ISSN 1120-1770

ITALIAN JOURNAL OF FOOD SCIENCE

Rivista italiana di scienza degli alimenti

IJFS

Volume XXI
Number 1
2009

Spedizione in ab. post. comma 26 - art. 2 - legge 549/95 n. 1/2009 - Torino

CHIRIOTTI EDITORI

ITALIAN JOURNAL OF FOOD SCIENCE

(RIVISTA ITALIANA DI SCIENZA DEGLI ALIMENTI)

Property of the University of Perugia
Official Journal of the Italian Society of Food Science and Technology
Società Italiana di Scienze e Tecnologie Alimentari (S.I.S.T.Al)
Initially supported in part by the Italian Research Council (CNR) - Rome - Italy
Recognised as a "Journal of High Cultural Level"
by the Ministry of Cultural Heritage - Rome - Italy

Editor-in-Chief:
Paolo Fantozzi
Dipartimento di Scienze Economico-Estimative e degli Alimenti, Università di Perugia,
S. Costanzo, I-06126 Perugia, Italy
Tel. +39 075 5857910 - Telex 662078 UNIPG - Telefax +39 075 5857939-5852067
E-mail: paolofan@unipg.it

Scientific Editor:
S. Mary F. Traynor, F.S.E., Ph.D.
Dipartimento di Scienze Economico-Estimative e degli Alimenti, Università di Perugia,
S. Costanzo, I-06126 Perugia, Italy
Tel. +39 075 5857912 - Telex 662078 UNIPG - Telefax +39 075 5857939-5852067
E-mail: ijfs@unipg.it

Publisher:
Alberto Chiriotti
Chiriotti Editori s.a.s., Viale Rimembranza 60, I-10064 Pinerolo, Italy
Tel. +39 0121 393127 - Telefax +39 0121 794480
E-mail: info@chiriottieditori.it - URL: www.chiriottieditori.it

Aim: The Italian Journal of Food Science is an international journal publishing original, basic and applied papers, reviews, short communications, surveys and opinions in food science (chemistry, analysis, microbiology), food technology (engineering, processing) and related areas (nutrition, safety, toxicity, physiology, dietetics, economics, etc.). Upon request and free of charge, announcements of congresses, presentations of research institutes, books and proceedings may also be published in a special "News" section.

Review Policy:
The Advisory Board with the Editor-in-Chief will select submitted manuscripts in relationship to their innovative and original content. Referees will be selected from the Advisory Board and/or qualified Italian or foreign scientists. Acceptance of a paper rests with the referees.

Frequency: Quarterly - One volume in four issues. Guide for Authors is published in each number and annual indices are published in number 4 of each volume.

Impact Factor: 0.518 published in the 2007 Journal of Citation Reports, Institute for Scientific Information

Subscription Rate: 2009: Volume XXI

PDF version	€ 40.00
Ordinary	€ 150.00
Supporting	€ 1,000.00

IJFS is abstracted/indexed in: Chemical Abstracts Service (USA); Foods Adlibra Publ. (USA); Gialine - Ensia (F); Institut Information Sci. Acad. Sciences (Russia); Institute for Scientific Information; CurrentContents®/AB&ES; SciSearch® (USA-GB); Int. Food Information Service - IFIS (D); Int. Food Information Service - IFIS (UK); EBSCO Publishing; Index Copernicus Journal Master List (PL).

IJFS has a page charge of € 20.00 up to 5 pages; extra pages are € 30.00.
Reprints (100) will be sent free of charge.

ITALIAN JOURNAL OF FOOD SCIENCE

IJFS

ADVISORY BOARD

REVIEW

ENZYMATIC BROWNING AND SOFTENING IN VEGETABLE CROPS: STUDIES AND EXPERIENCES

IMBRUNIMENTO ENZIMATICO E PERDITA DI CONSISTENZA DEGLI ORTAGGI: STUDI ED ESPERIENZE

R.N. BARBAGALLO*, M. CHISARI and G. SPAGNA*
Dipartimento di Orto-Floro-Arboricoltura e Tecnologie Alimentari (DOFATA),
Sez. Tecnologie Agroalimentari, Università di Catania,
Via S. Sofia 98, 98123 Catania, Italy
*Corresponding authors: Tel. +39 0957580220-213, Fax +39 0957141960,
e-mail: gspagna@unict.it and rbarbaga@unict.it

ABSTRACT

The main enzymes associated with browning and softening of "minimally processed vegetables" are reviewed. The enzyme activities considered are those endogenously present in plant tissues and that act during postharvest, processing and/or storage. The results shown are from research conducted by the Food Biotechnology Group of the University of Catania (Italy). The research results are compared with results obtained by other research groups in order to have a better under-

RIASSUNTO

Sono stati studiati i principali enzimi associati all'imbrunimento e alla perdita di consistenza degli "ortaggi trattati al minimo", considerando le attività endogene in post-raccolta, durante la preparazione industriale e/o la conservazione. I risultati mostrati si riferiscono alle ricerche svolte dal gruppo di Biotecnologie Alimentari dell'Università di Catania. Tali risultati sono stati confrontati con quelli ottenuti da altri gruppi di ricerca al fine di una migliore comprensione del-

- Key words: browning, endogenous enzymes, fresh-cut, softening, vegetables -

standing of problems related to extending the shelf-life of fruit and vegetable commodities.

le problematiche connesse all'estensione della shelf-life dei prodotti ortofrutticoli.

THE ISSUES

It is well known that enzymes are biological catalysts that act by lowering the activation energy and increasing the chemical reaction rate of specific physiological processes. As with every catalyst, these proteins are highly specific for each reaction, while not being changed themselves. They have highly complex structures and may be conjugated with metals, carbohydrates and/or lipids. Enzymatic activities are sensitive to several factors, mainly pH, temperature, substrate availability, inhibitors and activators. They are naturally present in vegetables (endogenous enzymes) and senescence phenomena and/or industrial processing put them in a condition to modify the physicochemical, sensory and nutritional properties of products (KOSHLAND, 1959; WHITAKER, 2003a and b).

Minimally processed or "fresh-cut" products are prepared and handled so as to maintain their fresh nature in order to provide "ready-to-eat" convenience products to the consumer especially in terms of saving time in cooking (VAROQUAUX and WILEY, 1994). These products were an important novelty in the food industry in the 1980s. The category is mainly made up of minimally processed vegetables (MPV) and, to a smaller degree minimally processed fruit (MPF). Producing MPV involves cleaning, trimming, peeling, coring, slicing, shredding and washing of raw vegetables, generally followed by packaging in plastic bags and storing at temperature <6°C, and being sold within 8-10 days. In MPV, discolouration and loss of turgidity result in a loss of sensory quality, particularly visual quality, due to browning reactions on leaf surfaces (russet spotting) and in bruised tissues (brown stain) and softening reactions. The main mechanism for these changes is the disruption and decompartmentalisation of cellular structures which allows enzymes and their substrates to come in contact with each other, leading to cascade reactions. Such phenomena are mainly initiated by endogenous enzymatic activity, followed by microbial spoilage that may lead to further product decay.

The enzymes involved in browning and softening reactions in vegetables such as tomatoes (*Solanaceae*), salad vegetables, including lettuce, baby romaine lettuce and endive (*Asteraceae*), Chinese cabbage (*Brassicaceae*) and fennel (*Apiaceae*) are mainly polyphenol oxidase (PPO; EC 1.14.18.8) followed in importance by phenylalanine ammonia-lyase (PAL; EC 3.1.5.1), pectin methylesterase (PME; EC 3.1.1.11) and polygalacturonases (Endo-PG; EC 3.2.1.15 -Exo-PG; EC 3.2.1.67 and EC 3.2.1.82). Phenylalanine ammonia-lyase activity is induced by cutting or bruising and leads to an increased phenolic content (HYODO *et al.*, 1978; KE and SALTVEIT, 1989) and these phenolics are specific substrates for polyphenol oxidase reactions.

In this review, the influence of some degradative enzymatic activities found in vegetables and in minimally processed products is reviewed. It is based on research conducted from 1998 to 2008 by the Food Biotechnology Group of the University of Catania (Italy). Detailed results of all the cited research can be obtained from the original articles.

THE PROTAGONISTS

Enzymes involved in degradation of vegetables

Evaluation of genotypes that have an extended shelf life is one of the most active areas of research aimed at improving vegetable quality. Such selection mainly depends on the use of some endogenous enzymatic activities as markers of quality loss (BARBAGALLO *et al.*, 2006; 2008). Among these activities, is polyphenol oxidase (PPO; EC 1.14.18.1) the primary enzyme responsible for the browning of damaged tissues due to the oxidation of phenolic compounds. Softening is a universal feature in the ripening of fleshy vegetables and is typically accompanied by the degradation of the middle lamella and loss of cell adhesion. This process derives from the solubilisation of cell wall pectin which involves the action of pectin methylesterase (PME; EC 3.1.1.11) and other pectinases.

Polyphenol oxidases or oxidases

Browning of vegetables and other alterations of the main sensory parameters can be due to enzymatic (enzymatic browning) or non-enzymatic reactions (mostly Maillard reactions). The oxidation substrates are oxygen and polyphenols (which are synthesised by phenylpropanoid metabolism, a pathway that is initiated by phenylalanine ammonia-lyase). Enzymatic browning consists of the oxidation of phenols to form reactive quinone compounds which can create radical oxidative coupling by interacting with the reactive residues of amino acids and peptides (amines and sulfhydryls) and with reducing sugars, to form brown oligomers of varying intensity. Quinone formation is reversible if reducing agents are applied (ascorbic acid, glutathione etc.); these are capable of generating colourless diphenols, while the subsequent process of oligomerization is irreversible (MAYER and HAREL, 1979; TAYLOR and CLYDESDALE, 1987; MC EVILY *et al.*, 1992). The oxidases principally involved are polyphenol oxidases, mostly classified into the following two groups (RAMÍREZ *et al.*, 2003) (Fig. 1):

1. Polyphenol oxidase, also known as catechol oxidase, tyrosinase, phenolase, catecholase, o-diphenol oxidase and monophenol oxidase, (PPO, EC 1.10.3.1) catalyses the hydroxylation of monophe-

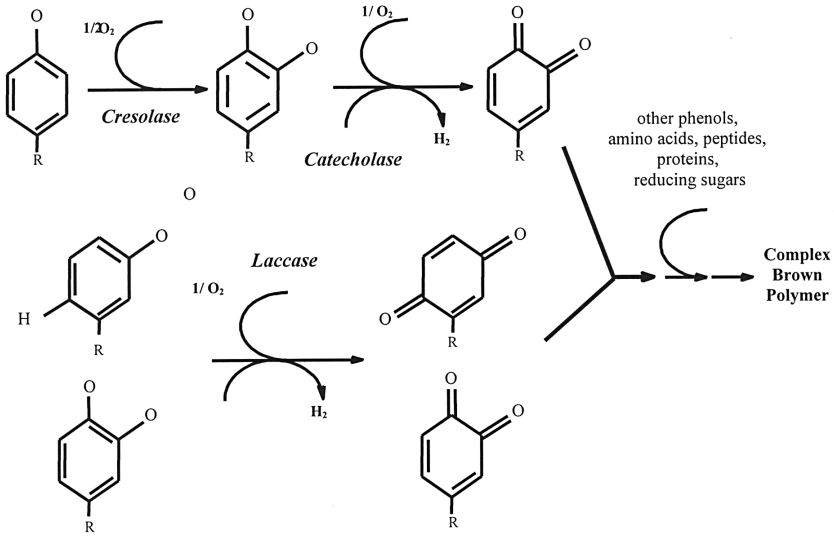

Fig. 1 - Reactions catalyzed by polyphenol oxidases.

nols into o-diphenols (monophenolase or cresolase activity) and subsequently oxidises them into o-quinones (o-diphenolase or cathecolase activity) at a ratio between 1 and 40. It is located in cell organelles such as chloroplasts, mitochondria and peroxisomes where it is firmly bound to the membrane and may even be found in the soluble fraction of the cell membrane. VAN LELYVELD *et al.* (1984) found increased PPO activity in response to mechanical shock.

2. Laccase (EC 1.10.3.2) oxidises *p*-diphenols more than o-diphenols and forms quinones. They are produced from wild mould strains (*Aspergillus*, *Botrytis*, etc.) during conidial formation. They also react strongly with anthocyanins and are found when fruit is spoiled by *Botrytis cinerea* (DUBERNET, 1974; MINASSI *et al.*, 2002).

Pectinases

Softening is a universal feature in the ripening of fleshy fruit and is typically accompanied by degradation of the middle lamella and loss of cell adhesion (FRAEYE *et al.*, 2007). This process derives from the solubilisation of cell wall pectin and involves the action of pectin methylesterase (PME, EC 3.1.1.11). PME causes cell autolysis and decompartmentalisation of cell wall components starting a series of cascade enzymatic reactions leading to: (i) an increased tissue respiratory ratio with consequent dehydration and senescence; (ii) increased ethylene biosynthesis and (iii) degradation of cell wall polysaccharides and tissue softening (VAN LINDEN *et al.*, 2008; PLAZA *et al.*, 2007). During fruit ripening PME cleaves the methyl esters from pectin to produce methanol, pectin with a low degree of esterification, and free acid. Decreasing levels of esterification are crucial for the softening of fruit; pectin is highly susceptible to degradation by endo-acting enzymes (HUBER, 1983; CRELIER *et al.*, 2001; FACHIN *et al.*, 2002).

Pectic hydrolases

One of the most important sensory characteristics of vegetables is turgidity related to the cell wall structure, which provides structure and rigidity (LONGO, 1996).

The composition of the cell wall can vary significantly among vegetables, but mainly consists of pectin, hemicellulose, cellulose, lignin, proteins and other components. Vegetables are particularly rich in pectin which forms the major structural polysaccharide component of the fruit lamella and cell wall. It is composed of linear polygalacturonic acid that is largely esterified with methyl or acetyl groups that alternate with rhamnose molecules ('smooth regions') with multiple side chains of various carbohydrates, including arabinan, xylan and galactan ('hairy regions'). Methoxylation affects the susceptibility of pectin to pectinases and is characteristic of different types of fruit. Apples, for example, usually have very high pectin methoxylation (BENEN and VORAGEN, 2003). Among the pectic hydrolases which degrade pectin, let us consider pectin methyl esterase and the polygalacturonases.

Pectin methyl esterase

Pectin methyl esterase (PME, EC 3.1.1.11) is produced by higher plants, mushrooms, some bacteria and yeasts (JAYANI *et al.*, 2005). This enzyme de-esterifies pectin by hydrolysing methoxyl groups to produce methanol and pectinic or pectic acids. PME starts with the non-reducing end of the chain and progresses to obtain alternating methoxylate and free carboxyl groups (Fig. 2).

De-esterification never reaches completion due to competitive inhibition by the product; in fact, 10% more esterification is needed to activate the enzyme. Furthermore, PME can hydrolyse other esters, ethyls, propyls and allyles, but less effectively.

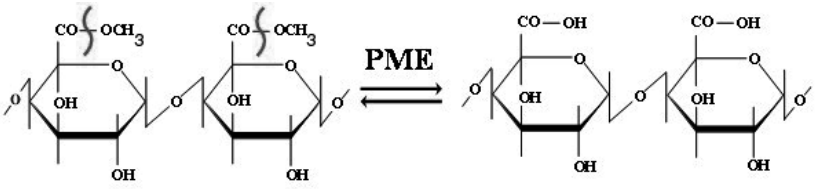

Fig. 2 - Reaction catalyzed by pectin methylesterase.

In many fruit PME accompanies polygalacturonase by depolymerising pectin substrate. Ca^{+2} stimulates the enzyme, because it removes competitive inhibition by liberating the enzyme from the complex with the substrate (HAGERMAN and AUSTIN, 1986). The first distinction between thermostable and thermosensitive enzymes was reported by VERSTEEG *et al.* (1980) when various isoenzymes of PME were isolated mostly from orange (SEYMOUR *et al.,* 1991; CAMERON and GROHMANN, 1996; CAMERON *et al.*, 1998; ANTHON and BARRETT, 2006). These isoenzymes can be differentiated, isoelectrically, by molecular weight and catalytic properties (kinetic, substrate affinity, pH and heat sensitivity) (CORREDIG *at al.*, 2000). In various fruit isoenzymes pI varies from 9-11 (LY-NGUYEN *et al.*, 2002; DENES *et al.,* 2000; LAATS *et al.,* 1997). The biochemical properties, thermal stability and food applications for PME are similar in many types of fruit like tomato, orange, apple, grapefruit and others. PME is inactivated in juices by pasteurization even though some thermostable isoenzymes remain untouched; in citrus processing, Ca^{+2} causes gelling with subsequent precipitation. PME from fruit can be inhibited by high sugar concentrations, but is restored in reconstituted juice (INGALLINERA *et al.*, 2005).

Polygalacturonases

The endopolygalacturonases catalyse the hydrolysis of the α-1,4-D-galacturonase bond, causing a rapid drop in substrate viscosity (Fig. 3). Even the hydrolysis of a small percentage of glycoside bonds can reduce viscosity by 50%. Endopolygalacturonases (Endo-PG, EC 3.2.1.15) randomly hydrolyse the polymer along the chain, while exopolygalacturonases (Exo-PG) are confined to cleaving off galacturonic acid monomers (EC 3.2.1.67) or digalacturonides (EC 3.2.1.82) from the non-reducing end. Endo-PGases are widely distributed throughout fungi, bacteria and many yeasts. In contrast, exo-PGases occur less frequently. Exo-PGases can be differentiated into two types: fungal exo-PGases, which produce monogalacturonic acid as the main end product, and bacterial exo-PGases, which produce digalacturonic acid as the main end product. The occurrence of PGases in vegetables has also been reported (BENEN and VORAGEN, 2003; BENEN and VISSER, 2003; TOIVONEN and BRUMMELL, 2008; TOMASSEN *et al.*, 2007).

PME and PG together with cellulases and peptidases are required for cellular demolition during the maceration in wine-making and extraction of fruit juices (DELLA PENNA *et al.*, 1990; HADFIELD *et al.*, 1998; SMITH *et al.,* 1990).

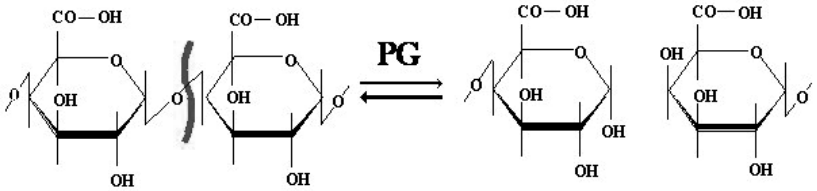

Fig. 3 - Reaction catalyzed by polygalacturonases.

Pectic lyases

Other pectinases belong to the lyases group; these are enzymes which catalyse chemical bond-breaking via hydrolysis and oxidation, often producing a new double-bond or a new aromatic compound. Pectic lyases are different with respect to others because they only need one substrate to catalyse the reaction in one direction and they need two in the other. While pectin and pectate lyases are necessary to depolymerise cell walls during sugar production, phenylalanine ammonia-lyase produces the substrates needed for oxidases to work. This is both nutritionally and sensorially deleterious. Pectic lyases, such as pectin lyase, pectate lyase and other pectinases occur less frequently than pectin hydrolases in beverages. Unfortunately no nucleotide sequences are available for many pectate and pectin lyases that have been characterised, so there is a large discrepancy between these enzymes (BENEN, 2003; BENEN and VORAGEN, 2003; ROMBOUTS *et al.,* 1982; HUBER, 1984; DE LORENZO *et al.*, 1991; SCAVETTA *et al.,* 1999; MARÍN-RODRÍGUEZ *et al.*, 2002). Pectin lyases are produced almost exclusively by mushrooms; in a few rare cases from some bacterial strains, they are extra-cellular. They can be distinguished by poly (methoxygalacturonide) endo pectin lyase (endo-PL, EC 4.2.2.10) and poly (methoxygalacturonide) exo pectin lyase exo-PL (exo-PL, EC 4.2.2.-), they break the glycoside bond by β-elimination of highly esterified pectin. The endo-PL reacts randomly on the substrate, while the exo-PL reacts at the non-reducing terminus. Pectin lyases are a valid alternative to polygalacturonase and pectin methylesterase since they directly de-polymerise pectins without altering their level of esterification. This prevents the release of methanol into the beverage and the formation of the colloidal precipitates of de-esterified pectin and endogenous Ca^{+2}.

Pectate lyases are mainly produced by microorganisms, although some may come from plants or pathogenic mushrooms. They are distinguished by poly β-1,4-D-galacturonide endopectate lyase (endo-PEL; EC 4.2.2.2) and poly-β-1,4-D-galacturonide exopectate lyase (exo-PEL; EC 4.2.2.9). They both break the glycoside bond of the chain by trans β-elimination of pectins with a low level of esterification, to form a double bond in the C_4-C_5 position. There is a substantial difference between the two types of enzymes; endo-PEL hydrolyses randomly, while exo-PEL reacts at the ends of the chain. Pectate lyase is less common than pectin lyase.

VEGETABLES AS SUBSTRATES OF ENZYMATIC DEGRADATION

Tomato

The shelf-life of tomatoes mainly depends on adopting strategies in order to select genotypes with low degradative enzyme expression. SPAGNA *et al.* (2005a) investigated the role of polyphenol oxidase on tomato quality loss and on lycopene degradation during storage. Polyphenol oxidase was extracted from five Sicilian varieties of tomato (*Pizzutello, Naomi* (Hazera), *F1 PS212* (Peto seed), *Rosa Maletto, PO228*) and assayed with a method using 3-methyl-benzothyazolinone hydrazone (MBTH) as a chromophore coupling agent, while 2-(3,4-dihydroxyphenyl)acetic acid (DOPAC) was used to determine PPO activity in tomato. The tomato PPO had a maximum activity at pH 4.8. The pH of juice in ripe fruit is between 4.1 and 4.4; in this range the PPO relative activity is between 74 and 87%. The optimum temperature for activity of tomato PPO is 40°C; the enzyme showed a good relative activity (55% of the maximum) at cold-storage temperature (4°C). PPO retained 82% of its relative activity at a NaCl concen-

tration of 0.1 M; it gradually became more inactivated as the concentration increased. The commercial tomato variety *Naomi* is more susceptible to enzymatic browning than the local varieties *Pizzutello*, *Rosa Maletto* and *PO228*, due to higher PPO activity levels. This result confirms the suitability of these local tomato varieties for the national markets. Results from storage tests show a relationship between the PPO activity and the colour changes associated with browning and lycopene degradation; lycopene is an antioxidant agent that reconstitutes the polyphenols that are oxidised by the action of PPO. The polymerisation of quinonic compounds due to the action of polyphenol oxidase is a major cause of fruit deterioration, that is then followed by microorganism development.

In an earlier study, BARBAGALLO *et al.* (2008) evaluated the effect of watering regime on the expression of PME and PPO enzymatic activities, as well as on the physico-chemical properties of typical long-term storage cherry tomatoes ('Pizzutello', 'Albicocca di Favignana', 'Rosso', 'Giallo piccolo a punta', 'Albicocca di Lipari', 'Percopara'), in order to propose a strategy suitable for Sicilian cultivation. Polyphenol oxidase seemed to be related to the water regime used; a maximum activity was observed under in normal watering conditions and pectin methylesterase activity greatly decreased as water stress increased (loss of activity ranging from 22.31 to 82.01%). Water stress conditions also induced an increased titratable acidity (up to 64.3% in cultivar 'Rosso') and an increase in dry matter and total soluble solids (increase ranging from 19.41 to 47.83% and from 5.60 to 29.03% for dry matter and total soluble solids, respectively). Different strategy solutions for the cultivation of tomato genotypes with an extended shelf life are possible: (a) selecting the local type that presents the lowest levels of pectin methylesterase and polyphenol oxidase ('Pizzutello') and (b) modifying the water regime in order to induce the commercial cultivars used in intensive cultivation, such as 'Naomi', to better 'protect themselves' from enzymatic degradative activities. The results suggested that high watering levels, leading to a more marked production of degradative enzymatic activities, could lead to products being more susceptible to undesirable browning and softening reactions.

Leafy vegetables

Leafy vegetables belong to a group of food commodities characterised by high amounts of vitamins, minerals and fibre and a reduced calorie content. Furthermore, they are relevant preventive agents against tumors and cardiovascular diseases, and inhibition agents against free radicals, responsible for the ageing phenomena (BAJPAI *et al.*, 2005). In the literature there are conflicting opinions as to the role of PAL and PPO in browning during storage of minimally processed lettuce. Some authors (HISAMINATO *et al.*, 2001; DEGL'INNOCENTI *et al.*, 2007) have proposed PAL activity as a marker for determining the browning potential of minimally processed lettuce. On the other hand, CANTOS *et al.* (2001) did not find any correlation between browning and PAL activity. The same authors found increased PPO activity due to enzyme activation from a latent to a fully active form following tissue bruising. HEIMDAL *et al.* (1995) reported a lack of correlation between PPO and browning phenomena. To date, no research has been published regarding the correlation between pectin methylesterase activity and softening of minimally processed lettuce.

Lettuce

Four botanical varieties of lettuce (*Lactuca sativa* L.) var. *longifolia*, *crispa*, *acephala* and *capitata* were studied by our research group (data not pub-

lished) in order to evaluate the impact of PAL and subsequent activities of PPO and PME in fresh-cut products. Colour changes during storage at 4°C, measured as the change in parameters L*, a*, b* and chroma, were taken as quality markers of minimally processed lettuce. A sharp variation of a* parameter, representing the green to red component, was noticed in all the varieties. Such variation can be taken as a browning index of lettuce (MARTIN-DIANA *et al.*, 2005). The a* value increased during storage in all varieties except *longifolia*; the Δa* values range from 2.2 (*crispa*) to 4.0 (*capitata*). All lettuce varieties examined showed similar PAL activity values immediately after cutting (day 0). The values increased during the first days of storage, but with different values; *longifolia* and *crispa* showed a maximum activity on day 4, while *capitata* showed a maximum on day 6 with a value 10 times higher than that on day 0. Different values of PPO activity were observed among the varieties already on day 0 of storage. In particular, *crispa* showed the highest activity (0.047 U/g) followed by *acephala*, *capitata* and *longifolia*. The higher PPO activity value on day 0 can be explained by the increased availability of phenolic substrates due to processing and cellular disruption. It can also be related to a defense mechanism of the plant against the stress induced by processing. The initial PME activity was different among the varieties, with *capitata* showing the highest value (4.74 U/g), followed by *crispa*, *longifolia* and *acephala*. By calculating the ratio between phenolics and PPO activity, it was possible to obtain some information about the enzymatic browning of fresh-cut lettuce. In *longifolia* the ratio was positive, indicating that PPO did not act effectively on the phenolics so browning did not occur. On the other hand, in *capitata* the ratio was more favourable to PPO, indicating a high activity towards phenolics with the formation of quinones, precursors of the brown legumes. In *acephala*, the phenolics/PPO ratio was similar to that of *capitata*, but the a* variation during storage was slightly lower. This was probably due to low PAL activity and consequently reduced phenolic formation.

In an earlier work (CHISARI *et al.*, 2007), oxidase activities and antioxidant capacity of minimally processed baby romaine lettuce (*Lactuca sativa* L. cv. Duende) cultivated under different salinity conditions were investigated. The two oxidases which were the object of the study were polyphenol oxidase and peroxidase (POD; EC 1.11.1.7). POD oxidises the hydrogen donors of peroxides and also accepts polyphenols as donors (THONGSOOK *et al.*, 2007; LOPEZ-SERRANO and ROS-BARCELÒ, 1995). POD is often associated with the appearance of undesiderable aromas but its role in browning is limited by the low level of H_2O_2 within plant cells (RICHARD-FORGET and GAUILLARD, 1997). Furthermore, physico-chemical analyses were performed to determine the total phenolic content, colour parameters and antioxidant capacity (assayed with ORAC method), during 10 days of storage at 4°C of minimally processed baby romaine lettuce cultivated under 3 different salinity conditions (2.8, 3.8, 4.8 dS/m), in order to determine the most suitable condition for further processing. Wounding of tissues during minimal processing activated both enzymatic activities which became, much more evident after 7 days of storage. The increase in PPO activity seemed to be correlated with the low salt availability, whereas no marked increase was observed at higher salt concentrations. The increase of polyphenol oxidase activity ranged from 2.3 to 21.0% at day 3, reaching 59.8% at day 7 in samples cultivated under low salinity conditions (2.8 dS/m). Peroxidase activity also increase, with a drastic increase up to day 7 (about 4 times higher than the initial value). Furthermore, POD extracts from samples cultivated

under low salinity conditions showed the highest activity (0.431 U/g fw at day 7) in comparison with the other two salinity conditions (0.361 and 0.404 U/g fw at day 7 for medium and high salinity conditions, respectively). The highest oxidase activity in extracts was recorded in samples cultivated under low salinity conditions. This result can be explained by the high availability of free water for the enzymatic reactions. A general decrease in the phenolic content was noticed in all the samples, reaching minimum values at day 7, in correspondence with the maximum PPO and POD activity values. A very similar decreasing trend was observed relative to the antioxidant capacity of the methanolic extracts of lettuce; the highest variation was found after 7 days of storage at 4°C in samples cultivated under low salinity conditions (-57% of the initial value); a general decrease in ORAC units was observed in all the samples. The oxidation of phenolic compounds inevitably led to browning of the samples; this was measured by the variations in the L*a*b* parameters. Colour degradation was linear up to day 10 in samples cultivated under low salinity, while there was no significant difference between day 7 and 10 in samples raised under with medium and high salinity conditions. Increasing levels of salinity (up to 4.8 dS/m) effectively reduced PPO and POD activities, colour changes and phenolic degradation, thus, preserving the antioxidant capacity of the product, immediately after cutting and throughout 7 days of storage.

Escarole

Escarole (*Cichorium endivia* var. *latifolium*) is one of the most highly regarded salads because of its colour, slightly bitter flavour and for its toning, purifying and diuretic properties in low-calorie diets. Furthermore, fresh-cut escarole has a long shelf-life because it resists browning. While not entirely understood, it may be due to low PPO activity, which is responsible for enzymatic browning (CASTANER *et al.*, 1999) and/or the low level of polyphenol substrate in the plant tissue. INGALLINERA and SPAGNA (2007) studied shelf-life and enzymatic oxidation in six fresh-cut escarole cultivars (*Salanca, Lauv, Davos, Perlita, Lorca, Elsa*). Samples were packed in both air atmosphere and nitrogen-or-argon saturated atmospheres with triple layer film. The phenol content, PPO activity and colour change were measured during storage for 14 days at 4°C. Colour variation during storage was significant. The lightness, L*, and red to green, a*, values showed a similar increase during storage in all the samples. There were no significant differences between the samples stored in air atmosphere and those stored in nitrogen-or-argon saturated atmosphere. The PPO values initially increased and reached a maximum after 2 days of storage. This result suggests that tissue wounding caused an increase in PPO activity due to a shift in the activation process from latent to fully active (CASTANER *et al.*, 1999). The subsequent decrease could have been due to the reduced availability of polyphenol substrate rather than to an effective reduction in enzyme. In fact, CANTOS *et al.* (2001) reported that PPO was active only in the initial phase and was not further synthesised. It may be deduced that escarole is well-suited as a fresh-cut salad vegetable with a long shelf-life due to its low PPO activity and browning resistance. Argon-and nitrogen-modified atmospheres can further improve shelf-life over a 14-day period due to reduced PPO biosynthesis and the absence of oxygen which prevents the enzyme from oxidizing which then leads to browning.

Chinese cabbage

The suitability of four cultivars of Chinese cabbage (*Brassica campestris* L. ssp. *Pekinensis* Lour) ('Bilko', 'Kasumi,

'Manoko' and 'Nikko') to be processed into a ready-to-eat product was evaluated by SCUDERI *et al.* (2007). Immediately after harvesting, cabbage samples were cut, washed with chlorinated water, packaged under ordinary atmospheric conditions and stored at 4°C for 8 days. The results indicate that Chinese cabbage is of interest as a ready-to-use product. In fact, there were few changes in the titratable acidity, soluble solids and weight loss values for *Manoko*; the CO_2 and O_2 percentage changes were the least in *Bilko*. The total PPO levels differed in the various cultivars during storage in the following order: *Manoko* < *Kasumi* < *Bilko* < *Nikko*. Generally, the green leaf tissue had a higher activity than the vascular ones, except for cv. *Nikko*. Variations in polyphenol were also found in the polyphenol content in the tissue; generally, the green leaf tissue had a higher content than the vascular ones. A good correlation was found between the PPO activity and browning, in agreement with other authors (SEVERINI *et al.,* 2003). These results indicate that *Manoko* is suitable for use as a ready-to-use product.

In an other study CATALANO *et al.* (2007) investigated the effect of harvest age and processing on the biochemical degradation of ready-to-eat Chinese cabbage during chilled storage. Chinese cabbage cultivar 'Manoko', harvested at 60 days, was taken from storage processed under the same conditions and packed under air and modified-active-packaging (MAP) with three mixtures of O_2 and CO_2 at different and increasing concentrations and with an anti-fog film for condensation control (O_2 permeability: 35 cc/m2/24h, CO_2 permeability: 135.8 cc/m^2/24h, H_2O permeability: 15 g/m^2/24h at 25°C). The gas mixtures used in MAP were those generally recommended in the literature for fresh-cut vegetables (CANTWELL, 1995), Mixture 1: 5% O_2, 5% CO_2; Mixture 2: 10% O_2, 5% CO_2; and Mixture 3: 10% O_2, 20% CO_2. Chinese cabbage samples were taken for analysis at 0, 4 and 12 days. Samples harvested at different ripening stages showed appreciable differences in all the parameters tested on the day of the production and during chilled storage. The samples wrapped in modified atmosphere only showed differences in PPO activity and browning during cold storage. The initial PPO activity, browning and total polyphenol content increased during ripening. Early harvested cabbage (40 days from sowing) maintained a good quality longer than those that were harvested later. This was due to a smaller PPO increase and less browning during the chilled storage, even though the initial phenolic content was lower. Five percent O_2 and 5% CO_2 was the most suitable packaging solution for preserving quality during chilled storage. The effect of reducing O_2 was more effective than increasing CO_2 in order to reduce browning during cold storage, although it is was very difficult to understand the exact effect that of MAP has on PPO activity and browning.

Fennel

Among minimally processed vegetables, fennel (*Foeniculum vulgare* Mill.) is not widely used due to the rapidity with which enzymatic browning occurs (ARTES *et al.,* 2002). The edible part is the bulb-like structure formed by the inflated leaf base, and the green stalks. In an attempt to extend the shelf-life of fresh-cut fennel, SPAGNA *et al.* (2005b) studied the effects of treating fennel with stabilising solutions and using different packaging conditions. PPO activity, colour changes and the levels of the main microbial groups were monitored during storage at 4°C in order to evaluate the effects of different stabilising treatments and packaging on the shelf-life of fennel. Washed and cut fennel samples were treated with two stabilising solutions (ethanol and SO_2) just before

packaging; this was carried out in ordinary and modified-atmosphere conditions using an impermeable PET (polyethylene terephthalate) film. The results obtained from the storage tests showed that pre-treatments with SO_2 extended the shelf-life of minimally processed fennel. In comparing untreated samples with those treated with ethanol, those treated with SO_2 showed a lower PPO activity during storage, less variation in L*a*b* variations and better hygienic-sanitary conditions. Modified atmosphere packaging showed positive results in terms of reducing microbial growth, while a clear correlation was not found between the tested packaging and enzymatic browning of the samples. Initial activity (day 0) ranged from 0.14 and 0.16 U/g fwt. Wounding of tissue caused an increase in PPO activity due to the activation process from latent to fully active PPO (CANTOS *et al.*, 2001). The increase was slight with a maximum reached at 7 days of storage for all the samples tested except for those treated with ethanol, whose PPO activity increased up to day 10. Samples without stabilising treatments, with both ordinary and modified atmosphere packaging, showed the highest PPO activity values (0.21 and 0.20 U/g fw, respectively).

CONCLUSIONS

Based on published literature regarding several types of vegetables, it is not possible to find a single explanation for the interactions between enzymatic activities and browning and softening phenomena. In fact, the deterioration and senescence processes in vegetables during the post-harvest period are extremely complex from a physiological point of view and involve several metabolic pathways. The "scientific reductionism" that leads to a simplification of biological systems to just a few variables, is not suitable when trying to explain specific situations of fresh-cut vegetables. In fact, the limiting factors in biochemical reactions that lead to degradation may differ according to the different botanical types and plant tissues. However, some general comments can be made on enzymatic browning, which essentially depends on PPO activity, even if other variables, such as PAL and PME activities, water content with the consequent solute concentration, play an important role. So, it is difficult to establish which parameter is most relevant in a particular vegetable type. However, the phenolics/PPO ratio and the presence of PME describe in part the degradation pattern of leafy vegetables during their shelf-life continuum.

REFERENCES

Anthon G.E. and Barrett D.M. 2006. Characterization of the temperature activation of pectin methylesterase in green beans and tomatoes. J. Agric. Food Chem. 54: 204.

Artes F., Escalona V.H. and Artes-Hernandez F. 2002. Modified atmosphere packaging of fennel. J. Food Sci. 67: 1550.

Barbagallo R.N., Chisari M., Branca F. and Spagna G. 2008. Pectin methylesterase, polyphenol oxidase and physicochemical properties of typical long-storage cherry tomatoes cultivated under water stress regime. J. Sci. Food Agric. 88: 389.

Barbagallo R.N., Chisari M., Branca F., Argento S. and Spagna G. 2006. Profilo enzimatico degradativo dei frutti in tipi locali di pomodoro. Italus Hortus 13: 622.

Benen J.A.E. and Visser J. 2003. Polygalacturonase. In: "Handbook of Food Enzymology", (Eds.), Whitaker J.R., Voragen A.G.J. and Wong D.W.S. p. 857. Marcel Dekker Inc., Basel, Switzerland.

Benen J.A.E. and Voragen A.G.J. 2003. Pectic Enzymes. In: "Handbook of Food Enzymology", (Eds.), Whitaker J.R., Voragen A.G.J. and Wong D.W.S. p. 845. Marcel Dekker Inc., Basel, Switzerland.

Benen J.A.E., 2003. Pectate and Pectin Lyases. In: "Handbook of Food Enzymology", (Ed.), Whitaker J.R., Voragen A.G.J. and Wong D.W.S. p. 1029. Marcel Dekker Inc., Basel, Switzerland.

Bajpai M., Mishra A. and Prakash, D., 2005. Antioxidant and free radical scavenging activities of some leafy vegetables. Int. J. Food Sci. Nutr., 56: 473.

Cameron R.G. and Grohmann K. 1996. Purification and characterization of a thermally tolerant pectin methylesterase from a commercial Valencia fresh frozen orange juice. J. Agr. Food Chem. 44: 458.

Cameron R.G., Baker R.A. and Grohmann K. 1998. Multiple forms of pectin methylesterase from citrus peel and their effects on juice cloud stability. J. Food Sci. 63: 253.

Cantos E., Espin J.C. and Tomàs-Barberàn F. 2001. Effect of wounding on phenolic enzymes in six minimally processed lettuce cultivars upon storage. J. Agric. Food Chem., 49: 322.

Cantwell M. 1995. Fresh cut products. Perish. Handli. Newsl. 81: 2.

Castaner M., Gil M.I., Ruiz M.V. and Artes F. 1999. Browning susceptibility of minimally processed Baby and Romaine lettuces. Eur. Food Res. Technol. 209: 52.

Catalano A.E., Spinale S., Peluso O., Barbagallo R.N., Spagna G., Scuderi D. and Noto G. 2007. Effect of harvest age and processing on some of the biochemical degradations of ready to eat chinese cabbage (*Brassica campestris* L. ssp. *Pekinensis* Lour.) during chilled storage. In: Acta Hort. (ISHS). International Conference on Quality Management of Fresh Cut Produce. Bangkok, Thailand. August 6-8, 2007. p. 281. Leuven, S. Kanlayanarat, P.M.A. Toivonen, K.C. Gros, Belgium.

Chisari M., Peluso O., Todaro A., Barbagallo R.N. and Spagna G. 2007. Oxidases activities and antioxidant capacity of minimally processed baby romaine lettuce (*Lactuca sativa* L. cv. Duende) cultivated under different salinity conditions. V Congreso Iberoamericano de Tecnología Postcosecha y Agroexportaciones. Cartagena, Murcia (España). May 29 - June 1, 2007. Grupo Postrecolección y Refrigeración. p. 342. UPCT y AITE, Spain.

