if DOCG	0.099	0.298
	DOC	1 if DOC	0.54	0.499
Product	% Aglianico varietal	% of Aglianico vine content	87.544	26.884
Expert quality ratings	AIS presence	1 if reviewed in AIS	0.254	0.435
	Gambero Rosso presence	1 if reviewed in GR	0.422	0.494
	Veronelli presence	1 if reviewed in VER	0.465	0.499
	AIS rating	AIS score	0.311	0.746
	Gambero Rosso rating	GR score	0.383	0.769
	Veronelli rating	VER score	0.7	1.07

Table 5 - Ordered Logit hedonic pricing results.

	Parameters	Coeff.	Sign.
Location	Campania	-0.476	**
	prov. AV	-0.551	***
Organization	Private company	0.818	***
	Specialized	0.222	
	Q bottled	-0.178	***
Certification	Organic	-0.009	
	DOCG	3.459	***
	DOC	0.856	***
Product	% Aglianico grape variety	0.007	***
Expert quality ratings	AIS presence	1.024	***
	Gambero Rosso presence	0.037	
	Veronelli presence	0.081	
	AIS rating	-0.024	
	Gambero Rosso rating	0.388	***
	Veronelli rating	0.328	***
Thresholds			*s.d.*
	k_1	-0.551	0.806
	k_2	1.742	0.807
	k_3	3.167	0.814

No. obs 1,053. Loglikelihood=-1,055.1; Wald $\chi^2(15)$=546.99 Prob > χ^2=0.000. Starred levels of significance are 10% (*), 5% (**), 1% (***).

Table 6 - Average marginal effect and derived implicit prices.

	Expected value	0–5€		5€-10€		10€-15€		>15€	
Campania	**-€ 1.04**	0.082	**	-0.012		-0.031	**	-0.038	***
AV prov	**-€ 1.14**	0.098	***	-0.027	***	-0.033	***	-0.039	***
Private company	**€ 1.63**	-0.151	***	0.051	***	0.049	***	0.051	**
Specialized	**€ 0.48**	-0.039		0.007	*	0.014		0.017	
Q bottled	**-€ 0.45**	0.031	***	-0.007	***	-0.011	***	-0.014	***
Organic	**-€ 0.02**	0.002		0		-0.001		-0.001	
DOCG	**€ 8.48**	-0.359	***	-0.244	***	0.115	***	0.489	***
DOC	**€ 1.79**	-0.154	***	0.041	***	0.051	***	0.062	***
% Aglianico grape variety	**€ 0.01**	-0.001	***	0	***	0	***	0.001	***
AIS presence	**€ 2.31**	-0.172	***	0.013		0.072	***	0.087	**
Gambero Rosso presence	**€ 0.08**	-0.007		0.001		0.002		0.003	
Veronelli presence	**€ 0.17**	-0.014		0.003		0.005		0.006	
AIS rating	**-€ 0.05**	0.004		-0.001		-0.002		-0.002	
Gambero Rosso rating	**€ 0.85**	-0.068	***	0.014	***	0.024	***	0.03	***
Veronelli rating	**€ 0.71**	-0.057	***	0.012	***	0.02	***	0.025	***

Starred levels of significance are 10% (*), 5% (**), 1% (***).

multiplied by the mean price of the corresponding price class of wines (Table 6).

Wine produced by private companies enjoys a higher market price than that of cooperatives. Location of a winery in the region of Campania negatively affects the average Aglianico selling price. DOCG certification has a great impact on price: the derived implicit price is 8 euros. As for the impact of the wine guides, inclusion in AIS, regardless of the assigned score, increases the average market price by around €2. Gambero Rosso and Veronelli show similar implicit prices, estimated to be around €0.80 for each additional assigned score. The effect of the three guides is comparable. Whereas few Aglianico wines are included in AIS, Gambero Rosso and Veronelli assess a larger number of Aglianico wines. While listing of the wine in the AIS catalogue is a sufficient condition to influence the selling price, the same does not hold for the other guides.

CONCLUSIONS

Our analysis sought to isolate and measure the role of some credence attributes on wine prices. Reputation of the production districts, provided by designation of origin certification, can enhance

a comparative, and collective, market advantage due to the role of terroir. Hedonic price estimation was performed on a specific Italian grape variety (Aglianico). Aglianico is a varietal native to southern Italy which has been awarded many designations of origin under two certifications: DOC and DOCG (the latter is more tightly regulated). Due to the unconventional nature of the dependent variable expressed in categories, ordered logit hedonic price modeling was implemented. Based on the empirical results, credence attributes greatly influence the market price of Aglianico. Among them, DOCG certification showed the highest influence on price. In some studies the role of DOC and DOCG were not clearly targeted. Some studies showed a strong relationship between such certification and price (CHAMBOLLE and GIRAUD-HÉRAUD, 2005; VEALE and QUESTER, 2008; BENFRATELLO *et al.*, 2009) while others showed that any such influence was weak, if non-existent. In our study the implicit price of DOC-DOCG certification is of extreme importance and, what is more, higher than that of the expert ratings. This result has two possible implications. The first is that grape variety homogeneity seems to be an important requisite when relations between extrinsic attributes and price heterogeneity are being examined. The second concerns the major role that this certification still performs. DOCG labeling gives access to a collective reputation and enables a premium price to be captured from consumers. Since the DOC-DOCG certifications became stricter for producers due to a new controlling system set in train by the new EU-COM, this study helps show that adoption of a collective strategy for quality wine will be rewarding for those firms that are willing to apply a strategy of brand equity to maintain or increase market power.

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REFERENCES

Akerlof G.A. 1970. The market for "lemons": Quality uncertainty and the market mechanism. Quarterly J. of Econ. 84: 488.

Anderson K. and Nelgen S. 2009. Global wine markets, 1961 to 2009: a statistical compendium, The University of Adelaide Press.

Angulo A.M., Gil J.M., Gracia A. and Sánchez M. 2000. Hedonic prices for Spanish red quality wine, British Food Journal. MCB University Press, 102(7): 481.

Benfratello L., Piacenza M. and Sacchetto S. 2009. Taste or reputation: what drives market prices in the wine industry? Estimation of a hedonic model for Italian premium wines. Applied Econ. 41(17): 2197.

Bombrun H. and Sumner A. 2003. What determines the price of wine? The value of grape characteristics and wine quality assessments. Agricultural Issues Center University of California 18: 16.

Caracciolo F. and Cembalo L. 2010. Traceability and demand sensitiveness: evidence from Italian fresh potatoes consumption. Int. Journal on Food System Dynamics 1(4): 352.

Carew R. and Florkowski W.J. 2010. The Importance of Geographic Wine Appellations: Hedonic Pricing of Burgundy Wines in the British Columbia Wine Market. Canadian Journal of Agricultural Economics/Revue canadienne d'agroeconomie 58(1): 93.

Cembalo L., Migliore G. and Schifani G. 2012. Consumers in postmodern society and alternative food networks: The organic food fairs case in Sicily. New Medit., 11(3): 41-49.

Cembalo L., Migliore G. and Schifani G. 2013. Sustainability and new models of consumption: The Solidarity purchasing groups in Sicily. Journal of Agricultural and Environmental Ethics. 26(1): 281-303.

Chambolle C. and Giraud-Héraud E. 2005. Certification of Origin as a non-tariff barrier. Review of Int. Econ. 13(3): 461.

Cicia G., Cembalo L., Del Giudice T., and Scarpa R. 2013. Country of origin effects on Russian wine consumers. J. of Food Products Marketing 4(19).

Combris P., Lecocq S. and Visser M. 1977. Estimation of a hedonic price equation for Bordeaux wine: does quality matter? The Econ. J. 107(441): 390.

Combris P., Lecocq S. and Visser M. 2000. Estimation of a hedonic price equation for Burgundy wine. Applied Econ. 32 (8): 961.

Costanigro M. and McCluskey J. 2011. Hedonic price analysis in food markets. In: Lusk J., Roosen J. and J. Shogren (Eds.), "The Oxford Handbook of the economics of food consumption and policy". Oxford University Press, Oxford.

Fogarty J. 2006. The return to Australian fine wine. European Review of Agric. Econ. 33(4): 542.

Goldstein R., Almenberg J., Dreber A., Emerson J.W., Herschkowitsch A. and Katz J. 2008. Do more expensive wines taste better? Evidence from a large sample of blind tastings. J. of Wine Econ. 3(1): 1.

Jones G.V. and Storchmann K.H. 2001. Wine market prices and investment under uncertainty: An econometric model for Bordeaux Crus Classés. Agric. Econ. 26(2): 115.

Landon S. and Smith C.E. 1998. Quality Expectations, Reputation, and Price. Southern Econ. J. 64(3): 628.

Lecocq S. and Visser M. 2006. What determines wine prices: objective *vs* sensory characteristics. J. of Wine Econ. 1(1): 42.

Mariani A., Pomarici E. and Boatto V. 2012. The international wine trade: Recent trends and critical issues. Wine Econ. and Policy. http://dx.doi.org/10.1016/j.wep.2012.10.001.

Oczkowski E. 2001. Hedonic Wine Price Functions and Measurement Error. Econ. Record. 77: 374.

OIV 2011. Report of the Director General of the OIV on the world vitiviniculture situation in 2010. International Organisation of Vine and Wine. Paris.

Panzone L. 2011. The lost scent of Eastern European wines in Western Europe: A hedonic model applied to the UK market. British Food J. 113(8): 1060.

Pomarici E., Boccia F. and Catapano D. 2012. The wine distribution systems over the world: An explorative survey. New Medit., 11(4): 23-32.

Pomarici E., Rocco L., Raia S. and Tedesco R. 2004. Aglianico in Campania: diffusione e importanza del vitigno e prospettive competitive. In: Moio L. (Ed.), "Colori, odori ed enologia dell'Aglianico", Regione Campania, SeSIRCA, Naples, Italy.

Rosen S. 1974. Hedonic prices and implicit markets: Product differentiation in pure competition. J. of Political Econ. 82: 34.

Schamel G. and Anderson K. 2003. Wine Quality and varietal, regional and winery reputations: Hedonic prices for Australia and New Zealand. The Econ. Record 79(246): 357-369.

Veale R. and Quester P. 2008. Consumer sensory evaluations of wine quality: The respective influence of price and country of origin. J. of Wine Econ. 3(1): 10.

Vecchio R. and Annunziata A. 2011. The role of Pdo/Pgi labelling in Italian consumers' food choices. Agric. Econ. Review. 12(2): 80.

Winkelmann R. and Boes S. 2006. Analysis of Microdata. Springer. Berlin.

Wittwer G. 2007. The global wine market in the decade to 2015 with a focus on Australia and Chile. General Working Paper No. G-166. Centre of Policy Studies. Monash University.

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PAPER

COMPOSITIONAL STUDIES OF SOME PEA (*PISUM SATIVUM* L.) SEED CULTIVARS COMMONLY CONSUMED IN PAKISTAN

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ABSTRACT

The present study was aimed at evaluating the composition and nutrition of some commonly consumed pea cultivars. The investigated parameters included proximate composition, vitamin contents, antinutritional factors (ANF), fatty acids, tocopherols, sterols, amino acid and mineral contents. Variability was observed among investigated cultivars in terms of amino acid and sugar contents. Despite variations among sugar contents, sucrose and raffinose were noted as being present in highest and lowest concentrations, respectively, in all cultivars. The distribution patterns of various amino acids in these cultivars suggested sulphur-containing amino acids as limiting amino acids. Analysis showed almost similar proportions of biochemical constituents among all cultivars. The data show that, in terms of both quality and quantity, the pea cultivars can serve as a significant source of essential amino acids, and bioactive constituents to meet the demand of populations of Pakistan.

- Keywords: pea cultivars, chemical composition, Pakistan -

INTRODUCTION

Pea (*Pisum sativum* L.) is an annual self-pollinated food legumes used throughout Pakistan in people of all income and age groups due to its nutritive value and pleasant taste. Short growing duration and relatively simple production stimulated its production making it a commercial commodity. It is cultivated on 10 thousand hectares with a total production of 82 thousand tons in Pakistan (ASHRAF *et al.*, 2011). The high cost of food crops coupled with the expensive and scarce source of animal proteins as well as the dwindling family income, has made pea a cheap source various food constituents for indigenous people of Pakistan. It is used in livestock feeds as a source of energy and protein and also in feeds for aquatic species.

Recent researches conducted by numerous authors (GDALA *et al.*, 1992; ZDUŃCZYK *et al.*, 1997; BASTIANELLI *et al.*, 1998) show that progress in pea breeding results not only in higher yields, but also in changes in the chemical composition of seeds. Further it is also recognized that genotypic variation and cultivation methods are two major factors influencing levels of chemical constituents in pea seeds (MARZO *et al.*, 1997).Various cultivars of pea are being used throughout Pakistan however no detailed study exists exploring its compositional and nutritional potential. So information is needed on the biochemical composition of pea cultivars to help understand their nutritional profiles from production and consumption points of view. In this context as part of our continuous studies on indigenous flora of Pakistan (KALEEM *et al.*, 2012, RIZWAN *et al.*, 2012, ZIA-UL-HAQ *et al.*, 2011 a, b; 2012 a, b; 2013 a, b) we have evaluated four commonly consumed pea varieties in Pakistan from compositional point of view.

MATERIALS AND METHODS

Material

The seeds of four pea (*Pisum sativum* L.) cultivars, Metvor, Samrina Zard, Climax Improved, and PF-400 were procured from Department of Agronomy, Bahauddin Zakariya University, Multan. Seeds of all the cultivars were divided into groups for storage in stainless-steel containers at 4°C prior to analysis.

Proximate analysis and vitamin contents

Moisture, crude fat, ash, protein and carbohydrates were determined according to AOAC International methods (AOAC, 1998) and results expressed in Table 1. Vitamin C were also measured by AOAC method (AOAC, 1998). Thiamine content was determined by the thiochrome method and riboflavin content by the fluorescence method (GSTIRNER, 1965). Niacin was determined by a reported method (ARINATHAN *et al.*, 2003) (Fig. 1).

Antinutritional factors (ANF) profile

A reported method of (ODUNFA, 1983) was used for separation of oligosaccharides by thin-layer chromatography. 50 µL of ethanol extract were spotted on precoated silica gel plates at 2 cm intervals along with 20 µL of reference standard mixture containing sucrose, raffinose, and stachyose. n-propanol, ethyl acetate and water (6:1:3 v/v) was used as solvent system. After 4 h development of the plates, the oligosaccharides were quantified by the guide strip technique of (SUGIMOTO and VANBUREN, 1970). The sugars content estimated according to the phenol-sulphuric acid method (DUBOIS *et al.*, 1956). Phytic acid was determined as reported previously (ZIA-UL-HAQ *et al.* 2013a) and results are shown in Table 2.

Mineral analysis

The pea samples were incinerated at 450°C for 12 h in a muffle furnace and acid digest was prepared by oxidizing each sub-sample with a nitric/perchloric acid (2:1) mixture. Aliquots were used to estimate Na and K by flame photometer (Flame Photometer Model-EEL). The minerals, such as calcium, manganese, magnesium, zinc, iron and copper, were determined with an atomic absorption spectrophotometer (Perkin-Elmer Model 5000) while Phosphorus

Table 1 - Proximate composition (%) of pea cultivars.

Contents	Metvor	Samrina Zard	Climax Improved	PF-400
Total protein	20.51b±1.71	23.80a±1.66	22.37a±1.60	20.60b±1.72
Total ash	3.16c±0.19	3.54b±.18	3.72a±0.19	3.52b±0.18
Total fat	2.35b±0.05	2.19c±0.05	2.63a±0.09	2.38b±0.09
Crude fiber	10.74a±1.7	9.14b±1.6	11.24a±1.2	10.99a±1.6
Total carbohydrates	56.54a±1.82	52.43a±1.73	50.86b±1.10	54.81a±1.75
Moisture	6.70c±0.31	8.90a±0.32	9.18a±0.28	7.7b±0.19

Data are expressed as means±standard; values having different letters differ significantly ($p<0.05$).

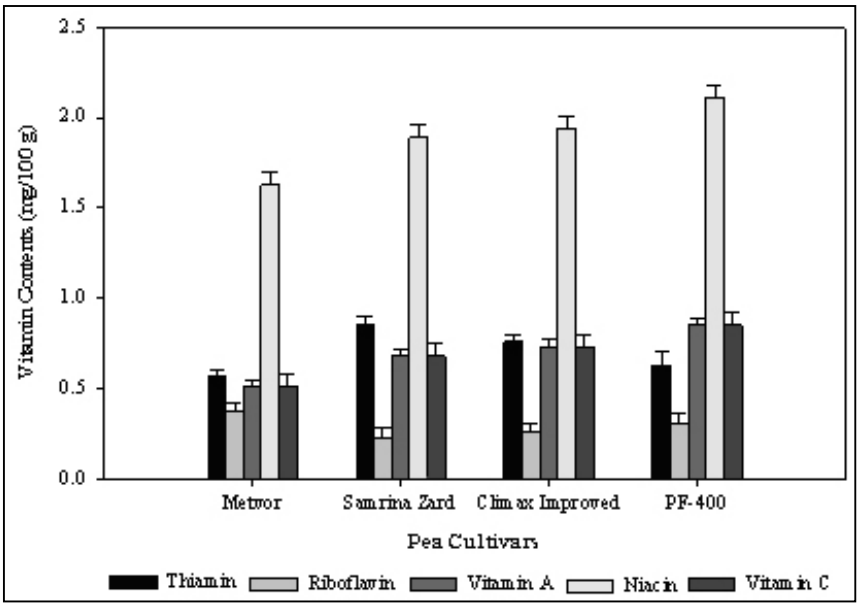

Fig. 1 - Vitamin contents (mg/100 g) of pea cultivars. Data are expressed as means±standard deviations; values having different letters differ significantly ($p<0.05$).

was determined by the phosphovanado-molybdate (yellow) method (AOAC, 1998) (Table 3). The samples were quantified against standard solutions of known concentration that were analyzed concurrently.

Amino acid analysis

Samples (300 mg), in triplicate from each cultivar, were hydrolyzed with 6 N HCl in an evacuated test tube for 24 h at 105°C. The dried residue was dissolved in citrate buffer (pH 2.2) after flash evaporation. Aliquots were analysed in an automatic amino acid analyser (Hitachi Perkin-Elmer Model KLA 3B), using the buffer system described earlier (ZARKADAS *et al.*, 1993). Methionine and cystine were analysed separately after performic acid treatment and subsequent hydrolysis with HCl (KHALIL and DURANI, 1990). Tryptophan was determined after

Table 2 - Anti-nutritional contents (g/ kg) of pea cultivars.

Cultivars	Sucrose	Raffinose	Stachyose	Verbascose	Phytic acid
Metvor	50.7b±0.10	8.43c±0.07	36.7a±0.09	14.80c±0.12	8.30a±0.13
Samrina Zard	46.4c±0.09	9.15b±0.06	33.8b±0.02	19.27a±0.15	7.25b±0.14
Climax Improved	55.9a±0.03	10.03a±0.04	27.1d±0.16	16.4b±0.17	6.19c±0.18
PF-400	48.3bc±0.08	9.29b±0.05	30.9c±0.12	18.1a±0.11	7.34b±0.06

Data are expressed as means±standard deviations; values having different letters differ significantly ($p<0.05$).

Table 3 - Mineral composition (mg/100) g of pea cultivars.

Minerals	Metvor	Samrina Zard	Climax Improved	PF-400	NRC/NAS pattern for infants (1989)
Sodium	111a±2.65	107b±2.38	106b±1.33	108b±1.89	113-200
Potassium	1014b±6.43	1017a±3.78	1021a±4.08	1019a±0.09	500-700
Phosphorus	284b±3.61	291a±2.13	282b±3.08	279b±2.92	500
Calcium	110a±6.24	107b±5.48	111a±4.73	108b±5.10	600
Iron	2.1ns±0.26	1.9ns±0.69	2.3ns±0.52	2.0ns±0.19	10
Copper	9.9b±0.10	10.9a±0.07	11.5a±0.04	10.2b±0.09	0.6-0.7
Zinc	3.4ns±0.20	3.1ns±0.17	3.6ns±0.11	3.2ns±0.07	5
Manganese	2.6ns±0.03	2.2ns±0.02	2.4ns±0.06	2.7ns±0.05	0.3-1
Magnesium	4.0ns±0.04	4.3ns±0.07	4.1ns±0.05	4.4ns±0.03	-

Data are expressed as means±standard deviations; values having different letters differ significantly ($p<0.05$).

Table 4 - Amino acid composition (%) of pea cultivars with FAO/WHO/UNU (1985) patterns of amino acid requirements for different age groups.

Amino acids	Metvor	Samrina Zard	Climax Improved	PF-400	[d]2-5 years	[d]10-12 years
Lysine	8.0a±0.03	7.7b±0.01	7.8b±0.08	8.2a±0.03	5.8	4.4
Histidine	2.4a±0.05	2.1b±0.02	2.0b±0.01	2.4a±0.02	1.9	1.9
Isoleucine	4.5a±0.05	4.4a±0.07	4.2b±0.05	4.0c±0.07	2.8	2.8
Leucine	7.2ns±0.05	7.3ns±0.03	7.4ns±0.04	7.1ns±0.01	6.6	4.4
Cystine	1.7bs±0.08	1.9a±0.04	1.5c±0.03	1.7b±0.08	2.5[a]	2.2[a]
Tyrosine	3.7b±0.01	3.4c±0.06	3.9a±0.02	3.7b±0.05	6.3[b]	2.2[b]
Threonine	3.6ns±0.04	3.8ns±0.04	4.0ns±0.03	3.7ns±0.04	3.4	2.8
Tryptophan	0.9a±0.03	0.7c±0.09	0.8b±0.02	0.8b±0.05	1.1	0.9
Valine	5.0a±0.05	4.8c±0.08	5.0a±0.04	4.9b±0.07	3.5	2.5
Arginine	7.2a±0.03	7.3a±0.04	7.0b±0.03	7.3a±0.05		
Methionine	1.3a±0.02	1.1c±0.05	1.2b±0.09	1.3a±0.02		
Phenylalanine	5.3a±0.12	4.9b±0.06	4.7b±0.07	5.2a±0.08		
Alanine	5.5a±0.03	5.2b±0.07	5.6a±0.05	5.4a±0.01		
Aspartic acid	11.2a±0.07	11.4a±0.08	10.5b±0.07	11.0a±0.05		
Glutamic acid	19.0b±0.05	20.5a±0.07	20.8a±0.09	20.2a±0.09		
Glyine	4.5b±0.04	4.6b±0.05	4.9a±0.04	4.2c±0.04		
Proline	3.9a±0.02	3.7a±0.03	3.5b±0.01	3.8a±0.07		
Serine	4.9ns±0.03	5.3ns±0.05	5.2ns±0.08	5.1ns±0.03		

Data are expressed as means±standard deviations; values having different letters differ significantly ($p<0.05$).
[d] Patterns of amino acid requirements for different age groups;
[b] =Tyr+phe;
[a] =cys+meth.

alkali (NaOH) hydrolysis by the colorimetric method (FREIDMAN and FINELY, 1971) (Table 4).

In vitro protein digestibility

A multienzyme technique was used to measure the in vitro protein digestibility (EKPENYONG and BORCHERS, 1979) as reported previously (ZIA-UL-HAQ *et al.*, 2013a) (Fig. 2).

Fatty acid (FA) composition

Fatty acid methyl esters (FAMEs) were prepared according to the standard of IUPAC method 2.301, and analyzed on a SHIMADZU gas chromatograph model 17-A with flame ionization detector (FID). Separation was done on a capillary column (30 m x 0.32 mm x 0.25 μm; Supelco; Bellefonte, Pa., USA). Nitrogen was used as a

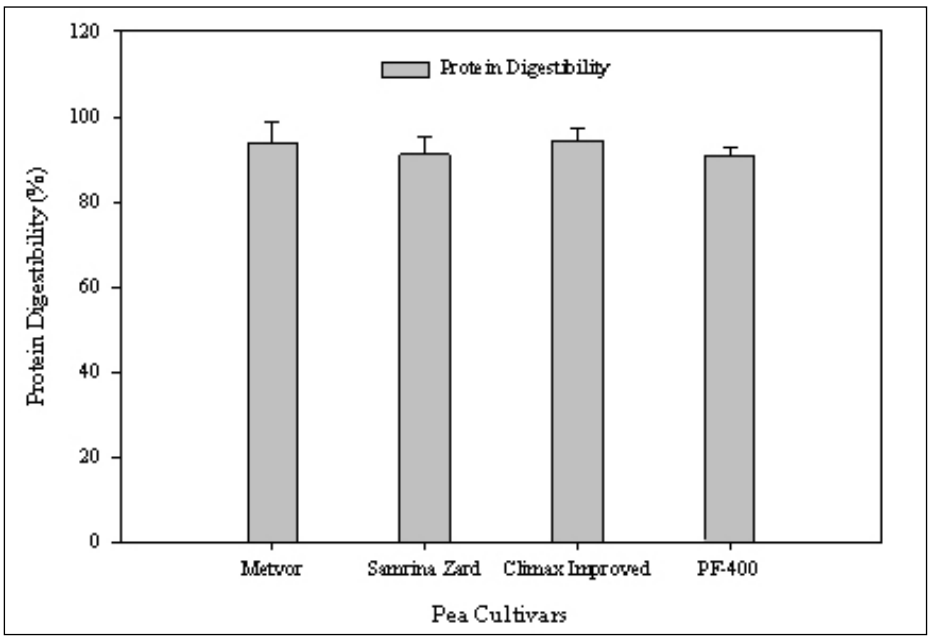

Fig. 2 - *In vitro* protein digestibility (%) of pea cultivars. Data are expressed as means±standard deviations; values having different letters differ significantly ($p<0.05$).

Table 5 - Fatty acid profile of pea cultivars.

Fatty acid	Metvor	Samrina Zard	Climax Improved	PF-400
16:0	10.57b±0.03	11.67a±0.05	12.21a±0.09	11.98a±0.07
16:1	0.09b±0.02	0.13a±0.04	0.07b±0.03	0.06b±0.09
17:0	0.19a±0.09	0.09b±0.02	0.21a±0.04	0.25a±0.06
18:0	3.04a±0.07	2.72b±0.08	2.95a±0.05	2.68b±0.01
18:1	28.41a±0.08	26.31b±0.05	25.05c±0.08	26.16b±0.07
18:2	47.49a±0.05	46.98b±0.03	47.77a±0.05	46.89b±0.05
18:3	9.77b±0.07	11.21a±0.2	10.99a±0.01	11.43a±0.06
20:0	0.24c±0.04	0.39a±0.01	0.31b±0.05	0.27bc±0.04
20:1	0.20c±0.01	0.50a±0.03	0.44a±0.07	0.28b±0.08

Data are expressed as means±standard deviations; values having different letters differ significantly ($p<0.05$).

carrier gas at a flow rate of 3.0 mL/min. Column temperature was programmed from 180° to 220°C at the rate of 3°C/min. Initial and final temperatures were held for 2 and 10 min, respectively. Injector and detector were kept at 230° and 250°C, respectively. A sample volume of 1.0 μL was injected with the split ratio of 1:75. FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards. The quantification was done by a Chromatography Station for Windows (CSW32) data handling software (Data Apex Ltd. CZ-158 00 Pague 5, the Czech Republic). The fatty acid composition was reported as a relative percentage of the total peak area and the results were calculated as mg/100 g of pea seeds (Table 5).

Tocopherol, sterols and squalene analysis

Samples were finely ground (1.0 mm mesh size) using a Moulinex Optiblend 2000 and 1 g of each sample was weighed into a 25×150 mm Pyrex culture tube with Teflon-lined screw cap. Samples were spiked with 2.5 mL internal standard (50 μg 6-ketocholesterol dissolved in 2.5 mL ethanol). Samples were hydrolysed under acidic conditions by a modification of a procedure previously described by (TOIVO *et al.*, 2001) briefly, absolute ethanol (1 mL) and HCl (6M, 5 mL) were added to each tube and samples were shaken vigorously. Tubes were then kept at 80°C for 1 h in a water bath, during which tubes were shaken every 10 min. The tubes were then cooled on ice and 5 mL ethanol, 10 ml hexane/diethyl-ether (1:1, v/v) were added to each sample. Tubes were vortexed (1 min) and then centrifuged at 1,000 rpm (10 min). The upper solvent layer was removed and the extraction repeated with a further 10 mL hexane/diethyl-ether. The combined extracts were dried under nitrogen and stored in a refrigerator until saponified by a procedure previously described (MAGUIRE *et al.*, 2004) for phytosterols, squalene and tocopherols analysis by HPLC. The HPLC system consisted of a Waters 510 pump and a Waters 717 plus autosampler (Waters Corporation, Milford, Massachusetts, USA). For phytosterol analyses, 20 μL sample was injected onto a Luna C8 (2) column (250×4.6 mm i.d.; Phenomenex, Cheshire, UK). Detection was done by a Waters 995 photodiode array detector. The mobile phase was 80% acetonitrile and 20% water at a flow rate of 1.6 mL/min. Column temperature was maintained at 50°C. The HPLC system used for squalene and tocopherol analysis was the same, except the column used was a Supelcosil LC-18-DB (250×4.6 mm i.d.; Supelco, Bellefonte, Pennsylvania, USA). Concerning tocopherol analysis, reverse phase chromatography does not distinguish between the β-and γ-isomers of tocopherol, thus the sum of these isomers is shown throughout as β+γ-tocopherol (Table 6 and Fig. 3).

Statistical analysis

Analyses were performed in triplicate and values marked by the same letter in same column of same class are not significantly different ($P < 0.05$). Data were analyzed by using the "MSTATC" statistical computer package.

RESULTS AND DISCUSSIONS

Compositional studies

Peas enjoy the distinction of being an important constituent of diets of the people of Pakistan as an excellent and inexpensive source of

Table 6 - Squalene, α-Tocopherol and β+γ-Tocopherol content (mg/100 g) of pea cultivars.

Cultivar	Squalene	α-Tocopherol	β+γ-Tocopherol
Metvor	1.7a±0.03	11.6b±0.05	5.5ns±0.02
Samrina Zard	0.5b±0.01	12.4b±0.01	4.9ns±0.04
Climax Improved	0.6b±0.02	10.9b±0.03	5.3ns±0.07
PF-400	0.9b±0.04	13.3a±0.02	5.1ns±0.05

Data are expressed as means±standard deviations; values having different letters differ significantly ($p<0.05$).

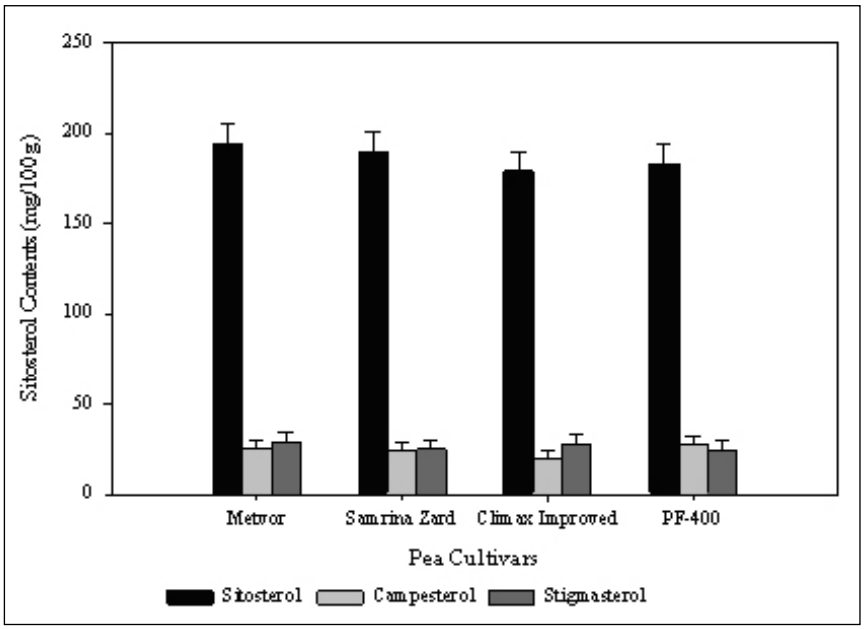

Fig. 3 - β-Sitosterol, campesterol, and stigmasterol content (mg/100 g) of pea cultivars. Data are expressed as means±standard deviations; values having different letters differ significantly ($p<0.05$).

protein, fatty acids, essential amino acids, vitamins and minerals. The data on the proximate composition, along with some of antinutritional factors is summarized in Table 1. The carbohydrates showed a range from 52 to 58%. As pea seeds have high content of high-quality starch (MCLEAN *et al.*, 1974), these can serve as a very good source of energy as well as protein for milk cows at a lower production level or in the declining stage of lactation period (KHORASANI *et al.*, 1992). The observed range for chemical composition is close to that reported earlier (COSTA *et al.*, 2006).

Vitamin contents (Fig. 1) differed from that already measured (VIDAL-VALVERDE *et al.*, 2003; URBANO *et al.*, 2005; MORYMA and OBA, 2008). The reason may be that measurements units differed from previous work. Similarly agrogeoclimatological conditions also affect vitamin contents percentage in legume seeds. Vitamin C contents are different from reported earlier (MICHIE and KAZUKO, 2008). Food levels of vitamin C and flavonoids not only vary greatly depending on species and variety, growing location, harvesting time, storage, processing, and other conditions, but also with respect to methodological differences (ADRIAN *et al.*, 2004).Vitamin C and the flavonoids are both very strong antioxidant agents and their biological activities are in part synergistic (ISLER *et al.*, 1988). Vitamin C is essential for connective tissue formation and maintenance, immune system stimulation, works as anti-oxidant, and enhances iron utilization among other roles (SHRIMPTON, 1993).

As for mineral substances (Table 2), potassium and phosphorus dominate while the calcium content is relatively low. Potassium content ranged from 1,014 mg/100 g in Metvor to 1,021 mg/100 g in Climax Improved. Sodium was found in lower quantity in Climax Improved (106 mg/100 g) while Climax Improved had the highest iron (2.3 mg/100 g) content. All cultivars contained good amounts of calcium, zinc and copper. The results correspond to those already reported for Pea in Pakistan (AMJAD *et al.*, 2006). These results revealed that peas may provide a sufficient amount of minerals to meet the human mineral requirement (NRC/NAS, 1989). However, excess of one mineral may prevent others being absorbed and utilized properly. A significant decrease of systolic blood pressure has been reported with calcium supplementation for the hypertensive persons, since magnesium works in conjunction with calcium to help in transmitting nerve impulse to the brain (ALLENDER *et al.*, 2006; HALLFRISCH *et al.*, 2000). Mineral supplementation can be used as an alternative approach to correct this imbalance.

The data (Table 4) indicated that all essential amino acids, except s-containing types and tryptophan, are present in excessive amounts in all the cultivars analyzed. Pea seeds have relatively favorable lysine content, but the content of methionine and tryptophan has to be are very low. Results are comparable to those of earlier workers (AMJAD *et al.*, 2006). These types of result are also obtained for chickpea. Amino acid deficiency can be met by consuming large amounts of legumes, or by taking a mixture of legumes, or by employing the complementarity that exists between high sulphur amino acid cereals and legumes, especially the soybean (ZIA-UL-HAQ *et al.*, 2007, 2011).

In vitro protein digestibility data (Fig. 2) revealed that values are lowest in PF-400 and highest in Climax Improved. A considerable variation has been reported for pea protein digestibility in the literature (VIDAL-VALVERDE *et al.*, 2003). Digestibility of legume proteins is poor. However, it can be improved through heat-treatments, e.g. cooking, autoclaving and roasting.

Data about the qualitative and quantitative composition of fatty acids are summarized in Table 5. Fatty acid profile of all pea cultivars reveals the lipids as a good source of the nutritionally essential linoleic and oleic acids. Linoleic acid, palmitic acid and oleic acid were the dominating fatty acids. The nutritional value of linoleic acid is due to its metabolism at tissue levels which produce the hormone-like prostaglandins. The activity of these prostaglandins includes lowering of blood pressure and constriction of smooth muscle (AURAND *et al.*, 1987). Linoleic and linolenic acids are the most important essential fatty acids required for growth, physiological functions and maintenance. Most of the fatty acids were unsaturated fatty acids, while saturated fatty acids (mainly, palmitic acid) contributed little of the total fatty acids content. The fatty acid composition and high amounts of unsaturated fatty acids make pea a special legume, suitable for nutritional applications. The presence of high levels of unsaturated fatty acids, in all the presently studied cultivars, is nutritionally desirable and results are comparable with some edible legumes (RYAN *et al.*, 2007).

Our results (Table 6 and Fig. 3) indicated that the pea seeds are exceptionally a rich source of tocopherols and sterols. Our values are in line with that reported earlier (RYAN *et al.*, 2007). Regional and cultivars variations for the distribution of campesterol, stigmasterol, β-sitosterol, Δ^5, avenasterol and clerosterol have already been reported in the literature. As with many of the other traits, no previously reported data on the tocopherol and sterol contents of pea seeds from Pakistan are available in literature. Phytosterols are supposed to have a wide range of effects like anti-inflammatory, anti-oxidative, and anticarcinogenic activities (BERGER *et al.*, 2004). Several studies have indicated that plant sterols inhibit the intestinal absorption of cholesterol, thereby lowering total plasma cholesterol and low-density lipoprotein (LDL) levels (DEJONG *et al.*, 2003). Squalene is believed to be an important dietary cancer chemopreventive agent (SMITH, 2000). It also has been shown to act as an antidote to reduce accidental drug-induced toxicities (SENTHILKUMAR *et al.*, 2006). It has been demonstrated to be a potent quencher of singlet oxygen, (KOHNO *et al.*, 1995). The tocopherol content in food is inversely associated with mortality from cardiovascular disease. In addition, tocopherols, due to their capacity to quench free radical damage, play a putative role in prevention of Alzheimer's disease and cancer (TUCKER and TOWNSEND, 2005).

Anti-nutritional contents

Pea seeds although highly digestible, contain some antinutritional substances that limit their consumption and utilization. Among these are the indigestible oligosaccharides, raffinose, stachyose, and verbascose. These sugars are not utilized by monogastric animals, including humans, who lack the specific α-galactosidase enzyme needed to digest those however their low concentration precludes any significance in human nutrition. Phytic acid and sugar contents (Table 2) are close to investigated earlier. **(**VIDAL-VALVERDE *et al.*, 2003). Intensive breeding efforts have helped to reduce the content and range of antinutritional substances. It is also recognized that varieties with a higher content of antinutritional substances produce higher yields, possibly due to the existence of mechanisms of higher resistance of these varieties to diseases and animal predators. Further theses antinutritional contents may be removed by different processing methods.

CONCLUSION

Current study indicated that peas are a good source of vitamins like thiamine, niacin and riboflavin and much needed iron, but relatively poor source of calcium and sulphur containing amino acids. Anti-nutritional contents present in pea seeds may be reduced by various treatments. In recent years, area, production and productivity of pea in Pakistan has showed an impressive positive annual growth. Concerted efforts are needed to evaluate and, introduce improved pea cultivars with high yield potential and disease resistance with passage of time. The widespread use and diversity of pea products bodes well for the crops future and attests to its versatility as food and feed.

REFERENCES

Adrian A.F., Laurie J.C., Christi, A. and Suzanne P.M. 2004. Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. J. Food Comp. Anal. 17: 1-35.

Allender P.S., Cutler J.A., Follmann D., Cappuccio F.P., Pryer J. and Elliott P. 1996. Dietary calcium and blood pressure: a meta-analysis of randomized clinical trials. Ann. Inter. Med. 124: 825-31.

Amjad L., Khalil A.L., Ateeq N. and Khan M. S. 2006. Nutritional quality of important food legumes. Food Chem. 97:331-335.

AOAC. 1998. "Official Methods of Analysis". 13th Ed. Association of Official Analytical Chemists, Washington, DC.

Arinathan V., Mohan V.R. and DeBritto J.A. 2003. Chemical composition of certain tribal pulses in South India. Int. J. Food Sci. Nut. 54: 209-217.

Ashraf I., Pervez M.A. Amjad M. and Ahmad R. 2011. Ef-

fect of varying irrigation frequencies on growth, yield and quality of pea seed. J. Agric. Res. 49:339-353.

Aurand L.W., Woods A.E. and Wells M.R. 1987. Food composition and analysis. Van Nostrand Reinhold Company, New York, USA.

Bastianelli D., Grosjean F., Peyronnet C., Duparque M. and Regnier J.M. 1998. Feeding value of pea (*Pisum sativum*, L.) 1. Chemical composition of different categories of pea. Anim. Sci. 67: 609-619.

Berger A., Jones P.J.H. and Abumweis S.S. 2004. Plant sterols: factors affecting their efficacy and safety as functional food ingredients. http://www.lipidworld.com/content/3/1/5.

Costa G., Queiroz-Monici K.S., Reis S.M.P.M., Oliveira A.C. 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean, chickpea and lentil legumes. Food Chem. 94: 327-330.

DeJong N., Plat J. and Mensink R.P. 2003. Metabolic effects of plant sterols and stanols. J. Nutr. Biochem. 4: 362-369.

Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F. 1956. Colorimetric method for determination of sugars and related substances. Ann. Chem. 28: 350-356.

Ekpenyong T.E. and Borchers R.L. 1979. Digestibility of proteins of winged bean seed. J. Food Sci. Tech. 16: 92-95.

FAO/WHO/UNU. 1985. Energy and Protein Requirements (Report of a Joint FAO/WHO/UNU Expert Consultation, Meetings Series No 724). WHO, Geneva, Switzerland.

Freidman M. and Finely J.W. 1971. Methods of tryptophan analysis. J. Agr. Food Chem.19: 626-631.

Gdala J., Buraczewska L. and Grala W. 1992. The chemical composition of different types and varieties of pea and the digestion of their protein in pigs. J. Anim. Feed Sci. 1: 71-79.

Gstirner F. 1965. Chemisch-phisikalische Vitaminbestimmungs-methoden. Ferdinand Enke Verlag, Stuttgart.

Hallfrisch J., Veillon C., Patterson K., Hall A.D., Benn I., Holiday B., Burns R., Zhonnie S., Price F. and Sorenson A. 2000. Bone-related mineral content of water samples collected on the Navajo reservation. Toxic.149: 143-148.

Hsu H.W., Vavak D.L., Satterlee L.D. and Miller G.A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42: 1269-1271.

Isler O., Brubacher G. and Kiss J. 1988. Skorbut, vitamin C und bioflavonoide. In: O. Isler, G. Brubacher, S. Ghisla and B. Krautler (Eds.), pp. 390-395.Vitamine II: Wasserlosliche vitamine. Thieme, Stuttgart.

Kaleem W.A., Nisar M., Qayum M., Zia-Ul-Haq M., Adhikari A. and DeFeo V. 2012. Anti-bacterial activities of cyclopeptide alkaloids from Zizyphus oxyphylla Edgew. Int. J. Mol. Sci. 13: 11520-11529.

Khalil L. A. and Durani F.R. 1990. Haulm and Hull of peas as a protein source in animal feed. Sarhad J. Agr. 6:219-225.

Khorasani G.R., Okine E.K., Corbett R.R. and Kenelly J.J. 1992. Peas for dairy cattle. In: "71st Annual Feeders Day Report", Animal Science Department, University of Alberta, Edmonton. pp.28.

Kohno Y., Egawa Y., Itoh S., Nagaoka S., Takahashi M. and Mukai K. 1995. Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radicals by squalene in n-butanol. Biochem. Biophys. Acta. 1256: 52-56.

Maguire L.S., OSullivan S.M., Galvin K., O'Connor T.P. and O'Brien N.M. 2004. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. Int. J. Food Sci. Nutr. 55:171-178.

Marzo F., Andres A., Maria V.C. and Ruben A.1997. Fertilization effects of phosphorus and sulfur on chemical composition of seeds of Pisum sativum L. and relative infestation by *Bruchus pisorum* L. J. Agric. Food Chem. 45:1829-1833.

McLean L.A., Sosulski F.W. and Youngs C.G. 1974. Effect of nitrogen and moisture on yield and protein in field peas. Can. J. Plant. Sci. 54: 301-305.

Michie M. and Kazuko O. 2008. Comparative study on vitamin c contents of legume seeds. J. Nutr Sci. Vitaminol. 54: 1-6.

Moryma M. and Oba K. 2008.Comparative study on vitamin c contents of legume seeds. J. Nut. Sci. Vitaminol. 54:1-6.