Corredig M., Kerr W. and Wicker L. 2000. Juice clarification by thermostable fractions of marsh grapefruit pectinmethylesterase. J. Agr. Food Chem. 48: 3238.

Crelier S., Robert M-C., Claude J. and Juillerat M-A. 2001. Tomato (*Lycopersicon esculentum*) pectin methylesterase and polygalacturonase behaviors regarding heat and pressure-induced inactivation. J. Agric. Food Chem., 49: 5566.

Degl'Innocenti E., Pardossi A., Tognoni F. and Guidi L. 2007. Physiological basis of sensitivity to enzymatic browning in lettuce, escarole and rocket salad when stored as fresh cut products. Food Chem., 104: 209.

De Lorenzo G., Cervone F., Hahn M.G., Darvill A. and Albersheim P. 1991. Bacterial endopectate lyase: evidence that plant cell wall pH prevents tissue maceration and increases the half-life of elicitor-active oligogalacturonides. Physiol. Mol. Plant Pathol. 39: 335.

Della Penna D., Lashbrook C.C., Toenjes K., Giovannoni J.J., Fisher R.L. and Bennett A.B. 1990. Polygalacturonase isoenzymes and pectin depolymerization in transgenic *rin* tomato fruit. Plant Physiol. 94: 1882.

Denes J.M., Baron A., Renard C.M., Pean C. and Drilleau J.F. 2000. Different action patterns for apple pectin methylesterase at pH 7.0 and 4.5. Carbohydr. Res. 327: 385.

Dubernet M. 1974. Recherches sur la tyrosinase de *Vitis vinifera* et la laccase de Botrytis cinerea. Applications technologiques. Thèse de 3 ème cycle. p. 1. Université de Bordeaux II, France.

Fachin D., Van Loey A.M., Ly Nguyen B., Verlent I., Indrawati and Hendrickx M.E. 2002. Comparative study of the inactivation kinetics of pectinmethylesterase in tomato juice and purified form. Biotechnol. Prog. 18: 739.

Fraeye I., De Roeck A., Duvetter T., Verlent, I., Hendrickx M. and Van Loey A. 2007. Influence of pectin properties and processing conditions on thermal pectin degradation. Food Chem., 105: 555.

Hadfield K.A., Rose J.K.C., Yaver D.S., Berka R.M. and Bennett A.B. 1998. Polygalacturonase Gene Expression in ripe melon fruit supports a role for polygalacturonase in ripening-associated pectin disassembly. Plant Physiol. 117: 363.

Hagerman A.E. and Austin P.J. 1986. Continuous spectrophotometric assay for plant pectin methylesterase. J. Agric. Food Chem. 34: 440.

Heimdal H., Kuhn B., Poll L. and Larsen L. 1995. Biochemical changes and sensory quality of shredded and MA-packaged iceberg lettuce. J. Food Sci. 60: 1265.

Hisaminato H., Murata M. and Homma S. 2001. Relationship between the enzymatic browning and phenylalanine ammonia-lyase activity of cut lettuce, and the prevention of browning by inhibitors of polyphenol biosynthesis. Biosci. Biotechnol. Biochem. 65: 1016.

Huber D.J. 1984. Strawberry fruit softening: the potential roles of polyuronides and hemicelluloses. J. Food Sci. 47: 1310.

Huber D.J. 1983 Polyuronide degradation and hemicellulose modifications in ripening tomato fruit. J. Amer. Soc. Hortic. Sci. 108: 405.

Hyodo H., Kuroda H. and Yang S.F. 1978. Induction of phenylalanine ammonia-lyase and increase in phenolics in lettuce leaves in relation to the development of russet spotting caused by ethylene. Plant Physiol., 62: 31.

Ingallinera B. and Spagna G. 2007. Shelf-life and enzymatic oxidation in fresh cut e scarole V Congreso Iberoamericano de Tecnología Postcosecha y Agroexportaciones. Cartagena, Murcia (España). May 29 - June 1, 2007. Grupo Postrecolección y Refrigeración. p. 607. UPCT y AITE, Spain.

Ingallinera B., Barbagallo R.N., Spagna G., Palmeri R. and Todaro A. 2005. Effects of thermal treatments on pectinesterase activity determined in blood orange juices. Enzyme Microbial Technol. 36: 258.

Jayani R.S., Saxena S. and Gupta R. 2005. Microbial pectinolytic enzymes: a review. Process Biochem. 40: 2931.

Ke D. and Saltveit M. 1989. Effect of calcium and auxin on russet spotting and phenylalanine ammonia-lyase activity in iceberg lettuce. Hort Science, 21: 1169.

Koshland D.E. Jr. 1959. Enzyme flexibility and enzyme action. J. Cell. Comp. Physiol., 54 (suppl. 1): 245.

Laats M.M., Grosdenis F., Recourt K., Voragen A.G.J. and Wichers, H.J. 1997. Partial purification and characterization of pectin methylesterase from green beans (*Phaseolus vulgaris* L.). J. Agric. Food Chem. 45: 572.

Longo C. 1996. Biologia vegetale: forme e funzioni (Eds.), UTET, Torino (Italy), p. 48.

Lopez-Serrano M. and Ros-Barcelò A. 1995. Peroxidase in unripe and processing-ripe strawberries. Food Chem. 52: 157.

Ly-Nguyen B., Van Loey A.M., Fachin D., Verlent I., Duvetter T., Vu S.T., Smout C. and Hendrickx E. 2002. Strawberry pectin methylesterase (PME): purification, characterization, thermal and high-pressure inactivation. Biotechnol. 18: 1447.

Marín-Rodríguez M.C., Orchard J. and Seymou, G.B. 2002. Pectate lyases, cell wall degradation and fruit softening. J. Exp. Bot. 53: 2115.

Martin-Diana A.B., Rico D., Barry-Rian C., Frias J.M., Mulcahy J., Henehan G.T.M. 2005. Calcium lactate washing treatments for salad-cut Iceberg lettuce: effect of temperature and concentration on quality retention parameters. Food Res. Int. 38: 729.

Mayer A.M. and Harel E. 1979. Polyphenol Oxidase in plants. Phytochem. 18: 193.

Mc Evily A.J., Iyengar R. and Gross A. 1992. Inhibition of polyphenol oxidase by phenolic compounds. In: "Phenolic Compounds and their Effects on Health". (Eds.) Anonymous. p. 318. American Chemical Society.

Minassi R.C., Pastore G.M. and Durán N. 2002. Potential applications of laccase in the food industry. Trend Food Sci. Technol. 13: 205.

Plaza L., Duvetter T., Monfort S., Clynen E., Schoofs L., Van Loey A.M. and Hendrickx M. 2007. Purification and thermal and high-pressure inactivation of pectinmethylesterase isoenzymes from tomatoes (*Lycopersicon esculentum*). A novel pressure labile isoenzyme. J. Agric. Food Chem., 55: 9259.

Ramírez E.C., Whitaker J.R. and Virador V.M. 2003. Polyphenol Oxidase. In: "Handbook of Food Enzymology", (Eds.), Whitaker J.R., Voragen A.G.J. and Wong D.W.S. p. 509. Marcel Dekker Inc., Basel, Switzerland.

Richard-Forget F. and Gauillard F.A. 1997. Oxidation of chlorogenic acid, catechins, and 4-methylcatechol in model solutions by combinations of pear (*Pyrus communis* cv. Williams) polyphenol oxidase and peroxidase: a possible involvement of peroxidase in enzymatic browning. J. Agr. Food Chem. 45: 2472.

Rombouts F.M., Geraeds C.C.J.M., Visser J. and Pilnik W. 1982. Purification of various pectic enzymes on crosslinked polyuronides. In: Affinity Chromatography and Related Techniques, (Eds.) Gribnau T.C.J., Visser J. and Nivard R.J.F. p. 266. Elseviers Scientific Comp. Amsterdam, Netherlands.

Scavetta R.D., Herron S.R., Hotchkiss A.T., Kita N., Keen N.T., Benen J.A., Kester H.C.M., Visser J. and Jurnak F. 1999. Structure of a plant cell wall fragment complexed to pectate lyase C. The Plant Cell 11: 1081.

Scuderi D., Catalano A.E., Chisari M., Restuccia C., Randazzo C., Caggia C., Barbagallo R.N. Spagna G. and Noto G. 2007. Shelf-life of Chinese cabbage (*Brassica campestris* L. ssp. *Pekinensis* Lour) packaged in ordinary atmosphere. Proceedings of Shelf Life International Meeting, Catania, Italy, June 21-23, 2006. Published as a special issue of Ital. J. of Food Sci., p. 411, Chiriotti Editori, Pinerolo, Italy.

Severini C., Baiano A., De Pilli T., Romaniello R. and Derossi A. 2003. Prevention of enzymatic browning in sliced potatoes by blanching in boiling saline solutions. Lebensm.-Wiss. U.-Technol. 36: 657.

Seymour T.A., Preston J.F., Wicker L., Lindsay J.A., Wei C.H. and Marshall M.R. 1991. Stability of pectinesterases of marsh white grapefruit pulp. J. Agric. Food Chem. 39: 1075.

Smith C.J.S., Watson C.F., Morris P.C., Bird C.R., Seymour G.B., Schuch W., Harding S. and Grierson D. 1990. Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. Plant Mol. Biol., 14: 369.

Spagna G., Barbagallo R.N., Chisari M. and Branca F. 2005a. Characterization of a tomato polyphenol oxidase and its role in browning and lycopene content. J. Agr. Food Chem., 53: 2032.

Spagna G., La Rosa R., Chisari M. and Giannone V. 2005b. Effects of stabilizing treatments and packaging on minimally processed fennels. Acta Horticolture 682: 1971.

Taylor A.J. and Clydesdale F.M. 1987. Potential of oxidised phenolics as food colorants. Food Chem. 24: 301.

Thongsook T., Whitaker J.R., Smith G.M. and Barrett D.M. 2007. Reactivation of broccoli peroxidases: Structural changes of partially denatured isoenzymes. J. Agric. Food Chem., 55: 1009.

Toivonen P.M.A. and Brummell D.A. 2008. Biochemical bases of appearance and texture changes fresh-cut fruit and vegetables. Postharvest Biol. Technol. 48:1.

Tomassen M.M.M., Barrett D.M., van der Valk H.C.P.M. and Woltering E.J. 2007. Isolation and characterization of a tomato non-specific lipid transfer protein involved in polygalacturonase-mediated pectin degradation. J. Exp. Bot. 58: 1151.

Van Lelyveld L.J., Gerrish C. and Dixon R.A. 1984. Enzyme activities and polyphenols related to mesocarp discoloration of avocado fruit. Phytochem. 2: 1531.

Van Linden V., Sila D.N., Duvetter T., De Baerdemaeker J. and Hendrickx M. 2008. Effect of mechanical impact-bruising on polygalacturonase and pectinmethylesterase activity and pectic cell wall components in tomato fruit. Posth. Biol. Technol., 47: 98.

Varoquaux P. and Wiley R.C. 1994. Biological and biochemical changes in minimally processed refrigerated fruits and vegetables. In: R.C. Wiley, "Minimally Processed Refrigerated Fruits and Vegetables". p. 226. Chapman and Hall, New York, USA.

Versteeg C., Rambouts F.M., Spaansen C.H. and Pilnik W. 1980. Thermostability and orange juice cloud destabilizing properties of multiple pectinesterases from orange. J. Food Sci. 45: 969.

Whitaker J.R. 2003a. Protein structure and kinetics of enzyme reactions: a historical perspective. In: "Handbook of Food Enzymology", (Ed.), Whitaker J.R., Voragen A.G.J., and Wong D.W.S. p. 1. Marcel Dekker Inc., Basel, Switzerland.

Whitaker J.R. 2003b. What enzymes do why they are highly specific and efficient catalysis. In: "Handbook of Food Enzymology", (Ed.), Whitaker J.R., Voragen A.G.J. and Wong D.W.S. p. 21. Marcel Dekker Inc., Basel, Switzerland.

Revised paper received September 11, 2008 Accepted November 18, 2008

PAPER

PRODUCTIVE CHARACTERISTICS AND VOLATILE COMPOUNDS OF SEVEN BROCCOLI CULTIVARS

CARATTERISTICHE PRODUTTIVE E COMPOSIZIONE VOLATILE DI SETTE VARIETÀ DI BROCCOLI

C. VIDAL-ARAGÓN[1], M. LOZANO[2], J. BERNALTE[3], C. AYUSO[3]*, I. GARCÍA[4], T. HERNÁNDEZ[2]; J. GARCÍA[2] and J.A. GONZÁLEZ[5]

[1]Escuela de Ingeniería Técnica Agrícola. Centro Universitario Santa Ana. Apdo 90, 06200 Almendralejo, Badajoz, Spain

[2]Instituto Tecnológico Agroalimentario de Extremadura, Apdo. 20107, 06071 Badajoz, Spain

[3]Escuela de Ingenierías Agrarias. Universidad de Extremadura, Ctra. Cáceres s/n, 06071 Badajoz, Spain

[4]Centro de Investigaciones Agrarias de Mabegondo, Ctr. de Betanzos-Santiago km 7,5, 15318 Abegondo, A Coruña, Spain

[5]Centro Investigación Finca "La Orden", 06187 Guadajira, Badajoz, Spain

*Corresponding author: Tel. +34 924 286200, Fax +34 924 286201, e-mail: cayuso@unex.es

ABSTRACT

The agronomical characteristics and the volatile fraction of seven broccoli cultivars were studied using GC/MS and dynamic head space as the isolation method. 'Parthenon' and 'Shena' were the most productive cultivars al-

RIASSUNTO

Sono state studiate, utilizzando come metodi di separazione la GC/MS e lo spazio di testa dinamico, le caratteristiche agronomiche e la frazione volatile di sette varietà di broccoli. Le varietà 'Parthenon' e 'Shena' sono risultate le

- Key words: fresh broccoli, GC/MS, head space analysis, production, quality, volatile compounds -

though their heads did not have the best quality characteristics. The fresh inflorescences were analysed to identify the most volatile compounds. A total of 45 compounds were identified. The sulphur compounds made up the majority of the volatile fraction, representing over 50% of the volatiles identified for all of the broccoli cultivars except 'Parthenon'. Dimethyl disulphide was the most abundant compound in all the cultivars. Discriminant analysis of the volatile fraction was carried out for descriptive purposes to distinguish the different broccoli cultivars. The first two discriminant functions explain 75.8% of the cumulative variance; seven groups were clearly identified.

più produttive, nonostante le loro infiorescenze non presentassero le migliori caratteristiche di qualità. Sono state analizzate le infiorescenze fresche per individuare le componenti più volatili. Sono stati identificati un totale di 45 composti. I composti solfurici costituivano la maggior parte della frazione volatile, rappresentando oltre il 50% dei volatili identificati per tutte le varietà di broccoli tranne che per la varietà 'Parthenon. Il dimetilsolfuro è risultato essere il composto più abbondante in tutte le cultivars. È stata condotta un'analisi discriminante, con finalità descrittive, della frazione volatile per individuare le differenti varietà di broccoli. Le prime due funzioni discriminanti giustificano il 75,8% della varianza cumulativa, sette gruppi sono stati chiaramente identificati.

INTRODUCTION

Broccoli, *Brassica oleracea* L. var. *italica* Plenck, belongs to the *Brassicaceae* family. The inflorescence is a hypertrophied mass of petioles and compressed foliate buds. Cultivar selection is complicated because there is a great variety of plant material available but very little specific information. In order to choose the most suitable cultivar, objective criteria such as yield and broccoli head quality should be used.

The most commonly used quality parameters for broccoli are granulometry, green colour, density of the head, stem diameter, shape and absence of hollow stem (RUFFIO-CHÂBLE and HERVÉ, 2001). Other characteristics, such as the volatile compounds, should also be considered as part of the quality criteria.

Recent studies on the products of secondary metabolism in plants have shown that these compounds called phytochemicals, generate a series of beneficial substances that vary in nature. Phytochemicals, ingested daily in gram quantities, can modulate human metabolism in such a way that they favour the prevention of tumours. Recent epidemiological studies have demonstrated that ingestion of moderate quantities of vegetables such as cabbage, broccoli, cauliflower, etc. provides a natural protection against cancerous agents. By eating brassicas the activation of precarcinogenic substances is inhibited, while the action of detoxifying enzymes is activated (CARRETERO, 2000). Brassicas are rich in glucosinolates, which are sulphur-containing molecules derived from protein and non-protein amino acids. They are precursors of anti-cancerous compounds such as isothiocyanates, nitriles, thiocyanates and organic cyanides (DI CE-

SARE *et al.*, 2001; FAHEY *et al.*, 2001; VALLEJO *et al.*, 2002).

From a nutritional point of view, brassicas are rich in vitamin C and certain minerals including iron and potassium. It should be emphasised that the high content of sulphur compounds, which are responsible for the unpleasant odour perceived when it is cooked, have certain effects that are beneficial to the health of the consumer (ENGEL *et al.*, 2002).

Broccoli heads deteriorate quickly after harvest. Modified atmosphere packaging reduces and delays all changes related to post-harvest quality loss. The choice of packaging film is a key factor for obtaining optimum modification of the atmosphere. It also helps avoid extremely low levels of O_2 and/or high levels of CO_2, that induce anaerobic metabolism which could generate off-flavours (SERRANO *et al.*, 2006). The volatile sulphur compounds that cause undesirable odours in broccoli during anaraerobic storage are derived through enzymatic pathways, but the enzyme activity does not appear to be the limiting factor (DERBALI *et al.*, 1998).

Many studies have been conducted on the volatile compounds found in brassicas. Information about the composition of these volatile constituents has been used to identify new volatile flavouring compounds, evaluate the effects of cooking methods and compare the volatile fractions in various brassicas (FORNEY *et al.,* 1991; HANSEN *et al.*, 1992; DI PENTIMA *et al.*, 1995; DERBALI *et al.,* 1998; DI CESARE *et al.,* 2001; VALETTE *et al.*, 2003; JACOBSSON *et al.,* 2004; SERRANO *et al.,* 2006). More information is needed about the compounds that contribute to the brassica flavour (VALETTE *et al.*, 2003). As in other products, the analysis of volatile compounds in broccoli could be used as a quality criterion (SOLÍS-SOLÍS *et al.*, 2007), as well as to distinguish different cultivars (CHIN *et al.,* 2007).

The aim of this study was to determine the agronomic characteristics and identify the volatile fraction of seven broccoli cultivars, by means of GC/MS, using dynamic head space as the isolation method.

MATERIALS AND METHODS

Plant material

The broccoli cultivars 'Marathon', 'Parthenon' and 'Samson' (Sakata), 'Merit' (Fito), 'Nubia' (Ramiro Arnedo), 'Serydan' (Intersemillas) and 'Shena' (Seminis) were grown during 2005-2006, at the "Finca La Orden" experimental farm, of the Extremadura, Regional Government, located in Badajoz, Spain. The alluvial soil has a sandy-loam texture, is slightly acidic, and has a low organic material content.

Broccoli plants, transplanted on September 21st, were grown with irrigation and top dressing fertiliser was applied in the irrigation water. Local techniques were used for the remaining cultivation processes. All samples were harvested at the same growth stage from January to March. The following data were recorded: harvest dates, broccoli production and some quality parameters (weight, head height, head and stem diameters, density and granulometry) of the broccoli heads.

Ten broccoli heads were taken to evaluate some quality parameters on three different dates during the harvest season. Broccoli inflorescences were weighed and the head height and head and stem diameters were measured. Density, the weight/volume ratio, was calculated using the formula: weight (kg) x 238.77/(diameter(cm)/2)^3. Shape is the head height/head diameter ratio. Granulometry was evaluated using a visual five point scale (thick, medium-thick, medium, medium-fine, fine); consumers consider fine grain to be the best quality.

Six inflorescences from each cultivar were rapidly transported to the laboratory and the volatile composition of each cultivar was immediately analysed.

Preparation and analysis of volatiles

Once flowering, the florets were cut into 1 cm^3 pieces weighing approximately 5 g and were placed in test tubes. The freshly prepared aliquot was placed in the Tekmar 2016 automatic head space analyzer (Teledyne Tekmar, Mason, Ohio, USA). Volatile isolation was carried out using a helium flow rate of 100 mL min^{-1}, and the purging time was 90 min at room temperature. The identification and quantification of the volatile compounds were performed on a Varian Star 3400 gas chromatograph coupled with a Varian Saturn 3 mass spectrometer detector (Varian Ibérica S.L., Madrid). The GC-MS system was equipped with an Agilent HP-5 column (Agilent, 50x0.32 mm x 1.05 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1 mL min^{-1} in a split ratio of 1:30. Injector and detector were held at 260°C. Mass spectra were obtained by ion trap and a spectra range of 15-400 m/z was used. The GC-MS apparatus was connected to a PC running Varian MS Workstation software. Identification of some compounds was confirmed by comparing the collected mass spectra with those of authenticated reference standards and all the spectra in the Nist 2.0d mass spectra library. The experimental retention index of some of the compounds was compared with those of authentic reference standards. Six replicates were performed for each broccoli cultivar.

Statistical analysis

Statistical analysis of the results was carried out by one-way analysis of variance (ANOVA) and a Tukey's test to compare the means ($p<0.05$). The multivariate stepwise discriminant analysis (Wilks' Lambda method) was also carried out using the SPSS 10.0 statistical package (SPSS Inc, Chicago, IL, USA).

Table 1 - Harvesting guide, number of days from transplant to first harvest, and production of the different broccoli cultivars.

Cultivar	January											February										March							Harvest		Production
	9	11	13	15	17	19	21	23	25	27	29	31	...	17	19	21	23	25	28	1	3	5	7	9	Days	n	days	(t/ha)[1]
Marathon																									112	11	58	11.16±1.05
Merit																									118	8	37	11.21±0.54
Nubia																									118	7	31	12.13±0.51
Parthenon																									112	10	51	14.51±1.13
Samson																									132	7	38	12.11±1.52
Serydan																									112	10	51	12.05±1.10
Shena																									112	9	43	13.38±2.27

[1] Mean value ± SD ($p>0.05$).

RESULTS AND DISCUSSION

Agronomic and quality characteristics

The broccoli cultivars studied in this work are widely distributed throughout Spain and in other European countries. The number of days (Table 1) from transplanting to first harvest, ranged from 112 to 118, except for 'Samson', that was first harvested on January 30 (132 days). The duration was longer than in previous seasons (GARCÍA *et al.*, 2005), probably because transplanting was carried out later and cold weather started when plants were in an early stage of development. The harvesting period for 'Nubia' lasted 31 days with 7 harvest dates, that of 'Marathon' lasted 58 days, with 11 harvest dates (Table 1).

Production was very similar for most of the cultivars; 'Parthenon' and 'Shena' had yields of almost 14 t ha^{-1} (Table 1).

Broccoli heads weighed (Table 2) about 500 g; 'Serydan' (600 g) was the heaviest cultivar. 'Marathon' had the smallest stem diameter (3.43 cm), as in previous seasons (GARCÍA *et al.*, 2005). 'Samson', 'Nubia' and 'Merit' had the highest density values while 'Marathon' and 'Parthenon' had the lowest. 'Marathon' had the lowest shape value while 'Parthenon' had the highest. Regarding granulometry, 'Marathon' and 'Shena' had a medium-thick grain while 'Merit' and 'Nubia' had a medium-fine grain.

Volatile compounds

The forty-five compounds identified in the seven broccoli cultivars are grouped into chemical families (Table 3). 'Merit' had the greatest aromatic richness; it had the highest peak area values of the identified compounds, while 'Serydan' had the lowest.

Sulphur compounds

The following sulphur compounds were identified: methanethiol, dimethyl sulphide, methyl thiocyanate, dimethyl disulphide, dimethyl trisulphide, dimethyl tetrasulphide, methyl-(methylthio)-methyl disulphide and methyl-(methylthio)-ethyl disulphide (Table 3). These sulphur compounds are considered by several authors to be the major odour impact compounds in brassica vegetables, and are usually used to compare cultivars (DI CESARE *et al.*, 2001) and to study the impact that various processing techniques have on the aroma (JACOBSSON *et al.*, 2004).

Over 50% of the volatiles identified in all cultivars were sulphur compounds with the exception of 'Parthenon'. In 'Shena', 72.6% of the total volatiles, ex-

Table 2 - Quality parameters of broccoli inflorescences.

Cultivar	Head weight[1] (g)	Stem diameter[1] (cm)	Density[1]	Shape[1]	Granulometry[2]
Marathon	508^{a}	3.43^{a}	0.263^{a}	0.500^{a}	Medium-thick
Merit	512^{a}	4.22^{b}	0.300^{abc}	0.527^{ab}	Medium-fine
Nubia	549^{ab}	4.07^{b}	0.307^{bc}	0.573^{bcd}	Medium-fine
Parthenon	532^{ab}	4.11^{b}	0.264^{a}	0.612^{d}	Medium
Samson	507^{a}	4.61^{c}	0.317^{c}	0.553^{ab}	Medium
Serydan	621^{b}	4.08^{b}	0.284^{ab}	0.584^{bcd}	Medium
Shena	534^{ab}	4.22^{b}	0.293^{abc}	0.583^{bcd}	Medium-thick

[1] Mean value of thirty determinations;
[2] Visual observation.
In columns, values followed by different letter are significantly different ($p<0.05$).

Table 3 - Volatile compounds identified in the aromatic fraction of seven fresh broccoli cultivars. [1] Retention time
ard error). In lines, values followed by different letter are significantly different (p<0.05).

RT[1]	Components[2]	Marathon	Merit	Nubia
3.69	Methanethiol	1116.91 (178.59)	2462.77 (690.23)	2330.30 (913.69)
5.31	Dimethyl sulphide	36.06 (8.34)	75.67 (18.15)	73.64 (6.35)
14.89	Methyl thiocyanate	21.16 (12.38)	33.27 (15.24)	40.99 (14.28)
17.51	Dimethyl disulphide	9618.37^{ab} (1972.08)	20143.33^{b} (5661.00)	5940.38^{a} (2509.11)
38.02	Dimethyl trisulphide	1077.08 (473.95)	1735.80 (663.84)	537.37 (140.15)
51.64	Methy l(methylthio)methyl disulphide	12.02 (5.61)	55.33 (25.66)	6.80 (3.12)
55.29	Methyl (methylthio)ethyl disulphide	ND	89.57 (33.28)	ND
59.29	Dimethyl tetrasulphide	78.52 (50.57)	75.62 (36.35)	17.18 (2.18)
Total area of sulphur compounds		**11960.11^{ab} (1962.05)**	**24671.36^{b} (6330.54)**	**8946.66^{a} (63.68)**
6.64	2-Methylpentane	713.51^{b} (181.19)	1192.53^{c} (114.52)	301.84^{ab} (46.01)
7.19	3-Methylpentane	883.51^{b}(227.82)	1609.12^{c} (148.37)	355.92^{a} (51.99)
7.89	Hexane	5394.17^{b}(1395.80)	9888.41^{c} (835.84)	1853.80^{a} (282.46)
11.39	Cyclohexane	151.03(12.49)	223.72 (13.10)	ND
22.01	Octane	47.18^{a}(9.53)	64.46^{ab} (14.04)	61.32^{ab} (4.92)
28.59	1,2-Dimethylbenzene	8.56^{ab}(2.25)	6.22^{a} (1.12)	18.57^{c} (0.55)
39.86	Trimethylbenzene	29.88(6.32)	ND	ND
42.64	Benzene-1-methyl-4(1-methylethyl)	ND	57.18 (1.49)	74.17 (22.24)
Total area of hydrocarbons		**7227.83^{b} (1802.92)**	**13041.62^{c} (1107.53)**	**2665.61^{a} (386.97)**
6.36	3-Buten-1-ol + 1-Propanol	17.65 (7.27)	278.57 (220.57)	79.31 (12.35)
9.39	2-Methylpropanol	298.36^{ab}(72.39)	542.58^{c} (46.11)	239.31^{a} (16.63)
12.64	1-Penten-3-ol	57.97(41.33)	98.46 (26.54)	144.92 (21.11)
13.86	3-Pentanol	207.63 (68.64)	442.63 (99.75)	295.05 (95.49)
16.54	3-Methylbutanol	59.43^{ab}(29.51)	124.26^{ab} (49.09)	230.63^{ab} (44.91)
19.29	1-Pentanol	ND	16.46 (1.72)	19.32 (2.53)
19.39	2-Penten-1-ol	4.78 (1.63)	12.62 (1.34)	10.76 (3.19)
20.94	3-Methyl-2-pentanol	61.68 (8.73)	ND	ND
27.14	3-Hexen-1-ol	99.84^{a} (43.88)	363.14^{ab} (91.33)	549.14^{b} (188.99)
28.27	Hexanol	7.13^{a} (2.99)	37.51^{c} (7.97)	36.13^{bc} (6.49)
Total area of alcohols		**814.47^{ab} (110.55)**	**1916.23^{c} (242.55)**	**1604.57^{c} (283.30)**
3.39	Ammonia	168.13 (43.28)	229.48 (105.45)	327.23 (90.65)
11.11	3-Butanenitrile	3.09 (1.23)	6.70 (0.69)	22.07 (2.51)
15.67	Ethenamine-N-methylene	3.44 (0.49)	1.98 (0.72)	3.76 (0.53)
Total area of nitrogen compounds		**174.66^{ab} (1.07)**	**238.16^{ab} (65.53)**	**353.07^{b} (44.21)**
10.86	3-Methylbutanal	4.17 (0.48)	6.10 (0.78)	17.84 (7.83)
11.44	2-Methylbutanal	ND	ND	74.45 (20.33)
18.49	2(E)-Hexenal	3.70^{a} (1.08)	44.97^{b} (2.81)	43.74^{b} (7.01)
31.34	Heptanal	ND	15.32 (13.14)	10.65 (5.13)
32.11	2,4-Hexadienal	2.03(0.21)	4.14 (0.72)	2.55 (0.32)
36.92	Benzaldehyde	4.19^{ab} (1.23)	3.92^{ab} (0.17)	8.86^{cd} (0.20)
49.21	Nonanal	2.91 (0.45)	7.80 (3.14)	4.66 (0.99)
Total area of aldehydes		**17.00^{a} (1.62)**	**82.25^{b} (5.85)**	**162.74^{d} (24.53)**
13.54	2,3-Pentanedione	14.36 (5.69)	50.05 (10.27)	23.78 (3.55)
16.24	3-Methyl-3-buten-2-one	6.47^{a} (3.81)	26.1^{abc} (5.00)	39.16^{c} (4.20)
18.07	3-Methyl-2-pentanone	16.79 (2.26)	ND	9.73 (1.31)
36.46	5-Ethyl-2-furanone-(SH)	2.56 (0.72)	3.27 (0.79)	4.00 (0.54)
Total area of ketones		**40.18^{ab} (5.73)**	**79.42^{ab} (12.47)**	**76.66^{ab} (6.11)**
34.54	Unknown terpene	10.12 (1.70)	221.13 (20.69)	217.52 (10.30)
41.41	3-Carene	ND	259.25 (23.26)	263.27 (14.96)
42.99	Limonene	70.03 (51.75)	ND	7.97 (4.35)
Total area of terpenes		**80.15^{a} (50.71)**	**480.38^{b} (43.61)**	**488.75^{b} (28.19)**
40.64	4-Hexen-1-ol-acetate	147.64 (68.61)	538.93 (402.86)	920.32 (699.31)
Total area of esters		**147.64 (68.61)**	**538.93 (402.86)**	**920.32 (699.31)**
4.64	Methyl isocyanate and others	678.78^{a} (284.50)	630.18^{a} (101.84)	457.38^{a} (60.24)
Total Area of Identified Compounds		**21165.60^{a} (1957.65)**	**41681.73^{b} (6044.45)**	**15675.75^{a} (3121.70)**

e in minutes; [2] Volatile compounds are listed by chemical family; [3] Results mean value of six replications (stand-

Area units x (10^{-4}) [3]			
Parthenon	Samson	Serydan	Shena
977.99 (298.34)	1259.51 (135.53)	1335.85 (347.54)	1349.16 (118.98)
ND	165.36 (13.65)	53.78 (42.40)	106.28 (13.28)
11.11 (3.43)	19.63 (5.26)	17.66 (6.05)	42.28 (21.93)
1867.09^{a} (802.20)	6345.86^{a} (2386.50)	4034.87^{a} (1048.00)	11034.95^{ab} (2213.19)
278.64 (137.47)	644.07 (358.46)	330.04 (127.72)	1501.38 (405.45)
ND	46.53 (22.92)	10.56 (5.02)	41.88 (8.54)
ND	24.30 (14.13)	ND	34.37 (6.24)
2.46 (0.25)	111.83 (95.15)	ND	95.10 (27.96)
3137.29^{a} (1227.76)	**8617.10^{a} (2731.27)**	**5782.76^{a} (1431.12)**	**14205.38^{ab} (2744.68)**
198.33^{a} (31.50)	423.62^{ab} (87.90)	65.96^{a} (12.45)	418.42^{ab} (43.35)
188.72^{a} (29.80)	473.14^{ab} (85.91)	58.43^{a} (10.93)	459.65^{ab} (45.44)
1631.13^{a} (238.58)	4081.26^{ab} (873.64)	1643.74^{a} (293.69)	2107.05^{ab} (200.20)
564.80 (46.10)	156.17 (27.10)	138.71 (53.20)	251.05 (158.05)
56.03^{a} (7.12)	110.08^{bc} (15.98)	129.02^{c} (16.92)	54.98^{a} (6.82)
12.01^{b} (0.67)	3.57^{a} (0.90)	5.00^{a} (0.89)	13.27^{bc} (1.11)
ND	ND	ND	ND
NF	ND	ND	ND
2651.03^{a} (393.11)	**5247.83^{ab} (1052.01)**	**2040.86^{a} (339.65)**	**3304.41^{ab} (134.63)**
41.50 (19.45)	193.01 (54.37)	59.53 (18.04)	154.98 (143.42)
251.30^{a} (38.01)	515.94^{bc} (83.45)	94.07^{a} (9.72)	138.17^{a} (12.13)
ND	ND	77.57 (10.62)	ND
257.36 (16.19)	243.73 (24.90)	139.92 (33.90)	145.68 (43.73)
2.84^{a} (0.14)	559.29^{b} (230.81)	70.68^{ab} (35.53)	41.20^{ab} (2.90)
11.26 (1.56)	14.33 (1.01)	8.73 (0.42)	ND
3.24 (0.45)	ND	6.20 (1.73)	3.03 (1.20)
78.41 (9.24)	ND	81.31 (15.06)	ND
76.07^{a} (29.49)	37.21^{a} (12.77)	162.80^{ab} (79.70)	150.92^{ab} (71.80)
8.63^{a} (2.27)	16.35^{abc} (3.92)	14.78^{ab} (2.90)	12.34^{a} (4.03)
730.61^{a} (56.40)	**1579.86^{bc} (186.29)**	**715.57^{a} (76.28)**	**646.31^{a} (68.14)**
166.95 (93.49)	253.94 (73.01)	551.47 (203.25)	ND
1.66 (0.12)	ND	ND	ND
2.84 (0.14)	7.96 (2.65)	7.29 (2.81)	2.08 (0.18)
171.45^{ab} (58.93)	**261.91^{b} (72.83)**	**558.76^{c} (28.14)**	**2.08^{a} (0.18)**
ND	18.68 (6.12)	ND	6.83 (0.58)
ND	80.59 (7.01)	45.01 (5.40)	50.74 (6.34)
5.52^{a} (2.84)	2.87^{a} (0.41)	9.05^{a} (3.88)	5.00^{a} (0.44)
8.01 (3.06)	16.09 (2.94)	4.98 (0.71)	1.24 (0.44)
ND	ND	ND	3.28 (0.63)
6.61^{bc} (0.76)	1.95^{a} (0.71)	1.88^{a} (0.29)	9.69^{d} (0.52)
ND	ND	ND	14.79 (5.26)
20.14^{a} (2.41)	**120.17^{c} (18.11)**	**60.91^{b} (10.57)**	**91.57^{b} (20.48)**
ND	ND	ND	ND
6.04^{a} (2.96)	32.18^{bc} (5.64)	12.17^{ab} (5.52)	12.30^{ab} (0.42)
37.22 (7.76)	22.37 (10.11)	ND	71.45 (9.28)
ND	2.37 (0.55)	ND	3.29 (0.71)
43.26^{ab} (10.23)	**56.93^{ab} (3.29)**	**12.17^{a} (5.52)**	**87.03^{b} (23.59)**
ND	ND	ND	ND
21.04 (5.23)	ND	148.48 (22.58)	ND
33.8 (8.76)	ND	392.65 (46.63)	ND
54.84^{a} (9.35)	**ND**	**541.13^{b} (58.17)**	**ND**
511.66 (336.53)	ND	362.95 (287.68)	276.97 (117.71)
511.66 (336.53)	**ND**	**362.95 (287.68)**	**276.97 (117.71)**
8687.29^{c} (753.59)	722.73^{a} (84.11)	2846.84^{b} (514.83)	952.75^{a} (186.08)
16129.24^{a} (1687.44)	**16610.48^{a} (2454.92)**	**12923.91^{a} (1460.48)**	**19572.19^{a} (2602.62)**

Table 4 - Percentage areas of chemical families, excluding ethanol peak.

Chemical family	Marathon	Merit	Nubia	Parthenon	Samson	Serydan	Shena
Sulphur compounds	56.51	59.19	57.07	19.45	51.88	44.74	72.58
Hydrocarbons	34.15	31.29	17.00	16.44	31.59	15.79	16.88
Alcohols	3.85	4.60	10.24	4.53	9.51	5.54	3.30
Nitrogen compounds	0.83	0.57	2.25	1.68	1.58	4.32	0.01
Aldehydes	0.08	0.20	1.04	0.12	0.72	0.47	0.47
Ketones	0.19	0.19	0.49	0.27	0.34	0.09	0.44
Terpenes	0.38	1.15	3.12	0.34	0.00	4.19	0.00
Esters	0.70	1.29	5.87	3.17	0.00	2.81	1.42
Methyl isocyanate and others	3.21	1.51	2.92	53.86	4.35	22.03	4.87
	99.88	99.99	100.00	99.87	99.98	99.98	99.97

cluding the ethanol peak, corresponded to the sulphur family (Table 4).

As can be observed in Table 3, dimethyl disulphide was the most abundant sulphur compound in all the broccoli samples. These results agree with those of JACOBSSON *et al.* (2004) for raw samples of the 'Marathon' broccoli cultivar. These authors carried out the extraction by means of dynamic head space. However, there is a discrepancy with the results reported by other authors in samples grown in Italy because different extraction techniques were used to study the volatile fraction and higher concentrations of the heavier sulphurs were obtained (DI CESARE *et al.,* 2001). Dimethyl disulphide originates from the rupture of the S-methyl-L-cysteine sulphoxide and its aroma is defined as the sulphureous aroma of cauliflower or cabbage (DI PENTIMA *et al.,* 1995). Its detection threshold is 0.01 ppb in water (HANSEN *et al.*, 1992). This compound was most abundant in 'Merit', and the values were significantly different from those of the other cultivars.

Methanethiol was the second most abundant compound in all the cultivars except 'Shena' (Table 3). The aroma of this compound is described as putrified, faecal, of cooked cabbage. In brassica vegetables, it originates from the rupture of S-methyl-L-cysteine sulphoxide. It is considered to be one of the sulphur compounds that has the strongest odour in foods. It has a very low detection threshold in water (0.02 ppb) and a high volatility (LINDSAY and RIPPE, 1986). In addition, it can be a precursor to other sulphur compounds (CHIN and LINDSAY, 1994). Under anaerobic conditions, broccoli produces severe off-flavours because metanethiol is one of the compounds found in the headspace. It is probably formed through an enzymatic pathway (DI PENTIMA *et al.*, 1995). The cultivars 'Merit' and 'Nubia' were the richest in methanethiol.

The third most abundant compound was dimethyl trisulphide, which can also be of the same origin. This compound was present in the highest percentage of all those identified by DI CESARE *et al.* (2001) in broccoli cultivars from Catania (Italy), the same as in other brassicas such as cauliflowers and cabbages. These differences may be due to the extraction methods used.

The rest of the sulphur compounds had smaller peak areas. The odour of dimethyl sulphide is described as cauliflower (ENGEL *et al.,* 2002) and can originate from S-methyl methionine (SCHWIMMER and FRIEDMAN, 1972). Its detection threshold in water is 0.3 ppb and it was most abundant in the 'Samson' cultivar. It has also been identified

by other authors in broccoli (JACOBSSON *et al.*, 2004) and cauliflower (ENGEL *et al.*, 2002).