NRC/NAS. 1989. Recommended dietary allowances. Washington DC, USA, National Academy Press, p. 302.

Odunfa S.A. 1983. Carbohydrate changes in fermenting locustbean (*Parkia filicoidea*) during iru preparation. Plant Food Human Nutr. 32: 3-10.

Rizwan K., Zubair M., Rasool N., Riaz M., Zia-Ul-Haq M., DeFeo V. 2012. Chemical and biological study of Agave attenuata. Int. J. Mol. Sci. 13: 6440-6451.

Ryan E., Galvin K., Connor T.P.O, Maguire A.R. and Brien N.M.O. 2007. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. Plant Food. Hum. Nutr. 62: 85-91.

Senthilkumar S., Devaki T., Manohar B.M. and Babu M.S. 2006. Effect of squalene on cyclophosphamide-induced toxicity. Clin. Chim. Acta 364:335-342.

Shrimpton D.H. 1993. Nutritional aspects of Vitamins. In: "The technology of Vitamins in food", P.B. Ottaway (Ed.), pp.2-25. Kluwer Academic Publishers.

Smith T.J. 2000. Squalene: potential chemopreventive agent. Exp. Opin. Invest. Drug. 9:1841-1848.

Sugimoto H. and VanBuren J.P. 1970. Removal of oligosaccharides from soymilk by an enzyme from *Aspergillus saito*. J. Food Sci. 35: 655-660.

Toivo J., Philips K., Lampi A.M. and Piironen V. 2001. Determination of sterols in foods: Recovery of free, esterified, and glycosidic sterols. J. Food Comp. Anal. 14: 631-643.

Tucker J.M. and Townsend D.M. 2005. Alpha-tocopherol: roles in prevention and therapy of human disease. Biomed. Pharm. 59: 380-387.

Urbano G., López-Jurado M., Frejnagel S., Gómez-Villalvaa E., Porres J.M., Frías J., Vidal-Valverde C. and Aranda P. 2005. Nutritional assessment of raw and germinated pea (*Pisum sativum* L.) protein and carbohydrate by in vitro and in vivo techniques. Nutr. 21: 230-239.

Vidal-Valverde C., Frias J., Hernandez A., Martın-Alvarez P.J., Sierra I., Rodrıguez C., Blazquez I. and Vicente G. 2003. Assessment of nutritional compounds and antinutritional factors in pea (*Pisum sativum*) seeds. J. Sci. Food Agric. 83:298-306.

Zarkadas C.G., Voldeng H.D., Yu Z. and Minero-Amador A. 1993. Assessment of the protein quality of new high-protein soya bean cultivars by amino acid analysis. J. Agri. Food Chem. 41:616-623.

Zduńczyk Z., Godycka I. and Amarowicz R. 1997. Chemical composition and content of antinutritional factors in Polish cultivars of peas. Plant Food Hum. Nutr. 50: 37-45.

Zia-Ul-Haq M., Ahmad S., Shad M.A., Iqbal S., Qayum M., Ahmad A., Luthria D.L. and Amarowicz R. 2011a. Compositional studies of some of lentil cultivars commonly consumed in Pakistan. Pak. J. Bot. 43: 1563-1567.

Zia-Ul-Haq M., Ahmad S., Calani L., Mazzeo T., Rio D.D., Pellegrini N. and DeFeo V. 2012a. Compositional study and antioxidant potential of *Ipomoea hederacea* Jacq. and *Lepidium sativum* L. seeds. Mol. 17:10306-10321.

Zia-Ul-Haq M., Ahmad S., Qayum M. and Ercişli S. 2013a. Compositional studies and antioxidant potential of Albizia Lebbeck (L.) Benth. Tur. J. Bio. 37:1-10.

Zia-Ul-Haq M., Ahmed S., Rizwani G.H., Qayum M., Ahmad S. and Hanif M. 2012b. Platelet aggregation inhibition activity of selected flora of Pakistan. Pak. J. Pharm. Sci. 25:863-865.

Zia-Ul-Haq M., Ćavar S., Qayum M., Imran I. and DeFeo V. 2011b. Compositional studies: antioxidant and antidiabetic activities of *Capparis decidua* (Forsk.) Edgew. Int. J. Mol. Sci. 12: 8846-8861.

Zia-Ul-Haq M., Landa P., Kutil Z., Qayum M. and Ahmad S. 2013b. Evaluation of anti-inflammatory activity of selected legumes from Pakistan: *In vitro* inhibition of cyclooxygenase-2. Pak. J. Pharm. Sci. 26:85-89.

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PAPER

SPECIES, SALT LEVEL, AND DIETARY FIBRE EFFECT ON FISH HAM

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ABSTRACT

With the purpose of preparing a cooked fish ham from gilthead sea bream, salmon, and hake and containing dietary fibre, three studies were made: gilthead sea bream *vs* hake batter, reduction of the brine salt content (3, 6, 20 %, w/w), and incorporation of DF: 2 % (w/w) carrageenan, 2 % (w/w) konjac glucomannan, and 2 % (w/w) of each. The incorporation of DF led to a better texture (hardness, cohesiveness, and springiness) and higher sensory scores. Furthermore, much thinner slices were achievable, particularly with the combination of 2 %, w/w, carrageenan and 2 %, w/w, konjac glucomannan.

- Keywords: farmed fish, sensory analysis, textural parameters -

INTRODUCTION

The consumption of vegetarian products and meat products with reduced fat content has been increasing over the past years (XIONG *et al.*, 1999; KUBBERØD *et al.*, 2002). Consumer preference for alternative healthier products is leading to the development of different meat systems (GIESE, 1996; COFRADES *et al.*, 2000). Besides, there is growing consumer interest in the development of meat analogues and fat substitutes using alternative sources of protein (BEGGS *et al.*, 1997; SHAND, 2000; YANG *et al.*, 2001). On the other hand, restructured fish products and the application of new food additives have been used as a way of reaching health-conscious consumers. In addition, fish can be an interesting alternative source of protein (SHAHIDI and VENUGOPAL, 1994).

Among the various traditional meat products in the market, cooked ham is specially suited – given its organoleptic and technological characteristics as well as its wide public acceptance – for experimenting fish as an innovative raw material. A cooked fish ham would be a novelty, since no scientific literature or patent on this subject is known. The most similar products are different fish sausages, prepared with total or partial replacement of livestock meat (MORRIS, 1988; CHUAPOEHUK *et al.*, 2001; LÓPEZ-CABALLERO *et al.*, 2005; CARDOSO *et al.*, 2008).

Gilthead sea bream (*Sparus aurata*) and salmon (*Salmo salar*) are commonly farmed fish species, whose market for whole fish presents signs of saturation. This creates a potential opportunity for ready-to-cook fillets. But, the production of value-added functional foods may also present an opportunity. Moreover, heat-induced gels prepared from gilthead sea bream showed an acceptable textural quality (CARDOSO *et al.*, 2011a). On the other hand, hake (*Merluccius capensis*) may add nutritional value and, combined with salmon, yield an interesting cooked fish ham appearance, due to the contrasting hake and salmon colours. Therefore, the preparation of a functional cooked fish ham with these three fish species may offer an outlet for these different raw materials.

Dietary fibre (DF), one of the food additives frequently used in the design of functional foods (PUUPPONEN-PIMÏA *et al.*, 2002), has a high nutritional value and its importance in health is well established (ANDERSON *et al.*, 1990, KRITCHEVSKY and BONFIELD, 1995). Knowledge of the beneficial effects of high dietary fibre diets – namely, regarding prevention of cardiovascular diseases and several types of cancer – has promoted the development of a large and profitable market for products enriched in DF. Besides, DF can offset the negative effect of some ingredients on the texture. For instance, some DFs, such as carrageenans (ORTIZ and AGUILERA, 2004) or also xanthan and guar gums (MONTERO *et al.*, 2000), have been used for technological purposes in fish products.

Among DFs, konjac flour, the generic name of the milled tuber from *Amorphophallus konjac* (PARK, 1996), has been used in the development of low fat products (LIN and HUANG, 2008) and its application to fish is receiving progressively more attention (PARK, 1996; XIONG *et al.*, 2009). It contains high molecular weight glucomannan, whose D-glucose and D-mannose units (molar ratio ~3:2) are bonded by β-1,4-linkages (TYE, 1991). Acetyl groups are randomly scattered along the essentially linear polymer with an occurrence of ~1 per 19 monomers (glucose or mannose). In the presence of alkali konjac glucomannan (KGM) will deacetylate and form a thermoirreversible and highly heat-stable gel, which is the basis of many traditional oriental foods (NISHINARI *et al.*, 1992). Regarding its application, it has been shown that konjac (at least 1%, w/w) can improve texture of fish products, namely reinforcing shear stress of gels in both whiting and pollock surimi (PARK, 1996). KGM can also generate a gel by synergistic interaction with other plant/algal hydrocolloids (carrageenan, starch and gellan gum) (FERNÁNDEZ-MARTÍN *et al.*, 2009). Namely, the combination of KGM and carrageenan markedly improved the quality of sea bass gel products (CARDOSO *et al.*, 2011b).

Carrageenan is another DF potentially beneficial for fish products. It is extracted from the red seaweeds and available in three main types, namely, kappa (κ), iota (ι) and lambda (λ). However, due to their chemical differences, only iota and kappa carrageenans have gelling ability (CARDOSO *et al.*, 2007). In spite of the fact that carrageenan addition to fish products is not a widespread practice, there have been some experiments involving presence of iota and kappa carrageenan in seafood (ORTIZ and AGUILERA, 2004; GÓMEZ-GUILLÉN and MONTERO, 1996; GÓMEZ-GUILLÉN and MONTERO 1997). Particularly, it was found that incorporation of at least 0.8-1.2% (w/w) of carrageenan into minced hake products hardened the heat-induced gels (CARDOSO *et al.*, 2007).

Accordingly, the objective of this work was to study the effect of fish species, salt level, and dietary fibre on the quality of a functional cooked fish ham.

MATERIALS AND METHODS

Raw materials and additives

Frozen South African hake (*Merluccius capensis*) fillets were bought from a local frozen fish processor. Each fish batch was kept frozen at –28°C and processed within three to four weeks after its arrival at the laboratory. Farmed gilthead seabream (*Sparus aurata*) were bought in a local supermarket and kept frozen at –28°C until

Table 1 - Relevant properties of the used dietary fibre products.

Properties	Carrageenan[a]	Konjac glucomannan[b]
Composition (D.M.)		
Total DF (%)	min. 80	min. 80
Granulometry (μm)	98 %< 250	< 250
Colour	pale yellow	tan
Taste	neutral	neutral
Water solubility	soluble	soluble

[a]The presented information regarding carrageenan was obtained from CEAMGEL1830® Product Sheet (2006).
[b]The presented information regarding konjac glucomannan was obtained from Nutricol GP 312 Product Sheet (2008).

processing. Individual weight varied between 300 and 400 g. Fish were processed (headed, tailed, gutted and filleted) at low temperature (< 10°C) within one week after purchase. Farmed salmon (*Salmo salar*) fillets were bought and kept frozen at –28°C until processing.

Regarding dietary fibre (DF) products, two were chosen for their favourable effect on the textural properties of the final products: carrageenan/CEAMGEL 1830 (Carr) by Ceamsa (Porriño, Spain) and konjac flour/Nutricol GP 312 (Kjc) provided by FMC Biopolymer (Philadelphia, USA). Their properties are presented in Table 1. Carr is a mixture of iota and kappa carrageenans (each approximately 50%, w/w) from red seaweeds, containing additionally potassium chloride and dextrose. Kjc contains KGM extracted from the konjac plant.

The other additives were all food grade materials manufactured by different companies: VATEL® Salt, sodium chloride from VATEL (Alverca, Portugal); TAROMA® Smoke, smoke aroma from BK Giulini (Ladenburg, Germany); and SIDUL® sucrose from SIDUL Açúcares (Santa Iria de Azóia, Portugal). Microbial transglutaminase TG-K (MTGase) ACTIVA® GS was supplied by Ajinomoto (Tokyo, Japan), presenting an activity of about 100 $U.g^{-1}$. All chemicals (sodium hydroxide and sodium tripolyphosphate) used were of analytical grade and from Merck (Darmstadt, Germany).

Experimental design

Based on the process used for the production of cooked pork ham (personal communication; BARAT *et al.*, 2005), three experiments were conducted with the purpose of developing a cooked fish ham (Fig. 1). As starting point, the mean textural and sensory parameters of cooked fish hams whose paste moiety was prepared from hake (code, HH20) and gilthead sea bream (GH20) were compared. For the fish chunks moiety, salmon and hake were always used. Afterwards, in a second step, the salt content of the brine solution was reduced from 20 (GH20) to 6% (GH6) and then to 3% (GH3), w/w. Finally, a third step was carried out in order to assess the textural and sensory effect of DF incorporation (2%, w/w, carrageenan, code GH3C; 2%, w/w, konjac, GH3K; 2%, w/w, carrageenan + 2%, w/w, konjac, GH3CK) in the paste moiety on the fish cooked ham products. Error assessment was derived from replication of the various analyses performed.

Production of cooked fish ham

The process for preparing cooked fish ham was partially based on the procedures usually applied to the production of pork hams in Southern Europe (personal communication; BARAT *et al.*, 2005) (Fig. 2). As such, cooked fish ham products were composed of two moieties, a fish paste and fish chunks, which were treated with a brine solution. Whereas, for the fish paste, hake and gilthead sea bream were tested, for the fish chunks, salmon and hake were used. Accordingly, frozen hake and gilthead sea bream fillets were thawed overnight in a refrigerator and minced once in a model 84145 meat grinder (Hobart, Troy, OH, USA), equipped with 2 cm grind blades and a metallic screen with 6 mm diameter circular holes. Frozen salmon and hake fillets were thawed over-

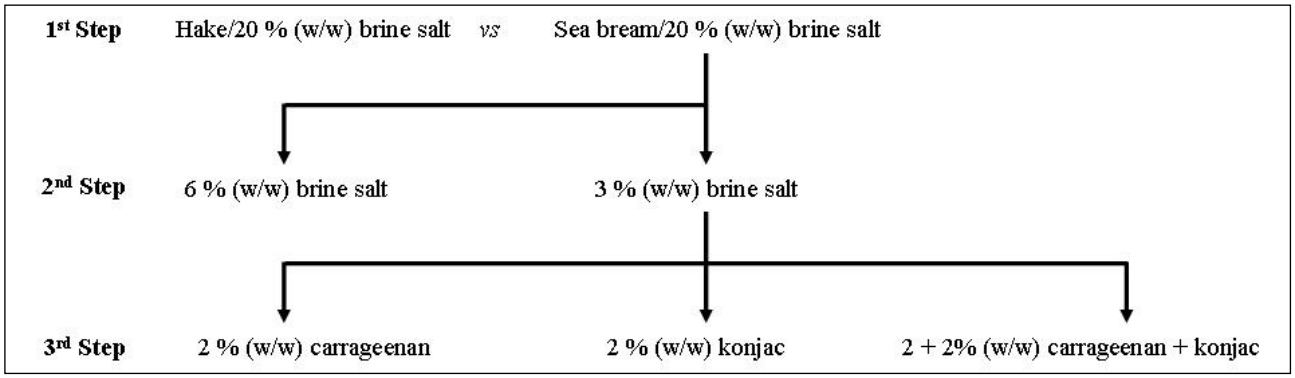

Fig. 1 - Experimental design diagram.

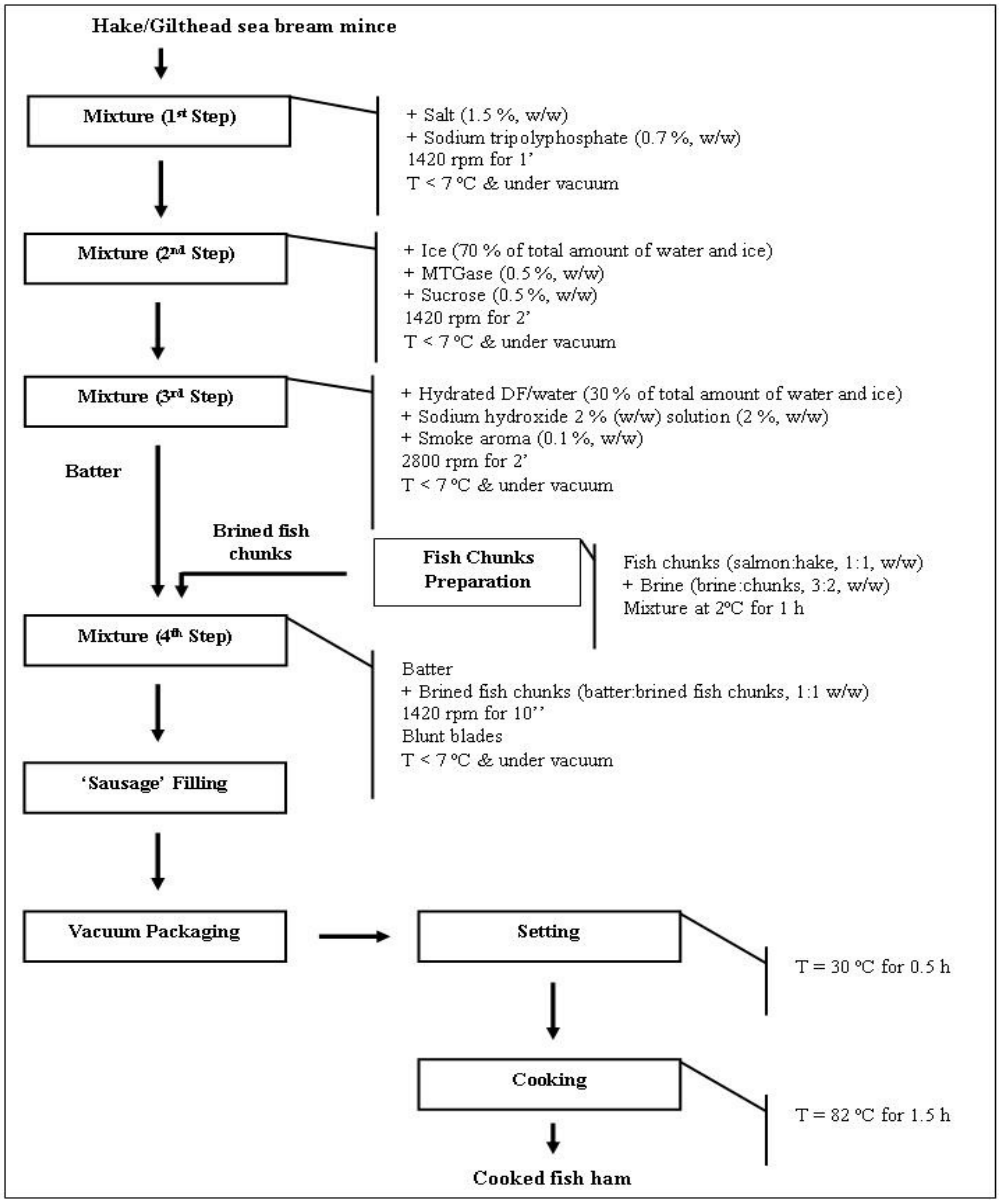

Fig. 2 - Flow sheet for the production of cooked fish ham.

night in a refrigerator and cut into chunks of between 1-2 cm size.

Regarding the pastes, the appropriate quantities of the various ingredients were weighed in order to produce 1.5 kg (Table 2) and three sequential steps were always followed. Firstly, hake/gilthead sea bream mince was mixed with salt and sodium tripolyphosphate for 1 min at 1420 rpm in a model UM12 refrigerated vacuum homogeniser (Stephan and Söhne, Hameln, Germany). Throughout all process, mixing was performed always under vacuum and refrigeration (temperature below 7°C). In a second step, ice (70% of the total amount of ice and water), MTGase, and sucrose were added and there was additional mixing for 1 min at the same speed. Thereafter, the last step involved the addition of the remaining ingredients, the hydrated (with water equivalent to 30% of the total amount of ice and water) fibre (in those products containing either carrageenan or konjac or, for the other products, only water equivalent to 30% of the total amount of ice and water), 2% (w/w) sodium hydroxide solution (required for the partial deacetylation of konjac glucomannan, but added to all products in order to avoid pH differences among the products), and smoke aroma, mixing all for 2 min at 2,800 rpm.

On the other hand, 1.4 kg of fish chunks (0.7 kg salmon + 0.7 kg hake) were immersed and mixed for 1 h with 2.1 kg of brine in a model SI-1101 Roto-shake genie shaker (Scientific Industries, Inc., Bohemia, NY, USA), which operated inside a refrigerator (2°±2°C). This mixing simulated the typical tumbling of the production process of cooked pork hams. The brine was previously prepared in the day before according to

the formulations shown in Table 3 and left in refrigeration (2°±2°C) overnight. Afterwards, 1.5 kg of the brined fish chunks were added to the paste and mixed for only 10 sec at 1420 rpm with blunt blades (instead of the sharp blades used in the previous mixing steps).

The attained mixture was put inside a model EB-12 hydraulic filler (Mainca Equipamientos Carnicos, S.L., Granollers, Spain) and encased under pressure into cellulose sausage casings mounted over the end of the stuffing horn. Afterwards, these cellulose casings were twisted and tied, thereby, shaping 'sausages' with a diameter of 9 cm and a length of about 25 cm. In the next step, the 'sausages' were vacuum-packed in plastic bags with a model A300/52 vacuum packager (Multivac Sepp Haggenmüller GmbH & Co. KG, Wolfertschwenden, Germany). Then, these packages were immersed in water at 30°C for 30 min (setting), moved to a model Combi-Master CM6 oven (Rational Grossküchen Technik GmbH, Landsberg am Lech, Germany) equipped with a digital thermometer and subjected to a steam cooking at 82°C for one and half hour (cooking). In order to guarantee these conditions even in the innermost part of the product, the oven's digital thermometer was placed in the centre of a product through a hole in the bag (approximately 9 minutes until reaching 82°C). Afterwards, products were immediately cooled in iced water and kept in a refrigerator (2°±2°C) overnight until further analysis.

Texture measurements

For the texture profile analysis (TPA), samples (cubes with 25 mm side were cut from the middle of the 'sausage') were compressed on the flat plate of the Instron 4301 texturometer (Instron Engineering Corp., Canton, MA, USA) with a cylindrical plunger (50 mm diameter) adapted to a 1,000 N load cell at a deformation rate of 50 mm/min. On the basis of preliminary trials to establish a compression limit that would

Table 2 - Batter formulations used in the preparation of cooked fish ham.

Ingredients (%, w/w)	1st Step		2nd Step		3rd Step		
	Hake Replacement		Brine Salt Content (%)		Dietary Fibre Added		
	No	Yes	6	3	Carrageenan (2%, w/w)	Konjac (2%, w/w)	Carrageenan + Konjac (2+2%, w/w)
Code	**HH20**	**GH20**	**GH6**	**GH3**	**GH3C**	**GH3K**	**GH3CK**
Hake mince	68.2	0.0	0.0	0.0	0.0	0.0	0.0
Gilthead sea bream	0.0	68.2	68.2	68.2	66.8	66.8	65.4
Water/Ice	26.5	26.5	26.5	26.5	25.9	25.9	25.3
Carrageenan	0.0	0.0	0.0	0.0	2.0	0.0	2.0
Konjac glucomannan	0.0	0.0	0.0	0.0	0.0	2.0	2.0
NaOH 2% (w/w) solution	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Sodium chloride	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sodium tripolyphosphate	0.7	0.7	0.7	0.7	0.7	0.7	0.7
MTGase	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sucrose	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Smoke aroma	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Total (%)	100	100	100	100	100	100	100

Table 3 - Brine formulations used in the preparation of cooked fish ham.

Ingredients (%, w/w)	1st Step		2nd Step		3rd Step		
	Hake Replacement		Brine Salt Content (%)		Dietary Fibre Added		
	No	Yes	6	3	Carrageenan (2 %, w/w)	Konjac (2 %, w/w)	Carrageenan + Konjac (2+2 %, w/w)
Code	**HH20**	**GH20**	**GH6**	**GH3**	**GH3C**	**GH3K**	**GH3CK**
Water/Ice	74.2	74.2	88.2	91.2	91.2	91.2	91.2
Sodium chloride	20.0	20.0	6.0	3.0	3.0	3.0	3.0
Sodium tripolyphosphate	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sucrose	1.5	1.5	1.5	1.5	1.5	1.5	1.5
MTGase	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Smoke aroma	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total (%)	100	100	100	100	100	100	100

ensure no cracking and recoverability of most samples, it was decided to compress samples to 60% of their height. In the double bite test, each sample was compressed twice. The following parameters were determined: hardness (N), maximum height of first peak on first compression (in terms of eating quality, food's resistance at first bite); cohesiveness (A_2/A_1), ratio of second-compression to first-compression positive areas (maintenance of food resistance during chew down); gumminess (N), product of hardness and cohesiveness (strength required in the chew down process); springiness (L_2/L_1), ratio of the detected height of the product on the second compression to the original compression distance (ability of food to reacquire its initial shape and size after a first bite); chewiness (N), product of gumminess and springiness (albeit expressed in N, a measure of the energy spent in the chew down process).

Concerning the slice thickness, a Weivo ham slicing device (Qingdao Weivo Machinery Corp., Shandong, China) with adjustable thickness screw was used. The minimal slice thickness ensuring the attainment of whole slices without cracks was registered for each of the prepared products.

Sensory analysis

Sensory evaluation was conducted by five panellists from INRB, I.P./L-IPIMAR, extensively trained with the sensory scheme for cooked ham evaluation and able to conduct a structured scaling of products (NIELSEN *et al.*, 2002). Panellists participated in preliminary trials and in the experiments that led to the development of cooked fish ham.

'Sausages' were taken out from the package, tempered to about 20°C and cut into 8 mm thick (for all products) slices with a 9 cm diameter. These slices were distributed in white plates and presented to the panellist in random order for evaluation. Mineral water was supplied to the panellists for rinsing between samples. The evaluation was performed in a room specifically conceived for sensory analysis and with adequate lighting.

Panellists were asked to score several sensory parameters of the product, using a 0-5 scale: color (0 - light to 5 - dark); fish, smoke and unpleasant aroma (0 - absent to 5 - excessive); elasticity (0 - plastic to 5 - elastic), hardness (0 - soft to 5 - hard), cohesiveness (0 - scarcely cohesive to 5 - very cohesive), succulence (0 - dry to 5 - succulent), oiliness (0 - slightly oily to 5 - very oily) and unpleasant texture (0 - absent to 5 - excessive); saltiness, fish, smoke and unpleasant flavour (0 - absent to 5 - excessive); and global opinion (0 - unpleasant to 5 - pleasant).

Statistical analysis

A general linear model – one-way ANOVA – was used to determine significant differences ($p<0.05$) among cooked fish ham products. This statistical methodology enabled to analyse each of the studied effects (paste fish species, brine salt content, and DF incorporation). The difference of means between pairs was resolved by using confidence intervals in a Tukey HSD test. All statistical treatment was done with the software STATISTICA© from StatSoft, Inc. (Tulsa, OK, USA), version 6.1, 2003.

RESULTS AND DISCUSSION

Comparison of gilthead sea bream and hake pastes

Texture evaluation

The utilization of hake mince or gilthead sea bream mince as the main constituent of the pastes was tested and the textural results are shown in Table 4 and Fig. 3.

Hardness, cohesiveness, springiness and the other TPA properties did not present any sig-

Table 4 - Mean textural properties of the cooked fish ham products*.

Experimental step	Product	Hardness (N)	Cohesiveness	Gumminess (N)	Springiness	Chewiness (N)
1st	HH20	11.5 ± 1.9^{bc}	0.37 ± 0.03^{bc}	4.2 ± 0.4^{bc}	0.61 ± 0.04^{cd}	2.6 ± 0.2^{b}
	GH20	10.1 ± 1.0^{abc}	0.35 ± 0.06^{abc}	3.5 ± 0.3^{abc}	0.51 ± 0.04^{abc}	1.8 ± 0.3^{ab}
2nd	GH6	7.5 ± 3.4^{ab}	0.29 ± 0.08^{ab}	2.4 ± 1.7^{ab}	0.44 ± 0.12^{a}	1.2 ± 1.1^{a}
	GH3	6.2 ± 2.7^{a}	0.28 ± 0.02^{a}	1.8 ± 0.9^{a}	0.47 ± 0.10^{ab}	0.9 ± 0.7^{a}
3rd	GH3C	11.7 ± 2.0^{bc}	0.40 ± 0.02^{cd}	4.6 ± 0.7^{c}	0.57 ± 0.02^{bcd}	2.6 ± 0.4^{b}
	GH3K	14.2 ± 1.3^{c}	0.47 ± 0.03^{de}	6.8 ± 1.0^{d}	0.66 ± 0.01^{d}	4.4 ± 0.7^{c}
	GH3CK	20.2 ± 1.7^{d}	0.55 ± 0.02^{e}	11.1 ± 1.3^{e}	0.65 ± 0.01^{d}	7.2 ± 0.8^{d}

*Presented values correspond to mean ± standard deviation.
Means within a column with different letters are significantly different ($p<0.05$).

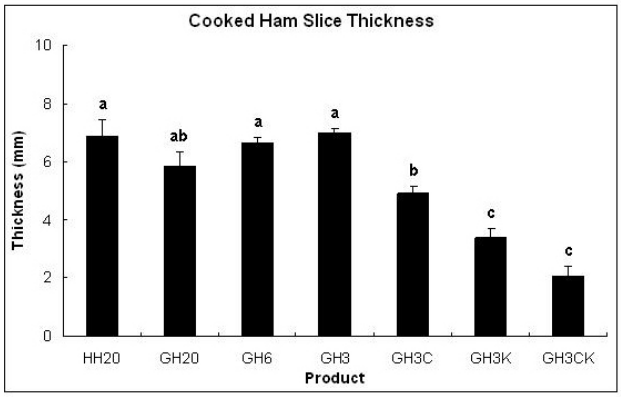

Fig. 3 - Minimum attained thickness of the cooked ham slices. Products whose bars present different letters are significantly different ($p<0.05$).

nificant difference ($p\geq0.05$). Moreover, the minimum slice thickness was unchanged by the substitution.

Sensory assessment

The main sensory evaluation results concerning gilthead sea bream substitution are presented in Table 5.

None of the studied sensory parameters was modified. Of course, panellists noticed some flavour change, but a 'hake flavour' *vs* a 'sea bream flavour' was of no relevance for this study. As a consequence, no sensory drawback can be ascribed to the complete replacement of hake mince by gilthead sea bream mince. On the other hand, the sensory panel considered both cooked fish hams of poor quality, since it assigned a low score to the global opinion, 1.8±1.3 and 2.0±0.7 (in a range from 0 to 5), for hake and sea bream ham, respectively. This was mainly due to the excessive saltiness of the products, 4.8±0.4 and 4.6±0.5, for hake and sea bream ham, respectively. Thus, the next step involved reducing the high salt content of the brine.

Regarding texture, the two tested species are interchangeable as main ingredient of the pastes. This contrasts with differences found in the textural parameters of gelled products prepared from hake and gilthead sea bream (CARDOSO *et al.*, 2007; 2011a). However, in this study, conditions were exactly the same for both. Hence, it can be concluded that a farmed fish species subjected to frozen storage can yield a textural profile similar to that of a wild fish species also frozen stored. Therefore, taking into account that gilthead sea bream are a fish species available at lower price than hake in Southern Europe, with acceptable gelation results (CARDOSO *et al.*, 2011a), and the economic advantages of finding new outlets (value-added functional products) for this farmed species, it was decided to carry out the remaining steps of this work with gilthead sea bream pastes.

Reduction of the brine salt content

Texture evaluation

Declining salt levels in the brine had no statistical effect on the TPA parameters (Table 4) and the product's minimal slice thickness (Fig. 3).

Sensory assessment

In general, salt has not only a technological function, but has also a sensory role in taste perception. In this regard, panellists associated a high saltiness level to an unpleasant effect (Table 5). The reduction of the brine salt content was detected by the panel, since saltiness significantly ($p<0.05$) declined from 4.6±0.5 (20%, w/w, brine salt content) to 2.8±1.6 (3%, w/w). But, despite the flavour improvement, global opinion of the fish hams remained low (2.8±1.3) as a result of the poor texture.

Taking into account the results, it can be assumed that the high brine salt content (20 %, w/w) typically used in the preparation of cooked pork hams has no technological function whatsoever in fish products, at least, for the cohesion of the fish chunks within the paste. In fact,

Table 5 - Main sensory assessment results of the cooked fish ham products*.

Experimental step	Product	Colour	Saltiness	Succulence	Oiliness	Global opinion
1st	HH20	2.2 ± 0.8^{a}	4.8 ± 0.4^{a}	4.0 ± 1.0^{a}	2.0 ± 1.7^{a}	1.8 ± 1.3^{a}
	GH20	3.0 ± 1.2^{a}	4.6 ± 0.5^{a}	4.0 ± 1.0^{a}	2.4 ± 1.7^{a}	2.0 ± 0.7^{a}
2nd	GH6	3.0 ± 0.7^{a}	3.6 ± 1.5^{ab}	4.2 ± 0.4^{a}	3.0 ± 1.4^{a}	2.4 ± 1.5^{ab}
	GH3	1.4 ± 0.5^{a}	2.8 ± 1.6^{b}	3.0 ± 1.0^{a}	1.6 ± 0.9^{a}	2.8 ± 1.3^{ab}
3rd	GH3C	1.8 ± 0.8^{a}	1.6 ± 1.1^{b}	2.4 ± 1.7^{a}	1.6 ± 0.5^{a}	3.4 ± 0.9^{ab}
	GH3K	1.4 ± 1.1^{a}	2.8 ± 0.8^{b}	3.6 ± 1.1^{a}	1.8 ± 1.3^{a}	3.6 ± 0.5^{ab}
	GH3CK	1.8 ± 1.3^{a}	2.0 ± 1.2^{b}	4.0 ± 0.7^{a}	2.8 ± 1.3^{a}	4.2 ± 0.4^{b}

*Presented values correspond to mean ± standard deviation.
Means within a column with different letters are significantly different ($p<0.05$).

several studies (URESTI *et al.*, 2004; RAMÍREZ *et al.*, 2007; CARDOSO *et al.*, 2010) have shown that salt is essential for the textural quality of restructured fish products, but its level can be reduced to the range of 1-3% (w/w) without significant deleterious effects on the hardness or the cohesiveness of the products (URESTI *et al.*, 2004; CARDOSO *et al.*, 2010). Particularly, it is possible to reduce salt and avoid the loss of quality (mainly texture and water holding capacity) through additives, such as, dairy proteins or MTGase (RAMÍREZ *et al.*, 2007). These authors showed that MTGase (0.3%, w/w) and a low salt concentration (1%, w/w) could improve the quality of restructured products from striped mullet (*Mugil cephalus*). Moreover, it has been reported that addition of 0.5% (w/w) MTGase to farmed sea bass gels allows for a reduction of the salt content to 1.0% (w/w) (CARDOSO *et al.*, 2010).

All the prepared products regardless of the brine salt content were of poor textural quality, displaying frailty and a low degree of mechanical resistance. Namely, the minimal slice thickness was always above 5 mm, since all slices thinner than 5 mm were not able to maintain their unity and easily crumbled into pieces. Moreover, the products were very soft (hardness below 11.5 N) and exhibited low cohesiveness (<0.40), especially if compared with similar restructured fish products, such as hake sausages, which presented a hardness of 29.0 N and a cohesiveness of 0.54 (CARDOSO *et al.*, 2008). Thus, significant textural improvements of the fish hams were required in order to achieve an acceptable product.

Incorporation of DF

Texture evaluation

The textural drawbacks of the products were overcome by the addition of DFs, which, besides improving texture, are functional additives, given their nutritional value and positive health effects (ANDERSON *et al.*, 1990, KRITCHEVSKY and BONFIELD, 1995). In fact, among DFs, two (carrageenan and konjac) were selected for their ability of combining health benefits with textural improvements. Accordingly, the textural properties of cooked fish ham containing DFs were measured (Table 4 and Fig. 3).

The incorporation of 2 % (w/w) carrageenan hardened the fish ham with respect to the product without DF (GH3), 11.7±2.0 *vs* 6.2±2.7 N. Likewise, the cohesiveness was increased ($p<0.05$), from 0.28±0.02 for GH3 to 0.40±0.02 for GH3C. However, all TPA properties remained low and similar to the values observed for the products without DF (HH20 and GH20). The minimal slice thickness was reduced ($p<0.05$) to below 5 mm, 4.9±0.3 mm.

The incorporation of 2% (w/w) konjac glucomannan also hardened the product in comparison with the no DF product (GH3), but failed to deliver any significant improvement with respect to the carrageenan-containing product (GH3C). On the other hand, the GH3K product presented a cohesiveness (0.47±0.03) well above ($p<0.05$) that of all the previously discussed products. Likewise, the gumminess (6.8±1.0 N) and the chewiness (4.4±0.7 N) were higher. Moreover, the addition of this DF enabled to reduce significantly ($p<0.05$) further the minimum slice thickness down to 3.4±0.3 mm.

The combination of 2%, w/w, carrageenan and 2%, w/w, konjac was tested in order to enhance the textural advantages accrued by both DFs. In fact, the hardness, cohesiveness, gumminess, springiness, and chewiness of the ham with carrageenan plus konjac (GH3CK) increased ($p<0.05$) with respect to the no DF ham (GH3). Namely, hardness augmented from 6.2±2.7 to 20.2±1.7 N, cohesiveness doubled from 0.28±0.02 to 0.55±0.02, and chewiness (which combines all the TPA parameters) had an eightfold increase from 0.9±0.7 to 7.2±0.8 N. This was the highest ($p<0.05$) measured value among all prepared cooked fish hams. However, no strong evidence supporting a synergistic relationship between these two DFs was found, since only gumminess and chewiness presented an increment with carrageenan and konjac exceeding the sum of the increases due to each DF *per se*. Namely, for gumminess, it augmented from 1.8±0.9 for GH3 to 11.1±1.3 N for GH3CK, a variation of 9.3 N, which surpassed the sum of the variations measured for each DF, 7.8 N (an increase of 2.8 N from GH3 to GH3C plus an increase of 5.0 N from GH3 to GH3K). Regarding the minimum slice thickness, it fell to only 2.1±0.4 mm.

Sensory assessment

The effect of DF on the main sensory parameters of cooked fish hams was also studied (Table 5).

For carrageenan and konjac *per se,* sensory scores of the main sensory parameters were unaltered, thereby remaining the global opinion relatively modest as a result of low succulence and creaminess/oiliness. However, the combination of both DFs enabled a significant ($p<0.05$) improvement of the global opinion, reaching 4.2±0.4, a value nearer to the maximum score, 5. Therefore, the 3% brine salt gilthead sea bream ham containing 2% (w/w) carrageenan and 2% (w/w) konjac (GH3CK) achieved a sensory quality deemed as good by the panellists, not only regarding its appearance (Fig. 4), but also its flavour (typical cooked pork ham flavour due to the smoke aroma ingredient) and texture.

The presented results show that it is technologically advantageous to include DFs in the cooked fish ham recipes. Therefore, any dilution effect (more important for carrageenan+konjac, since fish mince – the sole source of protein – in

Fig. 4 - External appearance of the produced cooked fish ham.

the paste lowered from 68.2 for GH3 to 65.4%, w/w, for GH3CK, Table 2) was more than outweighed by these two DFs. Consequently, higher incorporation levels of these DFs may improve even more the textural parameters. Though carrageenan is a mixture of iota and kappa carrageenans (CEAMGEL1830® PRODUCT SHEET, 2006) and konjac contains glucomannan (NUTRICOL GP 312 PRODUCT SHEET, 2008), both DFs have a similar positive effect, albeit slightly more intense with konjac. This effect is related to the ability of these DFs to form gels *per se* and, as such, act as gelling agents in food systems (FERNÁNDEZ-MARTÍN *et al.*, 2009; CANDOGAN and KOLSARICI, 2003). Therefore, carrageenan and konjac have an effect that goes beyond the sheer filling action and this may explain their favourable influence on the texture of fish ham products. Different hypothesis concerning this have been suggested. It may be that, besides the protein gel network, there is a second (polyssacharide) network enhancing hardness and related parameters as well as cohesiveness. Other authors found that iota carrageenan formed an independent network, which established connections between adjacent structures supporting the main structure formed by the fish protein (GÓMEZ-GUILLÉN *et al.*, 1996). But, this hypothesis does not exclude the existence of important polyssacharide-protein interactions, shown through the improvement of the gel-forming capacity of Alaska Pollock surimi by the addition of iota and kappa carrageenans as a result of the interaction of carrageenan sulphate groups and fish proteins (LLANTO *et al.*, 1990).

Finally, the combination of both DFs ensured a maximal positive effect on texture, leading to acceptable values in comparison with similar restructured fish products, such as hake sausages. Particularly, the cohesiveness (0.55±0.02) of the GH3CK ham was comparable to that of the hake sausage (0.54) and of a commercial pork sausage (0.60) (CARDOSO *et al.*, 2008).

CONCLUSIONS

Results showed that the use of gilthead sea bream did not alter the product, achieving an acceptable sensory assessment. The reduction of salt content had no negative textural effects and sensory evaluation was improved. The incorporation of DF led to a better texture (hardness, cohesiveness, and springiness) and higher sensory scores. Furthermore, much thinner slices were achievable, particularly with the combination of carrageenan and konjac glucomannan. On the whole, it was shown that it is possible to produce a texturally acceptable cooked fish ham from both the instrumental and sensory viewpoints, reaching an overall sensory quality similar to other restructured fish products. Moreover, this was achieved through the combination of multiple health promoting factors, namely, high dietary fibre intake, low fat intake, and fish nutrients, that is, a healthy low fat cooked ham containing dietary fibre.

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REFERENCES

Anderson J.W., Deakins D.A., Floore T.L., Smith B.M. and Whitis S.R. 1990. Dietary fiber and coronary heart disease. Crit. Rev. Food Sci. Nutr. 29: 95.

Barat J. M., Grau R., Ibáñez J.B. and Fito P. 2005. Post-salting studies in Spanish cured ham manufacturing. Time reduction by using brine thawing-salting. Meat Sci. 69: 201.

Beggs K.L.H., Bowers J.A. and Brown D. 1997. Sensory and physical characteristics of reduced-fat turkey frankfurters with modified corn starch and water. J. Food Sci. 62(6): 1240.

Candogan K. and Kolsarici N. 2003. The effects of carrageenan and pectin on some quality characteristics of low-fat beef frankfurters. Meat Sci. 64: 199.

Cardoso C., Mendes R. and Nunes M.L. 2007. Effect of transglutaminase and carrageenan on restructured fish products containing dietary fibres. Int. J. Food Sci. Technol. 42: 1257.

Cardoso C., Mendes R. and Nunes M.L. 2008. Development of a healthy low fat fish sausage containing dietary fibre. Int. J. Food Sci. Technol. 43: 276.

Cardoso C., Mendes R., Vaz-Pires P. and Nunes M.L. 2010. Effect of salt and MTGase on the production of high quality gels from farmed sea bass. J. Food Eng. 101: 98.

Cardoso C., Mendes R., Vaz-Pires P. and Nunes M.L. 2011a. Effect of MTGase, dietary fiber and UV irradiation upon heat-induced gilthead seabream (*Sparus aurata*) gels. Food Sci. Technol. Int. 17(2): 155.

Cardoso C., Mendes R., Vaz-Pires P. and Nunes M.L. 2011b. Production of high quality gels from sea bass: effect of MTGase and dietary fibre. Food Sci. Technol. 44: 1282.

CEAMGEL1830® Product Sheet 2006. Porriño, Spain: Ceamsa.

Chuapoehuk P., Raksakulthay N. and Worawattanamateekul W. 2001. Process development of fish sausage. Int. J. Food Prop. 4(3): 523.

Cofrades S., Guerra M.A., Carballo J., Fernández-Martín F. and Jiménez-Colmenero F. 2000. Plasma protein and soy fibre content effect on bologna-sausage properties as influenced by fat level. J. Food Sci. 65(2): 281.

Fernández-Martín F., López-López I., Cofrades S. and Jiménez-Colmenero F. 2009. Influence of adding Sea Spaghetti seaweed and replacing the animal fat with olive oil or a konjac gel on pork meat batter gelation. Potential protein/alginate association. Meat Sci. 83: 209.

Giese J.H. 1996. Fats, oils and fat replacers. Food Technol. 50(4): 78.

Gómez-Guillén M.C. and Montero P. 1996. Addition of hydrocolloids and non-muscle proteins to sardine (*Sardina pilchardus*) mince gels. Food Chem. 56(4): 421.

Gómez-Guillén M.C. and Montero P. 1997. Improvement of giant squid (*Dosidicus gigas*) muscle gelation by using gelling ingredients. Zeitschrift für Lebensmittel Untersuchung und Forschung A 204: 379.

Gómez-Guillén C., Solas T., Borderías J. and Montero P. 1996. Effect of heating temperature and sodium chloride concentration on ultrastructure and texture of gels made from giant squid (*Dosidicus gigas*) with addition of starch, iota-carrageenan and egg white. Zeitschrift für Lebensmittel Untersuchung und Forschung 202: 221.

Kritchevsky D. and Bonfield C. 1995. Dietary fibre in health and disease. Eagan Press, St. Paul, USA.

Kubberød E., Ueland Ø., Rødbotten M., Westad F. and Risvik E. 2002. Gender specific preferences and attitudes towards meat. Food Qual. Pref. 13(5): 285.

Lin K.W. and Huang C.Y. 2008. Physicochemical and textural properties of ultrasound-degraded konjac flour and their influences on the quality of low-fat Chinese-style sausage. Meat Sci. 79: 615.

Llanto M.G., Bullens C.W., Modliszewski J. and Bushway A.D. 1990. In Advances in Fisheries, Technology and Biotechnology for Increased Profitability, 34th Atlantic Fisheries Technological Conference and Seafood Biotechnology Workshop, Vol. VI.

López-Caballero M.E., Gómez-Guillén M.C., Pérez-Mateos M. and Montero P. 2005. A functional chitosan-enriched fish sausage treated by high pressure. J. Food Sci. 70(3): 166.

Montero P., Hurtado J.L. and Pérez-Mateos M. 2000. Microstructural behaviour and gelling characteristics of myosystem protein gels interacting with hydrocolloids. Food Hydrocolloids 14: 455.

Morris C. E. 1988. New products blend. Surimi and meat. Food Eng. 60(1): 49.

Nielsen J., Hyldig G. and Larsen E. 2002. 'Eating quality' of fish – A review. J. Aquatic Food Prod. Technol. 11(3/4): 125.

Nishinari K., Williams P.A. and Phillips G.O. 1992. Review of the physicochemical characteristics and properties of konjac mannan. Food Hydrocolloids 6: 199.

Nutricol GP 312 Product Sheet 2008. Philadelphia, USA: FMC Biopolymer.

Ortiz J. and Aguilera J.M. 2004. Effect of kappa-carrageenan on the gelation of horse mackerel (*T. murphyi*) raw paste surimi-type. Food Sci. Technol. Int. 10(4): 223.

Park J.W. 1996. Temperature-tolerant fish protein gels using konjac flour. J. Muscle Foods 7: 165.

Puupponen-Pimïa R., Aura A.M., Oksman-Caldentey K.M., Myllärinen P., Saarela M., Mattila-Sandholm T. and Poutanen K. 2002. Development of functional ingredients for gut health. Trends Food Sci. Technol. 13: 3.

Ramírez J.A., Del Ángel A., Uresti R.M., Velásquez G. and Vázquez M. 2007. Low-salt restructured products from striped mullet (*Mugil cephalus*) using microbial transglutaminase or whey protein concentrate as additives. Food Chem. 102: 243.

Shahidi F. and Venugopal V. 1994. Solubilization and thermostability of water dispersions of muscle structural proteins of Atlantic herring (*Clupea harengus*). J. Agric. Food Chem. 42: 1440.

Shand P.J. 2000. Textural, water holding and sensory properties of low-fat pork bologna with normal or waxy starch hull-less barley. J. Food Sci. 65(1): 101.

Tye R.J. 1991. Konjac flour: properties and application. Food Technol. 45: 88.

Uresti R.M., Téllez-Luis S.J., Ramírez J.A. and Vázquez M. 2004. Use of dairy proteins and microbial transglutaminase to obtain low-salt fish products from filleting waste from silver carp (*Hypophthalmichthys molitrix*). Food Chem. 86: 257.

Xiong Y.L., Noel D.C. and Moody W.G. 1999. Textural and sensory properties of low-fat beef sausages with added water and polysaccharides as affected by pH and salt. J. Food Sci. 64(3): 550.

Xiong G., Cheng W., Ye L., Du X., Zhou M., Lin R., Geng S., Chen M., Corke H. and Cai Y. Z. 2009. Effects of konjac glucomannan on physicochemical properties of myofibrillar protein and surimi gels from grass carp (*Ctenopharyngodon idella*). Food Chem. 116: 413.

Yang A., Keeton J.T., Beilken S.L. and Trout G. R. 2001. Evaluation of some binders and fat substitutes in low-fat frankfurters. J. Food Sci. 66(7): 1039.

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PAPER

PHYSICAL AND MICRO STRUCTURAL CHANGES IN CARROT POMACE-BASED EXTRUDATES

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ABSTRACT

The microstructural, colour and textural changes of carrot pomace based unfried, fried and seasoned extrudates were explored during storage for evaluating the product stability. The extrudates were analyzed to understand the changes in structure, colour and hardness. During storage, remarkable changes were observed in structural orientation of the fibre and cellular components of the unfried, fried and seasoned extruded. The photomicrograph revealed the presence of relatively much compact and dense structural orientation with numerous globules after six months of storage which may have lead to the increase in the hardness of the extrudates and may be the cause for the loss of crispiness of the extrudates. Hardness of the extrudates was increased from 13.78 to 45.80 N for unfried, 8.24 to 19.04 N for fried and 8.62 N to 21.85 N for seasoned extrudates, respectively. The L-value decreased from 66.22 to 62.53, 38.58 to 36.38 and 34.95 to 33.78 for unfried, fried and seasoned extrudates, respectively. The microstructural, colour and textural properties of unfried, fried and seasoned extrudates changed during storage of six month. Minimum change in crispiness and L-values was observed in fried extrudates.

- Keywords: carrot pomace, extrudates, microstructure, texture, colour -

INTRODUCTION

Fruit and vegetable wastes are inexpensive, available in large quantities, characterized by a high dietary fibre content resulting with high water binding capacity and relatively low enzyme digestible organic matter. A number of researchers have used fruits and vegetable by-products such as apple, pear, orange, peach, blackcurrant, cherry, artichoke, asparagus, onion, carrot pomace (GRIGELMO-MIGUEL and MARTIN-BELLOSO 1999; NAWIRSKA and KWASNIEVSKA 2005) as sources of dietary fibre supplements in foods. Cereal grains are generally used as major raw material for development of snack foods due to their good expansion characteristics because of high starch content. The broken rice is a by product of modern rice milling process. The rice portion can have varying percentages (5-7%) of broken kernels which contain nutritive value similar to whole rice and are available readily at relatively lower cost. Rice flour has become an attractive ingredient in the extrusion industry due to its bland taste, attractive white color, hypo allergenicity and ease of digestion (KADAN *et al.*, 2003). DE PILLI *et al.* (2011, 2012) investigated the effects of operating conditions (barrel temperature and water feed content) on biopolymer modification and quality of extrudates made up of rice starch and pistachio nut flour.

YAGCI and GOGUS (2009) incorporated durum clear flour, partially defatted hazelnut flour, fruit waste blend and rice grits for the development of extruded products. Other important applications and researches on extruded food concern the enrichment of cereal flour with protein and fatty flour with high nutritional values. For example, a snack food based on almond and wheat flour was studied by DE PILLI *et al.* (2001; 2004a; 2004b; 2005a; 2005b; 2007; 2008a; 2008b) to realize a functional food with high nutritional values.

Carrot (*Daucus carota*) is a rich source of β-carotene and possesses vitamins, like thiamine, riboflavin, vitamin B-complex and minerals (WALDE *et al.*, 1992). As the fiber-rich pomace is available in large quantity during juice production, it is worth exploiting the carrot insoluble fiber-rich fractions (IFRF) as a promising hypocholesterolemic ingredient to fulfill the increasing demand of functional ingredients in developing fiber-rich food products. It has been reported by the researchers that the incorporation of cellulose and IFRF into the fiber-free diet significantly reduces the serum total cholesterol levels by 17.3 and 33.5%, respectively (HSU *et al.*, 2006). However, dried carrot pomace has β-carotene and ascorbic acid in the range of 9.87 to 11.57 mg and 13.53 to 22.95 mg per 100 g respectively (UPADHYAY *et al.*, 2008). A promising way is to store the carrot pomace in dried form and utilize in the development of various food products specifically extrudates, which are becoming more popular (UPADHYAY *et al.*, 2008, 2010; KUMAR *et al.*, 2010a, 2010b).

In the extruded snacks, product microstructure can vary from porous and open celled to dense and fine celled. When high temperature short time (HTST) extrusion processing is employed, moisture content, shear in the extruder, die geometry and the rate of heating are the principal factors responsible for creating porous open celled structures and controlling product expansion. This factor contributes in formation of various textures in products having similar appearance and could result in differences in product microstructure. The information on structural changes of product due to extrusion processing conditions is limited.

Therefore this study was aimed to evaluate the colour, textural and microstructural changes in extrudates during storage.

MATERIALS AND METHODS

Commercial carrot was procured from local market, of Longowal, Dist. Sangrur, (Punjab, India). Rice broken and *pigeon* pea were procured from the local rice mill and *dhal* (pulse) mill respectively and these were ground using traditional *atta chakki* (burr mill) to get the desired particle size of the rice flour and was passed through 500 micron sieve. *Achari* (Pickled) mango-flavoured seasoning was procured from Symega Savoury Technology (Ernakulum, Kerala, India).

Dry carrot pomace powder preparation

The carrots were washed in running tap water number of times to remove extraneous material. Trashes were removed with a plane stainless steel knife and trimming was also done. A juice mixer grinder cum food processor was used to extract carrot juice. After the juice extraction pomace was collected and dried by following the method reported by KUMAR *et al.* (2011, 2012a).

A hot air oven (Osaw Industrial Products Pvt. Ltd., India) was used for drying carrot pomace, which could regulate drying air temperature up to 250°C with ±2°C accuracy. The dryer consisted of a preheating and heating chamber with thermostat based control unit, an electrical fan, and measurement sensors. The samples were spread over the trays and the temperature of the dryer was set to 60°C as reported by Kumar *et al.* (2012a). The drying procedure continued till the moisture content of the sample was reduced to about 5±1% (wet basis). The grinding was performed using the food processor with grinder attachment. The material was ground to pass through the

sieve of 2 mm size. The pomace powder so obtained was stored in sealed polythene bag for further use.

Extrudates preparation

The extrudates were prepared under the optimum conditions based on the extrusion studies (KUMAR *et al.*, 2010a). Ingredients were mixed and formulations were made as per the optimized conditions as rice to pomace and pulse ratio was kept 83.5:16.5 and the moisture content was kept 19.23%, screw speed 310 rpm and temperature 110°C for the carrot pomace based extrudates. Moisture was adjusted by sprinkling distilled water in dry ingredients. All the ingredients were weighed and then mixed in the same food processor for 10 minute. The mixture was then passed through a 2 mm sieve to reduce the lump formation due to addition of moisture. After mixing, samples were stored in bags at room temperature for 24 h (STOJCESKA *et al.*, 2008). Moisture content of samples was determined by hot air oven method (RANGANNA, 1995) prior to extrusion experiments. Extrusion of samples was performed using a co-rotating twin-screw extruder (Basic Technology Pvt. Ltd., Kolkata, India) consisting standard screw profile. The length to diameter (L/D) ratio of the extruder was 8:1. The barrel was provided with two electric band heaters and two water cooling jackets. The main drive of extruder was provided with a 7.5 HP motor (400 V, 3 ph, 50 cycles). The twin screw extruder was kept "ON" for 30 minutes to stabilize the set temperatures and samples were then poured in to feed hopper and the feed rate was adjusted to 4 kg/h for easy and non-choking operation. The die diameter was selected at 4 mm as recommended by the manufacturer for such product and recommended by STOJCESKA *et al.* (2008). The product was collected at the die end and kept at 60°±2°C in an incubator (Orbital Incubator, Macro Scientific Works, New Delhi) for 12 hour duration to remove extra moisture from the product.

Frying

Deep fat frying of the extrudates was carried out at 190°C for 30 seconds using rice bran oil. The frying operation was carried out in deep fryer (Sew-Euro Drive India Pvt. Ltd., Vadodhara, India) of 5L capacity. The moisture content of the product before frying was 1.3%.

Application of seasoning

Achari (Pickled) mango-flavoured seasoning procured from Symega Savoury Technology (Ernakulum, Kerala, India) consisted of ingredients like spices and condiments, salt, mango powder, corn starch, acidity regulator, onion powder, asafoetida, permitted anti- caking agents, hydrolyzed vegetable protein, yeast extract and flavour enhancers and salt. Seasoning was added to the extruded snack food by mixing the seasoning with rice bran oil (A P Solvex, Dhuri, India) (85:15 by weight) and then adding 23.95 g of the prepared slurry to 100 g of the extruded snack.

Packaging and storage

Packaging of the extrudates was carried out in metallized polypropylene at the PepsiCo-India Holdings Private Limited (A Frito-Lay Division), Channo, Bhawanigarh, Sangrur, Punjab (India). The packets of extruded snack food samples were stored in corrugated fibre box at temperature of 30°C and relative humidity of 50% in the research lab of Department of Food Engineering and Technology, Sant Longowal Institute of Engineering and Technology, Longowal, Sangrur, Punjab (India) for six months. The extrudates were analysed for textural parameters, colour values and changes in the structure after every month till six month period.

EVALUATION OF RESPONSES

Scanning electron microscopy

Scanning Electron microscopy (SEM) of the fibre rich snack food was conducted every month up to the 6 months. SEM (Jeol, Japan, Model-JSM 65100-LV) was used to illustrate the effect of storage on the microstructure of all the three samples (unfried, fried and fried with seasoning) stored using metalized polypropylene as packaging material.

Each extrudate was prepared for SEM examination by first drying it at 40°C in vacuum oven and further the extrudates were cut by razor blade to obtain an intact cross section. Samples were mounted on aluminium stubs using glue adhesive (Fewikwik, Pidilite, India) and sputter coated with a thick gold palladium layer with a sputter coater (Jeol, Japan, Model- JS31100). Photomicrographs of each extrudate sample up to six months of storage were taken at 700 and 1,500 magnifications. The magnifications were varied to get the more accurate and desirable results.

Texture analysis

Textural properties of the extrudates were determined by crushing method using a TA-XT2 texture analyzer (Stable Micro Systems Ltd., Goldming, UK) equipped with a 500 kg load cell. An extrudate about 40 mm long, was compressed with a probe (SMS - P/75 - 75mm diameter) at a crosshead speed 5 mm/s to 3 mm of 90% of diameter of the extrudate. The high-

est first peak value was recorded as this value indicated the first rupture of snack at one point and this value of force was taken as a measurement for hardness and the total number of peaks was taken as a measurement of crispiness (STOJCESKA *et al.* 2008).

Colour analysis

The colour of extrudates in terms of L, a, b values was determined using colorimeter (Model NP-3000, Nippon, Japan). Colour space is based on the concept that colours can be considered as combinations of colour plotted in a cubical form. The maximum and minimum for L-values are 100 and zero, indicates perfect reflecting diffuser and black respectively. The redness/greenness and yellowness/blueness are denoted by a-values and b-values, respectively (HUNTERLAB, 2008). Before measuring, the colorimeter was standardized with black and white calibration tiles provided with the instrument. Extrudate samples were cut longitudinally and the colour was measured in three places of each sample. Average values were recorded for the study (STOJCESKA *et al.* 2008). Colour analysis of the extrudates was conducted every month up to 6 months of storage.

Statistical analysis

The results were analysed by statistical software Statistca 7. The analysis of variance (ANOVA) for significance was conducted at a confidence level of 95% ($p \leq 0.05$) .The significance of difference between mean values was assessed with Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of magnification on the microstructural behaviour of extruded snack food

The photomicrographs at 700 and 1,500X magnification level confirm the pattern of changes in microstructure of the extrudates. It can be observed from the photomicrographs (Fig. 1) that no marked change in the pattern of microstructural was visualised during storage when compared to those observed at 700X. Hence, it was found sufficient to interpret the modifications of extrudate microstructure for studying the microstructural changes at one magnification level during storage.

Microstructural behaviour of the extruded snack food

The SEM (Fig. 2) shows the presence of protruding structures in control sample of the unfried extruded probably indicating the abundance of starch. The images of the cross-section show discrete cells. The structural integrity of the extrudates seems to have remained preserved. In the first month of storage hardly any change was observed in the structural orientation of the extrudates as compared to control sample, suggesting that the structural orientation of the extrudates was preserved and retained**.** In the second month of storage of the unfried extrudates, protruding has got diminished and the granular size seems to have reduced. The fibres seem to be structurally organised in ordered fashion resulting in the increase of hardness and decrease in the crispi-

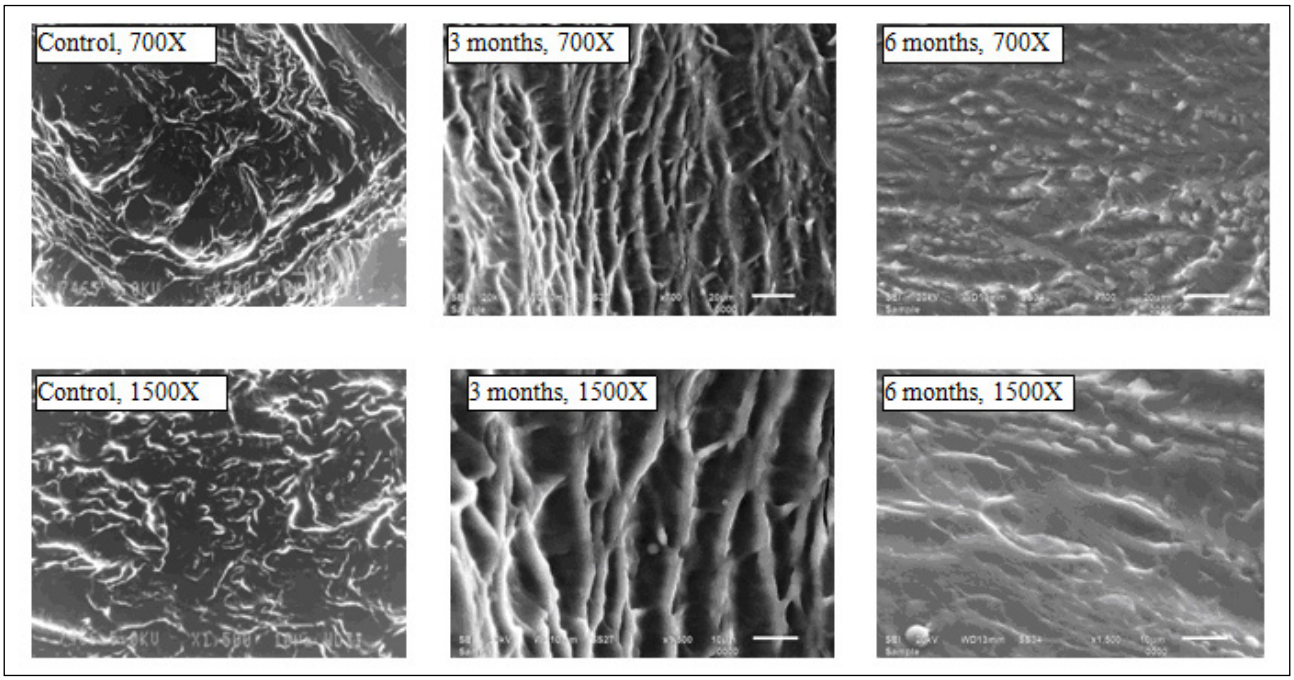

Fig. 1 - Effect of magnification level on unfried extruded snack at different storage period.

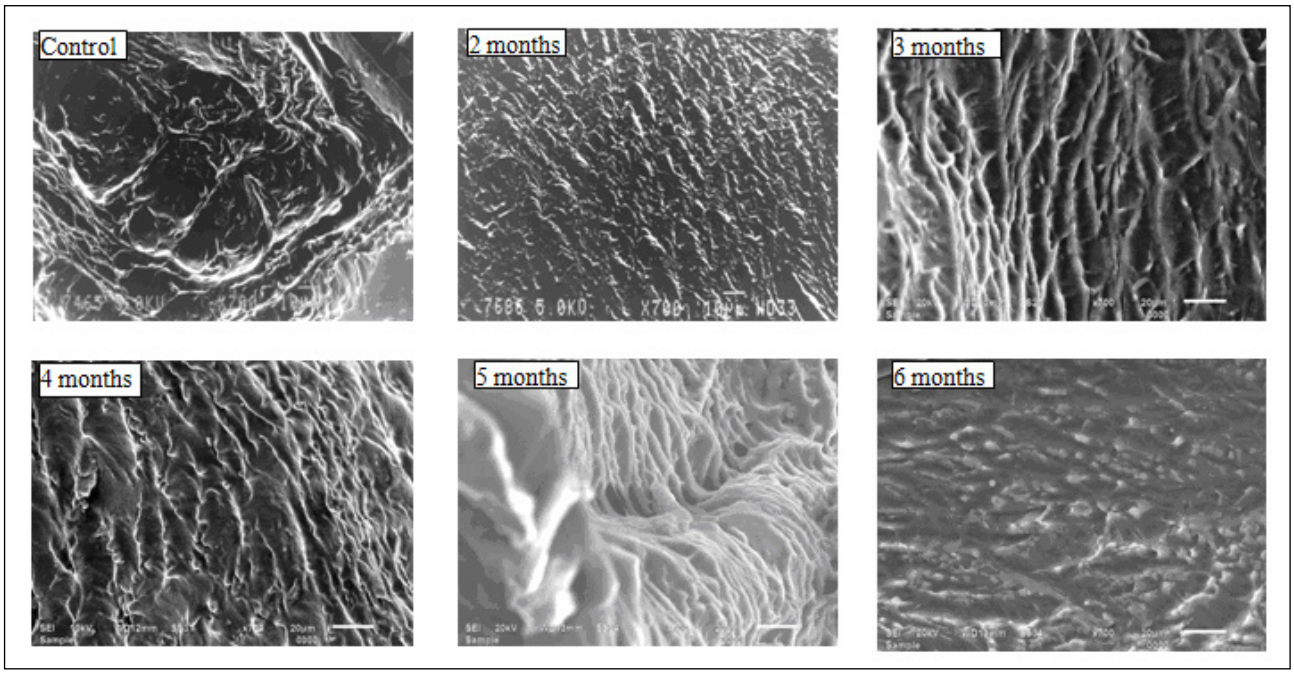

Fig. 2 - Microstructural changes in unfried extruded snack during storage.

ness of the extrudates, as revealed from Table 1. In the third month of storage unfried extruded snack food micrograph shows that the gaps have been developed between the fibres and the walls have become thinner and the granules have extended into long fibres as the accumulation and agglomeration of the cells seem to have resulted into the large fibres which in turn lead to the formation of organized structures. The photomicrographs for the fourth month revealed the presence of thinner long fibres that have got clubbed together to bigger size fibres with protruding, outfoldings and the cells have got restructured and are intact. These changes may be the reason for the increased hardness and marked reduction in the crispiness of the extrudates. The dense thin fibres were seen in the extrudates with thin walls and with very few open spaces and the restructuring arrangement of fibres and globular structure after the fifth month. The photomicrograph for the sixth month of storage revealed the presence of relatively much compact and dense structural orientation with numerous globules which may have lead to the increase in the hardness of the extrudates and may be the cause for the loss of crispiness of the extrudates can be verified from (Table 1). WANG *et al.* (1999) reported similar microstructural pattern for pasta like product and also exhibited a dense and compact structure.

Effect of frying on the microstructure of extruded snack

The photomicrographs (Fig 3) reveal the presence of compact mass of structures in the extrudate sample before the storage. Roughness can also be observed in the extrudates which reflects that the frying process has possibly brought the

Table 1 - Average hardness and crispiness of extrudates during storage.

Storage period	Hardness (N)			Crispiness		
	Unfried	Fried	Seasoned	Unfried	Fried	Seasoned
0	13.78±0.03^{g}	8.24±0.06^{g}	8.62±0.04^{g}	40.33±1.53^{a}	57.00±1.00^{a}	59.00±1.00^{a}
1	16.81±0.03^{f}	8.63±0.06^{f}	9.11±0.04^{f}	36.33±2.08^{b}	54.67±1.53^{b}	57.67±0.58^{b}
2	23.25±0.03^{e}	9.24±0.04^{e}	9.63±0.04^{e}	33.67±1.53^{c}	54.00±1.00^{b}	56.00±1.00^{c}
3	33.76±0.05^{d}	11.57±0.08^{d}	12.00±0.12^{d}	32.67±2.52^{cd}	46.33±0.58^{c}	49.00±1.00^{d}
4	40.18±0.07^{c}	14.02±0.05^{c}	13.92±0.03^{c}	31.33±1.53^{d}	42.33±1.53^{d}	46.33±1.53^{e}
5	41.55±0.08^{b}	15.35±0.06^{b}	18.17±0.05^{b}	28.67±1.53^{e}	38.00±1.00^{e}	45.00±1.00^{e}
6	45.80±0.08^{a}	19.04±0.07^{a}	21.85±0.02^{a}	22.00±2.00^{f}	33.00±1.00^{f}	41.33±0.58^{f}

$^{a-g}$ means within the same column with different letters are significantly different at ($p \leq 0.05$), number of samples analyzed-3.

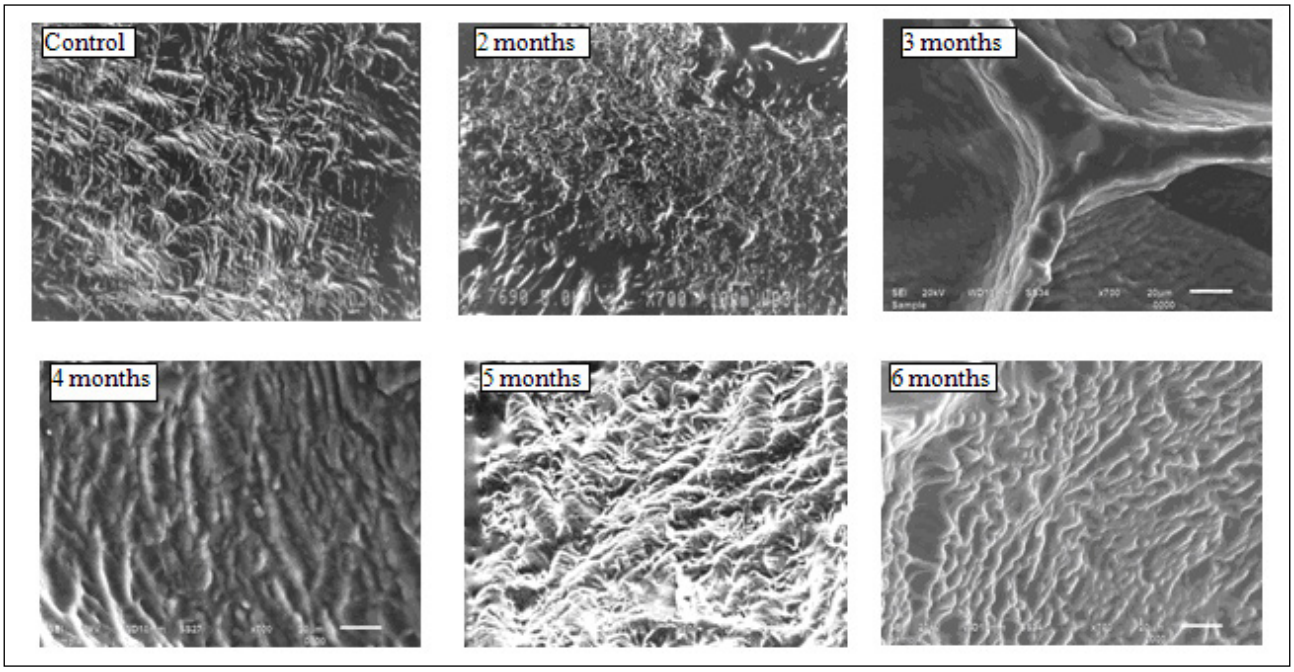

Fig. 3 - Microstructural changes in fried extruded snack during storage.

destruction of fibres which may have caused the decrease in hardness of extrudates which in turn may have lead to the increase in the crispiness of extrudates as revealed from Table 1. Similar structural pattern was observed in the first month of storage. In the second month of storage, surface roughness of the extrudates was observed which reveals that the frying process has caused a remarkable effect on the microstructural combination and composition of the fried extrudates and has lead to the development of uneven and rough surface as compared to unfried extrudates which may be the reason for the decrease in hardness and increased crispiness of the fried extrudate samples. In the third month of storage, the micrograph shows the roughness of the structures along with accumulation of oil globules. The structural derangement was seen in comparison to unfried extrudates. The decrease in the roughness of the structural components of the extrudates was visualised in the fourth month of storage but small protruding were observed, probably with the accumulation of oil globules which may lead to the increase in hardness and decrease in crispiness of the extrudate sample. The photomicrograph interprets the presence of the compact globular structure with the accumulation of globules in the fifth month of storage which apparently led to the development of globular structures of extrudates. The microstructural components of the fried extrudates have got recombined and small globules have lead to the formation of large sized globules after the sixth month of storage. The recombined structure may be the reason for the increased hardness of the extrudate sample within storage period (Table 1). JEAN *et al.* (1996) also have reported formation of fibres, definite orientation and organization of microstructure during extrusion.

Effect of seasoning on the microstructure of extruded snack

The photomicrographs of extrudates (Fig. 4) revealed the presence of more compact structures as compared to unfried and fried extrudates and with shinny structures which may be due to presence of oil and seasoning applied on the surface of extrudates. An uneven surface was observed during storage of seasoned samples. No prominent change in the structural, cellular and arrangement of fibres was observed in the first month of storage. The presence of globular structures with wave like structures was seen which clearly reflected the presence of oil globules with protruding and outfoldings in the second month of storage. In the third month of storage presence of thin structural components and long fibres in the extrudate with slight oily like surface was observed during storage. The presence of shinny outfoldings with a slight rough structure with numerous outfoldings was observed in the fourth month of storage.

The presence of complete globular and fibrillar structure with thin open spaces with the presence of oil within the globules was evident in the fifth month of storage as compared to unfried and fried extrudates. The micrograph reflected the presence of rough surface, probably due to deposition and accumulation of seasoning on the surface of extrudates, and the reori-

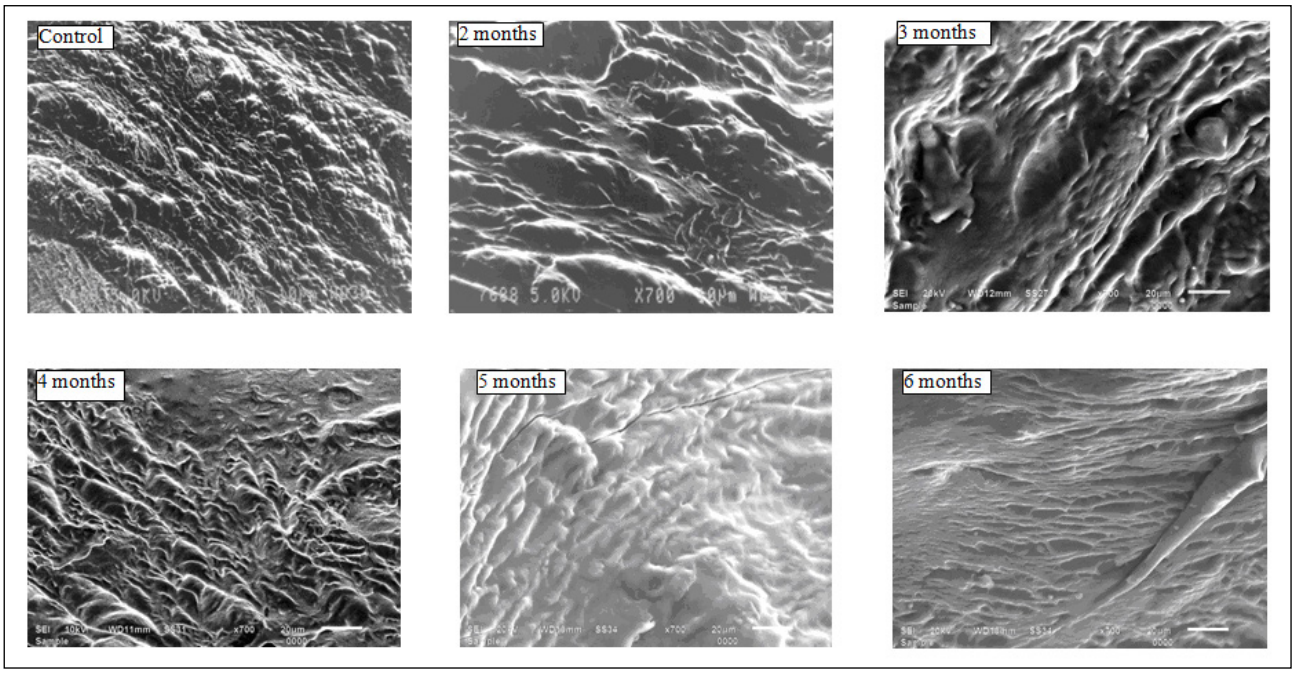

Fig. 4 - Microstructural changes in seasoned extruded snack during storage.

entation of fibres which has led to the formation of open space with a long single fibrous structure with a shinny surface in the sixth month of storage as compared to unfried and fried extrudates. Similar structural pattern in the extrudates was reported by JEAN *et al.* (1996) and revealed the presence of protruding with rough surface causing extrudates to be more dense and compact.

Hardness and crispiness

The changes in hardness and crispiness during storage of the extrudates are shown in Table 1. During the storage period, significant increase in the hardness of the unfried extrudates was observed from the first to sixth month and the hardness ranged from 13.78 to 45.80 N. The increase in hardness may be due to recombination of structure and strength enhancement as a result of moisture inclusion in structure of extrudates. Similar increase in hardness of the extrudates due to the moisture absorption has been reported by KUMAR *et al.* (2012b). In the fried extrudates, the significant change was observed in the hardness of the extrudates which varied from 8.24 to 19.04 N. The increase in hardness in fried extrudates was lesser as compared to the unfried extrudates because of the removal of moisture from the surface as well as from the inner cellular components of the extrudates during frying process (Table 1). In the fried extrudates with seasoning significant change was observed in the hardness of the extrudates during storage and ranged from 8.62 to 21.85 N, but the increase in hardness was lesser as compared to the unfried extrudates. Similar change in the hardness (corn flour and beef) with storage period was reported by BADDING-SMITHEY *et al.* (1995).

In the unfried extrudates, crispiness ranged from 40.33 to 22.00 and significant change in the crispiness was observed from first month of storage (Table 1). The decrease in crispiness may be due to the absorption of moisture during storage and the loss of crunchiness. However in the fried extrudates little change in the crispiness was observed during initial two months of storage. The decrease in crispiness was observed from third to sixth month, whereas the changes in crispiness varied from 57.00 to 33.00 during storage period of six month. These results are in line with the fact that frying of the extrudates had considerably improved the crispiness of the extrudates by removing the moisture from the surface and intercellular components of the extrudate sample as compared to unfried extrudates.

In the fried extrudate with seasoning significant change in the crispiness was observed during the storage which ranged from 59.00 to 41.33 means that the crispiness of the extrudates was retained (Table 1). The retention of the crispiness of extrudates may be due to the frying of extrudate sample which may have removed the moisture from the surface of extrudate sample and hence the crispiness of the extrudates was retained to a desired level. The fried extrudate with seasoning showed the better crispiness even after six months of storage as compared to fried and unfried samples. The better crispiness of seasoned samples as compared to fried and unfried extrudates may be due to absorption of

Table 2 - Average colour L, a and b values of extrudates during storage.

Storage period	L-value			a-value			b-value		
	unfried	fried	seasoned	unfried	fried	seasoned	Unfried	fried	seasoned
0	66.22±0.13^{a}	38.58±0.03^{a}	34.95±0.19^{a}	6.31±0.28^{a}	18.45±0.78^{e}	18.48±0.46^{d}	23.66±0.69^{a}	20.06±1.63^{a}	20.99±0.13^{a}
1	65.96±0.93^{a}	38.51±0.05^{a}	34.98±0.03^{a}	6.35±0.06^{a}	18.56±0.44^{e}	18.76±0.44^{cd}	23.58±0.39^{a}	19.28±1.40^{ab}	20.86±1.04^{a}
2	65.26±1.16^{a}	38.22±0.03^{b}	34.68±0.31^{a}	6.37±0.28^{a}	19.11±0.15^{d}	19.14±0.06^{c}	23.48±0.61^{a}	18.27±0.09^{b}	20.76±0.99^{a}
3	65.13±0.44^{a}	37.23±0.05^{c}	34.42±0.19^{ab}	6.40±0.17^{a}	19.31±0.08^{d}	19.20±0.01^{bc}	23.25±0.44^{a}	18.19±0.01^{b}	20.58±1.00^{a}
4	64.29±0.31^{ab}	37.07±0.05^{cd}	33.96±0.07^{b}	6.47±0.22^{a}	19.71±0.03^{c}	19.30±0.05^{b}	22.81±0.15^{ab}	18.20±0.06^{b}	20.51±1.01^{a}
5	63.67±0.55^{b}	36.74±0.05^{d}	33.82±0.06^{c}	6.52±0.23^{a}	21.11±0.03^{b}	19.35±0.02^{b}	22.45±0.90^{b}	18.17±0.05^{b}	19.07±0.19^{b}
6	62.53±0.49^{b}	36.38±0.51^{e}	33.78±0.09^{c}	6.59±0.13^{a}	22.15±0.06^{a}	20.21±0.03^{a}	22.28±0.35^{b}	17.59±0.26^{c}	19.08±0.12^{b}

$^{a-e}$ means within the same column with different letters are significantly different at $p \leq 0.05$, number of samples analyzed-3.

moisture by seasoning during storage thereby retaining the crispiness of the extrudates.

Effect of storage on colour values of the extruded snacks

The retention of colour after thermal treatments may be used to predict the quality deterioration of food. The unfried extrudates had significant change in the L-value of extrudates during storage (66.22 to 62.53), but no significant change was observed in the first four months of storage (Table 2). The decrease in L-value indicates the darkness in colour which may be attributed to the gradual increase in non enzymatic browning during storage. NEGI and ROY (2001) observed similar increase in non enzymatic browning of dehydrated carrots during long term storage. Similar result for unfried carrot pomace based extrudates has also been reported by KUMAR *et al.* (2012b). Similarly, in case of fried extrudates L-value decreased from 38.58 to 36.38 during the entire storage period. This could be due to the Maillard reaction and due to the thermal treatment in the form of frying and decrease in the moisture content of the fried extrudates. Similar decrease in L-value in carrot slices fried at 100°C for up to 15 min due to reducing moisture content was reported by SHYU *et al.* (2005). In case of fried extrudates with seasoning, minute changes in L-value of extrudates were observed during the initial three months of storage and the change varied from 34.95 to 34.42. The lower L-value as compared to fried and unfried extrudates indicates that the atmospheric frying and addition of seasoning caused darkness in the extrudates.

No significant change in the a-value of the unfried extrudates was observed during storage period (Table 2). In case of fried extrudates, non significant increase was observed during first month; however gradual increase was observed from second to fourth month. Significant increase was observed during fifth and sixth month of storage (19.71 to 22.15). In case of seasoned extrudates, the gradual increase was observed during first month. However, no significant increase in a-value was observed during third to fifth month of storage. The a-values for seasoned extrudates varied from 18.48 to 20.21. The a-value of the extrudates with seasoning followed similar pattern like fried extrudates. The increase in reddishness may be due to the destruction of heat sensitive pigments by thermal treatment in the form of frying and some non enzymatic browning reactions like Maillard reaction and retrogradation. Similar increase in redness was also reported by NEGI and ROY (2001) due to non enzymatic browning of dehydrated carrots during long term storage.

No significant change in the b-value of the unfried extrudates was observed till fourth month of storage period. The b-value of the unfried extrudates varied from 23.66 to 22.28 (Table 2). Significant decrease in b-values of fried extrudates was observed during initial two months; however, further decrease was not significant till fifth month of storage. The b-values for fried extrudates reduced from 20.06 to 17.59. In case of seasoned extrudates, decrease in b-value was observed during storage; however significant increase was noted only during fifth month of storage. The b-values for seasoned extrudates varied from 20.99 to 19.08. The lower decrease in the b-value of seasoned extrudates as compared to fried extrudates may be due to application of seasoning which may have profoundly changed the upper crust colour. The decrease in b-value reflected the decrease in yellowness which may be due to the degradation of beta carotene during storage period. Similar degradation of beta carotene due to air and light and storage time has also been reported by ORSET *et al.* (1999).

CONCLUSION

The study revealed the profound change in the structural orientation of the fibre and cellular components of the extrudates during storage in the unfried extruded snack foods. Similar pattern was observed in case of fried and seasoned

extruded snack foods during the storage period. It was reflected in the form of presence of open spaces and the wave like structures with protruding suggesting a marked effect on the microstructure of extrudates during storage. Hardness of the extrudates was increased for unfried, fried and seasoned extrudates respectively during storage and a consequently decrease in crispiness was observed. The maximum hardness was observed for unfried extrudates, however maximum crunchiness was observed for seasoned extrudates initially as well as after six month storage period. The colour L-value and b-value decreased and a-value increased for unfried, fried and seasoned extrudates respectively during storage. The minimum change in L-value was observed for seasoned extrudates, whereas minimum change in a-value and b-value was observed for unfried extrudates. The microstructural, colour and textural properties of unfried, fried and seasoned extrudates changed during storage of six months and least changes in hardness and crispiness occurred in seasoned extrudates.

REFERENCES

Badding-Smithey S.L., Huff H.E. and Hsieh F. 1995. Processing parameters and product properties of extruded beef and nonmeat cereal binders. Food Sci. Technol. - Lebensm Wiss Technol. 28(5), 386-394.

De-Pilli T., Severini C., Baiano A., Guidolin E., Legrand J. and Massini R. 2001. Application of extrusion flour fat: the case of the almond flour. Sci. Alim. 21(5), 519-536.

De-Pilli T., Severini C., Baiano A., Arhaliass A. and Legrand J. 2004a. Extrudability and stability of mixtures rich in fat in extrusion cooking - Comparison between single-screw and twin-screw extruders. Sci. Alim. 24(4), 307-322.

De-Pilli T., Severini C., Carbone B.F., Giuliani R. and Derossi A. 2004b. Improving of fatty extrudate structures with amylase and protease. J. Food Biochem. 28 (5), 387-403.

De-Pilli T., Severini C., Baiano A. Derossi A., Arhaliass A. and Legrand J. 2005a. Effects of operative conditions on oil loss and properties of products obtained by co-rotating twin-screw extrusion of fatty meal: preliminary study. J. Food Eng. 70 (1), 109-116.

De-Pilli T., Giuliani R., Carbone B.F., Derossi A. and Severini C. 2005b. Study on different emulsifiers to retain fatty fraction during extrusion of fatty flours. Cereal Chem. 82 (5), 494-498.

De-Pilli T., Carbone B.F., Fiore A.G. and Severini C. 2007. Effect of some emulsifiers on the structure of extrudates with high content of fat. J. Food Eng. 79(4), 1351-1358.

De-Pilli T., Carbone B.F., Derossi A., Fiore A.G. and Severini C. 2008a. Effects of operating conditions on oil loss and structure of almond snacks. Int. J. Food Sci. Tech. 43(3), 430-439.

De-Pilli T., Jouppila K., Ikonen J., Kansikas J., Derossi A. and Severini C. 2008b. Study on formation of starch-lipid complexes during extrusion-cooking of almond flour. J. Food Eng. 87, 495-504.

De-Pilli T., Derossi A., Talja A.R., Jouppila K. and Severini C. 2011. Study of starch-lipid complexes in model system and real food produced using extrusion-cooking technology. Innov. Food Sci. Emerg. Tech. 12, 610-616.