It should be noted that disulphidemethyl-(methyltio)-ethyl was not detected in the 'Marathon', 'Nubia', 'Parthenon' and 'Serydan' cultivars.

Hydrocarbon family

Hydrocarbons were the second most important group of compounds but are not of great interest from an aromatic point of view since they have high detection thresholds (VIDAL-ARAGÓN, 1996). Eight hydrocarbons, 4 linear and 4 cyclic, were identified (Table 3). The percentages of hydrocarbons ranged from 34% in 'Marathon' to 15.8% in 'Serydan' (Table 4). Within the hydrocarbons, hexane was the most abundant in all the cultivars, followed by 3-methylpentane and 2-methylpentane. The quantities of the last three compounds differed significantly in the cultivars analysed. 'Merit' and 'Marathon' had the most hydrocarbon compounds; 'Nubia', 'Parthenon' and 'Serydan' had lower levels. Trimethylbenzene was characteristic of 'Marathon', while benzene-1-methyl-4(1-methylethyl) was characteristic in 'Nubia' and 'Merit'. The 'Merit' cultivar was the richest in hydrocarbons.

Alcohols

Ten different alcohols besides ethanol were identified (Table 3). Not all of these were present in detectable quantities in all the cultivars. 2-Methylpropanol was the most abundant alcohol in 'Merit' and 'Marathon'. The 'Samson' and 'Nubia' cultivars had significantly higher quantities of 3-methylbutanol and 3-hexen-1-ol, respectively. The origin of alcohols in vegetables has been attributed to the reduction of the corresponding aldehydes. According to VIDAL-ARAGÓN *et al.* (2005), 3-methylbutanol comes from the reduction of 3-methylbutanal in pepper. The highest total quantity of alcohols was found in 'Merit', followed by 'Nubia' and 'Samson'. In percentage, the alcohol family ranges from 10.2% in 'Nubia' to 3.3% in 'Shena' (Table 4).

The ethanol peak in all the cultivars was very high (data not shown). During the purging time (90 min) the respiration rate increased because the vegetable material was chopped and kept at room temperature in the laboratory. With a lack of oxygen in the test tube, the process followed an anaerobic route as described by FORNEY *et al.* (1991).

Nitrogen compounds

Among the nitrogen compounds ammonia was the major compound in all cases except in the 'Shena' cultivar, in which it was not detected. The 'Nubia' cultivar had the highest 3-butanenitrile values. 'Serydan' had the most nitrogen compounds. The percentages of nitrogen compounds (Table 4) with respect to the total was low except in 'Serydan', in which nitrogen compounds made up 4.3% of the total.

Methyl isocyanate, a nitrogen compound, was very abundant in 'Parthenon'. However, since it elutes with other compounds, it was not included in this group; its values are shown separately (Table 3).

Aldehydes

Seven different aldehydes were isolated and identified (Table 3). Only the benzaldehyde and 2-hexenal values were significantly different in the samples studied. These compounds come from the oxidative degradation of unsaturated fatty acids (SALUNKHE and DO, 1976). The 'Merit' and 'Nubia' cultivars had significantly higher quantities of 2-hexenal with respect to the other cultivars, and 'Shena' had the greatest peak area of benzaldehyde. 2-Methyl butanal was only found in the 'Samson', 'Nubia', 'Shena' and 'Serydan' cultivars, and was the most abundant aldehyde. 3-Methyl butanal and 2-methyl butanal have also

been found in other fruits and vegetables such as sweet cherry or dried pepper. It originates from the Strecker degradation of the amino acid leucine (BERNALTE *et al.*, 1999; VIDAL-ARAGÓN *et al.*, 2005).

The aromatic fraction of aldehydes in brassica vegetables, expressed in percentage of area, was less than 1% (Table 4). The 'Nubia' cultivar had the highest amount and 'Marathon' the lowest. As aldehydes have characteristic aromatic notes, and a low olfactory detection threshold, this family could be responsible for a certain part of the broccoli aroma.

Ketones

Four ketones besides 2-propanone were identified. Since 2-propanone eluted with other compounds, it is included in the peak corresponding to methyl isocyanate and other compounds (Table 3). 'Shena' had the highest 3-methyl-2-pentanone content, and 'Merit' was the richest in 2,3-pentanedione, that was only identified in three cultivars. Ketones made up less than 0.5% of the total aromatic fraction of brassicas (Table 4).

Other compounds

The peak retention time of 4.64 min corresponded to the mixture of methyl isocyanate (ion 41-42), 2-methylpropanol (ion 43 and 59) and 2-propanone. This peak was quantitatively important in 'Parthenon' and 'Serydan', with values of 53.8% and 22.0%, respectively.

The 'Nubia', 'Merit' and 'Serydan' cultivars were relatively rich in terpenes, which are derived from carbohydrate degradation (ESKIN, 1990). Acetate from the glycolytic pathway could be the origin of isoprene units that generate terpene compounds (ESKIN, 1990).

The ester 4-hexen-1-ol-acetate was quantitatively important in the 'Nubia', 'Merit' and 'Parthenon' cultivars (Table 3).

Discriminant analysis results

According to the results obtained in the analysis of variance, no single variable can distinguish the seven broccoli cultivars, although some variables are able to distinguish 3 or 4 cultivars. Therefore it was of interest to combine the parameters by means of multivariate techniques. In this way the interrelation between all the parameters can be taken into account.

A stepwise discriminant analysis was carried out using 28 previously selected variables, and two discriminant functions were generated using only 11 variables. The first and second discriminant functions explained 62.2% (canonical correlation 0.995), and 13.6% (canonical correlation 0.978) of the variance, respectively. The cumulative variance was 75.8%. The results of the discriminant analysis (Fig. 1) show that the 'Shena', 'Merit', 'Parthenon' and 'Samson' cultivars were clearly distinguished from the rest. The other three cultivars are more similar to one another. The aim of this multivariate method was descriptive and was used to confirm differences in the volatile compounds among the cultivars.

It should be noted that the system did not use any sulphur compound to classify the samples, even though these made up the majority in the volatile fraction of all the broccoli cultivars analysed. To distinguish the samples, the system used four of the eight hydrocarbons identified: 2-methylpentane, 3-methylpentane, 1,2-dimethylbenzene and octane. The system also used the alcohols 2-methylpropanol and 3-hexen-1-ol. The aldehyde 2-hexenal was also used. This compound is present in appreciable quantities in most vegetables, and gives aromatic notes reminiscent of freshly cut grass and bitter almonds (RAVICHANDRAN and PARTHIBAN, 1998; ANGEROSA *et al.*, 2000). The discriminant analysis also used the ketone 3-methyl

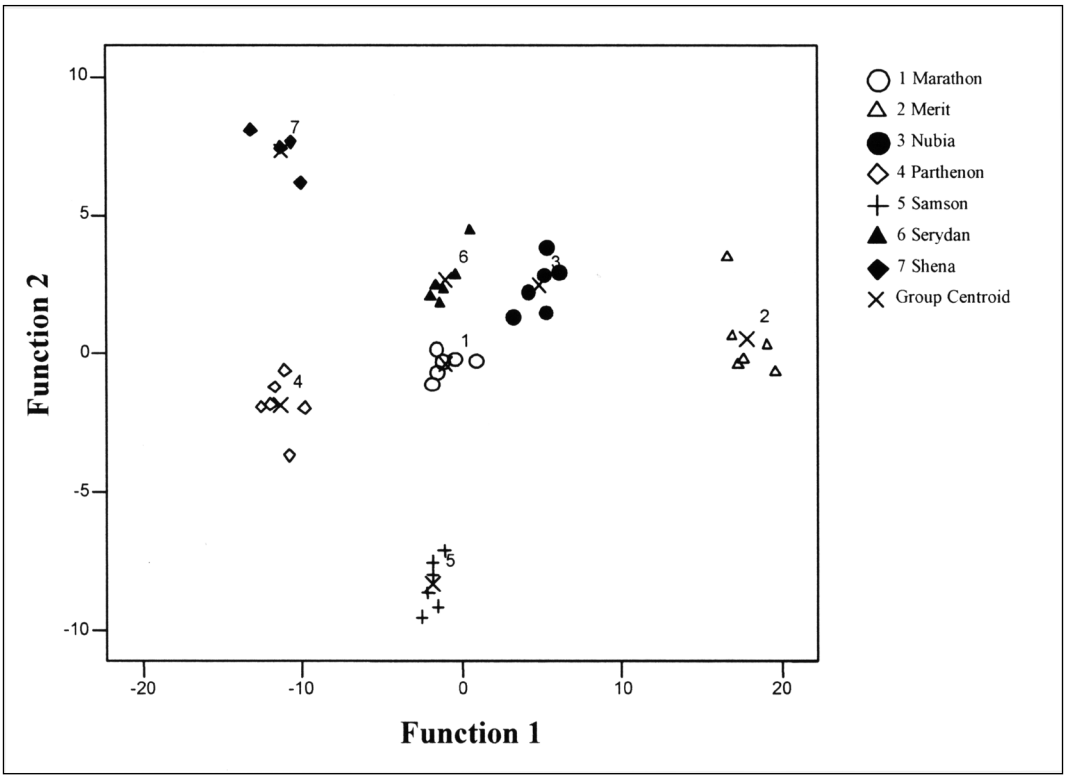

Fig. 1 - Distribution of broccoli cultivars on the discriminant plane.

2 pentanone, the 3-carene terpene and two nitrogen compounds, ammonia and the peak corresponding to methyl isocyanate plus others.

CONCLUSIONS

All the broccoli cultivars studied showed similar agronomical characteristics. 'Parthenon' and 'Shena' had the highest production, and 'Samson' and 'Nubia' had the highest density. 'Nubia' can be considered a good broccoli cultivar because it has a high production and density, and a medium-fine granulometry.

The 'Merit' cultivar had the highest volatile compound content while 'Serydan' had the lowest. Sulphur compounds were the most abundant in all the cultivars, ranging from 72.6% in 'Shena' to 19.5% in 'Parthenon', with dimethyl disulphide being the main sulphur compound in all cases. These compounds can be generated as a result of anaerobic conditions established in the analytical method.

Discriminant analysis of the volatile fraction may prove to be a useful tool for distinguishing different broccoli cultivars because it classifies them and establishes different, completely separated groups.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Junta de Extemadura for financing the project 2PR 03B014, that has allowed to carry out this study.

REFERENCES

Angerosa F., Mostallino R., Basti C. and Vito R. 2000. Virgin olive oil odour notes: their relationships with volatile compounds from the lipoxygenase pathway and secoiridoid compounds. Food Chem. 68: 283.

Bernalte M.J., Hernández M.T., Vidal-Aragón M.C. and Sabio E. 1999. Physical, chemical, flavor and sensory characteristics of two sweet cherry varieties grown in 'Valle del Jerte' (Spain). J. Food Qual. 22: 403.

Carretero M.E. 2000. Glucosinolatos. Panorama Actual Médico 24: 223.

Chin H.W. and Lindsay R.C. 1994. Mechanisms of formation of volatile sulfur compounds following the action of cysteine sulfoxide lyasses. J. Agric. Food Chem. 42: 1529.

Chin S.T., Nazimah S.A.H., Quek S.Y., Che Man Y.B., Abdul Rahman R. and Mat Hashim D. 2007. Analysis of volatile compounds from Malaysian durians (*Durio zibethinus*) using headspace SPME coupled to fast GC-MS. J. Food Comp. Anal. 20: 31.

Derbali E., Makhlouf J. and Vezina L.P. 1998. Biosynthesis of sulfur volatile compounds in broccoli seedlings stored under anaerobic conditions. Postharvest Biol. Technol. 13: 191.

Di Cesare L.F., Vitale R., Acciarri N. and Branca F. 2001. Composizione volatile caratteristica di alcune cultivar di broccolo, cavolo e verza coltivate in Italia. Industrie Alimentari 40: 508.

Di Pentima J.H., Rios J.J., Clemente A and Olías J.M. 1995. Biogenesis of off-odor in broccoli storage under low-oxygen atmosphere. J. Agric. Food Chem. 43: 1310.

Engel E., Baty C., Le Core D., Souchon I. and Martin N. 2002. Flavor-active compounds potentially implied in cooked cauliflower acceptance. J. Agric. Food Chem. 50: 6459.

Eskin N.A.M. 1990. Biochemical changes in raw foods: fruits and vegetables. In "Biochemistry of Foods". Academic Press, San Diego, CA.

Fahey J.W., Zalcmannn A.T. and Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56: 5.

Forney C.F., Mattheis J.P. and Austin R.K. 1991. Volatile compounds produced by broccoli under anaerobic conditions. J. Agric. Food Chem. 39: 2257.

García M.I., González J.A., Lozano M., Ayuso M.C., Bernalte M.J., Pacheco M., Calvo P., Martínez M.A. Benítez-Donoso R. and Campillo C. 2005. Comportamiento agronómico e industrial de cultivares de bróculi en las Vegas del Guadiana. Actas Portuguesas de Horticultura 5: 126.

Hansen M., Buttery R.G., Stern D.J., Cantwell M.I. and Ling, L.C. 1992. Broccoli storage under low oxygen atmosphere: identification of higher boiling volatiles. J. Agric. Food Chem. 40: 850.

Jacobsson A., Nielsen T. and Sjoholm I. 2004. Influence of temperature, modified atmosphere packaging, and heat treatment on aroma compounds in broccoli J. Agric. Food Chem. 52: 1607.

Lindsay R.C. and Rippe J.K. 1986. Enzymatic generation of methanethiol to assist in the flavor development of Cheddar cheese and other foods. In "Biogeneration of Aromas". R. Croteau and T. Parliment (Ed.), A.C.S. Symp. Ser. 317. Washington D.C.

Ravichandran R. and Parthiban R. 1998. The impact of processing techniques on tea volatiles. Food Chem. 62: 247.

Ruffio-Châble V. and Hervé Y. 2001. Coliflor y bróculi. In "Tecnología de las Hortalizas". Ed. Acribia. Zaragoza.

Salunkhe D.K. and Do J.Y. 1976. Biogenesis of aroma constituents of fruits and vegetables. CRC Crit. Rev. Food Sci. Nutr. 8: 161.

Schwimmer S. and Friedman M. 1972. Genesis of volatile sulphur containing food flavours. Flavour Industrie 3: 137.

Serrano M., Martínez-Romero D., Guillén F., Castillo S. and Valero D. 2006. Maintenance of broccoli quality and functional properties during cold storage as affected by modified atmosphere packaging. Postharvest Biol. Technol. 39: 61.

Solís-Solís H.M., Calderón-Santoyo M., Schorr-Galindo S., Luna-Solano G. and Ragazzo-Sánchez J.A. 2007. Characterization of aroma potential of apricot varieties using different extraction techniques. Food Chem. 105: 829.

Valette L., Fernandez X., Poulain S., Loiseau A.-M., Lizzani-Cuvelier L., Levieil R. and Restier L. 2003. Volatile constituents from Romanesco cauliflower. Food Chem. 80: 353.

Vallejo F., Tomás-Barberán F.A. and García-Viguera C. 2002. Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. Eur. Food Res. Technol. 215: 310.

Vidal-Aragón M.C. 1996. Influencia de la materia prima en la calidad y en el aroma del jamón curado de cerdo ibérico. PhD Thesis. Universidad de Extremadura.

Vidal-Aragón M.C., Lozano M. and Montero de Espinosa V. 2005. Evolution of the aromatic fraction in red pepper for paprika during La Vera region traditional drying process. J. Food Qual. 28: 211.

Revised paper received March 10, 2008 Accepted September 18, 2008

PAPER

CHARACTERIZATION OF BETA-GALACTOSIDASE FROM *ALTERNARIA ALTERNATA* ON SOLID-STATE CULTIVATION

CARATTERIZZAZIONE DI BETA-GALATTOSIDASI DA *ALTERNARIA ALTERNATA* SU TERRENO DI COLTURA SOLIDO

I. SEYIS BILKAY
Hacettepe University, Faculty of Science, Department of Biology (Biotechnology), Beytepe, 06532 Ankara, Turkey
Fax +90 (312) 299 2028, e-mail: iseyis@hacettepe.edu.tr

ABSTRACT

The effects of orange peel, lemon peel, apple peel, wheat-bran and watermelon peel on beta-galactosidase production from *Alternaria alternata* were studied. Beta-galactosidase (EC-3.2.1.23) was produced best by solid-state fermentation of *A. alternata* on wheat bran. The effects of pH and incubation temperature were studied. The highest enzyme activity was obtained at 50°C and pH 5.0. The enzyme was relatively stable in the pH range 3.0-5.0 and between 20°-50°C. The enzyme maintained 85%

RIASSUNTO

Sono stati studiati gli effetti della buccia di arancia, di limone, di mela, di cocomero e della crusca di grano sulla produzione di beta-galattosidasi da *Alternaria alternata*. La beta-galattosidasi (EC-3.2.1.23) veniva meglio sintetizzata dall'*A. alternata* per fermentazione su stato solido sulla crusca di grano. Sono stati studiati gli effetti del pH e della temperatura. L'attività enzimatica più elevata è stata osservata a 50°C e a pH 5,0. L'enzima era relativamente stabile a pH compresi fra 3,0-5,0 e ad

- Key words: *A. alternata*, beta-galactosidase, lactase, lemon peel, solid-state fermentation, stability, wheat bran -

of its activity at 20°-30°C and 70% at 50°C. Activity was more than 92% between pH 3.0-5.0 and 42% in 40 min and 50% in 30 min at 60°C. In addition, glucose did not have an inhibitory effect, and galactose had only a slight inhibitory effect.

temperature comprese fra 20° e 50°C. L'enzima manteneva l'85% della sua attività a 30°-50°C e il 70% a 50°C. L'attività era maggiore del 92% a valori di pH compresi fra 3,0-5,0 e risultava del 42% dopo 40 min e del 50% dopo 30 min a 60°C. Inoltre, il glucosio non presentava alcun effetto inibitorio, mentre il galattosio presentava solo un leggero effetto inibitorio.

INTRODUCTION

Many microorganisms, including bacteria, yeast and fungi produce different groups of enzymes. Enzymes are among the most important products obtained through microbial sources and many industrial processes use enzymes at some stage of the process (PANDEY *et al.*, 1999). Among these enzymes, beta-galactosidase (EC 3.2.1.23), also called lactase, catalyzes the hydrolysis of β-1,4-D-galactosidic linkages such as those in lactose. This enzyme can be found in plants, animals and microorganisms (PISANI *et al.*, 1990; FIEDUREK and ILCZUK, 1990; GUVEN *et al.*, 2007).

Lactose is the main component in milk and whey and the consumption of foods with a high content of lactose causes problems for lactose-intolerant people (GUVEN *et al.*, 2007; RICHMOND *et al.*, 1981). To avoid this problem, lactose needs to be hydrolyzed into simpler sugars such as glucose and galactose. The increasing demand for lactose-free dairy products is expected to create a world demand for beta-galactosidase (PETRIDES *et al.*, 1999). Beta-galactosidase, with the help of lactose hydrolysis, can also resolve the problem of pollution caused by whey disposal (CHOLLANGI and HOSSAIN, 2007).

The economics of enzyme-producing processes is one of the major factors that should be taken into account in a competitive market. The economical production of these enzymes has received increasing attention by many researchers. Solid-state fermentation has tremendous potential for producing enzymes economically; it is of special interest in processes where the crude fermented product can be used directly as the enzyme source (PANDEY *et al.*, 1999).

The aim of this study was to determine the optimum conditions that affect the beta-galactosidase enzyme activity produced by *Alternaria alternata* on solid-state cultivation medium; the stability of the enzyme was also investigated.

MATERIALS AND METHODS

Microbial strain

A. alternata strain from the Microbiology Laboratory of Hacettepe University was used to produce beta-galactosidase enzyme. Stock cultures were maintained on potato dextrose agar at +4°C.

Medium and inoculation

The medium described by FIEDUREK and ILCZUK (1990) was used with some modifications to produce and grow the enzyme. The medium contains (as g/L); 10.0 lactose, 1.5 peptone, 1.0 yeast-extract, 1.0 KH_2PO_4, 7.0 $(NH_4)H_2PO_4$, 1.0 $MgSO_47H_2O$ and 0.3 $CaCl_2$. Fifty mL of

media at pH 5.0 were placed in 250 mL flasks and sterilized in an autoclave at 121°C, 1.5 atm for 15 min. Wheat bran was used for solid-state cultivation. The enzyme was produced by solid cultivation using a 250 mL Erlenmeyer flask containing 10 g wheat bran and 10 mL distilled water. Solid medium was sterilized in an autoclave at 121°C, 1.5 atm for one h. The crude-extract was obtained after centrifugation. Wheat-bran, which is the substrate used in the solid-state media, was replaced by dehydrated lemon, orange, watermelon, apple and watermelon peel. The effect of lemon, orange, watermelon, apple, watermelon peel and wheat bran on beta-galactosidase production was studied. Spore suspensions containing $3 \bullet 10^6$ spores/mL were inoculated onto the media and incubated at 30°C for 6 d.

Enzyme activity assays

Beta-galactosidase activity was assayed by the method described by RECZEY *et al.* (1992). The culture was centrifuged at 7,200 rpm for 15 min and supernatant was used as the enzyme sample. The enzyme was assayed with 2.5 mg/mL o-nitrophenyl-β-D-galactopyranoside (ONPG) as substrate (Merck, Darmstadt), which was prepared in 0.1 M sodium-acetate buffer (pH 5.0). One mL of substrate solution and 0.2 mL enzyme sample were then added to the reaction tubes. The reaction mixture was incubated at 50°C for 15 min. Reaction was terminated by adding one mL of 10% sodium carbonate. The absorbance values were determined at 420 nm using a Jenway (Essex, UK), 6105 u.v., vis spectrophotometer. The amount of o-nitrophenol was calculated from the standard curve plotted in the 10-90 μg range. In addition, when lactose was used as substrate to determine lactase activity, the liberated glucose was estimated with Trinder Reagent (Sigma, USA).

One unit of beta-galactosidase activity was defined as the amount of enzyme that releases one μmole of o-nitrophenol or glucose per min in one mL medium at 50°C under standard assay conditions.

Characterization of beta-galactosidase enzyme

The medium was incubated 6 days at 30°C and the supernatant of *A. alternata* was used as the enzyme source. To investigate the effect of temperature on enzyme activity, the reaction medium was incubated at different temperatures between 20°-60°C. Similarly, in order to determine the optimum pH value, incubations were carried out at different pH values between 4.0-6.0. Enzyme activities were determined as relative activity.

Temperature stability

Enzyme stabilities at different temperatures (between 20°-60°C) were determined after preincubation for one hour, cooling and carrying out the standard assay procedure. In order to determine the stability of the enzyme at 60°C, enzyme activities were measured at 20, 30 and 40 min.

pH stability

Enzyme stabilities in the pH range 3.0-7.0 were determined with the standard assay procedure after preincubation for one hour. In this way, enzyme activities were determined as relative activity.

Effect of glucose and galactose on beta-galactosidase activity

One hundred mM glucose and galactose were prepared separately in 0.1 M sodium acetate buffer (pH 5.0). One mL of each solution was added to the reaction tubes containing 0.5 mL enzyme and 1 mL ONPG solution. The percent activity was determined based on the reference sample, which did not contain glucose or galactose. In this way, enzyme activities were determined as relative activity.

RESULTS AND CONCLUSIONS

Beta-galactosidase production from *A. alternata* in solid-state culture was determined with the different substrates. There are numerous reports in the literature on producing beta-galactosidase enzyme from different fungi such as *Aspergillus, Penicillium*, *Trichoderma* and *Alternaria* sp. In these studies, whey and liquid media were used for enzyme production (MACRIS, 1982; BAILEY and LINKO, 1990; FIEDUREK *et al.*, 1996; NAGY *et al.*, 2001; SEYIS and AKSOZ, 2004). In contrast, solid-state fermentation requires only simple fermentation equipment and offers numerous advantages including high productivity at relatively higher concentrations (PANDEY *et al.*, 1999).

Enzyme production in solid state and liquid cultivation was compared and the results show that solid-state gives higher yields (solid state: 6.99 U/mL; liquid: 4.90 U/mL). In a previous study carried out with *Rhizomucor*, the rate of enzyme production in solid-state medium was nine times faster compared with submerged fermentation (SHAIKH *et al.*, 1997). Therefore, the major objective of this study was to economically produce an industrial enzyme on solid-state production media. A similar approach has been followed in other studies on the same subject (PARK *et al.*, 1979; PASTORE and PARK, 1979; BARKER and SHIRLEY, 1980; BAILEY and LINKO, 1990; GONZALES and MONSAN, 1991; SOUZA *et al.*, 2001; HATZINIKOLAOU *et al.*, 2005; SZENDEFY *et al.*, 2006; SHANKAR and MULIMANI, 2007; XIONG *et al.*, 2007).

In liquid fermentation, lactose is used as an inducer and lactase is produced in the media. Lactase enzyme produced by solid-state fermentation from *A. alternata* shows that this enzyme is not induced by lactose but can be produced constitutively even if there is no lactose in the media.

The enzyme was then produced in different solid-state cultivation media and the results were compared. Agro-industrial residues are usually considered the best substrates for solid-state fermentation. The results show that medium containing wheat bran had the highest enzyme yield. The relatively low activities in the other media may have been due to the fact that *A. alternata* may synthesize more suitable enzymes in these media (Fig. 1). In a study to produce beta galactosidase from *Aspergillus oryzae* with submerged cultivation, the highest level of activity was obtained on the wheat bran medium (BAILEY and LINKO, 1990). To date, no studies have reported the use of substrates other than wheat bran. Therefore, the production of a considerable amount of beta galactosidase on lemon peel is a promising result.

Another objective of the study was to characterize the enzyme. To do this, the physiological conditions of the reaction medium were investigated. In lactase activity studies, chromogenic substrate ONPG was used in the reaction medium rather than lactose in order to color the product (DESHPANDE *et al.*, 1989; PISANI *et al.*, 1990). In the present study, ONPG was used as substrate for enzyme characterization. This enzyme is used in milk and dairy products and therefore lactose is the natural substrate of this enzyme. The Km value for lactose was calculated and found to be 6.87 mM.

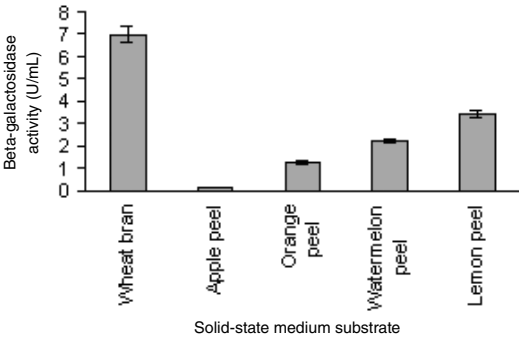

Fig. 1 - Beta-galactosidase production on alternative solid-state media.

When incubation temperatures between 20°-60°C were used, it was observed that the maximum activity was reached at 50°C, which can be considered the optimum temperature (Fig. 2). In previous studies, the optimum temperature for maximum beta-galactosidase activity was reported to be 50°C (RIOU *et al.*, 1992; SEYIS and AKSOZ, 2004; NAKKHARAT and HALTRICH, 2006; PANESAR *et al.*, 2006). Enzymes that can operate at higher temperatures have numerous advantages in biotechnological applications (YANO and POULOS, 2003). Therefore, a maximum activity at 50°C is an advantage of this enzyme, especially when used in commercial applications.

The effect of pH on beta-galactosidase activity was also determined at pH values between 4.0 and 6.0. The results of the experiments showed that the optimum pH was around 5.0 (Fig. 3). In similar studies carried out with fungal sources, the optimum pH values were between 4.0-5.0 (PARK *et al.*, 1979; FIEDUREK and ILCZUK, 1990; ISMAIL *et al.*, 1992; RIOU *et al.*, 1992; SEYIS and AKSOZ, 2004).

In a number of previous studies, the characteristics of enzymes produced in liquid culture were compared with those produced in solid-state cultures. The biochemical characteristics of the enzymes differed, which indicates that the culture method has a considerable effect on the biochemical characteristics of the enzyme (DESCHAMPS and HUET, 1985; ACUÑA-ARGÜELLES *et al.*, 1995; SARA SOLÍS-PEREIRA *et al.*, 1993; VINIEGRA-GONZALEZ *et al.*, 2003). In this study, temperature and pH characteristics of the enzyme produced in solid-state culture were compared with the results of a previous study (SEYIS, 2003), in which the enzyme was produced in liquid media; the temperature and pH optima for the enzyme were almost the same in both media.

The industrial use of an enzyme is often limited due to lack of stability. For enzymes used in commercial applications, the temperature and pH ranges at which the enzyme is relatively stable are important for the efficiency of the process in which they are used.

The temperature stability of the beta-galactosidase enzyme produced was studied and the results show that the enzyme kept more than 70% of its original activity between 20°-50°C (Fig. 4). When temperature stabilities were examined in liquid media, it was observed that the enzyme activity was approximately 50% at 50°C (SEYIS, 2003).

In a similar study, beta-galactosidase was produced from *P. notatum*. The results show that the stability of the enzyme was high between 20°-40°C and decreased above 50°C after 1 h of

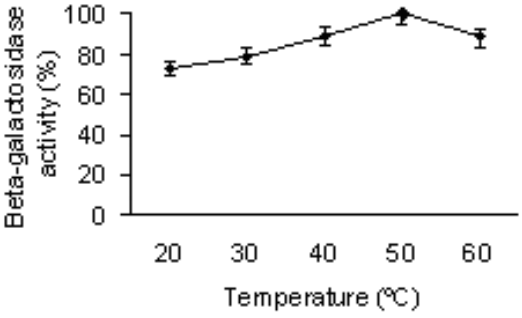

Fig. 2 - Effect of temperature on beta-galactosidase activity.

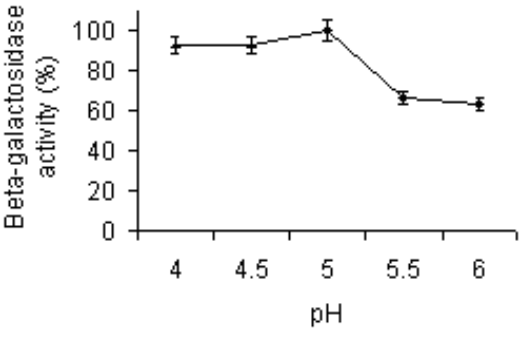

Fig. 3 - Effect of pH on beta-galactosidase activity.

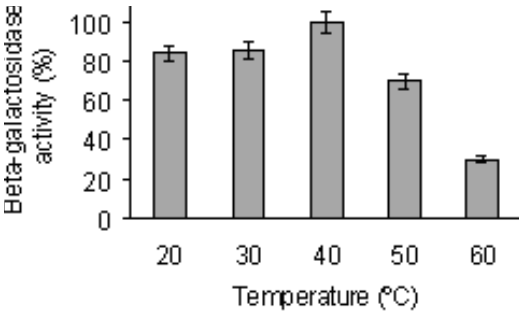

Fig. 4 - Stability of beta-galactosidase at 20°-60°C.

incubation (FIEDUREK and ILCZUK, 1990). It was reported that the activity of beta-galactosidase produced on *Aureobasidium pullulans* decreased rapidly above 45°C (DESHPANDE *et al.*, 1989). In another study carried out with *B. bassiana*, the activity decreased to 5.9% after 30 min at 60°C (MACPHERSON and KHACHATOURIANS, 1991). Results of the stability studies on *Aspergillus* and *Penicillium* at different incubation temperatures show that the stabilities of the enzymes change considerably after one h of pre-incubation (ROGALSKI *et al.*, 1994).

It was observed that at 60°C the enzyme maintained 50% of its original activity after 30 min and 42% after 40 min (Fig. 5). This is due to the fact that the structure of the enzyme changes at high temperatures. On the other hand, it was reported that the activities of enzymes produced from yeasts decrease markedly above 40°C when compared with the activities of the enzymes produced from fungi (SORENSEN and CRISAN, 1974). Therefore, beta-galactosidase enzyme production on yeasts is not suitable if they are to be used at high temperatures. When the enzyme activity at 60°C in liquid culture (SEYIS, 2003) was investigated, it was observed that after 40 min the activity was 53% of the original activity.

When pH stability of the enzyme was investigated, it was observed that the activity decreased at pH 6.0 and 7.0, while at pH 3.0 and 4.0 the enzyme activity was greater than 92% after one h. These results imply that this enzyme is highly stable with respect to pH (Fig. 6). In contrast, the stability of the enzymes changed considerably in studies carried out with *Aspergillus* and *Penicillium* at different pH values (ROGALSKI *et al.*, 1994).

Finally, the effect of glucose and galactose on beta-galactosidase activity was investigated. The results (Fig. 7) show that galactose has an insignificant inhibition effect and glucose does not have any. Similarly, in a previous study, in which the enzyme was produced on *Trichoderma viride*, the activity increased slightly with glucose and was not inhibited significantly with ga-

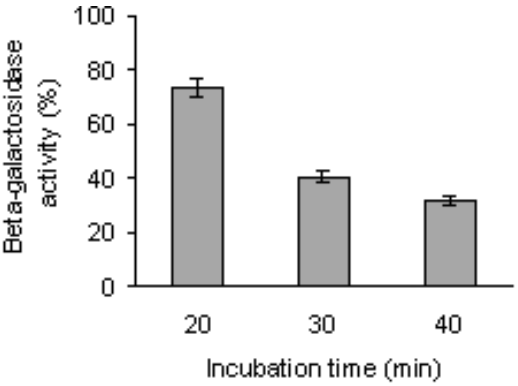

Fig. 5 - Effect of incubation time on beta-galactosidase activity at 60°C.

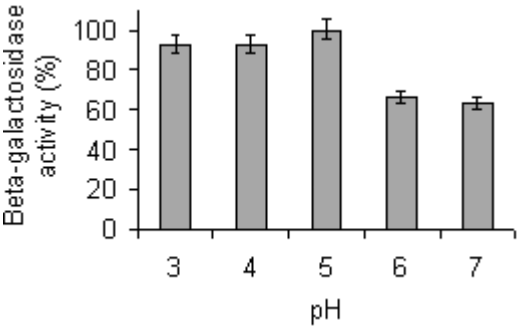

Fig. 6 - Stability of beta-galactosidase at pH 3-7.

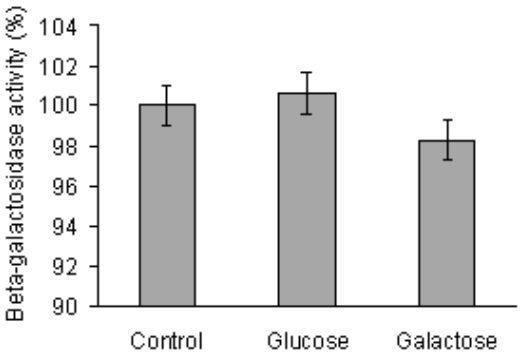

Fig. 7 - Effect of glucose and galactose on beta-galactosidase activity.

lactose (SEYIS, 2004). The literature reports that glucose and galactose act as inhibitors of beta-galactosidase enzyme produced from *Kluveromyces lactis* (CAVAILLE and COMBES, 1995). In studies carried out with *Bacillus* sp., the enzyme was strongly inhibited by galactose (CHAKRABORTI *et al.*, 2000; CHAKRABORTI *et al.*, 2003).

When the results from a solid-state medium are compared with those from a liquid medium (SEYIS, 2003), it can be concluded that solid-state cultivation is preferable. For example, when temperature stability is considered between 20°-50°C, enzyme activity was greater than 70% in solid state but approximately 50% in liquid medium. The enzymes were more stable at high temperatures and over a wide temperature range. This is very advantageous from an economical point of view for industrial applications. However, when pH stabilities were compared, the enzymes produced in liquid medium were more stable, which can be considered as a minor disadvantage of solid-state cultivation.

The optimum temperature and pH for beta-galactosidase activity were 50°C and 5.0, respectively. It was observed that, the enzyme was stable in the pH range 3.0-7.0 and between 20°-50°C. At 60°C the activity decreased to 42% after 30 min and to about 33% after 40 min.

REFERENCES

Acuña-Argüelles E., Gutiérrez-Rojas M., Viniegra-González G. and Favela-Torres E. 1995. Production and properties of three pectinolytic activities produced by *Aspergillus niger* in submerged and solid-state fermentation. Appl. Microb. Biotech. 43: 808.

Bailey M.J. and Linko M. 1990. Production of beta-galactosidase by *Aspergillus oryzae* in submerged bioreactor cultivation. J. Biotechnol. 16: 57.

Barker S.A. and Shirley J.A. 1980. Glucose oxidase, glucose dehydrogenase, glucose isomerase, β-galactosidase and invertase. In "Economic Microbiology; Microbial Enzymes and Bioconversion". p. 205, Rose, A.H. (Ed.). Academic Press, London.

Cavaille D. and Combes D. 1995. Characterization of β-galactosidase from *Kluveromyces lactis*. Biotechnol. Appl. Bioc. 22: 55.

Chakraborti S., Sani R.K. and Banerjee U.C. 2000. Purification and characterization of novel beta-galactosidase from *Bacillus* sp. *MTCCC 3088*. J. Ind. Microbiol. Biot. 24: 58.

Chakraborti S., Sani R.K., Sahoo D.K., Banerjee U.C. and Sobti R.C. 2003. Production and partial characterization of a novel β-galactosidase from a newly isolated *Bacillus polymyxa*. Scientia Iranica. 10: 279.

Chollangi A. and Hossain M. 2007. Separation of proteins and lactose from dairy wastewater. Chem. Eng. Process. 46: 398.

Deschamps F. and Huet M.C. 1985. Xylanase production in solid-state fermentation: A study of its properties. Appl. Microbiol. Biotechnol. 22: 177.

Deshpande M.S., Deopurkar R.L. and Rale V.B. 1989. Beta-galactosidase from *Auerobasidium pullulans*. Lett. Appl. Microbiol. 9: 21.

Fiedurek J. and Ilczuk Z. 1990. Screening of microorganisms for improvement of beta-galactosidase production. Acta Microbiol. Pol. 39: 37.

Fiedurek J., Gromada A. and Jamroz J. 1996. Effect of medium components and metabolic inhibitors on β-galactosidase production and secretion by *Penicillium notatum1*. J. Basic Microb. 36: 27.

Gonzales R.R. and Monsan P. 1991. Purification and some characterization of β-galactosidases from *Aspergillus fonsecaeus*. Enzyme Microb. Tech. 13: 349.

Guven R.G. and Guven K., Poli A. and Nicolaus B. 2007. Purification and some properties of a β-galactosidase from the thermoacidophilic *Alicyclobacillus acidocaldarius subsp. rittmannii* isolated from Antarctica. Enzyme Microb. Tech. 40: 1570.

Hatzinikolaou D.G., Katsifas E., Mamma D., Karagouni A.D., Christakopoulos P. and Kekos D.

2005. Modeling of the simultaneous hydrolysis ultrafiltration of whey permeate by a thermostable β-galactosidase from *Aspergillus niger*. Biochem. Eng. J. 24: 161.

Ismail S.A., Mabrouk S.S. and Mahoney R.R. 1997. Purification and characterization of beta-galactosidases from *Mucor pusillus*. J. Food Biochem. 21: 145.

Macpherson J.M. and Khachatourians G.G. 1991. Partial purification and characterization of beta-galactosidase produced *Beauveria bassiana*. Biotechnol. Appl. Bioc. 13: 217.

Macris B.J. 1982. Production of thermostable β-D-galactosidase by *Alternaria alternata* grown in whey. Appl. Environ. Microb. 44: 1035.

Nagy Z., Kiss T., Szentirmai A. and Biró S. 2001. β-Galactosidase of *Penicillium chrysogenum*: Production, Purification, and Characterization of the Enzyme. Protein Expres. Purif. 21: 24.

Nakkharat P. and Haltrich D. 2006. Purification and characterisation of an intracellular enzyme with β-glucosidase and β-galactosidase activity from the thermophilic fungus *Talaromyces thermophilus CBS 236.58*. J. Biotechnol. 123: 304.

Pandey A., Selvakumar P., Soccol C.R. and Nigam P. 1999. Solid state fermentation for the production of industrial enzymes. Curr. Sci. 77: 149.

Panesar P.S., Panesar R., Singh R.S., Kennedy J.F. and Kumar H. 2006. Microbial production, immobilization and applications of β-D-galactosidase. J. Chem. Technol. Biot. 81: 530.

Park Y.K., Santi M.S.S. and Pastore G.M. 1979. Purification and characterization of beta-galactosidase from *Aspergillus oryzae*. J. Food Sci. 44: 100.