De-Pilli T., Derossi A., Talja R.A., Jouppila K. and Severini C. 2012. Starch-lipid complex formation during extrusion-cooking of model system (rice starch and oleic acid) and real food (rice starch and pistachio nut flour). Eur. Food Res. Tech. 234(3), 517-525.

Grigelmo-Miguel N. and Martín-Belloso O. 1999. Comparison of dietary fibre from by-products of processing fruits and greens and from cereals. Food Sci. Technol.- Lebensm Wiss Technol. 32(8), 503-508.

Hsu P.K., Chien P.J., Chen C.H. and Chau C.F. 2006. Carrot insoluble fiber-rich fraction lowers lipid and cholesterol absorption in hamsters. Food Sci. Technol. - Lebensm Wiss Technol. 39(4), 338-343.

Hunterlab 2008. Application notes, Hunter Associates Laboratory Inc., Virginia 8(9), 1-4. www.hunterlab.com/appnotes/an08_96a.pdf - accessed on 15.11.2012.

Jean I.J., Work R., Camere M.E., Briggs J., Barrett A.H. and Bushway A.A. 1996. Selected properties of extruded potato and chicken meat. J. Food Sci. 61(4), 783-789.

Kadan R.S., Bryant R.J. and Pepperman A.B. 2003. Functional properties of extruded rice flours. J. Food Sci. 68(5), 1669-1672.

Kumar N., Sarkar B.C. and Sharma H.K. 2010a. Development and characterization of extruded product of carrot pomace, rice flour and pulse powder. African J. Food Sci. 4(11), 703-717.

Kumar N., Sarkar B.C. and Sharma H.K. 2010b. Development and characterization of extruded product using carrot pomace and rice flour. Int. J. Food Eng. 6 (3), 1-24.

Kumar N., Sarkar B.C. and Sharma H.K. 2011. Effect of air velocity on kinetics of thin layer carrot pomace drying. Food Sci. Technol. Int. 17(5), 459-469.

Kumar N., Sarkar B.C. and Sharma H.K. 2012a. Mathematical modelling of thin layer hot air drying of carrot pomace. J. Food Sc. Technol. 49(1), 33-41.

Kumar N., Sarkar B.C., Sharma H.K. and Jha S.K. 2012b. Colour kinetics and storage characteristics of pulse and rice by-product based extrudates. British Food J. 114(9), 1279-1296.

Nawirska A. and Kwasniewska M. 2005. Dietary fibre fractions from fruit and vegetable processing waste. Food Chem. 91(2), 221-225.

Negi P.S. and Roy S.K. 2001. The effect of blanching on quality attributes of dehydrated carrots during long term storage. Eur. Food Res. Technol. 212(4), 445-448.

Orset S., Leach G.C., Morais R. and Young A.J. 1999. Spray-drying of the microalga *Dunaliella salina*: Effects on beta-carotene content and isomer composition. J. Agric. Food Chem. 47(11), 4782-4790.

Ranganna S. 1995. Handbook of analysis and quality control for fruits and vegetable products, Tata Mc-Graw Hill Publishing Company Limited, New Delhi, 4-5.

Shyu S.L., Hau L.B. and Hwang L.S. 2005. Effects of processing conditions on the quality of vacuum-fried carrot chips. J. Sci. Food Agric. 85(11), 1903-1908.

Stojceska V., Ainsworth P., Plunkett A., Ibanoglu E. and Ibanoglu S. 2008. Cauliflower by-products as a new source of dietary fibre, antioxidants and proteins in cereal based ready-to-eat expanded snacks. J. Food Eng. 87(4), 554-563.

Upadhyay A., Sharma H.K. and Sarkar B.C. 2008. Characterization of dehydration kinetics of carrot pomace. Agric. Eng. Int.: CIGR J. 10(2), 1-9.

Upadhyay A., Sharma H.K. and Sarkar B.C. 2010. Optimization of carrot pomace powder incorporation on extruded product quality by response surface methodology. J. Food Quality 33, 350-369.

Walde S.G., Math R.G., Chakkarvarthi A. and Rao D.G. 1992. Preservation of carrots by dehydration techniques-A Review. Ind. Food Packer 46(6), 37-42.

Wang N., Bhirud P., Sosulski F. and Tyler R. 1999. Pasta-like product from pea flour by twin-screw extrusion. J. Food Sci. 64(4), 671-678.

Yagci S. and Gogus F. 2009. Effect of Incorporation of Various Food By-products on Some Nutritional Properties of Rice-based Extruded Foods. Food Sc. Technol. Int. 15, 571-581.

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PAPER

POLYPHENOL CONTENT AND ANTIRADICAL ACTIVITY OF "SARCONI" BEANS (*PHASEOLUS VULGARIS* L.) ECOTYPE

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ABSTRACT

The aim of this study was to establish the distribution and content of polyphenols (anthocyans, flavonols and hydroxycinnamic acids) in hulls and seeds of Sarconi beans having different colours and shapes. Sarconi beans are protected by the indication of geographic provenance (IGP) denomination and include different ecotypes. The seeds sampled in the study area (Basilicata, Val d'Agri) exhibited different colours from white (Riso Bianco) to dark yellow (Tabacchino), to green (Verdolino) and to red (San Michele Rosso) with shapes changing from small round to large round-ovoid. The seeds of the four ecotypes were collected from two farms in order to identify differences that could be caused by environmental conditions. Flavonols of some ecotypes of Sarconi beans have already been described, while the anthocyan composition and content have never been reported. In particular, Tabacchino beans contained the highest amount of both flavonols, such as kaempferol and quercetin derivatives (6.342-6.515 mg/g) and hydrxycinnamic acids (1.136-1.636 mg/g), while only San Michele contained anthocyans, such as cyanidin and pelargonidin derivatives (1.118-3.187 mg/g); Riso Bianco seeds contained only hydroxycinnamic acids (0.975-1.292 mg/g). Kaempferol derivatives were the most representative flavonols in the seed coat of Sarconi beans and in other Italian landraces they have only been found previously in Zolfino beans. This occurrence could distinguish Italian beans from Brazilian, Peruvian, and Mexican beans. The antiradical activity, as indicated in particular by EC_{50} values, ranged from 2.78 to 16.93 g sample/mg DPPH• (1,1-diphenyl-2-picrylhydrazil radical) for San Michele and Riso Bianco beans, respectively.

- Keywords: beans, flavonols, kaempferol derivatives, anthocyans, HPLC/DAD/MS -

INTRODUCTION

Common beans (*Phaseolus vulgaris*) are often regarded as nutraceutical food since their consumption has been linked to reduced risk of coronary heart disease (BAZZANO *et al.*, 2001), diabetes (OCHO-ANIN ATCHIBRI *et al.*, 2010), obesity (NASI *et al.*, 2009) and colon and breast cancer (CAMPOS-VEGA *et al.*, 2010; CUI *et al.*, 2007). Common bean seeds supply the diet with protein, complex carbohydrates, minerals and dietary fibres. They contain many bioactive substances such as enzyme inhibitors, lectines, phytates, oligosaccharides and polyphenols. Polyphenols have attracted increasing interest for their anticarcinogenic properties and play an important role in contrasting oxidative stress, which is defined as the imbalance between oxidants and antioxidants, identified as the cause of ageing and various diseases in humans (HEIM *et al.*, 2002; CARDADOR-MARTINEZ *et al.*, 2002).

Since the second half of the twentieth century, many species of agricultural interest once widely cultivated for human food, including common beans, have undergone a gradual contraction of their distribution. The causes contributing to this reduction may include the welfare state and changes in eating habits. Moreover, globalization of markets and uniformity in production through improved cultivars selected for their characteristics of stability may have also reduced the number of actual consumer preferences. A large number of local genotypes are still cultivated in marginal areas of Italy (LIMONGELLI *et al.*, 1996; MASI *et al.*, 1999; PIERGIOVANNI *et al.*, 2006; NEGRI and TOSTI, 2002) since consumers have acquired specific preferences for various combinations of bean size and shape. In this context the consumption of local ecotypes may afford a higher market value. In Italy such products are labelled by indication of geographic provenance (IGP).

The objective of the present study was to establish the distribution and content of polyphenols (anthocyans, flavonols and hydroxycinnamic acids) in hulls and seeds of Sarconi beans having different colours and shapes. Sarconi beans are protected by the IGP denomination and include different ecotypes. The seeds sampled in the study area (Basilicata, Val d'Agri) had different colours from white (Riso Bianco) to dark yellow (Tabacchino), to green (Verdolino) and to red (San Michele Rosso) and varying shapes from small round to large round-ovoid. The seeds of the four ecotypes were collected from two farms in order to identify differences that could be caused by environmental conditions (MARLES *et al.*, 2010). Flavonols of some ecotypes of Sarconi beans have already been described (DINELLI *et al.*, 2006), while the anthocyan composition and content have never been reported. In order to obtain functional information correlated with bean quality, antiradical activity with respect to the stable DPPH• radical was evaluated and EC_{50} values were considered.

EXPERIMENTAL

Plant material

Dry seeds of four landraces of *Phaseolus vulgaris* L. were collected from two different farms located in Val d'Agri (Basilicata, Southern Italy). The ecotypes considered were: Riso Bianco (white, large round-ovoid seed), Verdolino (green, round-ovoid seed), Tabacchino (yellow-orange, round-ovoid seed), San Michele Rosso (ruby red, small round seed) (Table 1).

Standards and solvents

Authentic standards of kaempferol 3-*O*-glucoside, rutin, pelargonidin, caffeic acid and gallic acid were purchased from Extrasynthèse S.A. (Lyon, France). All solvents used were of HPLC grade purity (BDH Laboratory Supplies, United Kingdom).

Extraction of polyphenols

A 500 mg sample of ground dry seeds was extracted with 40 mL of 70% ethanol, adjusted to pH 2.0 with formic acid to avoid degradation of anthocyanins, and kept for one night at room temperature. The seed coats of beans were removed and ground and 250 mg of seed coat powder was extracted with 20 mL of 70% ethanol adjusted to pH 2 with formic acid. The extracts were evaporated to dryness under vacuum at room temperature, and finally redissolved in $EtOH/H_2O$ (70:30) adjusted to pH 2.0 with formic acid, to a final volume of 2 mL.

HPLC/DAD analysis

Analyses of flavonols, anthocyanins and hydoxycinnamic acids were carried out using a HP 1100L liquid chromatograph equipped with a DAD detector and managed by a HP 9000 workstation (Agilent Technologies, Palo Alto, CA, USA). Flavonols and anthocyanins were separated using a 150 × 3 mm, 4 μm, Synergy MAX-RP80A column (Phenomenex, USA) operating at 26°C. Hydroxycinnamic derivatives were separated using a 150 × 4.6 mm, 5 μm, Luna RP18

Table 1 - Numbers of analysed samples.

Landraces	Farm 1	Farm 2
San Michele	1	2
Tabacchino	3	4
Riso Bianco	5	6
Verdolino	7	8

column (Phenomenex, USA) operating at 26°C. Three analytical methods were used: for flavonols a four-step linear solvent gradient system, starting from 5% up to 100% of CH_3CN for a 32-min period, at a flow rate of 0.4 mL/min; for anthocyanins a five-step linear solvent gradient system, starting from 5 up to 100% of CH_3CN for a 32-min period, at a flow rate of 0.4 mL/min; and for hydroxycinnamic acids a four-step linear solvent gradient system, starting from 0 up to 100% of CH_3CN for a 52-min period, at a flow rate of 0.6 mL/min. UV/Vis spectra were recorded in the 190-600 nm range and the chromatograms were acquired at 260, 280, 330, 350 and 520 nm.

HPLC/MS analysis

HPLC/MS analyses were performed using a HP 1100L liquid chromatograph linked to a HP 1100 MSD mass spectrometer with an API/electrospray interface (Agilent Technologies, Palo Alto, CA, USA). Spectra were recorded in negative ion mode, fragmentor 120, for flavonols and hydroxycinnamic acids, and in positive ion mode, fragmentor 120, for anthocyanins, applying the same chromatographic condition previously described. The mass spectrometer operating conditions were: gas temperature, 350°C; nitrogen flow rate, 10.0 L/min; nebulizer pressure, 30 psi; quadrupole temperature, 30°C; and capillary voltage, 3,500 V.

Identification and quantification of individual polyphenols

The identity of polyphenols was ascertained using data from HPLC/DAD and HPLC/MS analyses, by comparison and combination of their retention times, UV/Vis and mass spectra with those of authentic standards. Quantification of individual polyphenolic compounds was directly performed by HPLC/DAD using a five-point regression curve ($r^2 \geq 0.998$) on the basis of authentic standards. In particular, flavonols like kaempferol and quercetin derivatives were determined at 350 nm using kaempferol 3-*O*-glucoside and quercetin-3-*O*-rutinoside (rutin) as reference compounds, respectively. Cyanidin and pelargonidin derivatives were determined at 520 nm using pelargonidin as reference compound, while hydroxycinnamic derivatives were determined at 330 nm using caffeic acid as reference compound. In all cases, actual concentrations of the derivatives were calculated after applying corrections for differences in molecular weight.

Total phenolic content and antiradical activity

The total phenolic content was determined using the Folin-Ciocalteu method, described by SINGLETON *et al.* (1999) and slightly modified according to DEWANTO *et al.* (2002). To 125 μL of the suitably diluted sample extract, 0.5 mL of deionized water and 125 μL of the Folin-Ciocalteu reagent were added. The mixture was kept for 6 min and then 1.25 mL of a 7 % aqueous Na_2CO_3 solution were added. The final volume was adjusted to 3 mL with water. After 90 min, the absorption was measured at 760 nm against water as a blank. The amount of total phenolics is expressed as gallic acid equivalents (GAE, mg gallic acid/100 g sample) through the calibration curve of gallic acid. The calibration curve ranged from 20 to 500 μg/mL ($R^2 = 0.9969$).

Free radical scavenging activity was evaluated by DPPH• (1,1-diphenyl-2-picrylhydrazil radical) assay. The antiradical capacity of the sample extracts was estimated according to the procedure reported by BRAND-WILLIAMS *et al.* (1995) and slightly modified.

Two mL of the sample solution, suitably diluted with ethanol, was added to 2 mL of an ethanolic solution of DPPH• (0.025 g/100 mL) and the mixture was allowed to stand. After 20 min the absorption was measured at 517 nm *versus* ethanol, as a blank. Each day, the absorption of the DPPH• solution was checked. The antioxidant activity is expressed as EC_{50}, the antioxidant dose required to cause a 50% inhibition (CARDADOR-MARTINEZ *et al.*, 2002). EC_{50} was calculated plotting the ratio:

[DPPH• concentration at t = 20']/
/[DPPH• concentration at t = 0]

against the concentration of the antioxidant. EC_{50} is expressed as mg antioxidant/mg DPPH•.

RESULTS AND DISCUSSION

The present study compares the different phenolic classes in four varieties of *Phaseolus vulgaris* L. grown in different fields and different years. The extraction procedure for *Phaseolus* seeds ensures that all the polyphenol classes were obtained. Flavonols, hydroxycinnamic derivatives and anthocyanins were identified using data from HPLC/DAD and HPLC/MS analysis by comparison and combination of their retention times, mass spectrometry and UV spectra.

Fig. 1 shows the HPLC-DAD profile at 350 nm of seed coat extracts of the four Sarconi ecotypes. Riso Bianco does not contain flavonoids, while four flavonols were identified: kaempferol-3-*O*-xylosyl-glucoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-(6"-*O*-malonylglucoside and kaempferol aglycone. The first two compounds were found previously in Sarconi beans by DINELLI *et al.* (2006) and in Zolfino beans by ROMANI *et al.* (2004), while kaempferol-3-*O*-(6"-*O*-malonylglucoside) was identified in common beans (LIN *et al.*, 2008); kaempferol aglycone was isolated in seed

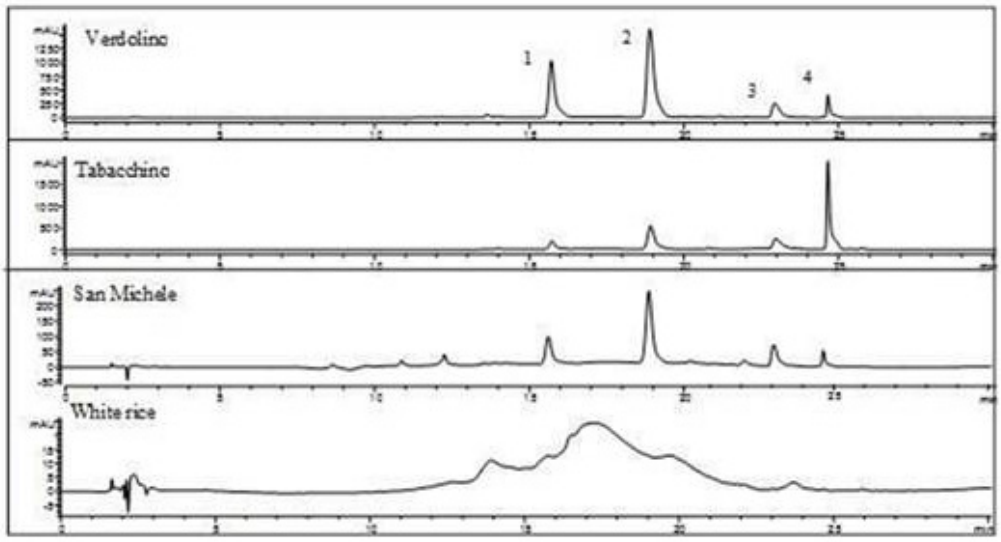

Fig. 1 - HPLC/DAD (350 nm) chromatographic profile of seed coats of four varieties. 1) kaempferol 3-*O*-xylosyl-glucoside, 2) kaempferol 3-*O*-glucoside, 3) kaempferol 3-*O*-(6"-*O*-malonylglucoside), 4) kaempferol.

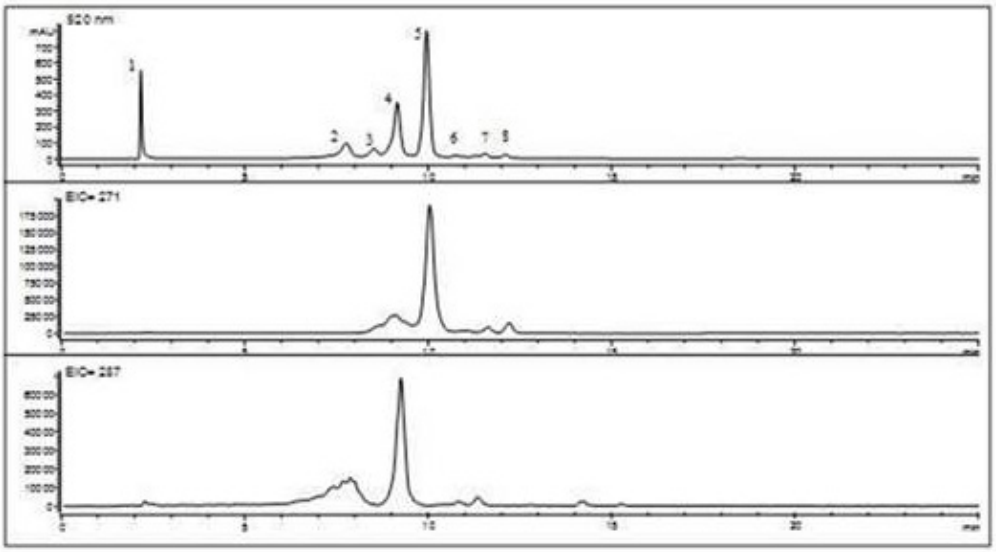

Fig. 2 - HPLC/DAD (520 nm) chromatographic profile and extract ions current (m/z 271, m/z 287) of San Michele extracts. 1) Cyanidin diglycosides, 2) Cyanidin 3,5-*O*-diglucoside, 3) pelargonidin 3,5-*O*-diglucoside, 4) cyanidin 3-*O*-glucoside, 5) pelargonidin 3-*O*-glucoside, 6) cyanidin 3-*O*-(6"-*O*-malonylglucoside), 7) Pelargonidin 3-*O*-(6"-*O*-malonylglucoside), 8) Pelargonidin derivative.

coats of Brazilian and Peruvian beans (RANILLA *et al.*, 2007). Verdolino contains traces of quercetin xylosyl-glucoside and quercetin aglycone. Differently from other cases (LIN *et al.*, 2008; RANILLA *et al.*, 2007; DÌAZ-BATALLA *et al.*, 2006; APARICIO-FERNANDEZ *et al.*, 2005; HEMPEL and BÖHM, 1996) where kaempferol and quercetin derivatives were found in comparable amounts, kaempferol derivatives were the most representative flavonols in the seed coat of Sarconi beans and in other Italian landraces they have only been found previously in Zolfino beans. This occurrence could distinguish Italian beans from Brazilian, Peruvian, and Mexican beans.

Anthocyans were found only in the seeds and seed coat of the red ecotype (San Michele Rosso). Fig. 2 reports the HPLC/DAD at 520 nm and TIC profiles of the extracted ion chromatograms for cyanidin (*m/z* 287) and pelargonidin (*m/z* 271). Cyanidin 3,5-*O*-diglucoside, pelargonidin 3,5-*O*-diglucoside, cyanidin 3-*O*-glucoside and pelargonidin 3-*O*-glucoside were found as previously reported (MACZ-POP *et al.*, 2006). Cyanidin 3-*O*-(6"-*O*-malonylglucoside) and pelargonidin 3-*O*-(6"-*O*-malonylglucoside) were detected and their presence was confirmed by fragmentation profiles (Fig. 3) in accordance with previous reports regarding red Mexican beans (LIN *et al.*, 2008). Cyanidin diglycoside and a pelargonidin derivative were also found in the extracts. Delphinidin, which is the most abundant aglycone in Mexican

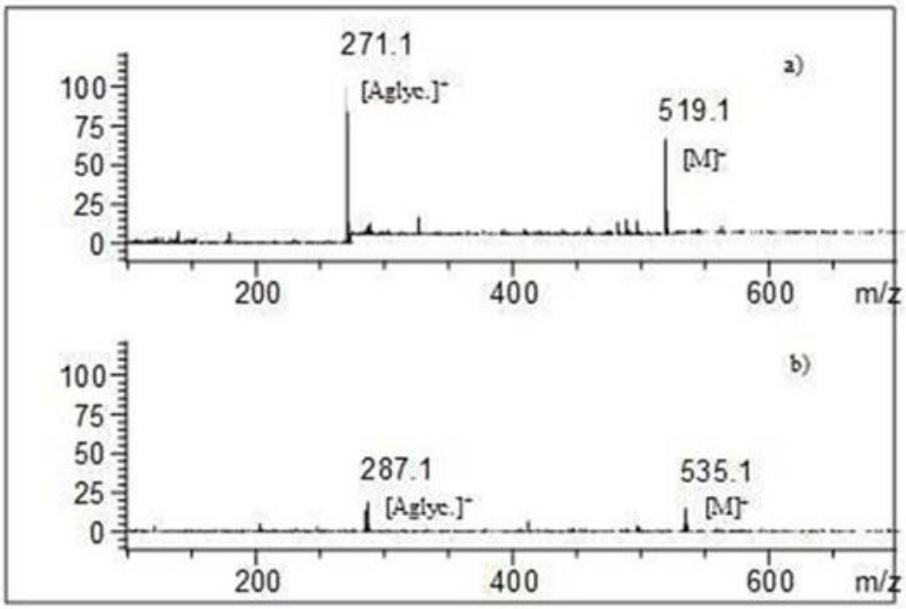

Fig. 3 - Positive ion mass spectrum acquired by API-electrospray of the San Michele seed coat extract. a) cyanidin 3-*O*-(6"-*O*-malonylglucoside) and b) pelargonidin 3-*O*-(6"-*O*-malonylglucoside).

and in Idaho (USA) black beans (APARICIO-FERNANDEZ *et al.*, 2005; ESPINOSA-ALONSO *et al.*, 2006; TAKEOKA *et al.*, 1997) was not found in our extracts. A quite similar pattern to that found in Sarconi beans has been reported for Singapore beans (MADHUJITH *et al.*, 2004), even if in this case a hydrolysis process was performed, only cyanidin and pelargonidin were found.

Fig. 4 shows the complex chromatographic profile at 330 nm of hydroxycinnamic acid derivatives of Riso Bianco. Caffeic (one compound), p-coumaric (two compounds) and ferulic (seven compounds) acid derivatives were identified. This pattern is different from those noted by other authors (LIN *et al.*, 2008; RANILLA *et al.*, 2007; DÌAZ-BATALLA *et al.*, 2006; ESPINOSA-ALONSO *et al.*, 2006; MADHUJITH *et al.*, 2006).

Quantitative data are reported in Tables 2 and 3. Table 2 refers to the seed coats of the samples: Tabacchino contained the highest amount both of flavonols and hydrxycinnamic acids, while only San Michele contained anthocyans. On the other hand, Riso Bianco contained only hydroxycinnamic acids. Marked differences were not noted between samples from the two farms. The anthocyan content was higher than that found in Korean kidney bean seed coats (CHOUNG *et al.*, 2003).

Table 3 lists the quantitative data for whole seed analysis. These data confirm the previous ones (Table 2) showing that polyphenols are present almost exclusively in seed coats and their amount is lower when the whole seed is analyzed. It should be noted that the anthocyan amount is of the same order of magnitude of black coloured genotypes determined using a spectrophotometric method and expressed as cyaniding-3-glucoside (AKOND *et al.*, 2011).

Table 3 also reports the EC_{50} data, i.e. the amount of whole bean which reduced to one half the activity of 1 mg DPPH. If the datum for San Michele beans is discharged, a very good correlation ($R^2 = 0.8952$) between polyphenol amount and EC_{50} value can be observed.

It emerges from Table 3 that the San Michele variety has the lowest EC_{50} value, i.e. the highest antiradical activity. This finding must be ascribed to the presence of anthocyans which contribute to total antiradical activity. A similar result was obtained by AKOND *et al.* (2011):

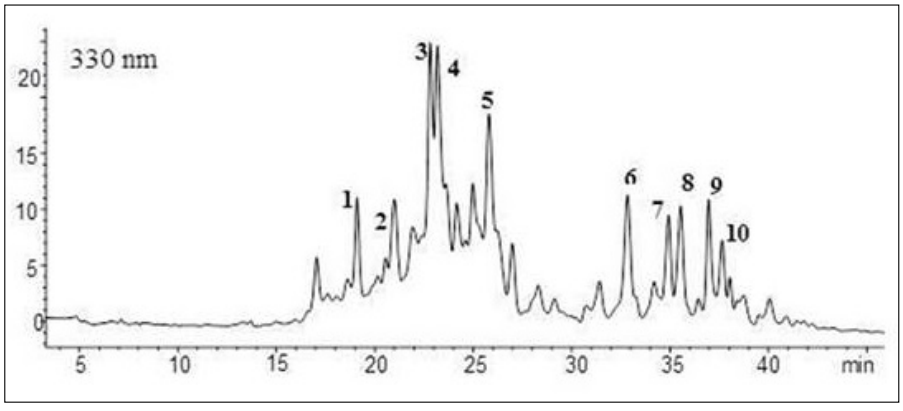

Fig. 4 - HPLC/DAD (330 nm) chromatographic profile of Riso Bianco extract. 1) Caffeic acid derivative; 2) and 8) p-cumaroyl derivatives; 3), 4), 5), 6), 7), 9) and 10) ferulic acid derivatives.

Table 2 - Seed coats polyphenols of the four varieties of *Phaseolus vulgaris* L. grown in two different locations (Table 1). The data are the mean values of three determination (standard deviation < 3%) and are expressed as mg/g (fresh weight).

	San Michele		Tabacchino		Riso Bianco		Verdolino	
Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
K 3-O-xyl.gluc.			0.494	0.589			1.147	1.230
K 3-O-gluc.	0.029	0.019	2.667	3.472			2.437	2.614
K 3-O-(6"-O-mal.gluc.)			1.293	1.172			0.602	0.646
K	0.007	0.01	1.888	1.282			0.342	0.306
Q xyl.gluc.							trace	trace
Q							trace	trace
Total flavonols	**0.036**	**0.029**	**6.342**	**6.515**			**4.528**	**4.796**
C diglyc.	0.385	0.214						
C 3,5-O-digluc.	0.406							
P	0.178	0.108						
C 3-O-gluc	0.750	0.333						
P 3-O-gluc.	1.354	0.414						
C 3-O-(6"-O-mal.gluc.)	0.042	0.027						
P 3-O-(6"-O-mal.gluc.)	0.051	0.018						
P derivative	0.021	0.004						
Total anthocyans	**3.187**	**1.118**						
Total hydroxy acids	0.919	0.579	1.136	1.636	1.292	0.975	0.587	0.626

K = kaempferol; Q = quercetin; C = cyaniding; P = pelargonidin.

Table 3 - Whole seed polyphenols and antiradical activity of the four varieties of *Phaseolus vulgaris* L. grown in two different locations (see Table 1).

	San Michele		Tabacchino		Riso Bianco		Verdolino	
Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
K 3-O-xyl.gluc.			0.061	0.073			0.138	0.213
K 3-O-gluc.	0.012	0.004	0.294	0.439			0.305	0.292
K 3-O-(6"-O-mal.gluc.)			0.134	0.136			0.087	0.085
K	0.002	0.002	0.238	0.155			0.046	0.027
Q xyl.gluc.							trace	trace
Q							trace	trace
Total flavonols	**0.014**	**0.006**	**0.727**	**0.803**			**0.576**	**0.617**
C diglyc.	0.059	0.065						
C 3,5-O-digluc.	0.039	0.036						
P	0.028	0.085						
C 3-O-gluc	0.065	0.044						
P 3-O-gluc.	0.116	0.051						
C 3-O-(6"-O-mal.gluc.)	0.008	0.006						
P 3-O-(6"-O-mal.gluc.)	0.004	0.003						
P derivative	0.001	0.001						
Total anthocyans	**0.320**	**0.291**						
Total hydroxy acids	**0.252**	**0.208**	**0.409**	**0.360**	**0.303**	**0.407**	**0.328**	**0.279**
EC50	**2.78**		**4.36**		**16.93**		**10.40**	

The data are the mean values of three determination (standard deviation < 3%) and are expressed as mg/g (fresh weight). EC_{50} values expressed as g sample/mg DPPH.
K = kaempferol; Q = quercetin; C = cyaniding; P = pelargonidin.

for coats containing anthocyans the correlation was better between antioxidant capacity and anthocyanin levels rather than total phenolics. A good correlation ($r^2 = 0.88$) between antioxidant capacity and total phenolic content has already been found in the case of Brazilian and Peruvian ecotypes (RANILLA *et al.*, 2007). In the present work, differences were noted between the samples of the two farms only in the case of seed coats, especially for anthocyan content of Tabacchino seed coats. In fact samples from farm 1 exhibited a much higher anthocyan content than

those from farm 2 and qualitatively the samples differ for cyanidin 3,5-*O*-diglucoside, which was present only in the samples from farm 1.

To conclude, the presence of kaempferol derivatives may be a possible marker of Italian landraces, while samples of the two farms may be discriminated on the basis of anthocyan content (from a quantitative standpoint) and the presence of cyanidin 3,5-*O*-diglucoside in the samples of only one farm in the case of the most pigmented seed. Furthermore, the polyphenol content of IGP food may become an important tool to characterize and increase the commercial value of niche products such as Sarconi beans, allowing consumers to chose food that contains these classes of nutraceutical compounds with antioxidant activity and chemoprotective properties.

Characterization and valorization of typical biodiversity is also in agreement with the interests and economic incentives of the main European programs.

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REFERENCES

Akond G.M., Khandaker L., Berthold J., Gates L., Peters K., Delong H. and Hossain K. (2011). Anthocyanin, total polyphenols and antioxidant activity of common bean. American Journal of Food technology, 6, 385-394.

Aparicio-Fernandez X., Yousef G., Loarca-Pina G., De Mejia E. and Lila M.A. (2005). Characterization and polyphenolics in the seed coat of black Jamapa bean (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry, 53, 4615-4622.

Bazzano L.A., He J., Ogden L.G., Loria C., Vapputuri S., Myers L. and Whelton P.K. (2001) Legume consumption and risk of coronary disease in US men and women: NHANES I epidemiologic follow-up study. Archives for Internal Medicine, 161, 2573-2578.

Brand-Williams W. and Cuvelier M.E. (1995). Use of a free radical method to evaluate the antioxidant activity. Lebensmittel- Wissenschaft Technologie, 28, 25-30.

Campos-Vega R., Guevara-Gonzalez R.G., Guevara-Olvera B.L., Oomah B. and Loarca Pina G. (2010). Bean (*Phaseolus vulgaris* L.) polysaccharides modulate gene expression in human colon cancer cells (HT-29). Food Research International, 43, 1057-1064.

Cardador-Martinez A., Loarca-Pina G. and Oomah B.D. (2002). Antioxidant Activity in Common Beans (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry, 50, 6975-6980.

Choung M.G., Choi B.R., An Y.N., Chu Y.H. and Cho Y.S. (2003). Anthocyanin profile of Korean cultivated kidney bean (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry, 51, 7040-7043.

Cui X., Dai Q., Tseng M., Shu X.-O., Gao Y.-T- and Zheng W. (2007) Dietary patterns and breast cancer risk in the Shanghai breast cancer study. Cancer Epidemiology, Biomarkers & Prevention, 16, 1443-1448.

Dewanto V., Wu X., Adom K.K. and Liu R.H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry, 50, 3010-3014.

Dìaz-Batalla L., Widholm J.M., Fahey Jr G.C., Castano-Tostado E. and Pardez-Lòpez O. (2006). Chemical composition with health implications in wild and cultivated Mexican common beans seeds (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry, 54, 2045-2052.

Dinelli G., Bonetti A., Minelli M., Marotti I., Catizone P. and Mazzanti A. (2006) Content of flavonols in Italian bean (*Phaseolus vulgaris* L.) ecotypes. Food Chemistry, 99, 105-114.

Espinosa-Alonso L.G., Lygin A., Widholm J.M., Valverde M.E. and Parede-Lopez O. (2006). Polyphenols in wild and weedy Mexican common beans (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry, 54, 4436-4444.

Heim K.E., Tagliaferro A.R. and Bobilya D.J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. Journal of Nutritional Biochemistry, 13, 572-584.

Hempel J. and Böhm H. (1996). Quality and quantity of prevailing flavonoid glycosides of yellow and green French beans (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry, 44, 2114-2116.

Limongelli G., Laghetti G., Perrino P. and Piergiovanni A.R. (1996). Variation of seed storage proteins in landraces of common bean (*Phaseolus vulgaris* L.) from Basilicata, Southern Italy. Euphytica, 92, 393-399.

Lin L.-Z., Harnly J.M., Pastor-Corrales M.S. and Luthria D.L. (2008). The polyphenolic profiles of common beans (*Phaseolus vulgaris* L.). Food Chemistry, 107, 399-410.

Macz-Pop G.A., Rivas-Gonzalo J.C., Perez-Alonso J.J. and González-Paramás A.M. (2006). Natural occurrence of free anthocyanin aglycones in beans (*Phaseolus vulgaris* L.). Food Chemistry, 94, 448-456.

Madhujith T., Amarowicz R. and Shadidi F. (2004). Phenolic antioxidants in beans and their effects on inhibition of radical-induced DNA damage. Journal of the American Oil Chemists Society, 81, 691-696.

Marles M.A.S., Balasubramanian P. and Bett K.E. (2010). Differential accumulation of polyphenolics in black bean genotypes grown in four environment. Journal of Agricultural and Food Chemistry, 58, 7001-7006.

Masi P., Figliuolo G. and Spagnoletti Zeuli P.L. (1999). Landraces of bean (*Phaseolus vulgaris* L.) collected in Basilicata, Italy. Plant Genetic resources Newsletter, 119, 51-55.

Nasi A., Picariello G. and Ferranti P. (2009). Proteomic approach to study structure, functions and toxicity of legume seed lectins. Perspectives for the assessment of food quality and safe. Journal of Proteomics, 72, 527-538.

Negri V. and Tosti N. (2002). Phaseolus genetic diversity maintained on-farm in central Italy. Genetic Resources and Crop Evolution, 49, 511-520.

Ocho-Anin Atchibri A.L., Brou K.D., Kouakou T.H., Kouadio Y.J. and Gnakri D. (2010). Screening for antidiabetic activity and phytochemical constituents of common bean (*Phaseolus vulgaris* L.) seeds. Journal of Medicinal Plant Research, 4, 1757-1761.

Piergiovanni A.R., Taranto G., Losavio P.D. and Pignone D. (2006). Common bean (*Phaseolus vulgaris* L.) landraces from Abruzzo and Lazio regions (Central Italy). Genetic Resources and Crop Evolution, 53, 313-322.

Ranilla L.G., Genovese M.I. and Lajolo F.M. (2007). Polyphenols and antioxidant capacity of seed coat and cotyledon from Brazilian and Peruvian bean cultivars (*Phaseolus vulgaris* L.). Journal of Agricultural and Food Chemistry, 55, 90-98.

Romani A., Vignolini P., Galardi C., Mulinacci N., Benedettelli S. and Heimler D. (2004). Germplasm characterization of Zolfino landraces (*Phaseolus vulgaris* L.) by flavonoid content. Journal of Agricultural and Food Chemistry, 52, 3838-3842.

Singleton V.L., Orthofer R. and Lamuela-Raventos R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of the Folin-Ciocalteu reagent. Methods in Enzymology, 299, 152-178.

Takeoka G.R., Dao L.T., Full G.H., Wong R.Y., Harden L.A., Edwards R.H. and Berrios J.D.J. (1997). Characterization of black beans (*Phaseolus vulgaris* L.) anthocyanins. Journal of Agricultural and Food Chemistry, 45, 3995-3400.

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PAPER

VOLATILE FINGERPRINT AND PHYSICO-MECHANICAL PROPERTIES OF 'MUSCAT BLANC' GRAPES GROWN IN MOUNTAIN AREA: A FIRST EVIDENCE OF THE INFLUENCE OF WATER REGIMES

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ABSTRACT

The volatile composition of aromatic grape varieties at harvest is a very important criterion in the choice of vinification technique to yield the optimal quality of the final product. The berry mechanical characteristics are important for assessing resistance to fungal attacks and for the estimation of shattering. In this study the effect of irrigation on the volatile fingerprint and the mechanical properties of the *Muscat blanc* (*Vitis vinifera* L.) grapes grown in mountain north-west region of Italy was investigated. Three water regimes were compared: standard irrigation, moderate irrigation and drought.

In the meteorological conditions of the considered season, a significant increase in the amounts of the most representative free volatile components of the *Muscat blanc* variety (linalool and geraniol markers), was observed in standard irrigation treatment. Significantly higher amounts of four C13-norisoprenoid bound compounds were observed in the drought treatment with respect to the standard treatment. Furthermore, no influence of irrigation treatment on berry skin hardness and thickness parameters was noted.

Therefore, in the considered alpine environment, on aromatic *Muscat blanc* variety, the optimum irrigation treatment is an important choose to improve the quality of the grapes.

- Keywords: *Muscat blanc*, volatile components, glycosides, skin hardness, water stress -

INTRODUCTION

Vitis vinifera L. *Muscat blanc* grape variety (synonym of white *Frontignan* and *Muscat* à petit grains blanc), a widespread aromatic grape variety, has been grown since the Middle Ages in the Aosta Valley, a mountain region located in the North-West of Italy. Its cultivation area is mostly concentrated in the South-facing, well exposed mountain slopes around the village of Chambave, at an altitude ranging from 500 to 700 m above sea level. Here it is used for producing the renowned dry wine sold under the Appellation of Origin Vallée d'Aoste - *Chambave Muscat*. The climatic conditions of the area are favourable for wine grape growing: low levels of average annual rainfall (less than 600 mm, even less than 400 mm during particularly dry years, with two seasonal peaks in spring and autumn), low relative humidity, low cloudiness and very high levels of solar radiation. The estimated annual mean temperature is 10°-11°C (MERCALLI, 2003).

Plant water status can greatly affect, either positively or negatively, vegetative growth, berry size, production yield, grape phenolic content and profile, grape aromatic potential, must composition, and wine sensory characteristics depending on its intensity level and timing (HEPNER *et al.*, 1985; MATTHEWS *et al.*, 1990; OJEDA *et al.*, 2001; DELOIRE *et al.*, 2004; CHAPMAN *et al.*, 2005; KOUNDOURAS *et al.*, 2006; QIAN *et al.*, 2009; VAN LEEUWEN *et al.*, 2009; GAMBACORTA *et al.*, 2011). Therefore, in environments where low rainfall is a limiting factor, irrigation practices may be a powerful tool for improving grape quality by modifying berry characteristics and composition; this can often be achieved by inducing moderate levels of water stress in specific stages of berry development and ripening. On the other hand, an excess of irrigation may easily result in poor grape quality (GLADSTONE, 1992). Thus, the assessment of the correct amount of irrigation and its scheduling is essential in order to reach a desired production target.

Volatile aroma compounds of the *Muscat blanc* have been less studied (USSEGLIO-TOMMASET and DI STEFANO, 1980; BUREAU *et al.*, 2000) than other aromatic *Muscat* varieties (RIBEREAU-GAYON *et al.*, 1975; GUNATA *et al.*, 1985; WIRTH *et al.*, 2001) and often many studies treating the aroma profile of the *Muscat* variety were related to aroma wines produced (SANCHEZ PALOMO *et al.*, 2007; del CARO *et al.*, 2012). The terpene content in the grape may be influenced by temperature, light and water availability during ripening (RIBEREAU-GAYON *et al.*, 2000b), thus a knowledge of free and bound precursors of this aromatic variety may help in the selection of best vineyard practices. The effect of the bioclimatic and agronomical conditions, in particular the water regimes, on the aromatic composition of white aromatic berries has not yet been deeply investigated, although the aromatic profile of the 'Muscat' grapes should be considered at least as important as the usual technological ripeness parameters (i.e. sugars content and acidic composition).

Texture Analysis is a analytical technique of survey used for measurement of the physical properties of wine grapes in order to assess their quality (ROLLE *et al.*, 2012). In fact, instrumental texture parameters can be used as phenols extractable markers and ripeness predictors (MAURY *et al.*, 2011; RÍO SEGADE *et al.*, 2011a). Knowing the characteristics of some mechanical parameters such as the ease of detachment of the pedicel, the hardness and thickness of the skin, is important because it is directly related to the phenomena of shattering, resistance to splitting and plant diseases (LANG and DURING, 1990; GABLER *et al.*, 2003), in particular during on-vine withering process (ROLLE *et al.*, 2010). At our knowledge, the effect of irrigation on the physico-mechanical characteristics of the berries has not yet been reported.

During the last ten years the irrigation, normally carried out by drip irrigation systems, has become increasingly popular among the grape growers of the Aosta Valley area. While it is considered a special measure to be adopted only when exceptionally dry conditions occur, it is now often become standard viticultural practice in Aosta Valley.

On the base of all these consideration, the aim of this work was to study the free and bound varietal volatiles and mechanical properties of berries of the *Vitis vinifera* L. grape aromatic variety *Muscat blanc* comparing the effect of three different water regimes on these physico-chemical parameters.

MATERIALS AND METHODS

Plant materials

The experimental site was located on a steep slope with a south aspect, at an altitude of 600 m above sea level, on a loamy sand soil. The vines (*Vitis vinifera* L., *cv Muscat blanc*, clone R6, grafted on 110 Richter rootstock) were 12 years old. The trellis system was Guyot. The vine and row spacing was 0.70 and 1.80 m respectively. The vineyard was equipped with a drip irrigation system using pressure-compensated emitters. The distance between emitters was 60 cm; their flow rate was 2.3 L h^{-1}.

Grapes were collected from the 2008 vintage season. A Randomized Complete Block Design with three replicates was adopted. Plots had three rows each of about 15 m long; only the central row was used for experimental purposes; the other two received the same water treatment and were used as border rows. On the cen-

tral row eight plants were selected and used for sampling berries.

Water treatments

Three water regimes were compared: standard irrigation (S), moderate irrigation (M), drought (D). Treatment D did not receive any irrigation, while S and M irrigation strategies were based primarily on pre-dawn water potentials (Ψ_{PD}). Treatment S was kept at Ψ_{PD} levels above -0.2 MPa (absence of water stress) until two weeks before harvest. Treatment M was kept above -0.2 MPa until veraison; after veraison a moderate water stress (-0.2 to -0.4 MPa) was allowed. No irrigation treatment was carried out during the last two weeks before harvest. Before veraison, even in the absence of water stress, treatments S and M received 50 and 25% of the estimated crop evapotranspiration (ET_c) respectively, at approximately 10 day intervals. ET_c was estimated from meteorological data logged by a weather station (Vantage Pro, Davis Instruments Corp., Hayward, California 94545 USA) situated in the experimental site, by multiplying the reference evapotranspiration (ET_0, directly calculated by the station) by a crop coefficient K_c (varying from 0.6 to 0.7 from the start of treatments to veraison).

Plant water status measurements

Plant water status was monitored by measuring the pre-dawn water potential Ψ_{PD} with a SKYE pressure chamber, model SKPM 1400 (SKYE Instruments, Llandrindod Wells, Powys LD1 6DF UK). Ten measurements were made from fruit set to harvest. For each plot, the Ψ_{PD} was estimated by measuring six fully expanded leaves taken from the primary shoots. Measurements took about two hours and terminated before dawn.

Cluster thinning

The crop load of the eight selected plants per plot was balanced by cluster thinning at veraison, aiming at a yield of 1.6-1.8 kg/vine, which is generally considered optimal for the usual local requirements, given the plant density of the experimental vineyard.

Technological parameters analysis

The total soluble solids concentration (°Brix, as SSC) was measured using an Atago 0-32 °Brix temperature compensating refractometer (Atago Co., Tokyo, Japan), pH was determined by potentiometry using a Crison electrode (Carpi, Italy) on the grape must. Titratable acidity (TA), expressed as gL^{-1} tartaric acid, was estimated using the official OIV method. The must contents of citric, tartaric and malic acid were analyzed using an HPLC system (P100-AS3000, Thermo Electron Corporation, Waltham, MA, USA) equipped with a UV detector (UV3000) set to 210 nm. The analyses were performed isocratically at 0.8 $mLmin^{-1}$ flow and 65°C column temperature, with a 300x7.8 mm i.d. Aminex HPX-87H cation exchange column and a Cation H^+ Microguard cartridge (Bio-Rad Laboratories, Hercules, CA, USA), using 0.0013 mol/L H_2SO_4 as mobile phase (GIORDANO *et al.*, 2009). Data treatment was carried out using the ChromQuest™ chromatography data system (ThermoQuest, Inc, San Jose, CA, USA).

Volatile analysis

Sample preparation

Analysis of free and glycosidically bound components was carried out according to the previous method proposed by DI STEFANO (1991), with some modifications. For each plot, two subsamples of 100 berries were taken and analysed separately. They were de-seeded and the pulp (about 200 g) was separated from the skin with the addition of $Na_2S_2O_5$ (80 mg). Skins were placed in 20 mL of methanol for 1 h to inactivate glycosidase enzymes. The pulps and skins were then separately crushed under a nitrogen atmosphere with a laboratory blender (Waring Laboratory, Torrington, USA). The skin suspension and pulp homogenate were then combined. The mixture was centrifuged twice (7,000 g; 10 min; 4°C), washing the solid pellet with tartaric acid buffer (pH=3.2) and the liquid extract was then clarified with pectolytic enzyme (100 mg) without secondary glycosidase activity (Rapidase X-Press, DSM, The Netherlands) at room temperature for 2 hours. Aliquots of 500 mL were collected from this juice. Afterwards, each replicate of the grape juice (100 mL) (n=2), added of 2-octanol as internal standard (200 µL of 41 mg L^{-1} solution in 10% ethanol), was loaded onto a 1g tC18 reversed-phase SPE cartridge (Sep-Pak, Waters, Ireland), previously activated with 10 mL of methanol and 20 mL of water, with a flow rate of ca. 3 mL min^{-1}. The cartridge was then rinsed with 30 mL of pure water to eliminate sugars, acids and other low molecular weight polar compounds. The free fraction was then eluted with 30 mL of dichloromethane. The eluate was dried over Na_2SO_4 and concentrated to 200 µL under a stream of nitrogen. The glycoconjugates were finally eluted from the cartridge with 50 mL of methanol and concentrated to dryness using a vacuum rotavapor (Buchi R-210, Switzerland) at 35°C. The dried glycosidic extract was dissolved in 3 mL of citrate-phosphate buffer (0.2 M, pH=5). The enzymatic hydrolysis was carried out using 50 mg of an AR-2000 commercial preparation with glycosidase side activities (DSM Oenology, The Netherlands) and incubating at 40°C for 24 h. After

addition of 200 µL of 2-octanol (41 mg L^{-1} solution in 10% ethanol), glycosidic precursors were then extracted following the SPE method previously described. The dichloromethane extract obtained was dried over anhydrous Na_2SO_4, concentrated to 200 µL under nitrogen and kept at -20°C until analysed. All analyses were performed in duplicate.

GC/MS analysis

Analysis was performed with a Shimadzu GC-2010 gas chromatograph equipped with a Shimadzu QP-2010 Plus quadrupole mass spectrometer. The gas chromatograph was equipped with a DB-WAX capillary column (30 m x 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific Inc., Folsom, CA, USA). The temperature program started at 35°C for 5 min; 2°C min^{-1} to 190°C; 3°C min^{-1} to 230°C for 5 min. Carrier gas (He) was at 1mL min^{-1}. Injections of 1 µL were performed in split mode 1:20. The injection port temperature was 230°C, the ion source temperature was 240°C and the interface temperature was 230°C (solvent delay of 4.5 min). The detection was carried out by electron impact mass spectrometry (70 eV) in total ion current (TIC) mode in a mass range *m/z* 30-350. Identification of compounds was carried out by comparing their mass spectra and retention indices (RI) (a mixture of a homologous series of C5-C28 was used) with those of standard compounds or by comparing their mass spectra and Kovats with those reported in literature or by comparing mass spectrum with mass spectral databases NIST12, NIST62 (National Institute of Standards and Technology, Gaithersburg, MD, USA), Adams and on line (http://webbook.nist.gov/chemistry/). Semiquantitative data ($\mu g L^{-1}$) were obtained by measuring the relative peak area of each identified compound in relation to that of the added internal standard.

Mechanical properties

A Universal Testing Machine (UTM) TAxT2i Texture Analyzer (Stable Micro Systems - SMS, Surrey, UK) equipped with a HDP/90 platform (perforated or not) and a 5 kg load cell was used. All the acquisitions were made at 400 Hz; data were evaluated using the Texture Expert Exceed software package (vers. 2.54 in Windows 2000). The operating conditions applied, probe and mechanical parameters measured, are summarized in Table 1, according to methods proposed by LETAIEF *et al.* (2008). Skin hardness was evaluated using the puncture test. The berries (n=30, MAURY *et al.*, 2009) were placed on the metal plate of the UTM with the pedicel in a horizontal plane in order to be consistently punctured in the lateral face. For the measurement of berry skin thickness, a piece of skin of almost 0.25 cm^2 was removed from the lateral side of all the 30 berries of each sample with a razor blade. Especial care was taken when removing the pulp from the skin and when positioning the skin sample on the UTM platform to prevent folds in the skin. After calibration of the instrumental probe position, the skin thickness was calculated as the distance between the point corresponding to the probe contact with the berry skin (trigger) and the platform base during a compression test. It was convenient to insert an instrumental trigger threshold equal to 0.05 N that enabled the plane surface of the probe to adhere completely to the skin sample before the acquisition started. It allowed a reduction or elimination of the 'tail' effect due to the postponement of the point. Peduncle detachment resistance was determined by a traction test (ROLLE *et al.*, 2009). In this test the peduncle was anchored to the pliers of the A/PS probe. During the traction, the peduncle passes through the perforated platform of the UTM (diameter of the hole 5mm), while the berry is blocked, permitting the determination of the force of peduncle detachment.

Statistical analysis

Statistical analyses were performed using the statistical software package SPSS (version 17.0; SPSS Inc., Chicago, IL, USA).

Table 1 - Operating conditions used in the texture analysis of grapes.

Test	Probe - Platform	Test speed	Force	Mechanical properties
Berry skin hardness	SMS P/2N Needle; HDP/90 platform	1 mm s^{-1}	compression 3 mm	F_{sk} = Berry skin break Force (N) W_{sk} = Berry skin break Energy (mJ) E_{sk} = Skin Young's modulus (N/mm)
Berry skin thickness	SMS P/2 Ø 2mm; HDP/90 platform	0,2 mm s^{-1}	-	Sp_{sk} = Berry skin thickness (µm)
Peduncle detachment resistance	SMS A/PS modified with rigid arm; HDP/90 perforated (Ø 5mm) platform	1 mm s^{-1}	traction 10 mm	F_{ped} = Peduncle detachment Force (N) W_{ped} = Peduncle detachment Energy (mJ)

RESULTS AND DISCUSSION

Meteorological conditions

The season was characterised by an unusually high amount of rainfall during spring and early summer followed by low precipitation levels until harvest. Total precipitation between flowering to veraison was 160 mm; only 32 mm were registered from veraison to harvest. Before veraison the pre-dawn leaf water potentials ψ_{PD} of all three treatments remained at relatively high levels (within the 0 to -0.2 MPa range), showing an absence of water stress even in the non-irrigated plots. After veraison, the ψ_{PD} of moderately irrigated and non-irrigated vines began to slowly drop, showing an effective differentiation of the relative water status. Only the D treatment reached pre-dawn ψ_{PD} below -0.6 MPa, which can be considered severe water stress threshold, but only in the last days before complete ripening, when these levels are not unusual. No statistically significant differences in ψ_{PD} were observed among treatments before 54 DAF (days after flowering). From 54 DAF until harvest treatment S showed significantly higher ψ_{PD} values than treatment D. Treatment M showed intermediate ψ_{PD}, values, often comparable or closer to D for most of the veraison-harvest period.

The S treatment received a total of 110 mm of additional water by drip irrigation distributed in ten applications from day 8 AF to day 90 AF; M treatment received 34 mm of additional water in five applications from day 8 to 68 AF. Differences in yield were not statistically significant; yields/vine ratios ranged from 1.67 kg (treatment D) to 1.82 (treatment M). Harvest date was 108 DAF.

Technological parameters of ripeness

Sugar content, pH, acid composition, yield and berry weight of grapes from the three different water treatments exhibited no significant statistical differences. At harvest, on average, the grape musts were characterized by soluble solid contents of 23.4 ± 0.6 as °Brix, titratable acidity expressed as tartaric acid of 7.53 ± 0.52 gL^{-1} and by a pH of 3.36 ± 0.05. The contents of the main organic acids were 0.26±0.04, 4.58 ±0.16, 3.90 ±0.51 for citric, tartaric and malic acid, respectively.

Free volatile compounds

A total of 33 free volatile compounds were detected (mean values of concentrations ± standard deviations) in *Muscat blanc* grape juices. They were classified into three chemical categories: four C6 compounds (alcohols and aldehydes), 27 monoterpenoids and two aromatic alcohols (Table 2). All C6 compounds and 18 of 27 monoterpenoids were always observed in all treatments.

Among monoterpenoids, free linalool was the most abundant compound found in all water regimes. Other components, that contribute with more than 5% to the terpenoids content, were geraniol, 3,7-dimethyl-1,5-octadien-3,7-diol (diendiol I) and 3,7-dimethyl-1,7-octadien-3,6-diol (diendiol II). Linalool, that provides characteristic sweet and flowery notes, together with geraniol, α-terpineol and ho-trienol are considered the marker compounds for the terpene-like character of Muscat wines (RIBEREAU-GAYON *et al.*, 1975; MARAIS, 1983). The content of linalool in *Muscat blanc* grape was found at similar average levels by other authors (BUREAU *et al.*, 2000; SANCHEZ-PALOMO *et al.*, 2006; LAMBRI *et al.*, 2012). Another terpene alcohol, geraniol, was found at high content in all treatments, above all in S treatment. This component is known as a floral, rose-like character in berries (LUND and BOHLMANN, 2006). Nerol was not found in any treatments in the free form, but at high concentration as bound-glycosidic precursor. Citronellol was present at low amount only in the S treatment, at amount similar to that of Muscat "a petit grains" grapes (SÁNCHEZ PALOMO *et al.*, 2007). Among polyoxygenated terpenes, diendiol I is predominant in grape juices, followed by diendiol II. Even if diendiol I is not considered to contribute directly to *Muscat* aroma due to its low sensory relevance, it is a precursor of monoterpenol odorants such as hotrienol and nerol oxide in wines (WILLIAMS *et al.*, 1980). Among C6 compounds, the major compounds of the *Muscat blanc* grape were the C6 alcohols in particular 1-hexanol and (E)-2-hexen-1-ol, known as responsible for the green and herbaceous aromas of grape and wines (GOMEZ *et al.*, 1995). A predominance of C6 alcohols and aldehydes has also been reported in musts of Semillon grapes, that presented a herbaceous note, when influenced by an excess of water (URETA and YAVAR, 1982).

Generally, the standard irrigation treatment seemed to increase the level of free and bound volatile compounds compared to the other treatments. In total, the content of five free varietal monoterpenoids (linalool, citronellyl formate, geraniol, (*Z*)-8-hydroxy-linalool and geranic acid), 2 C6-compounds (1-hexanol and (*E*)-2-hexen-1-ol) and three C13-norisoprenoids were significantly higher in the S treatment compared to the other water regimes. In fact linalool increased about 1.8 times compared to the D treatment ($P<0.05$ with ANOVA). Linalool represented about 28% of free monoterpenoids in the S treatment, about 29% in the M treatment and a low content in the drought regime (about 23%). The geraniol level in the S treatment was almost 2 times higher than in other treatments, accounting for 19% of all monoterpenoids ($P<0.05$ with ANOVA). Thus, the significant major contribution of linalool and geraniol, both the most distinctive Muscat-like varietal compounds, observed in the standard

Table 2 - Free volatile compounds (μgL^{-1})* in mountain *Muscat* grapes influenced by different irrigation treatments.

Compounds*	LRI$_{calc}$§ (LRI$_{lit}$@)	ID⊥	Concentration (μgL^{-1}) S	M	D	P ξ
C6 compounds						
(*E*)-2-Hexenal	1201 (1192)	I	4.0±0.6	6.3±8.5	2.8±1.6	NS
1-Hexanol	1352 (1356)	I	38.3±5.9^{a}	21±10.2^{b}	18.2±8.7^{b}	0.002
(*Z*)-3-Hexen-1-ol	1380 (1386)	I	6.8±1.9	5.2±2.0	4.0±1.9	NS
(*E*)-2-Hexen-1-ol	1404 (1409)	I	38.0±7.0^{a}	22.7±8.9^{b}	20.3±9.4^{b}	0.005
Total			87.1±15.4^{a}	55.2±29.6^{b}	45.3±21.6^{b}	0.006
Monoterpenoids						
β-Myrcene	1154 (1145)	I	2.2±2.1	2.8±3	0.7±0.8	NS
α-Phellandrene	1163 (1166)	I	ND	0.3±0.8	ND	NS
Limonene	1181 (1218)	I	3.3±2.4	3.5±4.5	2±0.6	NS
(*Z*)- β-Ocimene	1226 (1225)	II	1.2±1	1±1.7	ND	NS
γ-Terpinene	1229 (1238)	I	ND	0.2±0.4	ND	NS
(*E*)- β-Ocimene	1241 (1250)	II	2.3±1.9	2.5±3.3	ND	0.040
Terpinolene	1266 (1287)	I	1.5±1.6	1.8±2	ND	NS
(E)-Furan linalool oxide	1432 (1437)	I	4.8±2.2	4.2±1.9	2.7±0.8	NS
(Z)-Furan linalool oxide	1460 (1476)	I	6.5±1.9	6.5±2.3	5.3±2	NS
Linalool	1546 (1562)	I	211.2±60^{a}	167.7±41.7^{ab}	118.5±41.2^{b}	0.016
ho-Trienol	1605 (1623)	II	3.8±1.9	6.8±11.4	2.2±1.5	NS
α-Terpineol	1686 (1688)	I	4.2±1.2	3.5±0.5	3.2±0.8	NS
Ethoxy-diol I	1702	III	4.5±1	3±1.3	3±2.1	NS
(E)-Pyran linalool oxide	1728 (1710)	II	44.8±7.6	45±8.5	41.8±10.3	NS
(Z)-Pyran linalool oxide	1757 (1754)	II	4.8±1.2	6.3±2.3	5±1.3	NS
Citronellyl formate	1764	III	11.3±3.3^{a}	5±3.5^{b}	5.5±4^{b}	0.014
β-Citronellol	1765 (1775)	II	1.8±4.5	ND	ND	NS
Geraniol	1844 (1858)	I	142.7±33.2^{a}	75.5±23^{b}	82.5±53.3^{b}	0.016
Diendiol I	1948 (1949)	I	95±22.4	94.8±18.3	97.5±30.5	NS
Endiol	1980 (1986)	II	1.2±1.8	ND	ND	NS
Diendiol II	2126 (2134)	I	68.8±19.5	64.8±14.8	52.5±10.3	NS
Hydroxy citronellol	2205		12.3±2.8	ND	8.7±3	0.001
8-Hydroxy-6,7-dihydrolinalool	2208 (2220)	II	3.8±5.9	5.2±5.7	7.2±6.6	NS
(E)-8-Hydroxylinalool	2269 (2270)	II	18.2±5.5	16.5±5.3	14.5±3.9	NS
(Z)-8-Hydroxylinalool+ hydroxy-geraniol	2308 (2310)	II	35.5±6^{a}	17.7±14.3^{b}	35.8±9.9^{a}	0.014
Geranic acid	2348 (2329)	I	58±14.2^{a}	43.5±10.3^{ab}	34.5±16.5^{b}	0.032
Total			743.7±205.1^{a}	578.1±180.8^{ab}	522.6±199.4^{b}	0.020
Alcohols						
Benzyl alcohol	1862 (1882)	I	4.7±4.3	1.2±2.9	1.8±2.9	NS
2-Phenylethanol	1895 (1929)	I	3.8±2.8	1.7±2	ND	0.014
Total			8.5±7.1	2.9±4.9	1.8±2.9	0.053

Diendiol I: 3,7-Dimethyl-1,5-octadien-3,7-diol; Endiol: 3,7-dimethyl-1-octen-3,7-diol or 2,6-dimethyl-7-octen-2,6-diol; Diendiol II : 3,7-dimethyl-1,7-octadien-3,6-diol ; *(E)*-8-Hydroxylinalool: (*E*)-2,6-dimethyl-2,7-octadien-1,6-diol; Hydroxy citronellol: 3,7-dimethyl-octan-1,7-diol; 8-Hydroxy-dihydrolinalool: 2,6-dimethyl-7-octen-1,6-diol); *(E)*-8-Hydroxylinalool: (*E*)-2,6-dimethyl-2,7-octadien-1,6-diol; hydroxyl-geraniol: 3,7-dimethyl-2-octen-1,7-diol.

*Values are mean ± standard deviation (*n*=6 samples per treatment).

§LRI: Linear retention index on column DB-Wax calculated (@LRI literature).

⊥ID: Compounds identified by comparing I: their mass spectra and retention indices (RI) with those of standard compounds; II: their mass spectra and Kovat's index with those reported in literature; and III: mass spectrum with mass spectral database.

ξ P values are referred to one-way ANOVA: Values of means followed by the same letter are not significantly different (P≤0.05). NS= not significant.

ND, not detected.

irrigation treatment could help to differentiate this treatment from the other water regimes.

Glycosidically-bound compounds

A total of 46 bound volatile compounds were detected and classified into four chemical categories: three C6 compounds (alcohols), 33 monoterpenoids, eight C13-norisoprenoid and two aromatic alcohols (Table 3).

Among the monoterpenoids, high levels of geranic acid (accounting for about 17% of all terpenoids), followed by nerol (~16%), geraniol (~12%), linalool (~11%), (*E*)-8-hydroxy linalool (~11%) and hydroxy-geraniol (~7%), were found in all treatments.

It was observed, in all bound fraction, the presence of a cyclic ether, *cis*-rose oxide, at very low content. This compound is already known as a potent varietal odorant in Scheurebe and

Table 3 - Glycosidically-bound compounds (μgL^{-1}) * in the juice of mountain *Chambave Muscat* grapes influenced by different irrigation treatments.

Compound*	LRI_{calc} § (LRI_{lit} @)	ID⊥	Concentration (μgL^{-1})			P ξ
			S	M	D	
C6 compounds						
1-Hexanol	1352 (1356)	I	18.7±6.8	21.5±3	27.0±11.2	NS
(Z)-3-Hexen-1-ol	1380 (1386)	I	2±2.4	3±2	3.8±1.3	NS
(*E*)-2-Hexen-1-ol	1404 (1409)	I	1.5±1.6	3.3±0.5	3.5±1.8	NS
Total			22.2±10.8	27.8±5.5	34.3±14.3	NS
Monoterpenoids						
β-Myrcene	1154 (1145)	I	18±16.4	8.7±3.8	6.2±1.9	NS
α-Phellandrene	1163 (1166)	I	2.3±4.4	1.5±1	0.3±0.8	NS
Limonene	1181 (1218)	I	15.5±12.1	10.5±3.7	7.3±2.1	NS
β-Phellandrene	1189 (1287)	II	2.3±2.1	0.5±0.8	ND	0.011
(*Z*)-β-Ocimene	1226 (1225)	II	9.7±9.4	4.2±1.5	2.8±1.3	NS
γ-Terpinene	1229 (1238)	I	3.2±3.1	2±0	1±1.1	NS
(*E*)-β-Ocimene	1241 (1241)	II	17.2±15.4	8.3±3.1	5.7±2.2	NS
Terpinolene	1266 (1287)	I	12.5±13.7	4.5±1.9	3.2±0.8	NS
cis-Rose oxide	1339 (1355)	I	3.2±1.2	2.3±1.6	2.3±0.8	NS
(E)-Furan linalool oxide	1432 (1437)	I	68.2±19.7	78.2±31.7	74.3±28.7	NS
(Z)-Furan linalool oxide	1460 (1476)	I	50.8±28.4	41.2±14.4	46.7±21.1	NS
Linalool	1546 (1562)	I	539.8±100.7	640.3±223.2	468.2±161	NS
ho-Trienol	1605 (1623)	II	8.5±5.6	6±3	5.3±1.2	NS
α-Terpineol	1686 (1688)	I	37.5±14.3	35.3±14.5	32.8±14.5	NS
(E)-Pyran linalool oxide	1728 (1710)	II	108.5±26.7	127.8±52.3	132.2±37.9	NS
(Z)-Pyran linalool oxide	1757 (1754)	II	7.7±3.6	9.3±5.4	10.8±5.8	NS
β-Citronellol	1765 (1775)	II	39.8±7.9	38±12.8	39.2±5.4	NS
Nerol	1797 (1811)	I	788.2±187.2	699.2±139.2	690.5±190.9	NS
Geraniol	1844 (1858)	I	588±125.9	607.3±90.5	576.8±166.9	NS
exo-2-Hydroxy-1,8-Cineole	1850 (1860)	II	ND	ND	1.8±2.6	NS
Diendiol I	1948 (1949)	I	235.5±277.9	157±51.7	172.5±43.4	NS
Endiol	1980 (1986)	II	20.7±16.5	16±6.1	17.7±5.5	NS
Diendiol II	2126 (2134)	I	79±57.8	67±24.2	69±13.2	NS
Hydroxy citronellol	2206 (2143)	II	103±98.7	82.3±18.8	100.3±23.9	NS
8-Hydroxy-6,7-dihydrolinalool	2208 (2220)	II	127.2±89.3	96±23.5	115.5±20.7	NS
Hydroxy-nerol	2266	III	36±25.7^{ab}	30.2±5.8^{b}	45±8.1^{a}	0.030
(E)-8-Hydroxylinalool	2269 (2270)	II	565.5±471.8	421.8±122.3	446.5±55.9	NS
(Z)-8-Hydroxylinalool	2277 (2310)	II	16.8±17.7	15.2±3.3	18±1.7	NS
Hydroxy-geraniol	2308 (2310)	II	288.8±182.9	272.7±59	348.2±21.7	NS
Geranic acid	2348 (2329)	I	857.5±192.7	966.7±144.5	969.3±193.3	NS
p-Menth-1-ene-7,8-diol	2501 (2517)	II	295±583.1	48±19.6	50.2±7.2	NS
(Z)-8-Hydroxy-nerol	2582	III	39.3±23.5	30.8±6.2	37.3±8	NS
(E)-8-Hydroxy-nerol	2590 (2613)	II	82.2±62.8	63±17.4	79.8±14.7	NS
Total			5067.4±2698.2	4591.8±1107	4576.7±1064.3	NS
C13-norisoprenoids						
3-Hydroxy-β-damascone	2520 (2537)	I	ND	0.2±0.4	0.7±0.5	0.030
3-Oxo-α-ionol	2606 (2635)	I	54.7±17.4	70.5±22.6	66.3±19	NS
3,4-Dehydro-7,8-dihydro-β-ionone	2623	III	71.5±53.4	53.7±17.3	46.7±10.1	NS
3,9-Dihydroxy-megastigman-5-ene	2629	III	158±95.9	116.8±33.2	119.2±18.5	NS
6,7-Dehydro-7,8-dihydro-3-oxo-α-ionol	2660	III	1.7±4.1^{b}	12.3±4.8^{a}	22.8±14.7^{a}	0.006
3-Hydroxy-7,8-dihydro-β-ionol	2676 (2675)	II	ND	11.3±9.4	18.2±4.1	0.007
Homovanillic alcohol	2889 (2892)	II	ND	8.5±19.4	1±0	0.005
Dihydroconiferyl alcohol	-	III	38±37.7	24.5±18.7	24±10.3	NS
Total			323.9±208.5	297.8±125.8	298.9±77.2	NS
Alcohols						
Benzyl alcohol	1862 (1882)	I	23±6.4	27.3±4	25.7±11.6	NS
2-Phenylethanol	1895 (1929)	I	29.5±4.3	29.7±3.2	28±6.2	NS
Total			52.5±10.7	57±7.2	53.7±17.8	NS

Hydroxy-nerol: 3,7-dimethyl-2-octen-1,7-diol, (*m/z*: 69, 121, 136, 43, 59).

*Values are mean ± standard deviation (*n*=6 six samples per treatment).

§LRI: Linear retention index on column DB-Wax calculated (@LRI literature).

⊥ID: Compounds identified by comparing I: their mass spectra and retention indices (RI) with those of standard compounds; II: their mass spectra and Kovat's index with those reported in literature; and III: mass spectrum with mass spectral database.

ξ P values are referred to one-way ANOVA: Values of means followed by the same letter are not significantly different (P≤0.05). NS= not significant.

ND, not detected.

Gewürztraminer wines, and was even found recently, in the black Muscat Hamburg grape variety (FENOLL *et al.*, 2009). Its stereoselective biosynthesis in grape berry mesocarp has recently been demonstrated (LUAN *et al.*, 2005). Moreover, grapes from drought treatment were characterized by the presence of a low level of 2-*exo*-hydroxy-1,8-cineole too, a component of grape *cv* Sauvignon (BITTEUR *et al.*, 1990).

The different water regimes did not modify the levels of monoterpenoids except for the hydroxynerol that was found at a higher level ($P< 0.05$) in the D treatment, and for β-phellandrene that was not present in D water regimes. In contrast, compared to the S treatment, the drought treatment seemed to increase the levels of four C13-norisoprenoids.

Both positive and negative effects of water stress on the content of free and glycoconjugated aromatic components have been reported in previous investigations. KOUNDOURAS *et al.* (2006) compared three non-irrigated sites widely differing in their water status and found higher levels of bound volatile compounds in wines from stressed locations. However, in this case, the observed differences may also be due to the fact that three different environments were compared. On the other hand, on non-aromatic grape *cv* of Sauvignon blanc, non volatile S-cysteine conjugate precursors were assessed during optimising irrigation, and, according to our results, severe water deficit stress seemed to limit aroma potential (PEYROT DES GACHONS *et al.*, 2005).

Mechanical properties

Table 4 shows the mechanical properties of berry skins. Higher average values of F_{sk} were found in parcels S, although no statistical differences were observed. In this treatment, the berries skins also show a higher springiness (lower value of E_{sk}). No differences were found in skin thickness (Sp_{sk}). The skin of the grape plays a critical role, regulating gas exchange between the berry and the surrounding environment, serving as a protective barrier against fungal disease and protecting the grape from UV light and physical injuries (LANG and DURING, 1990; GABLER *et al.*, 2003). Therefore, skin hardness evaluated by break skin force, is a positive parameter for the grape quality. This characteristic can also be used to characterize the *Muscat blanc* cultivars because this properties is a varietal marker, even if the parameters are strongly conditioned to the climatic course of the year (ROLLE *et al.*, 2011) and growing locations (LE MOIGNE *et al.*, 2008; RÍO SEGADE *et al.*, 2011b; RÍO SEGADE *et al.*, 2011c). In this study on *Muscat blanc* variety, no influence of irrigation treatments on F_{sk} and Sp_{sk} parameters were found. Therefore, this practice of vineyard management would not seem to induce significant changes on the resistance at the splitting and plant diseases.

Instead, in spite of the high dispersion of the data, significant differences in peduncle detachment resistance were present among all three different levels of irrigation. In particular, F_{ped} showed the lowest average values in the treatments S (-0.377N) and M (-0.478) (Table 5). However, these values are higher in comparison with other varieties (ROLLE *et al.*, 2010) and, in fact, no problems of shattering were observed.

The high resistance to shattering is an important property for the Muscat blanc grapes, because of the severe environmental conditions of growing present in mountain areas. In young berries the shatter is caused by the hydrolysis of pectins of the middle lamella of the cell walls

Table 4 - Mechanical properties of berry skin.

	S	M	D	*P*ξ
F_{sk} (N)	0.522±0.122[a]	0.487±0.101[a]	0.492±0.112[a]	NS
W_{sk} (mJ)	0.589±0.195[a]	0.478±0.167[b]	0.479±0.170[b]	0.018
E_{sk} (N/mm)	0.202±0.029[b]	0.229±0.521[a]	0.236±0.050[a]	<0.001
Sp_{sk} (μm)	199±47[a]	196±34[a]	200±47[a]	NS

F_{sk} = Berry skin break force; W_{sk} = Berry skin break energy; E_{sk} = Young's modulus of Skin; Sp_{sk} = Berry skin thickness. Average value ± standard deviation (n=30). ξP values are referred to one-way ANOVA; NS= not significant. Values of means followed by the same letter are not significantly different (P≤0.05).

Table 5 - Peduncle detachment resistance.

	S	M	D	*P*ξ
F_{ped} (N)	2.393±0.592[ab]	2.292±0.550[b]	2.770±0.684[a]	0.024
W_{ped} (mJ)	2.402±1.077[a]	2.157±0.897[a]	2.616±1.104[a]	NS

F_{ped} = Peduncle detachment force; W_{ped} = Peduncle detachment energy. Average value ± standard deviation (n=30). Average value ± standard deviation (n=30). ξP values are referred to one-way ANOVA; NS= not significant. Values of means followed by the same letter are not significantly different (P≤0.05).

forming a separate layer at the base of pedicel (RIBEREAU-GAYON *et al.*, 2000a). During grape maturation adverse meteorological conditions and a varietal-specific sensitivity are responsible of this phenomenon (RIBEREAU-GAYON *et al.*, 2000a). Therefore, the force of detachment of the pedicel (F_{ped}) is an effective parameter that should be monitored to assess this characteristic. Although the irrigation effect on this parameter at harvest cannot be considered a problem, these grapes could not be adapted to on-vine drying process where the decrease of F_{ped} is high (ROLLE *et al.*, 2009).

CONCLUSION

The assessment of free volatile compounds and their precursors together with the mechanical properties in white aromatic grapes may be important in selecting the best irrigation strategy in order to obtain the highest grape quality.

In this study the contributions of free linalool and geraniol, the most characteristic volatile varietal compounds of Muscat grape, were more dominant in the standard than in the drought regime, with an increase in standard irrigation of about 78% for free linalool and 73% for free geraniol respect to drought treatment.

Berry mechanical characteristics are important for assessing the resistance to fungal attacks and for the estimation of shattering. In particular, no influence of irrigation treatment on F_{sk} and Sp_{sk} parameters was noticed. Therefore, apparently, this practice of vineyard management does not result in significant changes to the resistance towards splitting and spread of plant diseases. Finally, the values of technological parameters of ripeness as well as berry weight and production yield of grapes from the three different water treatments showed no significant differences.

REFERENCES

Adams R.P. 2001. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured Publishing Corporation, Carol Stream.

Bitteur S.M., Baumes R.L., Bayonove C.L., Versini G., Martin C.A. and Dalla Serra A. 1990. 2-exo-Hydroxy-1,8-cineole: a new component from grape var. Sauvignon. J. Agric. Food Chem. 38:1210.

Bureau S.M., Razungles A.J. and Baumes R.L. 2000. The aroma of Muscat of Frontignan grapes: effect of the light environment of vine or bunch on volatiles and glycoconjugates. J. Sci. Food Agric. 80: 2012.

Chapman D.M., Roby G., Ebeler S.E., Guinard J. and Matthews M.A. 2005. Sensory attributes of Cabernet sauvignon wines made from vines with different water status. Aust. J. Grape Wine Res. 11: 339.

Del Caro A., Fanara C., Genovese A., Moio L., Piga A. and Piombino P. 2012. Free and enzymatically hydrolysed volatile compounds of sweet wines from Malvasia and Muscat grapes (*Vitis vinifera* L.) grown in Sardinia. S. Afr. J. Enol. Vitic. 33: 115.

Deloire A., Carbonneau A., Wang Z.P. and Ojeda H. 2004. Vine and water: a short review. J. Int. Sci. Vigne Vin. 38:1.

Di Stefano R. 1991. Proposal for a method of sample preparation for the determination of free and glycoside terpenes of grapes and wines. Bull. OIV. 721-722: 219.

Fenoll J., Manso A., Hellín P., Ruiz L. and Flores P. 2009. Changes in the aromatic composition of the *Vitis vinifera* grape Muscat Hamburg during ripening. Food Chem. 114: 420.

Gabler M.G., Smilanick J.L., Mansour M., Ramming D.W. and Mackey B.E. 2003. Correlation of morphological, anatomical and chemical features of grape berries with resistance to *Botrytis cinerea*. Phytopathology. 93:1263.

Gambacorta G., Antonacci D., La Gatta M., Faccia M., La Gatta B., Pati S., Coletta A. and La Notte E. 2011. Phenolic composition of Aglianico and Nero di Troia grapes and wines as affected by cover cropping and irrigation. Ital. J. Food Sci. 23: 381.

Giordano M., Rolle L., Zeppa G. and Gerbi V. 2009. Chemical and volatile composition of three Italian sweet white passito wines. J. Int. Sci. Vigne Vin. 43:159.

Gladstone J.S. 1992. Viticulture and Environment, Winetitles, Adelaide.

Gomez E., Martinez A. and Laencina L. 1995. Changes in volatile compounds during maturation of some grape varieties. J. Sci. Food Agric. 67: 229.

Gunata Y.Z., Bayonove C., Baumes R. and Cordonnier R. 1985, The aroma of grapes. Localisation and evolution of free and bound fractions of some grape aroma components *cv* Muscat during first development and maturation. J. Sci. Food Agric. 36: 857.

Hepner Y., Bravdo B., Loinger C., Cohen S. and Tabacman H. 1985. Effect of irrigation and crop level on growth, yield and wine quality of Cabernet sauvignon. Am. J. Enol. Vitic. 36:132.

Koundouras S., Marinos V., Gkoulioti A., Kotseridis Y. and van Leeuwen C. 2006. Influence of vineyard location and vine water status on fruit maturation of nonirrigated *cv* Agiorgitiko (*Vitis vinifera* L.). Effects on wine phenolic and aroma components. J. Agric. Food Chem. 54: 5077.

Lambri M., Dordoni R., Silva A. and De Faveri 2012. Comparing the impact of bentonite addition for both must clarification and wine fining on the chemical profile of wine from Chambave Muscat grapes. Int. J. Food Sci. Sci. Tech. 47:1.

Lang A. and During H. 1990. Grape berry splitting and some mechanical properties of the skin. Vitis. 29:61.

Le Moigne M., Maury C., Bertrand D. and Jourjon F. 2008. Sensory and instrumental characterisation of Cabernet franc grapes according to ripening stages and growing location. Food Qual. Prefer. 19: 220.

Letaief H., Rolle L. and Gerbi V. 2008. Mechanical behaviour of winegrapes under compression tests. Am. J. Enol. Vitic. 59: 323.

Luan F., Mosandl A., Münch A. and Wüst M. 2005. Metabolism of geraniol in grape berry mesocarp of *Vitis vinifera* L. *cv* Scheurebe: demonstration of stereoselective reduction, E/Z-isomerization, oxidation and glycosilation. Phytochem. 66: 295.

Lund S.T. and Bohlmann J. 2006. The molecular basis for wine grape quality. A volatile subject. Science. 311: 804.

Marais J. 1983. Terpenes in the aroma of grapes and wines: A review. S. Afr. J. Enol. Vitic. 4: 49.

Matthews M.A., Ishii R., Anderson M.M. and Omahony M. 1990. Dependence of wine sensory attributes on vine water status. J. Sci. Food Agric. 51: 321.

Maury C., Madieta E., Le Moigne M., Mehinagic E., Siret R. and Jourjon F. 2009. Development of a mechanical texture test to evaluate the ripening process of Cabernet franc grapes. J. Text. Stud. 40: 511.

Mercalli L. 2003. Atlante climatico della Valle d'Aosta. Società Meteorologica Subalpina, Torino.

Ojeda H., Deloire A. and Carbonneau A. 2001. Influence of water deficits on grape berry growth. Vitis. 40:141.

Peyrot des Gachons C.P., Van Leeuwen C., Tominaga T., Soyer J.P., Gaudillere J.P. and Dubourdieu D. 2005. Influence of water and nitrogen deficit on fruit ripening and

aroma potential of *Vitis vinifera* L *cv* Sauvignon blanc in field conditions. J. Sci. Food Agric. 85: 73.

Qian M.C., Fang Y. and Shellie K. 2009. Volatile composition of Merlot wine from different vine water status. J. Agric. Food Chem. 57:7459.

Ribéreau-Gayon P., Boidron N. and Terrier A. 1975. Aroma of Muscat grape varieties. J. Agric. Food Chem. 23:1042.

Ribéreau-Gayon P., Glories Y., Maujean A. and Dubourdieu D. 2000a. Handbook of Enology, Vol. 1, The microbiology of wine and vinifications, John Wiley and Sons Ltd. Baffins Lane, Chichester.

Ribéreau-Gayon P., Glories Y., Maujean A. and Dubourdieu D. 2000b. Handbook of Enology, Vol. 2, The chemistry of wine stabilization and treatments, John Wiley and Sons Ltd. Baffins Lane, Chichester.

Río Segade S., Giacosa S., Gerbi V. and Rolle L. 2011c. Berry skin thickness as main texture parameter to predict anthocyanin extractability in winegrapes. LWT - Food Sci. Technol., 44: 392.

Río Segade S., Orriols I., Giacosa S. and Rolle L. 2011a. Instrumental texture analysis parameters as winegrapes varietal markers and ripeness predictors. Int. J. Food Prop. 14: 1318.

Río Segade S., Soto Vázquez E., Orriols I., Giacosa S. and Rolle L. 2011b. Possible use of texture characteristics of winegrapes as markers for zoning and their relationship with anthocyanin extractability index. Int. J. Food Sci. Technol. 46: 386.

Rolle L., Gerbi V., Schneider A., Spanna F. and Río Segade S. 2011. Varietal relationship between instrumental skin hardness and climate for grapevines (*Vitis vinifera* L.). J. Agric. Food. Chem. 59: 10624.

Rolle L., Siret R., Río Segade S., Maury C., Gerbi V. and Jourjon F. 2012. Instrumental texture analysis parameters as markers of table-grapes and winegrape quality: a review. Am. J. Enol. Vitic. 63, 11.

Rolle L., Torchio F., Cagnasso E. and Gerbi V. 2010. Evolution of mechanical variables of the winegrapes for icewine production during on-vine drying. Ital. J. Food Sci. 22:143.

Rolle L., Torchio F., Giacosa S. and Gerbi V. 2009. Modification of mechanical characteristic and phenolic composition in berry skins and seeds of Mondeuse winegrapes throughout the on-vine drying process. J. Sci. Food Agric. 89:1973.

Sánchez Palomo E., Díaz-Maroto M.C., González Viñas M.A., Soriano-Pérez A. and Pérez-Coello M.S. 2007. Aroma profile of wines from Albillo and Muscat grape varieties at different stages of ripening. Food Control. 18: 398.

Ureta C.F. and Yavar O.L. 1982. Influence de quelques pratiques culturales sur la qualite des raisins. Conn. Vigne Vin. 16:187.

Usseglio-Tommaset L. and Di Stefano R. 1980. Profilo aromatico del Moscato bianco del Piemonte. Riv. Vitic. Enol. 33: 58.

van Leeuwen C., Tregoat O., Choné X., Bois B., Pernet D. and Gaudiliére J.-P. 2009. Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? J. Int. Sci. Vigne Vin. 43:121.

Williams P.J., Strauss C.R. and Wilson B. 1980. Hydroxylated linalool derivatives as precursors of volatile monoterpenes of Muscat grapes. J. Agric. Food Chem. 28: 766.

Wirth J., Guo W., Baumes R. and Günata Z. 2001. Volatile compounds released by enzymatic hydrolysis of glycoconjugates of leaves and grape berries from *Vitis vinifera* Muscat of Alexandria and Shiraz cultivars. J. Agric. Food Chem. 49: 2917.

Zarrouk O., Francisco R., Pinto-Marijuan M., Brossa R., Santos R.R. Pinheiro C., Costa J.M., Lopes C. and Chaves M.M. 2012. Impact of irrigation regime on berry development and flavonoids composition in Aragonez (Syn. Tempranillo) grapevine. Agric. Water Manage. 114: 18.

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PAPER

AN EVALUATION OF FISH FRESHNESS: A PROPOSAL FOR A NEW INDEX

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ABSTRACT

The purpose of this study is to define a new system for assessing the freshness of marine bony fish based on measurable parameters. 151 fish were analysed to determine the concentrations of total volatile basic nitrogen, trimethylamine N-oxide, trimethylamine and malondialdehyde. The results of the determinations were included in an algorithm to calculate the value of the Freshness Index (FI). The most appropriate threshold value of FI that can distinguish fresh from not fresh fish was 0.33 (sensitivity 95.6%, specificity 73.6%). The results demonstrate the possibility of using the index for the evaluation, at low-cost, of consignments of fresh or presumed fresh fish both in the phase of official control and in self-verification systems.

- Keywords: freshness index, fish, Malondialdehyde, Total Volatile Basic Nitrogen, Trimethylamine, Trimethylamine N-Oxide -

INTRODUCTION

Fish are a very perishable product, and the maintenance of their freshness depends on three major factors: enzymatic autolysis, oxidation, and microbial proliferation.

These mechanisms related to perishability are determined by the intrinsic characteristics of the fish flesh, such as the low glycogen content, the high content of non-protein nitrogen, the high content of unsaturated fatty acids and the liability of the muscle tissue caused by a rather scarce connective tissue (ALASALVAR *et al.*, 2010).

After the death of the fish, the cascade of degradative processes occurs rapidly. Due to the low glycogen content and the enzymatic autolysis, the flesh does not acidify adequately and bacteria grow quickly. Moreover, the high water content and the small amount of connective tissue facilitate the rapid penetration of the microorganisms responsible for the short shelf life and the deterioration of the product quality. After the death of the fish, the microbiological profile changes rapidly, encouraging the proliferation of a bacterial flora consisting mainly of psychrophilic *Aeromonas* spp. and *Pseudomonas* spp. Bacterial activities are closely related to the storage temperature; the proliferation and penetration of microorganisms into the muscle appear to be very slow at temperatures below 8°C. Low temperatures also inhibit the Trimethylamine (TMA) activity, which allows the fish to maintain freshness for a longer period of time. On the contrary, the process of fat oxidation happens at low temperatures at a far greater rate than that of the bacterial degradation. The first stage involves the formation of peroxides that may promote formation of the aldehydes and ketones responsible for the characteristic odour of the degraded fish. For the first oxidative phase, an assessment of the Peroxide Value (PV) is more appropriate, while in the second phase, it is more appropriate to use the Thiobarbituric Acid (TBA) method (HUSS, 1988; 1995).