Pastore G.M. and Park Y.K. 1979. Screening of high β-galactosidase-producing fungi and characterizing the hydrolysis properties of a selected strain. J. Food Sci. 44: 1577.

Petrides D.P., Calandranis R.N.J. and Cooney C.L. 1999. Introduction to bioprocess simulation. In "Manual of Industrial Microbiology and Biotechnology". Demain, A.L. and David Wu, J.H.(Ed.).Washington, D.C.

Pisani F.M., Rella R., Raia C.A., Rozzo C., Nucci R., Gambacorta A., De Rossa M. and Rossi M.1990. Thermostable galactosidase from the archaebacterium *Sulfolobus solfataricus*. Purification and properties. Eur. J. Biochem. 187: 321.

Reczey K., Stalbrand H., Hahn-Hegerdal B. and Tijernal F. 1992. Mycelia-associated beta-galactosidase activity in microbial pellets of *Aspergillus* and *Penicillium* strains. Appl. Microbiol. Biot. 38: 393.

Richmond M., Gray J. and Stine C. 1981. Beta-galactosidase: review of recent research related to technological application, nutritional concerns and immobilization. J. Dairy Sci. 64: 1759.

Riou C., Freysinnet G. and Fevre M. 1992. Purification and characterization of a beta-galactosidase from *Sclerotinia sclerotium*. FEMS Microbiol. Lett. 95: 37.

Rogalski J., Dawidowicz A. and Leonowicz A. 1994. Lactose hydrolysis in milk by immobilized β-galactosidase. J. Mol. Catal. 93: 233.

Seyis I. 2003. Characterization of β-galactosidase from *Alternaria alternata*. Hacettepe J. Biol. Chem. 32: 105.

Seyis I. 2004. Lactase Enzyme Production from Some Fungal Sources. PhD Thesis, Hacettepe University, Ankara, Turkey.

Seyis I. and Aksoz N. 2004. Production of lactase by *Trichoderma* sp. Food Technol. Biotech. 42: 121.

Shaikh S.A., Khire J.M. and Khan M.I. 1997. Production of β-galactosidase from thermophilic fungus *Rhizomucor* sp. J. Ind. Microbiol. Biot. 19: 239.

Shankar S.K. and Mulimani V.H. 2007. α-Galactosidase production by *Aspergillus oryzae* in solid-state fermentation. Bioresource Technol. 98: 958.

Solís-Pereira S., Favela-Torres E., Viniegra-González G. and Gutiérrez-Rojas M. 1993. Effects of different carbon sources on the synthesis of pectinase by *Aspergillus niger* in submerged and solid state fermentations. Appl. Microbiol. Biot. 39: 36.

Sorensen S.G. and Crisan E.V. 1974. Thermostable lactase from thermophilic fungi. J. Food Sci. 39: 1184.

Souza D.F., Souza C.G.M. and Peralta R.M. 2001. Effect of easily metabolizable sugars in the production of xylanase by *Aspergillus tamarii* in solid-state fermentation. Process Biochem. 36: 835.

Szendefy J., Szakacs G. and Christopher L. 2006. Potential of solid-state fermentation enzymes of *Aspergillus oryzae* in biobleaching of paper pulp. Enzyme Microb. Tech. 39: 1354.

Viniegra-González G., Favela-Torres E., Noe Aguilar C., Rómero-Gomez S.J., Díaz-Godínez G. and Augur C. 2003. Advantages of fungal enzyme production in solid state over liquid fermentation systems. Biochem. Eng. J. 13: 157.

Xiong C., Jinhua W. and Dongsheng L. 2007. Optimization of solid-state medium for the production of inulinase by *Kluyveromyces S120* using response surface methodology. Biochem. Eng. J. 34: 179.

Yano J.K. and Poulos T.L. 2003. New understandings of thermostable and peizostable enzymes. Curr. Opin. Biotech. 14: 360.

Revised paper received March 12, 2008 Accepted June 16, 2008

PAPER

TURKISH CONSUMER ATTITUDES TOWARD FOOD PRODUCTS: THE CASE OF ERZURUM

LE ATTITUDINI DEI CONSUMATORI TURCHI NEI CONFRONTI DEI PRODOTTI ALIMENTARI: IL CASO DI ERZURUM

Y. TOPCU*, H.B. ISIK and A.S. UZUNDUMLU
Department of Agricultural Economics, Collage of Agriculture,
Ataturk University, 25240 Erzurum, Turkey
*Corresponding author: Tel. +90 442 2311393, Fax +90 442 2312678,
e-mail: ytopcu@atauni.edu.tr

ABSTRACT

Increasing global interactions and improvements in the Turkish economy in recent years have led to significant changes in the overall marketing sector and in the purchasing behaviour of Turkish consumers. The aim of this study was to determine the most salient food attributes that influence consumer food choices using factor analysis and then to identify consumer group profiles by k-means cluster analysis. The results showed that price was the most prominent food attribute that in-

RIASSUNTO

L'incremento delle interazioni globali e il miglioramento dell'economia turca negli ultimi anni hanno condotto ha significativi cambiamenti nel settore del mercato mondiale e nel comportamento d'acquisto dei consumatori turchi. Lo scopo di questo studio era quello di determinare, utilizzando un fattore d'analisi, i principali attributi degli alimenti che influenzano la scelta del consumatore e quello di identificare profili di gruppi di consumatori attraverso l'analisi dell'algoritmo di clustering. I risul-

- Key words: factor analysis, food attribute, k-means cluster analysis, market segmentation, Turkish consumer attitude -

fluenced the buying decisions of consumers in all groups. Consumer satisfaction with food products, brand loyalty and price were the most effective factors in cluster 1(C1), C2 and C3, respectively. The results of this study show the interrelationships among product attributes, consumption, preference values and attitudes of Turkish consumers. This information could be used to develop predominant strategies and policies for marketing food products in Turkey.

tati mostravano che il prezzo era l'attributo più importante degli alimenti che influenzava le decisioni d'acquisto dei consumatori di tutti i gruppi. La soddisfazione del consumatore riguardo i prodotti alimentari, la fedeltà al marchio e il prezzo erano i fattori più importanti nel cluster 1(C1), C2 e C3, rispettivamente. I risultati di questo studio mostrano l'interrelazione fra gli attributi del prodotto, il consumo, la preferenza e le attitudini dei consumatori turchi. Queste informazioni potrebbero essere utilizzate per lo sviluppo di strategie predominanti e politiche del mercato di prodotti alimentari in Turchia.

INTRODUCTION

Consumer food choice and purchasing are complex phenomena that are influenced by a myriad of factors. These factors can be roughly classified into three groups (BABICZ-ZIELINSKA, 1999): product-related (physical, chemical, sensory, functional and nutritional), consumer-related (personal, psychological and physiological) and environment-related (economical, cultural and social) factors. In this dynamic world, all of these factors are changing constantly, as are consumer attitudes and preferences.

Consumer behaviour in Turkey is also changing due to technological and socioeconomic advances. The average gross domestic product (GDP) per capita has increased in the last decade in Turkey. While the average GDP per capita was $2,888 in 1996, it reached $5,482 in 2006. The consumer price index (CPI) for food products, on the other hand, did not increase significantly during this same time period. Although the annual CPI for food products increased about 80% in 1996, the increase in 2006 was only about 10% (TUIK, 1996; 2006). These positive changes have led to better social welfare and higher standards of living. Therefore, consumer food choices and preferences are very different today than they were in the 1990s.

In today's consumer-based marketing, manufacturers and marketers must meet consumer expectations in order to maintain or increase their market share. To do this, they must first keep track of changes in consumer attitudes and preferences via detailed individual customer-based data and up-to-date marketing research. Based on this feedback, they can develop new marketing tactics and strategies, and design new products (TOPCU *et al.*, 2007). To design a new product, the manufacturers must consider both the intrinsic and extrinsic attributes of the product.

The effects of these two attributes on consumer attitudes have been widely studied (MALDONADO and VILLALBI, 1993; KOHYAMA, 1995; SHENG *et al.*,

1996; SHEPHERD and DENNISON, 1996; LENNERNÄS *et al.,* 1997; DELIZA *et al.,* 1999; MURRAY and DELAHUNTY, 2000; WĄDOŁOWSKA *et al.,* 2002; NEWBY *et al.,* 2006; HADDAD *et al.,* 2007), reflecting the role they play on consumer expectation. Studies on food-choice factors have shown that quality, freshness, taste, healthy diet, price, household preferences and habits are some of the most important choice factors.

Few studies have been conducted in Turkey regarding the effects that the intrinsic and extrinsic attributes of a product have on consumer purchasing decisions. TUZCUOGLU (1999), YURTTUT (2001) and AKPINAR (2004) examined store-brand food products in different regions, using descriptive analyses. They found that brand, price, packaging, food safety and hygiene of a food product highly influenced consumer choices and buying decisions. ARIKAN (2000), TOPCU (2006) and TOPCU and ISIK (2008), on the other hand, analyzed the most important factors affecting consumer purchasing decisions for dairy products such as brand, price, packaging, health concerns and product content, using conjoint models.

The aim of this study was to explore the factors influencing consumer food choices and purchasing decisions in Turkey. Factor and k-means cluster analyses were used to determine the salient attributes of the products and the clusters of consumers based on the relative homogeneity of their attitudes regarding food attributes. Very few marketing research studies in Turkey have used factor and cluster analyses. This paper has scientific merit in that both factor and cluster analyses were used for the different Turkish regions. Knowing the most important product attributes and consumer profiles for successful product design and innovation provides invaluable information for marketers, manufacturers, policy makers and academicians.

MATERIAL AND METHODS

The preliminary data of the present research were obtained from a survey conducted in the city centre of Erzurum[1], Turkey. In order to determine the sample size, while minimizing sample bias and representing the population correctly, the city centre was divided into four districts: the east-side Kazimkarabekir district with 17,976 households, the west-side Dadaskent district with 6,562 households, the north-side Palandoken district with 30,022 households, and the south-side Yakutiye district with 26,099 with households (ANONYMOUS, 2007).

To determine the sample size for each district, the following formula was used (YILDIZ *et al.,* 2006):

$$n = \frac{Z^2 * p * (1 - p)}{c^2} = 385$$

Where,
n = sample size;
Z = Z value (1.96 used for 95% confidence level);
p = percentage picking a choice, (0.5 used for sample size needed);
c = confidence interval, (0.05 = ±5 used).

Based on the population of each district, the weighted sample size and distribution of the surveys for each district were determined on a proportional basis. Out of 385, the number of questionnaires allocated to the Dadaskent, Kazimkarabekir, Yakutiye and Palandoken districts were 32, 89, 122 and 142, respectively.

Using information obtained from food science and marketing literature and based on the prior experience of the re-

[1] Erzurum has the geographic coordinates of 39°45'N latitude and 41°15'E longitude. It is on the Silk Road, and located at the north-east region of Turkey. Erzurum is one of the biggest provinces in the region with a population of 962,000 total and 402,000 in the city centre.

searchers, a draft questionnaire was prepared. In order to check for non-sampling error which occurs due to ambiguous definitions, unclear_instructions, questionnaire wording, format and length, a pre-test was carried out on 18 randomly selected (3, 4, 5 and 6) consumers in the target regions. The flow and naturalness of the questionnaire were tested, and the order and timing of the questions were re-arranged. The questionnaire was then modified and refined before starting the fieldwork.

To select consumer households in each district for this final questionnaire, a simple random sampling method (i.e., each member of the population has an equal chance of being chosen) was used. Each district was divided into sub-districts and then a face-to-face survey method was used with randomly selected heads of households (this could be either the wife or husband) in various parts of the sub-districts in order to represent the population adequately. The survey was conducted either in their home or at shopping centres.

Table 1 - Food attributes affecting consumer buying decisions, based on the 5-Likert scale.

Variables	Mean	Std. Deviation
Appearance of package	3.14	1.28
Advertising	3.07	1.28
Packaging material	4.06	1.03
Promotion and price discount	3.50	1.18
Product weight (kg)	3.46	1.18
New-design product	3.36	1.29
Product origin	3.70	1.17
Popularity of brand name	4.12	0.81
Natural/organic product	4.24	0.87
Brand penetration	3.83	1.05
Product quality	4.65	0.53
Price-quality (perceived value) relation	4.50	0.61
Prior experiences of consumers	4.50	0.72
Shelf life of product	4.77	0.59
Hygiene	4.73	0.55
Taste and freshness of food	4.59	0.64
Product price	4.12	0.94

Participants in the survey were asked to respond to each statement, indicating the significance level of the food attributes for them. A Likert-format 1-5 scale (where 1 refers to the least important and 5 refers to the most important attributes) was used. Of the 17 attributes (Table 1), 7 are related to physical attributes (packaging material and appearance, product weight (kg), organic product, shelf life, taste and freshness, hygiene), 5 are related to the sale-increasing activities (advertisement, promotion, price, brand diffusion, new design) and 5 are related to individual preferences (quality, price-quality relation (perceived value for food product), product origin, brand name, consumers' prior experiences).

Each question was reduced to a single statement to which responses were collected using the Likert scale described above. Demographic and socioeconomic information was also gathered (gender, age and occupation group, education, consumer food expenditure and income level) in the survey. Food expenditure and income were grouped into three levels.

After editing and coding, the data were first analyzed by factor analysis to determine the main factors affecting consumer food preferences, and then by cluster analysis to classify the consumer mass using their demographic and socioeconomic characteristics. SPSS statistical software was used to perform these two analyses.

These techniques have been used widely in many marketing studies on food attributes, including food products such as beef (OLIVER *et al.,* 2006), cheese (MURRAY and DELAHUNTY, 2000), conventional food (WADOLOWSKA *et. al.,* 2007), pasteurized milk (TOPCU, 2006), soft drinks (ENNEKING *et al.,* 2007) and yogurt (O'CONNOR *et al.,* 2006; HADDAD *et al.,* 2007).

Factor analysis is a data reduction technique that reduces the number of

Table 2 - Factors and correlated variable loadings.

Variables	Factor loadings*				
	F1	F2	F3	F4	F5
Appearance of package	**0.72**	0.22	-0.01	-0.03	-0.01
Advertising	**0.68**	0.19	0.09	-0.05	0.00
Packaging material	**0.68**	-0.05	0.00	0.33	-0.06
Promotion and price discount	**0.68**	-0.03	-0.11	-0.11	0.29
Product weight (kg)	**0.60**	0.10	0.24	0.06	0.04
New-design product	0.21	**0.77**	-0.11	0.06	0.03
Product origin	0.03	**0.75**	0.14	0.05	0.09
Popularity of brand name	0.15	**0.54**	0.33	-0.05	-0.34
Natural/organic product	0.08	**0.51**	0.06	0.45	0.25
Brand penetration	0.34	**0.50**	0.42	-0.05	-0.30
Product quality	-0.03	0.12	**0.72**	0.08	-0.04
Price-quality (perceived value) relation	0.01	-0.04	**0.67**	0.02	0.16
Prior experiences of consumers	0.17	0.09	**0.55**	0.20	0.02
Shelf life of product	0.01	0.10	-0.05	**0.74**	0.04
Hygiene	0.05	-0.14	0.24	**0.64**	-0.30
Taste and freshness of food	-0.04	0.08	0.38	**0.56**	0.20
Product price	0.17	0.06	0.19	0.03	**0.79**
Eigen-value	***3.48***	***1.94***	***1.45***	***1.18***	***1.09***
Share of explained variance (%)	***20.50***	***11.40***	***8.56***	***6.91***	***6.43***
Cumulative share of explained variance (%)	***20.50***	***31.90***	***40.46***	***47.37***	***53.80***
KMO (Kaiser-Meyer-Olkin) Statistic					**0.73**

* Bold numbers indicate the largest loading for each variable.

variables used in an analysis by creating new variables (called factors) that combine redundancy in the data (SPSS 15.0, 2006).

The first step in factor analysis is to determine the number of relevant factors. Therefore, the factor analysis conducted in this study reduced the number of food attributes from seventeen to five factors, having eigen-values greater than 1.0; this was done by Principal Component Analysis[2] using the Varimax rotation method[3]. Factor analysis was used initially to identify underlying aspects that could explain a correlation among a set of food attributes and the associated consumption values. The purpose of factor analysis in this study was to identify those attributes that accounted for a relatively large proportion of the variance in the sample. This subset could then be used for consumer segmentation.

In the second step of the analysis, a k-means cluster analysis was used. Clustering algorithms can be classified into two categories: hierarchical and non-hierarchical. Hierarchical clustering algorithms start with *n* clusters, equal to the

[2] A factor extraction method used to form uncorrelated linear combinations of the observed variables. The first component has the maximum variance. Successive components explain progressively smaller portions of the variance and are all uncorrelated with each other. Principal component analysis is used to obtain the initial factor solution. It can be used when there is a single correlation matrix.

[3] This method is an orthogonal rotation method that minimizes the number of variables that have high loading on each factor. It simplifies the interpretation of the factors.

number of observations, and then proceed until all observations are in one cluster. In non-hierarchical clustering, the researcher specifies the number of clusters in the data set *a priori.* In this study five consumption/preference value categories was identified (Table 3). Therefore the number of classes could be specified, thus non-hierarchical k-means clustering was used. The k-means procedure selects "m" random points from the data set. These are used as cluster seeds and all other points are assigned to the nearest cluster seed. Successive iterations involve replacing the current cluster seed by the cluster mean, and then reassigning all points to the nearest new cluster seed. The process continues until there is no change in cluster means from the previous interaction or the difference is very small. Hence, the consumer clusters were generated on the basis of a relative homogeneity of their attitudes towards food attributes based on consumer food expenditure and income levels. The pattern of consumption values associated with these attributes was identified by examining the outcome of the segments.

The final step was to use cross-tabulations to examine the distribution of the clusters in relation to the three food expenditure and income levels, and demographic and socioeconomic attitudes. Chi-square (χ^2) Goodness of Fit Tests (GFT) were conducted for each of the variables (gender, age, education, occupation, monthly food expenditure and income) in each group in order to measure whether each variable is different from each other or not. In order to measure if there is a relationship personal characteristics and their positions, a chi-square test of independence was used. If the p-value was not less than 0.05, the null hypothesis was accepted, which means that the demographic characteristics and their position levels were statistically independent (YILDIZ *et al.*, 2006).

RESULTS

Food attributes and their associated consumption/preference values

Kaiser normalization, which compares partial correlation coefficients with observed ones, was calculated as 0.73, which means that the data sets are at

Table 3 - Final cluster centres and the number of cases in each cluster.

Factor interpretation	Clusters[4]*		
	C1	C2	C3
Involvement (F1)	-0.57	**0.07**	-0.76
Brand satisfaction (F2)	-1.29	**0.51**	0.08
Satisfaction with food quality (F3)	**0.80**	0.27	-0.52
Food safety (F4)	**0.76**	-0.02	-2.55
Price (F5)	**1.49**	**0.85**	**1.50**
Number of cases in each cluster	***70***	***177***	***138***
% of total cases in each cluster	***18***	***46***	***36***

* Final cluster centre scores are at 0.01 significance levels.
* Bold numbers indicate the largest final cluster centre scores for each factor.
The total number of cases (n): 385.

[4] The numbers in this table indicate final cluster centres. The final cluster assignment and Euclidian distance between the case and cluster centre used to classify the case are given for each case.

the perfect level for factor analysis since the test score is greater than 0.50.

The results of principal component analysis for the first five factors are presented in Table 2. These first five factors accounted for 53.80% of the total variance. Factor 1, which accounted for 20.50% of the total variance, was dominated by attributes related to packaging, advertisement, promotion and product weight. Hence, Factor 1 is associated with involvement. Factor 2 accounted for 11.40% of total variance, and was dominated by new design, product origin, brand name and penetration, natural/organic product. Factor 2, therefore, is more associated with brand satisfaction.

Factor 3 accounted for 8.56% of the total variance, and was dominated by three attributes, quality, price-quality relation, and prior consumer experiences. Therefore, this factor includes consumer satisfaction with food quality. Factor 4 accounted for 6.91% of the total variance. Factor 4 was dominated by the attributes of shelf life, taste-freshness and hygiene. Factor 4, therefore, deals more with food safety. Factor 5 is only related to product price and accounted for 6.43% of the total variance.

These factors demonstrate different consumption and preference values in the decision to purchase food products. The question arises as to whether distinct consumer segments are associated with these values. If so, how many consumers are in each segment and how are these segments related to the consumption/preference values listed above? These questions are addressed in the following section of this paper, on the basis of the findings from the factor analysis above.

K-means clustering and consumer segmentations

Three clusters had group means that could be explained reasonably. The final cluster centres and the number of cases in each cluster are presented in Table 3. The total number of cases was 385. Cluster 1 (C1) was the smallest group with 70 consumers. Consumers in this group were more concerned about food quality satisfaction (F3) and food safety (F4). Cluster 2 (C2) was the largest group, containing 177 consumers. Involvement (F1) and brand satisfaction (F2) were the most dominant factors for consumers in this group. As for Cluster 3 (C3), there were 138 consumers in this group, and the price of the product was the most important factor in their purchasing decision, while it was also an important factor for the other two clusters.

Table 4 shows a cross-tabulation between the consumer demographic and socioeconomic characteristics and the three cluster groups. The results show that the ratio of male consumers in each group was higher than that of females. C2 had the highest male ratio (71%) while C1 had the highest female ratio (37%). One of the main reasons why the males were dominant could be due to their lifestyle and social activities practiced by those of the Islamic religion and oriental cultures. The females play a secondary role in social activities and personal interactions.

The distribution of consumers based on age indicates that the 35-54 age group and that over 55 had the highest and lowest portions in all of the clusters, respectively. While C2 had the largest ratio of the 15-24 age group (29%), C1 had the largest ratio of the 25-34 age group (36%).

As for the education levels of the consumers, the lowest ratio was made up of literate people (those who can read and write but do not have a diploma). Consumers with college degrees dominated in C1 (44%), while the ratio of high school graduates was the highest ratio in the other two clusters.

The distribution of occupation of the respondents among the clusters was as

Table 4 - Demographic characteristic and cluster number of cross-tabulation cases.

Demographical characteristics	Cluster number of cases							
	C1 Young high-income		C2 Mature low-income		C3 Mature mid-income		Total Consumer	
	Number	%	Number	%	Number	%	Number	%
Gender								
Male	44	**63**	126	**71**	94	**68**	264	**69**
Female	*26*	*37*	*51*	*29*	*44*	*32*	*121*	*31*
χ^2 GFT	***4.629***		***31.870****		***18.116****		***15.000****	
χ^2 test of independence	***(χ^2 = 1.636, df = 2, p: 0.441)***							
Age Groups								
15-24 age	16	23	52	29	21	15	89	23
25-34 age	*26*	***37***	*47*	*26*	*42*	*30*	*115*	*30*
35-54 age	25	36	69	**39**	63	**46**	157	**41**
55- + age	*3*	*4*	*9*	*6*	*12*	*9*	*24*	*6*
χ^2 GFT	***19.486****		***41.870****		***45.130****		***29.474****	
χ^2 test of independence	***(χ^2 = 12.020, df = 6, p: 0.062)***							
Education								
Literate	3	4	2	1	5	4	10	3
First school	*13*	*19*	*47*	*27*	*39*	*28*	*99*	*26*
High school	23	33	69	**39**	51	**37**	143	**37**
College	*31*	***44***	*59*	*33*	*43*	*31*	*133*	*34*
χ^2 GFT	***25.314****		***59.271****		***35.797****		***36.941****	
χ^2 test of independence	***(χ^2 = 7.545, d f= 6, p: 0.273)***							
Occupation								
White-collar state employ	21	**30**	61	**34**	53	**38**	135	**35**
Blue-collar state workers	6	*9*	10	*6*	9	*7*	25	6
Businessman	*18*	*25*	*35*	*20*	*27*	*20*	*80*	*21*
Pensioner	*3*	*4*	*14*	*8*	*11*	*8*	*28*	*7*
Housewife	9	13	38	20	24	17	71	19
Student	*13*	*19*	*19*	*11*	*14*	*10*	*46*	*12*
χ^2 GFT	***28.718****		***173.798****		***176.058****		***125.815****	
χ^2 test of independence	***(χ^2 = 8.994, df = 10, p: 0.533)***							
Food Expenditure								
Less than €73.5*	0	0	3	2	4	3	7	2
Between €74 and €103	*22*	*31*	*55*	*31*	*51*	*37*	*128*	*33*
More than €103	48	**69**	119	**67**	83	**60**	250	**65**
χ^2 GFT	***46.845****		***115.841****		***68.652****		***92.311****	
χ^2 test of independence	***(χ^2 = 3.840, df = 4, p: 0.428)***							
Income Groups								
*Less than €442****	*21*	*30*	*66*	***37***	*30*	*22*	*117*	*30*
Between €443 and €882	14	20	58	33	66	**48**	138	**36**
More than €882	*35*	***50***	*53*	*30*	*42*	*30*	*130*	*34*
χ^2 GFT	***46.963****		***109.881****		***89.237****		***74.741****	
χ^2 test of independence	***(χ^2 = 23.753, df = 4, p: 0.000)****							

*** The prices of the products were converted from New Turkish Lira (NTL) to Euro (€) using the exchange rate on November 15, 2007. The conversion rate used was 1.70 NTL/€;
** p<0.05; * p<0.01.

follows: the white-collar state employees made up the highest ratio in all of the clusters, blue-collar state workers and pensioners had the lowest. Businessmen had the second highest ratio in all of the clusters. Finally, the ratios of housewives and students were the lowest and highest in C1 (13% and 19%, respectively).

The results related to household food expenditures show that families whose monthly food expenditures exceed €103 dominate in all of the clusters (all have over 60% ratio). As for income levels, the highest ratio in C1, C2 and C3 correspond to more than €882 (50%), less than €442 (37%) and between €443 and €882 (48%), respectively.

With respect to chi-square GFT, the differences in relation to the distributions of demographic characteristics in C1, C2 and C3 are statistically significant. For example, the differences in the distribution of males and females in C1 (χ^2 = 19.49, $p<0.01$) are statistically significant. In the contrast, in accord with the chi-square test of independence, in which the variables in all the groups are more than p=0.05, all of the demographic characteristics (except for income) and their positions are statistically independent.

Characteristics of cluster groups

Based on the final cluster centres of the factors and demographic characteristics of the consumers in each cluster, the cluster profiles were determined. The main demographic characteristics of C1 are younger people (25-34 age group) with higher income (more than €882) and education (college degree) levels. This cluster is called "young high-income professionals". Food quality satisfaction, food safety and price were the most influential factors on purchasing decisions of respondents in C1. This means that the highest significance was ascribed to consumer satisfaction with the food product; a blend of food quality satisfaction, food safety and price influenced their food choice and buying decisions (FORBES *et al.*, 1986; PATTERSON *et al.*, 1997; HOMBURG and GIERING, 2001).

The dominant demographic characteristics of group C2 were mature (35-54 age group), high school graduates with a low income level (less than €442). C2 can be called "mature low-income professionals". The most important factors in group C2 were involvement, brand satisfaction and price. These factors indicate that respondents in C2 took into consideration brand loyalty connected with brand satisfaction, involvement and price. Involvement and brand satisfaction could cause the target customers to become the cognitive and affective loyalty approach toward food products (SIROHI *et al.,* 1998; YI and LA, 2004; OLSEN, 2007).

Finally, respondents in C3 were mostly mature (35-54 age group), high school graduates with a mid-income level (between €443 and €882). This group was called "mature mid-income professionals". Price was the most important factor in this cluster.

DISCUSSION

The results show that there are systematic cognitive and affective patterns among consumers in terms of their attitudes towards food product attributes. The consumption/preference values underlying these patterns clearly show that price plays the most important role in all clusters, but particularly in C3. On the other hand, consumer satisfaction with food products had the second greatest influence on food choice and buying decision for consumers in C1. It is believed that food satisfaction influences choice and the more willing is the consumer to pay for this food. When a consumer is very satisfied with a food product, she/he will increase her/his food expenditure share for this food. For example, participants in C1 had the highest income lev-

el; therefore, they took into consideration food satisfaction that includes safer and higher quality food products.

DREWNOWSKI and SPECTER (2004) stated that the effect of price and quality on food consumption is more complex. Many studies have found that price is the one of the most important factor affecting the choice of food (REICHHELD and TEAL, 1996; DRANSFIELD *et al.,* 1998; SIROHI *et al.,* 1998; REINARTZ and KUMAR, 1999; YI and JEON, 2003; WADOŁOWSKA *et al.,* 2007). Similarly, consumer satisfaction with higher quality, perceived value and safer foods has a positive effect on buying behaviour and food choice motivation. Consumer demand could, therefore, be increased considerably by these food products and consumers could allocate an important share of their expendable incomes to these foods (FORNELL and WERNERFELT, 1987; WESTBROOK and OLİVER, 1991; MARR and CROSBY, 1992; OLSEN, 2007).

In their evaluations of food attributes, consumers mainly check the factors that affect their satisfaction under effective cognitive involvement since they have limited processing capacity (LAROCHE *et al.*, 2001). The focal point of this decision-making mechanism constitutes their cognitive loyalty and this could turn into cumulative/action loyalty due to a positive relationship between the two constructs (ANDERSON *et al.,* 1994; OLIVER, 1993 and 1997; FAGERLI and WANDEL, 1999; ANDERSON and SIRINIVASAN, 2003; YI and LA, 2004; OLSEN, 2007).

In this study, even though brand loyalty including brand satisfaction and involvement based on visual food attributes had the lowest overall impact level on consumer purchasing decisions, the significance level of these factors was the highest in group C2. This could have been due to the consumer income levels. Group C2 had the lowest income level and consumers in this group tend to pay more attention to lower-price local-brand, organic products. Choosing of local-brand, organic foods could be due to cultural values, beliefs and tastes. Local-brand foods tend to be cheaper and meet the expectations (tastes) of more conservative people. In addition, knowing the origin leads consumers to trust the local brands more. As a result, the local people believe that the more people demand local-brand products, the more their local economy will grow. To develop the local economy, the people in the region should become intensively involved in supporting local-brand foods.

Traditionally, local consumers focus on moral criteria regarded as being basic to the norms of local economic improvement and on Turkish family dependability, such as filial submission, caring for the aged, righteousness, humility, good faith and loyalty. These values have begun to be affected by food satisfaction values due to recent socioeconomic and cultural changes in Erzurum have begun to reshape the norms of individual consumer behaviour.

In many situations, the motivation for food choice focuses on food attributes but depends mostly on the socioeconomic and demographic characteristics of the consumer. The demographic characteristic of the consumer affects and reflects his psychological state, cultural values and behaviour (DICKERSON and GENTRY, 1983; ARENI *et al.,* 1998; KALYANAM and PUTLER, 1997; REINARTZ and KUMAR, 1999; TOPCU, 2006). These characteristics, therefore, cause target consumer masses to behave differently toward food products.

The impact of gender on buying behaviour has been the topic of some research (SLAMA and TASHLIAN, 1985). The purchasing behaviour of women has been found to be strongly influenced by their evaluation of personal interaction processes. Compared to men, women are more involved in purchasing activities (SLAMA and TASHLIAN, 1985), and pay more attention to the consulting services of the sales personnel (GILBERT and

WARREN, 1995). In this study, however, fewer females participated in the purchasing activities due to local cultural norms, but those who participated paid more attention to food satisfaction. In contrast, the males were more involved in the buying activities and focused on food price and local-brand loyalty. These findings suggest that gender could influence the association between the dimensions of satisfaction and loyalty.

Age is another demographic characteristic that has attracted the attention of many researchers. Researchers have compared differences in the ability of young and elderly customers to process information needed to evaluate a product (ROEDDER and COLE, 1986; SMITH and BALTES, 1990). Most of these studies concluded that information processing declines with age (GILLY and ZEITHAML, 1985). Older people have restricted information-processing capabilities; therefore, they pay more attention to local-brands and prices. Hence, their reactions to satisfaction shifts might also change. On the other hand, younger participants take food satisfaction into more consideration since they have wider information-processing capabilities.

The income of a person is assumed to have a strong impact on choice decisions (LAROCHE and TOFFOLI, 1999; TOPCU and ISIK, 2008). In a general sense, it is assumed that people with higher income have achieved a higher level of education and better jobs. Thus, they usually engage more in information processing prior to the decision making process, and their choice is essentially based on the evaluation of the information given to them. Due to their cognitive capacities, they are supposed to feel more comfortable when dealing with and relying on new information inputs. In this study, consumers with higher income, better education and occupation pay more attention to food satisfaction on buying decisions. On the other hand, local-brand loyalty and food prices have important effects on the choices of the others.

Consequently, food satisfaction and local-brand loyalty, once measured by scholarly activity, has become more or less associated with socioeconomic and demographic characteristics. With the use of multiple group causal analysis, significant differences were found across subgroups concerning the relationship between satisfaction and loyalty for each of the moderators (demographic and socioeconomic characteristics). Thus, this research makes a significant contribution to a better understanding of the role of the link between main factors (i.e., food satisfaction, local-brand loyalty and price) and personal characteristics.

This research also indicates that the existing moral values of the oriental culture in Erzurum, i.e. educating youth and caring for the aged, affect the behaviour that is consonant with social identity. Instead of symbolizing the moral standards of a particular class, behaviour represents the wealth and privileges of the people. Many studies have attributed these changes in cultural values to an increased exposure of consumers in Erzurum to western cultural values that are materialistic (ALPMAN, 2005; MURGAN, 2006; BATI, 2007). Thus, consumer satisfaction with food products and local store brand loyalty could be the consequence of the gradual intermingling of local and western cultural values.

CONCLUSIONS

This paper estimates the major factors affecting food choices and buying decisions of consumers in Erzurum city, Turkey. Factor and k-means cluster analyses were used to determine the prominent attributes of the products and the clusters of consumers based on the relative homogeneity of attitudes towards food attributes. The results show

that among the factors influencing food choice for Turkish consumers, price is the most significant factor in all clusters. C3 has a strong correlation with consumer satisfaction including food quality and food safety attributes. Consumer satisfaction with food products was derived from a blend of these factors and was the second most important factor in C1 due to interactions among them. Thirdly, local-brand loyalty had a moderate affect but was not an important factor in food choice in C2.

The results of the study indicate that the moderating impacts of selected personal characteristics on consumer satisfaction, local store brand loyalty and price played a statistically significant role. Hence, this study makes a significant contribution to a better understanding of the association between main factors and personal characteristics. It also shows that the effectiveness of the nutritional policy in Turkey is currently related to various factors that influence food satisfaction and brand loyalty. The greatest interest of consumers in Erzurum is aroused by high quality and local-brand foods that are tasty, fresh, healthy, safe, and cheap.

The results also show there could be increased food satisfaction by choosing a local store brand, which could lead to the desired changes in Turkish nutrition. However, it is much more difficult to influence consumers who are not interested in food quality. Negative or neutral attitudes toward food quality were found more frequently in men, adults with elementary school education and individuals who described their economic condition as bad. Their nutritional habits could be improved through the influence of their families and the role played by women, and in the future, by improving their economic situation and through education, including an increased nutritional awareness. Policy makers, food manufacturers and marketers, on the other hand, could improve marketing policies, (i.e., marketing tactics and strategies) by basing them on food attributes and the attitudes of the Turkish consumer masses.

Even though this study has some scientific merit for the academician, policy maker and the food manufacturing community, there are some limitations. One limitation is that the survey was conducted in only one city. While the study does not represent all Turkish consumers of food products, it may give some indication of current trends.

REFERENCES

Akpinar M.G. 2004. The market analysis of store-brand (super and hypermarket) food products: the case study of Antalya. Ph.D. Dissertation, Cukurova Univ., Adana, Turkey.

Alpman P.S. 2005. Artistic installation and new opportunity of socialist art. J. Sociologist 8: 12.

Anderson E.W., Fornell C. and Lehmann D.R. 1994. Customer satisfaction, market share, and profitability: findings from Sweden. J. Market 58: 53.

Anderson R.E. and Sirinivasan S.S. 2003. E-satisfaction and e-loyalty: a contingency. Psych. and Market 20: 123.

Anonymous 2007. The data of documentation centre of Erzurum Metropolitan Municipality, Erzurum.

Areni C.S., Kiecker P. and Palan K.M. 1998. Is it better to give than to receive? Exploring gender differences in the meaning of memorable gifts. Psych. and Market 15: 81.

Arikan A.E. 2000. A study of milk consumption and consumer attitudes in Turkey. MS Thesis, Istanbul Univ., Istanbul, Turkey.

Babicz-Zielinska E. 1999. Food preferences among the Polish young adults. Food Qual. Prefer 10: 139.

Bati U. 2007. The social semiotics of shopping centres as consumption cathedrals: the sample of "forum Bornova shopping centre". J. Int. Soc. Sci. 4: 1.

Deliza R., Hedderley D., Rosenthal A., MacFie H.J.H. and Frewer L.J. 1999. The importance of brand, product information and manufacturing process in the development of novel environmentally friendly vegetable oils. J. Int. Food Agribusiness Market 10: 67.

Dickerson M.D. and Gentry J.W. 1983. Characteristics of adopters and no adopters of home computers. J. Consumer Res. 10: 225.

Dransfield E., Zamora F. and Bayle M. 1998. Con-

sumer selection of steaks as influenced by information and price index. Food Qual. Prefer. 9: 321.

Drewnowski A. and Specter S.E. 2004. Poverty and obesity: the role of energy density and energy cost. Am. J. Clin. Nutr. 79: 6.

Enneking U., Neumann C. and Henneberg S. 2007. How important intrinsic and extrinsic product attributes affect purchase decision. Food Qual. Prefer. 18: 133.

Fagerli R.A. and Wandel M. 1999. Gender differences in opinion and practices with regard to a healthy diet. Appetite 32: 171.

Forbes J.D., Tse D.K. and Taylor S. 1986. Towards model of consumer post-choice response behaviour. In "Advances in Consumer Research" R.L. Lutz (ed). Ann Arbor, MI: Assoc. Consumer Res. 13: 658.

Fornell C. and Wernerfelt B. 1987. Defensive marketing strategy by customer complaint management: A theoretical perspective. J. Market. Res. 24: 337.

Gilbert F.W. and Warren W.E. 1995. Psychographic constructs and demographic segments. Psych. and Market 12: 223.

Gilly M.C. and Zeithaml V.A. 1985. The elderly consumer and adoption of technologies. J. Consumer Res. 12: 353.

Haddad Y., Haddad J., Olabi A., Shuayto N., Haddad T. and Toufeili I. 2007. Mapping determinants of purchase intent of concentrated yogurt (Labneh) by conjoint analysis. Food Qual. Prefer. 18: 795.

Homburg C. and Giering A. 2001. Personal characteristics as moderators of the relationship between customer satisfaction and loyalty. Psych. Market. 18: 43

Kalyanam K. and Putler D.S. 1997. Incorporating demographic variables in brand choice models: an indivisible alternative framework. Market. Sci. 16: 166.

Kohyama K. 1995. Trend in food preference of modern Japanese. Report Nat. Food Res. Ins. 59: 1.

Laroache M. and Toffoli R. 1999. Strategic brand evaluation among fast-food franchises: a test of two frameworks. J. Business Res. 45: 221.

Laroache M., Teng L. and Kalamas M. 2001. Consumer evaluation of net utility: effect of competition on consumer brand selection. Japan. Psych Res. 43: 168.

Lennernäs M., Fjellström C., Becker W., Giachetti I., Schmitt A., Remaut de Winter A.M. and Kearney M. 1997. Influences on food choice perceived to be important by nationally representative samples of adults in the European Union. Euro. J. Clin. Nutr. 51: 8.

Maldonado R. and Villalbi J.R. 1993. Food preferences among schoolchildren. Anales Espan. Pediat. 39: 10.

Marr S.L. and Crosby L.A. 1992. Customer satisfaction measurement. American Marketing Association, Chicago.

Murgan M. 2006. Consumption market of modern era. Cited 15 December 2007. Updated: 26 October 2006. Available on the Internet: http://www.yaziyaz.com/kose/2006/10/26/.

Murray J.M. and Delahunty C.M. 2000. Mapping consumer preference for the sensory and packaging attributes of Cheddar cheese. Food Qual. Prefer. 11: 419.

Newby P. K., Weismayer C., Åkesson A., Tucker K.L. and Wolk A. 2006. Long-term stability of food patterns identified by use of factor analysis among Swedish women. J. Nutr. 136: 626.

O'Connor E., Cowan C., Williams G., O'Connell J. and Boland M.P. 2006. Irish consumer acceptance of a hypothetical second-generation GM yogurt product. Food Qual. Prefer. 17: 400.