In addition to being an index of preservation, the freshness of fish (as well as of other food products) greatly affects the product quality and market value; therefore, the control of food quality and freshness is of growing interest for both consumers and the food industry (NOH *et al.*, 2011). Moreover, current market strategies and dynamics of fresh fish require increasingly long storage times and, therefore, more effectiveness systems to ensure the preservation of fresh fish are been studying and applying. The assumption of this process are the initial conditions of the product at the time of the use of such techniques: fish that had already left much of its freshness does not reach the shelf life currently required by the market.

The assessment of freshness may be based on the determination of sensory methods as well as physical, chemical or microbiological parameters; some chemical parameters have already been identified. These parameters are related to the biochemical alterations of the lipid and protein components of the food, and they affect the texture, flavour, and aroma of the product. These three qualities are very important from a commercial point of view and help determine whether the product has reached an unacceptable condition in terms of food safety (HUSS, 1995; BARAT *et al.*, 2008; OCAÑO-HIGUERA *et al.*, 2011). The parameters that have been identified are Total Volatile Basic Nitrogen (TVB-N), Trimethylamine N-Oxide (TMAO), and Trimethylamine (TMA) for the protein component and Malondialdehyde (MDA) for the lipid component.

Over the years, different indices of freshness based on the sensory parameters have been proposed, such as the Quality Index Method (QIM) (BAIXAS-NOGUERAS *et al.*, 2003; CARDENAS BONILLA *et al.*, 2007), and biochemical parameters, such as the *K* index (DALGAARD, 2000). In general, these indices are related to the species of the fish and do not meet the requirements for sensory analysis as proposed by Council Regulation (EC) No 2406/96 and the measure of TVB-N proposed in Commission Regulation (EC) No 2074/05.

The purpose of this study is to define a new system for assessing the freshness of marine bony fish based on objective and measurable parameters, to be applied in the early/intermediate market stages that is when it is necessary that the products yet have the features needed to be storage for a prolonged periods.

MATERIALS AND METHODS

The specimens of fish used in the study belong to the Class Osteichthyes and were taken randomly during the retail distribution phase. The sampling criteria depended on the prevalence of the product on the local market.

The samples were transported under controlled temperatures and were evaluated with sensory methods as described in the Council Regulation (EC) No 2406/96. This evaluation was conducted by qualified and trained personnel.

The sample consisted of 151 fish belonging to the following families or groups:

- Sparidae (47 specimens);
- Merlucciidae (30 specimens);
- Pleuronectidae (36 specimens);
- Bluefish (38 specimens).

The Merlucciidae family was represented exclusively by the hake (*Merluccius merluccius*).

To assess the freshness, we identified some chemical parameters related to the biochemical alterations of lipid and nitrogenous compounds

in the food, such as the TVB-N, TMAO, TMA for the protein component, and MDA for the lipid component.

We used the method of Perna to determine TMA and TMAO (PERNA, 1992).

To assess the TVB-N, we used the official method reported in Annex II, Section II, Chapter III of Commission Regulation (EC) No 2074/2005.

For assessing the MDA, an HPLC method was used. This method is based on the derivatisation of MDA with thiobarbituric acid (TBA) extracted from the muscle tissue of fish in an acidic environment and was used for the determination of TMA and TMAO. The detection limit of this method was estimated at 0.01 g/mL (equal to 5 μg/100 g of muscle).

This method involved the homogenisation of 20 g of muscle tissue in the presence of 100 mL of 7.5% trichloroacetic acid solution in water. Next, 50 μL of the extract was added to 750 μL of 1% phosphoric acid, 250 μL of a thiobarbituric acid solution and 450 μL of water. After 60 minutes at 100°C, the reaction was stopped with an equal volume of NaOH solution, and the obtained mixture was injected directly into the chromatographic system. The analysis was conducted using a mobile reversed-phase consisting of a buffer mixture of phosphate and methanol (80:20). The spectrofluorometric detector used wavelengths of 525 nm for excitation and 550 nm for emission.

The results of the analytical determinations were included in an algorithm designed to determine the variation in the quality of the product both for the definite change of a single parameter and for small changes of two or more parameters simultaneously (CIANTI *et al.*, 2007).

The following algorithm was used:

$$FI = \sqrt[3]{a} / [(1+b)^* (1+d)^* \log(10 + c)]$$

where
a = TMAO mg/100 g of muscle;
b = TMA mg/100 g of muscle;
c = TVB-N mg/100 g of muscle;
d = MDA mg/100 g of muscle.

Statistical analysis

We used ROC (Receiver Operating Characteristic) curves to identify the threshold values of freshness that can distinguish fresh fish from fish that is not fresh (BOTTARELLI and PARODI, 2003; OBUCHOWSKI *et al.*, 2004; MANDREKAR, 2010). We chose the value with the highest sensitivity among the range of values associated with the highest percentage of the samples that were classified correctly. The area under the ROC curve (AUC) was a criterion of test performance and served as a measure of accuracy. The interpretation of the area under the curve followed the guidelines proposed by SWETS (1998):

- AUC=0.5: not an informative test;
- 0.5<AUC<=0.7: low accuracy;
- 0.7<AUC<=0.9: moderate accuracy;
- 0.9<AUC<1.0: high accuracy;
- AUC=1.0: a perfect test.

As a reference classification (gold standard), we used the method indicated in Council Regulation (EC) No 2406/96 by grouping the four freshness categories into two classes: categories A and Extra in the "good" fish class, and categories B and Not Admitted in the "not good" fish class. The decision to include in the same group the fish classified in category B with those Not Admitted is due to the aim to apply the method, in the future, in the early/intermediate market stages.

A ROC curve that included all the samples and 4 additional curves (one for each family or group) were developed.

The difference between the mean values of TMA, TMAO, TVB-N, MDA, and the FI value by freshness class ("good" or "not good") was assessed with the Student's t test (alpha level = 0.05).

The statistical analyses were performed with SPSS 18.0 and STATA 8.0.

RESULTS AND CONCLUSIONS

We analysed 151 samples, of which 45 (29.8%) were classified as "good" (category A or Extra) and the remaining 106 (70.2%) were classified as "not good" (B or Not Admitted). Table 1 shows the results of the descriptive values of TMA, TMAO, TVB-N, MDA, and FI.

In the overall sample, the mean of the individual parameters and the FI, with the exception of MDA, was significantly different between the two classes ($p<0.05$); in the "good" class, we detected higher values of FI and TMAO and lower values of TMA and TVB-N. The same situation has been observed for Pleuronectidae. The mean values of TVB-N for the bluefish and Sparidae and the TVB-N and the FI for Merlucciidae were not significantly different ($p>0.05$).

Fig. 1 shows the ROC curves for all of the samples combined, as well as curves for each family or group.

For all of the samples analysed, the most appropriate threshold level was 0.33 (sensitivity 95.6%, specificity 73.6%, 80.1% correctly classified cases). Therefore, fish with an FI greater than or equal to this value were "good" with a probability of 95.6%, and the test was highly accurate (AUC = 0.903).

The identified threshold was 0.2 for the Pleuronectidae samples (100% sensitivity, 92.9% specificity, 94.4% correctly classified cases), 0.52 for the Merlucciidae samples (75% sensitivity, 96.2% specificity, 93.3% correctly classified cas-

Table 1 - Trimethylamine (TMA), Trimethylamine N-Oxide (TMAO), Total Volatile Basic Nitrogen (TVB-N), Malondialdehyde (MDA), and Freshness Index (FI): descriptive analysis. Concentrations expressed in mg/100 g of muscle.

	"Good" (G)			"Not Good" (NG)		
	Mean (SD)	Median	Range	Mean (SD)	Median	Range
***All* G=45; NG=106**						
TMAO	165.41 (102.67)[a]	138.82	25.03-459.82	47.32 (73.34)[a]	7.98	0.01-309.30
TMA	10.19 (8.37)[a]	8.65	0.0-39.70	41.02 (33.28)[a]	28.65	2.02-132.50
MDA	0.10 (0.13)	0.06	0.01-0.64	0.07 (0.16)	0.03	0.01-1.27
TVB-N	19.89 (7.88)[a]	19.14	0.62-43.22	39.11 (32.38)[a]	26.24	8.64-197.57
FI	0.95 (0.74)[a]	0.62	0.20-3.04	0.21 (0.22)[a]	0.10	0.01-0.91
***Pleuronectidae* G=8; NG=28**						
TMAO	255.46 (145.02)[a]	244.78	44.70-459.82	24.89 (58.87)[a]	6.13	0.64-292.80
TMA	14.58 (12.52)[a]	12.85	1.02-39.70	75.85 (32.02)[a]	61.92	10.15-132.50
MDA	0.05 (0.05)	0.05	0.01-0.13	0.03 (0.02)	0.02	0.01-0.15
TVB-N	13.42 (6.42)[a]	13.65	6.25-25.00	72.61 (28.44)[a]	77.17	13.58-127.18
FI	0.73 (0.68)[a]	0.58	0.20-2.37	0.08 (0.12)[a]	0.05	0.02-0.57
***Merlucciidae* G=4; NG=26**						
TMAO	253.73 (117.64)[a]	250.37	129.20-385.00	44.74 (77.61)[a]	8.71	0.32-309.30
TMA	10.97 (7.04)[a]	11.62	2.20-18.45	34.09 (26.50)[a]	24.71	4.75-83.73
MDA	0.07 (0.08)	0.04	0.02-0.20	0.05 (0.04)	0.05	0.01-0.23
TVB-N	23.01 (8.27)	23.15	13.12-32.60	35.68 (39.63)	25.93	11.11-197.57
FI	0.70 (0.47)	0.53	0.34-1.39	0.18 (0.16)	0.11	0.03-0.68
***Bluefish* G=17; NG=21**						
TMAO	129.10 (65.76)[a]	115.34	49.57-312.26	70.48 (77.55)[a]	37.36	0.20-233.61
TMA	12.67 (6.90)[a]	11.90	2.20-24.45	32.03 (26.17)[a]	24.80	2.02-74.91
MDA	0.07 (0.06)	0.06	0.01-0.23	0.18 (0.35)	0.06	0.01-1.27
TVB-N	20.84 (4.55)	19.14	14.82-28.40	24.35 (8.39)	25.31	11.11-38.28
FI	0.65 (0.30)[a]	0.50	0.28-1.31	0.34 (0.26)[a]	0.28	0.03-0.91
***Sparidae* G=16; NG=31**						
TMAO	136.88 (70.41)[a]	141.36	25.03-254.33	54.07 (76.04)[a]	5.13	0.01-257.95
TMA	5.16 (5.30)[a]	2.73	0.01-16.55	21.46 (16.89)[a]	15.00	2.71-78.19
MDA	0.14 (0.19)	0.07	0.01-0.64	0.05 (0.05)	0.03	0.01-0.19
TVB-N	21.35 (9.94)	21.43	0.62-43.22	21.73 (7.97)	19.76	8.64-41.37
FI	1.44 (0.92)[a]	1.03	0.33-3.04	0.29 (0.24)[a]	0.25	0.01-0.80

[a]Student's t-test was used for the comparison of mean values by freshness class ("good" or "not good"): $p<0.05$.

es), 0.28 for the Bluefish samples (100% sensitivity, 47.6% specificity, 71.1% correctly classified cases), and 0.82 for the Sparidae samples (75.0% sensitivity, 100.0% specificity, 91.5% correctly classified cases).

The results of the tests were as follows:
- highly accurate (AUC = 0.98) for Pleuronectidae;
- highly accurate (AUC = 0.94) for Merlucciidae;
- moderately accurate (AUC = 0.77) for Bluefish;
- highly accurate (AUC = 0.93) for Sparidae.

The results of the ROC curves, as well as of the determinations of TMA, TMAO, TVB-N, and MDA, showed the differences in the assessment of species-specific fish families, which are related to the different nutrient contents and concentrations of catabolites. Based on this consideration, it is difficult to generate a threshold that can be valid for all the families we have considered, without losing sensitivity or specificity. On the other hand, the presence of a synthetic and objective index will limit the intra- and interoperator variability of a subjective evaluation, according to the application of Council Regulation (EC) No 2406/96. Table 2 shows the values for the sensitivity, specificity, and percentage of correctly classified cases for all the fish together and for each group individually, using the threshold value of 0.33 (the best one according to ROC curve for all samples).

In the absence of a clear definition of freshness, the decision to prefer high and absolute sensitivity rather than the specificity (BREMNER, 2000; BREMNER and SAKAGUCHI, 2000; NIELSEN *et al.*, 2002) allows for the assignment of the product to the different categories and minimises the misclassification of the categories at the greatest values (A or Extra). This consideration is related to the precautionary principle and the high consumer protection that benefits the European consumers, as expressed by the

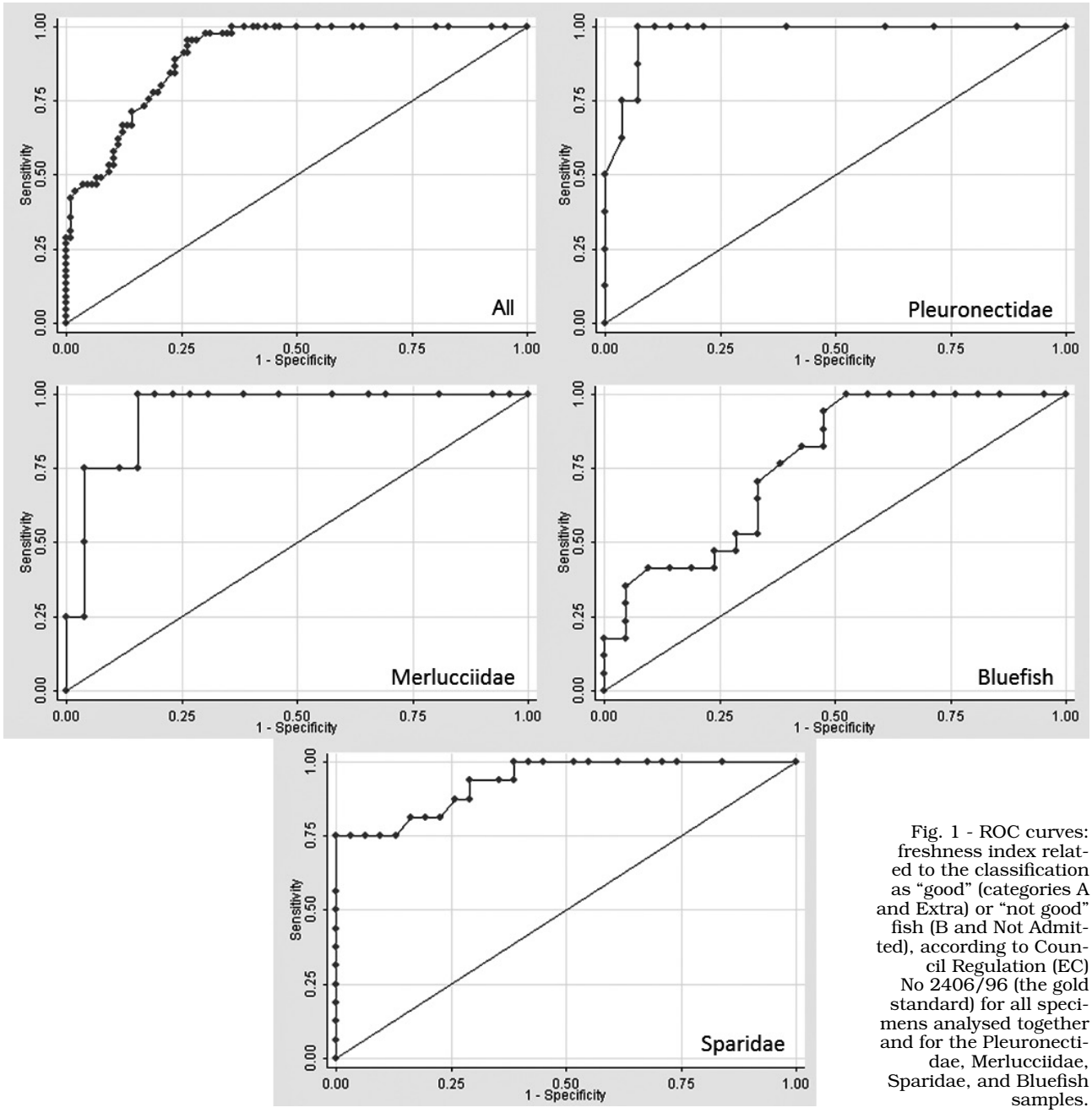

Fig. 1 - ROC curves: freshness index related to the classification as "good" (categories A and Extra) or "not good" fish (B and Not Admitted), according to Council Regulation (EC) No 2406/96 (the gold standard) for all specimens analysed together and for the Pleuronectidae, Merlucciidae, Sparidae, and Bluefish samples.

Table 2 - Freshness Index related to classification as "good" (categories A and Extra) or "not good" fish (B and Not Admitted), according to Council Regulation (EC) No 2406/96. The sensitivity and specificity were calculated using the 0.33 threshold value for the Freshness Index. The analysis included all specimens analysed together and separate analysis of the Pleuronectidae, Merlucciidae, Sparidae, and Bluefish samples.

	Sensitivity	Specificity	Correctly classified cases
All	95.6%	73.6%	80.1%
Pleuronectidae	87.5%	92.9%	91.7%
Merlucciidae	100.0%	84.6%	86.7%
Sparidae	100.0%	61.3%	74.5%
Bluefish	94.1%	52.4%	71.0%

EU Regulation 178/2002 and the White Paper on Food Safety.

The approach considered in this study does not allow for a comparison of these results with those of other authors, who generally do not describe freshness indices by sensitivity and specificity but use them to assess the quality changes over time (DUFLOS *et al.* 2010; BARAT *et al.*, 2008; CASTRO *et al.*, 2006). Moreover, other papers consider only frozen fish or compare different storage conditions (SONG *et al.*, 2012; COSTA *et al.*, 2012).

The biochemical analysis for the determination of the FI are low-cost, can be performed in laboratories with basic equipment, and can estimate the freshness of the fish quickly. Other biochemical methods, however, have a much

higher cost, can be performed only in specialized laboratories or are too generic. In particular, the exclusively measurement of TVB-N level, while less expensive than the determination of FI, generally reflect only later stages of advanced spoilage and is generally considered unreliable for the measurement of spoilage during the first ten days of chilled storage of several species. On the other hand, sensory methods require specially trained evaluators and must be performed scientifically under carefully controlled conditions so that the effects of test environment and personal bias may be reduced.

In conclusion, the preliminary results show that the freshness index provides a simple, straightforward, and objective method, corroborated and supported by biochemical tests, that can be used for the official control and self-verification systems. The Freshness Index can be used for the evaluation of consignments of fresh or presumed fresh fish to produce definite evidence for control purposes; this index can also monitor the production of fish up to the level of retail display.

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REFERENCES

Alasalvar C., Miyashita K., Shahidi F. and Wanasundara U. 2010. Handbook of Seafood Quality, Safety and Health Applications. UK: Wiley-Blackwell.

Baixas-Nogueras S., Bover-Cid S., Veciana-Nogués T., Nunes M.L. and Vidal-Carou M.C. 2003. Development of a Quality Index Method to Evaluate Freshness in Mediterranean Hake (*Merluccius merluccius*). J. Food Sci. 68: 1067.

Barat J.M., Gil L., García-Breijo E., Aristoy M.C., Toldrá F., Martínez-Máñez R. and Soto J. 2008. Freshness monitoring of sea bream (*Sparus aurata*) with a potentiometric sensor. Food Chem. 108: 681.

Bottarelli E. and Parodim S. 2003. Un approccio per la valutazione della validità dei test diagnostici: le curve R.O.C. (Receiver Operationg Characteristic). Ann. Fac. Medic. Vet. Parma 23: 49.

Bremner H. A. 2000. Toward practical definitions of quality for food science. Crit. Rev. Food Sci. Nutr. 40: 83.

Bremner H.A. and Sakaguchi M. 2000. A critical look at whether 'freshness' can be determined. J. Aquat. Food Prod. Technol. 9: 5.

Cardenas Bonilla A., Sveinsdottir K. and Martinsdottir E. 2007. Development of Quality Index Method (QIM) scheme for fresh cod (*Gadus morhua*) fillets and application in shelf life study. Food Control 18: 352.

Castro P., Carlos J., Padrón, P., Caballero Cansino M.J., Sanjuán Velázquez E., Millán De Larriva R. 2006. Total volatile base nitrogen and its use to assess freshness in European sea bass stored in ice. Food Control 17: 245.

Cianti L., Boccetti M., Pelatti E., Cellesi E., Catalano A, Perico A., Bavazzano P., Colzi A., Gravina M.T. and Boddi, V. 2007. L'indice di freschezza del pesce: proposta di un nuovo metodo di valutazione. Industrie Alimentari 46: 997.

Costa C., Antonucci F., Menesatti P., Pallottino F., Boglione C. and Cataudella S. 2012. An advanced color calibration method for fish freshness assessment: a comparison between standard and passive refrigeration modalities. Food Bioprocess Technol. DOI: 10.1007/s11947-011-0773-6.

Dalgaard P. 2000. Freshness, quality and safety in seafoods. Technical manual of the EU project CT 97.3014. Flair-Flow Europe F-FE 380A/00:5-31. Dublin, Ireland: The National Food Center.

Duflos G., Leduc F., N'Guessan A., Krzewinski F., Kol O. and Malle P. 2010. Freshness characterisation of whiting (*Merlangius merlangus*) using an SPME/GC/MS method and a statistical multivariate approach. J. Sci. Food Agric. 90: 2568.

Huss H.H. 1988. Fresh fish. Quality and quality changes. Roma: Food and Agriculture Organization of the United Nations.

Huss H.H. 1995. Quality and quality changes in fresh fish. FAO fisheries technical paper - 348. Rome: Food and Agriculture Organization of the United Nations.

Mandrekar J.N. 2010. Receiver operating characteristic curve in diagnostic test assessment. J. Thorac. Oncol. 5: 1315.

Nielsen J., Hyldig G. and Larsen E. 2002. 'Eating quality' of fish. A review. J. Aquat. Food Prod. Technol. 11: 125.

Noh D.H., Chung S.H., Choi S.J. and Hur S.J. 2011. A preliminary study on the development of an easy method for beef freshness using a cyclic voltammetric system. Food Control 22: 133.

Obuchowski N.A., Lieber M.L. and Wians F.H. 2004. ROC curves in clinical chemistry: uses, misuses, and possible solutions. Clin. Chem. 50: 1118.

Ocaño-Higuera V.M., Maeda-Martínez A.N., Marquez-Ríos E., Canizales-Rodríguez D.F., Castillo-Yáñez F.J., Ruíz-Bustos E., Graciano-Verdugo E.Z and Plascencia-Jatomea M. 2011. Freshness assessment of ray fish stored in ice by biochemical, chemical and physical methods. Food Chem. 125: 49.

Parisi G., Franci O. and Poli B.M. 2002. Application of multivariate analysis to sensorial and instrumental parameters of freshness in refrigerated sea bass (*Dicentrarchus labrax*) during shelf life. Aquaculture, 214: 153.

Perna A. 1992. La determinazione contemporanea di TMA, TMAO e DAM quale mezzo per stabilire lo stato di conservazione dei prodotti alimentari della pesca. Vet. Ital. 5: 48.

Song Y., Luo Y., You J., Shen H. and Hu S. 2010. Biochemical, sensory and microbiological attributes of bream (*Megalobrama amblycephala*) during partial freezing and chilled storage. J. Sci. Food Agric. 92: 197.

Swets J.A. (1989). Measuring the accuracy of diagnostic systems. Science 240: 1285.

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PAPER

EFFECT OF HARVESTING TIME AND STORAGE TEMPERATURE ON THE DURATION OF BALAH STAGE OF 'BARHI' DATES

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ABSTRACT

'Barhi' dates at Balah stage are characteristic with their attractive and pleasant flavor and texture. They are more acceptable by consumers and marketed with high prices at this stage of maturity compared with Rutab and Tamar stages of the same cultivar. The duration of Balah stage of 'Barhi' dates is about 4 weeks which is considered very short for successful marketing of 'Barhi' dates at this stage. This research work aimed at studying the possibility of prolonging the duration of Balah stage for 'Barhi' dates. Four harvesting dates and 3 storage temperatures were used in this study. The sensorial as well as the physico-chemical properties of the fresh and stored date samples were determined at specific intervals. The obtained results showed that the fresh 'Barhi' dates at Balah stage contain (on dwb) 2.8-4.2% ash, 20-35% Brix, 61-79% moisture, 0.18-0.20% acidity, 2.8-10.5% tannins, 14.1-49.4% fibre, 2.1-4.9% pectin, 80.6-87% total sugars, whereas the softening rate ranged between 0-5%. The sensory evaluation results revealed that date fruits stored at 0°C achieved the best results. The best combination of harvesting time and storage temperature was found to be 4th September and 0°C where a four weeks extra time were added to the Balah stage of 'Barhi' dates.

- Keywords: Dates, ripening stages, physical and chemical properties, sensory properties, harvesting, storage -

1. INTRODUCTION

Dates (*Phoenix dactylifera* L.) are considered a major fruit crop in the Middle East countries as more than 80% of the world production is produced in this area (ALSAED, 2010). They are rich in sugars (about 80% on dwb), particularly invert sugar, some essentially important vitamins and minerals, and fiber (YOUSIF *et al.*, 1991). After pollination, date fruits pass through five stages, namely, Habobouk, Kimri, Khalal or Beser or Balah, Rutab and Tamr (DAWSON and ATEN, 1962). Date fruits during the first two stages (Habobouk and Kimri) have high levels of tannins and accordingly are not consumed (EL-NAKHAL and AL-KAHTANI, 1986). At the Balah stage, they show the characteristic color of the cultivar, absence of stringent taste and appearance of sweet taste with a hard texture.

Growing and processing of dates in Jordan is new and belongs only to less than 30 years. There are three locations where dates could be successfully grown in the country i.e. Al-Azraq, Aqaba, and Jordan Valley. Commercial date palm farms have been recently spreading. The most popular commercial date cultivars in the country are 'Barhi', 'Madjoul', 'Degletnoor', 'Kahlas' and 'Hayani' (ALSAED, 2005). The annual production of date crop in Jordan reached to about 12,000 tones (JGSD, 2011), and is expected to increase significantly in the coming few years due to the many new date palm plantings.

'Barhi' dates at Balah stage are characteristic with their attractive and pleasant flavor and texture. They are more acceptable by consumers and marketed with high prices at this stage of maturity compared with Rutab and Tamr stages of the same cultivar. The duration of Balah stage of 'Barhi' dates is about 4 weeks which is considered very short for successful marketing of 'Barhi' dates at this stage.

Certain aspects of dates of Saudi Arabia were discussed by SAWAYA (1986). Ripening of five date cultivars at Balah stage at room temperature and at 5°C before and after freezing were studied by EL-NAKHAL and AL-KAHTANI (1986). It was found that ripening by freezing and thawing, then leaving date fruits at room temperature until they reach the desired stage of Rutab was the best treatment of ripening. The suitability of 8 date cultivars at Rutab stage grown at Al-Hassa area for preserving using refrigeration and freezing techniques was reported by YOUSIF and ABOU-ALI (1993). The results revealed that the Rutab of 'Um-Rhaim' and 'Hilali' dates proved to be superior on the other cultivars for freezing.

Freezing accelerates the diffusion of enzymes out of the date's fruit cell, which decompose the pectin and lignin in the fruit causing the tissues to become softened, as well as increasing the sugar content in the fruit. This mechanism accelerates the ripening process and makes dates more palatable for consumers (EL-NAKHAL and AL-KAHTANI, 1986).

There is a shortage in data about processing of the locally produced dates. Only some information were given recently about dates in Jordan regarding the cultivars, cultivation area and the quantities produced (ALSAED, 2005; GORN *et al.*, 2008). On the other hand, many papers are available on date cultivars grown in Saudi Arabia (YOUSIF *et al.*, 1985; YOUSIF and ABU-ALI, 1993; MIKKI and AL-TAISAN, 1993; YOUSIF, 1996; YOUSIF and ALGHAMDI, 1999, 2000; AL-REDHAIMAN, 2005; ALANSARI, 2008), United Arab Emirates (ABDULLAH and THOMPSON, 1998; AHMED *et al.*, 1995), Egypt (OSMAN, 2008), Oman (ELMARDI et al., 2002; AL-YAHYAI and AL-KHARUSI (2012), Iraq (YOUSIF *et al.*, 1982; BENJAMIN *et al.*, 1985), and United States (NORMAN *et al.*, 1976). DESROSIER and TRESSLLER (1977) found that there is a possibility of storing some date cultivars at the Balah stage at temperature of -29°C for two years . Also AL-OGADI (1983) found that the freezing technique is better than refrigerating for the preservation of Bahraini dates at the Balah stage. BENJAMIN *et al.* (1985) found that date cultivars of Zahdi and Ahmer Bathenjani in the Balah stage were successfully stored at temperature of -3°C. Regarding Saudi date cultivars, YOUSIF and ABU-ALI (1993) during their study on eight Saudi date cultivars at Rutab stage, found that refrigeration at 5°C can only preserve dates for one month. SWINGLE (1926) also reported about the suitability of 'Dejlaht Noor' date cultivar in the American environment for cold storage.

It was reported (ABDULLAH and THOMPSON, 1998) that 'Khnaize' dates at Rutab stage is more suitable for storage because it can keep its physical properties such as 'firmness' and chemical ones such as 'pH, TSS' value better than 'Kahlas' dates. Both the storage temperatures -10°C and -20°C could be used without greatly affecting the quality of the dates except the colour at -10°C which was darker and the texture was softer than -20°C. Also both maturity stages (early and middle of the Rutab) could be used since they did not greatly differ in their physical or chemical characteristics. The PE wrapping could have increased the storage period from the usual 2 months at 0°C and 94% relative humidity to more than 3 months.

The effects of prolonged freeze storage on Omani 'Khalas' dates physical and chemical quality attributes were reported by AL-YAHYAI and AL-KHARUSI (2012). Dates were collected at three ripening stages (i.e., Khalal, Rutab & Tamar) andsubjected to storage at -18°C in a conventional freezer. Results suggest that prolonged freeze-storage is a viable alternative that allows for the consumption of dates at three stages of ripening compared to conventional storage of only dry dates. The postharvest storage period possible for 'Barhi' dates at balah stage can be

markedly improved by exposure to elevated CO_2 up to 20% (AL-REDHAIMAN, 2005). It was suggested that modified atmosphere system can be developed for 'Barhi' dates at Balah stage to retard ripening and senescence and allow shipping of fruit to distant markets with acceptable quality.

Little data are available in the literature concerning the prolonging of Balah stage for 'Barhi' dates. Therefore, the main objective of this research is to study the possibility of increasing the duration of Balah stage for 'Barhi' dates. The suitability of cold storage techniques as a method for keeping 'Barhi' dates at Balah stage from spoilage and infestation is another objective.

2. MATERIALS AND METHODS

2.1 Samples collection and preparations

Samples of 'Barhi' date cultivar at the Balah stage were procured from a date palm farm located in the Jordan Valley area. Samples of Balah were collected four times, at the initial Balah stage (1st stage at 8/8/2011), at the early middle of the stage (2nd stage at 16/8/2011), at the middle of the stage (3rd stage at 24/8/2011) and at the end of Balah stage or the beginning of the Rutab stage (4th stage at 5/9/2011). Four cartons each filled with date bunches (about 5 kg) were chosen randomly. On each occasion, the fruits were brought to the laboratory on the day of harvesting and after the removal of sub-sample for analytical purposes, dates were filled in 1 kg plastic containers (Figs. 1 and 2) and stored in a refrigerating incubators at 5°, 0° and -5°C with a relative humidity of 65 to 70%.

Samples from each storage temperature were drawn at different intervals i.e. zero time, 2, 4, and 6 weeks. At the zero time (fresh initial Balah stage), Balah fruits were characterized with regard to their content of moisture, Brix, pH, acidity, fiber, ash, tannins, pectin, sugar composition (fructose, glucose, sucrose, total sugar), color, softening ratio, weight of whole fruit, flesh, calyx and seed, volume, length and circumference, as well as the sensory properties of the fruits. Samples from each storage temperature were taken every two weeks and analyzed for sugar composition, pectin, tannins, pH, Brix, moisture, and softening ratio.

2.2 Fruit physical measurements

Ten fruits in triplicate were taken randomly for each physical measurement; the obtained values were averaged and divided by 10 to have the measurement for one fruit. Weight of whole fruit, flesh, calyx and seed were determined using 4 digital electronic top loading balance model SB062, Germany. Volume of fruit was determined by water displacement; length and circumference were conducted using varnier caliber. Softening ratio was determined by observing the soft spots appearing on the fruits.

2.3 Chemical analysis

Moisture, ash, titrable acidity, pectin, fiber, tannins, pH and total soluble solids (Brix) were determined using AOAC method (1995).

Moisture content was determined following the AOAC method No. 934.06; ten intact dates were selected randomly, macerated in a high speed blinder and about 10 g sample in triplicate were taken and dried in a vacuum oven at 65°-70°C for about 48 hrs or until a constant weight was obtained. Ash content was determined according to the AOAC method No. 940.26; 2-3 g of a date sample were weighed in pre-weight porcelain crucible, placed in a muffle furnace (Thermolyne 600 Furnace) at 550°C. Pectin, fiber, and tannins were determined using AOAC official methods having the numbers 924.09, 993.21 and 955.35 respectively. Determination of pH was performed with pH meter model pH525. The acidity was determined (AOAC official method No. 954.07) by titration of samples with 0.1 N sodium hydroxide and completing titration to pH 8.1± 0.2, the acidity was expressed in gram as Malic acid per 100 g fruit. Total soluble solids (Brix) was determined by weighing 10 g sample then mixed well with 10 mL H_2O (distilled), followed by grinding in a mortar and mixing thoroughly then the Brix value was read using digital Abbe Refractometer model CETI, Belgium. Color was measured using the extraction procedure described by Maier and Schiller (1960). About 8 g of dates sample was transferred into a mortar and pestle and 40ml of 65% methanol was added. The content was transferred into a 100 mL beaker and stirred continuously for 3 minute; filtration through filter paper and washing 3 times with 18 mL of 65% methanol were performed. Then 2 mL of 4M acetate- citrate buffer were added to adjust pH to (6-6.3; the volume was adjusted up to 100 mL using distilled water in a volumetric flask.

The specific extinction was computed using the absorbance reading at 400 nm by the equation:

$$P = K*A*100/\text{sample weight}$$

where P= mg pigment/mL of extract, K= specific extinction coefficient = 0.65, A= Absorbance reading.

2.4 Sugar extraction and determination

The sugar composition (fructose, glucose, sucrose and total sugars) of the fresh and stored samples were determined using a method described by (LANGEMEIER and ROGERS, 1995), with slight modifications. Five g sample was

placed in 100 mL volumetric flask with 70% ethanol. The sample was homogenized in a homogenizer (Ultra turex mixer, Type 25, Germany) for 5 min at 15,000 rpm and then sonicated for 15 min at 45°C. The sample was then filtered using (Whatmon filter no. 1). The filtrate was micro filtered using 0.45 μm nylon-type membrane. Then the sample was ready for HPLC analysis. Using (NH2- R-P-Macherey-Angel-Germany) with 79:21 acetonitril: water at flow rate of 1 mL/min.

2.5 Sensory evaluation

The fresh and stored Balah samples were evaluated by a panel of assessors (10 semi-trained subjects) from the Department of Nutrition and Food Technology at the University of Jordan. Samples were evaluated using the hedonic scale test. Over all acceptability for the sensory attributes (texture, flavor, and color) were considered using a 9 point hedonic scale, with 9 indicating "like extremely" and 1 "dislike extremely". Each Balah sample were evaluated by 10 subjects, each scored the samples giving between 1 for dislike extremely and 9 for like extremely; so the Balah samples were evaluated on a scale of 90. Testing was conducted in one session in a well-lighted and odor-free environment with specific instructions for sample evaluators. The sensory evaluation for the fresh and stored Balah samples were done twice in a duration of 1 week. (OGUNRINOLA *et al.*, 1988; YOUSIF *et al.*, 1991).

2.6 Statistical analysis

The Analysis of Variance (ANOVA) was done using mixed procedure (Proc Mixed). Differences between treatment means were tested using protected Least Significant Difference (LSD) test at $p<0.05$. All statistical analyses were performed using the SAS software, version 9 (SAS Institute, 2002).

3. RESULTS AND DISCUSSIONS

3.1 Effect of harvesting time on the chemical properties

The effect of harvesting time on the chemical and physical properties of fresh 'Barhi' dates at Balah or Khalal stage of maturity are shown in Table 1. It is well known that dates pass through 5 stages of ripening i.e. Hababouk, Kimri, Balah or Khalal, Rutab and Tamer. For most date varieties, date fruits are consumed at Balah, Rutab and Tamer stages. Balah stage extends about 4 weeks and is described by 4 terms i.e. early, middle, proper and late (DAWSON and ATEN, 1962). The 1st harvest in this study was done early at the start of Balah stage (08/08/2011),

Table 1 - Chemical and physical properties of fresh "Barhi" dates at Balah stage as affected by harvesting time.

	Chemical and physical attributes				Harvesting time*		
	1st harvest	2nd harvest	3rd harvest	4th harvest	SD**	CV	LSD
Moisture% fwb	79.3a***	75.0a	64.4b	61.1b	2.3	3.4	4.4
Total sugar % dwb	87.0a	82.8ab	82.9ab	80.6b	2.4	2.9	4.5
Brix fwb	20d	23c	30b	35a	1.5	5.6	2.8
Fiber % dwb	49.4a	25.3b	14.4c	14.5c	2.6	10.0	4.9
Pectin % dwb	4.9a	4.4a	2.1b	2.3b	0.3	8.8	0.6
Tannins % dwb	10.5a	8.7b	5.0c	2.8d	0.5	0.7	9.0
Ash % dwb	4.2a	3.5b	2.8c	2.8c	0.3	9.0	0.6
Color (mg pigment/100 g fruit on dwb)	16.5a	12.4b	5.7c	5.5c	1.8	18.3	3.4
Color (sensorial)	Yellow to green	Yellow to orange	Yellow to orange	Yellow to orange	-	-	-
Titrable acidity as malic acid on fwb	0.18a	0.20a	0.20a	0.20a	0.02	9.4	0.03
PH	6.9a	6.9a	6.5b	6.3c	0.06	0.9	0.1
Weight (g)	7.3c	8.3b	8.6b	13.0a	2.7	2.9	5.0
Volume(cm^3)	9.0c	10.4b	10.6b	13.8a	3.0	2.8	5.7
Length (mm)	26c	29.3b	29.3b	30.7a	0.5	1.7	0.9
Diameter (mm)	22c	23b	23b	23.6a	0.3	1.3	0.5
Flesh weight (g)	6.3c	7.2b	7.7b	11.9a	2.8	3.3	5.2
Seed weight (g)	1.0a	1.1a	1.1a	0.9b	0.7	7.1	1.3
Calax weight (g)	0.4d	0.7c	1.0b	1.3a	0.1	10.1	0.2
Softening ratio %	0.0c	1.7bc	3.3ab	5.3a	1.2	-	2.3

*1st harvest was done at 8/8/2011; the 2nd at 16/8/2011, the 3rd at 24/8/2011 and the 4th at 5/9/2011;**SD: standard deviation; CV: coefficient of variation; LSD: least significant difference ***Means in each row followed by the same letter are not significantly different at 95% confidence.

Fig. 1 - 1st harvest of 'Barhi' dates at Balah stage (8/8/2011).

Fig. 2 - 2nd harvest of 'Barhi' dates at Balah stage (16/8/2011).

while the 4th harvest was conducted at the end of the stage (05/09/2011). Regarding the chemical properties, it can be seen from Table 1, that moisture, fiber, pectin, tannins, pH, pigments and ash levels tended to decline gradually by progressing of harvesting time. On the other hand, total soluble solids (Brix) tended to increase while titrable acidity and total sugars were almost stable by progressing of harvesting time. These results agree partly with those reported by GOLSHEN and FOOLADI (2006) who studied the physico-chemical properties of Iranian Shamasaei dates during progressive levels of maturity. However, those researchers reported lower tannins content (1.1%) and higher acidity levels (0.40%) for the same date variety at Balah stage. Moisture, ash, and total sugars contents of 'Barhi' dates at Balah stage grown in UAE were found to be close to those reported in this study (AHMED et. al.,1995). Furthermore, lower levels of fiber (1.5%) and tannins (1.25%) in Egyptian Sammany dates at Balah stage were reported by OSMAN (2008). Additionally, ELMARDI *et al.* (2002) found almost similar pectin results (1.8-2.3%) for Omani 'Fard' dates at Balah stage. Such differences in the chemical properties of date fruits at Balah stage might be attributed to variation in varieties and environmental conditions. Statistical analysis for data in Table 1 showed that the harvesting time affected significantly ($p \leq 0.05$) all of the studied chemical parameters except titrable acidity. Furthermore, the two statistical criteria given in Table 1 (SD and CV) reveal the acceptable accuracy of the results; it is well established that the acceptable coefficient variation (CV) should be less than 20; the maximum CV recorded for the chemical parameters was for color and was 18.3 which is considered acceptable.

3.2 Effect of harvesting time on the physical properties

Taking the physical properties into consideration, data in Table 1 and Figs. 1 and 2 show that the color of date fruit at Balah stage changed from yellow/green to yellow/orange by progressing of harvesting time. Remarkable increase in fruit weight, fruit volume, flesh weight, calyx weight and softening ratio was noticed by progressing of harvesting time. A sharp increase was clear for most studied physical properties at the 4th harvest which was conducted at the end of Balah stage. However, fruit length and diameter achieved a moderate increase by progressing of harvesting time whereas seed weight was almost stable at the 1st, 2nd and 3rd harvesting time and decreased by the fourth. Fruit weight, fruit volume, fruit length and seed weight of 'Barhi' dates at Balah stage ranged between 7.3-13.0 g, 8.9-13.8 cm^3, 26.0-30.7mm and 0.87-1.07 g, respectively. Statistical analysis for data related to the physical properties of Balah samples indicated the presence of significant effect ($p \leq 0.05$) of harvesting time on all those physical properties. Additionally, the calculated CV for all physical parameters was acceptable (less than 20).The obtained physical results are confirmed by earlier results for other date varieties at Balah stage reported by other researchers (OSMAN, 2008; GOLSHEN and FOOLADI, 2006; ELMARDI *et al.*, 2002; Ahmed *et al.*, 1995). However, lower fruit weight values (7.4 g) were reported for 'Barhi' dates at Balah stage grown under Indian environment (FAGERIA *et al.*, 1998).

3.3 Sugar composition results

Results of the sugar composition in fresh and stored 'Barhi' dates at Balah stage are presented in Tables 2-5. Total sugars ranged between 77.5-85.7% 0n dry weight basis (dwb). Higher values for total sugar were observed for fresh date fruits (80.6-85.7), whereas lower values (79.2-85.5) were characteristic of date fruits stored at 5°C for 4 weeks. It is clear from data in Tables 2-5 that the effect of harvesting time on the total sugars of fresh and stored Balah samples was not apparent. However, the higher total sugar content in 'Barhi' dates at Balah stage affects positively their sensory properties and enhance their acceptability for consumers specially for Muslims in the holy fastening month "Ramadan" where relatively huge quantities of dates at Balah, Rutab and Tamer stages are consumed. In spite of the little variation in total sugar content, they were affected significantly ($p \leq 0.05$) by both harvesting time and storage temperature. Howev-

Table 2 - Effect of harvesting time on the sugar compositions and proportions of fresh "Barhi" dates at Balah stage (on dwb).

Sample	Fructose	Glucose	Sucrose	Total sugars	Glucose/fructose ratio
Fresh 1st harvest*	38.6a**	41a	6.3ab	85.7a	1.1
Fresh 2nd harvest	32.8b	42.4a	7.2ab	82.8ab	1.3
Fresh 3rd harvest	33.9b	43.4a	5.4b	82.9ab	1.3
Fresh 4th harvest	31.9b	40.5a	7.9a	80.6b	1.3
SD***	2.4	1.5	1.0	2.2	-
CV	7.0	3.7	15.2	2.7	-
LSD	4.6	2.9	1.9	4.2	

*1st harvest was done at 8/8/2011; the 2nd at 16/8/2011, the 3rd at 24/11/2011 and the 4th at 5/9/2011; **Means in each column followed by the same letter are not significantly different at 95% confidence;***SD: standard deviation; CV: coefficient of variation; LSD: least significant difference.