Oliver R.L. 1993. Cognitive, affective, and attribute bases of the satisfaction response. J. Consumer Res. 20: 418.

Oliver R.L. 1997. "Satisfaction-a behavioural perspective on the consumer". McGraw-Hill, New York.

Oliver M.A., Nute G.R., Furnols F. M., Julián R. S., Campo M.M., Sañudo C., Cañeque V., Guerrero L., Alvarez I., Díaz M.T., Branscheid W., Wicke M. and Montossi F. 2006. Eating quality of beef, from different production systems, assessed by German, Spanish and British consumers. Meat Sci. 74: 435.

Olsen S.O. 2007. Repurchase loyalty: the role of involvement and satisfaction. Psych. and Market. 24: 315.

Patterson P.G., Johnson L.W. and Spreng R.A. 1997. Modelling the determinants of customer satisfaction for business-to-business professional services. J. Academy Market. Sci. 25: 4.

Reichheld F. and Teal F. 1996. The loyalty effect: the hidden force behind growth, profits and lasting value. Harvard Business Review 74: 56.

Reinartz W. J. and Kumar V. 1999. Store-market and consumer characteristics: the drivers of store performance. Market. Letters 10: 3.

Roedder J.D. and Cole C.A. 1986. Age differences in information processing: understanding deficits in young and elderly consumers. J. Consumer Res. 13: 297.

Schaninger C.M. and Sciglimpaglia D. 1981. The influence of cognitive personality traits and demographics on consumer information acquisition. J. Consumer Res. 8: 208.

Sheng M., Brochetti D., Duncan S.E. and Lawrence R.A. 1996. Hedonic ratings of reduced-fat food products: factors affecting ratings from female and male college-age students. J. Nutr. Recipe and Menu Develop. 2: 31.

Shepherd R. and Dennison C.M. 1996. Influences on adolescent food choice. Proceed. Nutr. Soc. 55: 345.

Smith J. and Baltes P.B. 1990. Wisdom-related knowledge: Age/cohort differences in response to life planning problems. Develop. Psych. 26: 494.

Sirohi, N., McLaughlin W. and Wittink D.R. 1998. A model of consumer perceptions and store loyalty intentions for a supermarket retailer. J. Retail. 74: 223.

Slama M. E. and Tashlian A. 1985. Selected socioeconomic and demographic characteristics associated with purchasing involvement. J. Market. 49: 72.

SPSS Base 15.0 2006. SPSS base 15 user's guide, Chicago, IL, 161.

Topcu Y., Isik H.B. and Dagdemir V. 2007. The changing role of agro-food marketing in new economy. J. Faculty Agric. 38: 207.

Topcu Y. 2006. Brand competition and consumer behaviours in dairy products: the case study in Erzurum. Ph.D. Dissertation, Ataturk Univ. Erzurum, Turkey.

Topcu Y. and Isık H.B. 2008. Determining the best product quality set for packed yogurt in Turkey. Ital. J. Food Sci. 20: 401.

TUIK 1996. Annual bulletin of consumer price index (CPI) and Gross Domestic Product (GDP) per capita. www.tuik.gov.tr/Turkish/sonist/tuketici/041296.html.

TUIK 2006. Annual bulletin of consumer price index (CPI) and Gross Domestic Product (GDP) per capita. www.tuik.gov.tr/Turkish/sonist/tuketici/041206.doc.

Tuzcuoglu K.S. 1999. Food products and related improvement on retailer market- buying decision of private label products. Ph.D. Dissertation, Istanbul Tech. Univ, Istanbul, Turkey.

Wądołowska L., Babicz-Zielińska E., Schlegel-Zawadzka M., Przysławski J. and Czarnocińska J. 2002. Preferences, consumption and choice factors for milk and its products among school children. Pol. J. Food and Nutr. Sci. 11: 81.

Wądołowska L., Babicz-Zielińska E. and Czarnocińska J. 2007. Food choice models and their relation with food preferences and eating frequency in the Polish population: POFPRES study. Food Policy Online 32:15. Cited 15 December 2007. Updated: 29 September 2007. Available on the Internet: http://www.sciencedirect. com/science/journal/03069192.

Westbrook R.A. and Oliver R.L. 1991. The dimensionality of consumption emotion patterns and consumer satisfaction. J. Consumer Res. 18: 84.

Yi Y. and Jeon H. 2003. Effects of loyalty programs on value perception, program loyalty, and brand loyalty. J. Academy Market. Sci. 31: 229.

Yi Y. and La S. 2004. What influences the relationship between customer satisfaction and repurchase intention? Investigating the effects of adjusted expectations and customer loyalty. Psych. and Market. 21: 351.

Yildiz N., Akbulut O. and Bircan H. 2006. "Statistics" 5th edition, p. 135. Aktif Press, Erzurum, Turkey.

Yurttut E. 2001. A study on retailer brand and recognition. MS Thesis, Marmara Univ., Istanbul, Turkey.

Revised paper received May 13, 2008 Accepted June 12, 2008

PAPER

CHANGES IN ANTIOXIDANT ACTIVITY AND THE PROANTHOCYANIDIN FRACTION OF RED WINE AGED IN CONTACT WITH PORTUGUESE (*QUERCUS PYRENAICA* WILLD.) AND AMERICAN (*QUERCUS ALBA* L.) OAK WOOD CHIPS

EVOLUZIONE DELLA ATTIVITÀ ANTIOSSIDANTE E DELLA FRAZIONE PROANTOCIANIDINICA DI UN VINO ROSSO INVECCHIATO A CONTATTO CON TRUCIOLI DI LEGNO DI QUERCIA PORTOGHESE (*QUERCUS PYRENAICA* WILLD.) ED AMERICANO (*QUERCUS ALBA* L.)

F.J. GONÇALVES and A.M. JORDÃO*
Instituto Politécnico de Viseu, Escola Superior Agrária,
Departamento das Indústrias Agro-Alimentares, Estrada de Nelas,
Quinta da Alagoa, Ranhados, 3500-606 Viseu, Portugal
*Corresponding author: Tel. +351232480600, Fax +351232426536,
e-mail: antoniojordao@esav.ipv.pt, antoniojordao@portugalmail.com

ABSTRACT

A red wine made from the *Syrah* grape variety was matured in contact with 5 and 10 g/L of oak wood chips from Portuguese (*Quercus pyrenaica* Willd.) or American (*Quercus alba* L.) oak species for 90 days, in order

RIASSUNTO

Un vino rosso della varietà *Syrah* è stato conservato per 90 giorni a contatto con 5 e 10 g/L di trucioli di legno di quercia portoghese (*Quercus pynenaica* Willd.) o americana (*Quercus alba* L.) con l'obiettivo di valutarne l'effetto

- Key words: antioxidant activity, chips, oak wood, proanthocyanidins, *Quercus alba*, *Quercus pyrenaica*, wine -

to evaluate the effects of the presence and concentration of these oak wood chip species on the antioxidant activity and the evolution of the proanthocyanidin fraction (monomeric, oligomeric and polymeric). The results showed an increase in the antioxidant activity in red wines aged in contact with the oak wood chip species during the first 52 days of storage followed by a slight decrease. The antioxidant activity values in the wine aged without oak wood chips remained nearly constant during the storage time. After 90 days, the presence of oak wood chips (especially a high concentration of the Portuguese oak species) had a positive effect on the antioxidant activity values and there was a high concentration of the monomeric proanthocyanidin fraction. For all the wines studied, the correlations between antioxidant activity and the monomeric fraction of proanthocyanidins were always high.

della presenza e della concentrazione sull'attività antiossidante e sull'evoluzione della frazione proantocianidinica (monomerica, oligomerica e polimerica). I risultati hanno evidenziato un incremento dell'attività antiossidante nei vini rossi invecchiati a contatto con trucioli di legno di quercia nei primi 52 giorni di stoccaggio, seguito da una sua leggera diminuzione. I valori dell'attività antiossidante nel vino invecchiato senza trucioli di legno di quercia rimanevano costanti durante l'invecchiamento. Dopo 90 giorni, la presenza di trucioli di legno di quercia (specialmente se ad alte concentrazioni della specie di quercia portoghese) presentava un effetto positivo sui valori di attività antiossidante e si osservava un alta concentrazione della frazione di proantocianidine monomeriche. Per tutti i vini studiati la correlazione fra attività antiossidante e frazione di proantocianidine monomeriche risultava sempre alta.

INTRODUCTION

Dietary habits have been correlated with the prevention of chronic diseases, such as cancer and coronary heart disease (BLOCK *et al.*, 1992; CORDER *et al.*, 2001). There is strong evidence indicating an inverse relationship between the moderate consumption of red wine, one of the major sources of dietary antioxidants, and the incidence of cardiovascular disease (FRANKEL *et al.*, 1995; MÉRILLON *et al.*, 1997). The antioxidant potential of foods and in particular in red wines is due to the presence of phenolic compounds, which may inhibit platelet aggregation (GRYGLEWSKI *et al.*, 1987 SHRIKHANDE, 2000; HOLLMAN, 2001), prevent oxidation of the low-density lipoproteins (LDL) (FRANKEL *et al.*, 1993) and decrease inflammatory and carcinogenic processes (TAPIERO *et al.*, 2002). Thus, it is of great interest to evaluate the antioxidant potential of grape beverages, especially red wine. According to BEECHER (2003), flavan-3-ols, flavonols and anthocyanins are the most important compounds that contribute to the antioxidant proprieties of red wine.

Proanthocyanidins are important phenolic compounds present in wines. These compounds are of unquestionable importance in oenology because they contribute to the sensory properties of wine and play an important role in the process of maturation and ageing of red wines. Proanthocyanidins are present in the

solid parts of the grape (skins, seeds and stems) with traces in the pulp (JORDÃO *et al.*, 1998; 2001). During wine ageing, these highly reactive molecules, undergo several reactions, including polymerisation and interactions with polysaccharides, proteins and other substances; they can also be oxidised (RICARDO DA SILVA *et al.*, 1991; CHEYNIER *et al.*, 1997). In addition, several authors (SÁNCHEZ-MORENO *et al.*, 2003; CIMINO *et al.*, 2007) have demonstrated that the peroxy radical-scavenging capacity of red wines is related to the amount of total proanthocyanidins.

Oak-barrels are currently used in the processing of natural red and white wines. The maturing of the wines in toasted oak-barrels causes changes in them and emphasises some of their characteristics. Many components can migrate from the oak wood staves during ageing_in barrels. Some examples are ellagitannins (VIRIOT *et al.*, 1993; JORDÃO *et al.*, 2005 a; 2007), tannins, gallic, ferulic, vanillic and ellagic acids, vanillin, coumarins and many volatile compounds (CHATONNET *et al.*, 1990; PÉREZ-COELLO *et al.*, 2000; ESCALONA *et al.*, 2002; JORDÃO *et al.*, 2005 b; 2006 a).

The phenolic contents that come from the wood increase during the maturation of red wine in oak casks or when oak wood chips are used, especially when toasted barrels or chips, are used. The amount of phenolics that migrate into the wine depends on the duration of ageing, the oak type, the size of the barrel and whether the barrel has been used previously (GONZÁLEZ-SAN JOSÉ and REVILLA, 2001; HO *et al.*, 2001; DE CONINCK *et al.*, 2006). The chemical differences that occur when different oak wood species are used for ageing wines (*Q. robur, Q. petraea* and *Q. alba*) and their influence on wine quality have been demonstrated (CHATONNET 1991; CADAHÍA *et al.*, 2001; JORDÃO *et al.*, 2005 a; b). The influence of Portuguese oak wood (*Q. pyrenaica* Willd.) on the evolution of the phenolic and sensorial properties of red wine has not been reported. Several authors (BELCHIOR *et al.*, 1998; CANAS *et al.*, 2000; JORDÃO *et al.*, 2005 a; b) have demonstrated that *Q. pyrenaica* contains large quantities of volatile compounds and ellagitannins; the content is similar to what has been quantified in the French oak species.

Several researchers have investigated the effect of oenological practices on the volatile fraction and phenolic content of red wines (GERBAUX *et al.*, 2002; POUSSIER *et al.*, 2003; DE CONINCK *et al.*, 2006) and on the antioxidant activity of wines (VILLAÑO *et al.*, 2006). In addition, BEER *et al.* (2006) showed that pre-fermentation maceration improved the colour of Pinotage wines, but did not affect the total antioxidant capacity. When, rotor juice/skin techniques were used, the wines had a higher antioxidant capacity and greater phenolic content. PRAJITNA *et al.* (2007) also showed that it is possible to manipulate the polyphenolic composition of wine and its antioxidant capacity by modifying production practices and using cluster thinning. Few oenological studies have been conducted on the possible influence of different oak wood chip species on the evolution of proanthocyanindins and antioxidant activity.

The aim of this study was to evaluate the time-dependent changes in the antioxidant activity and different proanthocyanidin fraction (monomeric, oligomeric and polymeric fraction) in a red wine matured in contact with Portuguese (*Q. pyrenaica* Willd.) and American (*Q. alba* L.) oak wood chips. All of the parameters studied in the wines aged with the different oak wood chips in different concentrations were compared with the values quantified in a standard wine (aged without oak wood chips). The correlation between the antioxidant activity and the different proanthocyanidin fraction contents in the wines was also analysed.

MATERIALS AND METHODS

Wine sample, reagents and equipment

Wine was made from a *Syrah* grape variety (*Vitis vinifera* L.) grown in the *Bairrada* region (north central Portugal) and harvested at the technological stage of ripeness in September 2006. The wine was made using classic winemaking technology, with a maceration time of 10 days. After fermentation (sugar content below 2.5 g/L), the wine was kept under controlled conditions and the free SO_2 level was analysed regularly. At the time of clarification, the main wine characteristics were the following: alcohol content, 13% (by volume), pH 3.84, total acidity, 6.0 g/L as tartaric acid, volatile acidity, 0.38 g/L as acetic acid, total SO_2, 45.0 mg/L and free SO_2, 20.0 mg/L.

DPPH (2,2-diphenyl-1-picrylhydrazyl) used to determine antioxidant activity and gallic acid were purchased from Fluka-Biochemika (Buchs, Switzerland) and Sigma-Aldrich (Steinheim, Germany), respectively. Spectrophotometric measurements were performed on a Perkin Elmer Lambda 25 UV/Vis spectrometer (Norwalk, CT, USA).

Oak wood chips

The Portuguese oak wood chips (*Q. pyrenaica* Willd.) came from the Gerês region (one of the most important Portuguese forests where *Q. pyrenaica* Willd. is present) and the American oak wood chips (*Q. alba* L.) came from the State of Missouri in the United States (particles were <2 mm), with a medium toasting (20 min at wood surface temperature of 160°-170°C) and grain (3.0 to 3.5 mm).

Experimental conditions

A total of five assays were carried out in this study; 10 L of red wine were used in each assay. The red wine was added to the *Q. pyrenaica* wood chips and the *Q. alba* wood chips at concentrations of 5 and 10 g/L wine. The control wine had no oak wood chips added. After the malolactic fermentation, the wines were aged in the dark for 90 days in contact with the oak wood chips at a temperature ranging from 16° to 18°C. The wine samples were filtered before analysis. All assays were carried out in duplicate.

Analysis of conventional oenological parameters

The wine used in this study was analysed for pH, titratable and volatile acidity, alcohol level, SO_2 level, total phenolic and total anthocyanin contents using the analytical methods recommended by the OIV (2006). The total phenolic and total anthocyanin contents are expressed in gallic acid (GAE) and malvidin-3-glucoside equivalents, respectively.

Antioxidant activity (DPPH method)

The procedure used to determine antioxidant activity is described by BRAND-WILLIAMS *et al.* (1995). Briefly, 0.1 mL of different sample concentrations were added to 3.9 mL of 2.2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution (25 mg/L). The DPPH solution was prepared daily and protected from the light. Absorbance at 515 nm was measured after 30 min of reaction at 20°C in a spectrophotometer. The reaction was carried out under shaking in closed Eppendorf tubes at 20°C. Methanol was used as a blank reference. The antioxidant activity is expressed as trolox equivalents (TEAC mM). All measurements were performed in duplicate.

Fractionation of proanthocyanidins according to the degree of polymerisation

Wine proanthocyanidins were fractionated according to their degree of

polymerisation; the catechins (monomers), oligomeric (degree of polymerisation ranging from 2 to 12-15) and polymeric (degree of polymerisation >12-15) fraction were separated using a C_{18} Sep-Pack column. The method is described by SUN *et al.* (1998a). Each sample was passed through two pre-conditioned neutral Sep-Pack cartridges connected in series. To eliminate phenolic acids, 4 mL of de-alcoholized medium was adjusted to pH 7.0 and then passed through the two connected Sep-Pack cartridges pre-conditioned with 10 mL of water adjusted to pH 7.0.

After drying the column with N_2, elutions were first carried out with 25 mL ethyl acetate to elute catechins and oligomeric proanthocyanidins; the polymeric fraction was then eluted with 10 mL methanol. To separate the monomeric fraction from the oligomeric fraction, both fractions were evaporated to dryness under vacuum at 25°C, dissolved in distilled water and then redeposited into the same connected cartridges pre-conditioned with distilled water. After drying the cartridges with N_2, catechins and oligomeric proanthocyanidins were first eluted with 25 mL diethyl ether (catechin fraction) and then with 10 mL methanol (oligomeric fraction).

For each fraction obtained, the flavanols were quantified by the modified vanillin assay described by SUN *et al.* (1998b). All quantifications were done in duplicate.

Statistics

To study the influence of the presence and concentrations of the different oak wood chip species used, an analysis of variance and comparison of treatment means (ANOVA, one-way) and LSD were performed using SPSS software program version 11.0 (SPSS Inc. Headquarters, Chicago, IL, USA).

RESULTS AND CONCLUSIONS

Antioxidant activity evolution

The evolution of antioxidant activity in wines aged in contact with the different oak wood chip species (Portuguese and American) at different concentrations, and in the absence of oak wood chips during 90 storage days is shown in Fig. 1. The antioxidant activity values in all of the wines increased up to 52 days of storage followed by a slight decrease. The increase in antioxidant activity in the first 52 days of storage was more pronounced at the 10 g/L oak wood chip concentration for both species. For standard wine (aged without oak wood chips) the antioxidant activity values remained nearly constant throughout the 90 day storage period. The wines aged in contact with the Portuguese oak species had higher antioxidant activity values than those aged in contact with the American oak species. The higher values were more evident after 52 storage days especially for the wine aged in contact with 10 g/L of the Portuguese species (POW10). The antioxidant activity values for the wines aged in contact with oak wood were less than those of the control wine during the first weeks of storage. They only reached higher values after 52 days (except for wine aged in contact with 5 g/L of American oak wood chips). These results suggest that the wine must be in contact with the oak wood for a certain length of time in order for the wines to have an antioxidant activity increase. This minimum time was longer when the wines were aged in contact with the 5 g/L of American oak wood chips.

The low antioxidant activity observed in the initial period could have been due to competition between the adsorption of wine phenolics in the oak matrix and the formation of oak phenolics in the wine. There may be an initial period where the adsorption of wine phenolics seemed to dominate; it was only af-

ter several weeks that the oak phenolic values were higher and more dominant. This dominance will induce the antioxidant activity in the wine.

It is thought that the antioxidant potential of red wines depends greatly on the flavanol content (BURNS *et al.*, 2000; SÁNCHEZ-MORENO *et al.*, 2000). Several authors have suggested that various components of wood diffuse into the wine during ageing in barrels; the organoleptic quality is improved and there are changes in the chemical composition and evolution of wine components, especially phenolic compounds. These compounds include tannins (PENG *et al.*, 1991; VIVAS *et al.*, 1996), lignin derivatives (PUECH *et al.*, 1996), and volatile components (CHATONNET and DUBOURDIEU, 1998; CHATONNET *et al.*, 1999). One of abovementioned compounds is ellagitannins. According to VIVAS and GLORIES (1996), these compounds play an important role in the wine oxidation process. They absorb the dissolved oxygen quickly and facilitate the hydroperoxidation of the wine components.

The general evolution (Fig. 1), suggests that during the initial storage period more phenolic compounds (e.g. ellagitannins) diffuse from the oak wood chips into the wine and the antioxidant activity present in the wine increases. After this initial period, the phenolic composition probably degrades and the antioxidant activity values decrease. There are several possibilities why the ellagitannin values decrease during the red wine ageing process. There could be polymerisation reactions among themselves, and condensation with proteins, peptides, polysaccharides and even with proanthocyanidins. JORDÃO *et al.* (2005

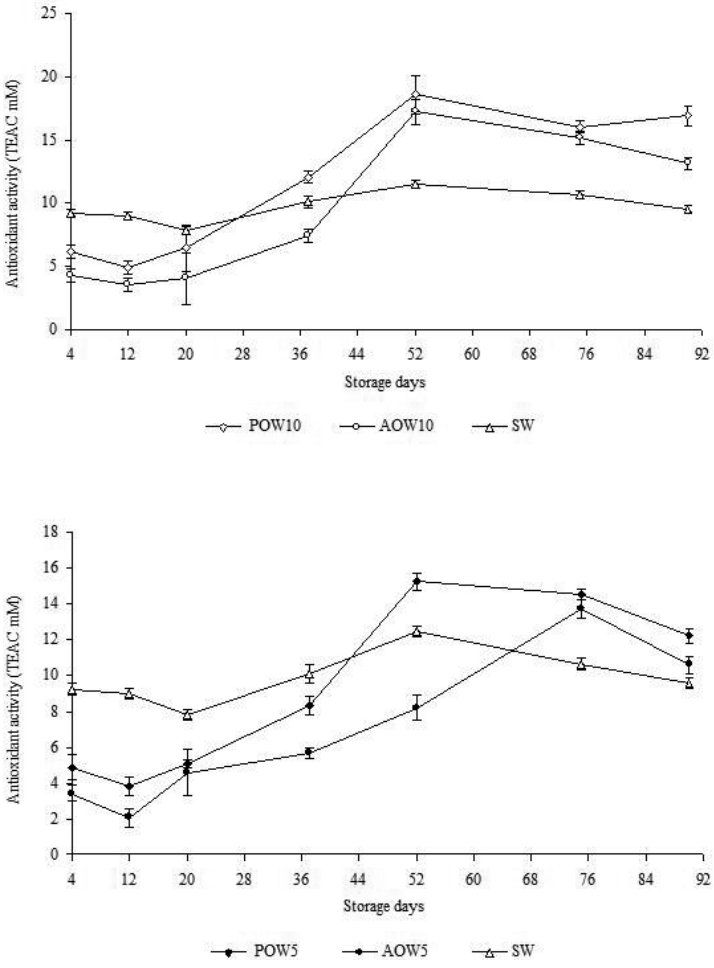

Fig. 1 - Evolution of antioxidant activity in red wine aged in contact with different oak wood chip species and at different concentrations.
–△– (SW) Standard wine; –◇– (POW10) Wine aged in contact with Portuguese oak wood chips (10 g/L); –O– (AOW10) Wine aged in contact with American oak wood chips (10 g/L); –◆– (POW5) Wine aged in contact with Portuguese oak wood chips (5 g/L); –●– (AOW5) Wine aged in contact with American oak wood chips (5 g/L).

a) used model wine solutions to show an increase in the amounts of ellagic acid and some ellagitannin diffusion from oak wood chips during the first weeks of storage followed by a decrease due to the degradation of ellagitannin.

At the end of ageing, an analysis of variance was carried out and the antioxidant activity treatment means were compared in all the wines studied (Fig. 2). The results obtained are similar to those for red wines described by other authors (FERNÁNDEZ-PACHÓN *et al.*, 2004; VILLAÑO *et al.*, 2006).

All of the wines aged in contact with oak wood chips had significantly higher (except AOW5 wine) antioxidant activity values than those for the standard wine (SW).

In recent years, some authors have studied the polyphenolic composition of *Q. pyrenaica* Willd. from Portugal and Spain and compared it with the composition of the American and French species (CHATONNET *et al.*, 1997; CADAHÍA *et al.*, 2001; FERNÁNDEZ DE SIMÓN *et al.*, 1998; 1999; 2006; JORDÃO *et al.*, 2007). In general, there were some quantitative differences, especially in the French (*Q. robur* and *Q. petraea*) and Iberian Peninsula species (including *Q. pyrenaica*, *Q. petraea* and *Q. robur*) with respect to the American species (*Q. alba* L., Missouri). All of these studies reported that European oak wood is richer than American in ellagitannins, although poorer in some low molecular weight phenolic compounds and some volatile compounds.

Thus, the difference in the polyphenolic composition between *Q. pyrenaica* Willd. and *Q. alba* L., especially with regard to the ellagitannin content, could explain the high antioxidant activity values found in wines aged in contact with Portuguese oak (especially when the highest oak wood chip concentration is used) compared with those of the American oak species; and taking into account the important role of these compounds in the wine oxidation process (VIVAS and GLORIES, 1996). These results are also important because they give supplementary information about the native oak wood species (*Q. pyrenaica* Willd.) from the Iberian Peninsula in comparison with the other oak wood species.

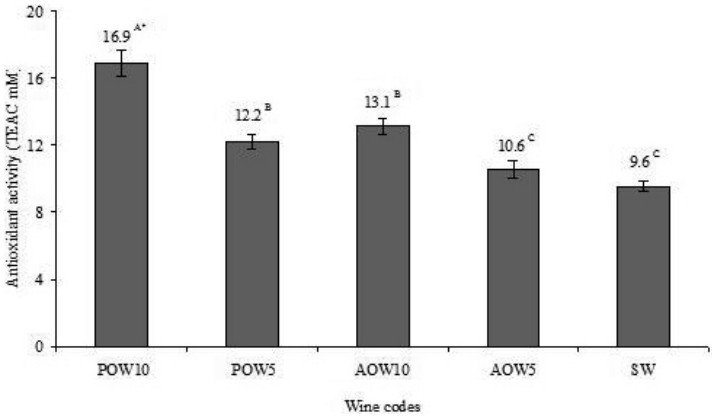

Fig. 2 - Influence of oak wood species and concentration on the mean wine antioxidant activity values in wine after 90 days of storage.
(POW10) Wine aged in contact with Portuguese oak wood chips (10 g/L); (AOW10) Wine aged in contact with American oak wood chips (10 g/L); (POW5) Wine aged in contact with Portuguese oak wood chips (5 g/L); (AOW5) Wine aged in contact with American oak wood chips (5 g/L); (SW) Standard wine.
Means were compared by the LSD method.
* Means followed by the same letter are not significantly different ($p<0.05$).
Error bars indicate standard deviation.

There was a correlation between the antioxidant activity, and the total phenols and each proanthocyanidin fraction (monomeric, oligomeric and polymeric) after 90 days of storage. There was a good correlation between the antioxidant activity and the monomeric fraction of proanthocyanidins ($y = 0.0144x+0.0759$, $R^2 = 0.9442$).

Based on these results, the monomeric proanthocyanidin fraction appears to have a major influence on the antioxidant potential of red wines. According to several authors (BURNS *et al.*, 2000; SÁNCHEZ-MORENO *et al.*, 2000; HEO *et al.*, 2007) the antioxidant potential of red wines depends to a great extent on the flavanol content; it is also influenced by the relative amounts of each flavanol.

The total phenol and total anthocyanin evolution during red wine ageing is shown in Table 1 for the different oak wood chip species and the control wine. In general, for all wines, the total phenol values varied slightly during the 90 days of storage. However, for the wines aged in contact with the higher concentration of the Portuguese oak wood species (POW10), the total phenol values were slightly higher during the storage period compared to the other wines, especially the control wine.

According to JINDRA and GALLENDER (1987), the increase in the total phenols in wines aged in contact with oak wood is due to the transfer of the phenols from the wood to the wine during the storage time. For other authors (GÓMEZ and GONZALEZ-SAN JOSÉ, 1995; POMAR and GONZALEZ-MENDOZA, 2001; DE CONINCK *et al.*, 2006), the effect on total phenols in wine that has been in contact with oak wood chips is not evident. This aspect could be due to a complex equilibrium between phenolic substances that diffused from the wood and coloured material that precipitated in the red wine during wine storage. The wine could adsorb the phenolic wine compounds from the wood. However, with a long storage time or a high oak wood chip concentration, there were marked differences between the wines aged in contact with the different oak chip species and the control wine.

The total anthocyanin content decreased slightly in all the wines during ageing (including the standard wine).

Table 1 - Evolution of the total phenol and total anthocyanin mean values in red wines aged in contact with

	4		12		20	
Wine code	Total Phenols*	Total Anthocyanins**	Total Phenols	Total Anthocyanins	Total Phenols	Total Anthocyanins
POW10	2211.7	160.0	2124.5	160.0	2213.5	154.4
AOW10	2119.8	143.9	2150.2	143.9	2163.2	154.4
POW5	2100.4	170.5	2120.9	170.5	2136.1	168.9
AOW5	2104.5	163.3	2110.8	163.3	2121.2	158.4
SW	2106.1	168.1	2094.5	165.2	2092.6	164.1

Wine code:
(POW10) Wine aged in contact with Portuguese oak wood chips (10 g/L); (AOW10) Wine aged in contact with American oak American oak wood chips (5 g/L); (SW) Standard wine.
* Expressed in mg GAE/L equivalents.
** Expressed in mg/L malvidin-3-glucoside equivalents.

This decrease was probably due to anthocyanin condensation and polymerisation reactions that occurred during wine ageing. The precipitation of these compounds is another reason for the observed decrease. This result is consistent with previous reports (POMAR and GONZALEZ-MENDOZA, 2001; DE CONINCK *et al.*, 2006).

The decrease in anthocyanin values was more evident in wines matured in contact with all of the oak wood chip species at the different concentrations. After 90 days of ageing, the wines that were matured in contact with oak wood chips had lower total anthocyanin values than the standard wine.

PÉREZ-PRIETO *et al.* (2003) suggested that the decrease of these pigments in wines matured in oak wood is probably due to anthocyanin degradation reactions that are different from the condensation reactions that lead to wine colour stabilisation. According to DE CONINCK *et al.* (2006), the decrease in the anthocyanin content in wines matured in contact with oak wood is more evident for some individual anthocyanins, namely malvidin-3-glucoside and malvidin-3-glucoside coumarate. Using model wine solutions JORDÃO *et al.* (2006b) reported that the malvidin-3-glucoside content was lower in solutions that had matured in contact with oak wood. At the same time this decrease in anthocyanin content corresponded to a decrease in the red colour measured by the a^* CIELAB coordinates. Other studies indicate that the potential decrease in anthocyanin content can be attributed to enzymatic or chemical degradation which occurs while the wine ages in contact with oak wood (CASTELLARI *et al.*, 2001; MATEJÍCEK *et al.*, 2005). While wine ages in contact with oak wood the red wine colour is stabilised by the formation of new anthocyanin-derived pigments. These new pigments usually have molar absorptivity coefficients at 520 nm (the same wavelength used in the total anthocyanin measurement) that are lower than native grape anthocyanins. Thus the formation of these new pigments could explain why the total anthocyanin value decreases in wines that have been aged in contact with oak wood.

The evolution of the monomeric, oligomeric and polymeric fractions of the

different oak wood chip species and at different concentrations.

Storage days							
37		52		75		90	
Total Phenols	Total Anthocyanins	Total Phenols	Total Anthocyanins	Total Phenols	Total Anthocyanins	Total Phenols	Total Anthocyanins
2226.3	116.4	2223.5	115.6	2226.3	102.6	2198.7	95.8
2218.6	129.3	2211.2	119.6	2194.6	109.9	2150.6	105.4
2120.9	128.5	2116.1	121.2	2120.9	108.3	2117.1	101.3
2114.8	134.2	2111.2	128.5	2114.8	109.9	2110.3	102.8
2098.9	154.7	2106.6	159.3	2110.5	145.1	2090.4	140.0

wood chips (10 g/L); (POW5) Wine aged in contact with Portuguese oak wood chips (5 g/L); (AOW5) Wine aged in contact with

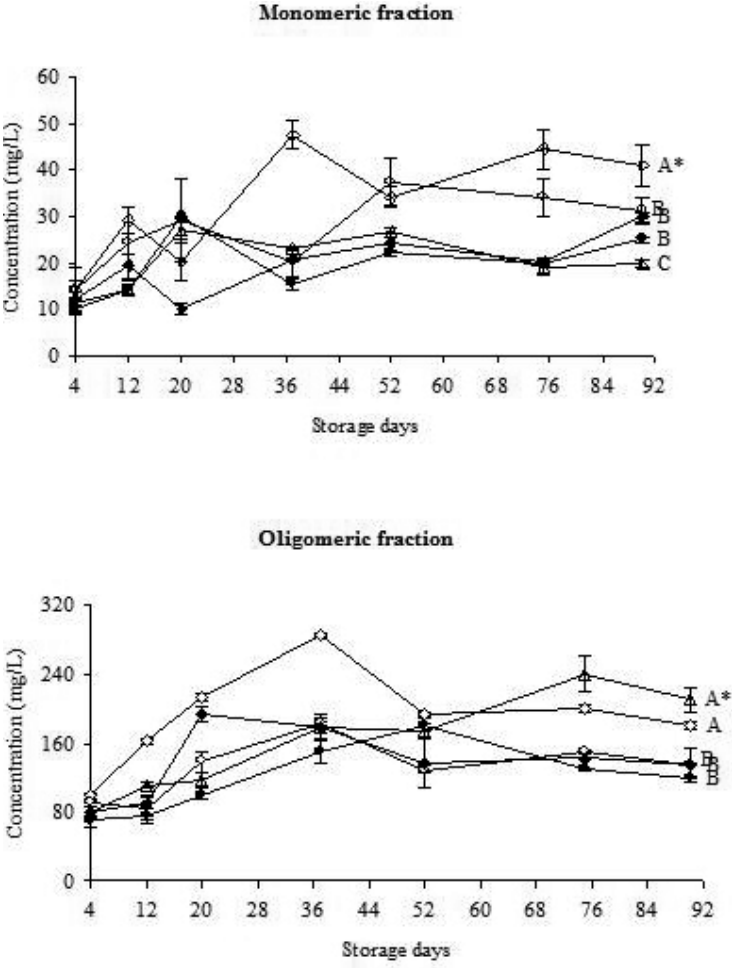

Fig. 3 - Evolution of the monomeric, oligomeric and polymeric proanthocyanidin fractions in red wines aged in contact with different oak wood chips species and at different concentrations.
–△– (SW) Standard wine; –◇– (POW10) Wine aged in contact with Portuguese oak wood chips (10 g/L); –o– (AOW10) Wine aged in contact with American oak wood chips (10 g/L); –♦– (POW5) Wine aged in contact with Portuguese oak wood chips (5 g/L); –●– (AOW5) Wine aged in contact with American oak wood chips (5 g/L). Means were compared by the LSD method.
* Means followed by the same letter are not significantly different ($p<0.05$).

proanthocyanidins in the red wines is shown in Fig. 3. In general, the monomeric and oligomeric proanthocyanidin fraction values increased in all the wines; this increase was characterised by small fluctuations. In contrast, the polymeric fraction values decreased in all wines during the ageing period. Recently, DE CONINCK *et al.* (2006) reported a similar evolution with barrels made from European oak species. However, after 10 weeks of storage, these authors noted a strong decrease in the values of the monomeric and oligomeric proanthocyanidin fractions.

There were appreciable quantitative differences in the monomeric and oligomeric fractions of the wines studied, especially before 52 days of storage. However there were no appreciable differences between the wines with respect to the polymeric fraction values.

After 90 days of storage (Fig 3), the monomeric fraction values of the wines matured in contact with oak wood chips and those of the standard wine differed significantly. The wine matured with Portuguese oak wood chips (POW10) had significantly higher values than the wine aged with American oak wood (AOW10). Thus, the highest chip concentration of the Portuguese oak species significantly affected the monomeric proanthocyanidin fraction content in the wines. Recently DE CONINCK *et al.* (2006) did not find any significant differences in the monomeric, oligomeric and polymeric fraction content in wines when a low concentration of Portuguese oak wood species (4 g/L) was used.

The standard wine (SW) and the wine matured in contact with Portuguese oak (PWO10) had the highest oligomeric fraction values. No significant differences in the oligomeric proanthocyanidin fraction content were detected in the other wines. After 90 days of storage, there were no appreciable quantitative differences in the polymeric proanthocyanidin content in the several wines studied; these results are in agreement with other authors (CHATONNET *et al.*, 1997). These authors did not detect any significant differences in the oligomeric and polymeric proanthocyanidin content in wines matured in different oak wood barrel species (*Q. petraea* and *Q. alba*). However, in other studies, in which different oak wood barrels species were used the polymerised phenolic values were high indicating that the monomeric phenols polymerised more rapidly in wines matured in oak wood barrels than the wines matured in tanks (CASTELLARI *et al.*, 2001).

In this study, information has been obtained regarding the effect that the use of Portuguese or American oak wood has on wine maturation especially with respect to antioxidant activity and the evolution of the proanthocyanidin fraction content. This knowledge could be useful for oenologists when it is necessary to select new sources of quality wood for cooperage and consequently for producing mature wines.

REFERENCES

Beecher G.R. 2003. Overview of dietary flavonoids: Nomenclature, occurrence and intake. J. Nutr. 133: 32485.

Belchior A.P., Caldeira I., Tralhão G., Costa S., Lopes C. and Carvalho E. 1998. Incidência da origem e queima da madeira de carvalho (*Q. pyrenaica*, *Q. robur*, *Q. sessiflora*, *Q. alba/stellata* + *Q. lyrata/bicolor*) e de castanho (*C. sativa*) em características físico-químicas e organolépticas de aguardentes Lourinhã em envelhecimento. Ciência Tec. Vitiv. 13: 97.

Beer D., Joubert E., Marais J. and Manley M. 2006. Maceration before and during fermentation: Effect on Pinotage wine phenolic composition, total antioxidant capacity and objective colour parameters. S. Afr. J. Enol. Vitic. 27: 137.

Block G., Patterson B. and Subar A. 1992. Fruit, vegetables and cancer prevention: a review of the epidemiological evidence. Nutrition and Cancer 18: 1.

Brand-Williams W., Cuvelier M.E. and Berset C. 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 28: 25.

Burns J., Gardner P.T., O'Neil J., Crawford S., Morecroft I., McPhail D.B., Lister C., Matthews D., MacLean M.R., Lean M.E., Duthie G.G. and Crozier A. 2000. Relationship among antioxidant activity, vasodilation capacity, and phenolic content of red wine. J. Agric. Food. Chem. 48: 220.

Cadahía E., Varea S., Muñoz L., Fernández de Simón B. and García-Vallejo M.C. 2001. Changes in low molecular weight phenolic compounds in Spanish, French and American oak woods during natural seasoning and toasting. J. Agric. Food Chem. 49: 1790.

Canas S., Conceição M.L., Spranger M.I. and Belchior A.P. 2000. Influence of botanical species and geographical origin on the content of low molecular weight phenolic compounds of woods used in Portuguese cooperage. Holzforschung 54: 255.

Castellari M., Piermatti B., Arfelli G. and Amati A. 2001. Influence of aging conditions on the quality of red sangiovese wine. J. Agric. Food Chem. 49: 3672.

Chatonnet P., Boidron J.N. and Pons M. 1990. Élevage des vins rouges en fûts de chêne: évolution de certains composés volatils de leur impact arômatique. Sci. Aliments 10: 565.

Chatonnet P. 1991. Incidence du bois de chêne sur la composition chimique et les qualités organoleptiques des vins. Aplications technologiques. Thèsis, Université de Bordeaux II.

Chatonnet P., Ricardo-da-Silva J.M. and Dubourdieu D. 1997. Influence de l'utilisation de barriques en chêne sessile européen (*Quercus petraea*) ou en chêne blanc américain (*Quercus alba*) sur la composition et la qualité des vins rouges. Rev. Fr. Oenol. 165: 44.

Chatonnet P. and Dubourdieu D. 1998. Comparative study of the characteristics of American white oak (*Quercus alba*) and European oak (*Quercus petraea* and *Q. robur*) for production of barrels used in barrel aging of wines. Am. J. Enol. Vitic. 49: 79.

Chatonnet P., Cutzach I., Pons M. and Dubourdieu D. 1999. Monitoring toasting intensity of barrels by chromatographic analysis of volatile compounds from toasted oak wood. J. Agric. Food Chem. 47: 4310.

Cheynier V., Rigaud J. and Ricardo-da Silva J.M. 1997. Structure of procyanidin oligomers isolated from grape seeds in relation to some of their chemical properties. In "Plant Polyphenols". (Ed. R. W. Hemingway and P.E. Laks), Plenum Press, New York.

Cimino F., Sulfaro V., Trombetta D., Saija A. and Tomaino A. 2007. Radical-scavening capacity of several Italian red wines. Food Chem. 103: 75.

Corder R., Douthwaite D., Lees D.M., Khan N.Q., Viseu dos Santos A.C., Wood E.G. and Carrier M.J. 2001. Endothelin-1 synthesis reduced by red wine. Nature 414: 863.

De Coninck G., Jordão A.M., Ricardo-da-Silva J.M. and Laureano O. 2006. Evolution of phenolic composition and sensory properties in red wine aged in contact with Portuguese and French oak wood chips. J. Int. Sci. Vigne Vin 40: 25.

Escalona H., Birkmyre L., Piggott J.R. and Paterson A. 2002. Effect of maturation in small oak casks on the volatility of red aroma compounds. Anal. Chim. Acta 458: 45.

Feuillat F., Moio L., Guichard E., Marinov M., Fournier N. and Puech J.L. 1997. Variation in the concentration of ellagitannins and *cis*- and *trans*-β-methyl-γ-octalactone extracted from oak wood (*Quercus robur* L., *Quercus petraea* liebl.) under model wine cask conditions. Am. J. Enol. Vitic. 48: 509.

Fernández de Simón B., Cadahia E., Conde E. and Garcia-Vallejo M.C. 1998. Les ellagitanins dans les bois de chênes espagnols. J. Sci. Tech. Tonnellerie 4: 83.

Fernández de Simón B., Cadahia E., Conde E. and Garcia-Vallejo M.C. 1999. Ellagitannins in woods of Spanish, French and American oaks. Holzforschung 53: 147.

Fernández-Pachón M.S., Villaño D., Garcia-Parrilla M.C. and Troncoso A.M. 2004. Antioxidant activity of wines and relation with their polyphenolic composition. Anal. Chim. Acta 513: 113.

Fernández de Simón B., Sanz M., Cadahia E., Poveda P. and Broto M. 2006. Chemical characterization of oak heartwood from Spanish forests of *Quercus pyrenaica* (Willd.). Ellagitannin, low molecular weight phenolic, and volatile compounds. J. Agric. Food. Chem. 54: 8314.

Frankel E.N., Kanner J., German J.B., Parks E. and Kinsella J.E. 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. The Lancet 341: 454.

Frankel E.N, Waterhouse A.L. and Teissedre P.L. 1995. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. J. Agric. Food. Chem. 43: 890.

Gerbaux V., Vincent B. and Bertrand A. 2002. Influence of maceration temperature and enzymes on the content of volatile phenols in Pinot Noir wines. Am. J. Enol. Vitic. 53: 131.

Gómez C.C. and González-San José M.L. 1995. Interpretation of colour variables during the aging of red wines: relationship with families of phenolic compounds. J. Agric. Food Chem. 43: 557.

González-San José M.L. and Revilla I. 2001. Effect of different oak woods on aged wine color and anthocyanin composition. Eur. Food Res. Technol. 213: 281.

Gryglewski R.J., Korbut R., Robak J. and Swies J. 1987. On the mechanism of antithrombotic action of flavonoids. Biochem. Pharmacol. 36: 317.

Heo H.J., Kin Y.J., Chung D. and Kim D-O. 2007. Antioxidant capacities of individual and combined phenolic in a model system. Food Chem. 104: 87.

Ho P., Silva M.C.M. and Hogg T.A. 2001. Changes in colour and phenolic composition during the early stages of maturation of port in wood, stainless steel and glass. J. Sci. Food Agric. 81: 1269.

Hollman P.C.H. 2001. Evidence for health benefits of plant phenolics: local or systemic effects ?. J. Sci. Food Agric. 81: 842.

Jindra J.A. and Gallender J.F. 1987. Effect of American and French oak barrels on the phenolic composition and sensory quality of Seyval blanc wine. Am. J. Enol. Vitic. 38: 133.

Jordão A.M., Ricardo-da-Silva J.M. and Laureano O. 1998. Evolution of anthocyanins during grape maturation of two varieties (*Vitis vinifera* L.): Castelão Francês and Touriga Francesa. Vitis 37: 93.

Jordão A.M., Ricardo-da-Silva J.M. and Laureano O. 2001. Evolution of catechin and procyanidin composition during grape maturation of two varieties (*Vitis vinifera* L.) Castelão Francês and Touriga Francesa. Am. J. Enol. Vitic. 52: 230.

Jordão A.M., Ricardo-da-Silva J.M. and Laureano O. 2005a. Extraction and evolution of some ellagic tannins and ellagic acid of oak wood chips (*Quercus pyrenaica*) in model wine solutions: Effect of time, pH, temperature and alcoholic content. S. Afr. J. Enol. Vitic. 26: 25.

Jordão A.M., Ricardo-da-Silva J.M. and Laureano O. 2005b. Comparison of volatile composition of cooperage oak wood of different origins (*Quercus pyrenaica vs. Quercus alba* and *Quercus petraea*). Mitt. Klosterneuburg 55: 31.

Jordão A.M., Ricardo-da-Silva J.M., Laureano O., Adams A., Demyttenaere J., Verhé, R. and De Kimpe N. 2006a. Volatile composition analysis by solid-phase microextraction applied to oak wood used in cooperage (*Q. pyrenaica* and *Q. petraea*) – Effect of Botanical Species and Toasting Process. J. Wood Sci. 52: 514.

Jordão A.M., Ricardo-da-Silva J.M. and Laureano O. 2006b. Effect of oak constituents and oxygen on the evolution of malvidin-3-glucoside and (+) catechin in model wine. Am. J. Enol. Vitic. 57: 377-381.

Jordão A.M., Ricardo-da-Silva J.M. and Laureano O. 2007. Ellagitannins from Portuguese oak wood (*Quercus pyrenaica* Willd.) used in cooperage: influence of geographical origin, coarseness of the grain and toasting level. Holzforschung 61: 155.

Matejícek D., Mikes O., Klejdus B., Sterbova D. and Kubán V. 2005. Changes in contents of phenolic compounds during maturing of barrique red wines. Food Chem. 90: 791.

Mérillon J.M., Fauconneau B., Teguo P.W., Barrier L., Vercauteren J. and Huguet F. 1997. Antioxidant activity of the stilbene astringin, newly extracted from *Vitis vinifera* cell cultures. Clinical Chem. 43: 1092.

OIV (Organisation International de la Vigne et du Vin). 2006. Recueil des méthodes internationales d'analyse des vins et moûts. Edition Officielle. Paris.

Peng S., Scalbert A. and Monties B. 1991. Insoluble ellagitannins in *Castanea sativa* and *Quercus petraea* woods. Phytochem. 30: 775.

Pérez-Coello M.S., González-Viñas M.A., García-Romero E., Cabezudo M.D. and Sanz J. 2000. Chemical and sensory changes in white wines fermented in the presence of oak chips. Int. J. Food Sci. Technol. 35: 23.

Pérez-Prieto L.J., Hera-Orts M.L., López-Roca J.M., Fernández- Fernández J.I. and Gómez-Plaza E. 2003. Oak-matured wines: influence of the characteristics of the barrel on wine colour and sensory characteristics. J. Sci. Food Agric. 83: 1445.

Poussier M., Guilloux-Benatier M., Torres M., Heras E. and Adrian M. 2003. Influence of different maceration techniques and microbial enzymatic activities on wine stilbene content. Am. J. Enol. Vitic. 54: 261.

Pomar M. and Gonzalez-Mendoza L.A. 2001. Changes in composition and sensory quality of red wine aged in American and French oak barrels. J. Int. Sci. Vigne Vin 35: 41.

Prajitna A., Dami I.E., Steiner T.E., Ferree D.C., Scheerens J.C. and Schwartz S.J. 2007. Influence of cluster thinning on phenolic composition, resveratrol, and antioxidant capacity in Chambourcin wine. Am. J. Enol. Vitic. 58: 346.

Puech J.L., Feuillat F., Mosedale J.R. and Puech C. 1996. Extraction of ellagitannins from oak wood of model casks. Vitis 35: 211.

Ricardo-da-Silva J.M., Cheynier V., Souquet J.M. and Moutounet M. 1991. Interaction of grape seed procyanidins with various proteins in relation to wine fining. J. Agric. Food Chem. 57: 111.

Sánchez-Moreno C., Satué-Garcia M.T. and Frankel E.N. 2000. Antioxidant activity of selected Spanish wines in corn oil emulsions. J. Agric. Food. Chem. 48: 5581.

Sánchez-Moreno C., Cao G., Ou B. and Prior R.L. 2003. Anthocyanin and proanthocyanidin content in selected white and red wines. Oxygen radical absorbance capacity comparison with non-traditional wines obtained from high bush blueberry. J. Agric. Food Chem. 51: 4889.

Shrikhande A.J. 2000. Wine by-products with health benefits. Food Res. Int. 33: 467.

Sun B.S., Leandro C., Ricardo-da-Silva J.M. and Spranger I. 1998 a. Separation of grape and wine proanthocyanidins according to their degree of polymerization. J. Agric. Food Chem. 46: 1390.

Sun B.S., Ricardo-da-Silva J.M. and Spranger I. 1998 b. Critical factors of vanillin assay for catechins and proanthocyanidins. J. Agric. Food Chem. 46: 4267.

Tapiero H., Tew K.D., Nguyen B.A.G and Mathé G. 2002. Polyphenols: do they play a role in prevention of human pathologies? Biomed. Pharmacother. 56: 200.

Villaño D., Fernández-Pachón M.S., Troncoso A.M. and García-Parrilla M.C. 2006. Influence of enological practices practices on the antioxidant activity of wines. Food Chem. 95: 394.

Viriot C., Scalbert A., Lapierre C.L. and Moutounet M. 1993. Ellagitannins and lignins in aging of spirits in oak barrels. J. Agric. Food. Chem. 41: 1872.

Vivas N. and Glories Y. 1996. Role of oak wood ellagitannins in the oxidation process of red wine during aging. Am. J. Enol. Vitic. 7: 103.

Vivas N., Glories Y., Bourgeois G. and Vitry C. 1996. The heartwood ellagitannins of different oak (*Quercus* sp.) and chestnut species (*Castenea sativa* Mill.). Quantity analysis of red wines aging in barrels. J. Sci. Tech. Tonnellerie 2: 51.

Revised paper received March 21, 2008 Accepted May 26, 2008

PAPER

CHANGES IN CHEMICAL COMPOSITION, ANTIOXIDANT ACTIVITIES AND TOTAL PHENOLIC CONTENT OF *ARBUTUS ANDRACHNE* FRUIT AT DIFFERENT MATURATION STAGES

CAMBIAMENTI DI COMPOSIZIONE CHIMICA, ATTIVITÀ ANTIOSSIDANTE E CONTENUTO IN COMPOSTI FENOLICI TOTALI DI FRUTTI DELL'*ARBUTUS ANDRACHNE* A DIFFERENTI STADI DI MATURAZIONE

M. ÖZGEN[1]*, A.A. TORUN[2], S. ERCIŞLI[3] and S. SERÇE[4]

[1]Department of Horticulture, University of Gaziosmanpasa, Taşlıçiftlik, 60240 Tokat, Turkey

[2] Department of Soil Sciences, Çukurova University, 01330 Adana, Turkey

[3] Department of Horticulture, Ataturk University, 25240 Erzurum, Turkey

[4] Department of Horticulture, Mustafa Kemal University, Antakya, 31034 Hatay, Turkey

*Corresponding author: Tel. +90 356 2521616-ext 2110, Fax +90 356 2521488, e-mail: mozgen@gop.edu.tr

ABSTRACT

Several characteristics of *Arbutus andrachne* L. fruit harvested at four maturation stages (green, green-orange, orange-red and red) have been examined. Soluble solids, pH, acidity, organic acids, specific sugars, and several nutrient element concentrations (N, P, K, Ca, Mg, Fe, Zn, Mn, Cu) have been deter-

RIASSUNTO

Sono state studiate numerose caratteristiche dei frutti dell'*Arbutus andrachne* L. raccolti a 4 differenti stadi di maturazione (verde, verde-arancio, arancio-rosso e rosso). Sono state determinate le concentrazioni di solidi solubili, di acidi organici, di zuccheri specifici, di numerosi elementi nutrizionali (N, P, K,

- Key words: Antioxidant activities; *Arbutus andrachne*; mineral elements; organic acids; phenolic content; soluble sugars -

mined. The total phenolic content along with antioxidant activity, determined by FRAP and TEAC methods, were also measured. There were significant differences among most of the traits tested. The average soluble solids increased from 16.0 to 20.6% going from the green to the red stages, while the acidity decreased from 0.54 to 0.45%. All of the element concentrations were statistically different among the various stages, with N and Ca being the most variable. The total acidity was found to be the highest in the orange-red and red stages (0.52 and 0.50 g/100 g, respectively). The main organic acid was malic acid (0.32 g/100 g) followed by ascorbic and citric acids (0.09 and 0.03 g/100 g) respectively. The red (17.84 g/100 g) and orange-red (16.25 g/100 g) stages had the highest total sugar values. Fructose was the main sugar in the fruit with an overall average of 8.91 g/100 g. The total phenolic content was highest at the red stage (3,904 mg gallic acid (GA)/kg fresh weight (fw)), followed by the green stage (3,589 mg GA/kg fw). The antioxidant activities were highest at the red stage as determined by both the TEAC and FRAP methods (26.5 and 21.8 μmol TE/g fw).

Ca, Mg, Fe, Zn, Mn, Cu), il pH e l'acidità. Sono stati inoltre determinati il contenuto in composti fenolici totali e l'attività antiossidante, attraverso i metodi FRAP e TEAC. Si sono osservate differenze significative per la maggior parte delle caratteristiche considerate. La media dei solidi solubili aumentava dal 16,0 al 20,6% passando dallo stadio verde al rosso, mentre l'acidità diminuiva dal 0,54 al 0,45%. Le concentrazioni di tutti gli elementi nutrizionali erano statisticamente differenti fra i diversi stadi di maturazione, fra questi l'N e il Ca sono risultati i più variabili. L'acidità totale più alta è stata rilevata nei frutti allo stadio arancio-rosso e rosso (0,52 e 0,50 g/100 g, rispettivamente). Gli acidi organici principali erano l'acido malico (0,32 g/100 g) seguito dall'ascorbico e dal citrico (0,09 e 0,03 g/100 g) rispettivamente. I frutti allo stadio rosso (17,84 g/100 g) e arancio-rosso (16,25 g/100 g) presentavano i valori più alti di zuccheri totali. Il fruttosio era lo zucchero principale nei frutti, con un contenuto medio di 8,91 g/100 g. Il contenuto in composti fenolici totali era più alto nei frutti allo stadio rosso (3.904 mg acido gallico (GA)/kg peso fresco (pf)), seguito dallo stadio verde (3.589 mg GA/kg pf). Come determinato con entrambi i metodi TEAC and FRAP, le attività antiossidanti erano più alte allo stadio rosso (26,5 and 21,8 μmol TE/g pf).

INTRODUCTION

During the last decade, highly colored fruit has emerged as a natural alternative in modern medicine. Natural plant pigments such as carotenoids, anthocyanins, chlorophylls, and betalains are considered to have bioactive (functional food) value, because they can help maintain human health by protecting against chronic diseases, or by restoring wellness by repairing damaged tissues (BEATTIE *et al.*, 2005; KAUR and KOOPER, 2001; LILA, 2007). Current thinking links the high antioxidant content of fruit and vegetables to the inhibition of oxidative damage correlated to diseases such as cancer, coronary heart disease and stroke.

Arbutus andrachne L. is a small evergreen tree of the *Ericaceae* family that is native to the Mediterranean region and southwestern Asia. Many accessions of this fruit have also been reported in several locations of Anatolia (DAVIS, 1982). In the Mediterranean area of Turkey, the tree flowers from March to April and the small fruits ripen in December. Their fruit is juicy with an attractive red-orange color when ripe; they are consumed locally in both fresh and processed forms (BAYTOP, 1999). Generally, the fruit is sweet but has an insipid flavor. It contains large amounts of tannins, anthocyanins and carotenoids with attractive deep red to orange color.

Historically, *A. andrachne* fruit has been used medicinally by many indigenous cultures. However, scientific literature confirming the effects of *A. andrachne* fruit is very limited. Only a few studies have been conducted in an attempt to characterize some of the chemical and antioxidant properties of *A. andrachne* fruit (TAWAHA *et al.,* 2007). These studies reported high amounts of total phenolic and antioxidant capacity in mature fruit. Antioxidant activity and chemical composition of the fruit can be influenced by genetics, environment, postharvesting conditions and maturation stages (CELIK *et al.,* 2008; CONNOR *et al.,* 2005; OZGEN *et al.,* 2006a; SIRIWOHARN *et al.,* 2004; WANG and LIN, 2000). For optimum medicinal value, it is important to determine at which ripening stage the phytonutrient composition is at a maximum. The aim of this study was to determine the changes of chemical composition, antioxidant capacity and total phenolic content of *A. andrachne* fruit at four different stages of maturity.

MATERIALS AND METHODS

The *A. andrachne* fruit was harvested at four different maturity stages (green, green-orange, orange-red and dark red) in mid-December 2007 from eight different sites in the Mediterranean region of Turkey. The plants were all growing wild. The fruit was transferred to the laboratory within two days for physical and phytochemical analyses. The fruit from each stage collected from the different sites was combined into large lots, and then sub-divided into three replications. The fruit color was measured using a Minolta portable chromameter (Minolta, Model CR-400, Tokyo, Japan) which provided CIE L*, a* and b* values. About 100 g fruit were frozen at -20°C for the three replicates for each of the four maturity stages. At the time of analysis the fruit was thawed and homogenized in a standard food blender. Slurries were used to determine total soluble solids (TSS) content by refractometery (Atago, Pal-1, Tokyo, Japan) and titratable acidity (TA) using standard methodology (PERKINS-VEAZIE and COLLINS 1993). The chemical analyses were completed within two weeks of storage.

A single extraction procedure designed to assay phenols (SINGLETON and ROSSI, 1965) was used to determine the total soluble phenolic content (TP) and the antioxidant capacity of all the samples. For each replicate, a 3 g aliquot of slurry was transferred to polypropylene tubes and extracted with 20 mL of extraction buffer containing acetone, water and acetic acid (70:29.5:0.5 v/v) for two h. After filtration, acetone was removed by rotary evaporation and the concentrated samples were brought to a final volume of 20 mL with de-ionized water. To determine TP levels, 0.5 mL of each extract was combined with Folin-Ciocalteu's reagent and water 1:1:20 (v/v) and incubated for eight min; 5 mL of 7% (w/v) sodium carbonate were then added. After two h, the absorbance was measured by an automated UV-vis spectrophotometer (Model T60U-UV/visible, PG Instruments, London, UK) at 750 nm. Gallic acid was used as standard. The results are expressed as mg gallic

acid equivalent in g fresh weight basis (GAE/g fw).

Total antioxidant activity was estimated by two standard procedures: FRAP (Ferric Reducing Ability of Plasma) and TEAC (Trolox Equivalent Antioxidant Capacity). FRAP was determined according to the method of BENZIE and STRAIN (1996). The assay was conducted using three separate stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid, and 20 mmol/L ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1 v/v/v) to form the FRAP reagent just prior to analysis. For each assay, laboratory duplicate 2.95 mL of FRAP reagent and 50 μL of sample extract were mixed. After 10 min, the absorbance of the reaction mixture was determined at 593 nm on the spectrophotometer (T60U-UV/visible, PG Instruments, London, UK).

For the standard TEAC assay, ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was dissolved in acetate buffer and prepared with potassium persulfate as described by OZGEN *et al.* (2006b). The mixture was diluted in an acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700±0.01 at 734 nm for longer stability. For the spectrophotometric assay, 2.95 mL of the $ABTS^{+}$ solution and 50 μL of fruit extract were mixed and incubated for 10 min and the absorbance was measured at 734 nm. Trolox was used as standard for both assays. The results are expressed as mmol Trolox equivalent in g fresh weight basis (TE/g fw).

Fruit slurries (5 g) were diluted with purified water or meta-phosphoric acid (2.5%) solution for individual sugar and organic acid analyses, respectively. The homogenate was centrifuged at 6,000 rpm for 5 min. Supernatants were filtered through a 0.45 μm membrane filter (Iwaki Glass, Perkin Elmer, Waltham, MA, USA) before HPLC analysis, and the mobile phase solvents were degassed before use. All the samples and standards were injected three times each and the mean values were used.

The HPLC analysis were carried out using a Perkin Elmer HPLC system with Totalchrom Navigator 6.2.1 software, a pump and UV detector (Perkin Elmer Series-200, Waltham, MA, USA). Separation and determination of organic acids was based on SHUI and LEONG (2002) methods with modifications. The separation was carried out on a SGE wakosil C18RS 5μm column (250x4.6 mm I.D.). Optimum efficiency of separation was obtained using pH 2.5 sulfuric acid solution (solvent A) and methanol (solvent B). Other parameters adopted were as follows: injection volume, 20 μL, column temperature 30°C, and detection wavelength 215 nm.

Analysis of the sugars was performed according to the method described by BARTOLOME *et al.* (1995) using a refractive index (RI) detector (Perkin Elmer, Waltham, MA, USA). The separation was carried out on a SGE SS Exsil amino column (250x4.6 mm I.D., Perkin Elmer, Waltham, MA, USA). The elution solvent used was 80% acetonitrile and 20% deionised water. The column was operated at 30°C with 0.9 mL/min flow rate. Sample injection volume was 20 μL.

All fruit samples were dried at 70°C for 48 h. After grinding, samples were digested in a H_2O_2-HNO_3 mixture in sealed tubes in a microwave oven (Varian Milestone, 1200 Mega, Palo Alto, CA, USA) prior to analysis by atomic absorption spectrometry (Varian, FS 220, Palo Alto, CA, USA). Reference leaf samples from the National Institute of Standards and Technology (Gaithersburg, MD, USA) were used to check the nutrient element measurements. All the values for each parameter are the means of three independent determinations.

Data were analyzed using SAS procedures and software (SAS, 2005). Means and standard deviations were obtained using the TABULATE procedure. Analyses of variance were carried out by GLM procedure. The means were separated using the least significant difference (LSD) method at the 0.05 significance level. The coefficients of variation (C.V.) were calculated by dividing relevant standard deviations by the means and multiplying by 100.

RESULTS AND DISCUSSION

Some characteristics of *Arbutus andrachne* fruit at different stages of maturity are presented in Table 1. The TSS, pH and TA values differed significantly among the different maturation stages. The average TSS was 18.4 % and the maturation stages could be separated into two groups: green and green-orange stages with 16.0% and 17.1%, respectively, and orange-red and red stages with 19.9 and 20.6%, respectively. These results are the indicative of the increase in soluble solids as the fruit matures. As expected, the acidity decreased from the green to the green-orange stages; the highest acidity value was recorded in the green stage (0.54%).

The color measurements of L, a and b are presented in Table 2. L indicates lightness, from 0 (black) to 100 (white); a, from -60 (green) to 60 (red); and b, from -60 (blue) to 60 (yellow). All of the variables significantly different; as ex-

Table 1 - The soluble solids, pH and acidity of *Arbutus andrachne* L. fruit.

Stage	Soluble solids (%)	pH	Acidity (%)
Green	16.0±0.2 b	8.37±0.02 b	0.54±0.01 a
Green-orange	17.1±1.0 b	8.38±0.11 b	0.44±0.01 b
Orange-red	19.9±0.8 a	8.71±0.05 a	0.39±0.01 b
Red	20.6±0.4 a	8.41±0.03 b	0.45±0.02 b
Mean	18.4±2.1	8.47±0.15	0.45±0.06
$LSD_{0.05}$	0.6	0.06	0.08
C.V. (%)	11.3	1.8	12.9

Values represent the means of a triplicate determination (n=3) ± SD. Values in a column with different letters are significantly different at p<0.05.

Table 2 - Color measurements of *Arbutus andrachne* L. fruit.

Stage	L	a	b
Green	51.1±0.5 a	-0.1±1.8 d	50.4±0.6 a
Green-orange	42.1±0.6 b	14.7±1.3 c	36.3±1.0 b
Orange-red	53.6±1.5 a	26.8±0.9 b	54.6±2.7 a
Red	41.7±2.0 b	34.7±2.6 a	38.6±4.0 b
Mean	47.1±5.6	19.0±13.8	45.0±8.3
$LSD_{0.05}$	2.4	3.3	4.7
C.V. (%)	12.0	72.5	18.5

Values represent the means of a triplicate determination (n=3) ± SD. Values in a column with different letters are significantly different at p<0.05.

pected, the variation in L and b was much less than that of a (12.0 and 18.5% to 72.5%). This trend was expected as *A. andrachne* fruit matures from green to red and the results confirm that the sampling of the maturation stages based on their fruit skin color was carried out accurately.

The nutrient element concentrations in the different maturation stages are reported in Table 3; the concentrations of all the elements were significantly different among the stages. The C.V. values, ranging from 5.7 to 18.4% indicate that the concentrations have differential variation patterns. The most variable elements were N and Ca which decreased with maturation. N values decreased from 0.70 to 0.44% from the green to the red stage and Ca concentration decreased from 5.05 to 3.41 g/kg. Similar trends were obtained for the Zn and Mn concentrations.

The organic acid measurements are shown in Table 4. The total acidity value was the highest in the orange-red and red stages (0.52 and 0.50 g/100 g respectively). The main organic acid was malic acid (0.32 g/100 g) followed by ascorbic and citric acids (0.09 and 0.03 g/100 g respectively). The malic and ascorbic acid concentrations were higher in the orange red- and red stages, while citric acid patterns were different. The highest citric acid content was measured in the fully-mature red stage, while the lowest amount recorded was at the green stage.

The results of the sugar analyses are also presented in Table 4. The average total sugar was 15.25 g/100 g, and all of the stages had significantly different means. The red (17.84 g/100 g) and orange-red (16.25 g/100 g) stages had the highest values. The total sugars and % soluble solids were significantly correlated (R^2 = 0.90 and P<0.001). The main sugar was fructose with the overall average of 8.91 g/100 g. Similar to the total sugar values, all of the stages

Table 3 - Several macro and micro nutrient concentrations of *Arbutus andrachne* L. fruit sampled from the Mediterranean region of Turkey.

Stage	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
	(%)	(g / kg)				(mg / kg)			
Green	0.70±0.05 a	1.10±0.01 a	9.26±0.04 c	5.05±0.08 a	1.61±0.08 a	21.19±0.68 a	17.46±0.78 a	5.70±0.01 a	4.32±0.12 b
Green-orange	0.69±0.02 a	0.94±0.01 c	10.36±0.15 a	5.02±0.11 a	1.68±0.04 ab	22.85±2.43 a	15.32±1.08 b	5.60±0.11 a	5.12±0.05 a
Orange-red	0.59±0.04 b	0.98±0.02 b	9.50±0.10 b	4.97±0.14 a	1.77±0.04 a	18.33±0.94 b	15.57±0.39 b	5.93±0.27 a	3.67±0.09 c
Red	0.44±0.03 c	0.72±0.00 d	8.99±0.06 d	3.41±0.11 b	1.31±0.02 c	22.72±0.78 a	15.55±0.35 b	4.90±0.24 b	3.64±0.28 c
Mean	0.60±0.11	0.93±0.14	9.53±0.54	4.61±0.73	1.59±0.18	21.28±2.24	15.98±1.09	5.53±0.43	4.19±0.64
$LSD_{0.05}$	0.07	0.02	0.18	0.21	0.09	2.63	1.34	0.35	0.29
C.V. (%)	18.4	15.2	5.7	15.9	11.5	10.6	6.8	7.8	15.4

Values represent the means of a triplicate determination (n=3) ± SD. Values in a column with different letters are significantly different at p<0.05.

Table 4 - Organic acids and sugars of *Arbutus andrachne* L. fruit.

Stage	Organic acids (g/100 g)				Sugars (g/100 g)			
	Malic	Ascorbic	Citric	Total	Fructose	Glucose	Sucrose	Total
Green	0.27±0.00 c	0.08±<0.01 c	0.01±<0.01 d	0.36±<0.01 b	6.48±0.24 d	3.47±0.11 c	2.81±0.07 a	12.76±0.36 d
Green-orange	0.27±0.02 c	0.06±<0.01 d	0.03±0.01 b	0.36±0.02 b	8.38±0.02 c	3.34±0.09 c	2.44±0.06 b	14.17±0.34 c
Orange-red	0.38±0.01 a	0.11±<0.01 a	0.02±<0.01 c	0.52±0.01 a	9.86±0.12 b	5.14±0.24 b	1.25±0.09 c	16.25±0.44 b
Red	0.36±0.01 b	0.10±<0.01 b	0.05±<0.01 a	0.50±0.01 a	10.92±0.12 a	6.19±0.09 a	0.73±0.12 d	17.84±0.33 a
Mean	0.32±0.05	0.09±0.02	0.03±0.01	0.43±0.08	8.91±1.75	4.53±1.25	1.81±0.89	15.25±2.05
$LSD_{0.05}$	0.02	<0.01	0.01	0.02	0.33	0.27	0.16	0.70
C.V. (%)	16.7	25.8	44.5	18.4	19.6	27.5	49.2	13.4

Values represent the means of a triplicate determination (n=3) ± SD. Values in a column with different letters are significantly different at p<0.05.

had significantly different means; all of the values increased as the fruit matured. A similar pattern was noted for glucose except that there was no difference between the green and green-orange stages. The sucrose concentration showed a converse pattern. Although all of the stages had different means, the sucrose content decreased as the fruit matured. The green stages had an average of 2.81 g/100 g, while that of the fully-mature red stage was 0.73 g/100 g.

The total phenolic and antioxidant contents were determined by two methods and the results are presented in Table 5. Almost all of the stages differed with respect to all of the variables. The total phenolic content and the two measurements of antioxidant activity, TEAC and FRAP, were highly correlated with each other ($R^2 > 0.90$ and $P<0.001$). The total phenolic values were the highest at the red stages (3,904 mg GAE/kg fw), followed by the green stage (3,589 mg GAE/kg fw). Green-orange and orange-red stages had similar phenolic contents, but the values were less than those for the green and red stages. The antioxidant activities were highest at the red stages for both the TEAC and FRAP methods (26.5 and 21.8 μmol TE/g fw). The FRAP method indicated that there was no significant difference in antioxidant activity between the green (24.9 μmol TE/g fw) and the red (25.8 μmol TE/g fw) stages.

In this study, some of the chemical properties and antioxidant capacities of *A. andrachne* fruit were characterized at different stages of maturity. The total phenolic, antioxidant capacity and chemical composition values varied significantly at the four maturation stages. Red fruit had the highest antioxidant capacity. Although *A. andrachne* fruit is not grown commercially, the rich phytonutrient composition may appeal to people and lead to an increased consumption of this fruit.

Table 5 - Total phenolics and antioxidant capacities determined by Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Reducing Antioxidant Power (FRAP) of *Arbutus andrachne* L. fruit.

Stage	TP (mg Gallic acid/kg fw)	Total Antioxidant Activity	
		TEAC (µmol TE/g fw)	FRAP (µmol TE/g fw)
Green	3,589±98 b	28.7±0.6 b	24.9±0.5 a
Green-Orange	2,953±51 c	23.9±0.3 c	19.1±0.3 b
Orange-Red	2,858±60 c	22.1±1.4 d	17.6±0.4 c
Red	3,904±54 a	31.2±0.8 a	25.8±1.0 a
Mean	3,326±460	26.5±3.9	21.8±3.7
$LSD_{0.05}$	130	1.6	1.2
C.V. (%)	13.8	14.6	17.1

The measurements were conducted in three replicates. The stages were sampled from bulk fruit of eight samples with green, green-orange, orange-red and red fruit skin colors.
Values represent the means of a triplicate determination (n=3) ± SD. Values in a column with different letters are significantly different at $p<0.05$.

ACKNOWLEDGMENT

The authors are thankful for the partial support from COST 863 (TÜBİTAK-TOVAG 105O354).

REFERENCES

Bartolome A.P., Ruperez P. and Fuster C. 1995. Pineapple fruit: morphological characteristics, chemical compositions and sensory analysis of Red Spanish and Smooth Cayenne cultivars. Food Chem. 53: 75.

Baytop T. 1999. "Therapy with Medicinal Plants in Turkey (Past and Present)". Nobel Medical Press, Istanbul.

Beattie J., Crozier A. and Duthie G.G. 2005. Potential health benefits of berries. Curr. Nutr. Food Sci. 1: 71.

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. Annals Biochem. 239: 70.

Celik H., Özgen M., Serçe S. and Kaya C. 2008. Phytochemical accumulation and antioxidant capacity at four maturity stages of cranberry fruit. Sci. Hortic. 117: 345.

Connor A.M., Finn C.E., McGhie T.K. and Alspach P.A. 2005. Genetic and environmental variation in anthocyanins and their relationship to antioxidant activity in blackberry and hybridberry cultivars. J. Amer. Soc. Hort. Sci. 130: 680.

Davis P.H. 1982. "Flora of Turkey and the East Aegean Islands" (Vol. 4) p. 172. University Press, Edinburgh.

Kaur C. and Kapoor H.C. 2001. Antioxidants in fruits and vegetables - the millennium's health. Int. J. Food Sci. Techn. 36: 703.

Lila M.A. 2007. From beans to berries and beyond: teamwork between plant chemicals for protection of optimal human health. Ann. NY Acad. Sci. 1114: 372.

Ozgen M., Tulio A.Z. Jr., Chanon A.M., Janakiraman N., Reese R.N., Miller A.R. and Scheerens J.C. 2006a. Phytonutrient accumulation and antioxidant capacity at eight developmental stages of black raspberry fruit. HortSci. 41: 1082.

Ozgen M., Reese R.N., Tulio A.Z., Miller A.R. and Scheerens J.C. 2006b. Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to Ferric Reducing Antioxidant Power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. J. Agric. Food Chem. 54: 1151.

Perkins-Veazie, P.M. and Collins J.K. 1993. Quality evaluation and postharvest procedures for small fruits. USDA-ARS South Central Agricultural Research Lab., Lane, OK.

SAS Institute. 2005. SAS Online Doc, Version 8. SAS Inst., Cary, N.C.

Shui G. and Leong L.P. 2002. Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by high-performance liquid chromatography. J. Chromatogr. A 977: 89.

Singleton V.L. and Rossi J.L. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Amer. J. Enol. Viticult. 16: 144.

Siriwoharn T., Wrolstad R.E., Finn C.E and Pereira C.B. 2004. Influence of cultivar, maturity, and sampling on blackberry (*Rubus* L. hybrids) anthocyanins, polyphenolics, and antioxidant properties. J. Agric. Food Chem. 52: 8021.

Tawaha K., Alali F.Q., Gharaibeh M., Mohammad M. and El-Elimat T. 2007. Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chem. 104: 1372.

Wang S.I. and Lin H.S. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. J. Agric. Food Chem. 48: 140.

Revised paper received June 21, 2008 Accepted September 11, 2008

PAPER

EFFECTS OF DIET AND ABOMASUM PARTS ON ENZYMIC PROPERTIES OF LIQUID LAMB RENNET

EFFETTO DELL'ALIMENTAZIONE E DI PARTI DIVERSE DELL'ABOMASO SULLE PROPRIETÀ ENZIMATICHE DEL CAGLIO LIQUIDO DI AGNELLO

E. MOSCHOPOULOU*, E. ONOUFRIOU and I. KANDARAKIS
Laboratory of Dairy Science and Technology,
Department of Food Science and Technology, Agricultural University of Athens,
Iera Odos 75, 118 55 Athens, Greece
*Corresponding author: Tel. +302105294630, Fax +302105294672,
e-mail: catmos@aua.gr

ABSTRACT

The enzymic properties of liquid artisanal rennet made using whole abomasa or abomasal tissues or abomasal contents from lambs fed mainly with milk (A) or mainly with grass (B) were studied. The results showed that the yield in milk clotting activity (MCA) was affected by both the diet and the abomasum part. Rennet made from whole abomasums of A lambs showed the maximum yield in MCA. Chymosin activity was only affected by the diet. Regardless of the part of the abomasum, rennets from

RIASSUNTO

Sono state studiate le proprietà enzimatiche di differenti tipologie artigianali di caglio liquido di agnello prodotte da abomasi interi, o da tessuti abomasali o dal contenuto dell'abomaso di agnelli alimentati principalmente con latte (A) o principalmente con foraggio (B). I risultati evidenziavano che l'attività coagulante del caglio era influenzata da entrambe le diete e dalla parte di abomaso utilizzato. Il caglio liquido ottenuto dagli abomasi interi degli agnelli alimentati principalmente con latte (A) ha mostrato

- Key words: abomasum contents, abomasum tissue, enzymic properties, liquid lamb rennet, ruminant diet -

A lambs showed over 80% chymosin activity which was significantly higher (P<0.05) than chymosin activity of rennets from B lambs (47.3%-66%). Esterolytic activity was affected by the abomasum part. Rennets made from abomasal tissues showed the highest esterolytic activity.

l'attività coagulante più elevata. L'attività della chimosina era influenzata solamente dalla dieta. Indipendentemente dalla parte di abomaso utilizzata, l'attività della chimosina è risultata significativamente più alta (80%, P<0,05) nei cagli liquidi ottenuti dagli agnelli alimentati principalmente con latte (A) rispetto ai cagli prodotti dagli agnelli alimentati principalmente con foraggio (47,3%-66%). L'attività esterasica risultava influenzata dalla parte di abomaso utilizzata. I cagli liquidi preparati utilizzando il tessuto abomasale mostravano attività esterasica più alta.

INTRODUCTION

In Mediterranean countries, typical products such as cheese and rennet paste are produced from small ruminants. The ovine and caprine cheese sector is well developed in France, Italy, Spain and Greece, while the sector of rennet pastes and cheeses made with this kind of rennet is restricted mainly to Italy and Spain (RUBINO *et al.,* 1999). In Greece, ovine or caprine rennet is an artisanal liquid product that is used in the production of traditional cheeses; it is made locally by the cheesemakers themselves using whole abomasa. To make this liquid rennet the salted and dried whole abomasa that have been stored for about one year at 4°C, are minced and macerated three to four times from 30 min to 24 h with water and salt in various ratios. The final product (liquid rennet) is the combined extracts with additional salt. In another preparation that is used only in home-made cheeses, only the abomasal contents are used.

It has been proven that the enzymic composition of rennet from small ruminants is of major importance, because it affects the properties of the cheese produced (BARZAGHI *et al.,* 1997; LARRAYOZ *et al.*, 1999; IRIGOYEN *et al.,* 2002; MOATSOU *et al.*, 2004; ADDIS *et al.*, 2005a, b). Chymosin and pepsin, the two main enzymes found in the abomasum of a ruminant, are secreted in the abomasal mucosa in the form of zymogens and their ratio is greatly affected by age and diet (ANDREN, 1992; PIREDDA and ADDIS 1998; BUSTAMANTE *et al.,* 2000). The abomasa of milking and unweaned lambs are characterized by high contents of chymosin and pregastric lipase (PIREDDA and ADDIS, 1998; BUSTAMANTE *et al.,* 2000). However, slaughtering of young milk- fed ruminants to produce rennet is no longer economically feasible. Therefore, more and more ruminants that are also fed with grass are used to produce rennet.

There have been several reports concerning the enzymic properties of ovine or caprine rennets made from whole abomasa of milking or grazing animals (FULCO *et al.*, 1987; BARZAGHI and RAMPILLI, 1996; PIREDDA and ADDIS, 1997; PIREDDA and ADDIS, 1998; BUSTAMANTE *et al.*, 2000; ROSSANO *et al.*, 2003; MOSCHOPOULOU *et al.*, 2007). In contrast, information on the enzymic prop-

erties of rennet made only from abomasal tissue or abomasal contents is limited to those made from abomasal tissue (ANIFANTAKIS and GREEN, 1980; ZHANG *et al.,* 2005). The aim of this work was to examine the enzymic properties of liquid rennet made from whole abomasum, or abomasal tissue or the abomasal contents of lambs fed mainly with grass and to compare them with those from lambs fed mainly with milk.

MATERIALS AND METHODS

Samples

Twenty-eight fresh lamb abomasa were obtained from a slaughtering house in the region of Ioannina, Greece. Half of them were from lambs fed mainly with milk (group A) and the other half from lambs fed mainly with grass (group B). All animals were about 40 days old. In each group, half of the abomasa were used whole, while in the other half the abomasal tissue was separated from the abomasal contents and rinsed with water in order to eliminate other residues. The whole abomasa and the abomasal tissues were salted slightly, air-dried at 25°C for 25 and 15 days, respectively and kept at -28°C. The abomasal contents were not dried and were kept at -28°C.