Table 3 - Effect of harvesting time on the sugar compositions and proportions of "Barhi" dates at Balah stage stored at 5° C for 4 weeks (on dwb).

Sample	Fructose	Glucose	Sucrose	Total sugars	Glucose/fructose ratio
1st harvest*	32.4b**	39.4a	1.9b	79.2b	1.2
2nd harvest	37.3a	39.3a	3.5a	79.6b	1.1
3rd harvest	34.2b	41.4a	3.8a	79.9b	1.2
4th harvest	39.5a	42.5a	3.5a	85.5a	1.1
SD***	1.6	1.8	0.8	1.5	-
CV	4.4	4.4	23.8	1.8	-
LSD	3.0	3.4	1.4	2.8	-

*1st harvest was done at 8/8/2011; the 2nd at 16/8/2011, the 3rd at 24/11/2011 and the 4th at 5/9/2011; **Means in each column followed by the same letter are not significantly different at 95% confidence;***SD: standard deviation; CV: coefficient of variation; LSD: least significant difference.

Table 4 - Effect of harvesting time on the sugar compositions of "Barhi" dates at Balah stage stored at 0°C for 4 weeks (on dwb).

Sample	Fructose	Glucose	Sucrose	Total sugars	Glucose/fructose ratio
1st harvest*	38.0a**	43.6a	8.5a	81.9ab	1.1
2nd harvest	33.3a	40,1b	11.1a	84.8a	1.2
3rd harvest	32.8a	38.5b	11.5a	83.1ab	1.2
4th harvest	30.9a	39.2b	10.6a	80.7b	1.3
SD***	4.1	1.8	1.6	1.9	-
CV	12.1	4.5	18.2	2.3	-
LSD	7.7	3.4	3.0	3.6	-

*1st harvest was done at 8/8/2011; the 2nd at 16/8/2011, the 3rd at 24/11/2011 and the 4th at 5/9/2011; **Means in each column followed by the same letter are not significantly different at 95% confidence;***SD: standard deviation; CV: coefficient of variation; LSD: least significant difference.

Table 5 - Effect of harvesting time on the sugar compositions of "Barhi" dates at Balah stage stored at -5° C for 4 weeks (on dwb).

Sample	Fructose	Glucose	Sucrose	Total sugars	Glucose/fructose ratio
1st harvest*	32.3b**	41.6bc	2.8b	77.5b	1.3
2nd harvest	36.3a	39.5c	5.2a	80.6ab	1.1
3rd harvest	35.2ab	45.1ab	3.5ab	83.4ab	1.3
4th harvest	34.4ab	46.4a	3.4ab	84.3a	1.4
SD***	2,0	2.2	1.2	3.3	-
CV	5.8	5.2	32.2	4.0	-
LSD	3.7	4.2	2.3	6.2	-

*1st harvest was done at 8/8/2011; the 2nd at 16/8/2011, the 3rd at 24/11/2011 and the 4th at 5/9/2011; **Means in each column followed by the same letter are not significantly different at 95% confidence;***SD: standard deviation; CV: coefficient of variation; LSD: least significant difference.

er, the CV values for total sugar contents in the four treatments i.e. fresh, stored at 5°, 0°, and -5°C ranged between 1.8-4.0 indicating an acceptable accuracy for total sugar results.

Glucose percentages in fresh Balah samples (Table 2) ranged between 40.5-43.4%, while fructose ranged between 31.9-38.6% and sucrose ranged between 5.4-7.9%. Furthermore, glucose/fructose ratio ranged between 1.1-1.3. Data in Table 2 show that glucose was the 1st sugar followed by fructose and sucrose. Lower quantities of sucrose in 'Barhi' dates (5.4-7.9%) classifies 'Barhi' dates as soft dates (ALSAED, 2005). Glucose was not affected significantly ($p \leq 0.05$) by the harvested time while the other sugars were affected. Similar results for glucose/fructose ratio in 'Barhi' dates at Balah stage was reported by AHMED *et al.* (1995). The same authors reported that fresh 'Barhi' dates at Balah stage contain 78, 32.8, 29.5 and 15.5% total sugars, glucose, fructose and sucrose respectively on dwb. Those sugar composition results are slightly lower than what have been reported by this study with an exception for sucrose. Such variation might be attributed to differences in environmental conditions. However, results for sucrose in this study differ than those reported by ALMARDI *et al.* (2002) during their work on Omani 'Fard' dates. Such variation might be ascribed to varietal differences.

The same trend for sugar composition in Balah samples stored at 5°C for 4 weeks can also be observed (Table 3), i.e. no significant effect for the harvesting time on glucose content while the other sugars were affected. The glucose to fructose ratio was also between 1.1-1.2. Furthermore, Lower sucrose levels were observed in Balah samples stored at 5 C°. Such results might be attributed to the increased invertase activity at 5°C (BENJAMIN *et al.*, 1985).

It can be seen from data in Table 4 that fructose and sucrose were not affected significantly ($p \leq 0.05$) by harvesting time when samples were stored at 0°C for 4 weeks. Glucose to fructose ratio was almost the same as those for fresh Balah samples and those stored at 5°C (1.1-1.3). Something of interest in these results is the higher sucrose levels (8.5-11,5%) compared with the other two storage temperatures (5° and -5°C). Such results might be due to the inactivation of invertase at 0°C (BENJAMIN *et al.*, 1985).

Regarding the effect of harvesting time and storage at -5°C on the sugar composition of Balah samples, data in Table 5 reveal that all sugars were affected significantly ($p \leq 0.05$) by the harvesting time. More glucose than fructose was present (1.1-1.4) and relatively low quantities of sucrose were found.

Taking the statistical analysis of the sugar composition for stored Balah samples (stored at 0°, 5° and -5°C) into consideration, the statistical indicators in Tables 3-5 reveal that the CV values were less than 20% except for sucrose at 5° and -5°C storage treatments where the CV values exceeded 20% (23.8 and 32.2%). Such higher CV values which mean large variability might be due to personnel error during the extraction process for sugar analysis.

3.4. Effect of storage temperature and harvesting time on some Balah quality parameters

In Jordan, date producers used to get better prices for dates at Balah stage compared with Rutab stage. Accordingly, they try to find means and methods to extend the Balah stage and minimize the softening ratio or the change to Rutab.

It is clear from data in Table 6 that softening ratio was very low (less than 2 %) in fresh dates at the 1st and 2nd harvest; a significant increase ($p \leq 0.05$) in softening ratio occurred at the 3rd and 4th harvesting times. However, this increase is considered acceptable since it is less than 7 % (YOUSIF and ABOU-ALI, 1993). Storage results (Table 6) of date samples for 4 weeks at three temperatures (5°, 0°, -5°C) revealed that remarkable and significant ($p \leq 0.05$) increase in softening ratio (33-90%) was characteristic of the 1st harvest, with moderate one (20-30%) for the 2nd harvest and relatively low one for the 3rd and 4th harvest. Extreme softening results could be noticed for date samples stored at -5°C. As a result, date samples taken at the 3rd harvest could be stored successfully at 5° or 0°C for 4 weeks without softening. In other words, the 1st and 2nd harvest were not suitable to extend the duration of Balah stage.

Pectin content in 'Barhi' dates at Balah stage tended to decrease significantly ($p \leq 0.05$) by progressing of harvesting time. It ranged between 4.9% (on dwb) in the 1st harvest and 2.3% in the 4th harvest. Such decrease might be ascribed to the increased activity of pectinases. These results agree with those reported by ELMARDI *et al.* (2002). Storage at 5° and 0°C for 4 weeks had no significant effect ($p \leq 0.05$) on pectin content, whereas this effect was significant for date samples stored for 4 weeks at -5°C. Compared with other fruits, dates might be considered relatively a rich source for pectin (ALSAED, 2005). Such high pectin content confirm the distinguished nutritive value of dates (ALSHAHABI and MARSHALL, 2003).

Results in Table 6 also show that tannins content in 'Barhi' dates at Balah stage have the same trend as for pectin, i.e. there was a significant effect ($p \leq 0.05$) for both harvesting time and storage temperature on tannin content. The 3rd and 4th harvesting time were characteristic with low tannins (less than 5% on dwb). However, GOLSHAN TAFTI and FOOLADI (2006) reported lower values for tannins (1.3% dwb) in Iranian Shamsaei dates at Balah stage.

Table 6 - Some quality properties of 'Barhi' dates at Balah stage as affected by harvesting, storage time and temperature.

Chemical attributes	Harvesting time			
	1st harvest*	2nd harvest	3rd harvest	4th harvest
		Softening ratio		
Fresh	0c**	1.7bc	3.3ab	5.3a
stored at 5°C for 4 wks	33.3a	20a	10a	10a
stored at 0°C for 4 wks	33.3a	23.3ab	13.3bc	6.7c
stored at -5°C for 4 wks	90a	30bc	46.7b	13.3c
		Pectin % (on dwb)		
Fresh	4.9a	4.4b	2.1b	2.3b
stored at 5°C for 4 wks	5.3a	4.1a	4.7a	4.4a
stored at 0°C for 4 wks	5.2a	4.5a	4.9a	4.3a
stored at -5°C for 4 wks	3.7b	5.5a	4.9ab	4b
		Tannins % (dwb)		
Fresh	10.5a	8.73b	5.0c	2.8d
stored at 5°C for 4 wks	6.5a	7.3a	3.97b	2.0c
stored at 0°C for 4 wks	1.3c	3.7a	2.5b	1.6c
stored at -5°C for 4 wks	4.8a	3.9b	3.3b	1.77c
		Brix		
Fresh	20d	23c	30b	35a
stored at 5°C for 4 wks	22.7c	23c	32b	38a
stored at 0°C for 4 wks	23d	28c	36b	40a
stored at -5°C for 4 wks	25b	28b	38a	42a
		pH		
Fresh	6.9a	6.9a	6.5b	6.3c
stored at 5°C for 4 wks	6.6a	6.1c	6.3b	6.5a
stored at 0°C for 4 wks	6.1c	6.0c	6.9b	7.3a
stored at -5°C for 4 wks	7.4a	6.9c	7.2b	7.2b

*1st harvest was done at 8/8/2011; the 2nd at 16/8/2011, the 3rd at 24/11/2011 and the 4th at 5/9/2011; **Means in each row of each property (pectin, tannins, etc.) followed by the same letter are not significantly different at 95% confidence.

Concerning Brix and pH, results in Table 6 indicate a significant increase in Brix or total soluble solids by progressing of harvesting time and the opposite was true for the pH, i.e. it was decreased by progressing of harvesting time. These results were confirmed with those reported by ALMARDI *et al.* (2002) and GOLSHAN TAFTI and FOOLADI (2006).

3.5 Sensorial results

Sensory scores could be considered the most important quality parameter for dates at Balah stage from the consumer point of view. It is clear from Data in Table 7, that Balah samples taken at the 1st, 2nd and 3rd harvest either fresh or stored for 4 weeks at 5°, 0°, -5°C achieved the lowest sensory

Table 7 - Sensory evaluation results of fresh and stored "Barhi" dates at Balah stage as affected by harvesting time.

	Harvesting time						
	1st harvest	2nd harvest	3rd harvest	4th harvest	SD*	CV	LSD
Panelists scores			Fresh dates				
Average of total scores	53c**	58c	65b	73a	2.4	3.8	6.5
Panelists scores*			Dates stored at 5°C for 4 wks				
Average of total scores	56b	61a	64a	61a	1.5	2.4	4.1
Panelists scores*			Dates stored at 0°C for 4 wks				
Average of total scores	50.5d	57.5c	67.5b	82.5a	0.7	1.1	2.0
Panelists scores*			Dates stored at -5°C for 4 wks				
Average of total scores	68b	60.5c	67.5b	79a	1.5	2.2	4.2

*SD: standard deviation; CV: coefficient of variation; LSD: least significant difference; ** Means in each row followed by the same letter are not significantly different at 95% confidence

scores (between 50.5 and 68 on a scale of 90), indicating an inferior quality that might be ascribed to high tannin and pectin and low total solid contents. On the contrary, higher sensory scores were achieved by Balah samples taken at the 4th harvest. This indicates that the 4th harvesting time (5 September) was the optimum with regard to dates quality at Balah stage. Taking the storage temperature for Balah samples taken at the 4th harvest, it is apparent from data in Table 7 that Balah samples stored at 0° and -5°C achieved the best sensory scores (82.5/90 and 79/90 respectively). Furthermore, the lowest sensory score was given to date samples stored for 4 weeks at 5°C. This might be due to the bad effect of the relatively high storage temperature(5°C) and increased enzyme activity on some of the quality parameters of Balah specially color, flavor and texture.

In conclusion, the Balah stage for 'Barhi' dates can be successfully extended for 4 weeks if they were harvested at the end of the stage (4th harvest) and stored at 0° or -5°C for 4 weeks.

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REFERENCES

Abdullah A. and Thomson A.K. 1998. Effect of temperature on the storage of Rutab dates harvested at different maturity stages. The 1st International Conference on Date Palm, March 8-10, Al-Ain, UAE.

Ahmed I.A., Ahmed A.K. and Robinson R.K.1995. Chemical composition of date varieties as influenced by the stage of ripening. Food Chemistry, 54, 305-309.

Alansari A.M. 2008. Hydrocooling rates of "Barhi" dates at the Khalal stage. Postharvest Biology and Technology, 48, 402-407.

Alfarsi M.A. Morris E. Baron C. and Alasalvar. 2003. Comparison of antioxidant activity, phenolics, carotenoids and Anthocyanins of 3 native fresh and dried Omani dates. Fourth Int. Conference & Exhibition on Nutraceuticals and Functional foods, Sep. 28-1 Oct. 2003 Las Vegas, USA.

Al-Ogadi H.K. 1983. A Preliminary report on the results of freezing experiment of Bahraini dates(Beser). (Unpublished report).

Al-Redhaiman K.N. 2005. chemical changes during storage of "Barhi" dates under controlled atmosphere conditions. Hortscience, 40 (5): 1413-1415.

Alsaed A.K. 2005. Date Processing. Jordanian Association for Agricultural Engineers, pp42, Amman, Jordan (Arabic).

Alsaed A.K. 2010. Fruit and vegetables processing. 1st Edition, pp. 500, Scientific Research Council, University of King Saud, Saudi Arabia (Arabic).

Alshahabi, W. and Marshall R.J. 2003. The fruit of date palm: its possible use as the best food for the future. Int. J. Food Sci. Nutr., 54 (4), 247-259.

Al-Yahyai R. and Al-Kurusi L. 2012. Physical and chemical quality attributes of freeze-stored dates. Int. J. Agric. Biol., 14:97-100.

AOAC. 1995. Association of Official Analytical Chemists. Official Methods of Analysis. 16th Ed., Virginia.

Benjamin N.D. Shaban H.R., Al-Shaker S.A., Maisore M.S and Ibrahim T.K. 1985. Cold storage of date fruit at rutab stage. I. Determination of suitable cultivars and storage duration . Journal of Agriculture and Water resources Research Canter of Iraq (JRAWR). 4(2), 209-299.

Dawson W. And Aten A. 1962. Dates handling, processing and packaging. Food and Agriculture Organization of the United Nations, Rome, Italy.

Desroister N and Tressler D.K. 1977. Fundamentals of food freezing. Westport, Connecticut: The AVI Publishing Company, INC.

Elmardi M.O., Esechie H., Al-Kharousi L.M. and Abdelbasit K.M. 2002. Effect of pollination method on changes in physical and chemical characteristics of date fruit during development. Agriculture Sciences, 7, 21-27.

El-Nakhal H. and Al-Kahtani M.S. 1986. Ripening of date fruits by freezing. J. Coll. Agric., King Saud Univ., 8 (2), 325-335.

Fageria M.S. Dhaka R.S. and Chaudhary N.L. 1998. Determination of maturity standards of dates. Proceedings of the 1st International Conference on Date Palm, March 8-10, Al-Ain, UAE.

Golshan Tafti A. and Fooladi M.H. 2006. A study on the physic-chemical properties of Iranian 'Shamasaei' date at different stages of maturity. World J. of Dairy & Food Sciences, 1, 28-32.

Gorn Kh., Alrawabdeh F. and Alquasim M. 2008. Agricultural calendar of date palm in Jordan. Ministry of Agriculture, Amman, Jordan.

JGSD. 2011. Jordanian General Statistics Directorate. Amman, Jordan.

Langemeier J. and Rogers D. 1995. Rapid method for sugar analysis of dough and baked products. Am. Assoc. Cereal Chem., 72: 349-351.

Maier V.P. and Schiller F.H. (1960). Study on domestic dates. I. Methods of evaluating darkening. Food Technol., 14, 139-142.

Mikki M., and Al-Taisan S. 1993. Physico-chemical changes associated with freezing storage of date cultivars at their Rutab stage of maturity. Proceedings of the 3rd Date Palm Symposium,17-20 Jan., Hofuf, Saudi Arabia.

Norman S.M., Houck L.G., Fouse D.C., Sinder J.W., Burkner P.F., Perkins R.M. and Nash P.A. 1976. Changes in quality of field run dates under various combinations of outdoor and refrigerated storage. Date Grower's Inst. Rpt. 53: 9-17.

Ogunrinola, O., Jeon J. and Ponte, G. 1988. Functional properties of hydrolyzed whey permeate syrups in bread formulation. J. Food Sci., 53: 215-217.

Osman S.M. 2008. Fruit quality and general evaluation of 'Zaghloul' and 'Samany' date palms cultivars grown under conditions of Aswan. American-Eurasian J. Agric. & Environ. Sci., 4, 230-236.

SAS. 2002. SAS-User's Guide, Statistics, Version 9. SAS Institute Inc., Carry, NC., USA.

Sawaya W.N. 1986.Dates of Saudi Arabia. Ministry of Agriculture and Water, Riyadh, Saudi Arabia.

Swingle L.1926. Cold storage of dates. Date Grower's Inst. Rpt. 3:3-6.

Yousif A.K. 1996. Processing, shelf-life and evaluation of plain and chocolate coated date bars. Basrah J. of Agricultural Science, 9,(1).

Yousif A.K. and Abou Ali M. 1993. Suitability Of some Saudi dates at Rutab stage for storage by cooling and freezing techniques. Proceedings of the 3rd Date Palm Symposium,17-20 Jan., Hofuf, Saudi Arabia (Arabic).

Yousif A.K. and Alghamdi A.S. 1999. Suitability of some date cultivars for jelly making, J. Food Sci. & Technol., 36,515-518.

Yousif A.K. and Alghamdi A.S. 2000. Suitability of some Saudi date cultivars for jam making. J. King Saud Univ., 12,41-50.

Yousif A.K., Benjamin N.D., Kado A., Mehi-Alddin Sh. and Ali S.M. 1982. Chemical composition of Iraqi dates. Date Palm J. 1,(2),285-294.

Yousif A.K., Hamad A.M. and Mirandella W.A. 1985.Pickling of dates at the early khalal stage. J. Food Technology, 20, (6), 697-702.

Yousif A.K., Morton I.D. and Mustafa A.I. 1991. Effect of storage and packaging on the chemical and physical properties of date paste. Tropical Science,31,159169.

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PAPER

ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT OF MAPO TANGELO

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ABSTRACT

The antioxidant properties of Mapo Tangelo fruit and leaves extracts were investigated. The content of health-promoting components (total phenolics, flavonoids and ascorbic acid) were evaluated by spectrophotometric assays. Higher concentrations of total phenolics, flavonoids and ascorbic acid were found in peel samples extracted with boiling water, followed by leaves extracted with boiling ethanol. The radical-scavenging activity of the extracts was evaluated by different *in vitro* tests (total antioxidant activity, DPPH, ABTS and β-carotene bleaching tests). The trend observed for bioactive compounds was confirmed by antioxidant activity assays and good correlation values were obtained with total phenolics, flavonoids and ascorbic acid.

- Keywords: antioxidant, leaves, Mapo Tangelo, peel, pulp, radical-scavenging activity -

INTRODUCTION

Mapo tangelo (*Citrus deliciosa Ten. x C. paradisi Macf.*) is widely grown in Italy where it is valued for its earliness, juiciness and taste. It is a hybrid between Avana mandarin and Duncan grape fruit obtained in 1950 at the Citrus Experimental Institute of Acireale (Italy) and released for cultivation in 1972. Considerable efforts have been devoted to the determination of the antioxidant activity of several citrus fruits using either the fruit itself, its extracts or juice and it has been found to possess antioxidant, anti-inflammatory, anti-tumor and anti-fungal activities (WANG *et al.*, 2011). In this context, the aim of this research was the evaluation of antioxidant properties of the Mapo tangelo pulp, peel and leaves. Different extraction protocols and solvent mixtures have been considered, in relation to the polarity of the compounds to be extracted. Bioactive compounds were evaluated in terms of total phenolic compounds, flavonoids and ascorbic acid content, while *in vitro* antioxidant activities were assessed by total antioxidant activity, DPPH and ABTS assays. Free radical scavenging properties of sample extracts were also measured by β-carotene bleaching kinetic reaction and the correlation between antioxidant properties and antioxidant compounds was reported.

MATERIALS AND METHODS

Materials and instrumentation

All chemical used were of high analytical grade, product of Sigma-USA. Solvents were HPLC-grade and provided by Fluka Chemika-Biochemika. UV-Vis spectra were recorded with a Jasco V-530 UV/Vis spectrometer (Jasco, Japan).

Plant materials and extraction procedure

To obtain a representative sample, Mapo tangelo fruits (*Citrus deliciosa Ten. x C. paradisi Macf.*) were randomly harvested from 20 plants in Sicily (South Italy). All samples were examined for integrity and absence of dust and insect contamination, and were freeze-dried and stored at -20°C until analysis. Fruits were washed with tap water and then in distilled water for three times before the peel and pulp fractions were carefully separated. The Mapo tangelo peel, composed of flavedo and albedo, were removed from pulp and powered in a conventional food mixer. The pulp was cut crosswise and powered in a conventional food mixer. Fresh leaves from Mapo Tangelo trees were manually picked on and washed with tap water and then with distilled water for three times and then powered in a conventional food mixer. A portion of the sample was extracted applying the procedures reported in Table 1. To fully understand the impact of the extraction method and to compare the solvent extraction efficiency, the same extraction protocol was repeated using water, ethanol and water/ethanol mixture (50/50, v/v) as extraction solvents. In a standard procedure, 50 g of sample were continuously extracted using a Soxhlet apparatus (400 mL, 10 h). Alternatively, a second extraction procedure was performed on pulp, peel and leaves for 96 h at room temperature. The recovery yields of the extraction procedure were also considered and the determination of total phenolic compounds was reported for all solvents and extraction methods (Table 1). All fractions were evaporated under reduced pressure and re-dissolved in a known volume of the solvent originally used for their extraction.

Determination of total antioxidant capacity (TAC)

The TAC of extracts was evaluated according to the method reported in literature (SPIZZIRRI *et al.*, 2011). Briefly, in five test tube, 0.3 mL of five different extract solutions were mixed with 1.2 mL of reagent solution (0.6 mol·L^{-1} H_2SO_4, 28.0 mol·L^{-1} Na_3PO_4, and 4.0 mol·L^{-1} $(NH_4)_2MoO_4$) to rise the final concentrations of 0.02, 0.05, 0.08, 0.11, 0.16 mg·mL^{-1}. The reaction mixture was incubated at 95°C for 150 min and, after cooling to room temperature, the absorbance of the mixture was measured at 695 nm to record the calibration curve. The TAC of the extracts was expressed as micromoles of gallic acid equivalents per gram of polar extract by using the equation obtained from the calibration curve of the antioxidant.

Evaluation of total phenolic content by Folin-Ciocalteu procedure

The amount of total phenolic equivalents was determined using Folin-Ciocalteu reagent procedure, according to the literature with some modifications (RESTUCCIA *et al.*, 2011). A 2.0 mL aliquot of four different extracts (0.02, 0.05, 0.08 and 0.20 mg·mL^{-1}) was mixed thoroughly with 1.0 mL of Folin-Ciocalteu reagent in a volumetric flask. After 3 min, 1.0 mL of Na_2CO_3 (7.5% w/w) were added, and then the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm to record a calibration curve and the correlation coefficient (R^2), slope and intercept of the regression equation were calculated by the method of least square. The amount of total phenolic compounds in the extracts was expressed as micromoles of gallic acid equivalents per gram of polar extract by using the equation obtained from the calibration curve (R^2 = 0.996) of the antioxidant.

Determination of total flavonoids (TF)

A slightly modified version of the spectrophotometric method was used to determine the TF concentrations of samples (SPIZZIRRI *et al.*, 2011). Briefly, in a graduate flask, 0.7 mL of five different alcoholic extract solutions (0.10, 0.25, 0.030, 0.045 and 0.060 mg·mL^{-1}) were mixed with 2.0 mL of distilled water followed by addition of 0.3 mL of a $NaNO_2$ solution (5% w/w). After 6 min, 600 µL of a $AlCl_3 \cdot 6\ H_2O$ solution (10% w/w) were added and allowed to stand for another 5 min before 2.0 mL of 1.0 mol·L^{-1} NaOH was added. The mixture was brought to 10.0 mL with distilled water and mixed well. The absorbance was measured immediately against the blank at 510 nm to record the calibration curve. The amount of TF in the starting material was expressed as micromoles of catechin equivalents per gram of initial material by using the equation obtained from the calibration curve of the antioxidant (R^2).

Ascorbic acid assay

A slightly modified version of the spectrophotometric method was used to determine the ascorbic acid concentrations of samples (ZHANG *et al.*, 2009). Briefly, in a graduate flask 1.0 mL of the polar extract was mixed with 1.0 mL of $1.5 \cdot 10^{-2}$ mol·L^{-1} ferric chloride and 1.0 mL of $1.5 \cdot 10^{-2}$ mol·L^{-1} potassium ferricyanide. The mixture was brought to 10.0 mL with distilled water and mixed well. Then, it was let to stand at 30 min at 20°C. Afterward, the solution's absorbance was measured at 735 nm against a reagent blank prepared with the same reagent concentrations except for ascorbate. The amount of ascorbic acid in the starting material was expressed as mass of ascorbic acid per gram of initial material by using the equation obtained from the calibration curve of the antioxidant (R^2).

Determination of scavenging effect on DPPH radical

In a standard procedure, extracts was allowed to react with the stable free radical DPPH with the aim of evaluating their free radical scavenging properties (SPIZZIRRI *et al.*, 2011). Six different extract concentrations (2.0, 4.0, 10.0, 20.0, 30.0 and 40.0 mg·mL^{-1}), were mixed with DPPH solution and, after 30 min, the absorbance of the remaining DPPH was determined at 517 nm. The scavenging activity was expressed as percent inhibition of DPPH radicals calculated according to the following equation:

$$Inhibition\% = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of a standard that was prepared in the same conditions, but without extracts, and A_1 is the absorbance of extract samples.

Determination of scavenging effect on ABTS radical cation

The scavenging activity towards the ABTS radical cation was assessed according to the literature with slight modifications (RE *et al.*, 1999). Radical cation ($ABTS^{\bullet+}$) was produced according to the literature and an aliquot of sample was mixed with 25.0 mL of ABTS radical solution. The mixture, protected from light, was incubated in a water bath at 37°C for 5 min. The decrease of absorbance at 734 nm was measured at the endpoint of 5 min. The antioxidant activity was expressed as a percentage of scavenging activity on ABTS radical according to equation (1).

β-Carotene-linoleic acid assay

The antioxidant properties of extracts were evaluated through measurement of percent inhibition of peroxidation in linoleic acid system by using the β-carotene bleaching test (RESTUCCIA *et al.*, 2011). Briefly, 1.0 mL of β-carotene solution (3.0 mg·mL^{-1} in chloroform) was added to 7.0 µL of linoleic acid and 70.0 µL of Tween 20. The mixture was then evaporated at 40°C for 10 min in a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately emulsioned with 35.0 mL of distilled water. The emulsion (5.0 mL) was transferred to five different test tubes, 1.5 mL of extract (0.015, 0.050, 0.090, 0.130 and 0.180 mg·mL^{-1}) was added to each tube and shaken in a water bath at 45°C for 60 min. The absorbance of the filtered samples and control was measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The measurement was carried out at the initial time (t = 0) and successively at 60 min. The antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene using the following equation:

$$AA > (1 - \frac{A_0 - A_{60}}{A_0^o - A_{60}^o})$$

where A_0 and A_{0° are the absorbance values measured at the initial incubation time for samples and control, respectively, while A_{60} and A°_{60} are the absorbance values measured in the samples and in control, respectively, at t = 60 min.

Statistical analysis

The results are presented as the average of five experiments and standard deviation (± SD). Data were analysed using one-way analysis of variance (ANOVA), and differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The extraction protocols, the recovery yields (%) related to the extraction procedures and total phenolic content are reported in Table 1. For all samples, higher yields and amount of total phenols was obtained by Soxhlet method respect to the maceration at room temperature, irrespective of the used solvent. This is due to the higher temperature of the Soxhlet protocol which improves the extraction efficiency by increasing the cell walls permeability, the solubility and thus the diffusion coefficients of the molecules of interest, and by decreasing the viscosity of the solvent (WANG *et al.*, 2011). Generally speaking, soluble phenolic compounds are extracted from plant materials using water, methanol, ethanol or acetone. Although methanol and methanol-water mixtures have been most commonly used for phenols extraction from citrus fruits, in this study ethanol was selected because it is environmentally benign and relatively safe to human health. Table 1 shows that pulp and peel extracts possess decreasing yields and total phenols content moving from pure water to pure ethanol, with both Soxhlet and maceration procedure, while data obtained for leaf samples by Soxhlet method show an opposite trend. On the other hand, the same samples extracted by maceration at room temperature produce higher values of yields and total phenols with enhancing ethanol concentration. These results are related with the composition of the sample, because crude phenolic extracts contain a complex mixture of compounds differing in their degree of polymerization and in the number and arrangement of both hydroxyl and methoxy groups on the aromatic rings. Moreover, pure ethanol showed the best results in terms of total phenols concentration in leaf samples while the better results for pulp and peel samples extracted by boiling water, this effect being related once again with the polarity of the extracted compounds. In *Citrus* species naringenin and hesperetin widely distribute as their glycoside form (i.e. naringin, neohesperidin, narirutin, and hesperidin). This was confirmed also for Tangelo fruits by RAMFUL *et al.* (2011) reporting the LC-DAD profile of the pulp extract where the most abundant flavonoids were narirutin, hesperidin, neoeriocitrin, isorhoifolin, didymin, rutin and poncirin, while the same experiment dealing with Tangelo flavedo samples showed in decreasing order hesperidin, narirutin, neoeriocitrin, rutin, neohesperidin, isorhoifolin, poncirin, diosmin, didymin and rhoifolin as the most abundant phenols (RAMFUL *et al.*, 2010). As the presence of attached sugars tends to render the phenolic compounds more water soluble, the obtained results for peel and pulp samples reflect the higher affinity of the extracted compounds for water in comparison with pure ethanol. The difference among the concentrations of phenolic compounds between pulp, peel and leaf samples was significative, and higher phenolic compounds were always found in peel samples extracted with boiling water (12.45 μmol of gallic acid equivalent g^{-1}), followed by leaves extracted with boiling ethanol (9.11 μmol of gallic acid equivalent g^{-1}) and finally by pulps extracted with boil-

Table 1 - Extractive procedures applied to pulp, peels and leaves samples of Mapo Tangelo fruit. Recovery yields and total phenols measured in pulp, peel and leaves extracts of Mapo Tangelo fruit. Data expressed as mean value ± standard error (n = 5).

Matrix	Weight (g)	Extraction solvent	Extraction method	Time	Recovery yield*	Total phenolic content**	
		Water (mL)	*Ethanol (mL)*		(h)	[%]	(μmol·g^{-1})
Pulp	50.0	400.0	/	Soxhlet extraction	10.0	8.26±0.30	6.00±0.57
Pulp	50.0	200.0	200.0	Soxhlet extraction	10.0	4.51±0.28	4.34±0.39
Pulp	50.0	/	400.0	Soxhlet extraction	10.0	2.82±0.17	1.41±0.28
Pulp	50.0	400.0	/	Maceration at r.t.	96.0	0.65±0.09	0.21±0.03
Pulp	50.0	200.0	200.0	Maceration at r.t.	96.0	0.25±0.03	0.18±0.02
Pulp	50.0	/	400.0	Maceration at r.t.	96.0	0.23±0.04	0.04±0.01
Peels	50.0	400.0	/	Soxhlet extraction	10.0	3.46±0.31	12.45±0.80
Peels	50.0	200.0	200.0	Soxhlet extraction	10.0	4.64±0.43	8.21±0.48
Peels	50.0	/	400.0	Soxhlet extraction	10.0	3.38±0.27	5.38±0.40
Peels	50.0	400.0	/	Maceration at r.t.	96.0	0.36±0.07	0.57±0.06
Peels	50.0	200.0	200.0	Maceration at r.t.	96.0	0.23±0.03	0.18±0.03
Peels	50.0	/	400.0	Maceration at r.t.	96.0	0.13±0.02	0.09±0.02
Leaves	50.0	400.0	/	Soxhlet extraction	10.0	6.98±0.32	5.43±0.44
Leaves	50.0	200.0	200.0	Soxhlet extraction	10.0	3.12±0.34	8.17±0.53
Leaves	50.0	/	400.0	Soxhlet extraction	10.0	1.60±0.12	9.11±0.68
Leaves	50.0	400.0	/	Maceration at r.t.	96.0	0.06±0.01	0.27±0.05
Leaves	50.0	200.0	200.0	Maceration at r.t.	96.0	0.33±0.02	0.38±0.06
Leaves	50.0	/	400.0	Maceration at r.t.	96.0	0.59±0.08	0.69±0.05

*Weight of extract per weight of matrix, **μmol of gallic acid equivalent per 1 g of matrix.

Table 2 - Flavonoids, ascorbic acid content and antioxidant activities measured in pulp, peel and leave extracts of Mapo Tangelo fruit. Data expressed as mean value ± standard error (n=5).

Extracts	Total flavonoids**	Total antioxidant capacity*	Ascorbic acid content	Scavenging effect IC_{50} (mg/mL)		β-Carotene-linoleic acid assay
	μmol·g⁻¹	*μmol·g⁻¹*	*μmol g⁻¹*	*DPPH radical*	*ABTS radical*	*IC_{50} (mg/mL)*
Pulp[1,a]	0.46±0.05	9.28±0.60	2.56±0.04	0.18±0.01	0.19±0.01	2.12±0.09
Pulp[2,a]	0.23±0.05	6.70±0.09	1.82±0.07	0.23±0.08	0.32±0.08	3.75±0.18
Pulp[3,a]	0.11±0.01	3.53±0.08	1.08±0.09	0.27±0.10	0.40±0.10	4.23±0.23
Peels[1,a]	0.90±0.04	15.94±0.75	5.39±0.05	0.04±0.01	0.06±0.02	1.08±0.19
Peels[2,a]	0.53±0.07	11.31±0.80	4.09±0.08	0.12±0.03	0.18±0.03	1.21±0.08
Peels[3,a]	0.38±0.01	7.98±0.39	1.99±0.10	0.21±0.08	0.26±0.08	2.38±0.10
Leaves[1,a]	0.39±0.06	9.03±0.42	1.70±0.02	0.19±0.06	0.18±0.06	2.85±0.20
Leaves[2,a]	0.58±0.04	11.07±0.19	3.24±0.07	0.13±0.04	0.23±0.04	1.97±0.15
Leaves[3,a]	0.63±0.06	12.48±0.51	4.43±0.10	0.10±0.03	0.29±0.03	1.32±0.16

[1]Water; [2]Water/ethanol 50/50 (v/v); [3]Ethanol.
[a]Hot continuous extraction.
*μmol of gallic acid equivalent per 1 g of matrix; **μmol of catechin equivalent per 1 g of matrix.

ing water reaching the maximum value of 6.00 μmol of gallic acid equivalent g^{-1}. The higher values of phenolics obtained in peels, in comparison with those found in pulps, confirm the data obtained in previous studies on citrus fruits (GORISTEIN *et al.*, 2004a; BARRECA *et al.*, 2011; WANG *et al.*, 2011). However, RAMFUL *at al.* (2011) reported, for five Tangelo peels, concentrations of phenolic compounds ranging from 3,000 to about 6,200 μg of gallic acid equivalents g^{-1} which are much higher than those reported in this study. However, it should be considered that in the cited research, a significant concentration of phenols was accomplished before extraction, because portions of the peripheral peels of each variety were lyophilised for 48 h. Moreover it should be underlined that, besides analytical parameters, other factors can influence the phenolic content such as genetic differences, geographical origin and agronomic practices. As far as pulps are concerned, concentrations found in mesocarpes are in accordance with those obtained for pulps of other Tangelo fruits by RAMFUL *et al.* (2011) generally exceeding 1,000 μg g^{-1}. In Table 2 the values of total flavonoids (TF), ascorbic acid content and total antioxidant capacity (TAC) of Mapo Tangelo pulp, peel and leaf samples extracted by Soxhlet method are reported. Data highlight that the same trend observed for total phenols are confirmed for TAC, TF and ascorbic acid content: higher values for pulp and peel samples are obtained with water, while pure ethanol shows the best results for leaf samples. WANG *et al.* (2011) found that flavonoid content in different citrus fruit pulps ranged from 8.41 to 21.6 mg rutin equivalent g^{-1} DW, while GORINSTEIN *et al.* (2004a) found a total flavonoid content of 47.12±4.1 in peeled hybrids and 37.7±3.2 in peeled white grapefruits (mg catechin/100 g FW). More recently, RAMFUL *et al.* (2011) reported for Tangelo pulps a range of about 480-620 μg of quercetin g^{-1} FW of plant material. Higher levels of total flavonoids were obtained in extracts of a Tangelo peels (5,615±93 μg of quercetin g^{-1} FW) (RAMFUL *et al.*, 2010) which is once again much higher than flavonoid concentrations obtained for peel samples in our research. TF in leaf samples are comparable with those (178±13 mg kg^{-1} FW) obtained by LC-MS by BARRECA *et al.* (2011) for Chinotto (*Citrus myrtifolia Raf.*) leaves, while MENICHINI *et al.* (2011) found for *C. medica cv Diamante* a concentration of TF of 97.5±2.8 mg 100 g^{-1} FW (expressed as quercetin equivalents). Table 2 shows that ascorbic acid contents for Mapo tangelo pulps are comparable with those obtained by RAMFUL *et al.* (2011) for Tangelo fruits, ranging from about 0.25 to about 0.52 mg mL^{-1}. Higher ascorbic acid concentration was found in peel (5.39 μmol g^{-1} FW). This data is comparable with that found in other tangelos evaluated by RAMFUL (2011) reporting a range of ascorbic acid concentration from 2.84 to 5.68 μmol g^{-1}. In addition, to investigate the TAC of the extracts, the phospho-molybdenum method have been applied; by taking into account values of the total phenolics reported in Table 2, it is possible to underline that 65, 78 and 73% of TAC of pulp (Soxhlet, 100% water), peel (Soxhlet, 100% water) and leaves (Soxhlet, 100% ethanol) samples can be related with molecules possessing a phenolic moiety. The radical-scavenging activity of the extracts was assessed by means of DPPH and ABTS assays. The IC_{50} values for both assays are given in Table 2, and the scavenging activities are depicted in Fig. 1 A-F. Peels extracted by water, possess the highest ability to scavenge DPPH radicals (IC_{50} of 0.04 mg mL^{-1}) followed by leaves extracted by boiling ethanol (IC_{50} 0.10 mg mL^{-1}) and pulps extracted by boiling water (IC_{50} 0.18 mg mL^{-1}). DPPH results of peel and pulp aqueous extracts were almost the same than the corresponding ABTS assay (IC_{50} 0.06 and 0.18 mg mL^{-1}). Hence, both

methods could be equally useful for assessing antioxidant activities of natural extracts at physiological pH and where colour interference is not significant. It has been reported that the effect of antioxidants on both DPPH and ABTS radical scavenging is related with their hydrogen-donating ability but, while DPPH is a useful reagent for the evaluation of antioxidant properties of compounds in organic media, the ABTS radical is widely applied to test food and natural water-soluble phenolics. Moreover, poor selectivity of ABTS in the reaction with H-atom donors is considered a limitation of the method as it reacts with any hydroxylated aromatics independently of their real antioxidative potential. In contrast, DPPH is more selective as it does not react with flavonoids, which contain no OH-groups in B-ring as well as with aromatic acids containing only one OH-group. Data shown in Table 2 seem to indicate hydrophilic features of compounds extracted from peel and pulp samples. On the contrary, water leaf extract seem to scavenge ABTS radicals better than the corresponding alcoholic extract, in contrast with DPPH assay results. These differences can be ascribed either to different phenols distribution in the diverse solvent mixtures or, if the extracts should contain the same phenols, to their different antioxidant action in relation with the environment. In fact, according to FINOTTI and DI MAJO (2003), all *Citrus* flavonoids have an antioxidant action in a hydrophilic environment while, in a lipophilic environment, some molecules (neohesperidin, hesperetin, didymin and isosakuranetin) show a reduced antioxidant capacity, and other (naringin, narirutin, naringenin, neoeriocitrin, heridictyol invert their behavior, becoming pro-oxidants. All extracts were also able to inhibit the discoloration of β-carotene. The results (Fig. 1 G-I) of effect of the samples on the autoxidation of linoleic acid are shown in Table 2. With a view to rationalizing the antioxidant po-

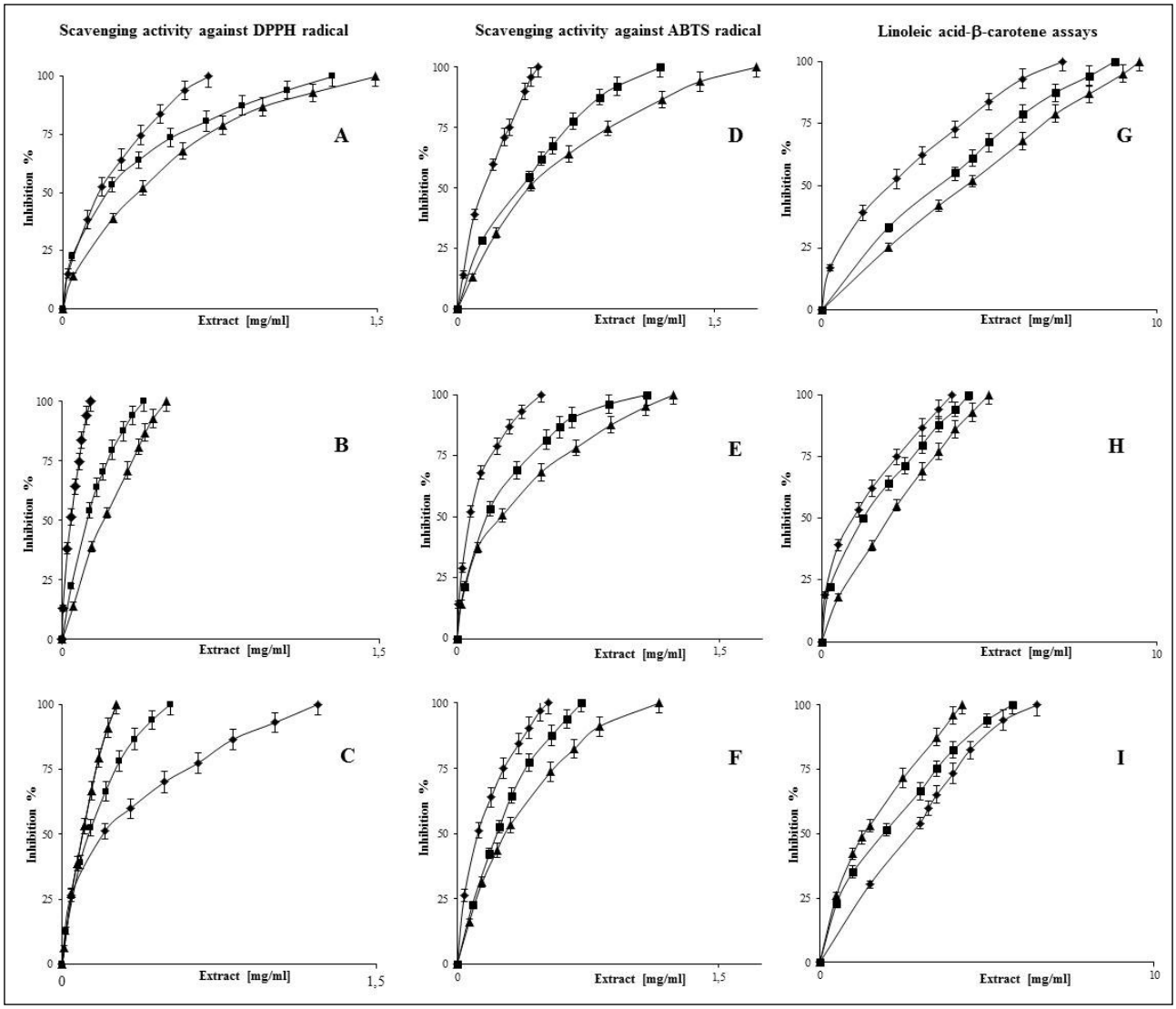

Fig. 1 - Scavenging activity against DPPH radical, ABTS radical and linoleic acid- β-carotene assays of Mapo Tangelo pulp (A, D, G), peels (B, E, H) and leaves (C, F, I) extracts with water (◆), ethanol (▲) and water/ethanol 50/50 (v/v) mixture (■).