Rennet preparation from whole dried abomasum

Each abomasum (n = 14 whole dried abomasa, 7 for group A and 7 for group B) was cut into small pieces and was extracted individually with a 6% NaCl -2% H_3BO_3 solution by volume and 8 times its weight. The pH was adjusted to 5.3 with 1 N NaOH and the extraction was carried out under continuous stirring at 4°C for 24 h. Following traditional technology, the extract was filtered through a cheese cloth, while another volume of the extraction solution was added to the remaining abomasum for a second extraction under the same conditions. Eventually, the two extracts were pooled to give the final rennet product.

Rennet preparation from dried abomasal tissue

Each abomasal tissue (n = 7 dried abomasal tissues per group) was extracted individually with the extraction solution with a volume 12 times its weight and under the same conditions described above. A second extract was also obtained.

Rennet preparation from fresh abomasal contents

Following the same procedure as above, the contents from each fresh abomasum (n = 7 fresh abomasal contents per group) were extracted with an extraction solution with a volume that was twice its weight.

Analyses

The weights of the individual whole abomasa and abomasal tissues before and after drying, as well the fresh contents, were recorded. The milk clotting activity (MCA) of both the first and the second extracts was determined according to the IDF method (1997a). The results are expressed as U/mL, where U=International Milk Clotting Unit (IMCU). Moreover, the MCA of the pooled first and second extracts was determined with the same method.

The presence of the zymogens, prochymosin and pepsinogen in the rennet samples was checked by activating them as follows: the pH of a 50 mL extract was adjusted to 2.0 with 1N HCl, the extract was kept at this pH for 1 h at room temperature and then the pH was increased to 5.5 with 1N NaOH. The MCA of the activated rennet sample was measured as above.

The percentage content of chymosin

activity was determined according to the IDF (1997b) chromatographic method. Before chromatographic separation, the rennet sample was filtered through a Whatmann N. 1 filter paper to eliminate the solid particles and was then desalted through a PD-10 column (Pharmacia, Uppsala, Sweden).

The esterolytic activity (EA) was measured according to the method described by PIREDDA and ADDIS (1998) and the results are expressed as U/mL where U is the enzyme activity that is liberated from 1 μmole α-naphthol per min under the experimental conditions.

All analyses were carried out in triplicate. Results were statistically analysed using Statgraphics Centurion v.XV software. The model included the effects of diet (two levels) and the effect of the abomasum part (three levels).

RESULTS AND DISCUSSION

Enzymic properties of rennet made from whole abomasum

The colour of rennet A was whitish, while that of rennet B was greenish. Both contained fine particles of curd, foam and, in general, they were not homogeneous. The mean weight and the mean pH value of the fresh whole abomasa used from the lambs fed mainly with milk (group A) did not differ from those from the lambs fed mainly with grass (group B) (Table 1). In addition, the loss of weight during drying was about 53% for both groups and therefore a quantitative comparison could be made between the enzymic properties of the artisanal produced rennets.

In the abomasum, chymosin and pepsin are secreted as inactive prochymosin and pepsinogen which are then converted to active enzymes at low pH. Since, the abomasum extract may contain both enzymes and zymogens, the activation procedure usually occurs in the industrial production of liquid rennet in order to maximise the MCA. In this study, the activation procedure did not increase the MCA in any of the extracts (data not shown) and this means that there was no zymogen in them. This fact was attributed to the low initial pH of about 3.8 (Table 1) of the whole abomasa, abomasal tissues and abomasal contents used. At such a low pH, the zymogens are slowly activated (ANIFANTAKIS and GREEN, 1980; FOLTMANN, 1993).

Table 1 - Mean pH ± s.d. and mean weight ± s.d. (n=7) of the different parts of abomasum from lambs fed mainly with milk (A) or grass (B).

Rennet	Weight of whole fresh abomasum	pH of whole fresh abomasum	Weight of whole dried abomasum	Weight of fresh abomasal tissue	pH of fresh abomasal tissue	Weight of dried abomasal tissue	Weight of fresh abomasal contents	pH of fresh abomasal contents
A	261.4 (±55.5)	3.88 (±0.16)	122.1 (±28.1)	65.7 (±9.8)	4.47 (±0.29)	22.6 (±5.6)	198.0^{a*} (±63.3)	3.25 (±0.15)
B	245.7 (±44.3)	3.73 (±0.2)	113.9 (±18.7)	64.3 (±13.9)	4.75 (±0.24)	21.3 (±3.7)	99.0^{b} (±38.5)	3.33 (±0.12)

*Means in the same column with different letters differ significantly ($P<0.05$).

The pH value is a critical characteristic of liquid rennet, because it not only affects rennet stability but also the milk-clotting activity due to the association of chymosin to the casein micelles of the curd particles present (MOSCHOPOULOU, 2003). In this study, both rennets A and B were adjusted to have pH values of about 5.3; any difference between them could then be attributed to the animal feeding.

In rennets A and B, the MCA yield, expressed as total MCA units, of the second extraction was about 12 and 9%, respectively, of the total yield (the two pooled extracts) (Fig. 1). The first and second extracts were pooled in all cases and considered as the final rennet product for all successive analyses.

The MCA of rennet A (29.6 U/mL) was significantly higher ($P<0.05$) than the MCA (16.3 U/mL) of rennet B (Table 2). As a result, yield in MCA of rennet A was significantly higher ($P<0.05$) than that of rennet B (Table 2). In addition, rennet A showed a significantly higher ($P<0.05$) chymosin activity (80.3% of the total activity) than rennet B (66%). These results clearly show the influence of diet on the enzymic properties of the rennets produced, when whole abomasa are used. The results are in agreement with those reported by PIREDDA and ADDIS (1998) for lamb rennet pastes and by ROSSANO *et al.* (2003) for kid goat rennet pastes.

Regarding lipolytic properties, rennet B showed higher esterolytic activity (EA) than rennet A (Table 2). The assay for EA used in this study was based mainly on gastric and microbial lipases/esterases. The fact that rennet A showed lower EA than rennet B could be due to a greater incidence of gastric and/or microbial esterases in the rennet from whole abomasa B collected from the lambs that had grazed.

Finally, a comparison of the enzymic characteristics, based on the weight of the material used, showed that MCA and EA per gram differed significantly ($P<0.05$) among the different sources of rennet (Table 3). The MCA (481.4U per gram) of whole abomasum A was significantly higher ($P<0.05$) than the MCA (269.3U per gram) of whole abomasum B and was also significantly lower ($P<0.05$) than the

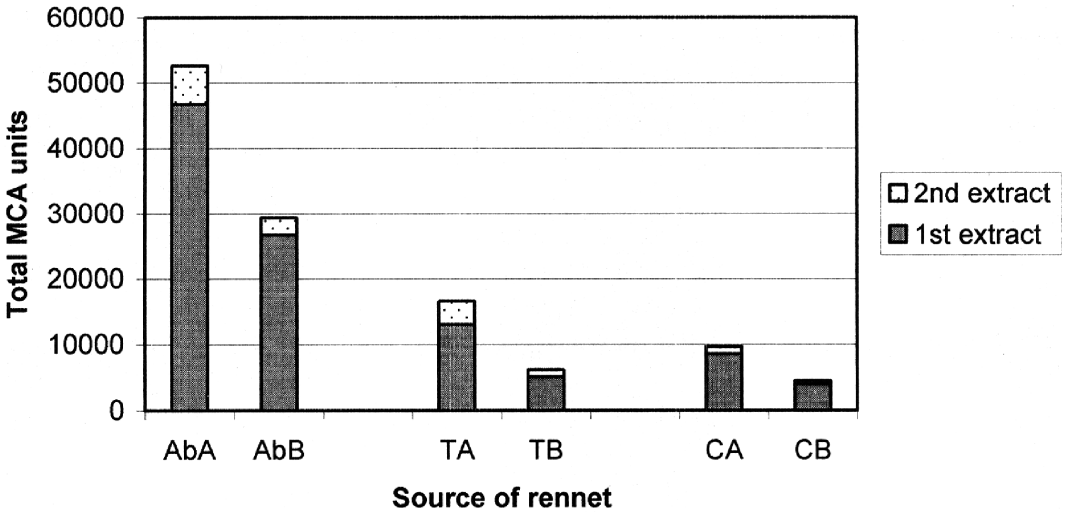

Fig. 1 - Yield in milk clotting activity (MCA) of lamb rennet made from different parts of abomasa of lambs fed mainly with milk (A) or grass (B): Ab (whole abomasum), T (abomasum tissue), C (abomasum contents). Average of 7 trials.

respective value of abomasal tissue and significantly higher (P<0.05) than the respective value of abomasal contents. In both cases, the values of MCA per gram of whole abomasum were higher than those reported per gram of lamb rennet paste by PIREDDA and ADDIS (1998). This difference was attributed to the different extraction conditions. In contrast, the EA (27.7 mU per gram) of whole abomasum A and that of whole abomasum B (40.3 mU per gram) were much lower than those reported by PIREDDA and ADDIS (1998) for lamb rennet paste. This was attributed to the low pH of whole abomasum before the pH adjustment during extraction. According to PIREDDA and ADDIS (1997), irreversible denaturation of lipases takes place at pH<4.6.

Enzymic properties of rennet made from abomasal tissue

Both rennets had a transparent yellowish colour with no particles. As shown in Table 1, the mean pH value and the mean weight of the fresh abomasal tissues did not differ between groups A and B. Both groups lost about 66% of their initial weight during drying and therefore there was no difference between the weights of the dried abomasal tissues that were finally used. The mean yields in MCA of the second extracts in rennets A and B were about 25% and 20%, respectively of the mean total yield (Fig. 1). The first and the second extracts were therefore pooled in all cases for the successive analyses.

Table 2 - Enzymic properties (mean ± s.d. of 7 trials) of liquid rennet made from different parts of abomasum from lambs fed mainly with milk (A) or grass (B).

	MCA(U/mL)		Yield in MCA (Total U)		% chymosin activity		EA(mU/mL)	
	A	B	A	B	A	B	A	B
Whole abomasum	29.6^{a} (±8.2)	16.3^{b*} (±3.9)	56,713^{a} (±9,866)	30,308^{b} (±7,464)	80.3^{a} (±12.8)	66^{b} (±9.4)	1.74 (±0.9)	2.54 (±0.8)
Abomasal tissue	36.42^{a} (±8.5)	14.31^{b} (±7.9)	17,825^{a} (±4,778)	6,952^{b} (±4,080)	86^{a} (±15.7)	63^{b} (±15.6)	2.41 (±1.1)	2.71 (±0.5)
Abomasal contents	10.7 (±7.2)	10.0 (±2.9)	9,897^{a} (±6,258)	4,736^{b} (±1,127)	80^{a} (±19.6)	47.3^{b} (±16.8)	1.6 (±0.8)	1.6 (±0.7)

*Means in the same row with different letters differ significantly (P<0.05).

Table 3 - Enzymic properties (mean ± s.d. of 7 trials) of the different parts of abomasum from lambs fed mainly with milk (A) or grass (B).

	MCA (U/g)		% chymosin		EA (mU/g)	
	A	B	A	B	A	B
Whole abomasum	481.4^{a,1*}	269.3^{b,1}	80.3^{a}	66^{b,1}	27.7^{a,1}	40.3^{b,1}
Abomasal tissue	815.1^{a,2}	319.5^{b,1}	86^{a}	63^{b,1}	51.8^{2}	59.3^{2}
Abomasal contents	53.6^{3}	51.1^{b,2}	80^{a}	47.3^{b,2}	7.7^{3}	7.1^{3}

*Means in the same row with different letters differ significantly (P<0.05). Means in the same column with different numbers differ significantly (P<0.05).

MCA of rennet A (36.42 U/mL) was significantly higher ($P<0.05$) than that of rennet B (14.31 U/mL) (Table 2). Consequently the yield (total units) was also higher in rennet A than in rennet B. Furthermore, the mean chymosin activity of rennet A was 86% of the total activity. This was significantly higher ($P<0.05$) than the chymosin activity (63%) of rennet B. This result is in agreement with that reported by ZHANG *et al.* (2005), who used abomasal mucosa from kid goats and found that chymosin activity in suckling kids had the highest activity, while the weaned group had the lowest.

On the contrary, the esterolytic activities of rennets A and B were similar to each other (Table 2). This result is reasonable since gastric esterases are secreted by the gastric tissue (RICHARDSON et al., 1971) and the mean pH value of the abomasal tissues was not as low as in the abomasal contents or in whole abomasa (Table 1).

Compared to the two other rennet sources, abomasal tissue showed the highest MCA per gram in any case (Table 3). The MCA of abomasal tissue A (815.1U per gram) was significantly higher ($P<0.05$) than that of abomasal tissue B (319.5U per gram) and both were significantly higher ($P<0.05$) than the respective values of whole abomasum and abomasal contents. The same tendency was observed for EA. The abomasal tissues showed a higher gastric esterase activity compared to that of the abomasal contents or whole abomasums (Table 2).

Enzymic properties of rennet made from abomasal contents

Abomasal contents of group A was significantly heavier ($P<0.05$) than abomasal contents from group B, probably due to the presence of milk contents A (Table 1). In both rennets, the yield of the second extraction was about 14% of the total yield in MCA (Fig. 1). Therefore, the extracts were pooled for the successive analyses. MCA of rennet A (10.7 U/mL) was similar to that of rennet B (10.0 U/mL), but the total MCA units of rennet A were higher than those of rennet B (Table 2) as more rennet A was produced. In addition, chymosin activity of rennet A (80%) was significantly higher ($P<0.05$) than the chymosin activity of rennet B (47.3%). Chymosin activity of rennet A made from the abomasal content was similar to those of rennets A made from whole abomasum or abomasal tissue. This is probably due to the presence of milk which enhances the chymosin secretion. No difference was detected in esterolytic activity between the two groups of rennets. However, these values were as low as those of rennets from whole abomasa A. This was probably due to the fact that the incidence of abomasal tissue or mucosa in abomasal contents was very low with respect to that of the other rennet sources and presumably the A and B rennets. Compared to the other rennet sources, abomasal contents had the lowest MCA (51.1-53.6 U) and EA (7.1-7.7 mU) per gram independent of the diet (Table 3).

CONCLUSIONS

The results show that there is no benefit of using only abomasal contents of lambs fed with either milk or grass instead of whole abomasa to produce liquid rennet, with respect to MCA yield, chymosin content and EA. In this case, the most significant benefits would be that the label could claim that the rennet does not contain animal tissue and it is easy to handle by just emptying and keeping the contents without salting, drying or cutting. In contrast, when liquid rennet was made only from abomasal tissues of lambs fed mainly with milk, the chymosin content was over 85% and the EA/mL was higher than that of the

rennet made from whole abomasa. Other benefits include decreased storage volume of the raw material and easy production. A clean product, without particles, can be obtained without having to use centrifugation.

REFERENCES

Addis M., Piredda G., Pes M., Di Salvo R., Scintu M.F. and Pirisi A. 2005a. Effect of the use of three different lamb paste rennets on lipolysis of the PDO Pecorino Romano Cheese. Int. Dairy J. 15: 563.

Addis M., Pirisi A., Di Salvo R., Podda F. and Piredda G. 2005b. The influence of the enzymatic composition of lamb rennet paste on some properties of experimentally produced PDO Fiore Sardo cheese. Int. Dairy J. 15: 1271.

Andren A. 1992. Production of prochymosin, pepsinogen and progastricsin and their cellular and intracellular localization in bovine abomasal mucosa. Scand. J. Clin. Lab. Invest. 52: 59.

Anifantakis E. and Green M. 1980. Preparation and properties of rennets from lamb's and kid's abomasa. J. Dairy Res. 47: 221.

Barzaghi S. and Rampilli M. 1996. Evaluation of the enzymatic characteristics of commercial rennet pastes. Sci. Tecn. Latt. Cas. 47: 171.

Barzaghi S., Davoli E., Rampilli M. and Contarini G. 1997. Lipolysis in Provolone cheese: effect of rennet paste. Sci. Tecn. Latt. Cas. 48: 146.

Bustamante M., Chavarri F., Santisteban A., Ceballos G., Hernandez I., Miguelez M.J., Aranburu I., Barron L., Virto M. and de Renobales M. 2000 Coagulating and lipolytic activities of artisanal lamb rennet pastes. J. Dairy Res. 67: 393.

Foltmann B. 1993. General and molecular aspects of rennets. In Cheese: "Chemistry, Physics and Microbiology", Vol. 1, 2nd ed, edited by Fox P.F. Chapman & Hall, London.

Fulco A., Candido A., Nuccio M.G. and Imbro M. 1987. Characteristics of lamb's rennet paste used in Sicilian cheesemaking. Sci. Tecn. Latt. Cas. 38: 146.

IDF. 1997a. International standard 157A:1997. Bovine rennets: Determination of Total milk-clotting activity. Brussels.

IDF. 1997b. International standard 110B:1997. Calf rennet and adult bovine rennet: Determination of Chymosin and Bovine Pepsin Contents (Chromatographic method). Brussels.

Irigoyen A., Izco J.M., Ibanez F.C. and Torre P. 2002. Influence of calf or lamb rennet on the physicochemical, proteolytic and sensory characteristics of an ewe's-milk cheese. Int. Dairy J. 12: 27.

Larrayoz P., Martinez M.T., Barron L.J.R., Torre P. and Brcina Y. 1999. The evolution of free fatty acids during the ripening of Idiazabal cheese: influence of rennet type. Europ. Food Res. Int. 210: 9.

Moatsou G., Moschopoulou E., Georgala Aik., Zoidou E., Kandarakis I., Kaminarides S. and Anifantakis E. 2004. Effect of artisanal liquid rennet from kids and lambs abomasa on the characteristics of Feta cheese. Food Chem. 88: 517.

Moschopoulou E. 2003. Study of traditional rennet and of chymosin and pepsin from kid abomasa. Ph. D. Thesis. Agricultural University of Athens (In Greek).

Moschopoulou E., Kandarakis I. and Anifantakis E. 2007. Characteristics of lamb and kid artisanal liquid rennet used for traditional Feta cheese manufacture. Small Rum. Res. 72: 237.

Piredda G. and Addis M. 1997. The enzymatic characteristics of lamb rennet pastes. In Proceedings of the joint plenary of AIR concerted action no. 3-CT94-2039 Microflora, pp. 113 Portugal May 26-27.

Piredda G. and Addis M. 1998. Diet influence on enzymatic activities in lamb rennet pastes. Sci. Tecn. Latt. Cas. 49: 129.

Richardson G.H., Nelson J.H. and Farnham M.G. 1971. Gastric lipase characterization and utilization in cheese manufacture. J. Dairy Sci., 54: 643.

Rossano R., D'Ambrosio A., Ferrara V., D'Elia A., Pizzillo M. and Riccio P. 2003. Influence of diet and age of kids on enzymatic activities of kid rennet pastes. Ital. J. Food Sci. 15: 585.

Rubino R., Morand-Fehr P., Renieri C., Peraza C. and Sarti F.M. 1999. Typical products of the small ruminant sector and the factors affecting their quality. Small Rum. Res. 34: 289.

Zhang F.X., Chen J.P., Yang F. and Li L.Q. 2005. Effects of age and suckling on chymosin and pepsin activities in abomasums of goat kids. Int. J. Dairy Techn. 58: 115.

Revised paper received July 1, 2008 Accepted September 25, 2008

SHORT COMMUNICATION

THE EFFECT OF FLUID GOAT MILK WHEY ON SOME QUALITY CHARACTERISTICS OF CHEVON NUGGETS

EFFETTO DEL SIERO FLUIDO DI LATTE DI CAPRA
SU ALCUNI PARAMETRI DI QUALITÀ DI CUBETTI DI CAPRA

A.K. DAS* and R.B. SHARMA
Goat Products Technology Laboratory
Central Institute for Research on Goats, Makhdoom,
Farah-281 122, Mathura, India
*Corresponding author: Tel. +91 565 2763380, Fax +91 565 2763246,
e-mail: arunlpt@gmail.com

ABSTRACT

Fluid goat milk whey was used to replace ice in chevon nugget formulations and the physico-chemical, textural and sensory properties of the nuggets were evaluated. Liquid goat milk whey was used to substitute for 0, 25, 50, 75 and 100% of the ice in nugget formulations. There were no significant differences in most physico-chemical, textural and sensory attributes of the nuggets. The emulsion stability was significantly increased by adding liquid whey to the formulation and consequently the yield

RIASSUNTO

Il siero fluido di latte di capra è stato utilizzato per rimpiazzare il ghiaccio nella formule di cubetti di capra. Sono state quindi valutate le proprietà fisico-chimiche, la consistenza e le proprietà sensoriali dei cubetti. Il siero fluido di latte di capra è stato utilizzato per sostituire per lo 0, 25, 50, 75 e 100% il ghiaccio nelle formule dei cubetti. Non sono state evidenziate differenze significative nelle proprietà chimico-fisiche, nella consistenza e nelle proprietà sensoriali dei cubetti. La sta-

- Key words: chevon nuggets, fluid whey, physico-chemical, sensory attributes -

of the final product. The ash content and pH value increased slightly with addition of liquid goat milk whey in the nuggets. Even 100% whey replacement did not produce any adverse effect in the sensory properties and the product was highly acceptable to the panelists. There was no adverse flavour in the nuggets prepared with fresh liquid whey. Natural fresh liquid goat milk whey, a valuable often wasted animal product can be used successfully in chevon nugget formulations to develop a highly acceptable and valuable meat product at a minimal cost.

bilità dell'emulsione risultava significativamente incrementata dall'aggiunta di siero liquido di latte di capra alla formula e conseguentemente risultava migliore anche la resa del prodotto finito. Il contenuto in ceneri ed il pH aumentavano leggermente con l'aggiunta di siero liquido di latte di capra ai cubetti. Anche il 100% di siero non causava alcun effetto negativo sulle caratteristiche sensoriali e il prodotto risultava altamente accettabile dal panel sensoriale. Non sono stati evidenziati effetti negativi sull'aroma dei cubetti preparati con siero liquido fresco. Il siero liquido fresco di latte di capra, un prezioso e spesso sprecato prodotto animale, può essere utilizzato nella formulazione di cubetti di capra per sviluppare un prodotto a base di carne altamente accettabile e prezioso ad un costo ridotto.

INTRODUCTION

Adequate binding of processed meat is essential in marketing high quality products. The term "binding" is used to describe both the adhesion of meat particles and water binding (holding) by the meat proteins (HONGSPRABHAS and BARBUT, 1999). High quality non-meat proteins such as soy, milk proteins and egg white are often used in comminuted meat products to improve yield and textural and nutritional properties and potentially to reduce the cost of the meat formulation (DAS *et al.* 2006).

Different milk proteins are being used in meat processing for their good water binding and good emulsion stabilising properties. Whey is the non-solid or yellow green liquid that separates from the curd during natural cheese production. Fresh liquid whey is composed primarily of lactose (5%), water (93%) and minerals (0.53%) with minimal fat (0.36%) and protein (0.85%) (CHANDAN *et al.*, 1982). Whey proteins are an excellent source of essential and branched-chain amino acids, particularly lysine. These proteins may be beneficial in the production of frankfurters, resulting in a more balanced amino acid composition compared with full meat controls (LEE *et al.*, 1980). There is a large body of research on the use of more expensive whey protein concentrates (WPC) or dried whey powders, but few studies have used fluid whey in comminuted meat products. Due to the high biological oxygen demand (BOD), the disposal of whey is very expensive and poses a problem for the environment.

Due to the cost of concentrating or drying, whey is often dumped because

of the lack of technology to process it. Huge amounts of whey are generated by the dairy industry but little of this liquid is currently being used in the production of food or feed. Hence, there is a tremendous need to find a way to use whey proteins or products in food processing in order to prevent wasting this valuable protein and mineral source (YETIM *et al.,* 2001). To date, limited research has been conducted regarding the feasibility of using unconventional fluid whey, especially goat milk whey, to replace ice in comminuted meat products (ZORBA *et al.,* 1998; YETIM *et al.,* 2001; YETIM *et al.,* 2006).

The objectives of this study were to explore the possibility of using liquid goat milk whey in goat meat (chevon) nugget formulation and to evaluate their physico-chemical, textural and sensory attributes.

MATERIAL AND METHODS

Meat samples

The meat samples, comprised mostly of *Longissimus dorsi*, *Semimembranosus* and *Semitendinosus* muscles from male (12-16 months old) goats slaughtered according to standard methods in the Institute slaughterhouse, were collected within 5 h of slaughter and packed in low density polyethylene (LDPE) bags. The samples were placed in a refrigerator at 4°C for about 12 h. After conditioning, the separable fat and connective tissue of the meat samples were trimmed off and then kept at -20°C till further use. On the day of the experiment, the meat was partially thawed for 15 h at 4°C. It was then cut into small cubes and minced using an automatic meat mincer (Tallers Ramon Model P-22, Barcelona) by passing it through 5 mm plates. The minced goat meat (2.35% fat) was then used in the product formulation.

Goat meat nugget processing

A 5 kg formulation was made for each treatment. The nuggets were processed as outlined by DAS *et al.* (2008b). In the control formulation 10% ice flakes were used, in the treated formulations all of the ingredients were the same as in the control expect for ice flakes or liquid whey. The formulations and processing of the control and treated goat meat nuggets were standardised in preliminary trials (Table 1). Sodium nitrite (150 ppm) was also added to the above formulations. Goat milk whey was used to substitute for 0, 25, 50, 75 and 100% of the ice flakes. The goat milk whey contained 4.93% total solids (0.27% fat, 0.68% protein, 0.54% ash and 3.35% lactose).

The emulsion was made in a bowl chopper, (Model K 20, Seydelmann, Germany) and the meat emulsion (~750 g) was put into stainless steel moulds (18x12x4 cm), packed compactly and covered. The emulsion-filled moulds from all of the treatments were clipped and cooked in a steam oven without pressure for 35 min. The internal temperature (90°C) of the cooked meat blocks was measured using a probe type thermometer (Fisher Scientific, Fremount CA, USA). The meat blocks were cooled to room temperature, chilled overnight at 4°C and cut into slices of 15 mm thickness. The slices were manually cut into nuggets. About 200 g of nuggets were packed for further study. The experiment was replicated three times.

pH determination

The pH values of the emulsion and nuggets were determined by blending 10 g of sample with 50 mL of distilled water for 1 min using a tissue homogeniser (Model PT-MR-2100, Kinematica AG, Luzernerstrasse, Switzerland) at 8,000 rpm for 1 min. The pH was recorded by inserting the combined glass electrode

Table 1 - Formulations* for control and treated goat meat nuggets.

Ingredients (g)	% Liquid whey used in ice flakes				
	0	25	50	75	100
Minced goat meat	3,500	3,500	3,500	3,500	3,500
Ice flakes	500	375	250	125	-
Liquid goat milk whey	-	125	250	375	500
Refined mustard oil	500	500	500	500	500
Refined wheat flour	150	150	150	150	150
Dry spice mix[a]	90	90	90	90	90
Condiments[b]	150	150	150	150	150
Salt	80	80	80	80	80
Tripolyphosphate	15	15	15	15	15
Sugar	15	15	15	15	15

[a]Dry spice mix-Anise seed, black pepper, capsicum, caraway seed, cardamom, cinnamon, cloves, coriander powder, cumin seed, turmeric and dried ginger. [b]Condiments - garlic and onion (4:1).
* = 5 kg formulation; sodium nitrite (150 ppm) was added to the above formulations.

of the digital pH meter (Systronics, μ pH system 361, Delhi, India) into the suspension.

Emulsion stability and cooking yield

The emulsion stability of the samples was estimated by heating approximately 25 g of emulsion in a polypropylene bag at 80°C for 20 min using a thermostatically controlled water bath. After draining off the exudate, the cooked mass was cooled and weighed (DAS *et al.,* 2006). The weight of each meat block was recorded before and after cooking. The cooking yield was calculated and is expressed as percentage by weight of cooked meat block/ weight of raw meat block x 100.

Proximate composition and expressible water

The moisture, protein fat and ash content of the cooked goat meat nuggets were determined by the AOAC (1995) methods.

Expressible water was estimated using the method of RAMIREZ *et al.* (2002) with slight modification. About 5 g of cooked samples were weighed and placed on 2 layers of Whatman N. 1 filter paper. The samples were placed at the bottom of 50 mL centrifuge tubes and centrifuged at 1,500 g (Biofuge Primo R, Heraeus, DE) for 15 min. Immediately after centrifugation, the meat samples were re-weighed and the amount of expressible water was calculated as initial weight - final weight/initial weight x 100. Duplicate samples were analysed and the averages are reported for each treatment.

Texture profile analysis

The textural properties of the nuggets were evaluated according to the procedure described by DAS *et al.* (2006) using the texturometer (Stable Micro System Model TA.XT 2i/25, Godalming, Surrey, UK). Texture profile analysis (BOURNE, 1978) was performed using the central cores from five pieces of each sample (1.5x1.5x1.5 cm), which were compressed twice to 80% of the original height. A crosshead speed of 2 mm/s was used applying 25 kg load cell and 75 mm compression plate probe (P75).

The shear force of samples was estimated with a Warner-Bratzler blade attached to the same texture analyser. Five cores (1.5 cm height and 1.5 cm diameter) were taken from the nugget of each treatment. The crosshead speed was 2 mm/s. Maximum force required to cut the sample (shear force) was recorded.

Sensory evaluation

A sensory evaluation method (KEETON, 1983) using a 9-point hedonics scale was followed with some modification (9 = excellent; 1 = extremely poor). The sensory panel consisted of ten experienced scientists from the Institute. The panelists were told about the nature of the experiments without disclosing the identity of the samples and were asked to rate their preference on a 9-point hedonics scale on the sensory evaluation form for different traits. Samples were warmed in a microwave oven for 1 min and served to the panelists. Water was provided to rinse their mouths between the samples. The panelists judged the samples for overall appearance and colour, flavour, juiciness, texture and overall acceptability.

Statistical analysis

Three trials were conducted and data were subjected to analysis of variance (ANOVA). Significant treatment and interaction means were separated using Duncan's Multiple Range Test (SNEDECOR and COCHRAN, 1995).

RESULTS AND DISCUSSION

Physico-chemical properties

The physico-chemical properties of the goat meat nuggets with different levels of liquid whey are presented in Table 2. Results showed that there was no significant difference in the pH of the control and treated emulsions that ranged from 6.05 to 6.11. Similarly, YETIM *el al.* (2006) observed no statistical difference in the pH of sausage batters. All of the emulsions had acceptable stability and the whey-incorporated emulsions had more acceptable stability in terms of yield of cooked mass. Emulsions with 100%, 75% and 50% liquid whey replacement had significantly ($P<0.05$) higher emulsion stability than that of the control and the 25% replacement group. This result could be attributed to gelation and the higher water and fat binding properties of whey protein. YETIM *et al.* (2001, 2006) reported that as the liquid whey increased in the formulation, the separated water and fat generally decreased causing the stability to increase. Similarly, in a model system, ZORBA *et al.* (1998) observed that liquid whey had a positive effect on emulsion characteristics and fluid whey had the highest emulsion stability.

The cooking yield of goat meat nuggets improved significantly ($P<0.05$) with the 75% and 100% whey replacement groups compared to the others. EL-MAGOLI *et al.* (1996) also reported that the addition of whey protein concentrates (WPC) improved fat, moisture retention and cooking yield in ground beef patties. There was a slight variation in the proximate composition between the control and the treated goat meat nuggets. The moisture content was significantly ($P<0.05$) lower for the 100% whey replacement treatment whereas the percent of protein, fat, ash and pH were not significantly different but showed an increasing trend as the level of whey increased. These findings may be due to the protein (0.68%), fat (0.27%) and ash (0.54%) content from the liquid goat milk whey. Similar results were reported by YETIM *et al.* (2001). Water-holding capacity (WHC) is directly associated with the percent water expressed by centrifugation (the lower the percent of water extracted, the higher the WHC) (DAS *et al.*, 2008a). The slight

but not significant increase in WHC of the higher whey treatments may be due to a higher protein content (Table 2) and formation of good gel matrix during cooking that entraps more water. HONGSPRABHAS and BARBUT (1999) found that the pre-heated whey protein to replace 2% of the meat protein was beneficial in increasing WHC, reducing cooking loss and increasing gel strength of the raw and cooked products.

Nugget texture with liquid whey

There was no significant difference in the texture of nuggets between the control and the groups treated with liquid goat milk whey (Table 2). The nuggets with whey required slightly more force for compression and shear which are indicated in the hardness and shear force values. YETIM *et al.* (2006) reported that the control and 100% whey-containing sausages were similar in hardness and gumminess. Chewiness increased linearly as the liquid whey content increased but the difference was not significant ($P<0.05$). HUFFMAN (1996) noted that whey protein concentrate (WPC) are used to modify the textural characteristics such as hardness, chewiness and elasticity in meat products. Similar to the results in the present study, HUNG and ZAYAS (1992) did not find any difference in shear strength when whey protein concentrate was added to meat loaf and frankfurters. In contrast, ENSOR *et al.* (1987) reported an increased firmness in comminuted beef products when whey protein concentrate was added. The results of this study show that the firmness and shear force values depend on the type of meat product processing and the type of milk proteins used.

Sensory attributes of nuggets with liquid whey

Fluid goat milk whey did not affect the appearance or colour of the meat

Table 2 - Physico-chemical and textural properties of goat meat nuggets with liquid goat milk whey.

Parameters	% Liquid whey used in ice flakes				
	0	25	50	75	100
Emulsion pH	6.05	6.06	6.05	6.09	6.11
Emulsion stability (%)*	92.11^{b}	92.58^{b}	94.27^{a}	95.13^{a}	96.53^{a}
Cooking yield (%)	92.68^{b}	93.17^{b}	95.62^{ab}	96.23^{a}	97.69^{a}
pH	6.25	6.26	6.28	6.32	6.38
Moisture (%)	62.34^{a}	62.63^{a}	62.58^{a}	62.08^{a}	61.14^{b}
Protein (%)	14.86	14.88	14.93	14.97	15.01
Fat (%)	14.22	14.32	14.57	14.62	14.73
Ash (%)	2.62	2.53	2.71	2.74	2.93
Expressible water (%)	13.05	12.98	12.56	11.91	11.62
Textural attributes (n=15)**					
Hardness (N/cm^2)	51.74	51.86	52.93	54.04	55.46
Springiness (cm)	0.802	0.806	0.801	0.808	0.810
Chewiness (N/cm)	10.51	10.58	10.71	10.82	11.01
Shear force (N)	10.68	10.70	10.98	11.23	11.54

Means in the same row with the different superscripts are significantly different ($P<0.05$).
* n=18.
** There were no significant changes in texture.

Table 3 - Sensory attributes[a] of nuggets with different levels of goat milk whey.

Attributes	% Liquid whey used in ice flakes				
	0	25	50	75	100
Appearance & colour	8.53	8.62	8.66	8.54	8.63
Flavour	7.23	7.09	7.18	7.01	6.98
Texture	6.80	6.79	7.08	7.11	7.19
Juiciness	8.28	8.35	8.26	8.32	8.17
Overall acceptability	7.67	7.72	7.66	7.92	7.83

[a]Based on 9-point hedonic scale, where 9=excellent and 1=extremely poor.
No significant difference at the 5% level for any sensory attribute, n=30.

products and the nuggets had a desirable surface colour. There were no significant differences in texture, flavour, juiciness or overall acceptability between the control and the whey treatment groups (Table 3). These results are in agreement with YETIM *et al.* (2001), who reported no potential off-flavour development in the products that contained a higher amount of liquid whey and the panelists did not detect any significant differences in the presence of off-flavours. MARRIOTT *et al.* (1998) reported that boneless hams containing liquid whey did not have any whey flavour. Goat meat nuggets received favourable ratings for overall acceptability.

CONCLUSION

Natural fresh liquid goat milk whey can be used successfully in goat meat nugget formulation to obtain more desirable emulsion stability and positive physico-chemical, textural and sensory attributes. The development of comminuted meat products with liquid whey offers a potential response to the environmental concerns by utilizing a valuable often wasted animal product to develop a highly acceptable and valuable fortified meat product at a minimal cost.

ACKNOWLEDGEMENTS

The authors are grateful to the director of the Institute for providing necessary facilities and to Mr. Radhey Shyam and Mohd Sarfaraz for their technical help.

REFERENCES

AOAC. 1995. "Official Methods of Analysis". 16th ed. Association of Official Analytical Chemists. Alington, V A.

Bourne M.C. 1978. Texture profile analysis. Food Technol. 32:62.

Chandan R.C., Uebersax M.A. and Saylock M.J. 1982. Utilization of cheese whey permeate in canned beans and plums. J. Food Sci. 47:1649.

Das A.K., Anjaneyulu A.S.R. and Kondaiah N. 2006. Development of reduced beany flavour full-fat soy paste for comminuted meat products. J. Food Sci. 74:S395.

Das A.K., Anjaneyulu A.S.R., Gadekar Y. P., Singh R.P. and Pragati H. 2008b. Effect of full-fat soy paste and textured soy granules on quality and shelf-life of goat meat nuggets in frozen storage. Meat Sci. 80:607.

Das A.K., Anjaneyulu A.S.R., Kondaiah N. and Veram A.K. 2008a. Physico-chemical, textural, sensory characteristics and storage stability of goat meat patties extended with full-fat soy paste and soy granules. Int. J. Food Sci. Technol. 43:383.

El-Magoli S.B., Laroia S. and Hansen P.M.T. 1996. Flavour and texture characteristics of low-fat ground beef patties formulated with whey protein concentrates. Meat Sci. 42:179.

Ensor S.A., Mandigo R.W., Calkins C.R. and Quint L.N. 1987. Comparative evaluation of whey protein concentrate, soy protein isolate and calci-

um reduced NFDM as binders in an emulsion type sausage. J. Food Sci. 52:1155.

Hongsprabhas P. and Barbut S. 1999. Effect of preheated whey protein level and salt on texture development in poultry meat batters. Food Res. Int. 32:145.

Huffman L.M. 1996. Processing whey protein for use as food ingredients. Food Technol. 50:49.

Hung S.C. and Zayas J.F. 1992. Functionality of milk proteins and corngerm protein flour in comminuted meat products. J. Food Quality 15:139.

Lee A., Cannon R.Y. and Huffman D.L. 1980. Whey protein concentrates in a processed meat loaf. J. Food Sci. 40:736.

Keeton J.T. 1983. Effect of fat and NaCl/phosphate levels on the chemical and sensory properties of pork patties. J. Food Sci. 48:878.

Marriott N.G., Wang H., Claus J.R. and Graham P.P. 1998. Evaluation of restructured low-fat ham containing whey. J. Muscle Foods 9:201.

Ramirez J., Uresti R., Tellez M. and Vazquez M. 2002. Using salt and microbial transglutaminase as binding agents in restructured fish products resembling hams. J. Food Sci. 67:1778.

Snedecor G.W. and Cochran W.G. 1995. "Statistical Methods". 8th ed., IBH Publ Co, Oxford, New Delhi.

Yetim H., Muller W.D. and Eber M. 2001. Using fluid whey in comminuted meat products: effects on technological, chemical and sensory properties of frankfurter-type sausage. Food Res. Int. 34:97.

Yetim H., Muller W.D., Dogan M. and Klettner P.G. 2006. Using fluid whey in comminuted meat products: effects on textural properties of frankfurter-type sausages. J. Muscle Food. 17:354.

Zorba O., Ozdemir S. and Gokalp H.Y. 1998. Stability of model emulsions prepared using whey and muscle proteins. Die Nahrung 42:16.