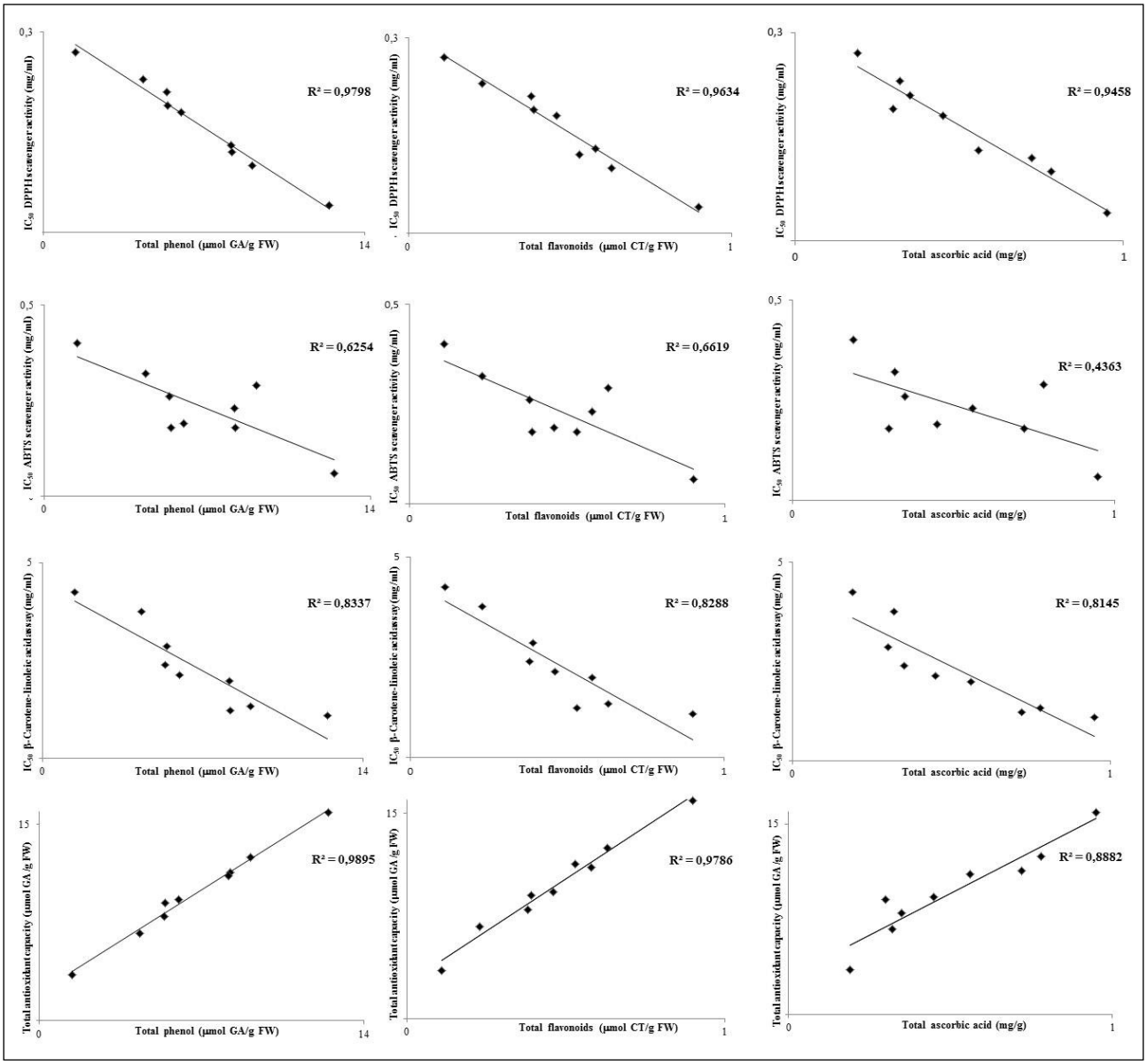

Fig. 2 - Linear regression plots of IC_{50} values of scavenging activity against DPPH and ABTS radicals and linoleic acid-β-carotene assays with respect to total phenols, flavonoids, ascorbic acid contents and total antioxidant activity of Mapo Tangelo pulp, peels and levees extract.

tential of the extracts in terms of their phytophenolic constituents, linear regression plots were generated (Fig. 2). A strong correlation between total phenols and antioxidant capacity extracts was noted (TAC, R^2 = 0.9895; DPPH, R^2 = 0.9798; β-carotene, R^2 = 0.8337) except for ABTS (R^2 = 0.6254). It has been demonstrated that ABTS and DPPH assays, even though they belong to the hydrogen atom transfer rather than electron transfer type of antioxidant tests, may yield different results, in relation with their individual mechanisms and kinetics of radical inactivation as well as with the solvent used for extraction. Extracts with the highest phenolic contents had the highest antioxidant potential in all assayed systems, whilst extracts characterised by low total phenolic levels exhibited poor antioxidant capacities as already reported by other studies (GORINSTEIN *et al.* 2004b; RAMFUL *et al.*, 2011; RAPISARDA *et al.*, 1999) thus suggesting that the antioxidant activity of the citrus extracts is not likely to be ascribed to the property of an individual compound but rather to the synergistic actions of several phytochemicals. The flavonoid levels also showed good influence on the antioxidant capacities of the extracts in all antioxidative systems as evidenced by the correlation coefficient values (TAC, R^2 = 0.9786; DPPH, R^2 = 0.9634; β-carotene, R^2 = 0.8288) (Fig. 2). Once again, moderate correlation was found for ABTS (R^2 = 0.6619). Finally, Fig. 2 also shows high correlations between ascorbic acid levels and antioxidant capacity of the extracts (TAC, R^2 = 0.8882; DPPH, R^2 =

0.9458; β-carotene, R^2 = 0.8145, ABTS, R^2 = 0.4363) supporting previous findings of GARDNER *et al.* (2000) and SÁNCHEZ-MORENO *et al.* (2003). On the other hand, several studies do not support these results, assessing that ascorbic acid contribution to antioxidant activity is small as phenolics of fruits are more active antioxidants than ascorbate and thus considering vitamin C a minor component compared with the free phenols of fruits (RAPISARDA *et al.*, 1999; RAMFUL *et al.*, 2010; RAMFUL *et al.*, 2011).

In conclusion this study this study evaluated the antioxidant potentials of pulp, peel and leaf extracts of Mapo Tangelo fruit. After the optimization of the extraction parameters, it was found that tissues generally contain more antioxidant compounds than pulp. These results have been confirmed by the antioxidant activities of the samples measured by three independent methods (DPPH assay, ABTS assay and β-carotene bleaching test). Obtained data showed that high total phenols content increases antioxidant activity and there is a linear correlation between the concentrations of phenols, flavonoids and ascorbic acid and the antioxidant activity. Considering the nutritional importance of the subject, further studies should be accomplished on the isolation and characterisation of individual compounds in Mapo Tangelo fruit and tissues to elucidate their different antioxidant mechanisms and the existence of possible synergism among them.

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REFERENCES

Barreca D., Bellocco E., Caristi C., Leuzzi U. and Gattuso G. 2011. Elucidation of the flavonoid and furocoumarin composition and radical-scavenging activity of green and ripe chinotto (*Citrus myrtifolia Raf.*) fruit tissues, leaves and seeds. Food Chem. 129: 1504.

Finotti E. and Di Majo D. 2003. Influence of solvents on the antioxidant property of flavonoids. Die Nahrung 47: 186.

Gardner P.T., White T.A.C., McPhail D.B. and Duthie G.G. 2000. The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. Food Chem. 68: 471.

Gorinstein S., Cvikrova M., Machackova I., Haruenkit R., Park Y.S. Jung S.T., Yamamoto K., Martinez Ayala A.L., Katrich E. and Trakhtenberg S. 2004a. Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. Food Chem., 84: 503.

Gorinstein S., Zachwieja Z., Katrich E., Pawelzik E., Haruenkit R., Trakhtenberg S. and Martin-Belloso O. 2004b. Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid. Leb. Wissen. Technol. 37: 337.

Menichini F., Loizzo M.R., Bonesi M., Conforti F., De Luca D., Statti G., de Cindio B., Menichini F. and Tundis R. 2011. Phytochemical profile, antioxidant, anti-inflammatory and hypoglycaemic potential of hydroalcoholic extracts from *Citrus medica L. cv* Diamante flowers, leaves and fruits at two maturity stages. Food Chem. Toxicol. 49: 1549-1555.

Ramful D., Bahorun T., Bourdon E., Tarnus E. and Aruoma O.I. 2010. Bioactive phenolics and antioxidant propensity of flavedo extracts of Mauritian citrus fruits: Potential prophylactic ingredients for functional foods application. Toxicol., 278: 75.

Ramful D., Tarnus E., Aruoma O.I., Bourdon E. and Bahorun T. 2011. Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. Food Res. Int. 44: 2088.

Rapisarda P., Tomaino A., Lo Cascio R., Bonina F., de Pasquale A. and Saija A. 1999. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. J. Agr. Food Chem. 47: 4718.

Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med. 26: 1231.

Restuccia D., Spizzirri U.G., Chiricosta S., Puoci F., Altimari I. and Picci N. 2011, Antioxidant activity of EVOO extracts from *Cerasuola cv* olive fruit. Ital. J. Food Sci. 123: 62.

Sánchez-Moreno C., Plaza L., de Ancos B. and Cano M.P. 2003. Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juices. J. Sci. Food Agr. 83: 430.

Spizzirri U.G., Restuccia D., Chiricosta S., Parisi O.I., Cirillo G., Curcio M., Iemma F., Puoci F. and Picci N. 2011. Olive stones as source of antioxidants for food industry. J. Food Nutr. Res. 50: 57.

Wang A.Y., Zhou M.Y. and Lin W.C. 2011. Antioxidative and anti-inflammatory properties of *Citrus sulcata* extracts. Food Chem. 124: 958.

Zhang H., Li J., Wang K., Du X. and Li Q. 2009. A simple and sensitive assay for ascorbate using potassium ferricyanide as spectroscopic probe reagent. Anal. Biochem. 388: 40.

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PAPER

POST-HARVEST QUALITY, PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY IN ORGANIC AND CONVENTIONAL KIWIFRUIT (*ACTINIDIA DELICIOSA*, CV. HAYWARD)

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ABSTRACT

The study provides original data on quality attributes and nutritional profile of organic and conventional kiwifruit grown in Italy (Lazio region). Data on macronutrients (protein, lipid, carbohydrate) total dietary fiber, minerals, trace elements, organic acids (citric, malic, oxalic) and bioactive molecules including ascorbic acid, carotenoids (lutein and β-carotene), tocopherols (α-tocopherol, γ-tocopherol, γ-tocotrienol) content are reported.Organic orchards displayed a lower yield but higher fruit performances (flesh firmness, dry matter, soluble solids) than conventional ones. Ascorbic acid content was significantly higher in organic kiwifruit (66 mg/100 g) than in conventional one (53 mg/100 g). Both lutein and β-carotene content was higher in organic kiwifruit than in conventional one. No significant differences in tocopherol content between cultivation systems were observed. Antioxidant activity was significantly higher ($p < 0.001$) in organic than in conventional fruit mirroring the trend reported for ascorbic acid.

- Keywords: organic kiwifruit, minerals, organic acids, oxalic acid, ascorbic acid, carotenoids, tocopherols, antioxidant activity -

INTRODUCTION

The cultivation of kiwifruit [*Actinidia deliciosa*, (A. Chev.) C.F. Liang et A.R. Ferguson var. deliciosa, cultivar Hayward] is spread throughout the world. Since the early '60s, when kiwi began to be cultivated in Italy, the cultivation of this fruit increased steadily, so far Italy has become the world's largest producer of Kiwifruit (TESTOLIN and FERGUSON, 2009). Kiwifruit is extensively grown in central Italy (Lazio region), about 37% of the Italian kiwifruit production is in the Lazio region that is about 10% of the world's production (TESTOLIN and FERGUSON, 2009). The high quality of kiwifruit grown in this area has led to the designation of Protected Geographical Indication (PGI) to the "Kiwi Latina" by the European Union (EU) since 2004. About 10% of the PGI kiwifruit orchards are cultivated according to organic agricultural practice, this geographical area indeed has favourable environmental conditions suitable to kiwifruit cultivation, this makes easier to apply low input cultivation systems.The physico-chemical features (BELTRAMO *et al.*, 2007) and the nutritional quality in relation to vine training system and genotype (D'EVOLI *et al.*, 2009) of conventional kiwifruit in the Lazio region (area of PGI "Kiwi Latina") have already been described. By contrast few comparative studies dealing with the postharvest quality of organic and conventional kiwifruit are available (HASEY *et al.* 1997; BENGE *et al.* 2000; AMODIO *et al.* 2007), but no data describing the nutritional quality of organic kiwifruit in the PGI "Kiwi Latina"area are available.

This study was designed to assess the physicochemical characteristics (weight, firmness, soluble solids, and titrable acidity) and the nutritional quality of organic and conventional kiwifruit (*cv* Hayward) grown in the same pedoclimatic condition: the orchards were in Cori locality in the"Kiwi Latina" PGI area. Fruit were analysed for their compositive profile, including macronutrients, total dietary fiber, minerals and trace elements, organic acids (citric, malic) and oxalic acid content. Furthermore, the concentration of bioactive molecules like ascorbic acid, carotenoids (lutein and β-carotene), tocopherols (α-tocopherol, γ-tocopherol, γ-tocotrienol) was quantified. Kiwifruits from both cultivation systems were also evaluated for the expression of their antioxidant activity.

MATERIALS AND METHODS

Vine and orchard characteristics and management

The study was carried out in two neighbouring farms growing with organic and conventional kiwifruit orchards in the PGI "Kiwi Latina" area (Cori). The area is suitable for the cultivation of kiwifruit because the climate is mild (frost is rare during spring and autumn) and temperatures during winter are never dangerous for the vines. Yearly rainfall ranges from 800 to 1,200 mm. The wind speed is rarely dangerous. Soils of both orchards had a clay texture, pH around neutrality, limestone in trace amount, about 2.7% organic matter and medium-high nutrient (N, K, P, Mg, Ca, Fe) content. Both organic and conventional orchards consisted of mature vines of the *cv* Hayward, with *cv* Matua as pollenizer (500 vines/ha). Vines were trained to the pergola system. In both orchards, vines were irrigated with a drip irrigation system, ensuring full resupplying of evapo-transpired water (etc.). Cultural practices differed for the kind of fertilisers used (chemical fertilizers in the conventional orchard and organic fertilizer in the organic one) and for the use of "Dormex" (hydrogen cyanamide), to enhance bud breakingin the conventional cultivation system.

Harvesting and post-harvest storage of kiwifruit

At harvesting (end of October), the yield (t/ha) was estimated from the actual crop production and recorded when the fruit soluble solids content was around 7°Brix. Fruit was immediately stored in normal atmosphere at T = 0°±0.5°C and RH > 90% for about 5 months. Kiwifruit were than sampled following the outlines of GREENFIELD and SOUTHGATE (2003). About 5 kg of fruit from each orchard were transported to the laboratory, equal amount of defect-free kiwifruit were randomly grouped into 4 batches per orchard. Each batch was homogenized and aliquots were taken for subsequent analyses.

Fruit weight, flesh firmness and soluble solids content

Fruit weight was determined by weighing 100 fruits per orchard. Fruit flesh firmness was determined on 100 fruits per orchard with a hand-held penetrometer (Effe.gi, Ravenna, Italy) with an 8 mm plunger, after removal of about 1 cm^2 of skin. The same fruits were used to determine the soluble solids content, as °Brix, by taking a juice sample from the equatorial part of each fruit using a hand-held refractometer (Model M, Atago, Japan). Fruit dry matter content was determined on 40 fruits by drying them at 105°C in a forced air oven to constant weight (AOAC, 1996).

Preparation of a fruit powder

Fruit powder was prepared for titratable acidity, carbohydrates, and organic acids analyses. Four samples per orchard were prepared, each composed of sub-samples (segments) of 8 fruits: hairs and skin were removed by scraping the

surface of each fruit with a sharp knife, then a segment representative of all the fruit tissues (outer and inner pericarp and columella) was removed from each fruit and rapidly frozen in liquid N_2. Samples were stored at -80°C. The frozen samples were ground to a fine powder under liquid N_2 (nitrogen powder) in a pre-cooled mortar and stored at -80°C until analysis.

Titratable acidity

Five grams of fruit powder were dissolved in 10 mL of distilled water. Titratable acidity was determined by titrating the solution with 0.1 N NaOH to pH 8.2. Results are expressed as g of citric acid per kg of fruit.

Proximate analysis

Moisture, protein, lipid and ash were determined according to AOAC methods (1996). The analyses were carried out on triplicate.

Carbohydrates and starch

Fifty mg of the fruit powder were extracted as described by FAMIANI *et al.* (2009), centrifuged and the supernatant analysed for glucose, fructose and sucrose content as described by JONES *et al.* (1977). The pellet, containing starch, was re-suspended and processed as described by ANTOGNOZZI *et al.* (1996) to completely hydrolyse the starch to glucose.

Total dietary fiber

Total dietary fiber was determined following the method of PROSKY *et al.* (1988). The analyses were carried out on triplicate.

Oxalic acid

One hundred mg of fruit powder were extracted in 1.5 mL of distilled water. Samples were then processed as described by PROIETTI *et al.* (2009) to extract both soluble and insoluble oxalic acid. Oxalic acid was determined following the method of BEUTLER *et al.* (1980).

Citric and malic acids

Citric and malic acid content was determined in the fruit extracts used to determine the soluble sugars content, by the enzyme-coupled spectrophotometric method described by LOWRY and PASSONNEAU (1972).

Ascorbic acid

Ascorbic acid was quantified according to the method of VALLS *et al.* (2002) by HPLC on an Alltima NH_2 column (0.46x25 cm, Alltech) at 248 nanometers with a photodiode array detector (HPLC/PDA) referring to the ascorbic acid standard calibration curve.

Minerals and trace elements

Minerals (Ca, Mg, Na, K, P) and trace elements (Fe, Zn, Cu, Mn) content was determined by ICP-Plasma (Optima 3200XL - Perkin-Elmer) following liquid ashing (4 mL HNO_3+1 mL H_2O_2) of the samples in a microwave digestion system (Milestone, 1200 Mega). Standard Reference Materials:Mixed diet (NBS 8431, National Bureau of Standards, Gathersburg, MD 20899) and Wholemeal flour (BCR 189, Community Bureau of Reference, Brussels) were analysed as a check on the accuracy of the analysis.

Carotenoids

β-carotene and lutein were extracted and quantified by HPLC following the method of SEYBOLD *et al.* (2004).

Tocopherols

α-tocopherol, γ-tocopherol, γ-tocotrienol were extracted and quantified using a HPLC/fluorescence detector as described by BALTZ *et al.* (1992).

Antioxidant activity

Kiwifruit crude extracts were prepared as described by MEYERS *et al.* (2003) and their antioxidant activity determined by FRAP assay (BENZIE and STRAIN, 1996). Results are expressed as mmol Trolox equivalent (TE) kg.

Statistics

Data are reported as the Mean±Standard Deviation of at least three analyses. Organic and conventional kiwifruits were compared by means of Student's *t*-test.

RESULTS AND DISCUSSION

Yield, physico-chemical characteristics and kiwifruit compositional profile of both organic and conventional orchards are reported in Table 1. The organic orchards showed a lower yield compared to the conventional one: 17.0 *vs* 33.5 t ha^{-1} (Table 1). The lower yield exhibited by organic orchard may be attributed to the restriction in the use of agronomical inputs in this cultivation. Indeed, in the present study the use of Dormex in conventional orchard (not allowed in organic cultivation) probably improved fruit production by increasing bud-breaking (INGLESE *et al.*, 1998). A lower yield of organic kiwifruit orchard was already observed by AMODIO *et al.* (2007). Organic kiwifruit showed flesh

Table 1 - Yield, physiso-chemical characteristics, macronutrients, starch, total dietary fiber, oxalic acid, organic acids, of organic and conventional kiwifruit (fresh weight).

		Cori orchards	
		Organic M±SD	Conventional M±SD
Yield	t ha^{-1}	17.0	33.5
Fruit weight	g	104±20	102±13
Flesh firmness	N	36±8a	28±9b
SSC	°Brix	13.8±1.3a	12.7±0.4b
Titratable acidit	mg/100 g	1.29±0.1a	1.18±0.04b
Moisture	mg/100 g	84±0.4a	86±0.2b
Ash	mg/100 g	0.8±0.09a	0.7±0.04a
Protein	mg/100 g	0.9±0.1a	0.9±0.08a
Lipid	mg/100 g	0.1±0.01a	0.1±0.01a
Carbohydrates	g/100g	11±0.4a	10±0.2b
Glucose	g/100 g	5±0.3a	4±0.1b
Fructose	g/100 g	5±0.3a	4±0.1b
Sucrose	g/100 g	1±0.1a	1±0.04 b
Starch	mg/100 g	0.07±0.02a	0.04±0.02b
Total diet. fiber	mg/100 g	2.4±0.1a	2.4±0.2a
Oxalic acid	mg/100 g	8.5±0.2a	8.5±1.6a
Soluble	mg/100 g	1.2±0.8a	1.8±0.6a
Insoluble	mg/100 g	7.4±1.8a	6.6±1.6a
Organic acids			
Citric acid	mg/100 g	1.4.±0.1a	1.2±0.2b
Malic acid	mg/100 g	0.3.±0.03a	0.2±0.02b
Ascorbic acid	mg/100 g	66±1.1a	53±1.2b

Values are the M±SD of three determinations.
Values followed by different letters are significantly different (*a vs b p<0.05)*

firmness, soluble solids and titratable acidity significantly higher than conventional one (Table 1). AMODIO *et al.* (2007) found in organic kiwifruit a soluble solids content similar to that found in this study, on the other hand previous studies (HASEY *et al.*, 1997; BENGE *et al.*, 2000; AMODIO *et al.*, 2007) did not find differences in flesh firmness between organic and conventional kiwifruit. Fruit firmness can be affected by several factors, such as fertilization (JOHNSON *et al.*, 1997), light exposure (ANTOGNOZZI *et al.*, 1995), calcium content (FRANCESCHI and NAKATA, 2005) and dry matter content (FAMIANI *et al.*, 2012). The highest flesh firmness showed by organic kiwifruit could be due to the lower moisture content compared to the conventional one (Table 1) and to the better lightening as a result of lower bud-breaking with a consequent lower number of shoots/vine due to the restriction in Dormex use. Flesh firmness is also considered an index of fruits storability, value over 2.5 N ensures higher fruit quality during storage. Thus our data (Table 1) indicate a high quality of PGI kiwi Latina in terms of storability.

Macronutrients profile of kiwifruit is reported in Table 1. Moisture content was significantly lower ($p<0.001$) in organic kiwifruit compared to the organic one. Several studies have already confirmed a trend toward a low moisture content with a concomitant higher nutrient content in organic products (WORTINGTON *et al.*, 2001; BOURNE and PRESCOTT, 2002). No significant differences in ash, protein, lipid, total dietary fiber content between organic and conventional kiwifruit were detected (Table 1). Conversely, carbohydrate content was significantly higher ($p<0.05$) in the organic kiwifruit: glucose, fructose and sucrose content was significantly higher ($p<0.05$) compared to the conventional ones (Table 1). AMODIO *et al.* (2007) did not find differences in simple sugar content between organic and conventional kiwifruit. Starch content was very low, indicating the ripened stage of the fruit after 5 months of storage (Table 1). Total dietary fiber content was similar in kiwifruit of both the cultivation systems (Table 1).Oxalic acid content in organic kiwifruit was reported for the first time in this study. Oxalic acid and its soluble and insoluble forms, were detected in very low amount in both organic and conventional kiwifruit with the insoluble form detected in much higher amount than the soluble form (Table 1). Therefore at the end of storage oxalic acid, especially the soluble form, do not adversely affect the quality of the fruit. Among organic acids, citric acid content was similar in both organic and conventional fruit, whereas malic acid content was significantly higher ($p<0.05$) in the organic fruit (Table 1). AMODIO *et al.* (2007) did not find differences in both citric and malic acids content between organic and conventional kiwifruit. Ascorbic acid content in organic kiwifruit was significantly higher ($p<0.05$) than in conventional fruit (66 *vs* 53 mg/100 g) (Table 1). The data reported in this study about the ascorbic acid content in conventional kiwifruit were in the range already reported in other studies (LEONG and SHUI, 2002; NISHIYAMA *et al.*, 2004; DU *et al.*, 2009; D'EVOLI *et al.*, 2009). In this study ascorbic acid appears to follow the trend of sugar content, in particular of glucose, which represents an important sugar of the metabolic pathway of ascorbic acid synthesis (LOEWUS, 1999). A number of organically grown fruit species have been found to contain higher amount of ascorbic acid compared to the conventional ones (WHORTINGTON, 2001; AMODIO *et al.*, 2007; DUARTE *et al.*, 2010). The use of 'compost' (rich in organic not easily available nitrogen) as a soil supplement has been shown to enhance ascorbic acid synthesis in organic fruit compared to those produced conventionally (ASAMI *et al.*, 2003; WANG and LIN, 2003), probably because it induces plants to first synthesize non-nitrogen-containing compounds. No significant differences in minerals and trace elements content between organic and conventional kiwifruit were observed (Table 2). Among carotenoids only lutein and β-carotene were detectable (Fig. 1). Lutein was the most abundant carotenoids in kiwifruit, its content in organic fruit was 0.19 mg/100 g,

Table 2 - Minerals and Trace Elements content in organic and conventional kiwifruit (f.w.).

		Cori orchard	
		Organic M±SD	Conventional M±SD
Ca	mg/100 g	23±1.8	21±4.3
P	mg/100 g	25±2.9	25±1.8
Na	mg/100 g	4.2±1.4	4.2±0.4
Mg	mg/100 g	12.5±0.5	11.4±1.0
K	mg/100 g	281±11	257±11
Fe	mg/100 g	0.23±0.4	0.19±0.02
Zn	mg/100 g	0.08±0.003	0.07±0.003
Mn	mg/100 g	0.04±0.003	0.03±0.004
Cu	mg/100 g	0.13±0.003	0.14±0.002

Values are the M±SD of three determinations.
No differences between means were observed.

a slightly lower amount was detected in conventional fruit (0.16 mg/100 g). β-carotene content was 0.05 mg/100 g in the organic fruit, a significantly higher amount ($p<0.05$) compared to that found in the conventional one (0.04 mg/100 g) (Fig. 1). Previous studies reported a carotenoids content in kiwifruit similar to that found in this study (CANO, 1991; D'EVOLI *et al.*, 2009), by contrast higher values were found by NISHIYAMA (2007); on the other hand a large variation especially in lutein content in fruit was known (HART and SCOTT, 1995). No differences in tocopherols content between organic and conventional kiwifruit were found (Fig. 2). Among tocopherols, α-tocopherol was the most represented (0.8 mg/100 g in both cultivations) followed by γ-tocotrienol (average 0.12 mg/100 g in both cultivations) and γ-tocopherol (average 0.04 mg/100 g in both cultivations) (Fig. 2). Finally, kiwifruit was also analyzed for expression of the antioxidant activity, measured in the hydrophilic extracts of the fruit as FRAP (mmol Trolox/100 g). The antioxidant activity expressed by organic fruit was significantly higher ($p<0.001$) than that of the conventional fruit (0.62 and 0.51 mmol Trolox/100 g, respectively) (Fig. 3). The differences in antioxidant activity observed in the two cultivation systems mirrored the trend reported for their respective ascorbic acid content (Table 1). Previous studies (DU *et al.*, 2009; PARK *et al.*, 2011) carried out on several *Actinidia* genotypes already highlighted a high direct correlation between antioxidant activity and both vitamin C and polyphenols content.

CONCLUSION

In this study a direct comparison of the nutritional quality of organic and conventional kiwifruit has been possible because both the cultivations were carried out in the same locality; this allowed the effect of the pedoclimatic conditions to be overcome. Our findings suggest that organic cropping system enhances the qualitative properties of kiwifruit and its storability. The nutritional traits of the kiwifruit analyzed in this study indicate that this fruit is an excellent vehicle in the diet of many bioactive molecules (e.g. ascorbic acid, carotenoids, to-

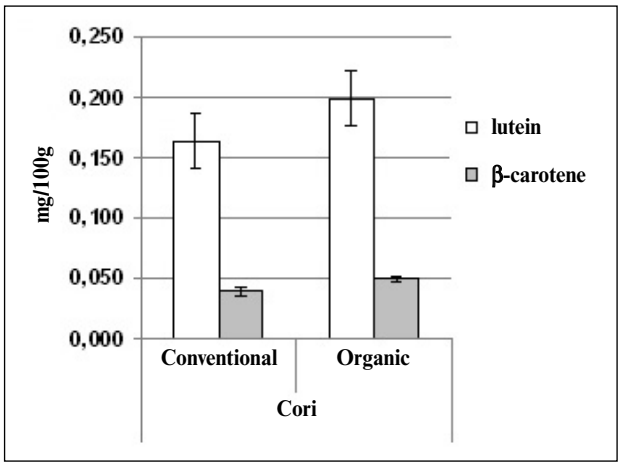

Fig. 1 - Lutein and β-carotene contents in organic and conventional kiwifruit.

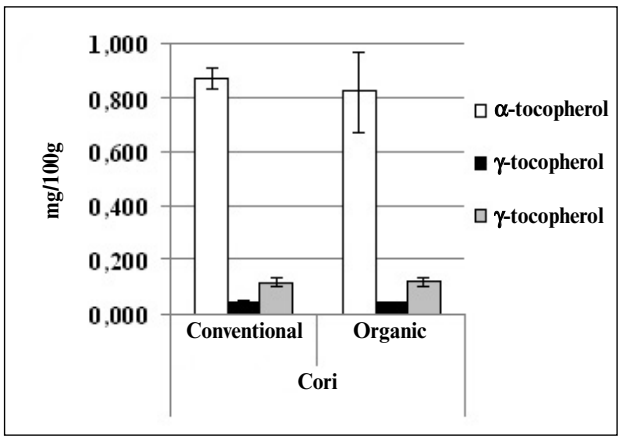

Fig. 2 - Tocopherol contents in organic and conventional kiwifruit.

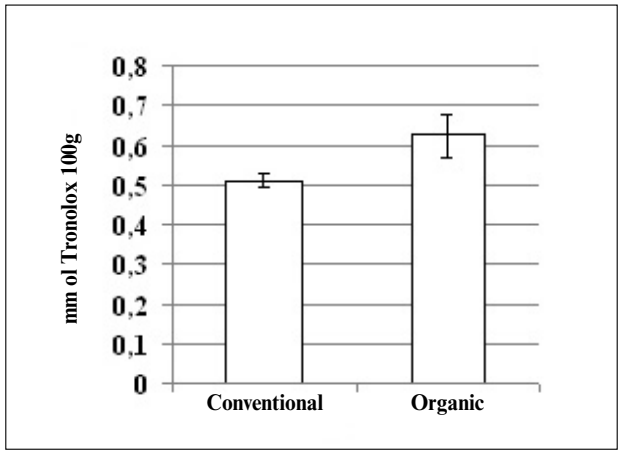

Fig. 3 - Antioxidant activity (FRAP) in organic and conventional kiwifruit.

copherols). In particular the results confirmed that kiwifruit is among the fruits with the highest content of lutein and, furthermore, its daily consumption would provide an adequate vitamin C intake, consistent with the Recommended Dietary Allowance (LARN, 1996). The antioxidant activity of fruit is highly dependent on the biological activity of a number of molecules in foods. In this study the antioxidant activity was measured in the hydrophilic extracts of kiwifruit, ascorbic acid mostly contributed the highest antioxidant activity expressed by organic fruit compared to the conventional one, thus representing the most important nutritionally active fraction of kiwifruit.

Indeed fruit is an important part of the daily human diet and its consumption plays an important role in the prevention of chronic-degenerative diseases (RIBOLI and NORAT, 2003). The knowledge of the phytochemical profile in fruit can be an important tool either from an agronomic point of view, in order to obtain more valuable productions (the management of crops, the choice of the genotype, are all factors that can influence the accumulation bioactive molecules), or from a nutritional point of view because it provides reliable information to better understand the relationship between diet composition and the risk of disorders of recognized nutritional etiology, allowing to plan strategies to achieve the recommended dietary intake of molecules important to health.

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REFERENCES

Amodio M.L, Colelli G., Hasey J.K. and Kader A.A. 2007. A comparative study of composition and postharvest performance of organically and conventionally grown kiwifruits. J. Sci. Food Agric. 87:1228.

Antognozzi E., Battistelli A., Famiani F., Moscatello S., Stanica F. and Tombesi A. 1996. Influence of CPPU on carbohydrate accumulation and metabolism in fruits of *Actinidia deliciosa* (A. Chev.). Sci. Hortic. 65:37.

AOAC 1996. "Official Methods of Analysis", 16th Ed. Association of Official Analytical Chemists, Arlington, VA.

Asami D.K., Hong Y., Barret D.M. and Mitchell A.E. 2003. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried Marionberry, Strawberry, and corn grown using conventional, organic and sustainable agricultural practices. J. Agric. Food Chem. 51: 1237.

Baltz M., Schulte E. and Thier H.P. 1992. HPLC separation of tocopherols and tocotrienols [Trennung von Tocopherolen und Tocotrienolen durch HPLC] Fat Sci. Technol. 94: 209.

Beltramo C., Sartor C., Cavanna M., Beccaro G.L., Mellano M.G. and Botta R. 2007. Valutazione dei parametri qualitativi durante il post-raccolta di campioni di actinidia provenienti da diverse regioni italiane. Proceedings "8° Convegno Nazionale - Actinidia 2007", Cuneo/Torino, Italy, 27-29 November, pp. 424-430.

Benge J.R., Banks N.H., Tillmann R and Nihal de Silva H. 2000. Pairwise comparison of the storage potential of kiwifruit from organic and conventional production systems. New Zealand J. Crop Hortic. Sci. 28:147.

Benzie I.F.F. and Strain J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP Assay. Anal. Biochem. 239: 70.

Beutler H.O., Becker J., Michal G. and Walter E. 1980. Rapid method for determination of oxalate. Fresenius Zeitschrift für Analytische Chemie 301:186.

Bourne D. and Prescott J. 2002. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. Crit. Rev. Food Sci. Nutr. 42(1): 1.

Cano M.P. 1991. HPLC separation of chlorophyll and carotenoid pigments of four kiwifruit cultivars. J. Agric. Food Chem. 39:1786.

D'Evoli L., Lucarini M., Gabrielli P., Aguzzi A. and Lombardi-Boccia G. 2009. Profilo compositivo e marcatori molecolari della qualità dei frutti dell'area IGP "Kiwi Latina" (*Actinidia deliciosa)* in relazione ai metodi di coltivazione ed ai genotipi. Italus Hortus 16 : 274.

Du G., Li M., Ma F. and Liang D. 2009. Antioxidant capacity and the relationship with polyphenols and Vitamin C in *Actinidia* fruits. Food Chem. 113: 557.

Duarte A., Caixeirinho D., Miguel M.G., Sustelo V., Nunes C., Mendes M. and Marreiros A. 2010. Vitamin C content of citrus from conventional versus organic farming system. Acta Hort. 868: 389.

Famiani F., Baldicchi A., Battistelli A., Moscatello S. and Walker R.P. 2009. Soluble sugar and organic acid contents and the occurrence and potential role of phosphoenolpyruvate carboxykinase (PEPCK) in gooseberry (*Ribes grossularia* L.). J. Hort. Sci. Biotechnol. 84: 249.

Famiani F., Baldicchi A., Farinelli D., Cruz-Castillo J.G., Marocchi F., Mastroleo M., Moscatello S., Proietti S. and Battistelli A. 2012. Yield affects qualitative kiwifruit characteristics and dry matter content may be an indicator of both quality and storability. Sci. Hortic. 146: 124-130.

Franceschi V. and Nakata P.A. 2005. Calcium oxalate in plants: formation and function. Annu. Rev. Plant Biol. 56: 41.

Greenfield H. and Southgate D.A.T. 2003. Food Composition Data. Production. Management and use. FAO Ed.

Hart D.J. and Scott J. 1995. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurements of the carotenoid content of vegetables and fruits commonly consumed in UK. Food Chem. 54: 101.

Hasey J.K., Johnson R.S., Meyer R.D. and Klonsky K. 1997. An organic versus a conventional farming system in kiwifruit. Acta Hort. 444: 223.

Inglese P., Gullo G. and Pace L.S. 1998. Effect of cyanamide on budbreak and cane fruitfulness for 'Hayward' kiwifruit in relation to cane length and time of application. N.Z. J. Crop Hortic. Sci. 26: 45-53.

Johnson R.S., Mitchell F.G., Crisosto C.H., Olson W.H. and Costa G. 1997. Nitrogen influences kiwifruit storage life. Acta Hort. 444(1): 285.

Jones M.G.K., Outlaw W.H. and Lowry O.H. 1977. Enzymic assay of 10^{-7} to 10^{-14} 615 moles of sucrose in plant tissues. Plant Physiology 60: 379.

LARN. 1996. Livelli Raccomandati di Assunzione di Nutrienti per la Popolazione Italiana. S.I.N.U. Ed.

Leong L.P. and Shui G. 2002. An investigation of the antioxidant capacity of fruits in Singapore markets. Food Chem. 76: 69.

Loewus F.A. 1999. Biosynthesis and metabolism of ascorbic acid in plants and of analogs of ascorbic acid in fungi. Phytochemistry 52:193.

Lowry O.H. and Passonneau J.V., 1972. A Flexible System of Enzymatic Analysis. Academic Press, New York, USA.

Meyers K.J., Watkins C.B., Pritts M.P. and Liu R.H. 2003. Antioxidant and antiproliferative activities of strawberries. J. Agric. Food Chem. 51, 6887-92.

Nishiyama I., Yamashita Y., Yamanaka M., Shimohashi A., Fukuda T. and Oota T. 2004. Varietal difference in Vitamin C content in the of kiwifruit and other *Actinidia* species. J. Agric. Food Chem. 52: 5472.

Nishiyama I. 2007. Fruits of the *Actinidia* genus. Adv. Food Nutr. Res. 52: 293.

Park Y.-S., Leontowicz H., Leontowicz M., Namiensnik J., Suhaj M., Cvikrovà M., Martincovà O., Weisz M. and Gorinstein S. 2011. Comparison of the contents of bioactive compounds and the level of antioxidant activity in different kiwifruit cultivars. J. Food Comp. Analysis 24:963.

Proietti S., Moscatello S., Famiani F. and Battistelli A. 2009. Increase of ascorbic acid content and nutritional quality in spinach leaves during physiological acclimation to low temperature. Plant Physiol. and Biochem. 47:717.

Prosky L., Asp N.G., Schweizer T.F., De Vries J.W. and Furda I. 1988. Determination of insoluble, soluble and total dietary fiber in foods and food products: interlaboratory study. Ass. Off. Anal. Chem. 71: 1017.

Riboli E. and Norat T. 2003. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. Am. J. Clin. Nutr. 78 (3 Suppl.): 559S.

Seybold C., Fröhlich K., Bitsch R., Otto K. and Böhm V. 2004. Changes in contents of carotenoids and vitamin E during tomato processing. J. Agric. Food Chem. 52: 7005.

Testolin R. and Ferguson A.R. 2009. Kiwifruit (*Actinidia* spp.) production and marketing in Italy. New Zealand J. Crop Hortic. Sci. 37:1.

Valls F., Rancho M.T., Fernandez-Muino M., Alonso-Torre S. and Checa M.A. 2002. High pressure liquid chromatographic determination of ascorbic acid in cooked sausages. J. Food Protect. 65: 1771.

Wang S.Y. and Lin H.S. 2003. Compost as soil supplement increases level of antioxidant compounds and oxygen radical absorbance capacity in strawberries. J. Agric. Food Chem. 51: 6844.

Worthington V. 2001. Nutritional quality of organic versus conventional fruits, vegetables, and grains. J. Altern. Complem. Med. 7(2):161.

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PAPERS

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Anonymous. 1982. Tomato product invention merits CTRI Award. Food Technol. 36(9): 23.

(Book)

AOAC. 1980. "Official Methods of Analysis" 13th ed. Association of Official Analytical Chemists, Washington, DC.

Weast, R.C. (Ed.). 1981 "Handbook of Chemistry and Physics" 62nd ed. The Chemical Rubber Co. Cleveland, OH.

(Bulletin, circular)

Willets C.O. and Hill, C.H. 1976. Maple syrup producers manual Agric. Handbook No. 134, U.S. Dept. of Agriculture, Washington, DC.

(Chapter of book)

Hood L.F. 1982. Current concepts of starch structure. Ch. 13. In "Food Carbohydrates". D.R. Lineback and G.E. Inglett (Ed.), p. 217. AVI Publishing Co., Westport, CT.

(Journal)

Cardello A.V. and Maller O. 1982. Acceptability of water, selected beverages and foods as a function of serving temperature. J. Food Sci. 47: 1549.

IFT Sensory Evaluation Div. 1981a. Sensory evaluation guide for testing food and beverage products. Food Technol. 35 (11): 50.

IFT Sensory Evaluation Div. 1981b. Guidelines for the prepaation and review of papers reporting sensory evaluation data. Food Technol. 35(4): 16.

(Non-English reference)

Minguez-Mosquera M.I., Franquelo Camacho A, and Fernandez Diez M.J. 1981. Pastas de pimiento. Normalizacion de la medida del color. Grasas y Aceites 33 (1): 1.

(Paper accepted)

Bhowmik S.R. and Hayakawa, K. 1983. Influence of selected thermal processing conditions on steam consumption and on mass average sterilizing values. J. Food Sci. In press.

(Paper presented)

Takeguchi C.A. 1982. Regulatory aspects of food irradiation. Paper No. 8, presented at 42nd Annual Meeting of Inst. of Food Technologists, Las Vegas, NV, June 22-25.

(Patent)

Nezbed R.I. 1974. Amorphous beta lactose for tableting U.S. patent 3,802,911, April 9.

(Secondary source)

Sakata R., Ohso M. and Nagata Y. 1981. Effect of porcine muscle conditions on the color of cooked cured meat. Agric. & Biol. Chem. 45 (9): 2077. (In Food Sci. Technol. Abstr. (1982) 14 (5): 5S877).

Wehrmann K.H. 1961. Apple flavor. Ph. D. thesis. Michigan State Univ., East Lansing. Quoted in Wehrmann, K.H. (1966). "Newer Knowledge of Apple Constitution", p. 141, Academic Press, New York.

(Thesis)

Gejl-Hansen F. 1977. Microstructure and stability of Freeze dried solute containing oil-in-water emulsions Sc. D. Thesis, Massachusetts Inst. of Technology, Cambridge.

(Unpublished data/letter)

Peleg M. 1982. Unpublished data. Dept. of Food Engineering., Univ. of Massachusetts, Amherst.

Bills D.D. 1982. Private communication. USDA-ARS. Eastern Regional Research Center, Philadelphia, PA.

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