Revised paper received May 23, 2008 Accepted June 24, 2008

SHORT COMMUNICATION

DETERMINATION OF WATER CONTENT IN MEAT PÂTÉ BY KARL FISCHER TITRATION AND ITS MOISTURE SORPTION CHARACTERISTICS

DETERMINAZIONE, ATTRAVERSO IL METODO DI TITOLAZIONE KARL FISCHER, DEL CONTENUTO IN ACQUA DEL PATÉ DI CARNE E DELLA SUA CAPACITÀ DI ASSORBIRE UMIDITÀ

M. ADAM, L. CERVENKA*, S . REZKOVA, K. VENTURA and J. KRALOVSKY
Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Nam. Cs. legii 565, 532 10 Pardubice, Czech Republic
*Corresponding author: Tel. +420 466 037718, Fax +420 466037068, e-mail: libor.cervenka@upce.cz

ABSTRACT

The water content of two chicken meat pâté samples using the classical Karl Fischer (KF) titration method together with oven drying was analysed. Based on the experimental optimization of individual conditions the most suitable solvents, extraction time, titration agent, and sample size were selected. The KF titration in a methanol/chloroform solvent mixture (1:1, v/v) at 25°C is the most suitable for determining the water content in this type of meat product. The applicability of KF

RIASSUNTO

È stato determinato il contenuto in acqua di due campioni di paté di pollo utilizzando il metodo classico di titolazione Karl Fischer e l'essiccamento in stufa. Sono stati selezionati, in base a ottimizzazioni sperimentali, i solventi più adatti, i tempi di estrazione, l'agente titolante e la quantità di campione. La titolazione di Karl Fischer in una miscela solvente metanolo/cloroformio (1/1, v/v) a 25°C è risultata la più idonea per determinare il contenuto in acqua di questa tipologia di pro-

- Key words: Karl Fischer titration, meat pâté, moisture content, water content -

titration agents purchased from various producers was compared but these was no variation in the results. The water content data obtained by KF titration and that of the moisture content determined by oven-drying were compared with those obtained by moisture measurements where the appropriate sorption isotherm was created.

dotti a base di carne. È stata valutata l'applicabilità di agenti titolanti Karl Fischer forniti da produttori diversi, ma non sono state osservate differenze nei risultati ottenuti. I dati ottenuti con la titolazione Karl Fischer per quanto riguarda il contenuto in acqua e quelli del contenuto in umidità determinati in stufa sono stati confrontati con quelli ottenuti dalle misurazioni di umidità ricavate appropriate isoterme di assorbimento.

INTRODUCTION

Water is an essential compound that directly or indirectly enters into many chemical reactions that occur in food. Determination of water content is one of the most frequently performed analyses in a food industry laboratory. The water content of foodstuffs is very important because it affects the physical characteristics, technological processes, microbiological stability and shelf life of food; it also has legal and economic ramifications (ISENGARD, 1995). To maintain the required quality and storage stability, it is necessary to determine the water content quickly and precisely. Although numerous methods have been suggested for determining the water content in food (SINGH *et al.*, 2004; MUIK *et al.*, 2004; ISENGARD and PRAGER, 2003; FOUSKAKI *et al.*, 2003), the Karl Fischer (KF) titration method is among the most frequently used chemical methods that is successfully applied to a variety of foodstuffs (SCHMITT and ISENGARD, 1998; ISENGARD *et al.*, 2006; MATHLOUTHI, 2001; REH *et al.*, 2004). This method is based on titration by agents containing I_2, SO_2, organic base (pyridine or the more frequently used imidazole) and reaction solvent (usually methanol). It is used as an arbitrary method together with oven-drying. However, drying of food may cause a loss of mass in the product during the heating process (ISENGARD, 2001). Therefore, instead of using the term water content, it would be more correct to say mass loss.

Water content and water activity are correlated via sorption isotherms which are very product-specific. If the sorption isotherm of the product is known and the water activity is measured, the moisture content can be read from the isotherm and *vice versa*. Moreover, the concept of water activity has been very valuable in physiological studies of microorganisms, principally because the values measured generally correlate well with the growth and metabolic activity of spoilage microorganisms (CHIRIFE and BUERA, 1996).

Meat pâté is a ready-to-eat product with high nutritive value for humans. In the Czech Republic, meat pâté-type products are sterilised and stored at ambient temperature. Despite temperature treatments, this kind of product has been associated with several outbreaks of food poisoning (MORRIS and RIBEIRO, 1991; DE VALK *et al.*, 2001).

The aim of this study was to determine the water content in meat pâté using

the Karl Fischer titration method and to compare the results with those obtained by the oven-drying method and sorption measurements.

METHODS AND MATERIALS

Two samples of meat pâté in tin-cans were purchased in the local market. According to the manufacturer's information, the samples were characterised as follows: chicken pâté (sample VSP) contained chicken meat (30% w/w), water, lard, pork liver, starch, milk protein, salts, preservative agents and condiments. Poultry pâté (sample VSE) contained poultry meat, water, lard, vegetable protein, salt, preservative agents and condiments.

KF titrations were carried out with a KF Titrator AF8 (Orion Research, Boston, MA, USA) with various titration agents as follows; Hydranal® Composite 5 (Riedel-de Haën, Seelze, Germany), Apura® CombiTitrant 5 (Merck, Darmstadt, Germany) and Hydra-Point® Composite 5 (J.T. Baker, Deventer, The Netherland).

A 0.25 g portion of the sample was extracted by sonication in 25 mL of a methanol/chloroform mixture (1:1 v/v) for 10 min in an ultrasonic bath. An aliquot of 0.5 mL was then introduced into the KF titration cell and the water content was determined in 5 replications. All solvents were of analytical grade with a declared water content below 0.1% and were purchased from Merck (Darmstadt, Germany). Prior to the analysis of real samples, the whole procedure was conducted with pure solvents (negative control). This procedure was performed for all solvent mixtures tested.

Novasina AW Sprint TH 500 thermoconstanter (Axair Ltd., Pffaffikon, Switzerland) was used to measure the water activity of the samples at 25°C. An isopiestic method was employed to determine the sorption isotherm (KAYA *et al.*, 2002). Standard salt slurries were prepared according to STOLOFF (1978) as follows: LiCl (a_w = 0.11), $MgCl_2 \cdot 6H_2O$ (a_w = 0.32), $Mg(NO_3)_2 \cdot 6H_2O$ (a_w = 0.53), NaCl (a_w = 0.75), $(NH_4)_2SO_4$ (a_w = 0.79), $BaCl_2 \cdot 2H_2O$ (a_w = 0.90), K_2SO_4 (a_w = 0.97) and $K_2Cr_2O_7$ (a_w = 0.98). All reagents were analytical grade and were purchased from Lach-Ner (Neratovice, the Czech Republic).

Duplicate samples were weighed (0.2 g) in small crucibles of aluminium foil and placed on tripods in glass weighing dishes above the salt slurry. Tightly closed weighing dishes were kept in controlled temperature incubators at 25°C to equilibrate the samples. Equilibrium was reached when the weight differences did not fluctuate by more than 0.005 g (0.5%) in three consecutive weighings. When the samples reached equilibrium, their moisture contents were determined by the oven-drying method (105°C). At high water activities (a_w > 0.70), a small amount of potassium sorbate (Lach-Ner, Neratovice, the Czech Republic) was added to prevent microbial spoilage of analyzed meat pâté samples.

The moisture content in meat pâté samples (1.0 g) was also determined after drying the sample to a constant weight at 105°C using a UM 300 drying oven with forced ventilation (Memmert, Schwabach, Germany) for 3 h. Analyzed samples were weighed on the glass weighing dishes (i.d. 5 cm) and mixed with 1.0 g of freshly dried sea sand. These analyses were performed in duplicate in three independent measurements (n = 6). Analysis of variance (ANOVA) was applied for statistical treatment of the data at a probability level of p = 0.05.

Proper working conditions must be selected when the KF titration method is used. The assay conditions were optimized and the most suitable extraction solvent, extraction time, sample size and titration agent were used.

Standard working media for Karl Fischer titration contains methanol as solvent. To improve the solubility of substances with low polarity, a non-polar

solvent miscible with methanol must be added and organic solvent should also be selected that can sufficiently extract water from the sample in a relatively short period of time. In this study, various organic solvent, methanol mixtures were tested, including methanol/diethyl ether (1:1, v/v), methanol/chloroform (1:1, v/v) methanol/n-hexane (2.5:1, v/v) and methanol/toluene (1:1, v/v). The results obtained are summarized in Tables 1 and 2 for the VSP sample and VSE samples, respectively.

Based on the ANOVA results (not shown), there were no statistically significant differences among extraction solvents and titration agents used. Only Apura® CombiTitrant 5 was selected for further experiments.

After putting the sample into the titration cell, it is necessary to allow some time for equilibrium to be reached inside the cell. The appropriate equilibrium time was determined for the meat pâté samples dissolved in the methanol/chloroform (1:1) or methanol/toluene (1:1) mixtures. The various extraction times ranged from 0 to 10 min for methanol/chloroform and from 0 to 15 min for methanol/toluene. The optimal extraction times were 2.0 minutes for methanol/chloroform and 7.0 min. for methanol/toluene. The water contents at various extraction times for both samples are depicted in Fig. 1, where differences between the methanol/chloroform and methanol/toluene mixtures can be seen. Similar experiments were performed for the methanol/diethyl ether mixture (1:1), and the appropriate extraction time was determined to be 0.5 min (results not shown).

The influence of sample size ranging from 0.2 to 0.4 g in 0.05 g increments in relation to the water content was evaluated using methanol/chloroform (1:1) or methanol/diethyl ether (1:1) as the extraction solvents. When sample size exceeded 0.3 g, the water content of the meat pâté samples decreased (Fig. 2). The volume (25 mL) of the organic solvents used in this study has a limited dissolving capacity for this kind of product. A sample size of 0.25 g was therefore chosen for determining the water content in the meat pâté samples. The water contents in the samples obtained at optimized KF conditions are summa-

Table 1 - Water content (%) of chicken meat pâté (VSP) at 25°C with different extraction solvents and titration agents, sample size 0.25 g, 5 replicates each.

Extraction solvent	Hydranal®	Apura®	Hydra-Point®
methanol/diethyl ether (1:1, v/v)	64.07±0.50	63.92±0.59	63.20±0.44
methanol/n-hexane (2.5:1, v/v)	64.97±0.64	63.97±0.62	64.82±1.12
methanol/chloroform (1:1, v/v)	63.90±0.85	63.64±0.68	64.27±0.39
methanol/toluene (1:1, v/v)	63.85±0.62	63.22±0.64	62.25±0.80

Table 2 - Water content (%) of poultry meat pâté (VSE) at 25°C with different extraction solvents and titration agents, sample size 0.25 g, 5 replicates each.

Extraction solvent	Hydranal®	Apura®	Hydra-Point®
methanol/diethyl ether (1:1, v/v)	69.65±0.98	70.06±0.56	69.98±0.65
methanol/n-hexane (2.5:1, v/v)	71.94±0.70	70.79±0.73	71.54±1.24
methanol/chloroform (1:1, v/v)	70.49±0.87	70.29±0.76	70.85±0.60
methanol/toluene (1:1, v/v)	70.80±0.75	69.69±1.05	70.17±0.77

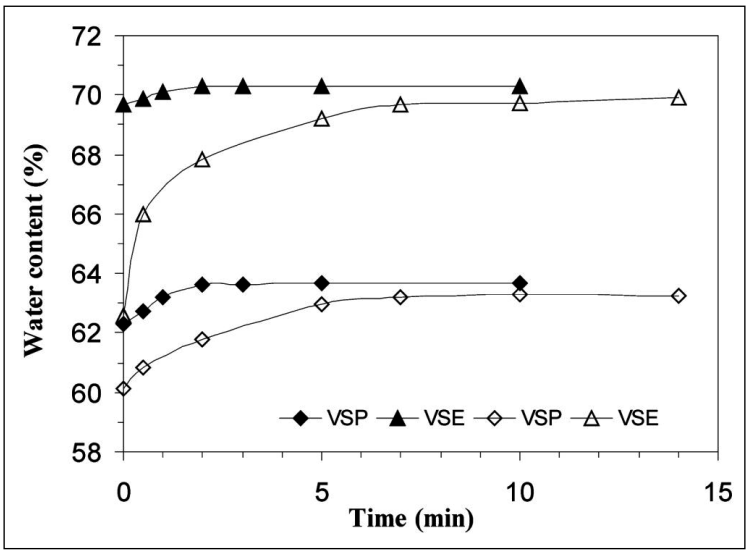

Fig. 1 - Influence of organic solvents on extraction time. Methanol/chloroform (1:1, v/v) - closed symbols; methanol/toluene (1:1, v/v) - open symbols; VSP - chicken meat pâté; VSE - poultry meat pâté.

rized in Table 3 along with the moisture contents obtained by the oven-drying method.

The water activities (a_w) at 25°C were 0.975±0.003 and 0.977±0.005 for VSP and VSE, respectively. The meat pâté tested in this study is classified as a high-moisture food. This means that there is a high amount of available water that can be used by microorganisms for their own proliferation and to form toxic substances (ROSS, 2001). A temperature treatment (below 12°C) and the addition of antimicrobial agents, such as nitrite and isoascorbate salts, improve shelf-life stability during storage.

The isotherms have a typical type II sigmoid shape that is common to most food materials (Fig. 3a, b) (BRUNAUER *et al.*, 1938). The equilibrium moisture content rose gradually at lower a_w values followed by a steep increase above a_w =

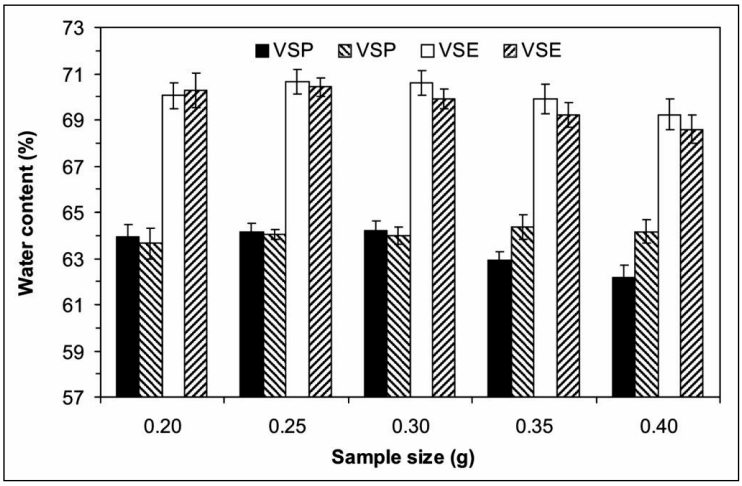

Fig. 2 - The influence of sample size on water content and extraction solvents. Methanol/diethyl ether (1:1, v/v) - solid columns; methanol/chloroform (1:1, v/v) – pattern columns); n = 5.

Table 3 - Water content (%) of analysed meat pâté samples obtained by various methods.

Analysed sample	KF titration	Oven drying	Moisture measurements
VSP (chicken pate)	64.05±0.22	63.92±0.24	62.0±1.5
VSE (poultry pate)	70.42±0.40	70.73±0.25	69.2±2.7

0.8. Several equations are available in the literature for relating moisture content and equilibrium a_w, which may be used to predict isotherms (SCHMITT and ISENGARD, 1998). In this study, the GAB equation was used to create the sorption isotherm of chicken meat pâté (SAMANIEGO-ESGUERRA *et al.*, 1991). The GAB equation fit the experimental data for VSE and VSP well. The coefficients of determination (R^2) were 0.979 and 0.987, respectively. The monolayer moisture contents of VSE and VSP at 25°C were computed from the GAB constants and were 9.5±1.3 for VSE and 11.2±1.8% for VSP. These values were higher than those obtained by authors who studied moisture sorption properties of chicken sausages (SINGH *et al.*, 2001). Using the known sorption isotherms and water activity values of the VSP and VSE samples, the moisture content was calculated to be 62.0 and 69.2%, respectively. The water content values obtained by KF titration and the moisture content values determined by the oven-drying method and sorption measurement were compared. There were no statistically significant differences among the water and moisture content values obtained by the different methods (Table 3). According to the literature reviewed in this article, this finding seems to be product-specific. In a comparative study to determine the water content in cereal products, MATHLOUTHI (2001) reported consistent results using both the oven-drying method and Karl Fischer titration. In contrast, it has been suggested that the drying methods should be rejected as the basic reference method for determining water content in milk powders (REH *et al.*, 2004; ISENGARD, 2001).

In this study the most suitable solvents, titration agent, extraction time and sample size were selected. The Karl Fischer titration in a methanol/chloroform (1:1, v/v) solvent mixture and Apura® titration agent at 25°C are the most suitable for determining the water con-

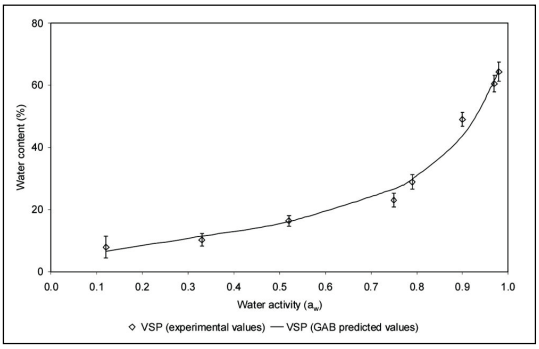

Fig. 3a - Sorption isotherm of chicken meat pâté (sample VSP) at 25°C.

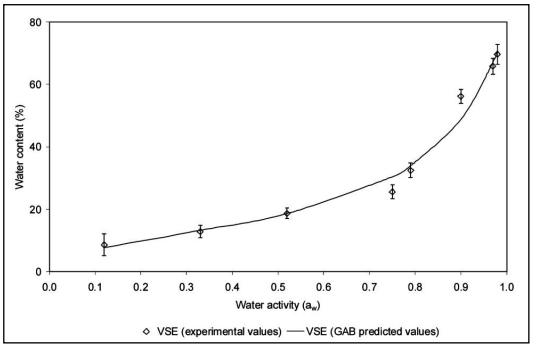

Fig. 3b - Sorption isotherm of poultry meat pâté (sample VSE) at 25°C.

tent in this type of product. The reproducibility of this method is similar to the oven-drying method and sorption measurements under optimal conditions. This could indicate that the weight loss is predominantly due to loss of water during the heating of the chicken meat samples. While the results from the current study do not give a priority to KF titration, this fast and widely used technique for determining the total water content is still recommended as a basic reference method for meat pâté-type products.

ACKNOWLEDGEMENTS

The authors thank the Ministry of Education, Youth and Sports of the Czech Republic (Project MSM 0021627502) and from the Czech Science Foundation (Project No. 203/08/1536) for financial support.

REFERENCES

Brunauer S., Emmet P.H. and Teller E.J. 1938. Adsorption of gases in multimolecular layers. J. Am. Chem. Soc. 66: 309.

Chirife J. and Buera M.D. 1996. Water activity, water glass dynamics, and the control of microbiological growth in foods. Crit. Rev. Food Sci. Nutr. 36: 465.

de Valk H., Valliant V., Jacquet C., Rocourt J., Le Querrec F., Steiner F., Quelquejeu N., Pierre O., Pierre V., Desenclos J.C. and Goulet V. 2001. Two consecutive nationwide outbreaks of listeriosis in France, October 1999-February 2000. Am. J. Epidemiol. 154: 944.

Fousaki M., Karametsi K. and Chaniotakis N.A. 2003. Method for the determination of water content in sultana raisins using a water activity probe. Food Chem. 82: 133.

Isengard H.-D. 1995. Rapid water determination in foodstuffs. Trends Food Sci. Tech. 6: 155.

Isengard H.-D. 2001. Water content, one of the most important properties of food. Food Control 12: 395.

Isengard H.-D. and Prager H. 2003. Water determination in products with high sugar content by infrared drying. Food Chem. 82: 161.

Isengard H.-D., Kling R. and Reh C.T. 2006. Proposal of a new reference method to determine the water content of dried dairy products. Food Chem. 96: 418.

Kaya A., Fehrettin G. and Medeni M. 2002. Moisture sorption isotherm of grape pestil and foamed grape pestil. Nahrung 46: 73.

Mathlouthi M. 2001. Water content, water activity, water structure and the stability of foodstuffs. Food Control 12: 409.

Morris I.J and Ribeiro C.D. 1991. The occurrence of *Listeria* species in pâté: the Cardiff experience 1989. Epidemiol. Infect. 107: 111.

Muik B., Lendl B., Molina-Diaz A., Perez-Villarejo L. and Ayora-Canada M.J. 2004. Determination of oil and water content in olive pomace using near infrared and Raman spectroscopy: a comparative study. Anal. Bioanal. Chem. 379: 35.

Reh C., Bhat S.N. and Berrut S. 2004. Determination of water content in powdered milk. Food Chem. 86: 457.

Ross Y.H. 2001. Water activity and plasticization. In "Food Shelf Life Stability". N.A.M. Eskin and D.S. Robinson (Ed.), p. 3. CRC Press, Boca Raton, Florida.

Samaniego-Esguerra C., Boag I.F. and Robertson G.L. 1991. Comparison of regression methods for fitting the GAB model to the moisture isotherms of some dried friut and vegetables. J. Food Eng. 13: 115.

Schmitt K. and Isengard H.-D. 1998. Karl Fischer titration: A method for determining the true water content in cereals. Fresen. J. Anal. Chem. 360: 465.

Singh B., Wang N., Prasher S. and Ngadi M. 2004. A spectroscopic technique for water content determination in potato, paper 046116 presented at Annual Meeting ASABE, Ottawa, Canada, August 1-4.

Singh R.R.B., Rao K.H., Anjaneyulu A.S.R. and Patil G.R. 2001. Moisture sorption properties of smoked chicken sausages from spent hen meat. Food Res. Int. 34: 143.

Stoloff L. 1978. Calibration of water activity measuring instruments and device: collaborative study. J. Assoc. Off. Anal. Chem. 61: 1166.

Revised paper received May 6, 2008 Accepted July 1, 2008

SHORT COMMUNICATION

EVALUATION OF FINING EFFICIENCY OF CORN ZEINS IN RED WINE: A PRELIMINARY STUDY

VALUTAZIONE DELL'EFFICIENZA CHIARIFICANTE DI ZEINE DI MAIS IN VINO ROSSO: STUDIO PRELIMINARE

B. SIMONATO, F. MAINENTE, I. SUGLIA[1], A. CURIONI[1] and G. PASINI[1,*]
Dipartimento di Scienze, Tecnologie e Mercati della Vite e del Vino,
Università di Verona, Via della Pieve 70, 37029 San Floriano (VR), Italy
[1]Dipartimento di Biotecnologie Agrarie, Università degli Studi di Padova,
Viale dell'Università 16, 35020 Legnaro (PD), Italy
*Corresponding author: e-mail: gabriella.pasini@unipd.it

ABSTRACT

The use of corn zeins, extracted from commercial maize flour under both reducing (RZ) and non-reducing (NRZ) conditions, in fining a red wine was investigated and compared with two commercial animal gelatins (G1, non-hydrolyzed and G3, hydrolyzed). RZ, but not NRZ, showed a good fining action in terms of decreasing turbidity and removing phenolic compounds such as anthocyanins and proanthocyanidins, indicating that protein reduction is needed for fining ability. The results

RIASSUNTO

È stato valutato l'effetto chiarificante su un vino rosso di zeine estratte da farina commerciale di mais in condizioni riducenti (RZ) e non riducenti (NRZ), in confronto con due gelatine animali commerciali (G1 non idrolizzata e G3 idrolizzata). Solo RZ ha mostrato una buona capacità chiarificante relativa alla diminuzione della torbidità e alla rimozione di composti fenolici come antocianine e proantocianidine, indicando la necessità di utilizzare le proteine in condizioni ridotte nel

- Key words: corn, fining, plant proteins, polyphenols, wine, zeins -

indicate that zeins, if extracted from corn under reducing conditions, could be a good substitute for animal proteins in red wine fining.

processo di chiarifica. I risultati indicano pertanto che le zeine, estratte dal mais in condizioni riducenti, possono rappresentare un buon sostituto delle proteine animali.

INTRODUCTION

Clarification treatments (fining) are often necessary in red wines to prevent colloidal precipitation which involves phenolic substances. The purpose of fining is to clarify the wine and make it stable by lowering the polyphenol content (YOKOSUKA and SINGLETON, 1995). This is commonly done by adding proteins such as gelatin, isinglass, albumin or casein to the wine (BOULTON *et al.*, 1996). These proteins interact with polyphenols and cause their flocculation which clarifies and stabilizes the red wine (YOKOSUKA and SINGLETON, 1995; VERSARI *et al.*, 1999; MARCHAL *et al.,* 2002). Fining treatments also improve the organoleptic characteristics of the wine by lowering the tannin concentration (MAURY *et al.*, 2003).

Animal gelatins are the most widely used fining agents due to their low cost, ability to clarify wine and reduce its astringency. However there is now great concern about using these products because of the cases of Bovine Spongiform Encephalopathy (BSE). It has become necessary to find other fining agents that could replace animal proteins and these substitutes are being sought among plant proteins (MARCHAL *et al.*, 2002; MAURY *et al.*, 2003).

The Food Standards of Australia and New Zealand (FSANZ, 2004), the agency responsible for health protection and food safety in Australia and New Zealand, permits the use of plant proteins obtained from wheat, rice, peas, lupins and maize as wine fining agents. EC REGULATION N. 2165 (2005), which concerns the organization of the viticultural and enological market, has authorized the use of plant proteins for wine treatment.

A previous study showed that hydrolized and deaminated glutens have a good clarification efficiency, but the gluten clarification rates were lower than those obtained with egg proteins (MARCHAL *et al.,* 2002). Moreover, it has been demonstrated that wheat gluten and a protein preparation made from white lupin selectively precipitated condensed tannins from red wine and from a model one (MAURY *et al.*, 2003). It has also been shown that the molecular weight of the proteins is an important factor in tannin precipitation which has a significant effect on fining efficiency (SARNI-MANCHADO *et al.*, 1999; MAURY *et al.*, 2003).

Among the proteins derived from plant seeds, the alcohol-soluble storage proteins of maize (zeins) could be potentially useful in wine fining. Zeins have some chemical-physical characteristics that make them suitable for the interactions with phenolic compounds, including a high content of non-polar amino acids (SHUKLA and CHERYAN, 2001). Zeins, constituting about 60-70% of the maize endosperm proteins (LANDRY *et al.*, 2000), occur in the seed as a heterogeneous protein mixture, made up of various polypeptides that differ in molecular weight, isoelectric point (WILSON *et al.*, 1981) and aggregation state (SHUKLA and CHERYAN, 2001). Moreover, commercial corn gluten meal, which contains the storage proteins of the maize kernel, is produced in large quantities

as the main by-product of the starch industry and can be purchased at a very low price.

In this preliminary study, the use of maize zeins, extracted from commercial maize flour under both reducing and non-reducing conditions was investigated for use in fining a red wine and the results were compared with those obtained with two commercial animal gelatins.

MATERIAL AND METHODS

Materials

The red wine (cv. Merlot) used for the fining experiments was produced during the 2006 vintage. The wine was obtained before fining. The analytical characteristics of the wine are reported in Table 1.

The fining agents used in the experiments were two commercial porcine powdered gelatins (G1 and G3) (Enologica Vason S.r.l., Verona, Italy) and two different protein extracts prepared in our laboratory from a commercial maize flour and commercial corn gluten (Cargill s.r.l. Div. Cerestar, Castelmassa, RO, Italy).

Protein extraction from maize flour and commercial corn gluten

Maize flour (250g) was extracted with 1 L of 0.5 M NaCl by stirring for 2 h at 4°C. After centrifugation, the supernatant containing the salt-soluble compounds was discarded. The residue was washed with 500 mL of 0.5 M NaCl and centrifuged again. This procedure was repeated and followed by a final wash with distilled water. The residue was extracted overnight, at room temperature with continuous stirring, with three different solvents: 1 L of 70% (v/v) ethanol, 70% (v/v) ethanol containing 40 mM Na_2SO_3 and 70% (v/v) ethanol containing 20 mM dithiothreitol (DTT). The suspensions obtained were then centrifuged at 12,000 *g* for 20 min at 4°C.

Corn gluten (250 g) was directly extracted overnight with 1 L of 70% (v/v) ethanol at room temperature and centrifuged as above. In all cases, the supernatants were treated with 6 volumes of cold acetone. After standing for 2 h at room temperature, the precipitated proteins were collected by centrifugation and dried under a N_2 stream.

Table 1 - Analytical characteristics of the Merlot red wine before fining.

Total acidity (g/L tartaric acid)	5.25
Volatile acidity (g/L acetic acid)	0.42
pH	3.50
Alcohol content (% v/v)	12.9
Free SO_2 (mg/L)	20
Total SO_2 (mg/L)	31
Turbidity (NTU)	60
Anthocyanins (mg/L)	345
Proanthocyanidins (mg/L)	1895

Nitrogen quantification

Samples were mineralized according to the method of HACH *et al.* (1985) and nitrogen was quantified by the AOAC method 33.056 (1984).

Electrophoresis

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed according to LAEMMLI (1970) in a Mini Protean II cell (Bio-Rad, Milan, Italy) with a gel containing a total polyacrylamide concentration of 16%.

Ten mg of each sample were solubilized in 1 mL of 0.3 M Tris-HCl, pH 7.4, containing 2% (w/v) SDS and 8% (w/v) glycerol and 15 μL of the resulting protein solution were loaded on the gels. Electrophoresis was run at 50 mA constant current until the tracking dye bromophenol blue, reached the bottom of the gel. Gels were stained with Comassie Brilliant Blue and de-stained with 7.5% acetic acid. Molecular weight standard proteins (Bio-Rad, Milan, Italy) were phosphorylase B (97.4 kDa), bovine

serum albumin (66.2 kDa), ovoalbumin (45 kDa), carbonic anhydrase (31 kDa), soy trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa).

Fining experiments

The two powdered preparations, obtained from the maize flour derived from ethanol (non-reduced zeins, NRZ) and ethanol/Na_2SO_3 extraction (reduced zeins, RZ), were used in wine fining experiments.

Each fining agent (G1, G3, NRZ and RZ) was suspended in water at a final concentration of 5% (w/v) and stirred for 12 h at room temperature before use. The suspensions were added to 500 mL of wine to a final concentration of 5, 10 and 15 g/hL. After settling for 48 h at 20°C, wine samples were centrifuged at 1,900 *g* for 10 min (MAURY *et al.*, 2003). The turbidity of the samples was measured before and after the fining treatment, using a Hach 2100N turbidimeter (Hach Company, Loveland, Colorado, USA) calibrated with Formazin turbidity standard 4000 NTU (Hach Company, Loveland, Colorado, USA). Turbidity was expressed in Nephelometric Turbidity Units (NTU).

Anthocyanin quantification

Anthocyanins were determined with a method based on their discoloration in the presence of SO_2. One mL of wine was added to 1 mL of a solution made up of 96% ethanol and 37% HCl (99:1 v/v). Twenty mL of 2% HCl were then added and 5 mL of the resulting solution were put in flasks A and B. Two mL of water were added to flask A and 2 mL of 15% $NaHSO_3$ to flask B. After 30 min in the dark, the absorbance at 520 nm was measured. The anthocyanin concentration (mg/L) was calculated with the following formula: $C\ (mg/L) = DA \cdot 875$, where DA is the difference between the absorbance values of the solutions in flasks A and B, (RIBEREAU-GAYON and STONESTREET, 1965).

Proanthocyanidin quantification

Proanthocyanidins were determined after their conversion to anthocyanidins in a hot, acid environment. Briefly, 20 mL of wine (diluted 50-fold with water) were placed in a flask with 0.2 g of polyvinylpolypyrrolidine (PVPP). After mixing for 5 min, the suspension was filtered with Whatman n° 1 filter paper under vacuum. The filter was put in a flask and 25 mL of a solution made up of 150 mg/L ferrous sulphate in n-butanol and 37% HCl (50:50 v/v) (sol A) were added. The flask was put in a boiling water bath for 50 min and then rapidly cooled under running tap water. The absorbance of the solution was then read at 550 nm, using the absorbance of sol A as the blank. The concentration of proanthocyanidins in mg/L was calculated with the following formula: $C\ (mg/L) = A \cdot d \cdot 1.217 \cdot 50$, where A is the absorbance at 550 nm, and *d* is the dilution factor of the wine (POMPEI *et al.*, 1971).

Wine hue and color

Wine sample hue was quantified as the ratio of absorbances at 420 and 520 nm (GLORIES, 1984). Wine color was quantified as the addition of absorbances at 420, 520 and 620 nm (GLORIES, 1984).

Wine analyses

Alcohol, pH, titratable acidity, volatile acidity and SO_2 content were determined using the OIV methods (1990).

RESULTS

Characterization of the fining agents

Zeins were extracted from both the maize flour, previously treated for the removal of water/salt-soluble compounds (essentially starch and water/salt-soluble proteins), and directly from a commercial corn gluten, whose main component is the maize prolamine fraction. Corn gluten is a by-product of wet corn

milling. It contains (on a dry basis): 67-71% proteins, 21-26% carbohydrates of which 12-15% is starch, 3-7% fat, 1-2% fiber and 1-2% ash (DOMBRINK-KURTZMAN and BIETZ, 1993). The main protein fractions of corn gluten consist of approximately 65% zein and 30% glutelin. The zein fraction can be extracted with aqueous aliphatic alcohols of low molecular weight, leaving an insoluble residue mainly consisting of starch and glutelin. A gluten-rich fraction can then be prepared by removing the starch by enzymatic digestion with α-amylase (CAO *et al.*, 1996). In order to recover the zein fraction from the flour, which is typically water/salt-insoluble (ESEN, 1987), the water/salt-soluble components (starch and water/salt soluble proteins) had to be removed by pre-treating the flour with a salt solution. In contrast, the commercial corn gluten, whose main component is the water-salt-insoluble zein fraction, was directly extracted for zeins. The extraction with 70% ethanol (alone or containing a reducing agent) allowed a solution to be obtained from which zeins were recovered by acetone precipitation. In this way it was possible to obtain a dry powder, which, in all cases, was made up of 100% protein, as were both gelatin samples (G1 and G3) (not shown).

The protein composition of the zein samples extracted in non-reducing (70% ethanol alone) and reducing (70% ethanol plus reducing agent) conditions was analyzed by SDS PAGE.

Samples extracted with 70% ethanol from both the maize flour (NRZ) and corn gluten, showed the same protein pattern, which is typical of native zein (Fig. 1a) (SHEWRY and TATHAM, 1990). In addition to the resolved bands, a major protein fraction blocked at the top of the gel was observed. This fraction was comprised of protein aggregates with a molecular weight that was too high to enter the gel pores. The components with an electrophoretic mobility corresponding to ~24 and ~22 kDa were identified as α-zeins (PAULIS, 1981). Dimers of ~50 kDa and trimers of ~75 kDa, formed by α-zein monomers (PAULIS, 1981), were also evident.

The SDS PAGE analysis of proteins obtained from maize flour with a reducing agent (i.e. from 70% ethanol containing Na_2SO_3 or DTT) followed by acetone precipitation, revealed a pattern in which the high-molecular-weight aggregates were absent (Fig. 1b), which confirmed that they are stabilized by S-S bonds. In addition to the heavy stained bands with molecular weights of ~17 kDa (β-zein), ~22 - 25 kDa (α-zeins) and ~27 kDa (γ-zein), which derive from the reduction of the aggregates (PAULIS, 1981), a 50 kDa protein band appeared, which was identified as Reduced-Soluble-Protein (RSP), a protein that can be extracted from maize only in the presence of a reducing agent (VITALE *et al.*, 1982; PASINI *et al.*, 2002). Since treatments with DTT and Na_2SO_3 gave almost identical protein patterns and taking into account

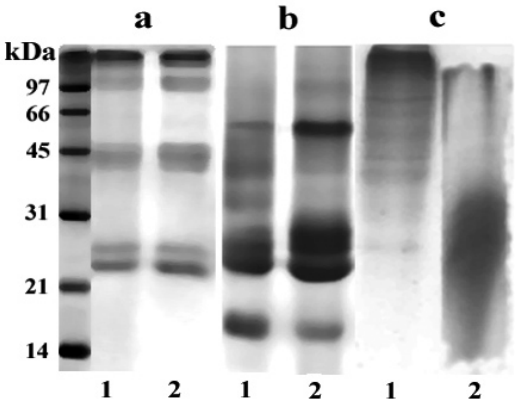

Fig. 1 - SDS-PAGE analysis of the proteins used in the fining experiments.
a: Maize flour (lane 1) and commercial corn gluten (lane 2) proteins extracted with 70% ethanol under non-reducing conditions.
b: Maize flour extracted with 70% ethanol in the presence of Na_2SO_3 (lane 1) and DTT (lane 2).
c: Non-hydrolyzed G1 (lane 1) and hydrolyzed G3 (lane 2) gelatins.
Molecular weight standard proteins are on the left.

the toxicity of DTT versus the possibility of using Na_2SO_3 in enology, Na_2SO_3 was used to prepare the samples for the fining experiments.

The electrophoretic patterns of the two porcine gelatins (G1 and G3) were also analyzed. G1, a commercial gelatin derived from non-hydrolyzed collagen, showed poorly resolved protein bands with molecular weights that were greater than 31 kDa, most of which were located at the top of the gel (Fig.1c, lane 1). In contrast, G3, which was obtained by hydrolysis of porcine collagen, showed unresolved protein bands of molecular weights less than 31 kDa (Fig.1c, lane 2).

Fining treatments

Experimental wine fining treatments were performed with the non-reduced (NRZ) and reduced (RZ) zeins extracted from the maize flour. This material was chosen, instead of the commercial gluten meal, in order to use a standardized material (the maize flour), and avoid using commercial corn products that could have been subjected to different industrial treatments.

The wine fining properties of RZ and NRZ at 5, 10 and 15 g/hL, in comparison with G1 and G3 at the same concentrations, were evaluated 48 h after the addition (Fig. 2). The turbidity of the original wine (before fining) was around 60 NTU, while after centrifugation the turbidity of the same wine was reduced to 36 NTU. About 40% of the turbidity was due to suspended material which could be physically removed by centrifugation. This centrifuged wine was then used as the control wine for the fining experiments.

Treatments with NRZ resulted in low wine clarification. The best result obtained with this protein preparation was a reduction in wine turbidity of only about 22%. In contrast, the use of the reduced protein preparation (RZ) reduced the turbidity by 88.2% (Fig. 2 – 10 g/hL). This value was similar to that obtained for the wine processed with gelatin G1 at the same dose (87.7%). Treatment with gelatin G3 gave the best result at 5 g/hL, with a turbidity decrease of 85%; higher concentrations resulted in increased turbidity, which could have been due to an unfavorable protein/phenolic ratio (SIEBERT *et al.*, 1996).

Since RZ performed better than NRZ, with respect to decreasing wine turbidity, only RZ was used for the characterization of the maize zein as a wine fining agent.

Effects on phenolic compounds

The effect of the anthocyanin content on the wine derived from fining with RZ was evaluated in comparison to that of the G1 and G3 treatments (Table 2). For all the fining agents tested, the percentage of anthocyanins removed increased with the dose of fining agent used.

Depending on the quantity added to the wine, RZ removed from 4 to 11.6% of the anthocyanins; these values are very simi-

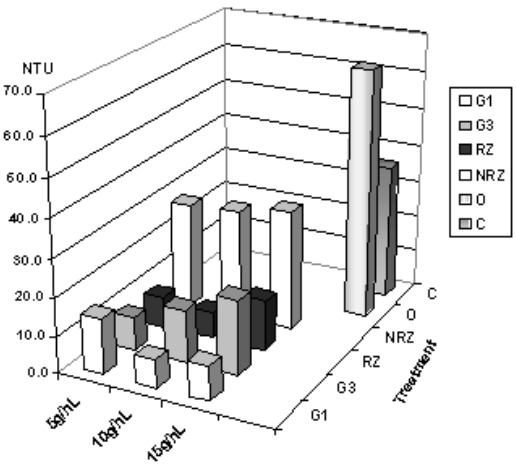

Fig. 2 - Effect of different fining treatments on the turbidity of Merlot red wine expressed as Nephelometric Turbidity Units (NTU).
O: original wine; C: centrifuged (control) wine; NRZ: non-reduced zeins; RZ: reduced-zeins; G3: hydrolyzed gelatin; G1: non-hydrolyzed gelatin.

Table 2 - Anthocyanin quantification (mg/L) after fining treatment. Means of three determinations. Standard Deviation <0.4%. C: centrifuged (control) wine.

Treatment	0 g/hL	5 g/hL	10 g/hL	15 g/hL
RZ		335	325	305
G1		333	318	307
G3		331	328	316
C	345			

lar to those obtained with G1 (from 3.5 to 11%). G3 was the least effective, with 4 to 8.4% of the anthocyanins being removed.

Proanthocyanidins, which are responsible for wine astrigency and haze, can interact with fining proteins leading to co-precipitation (SARNI-MANCHADO *et al.*, 1999; MAURY *et al.*, 2003). The quantities of proanthocyanidins before and after the fining treatments with RZ, G1 and G3 are reported in Table 3. Depending on the dose used, RZ removed from 12.8 to 19.3% of the proanthocyanidins. These values are intermediate between those obtained using G1 (from 6.3 to 11.7%) and those using G3 (from 19.5 to 23.7%).

Effect on wine hue and color

Wine hue and color values were unaffected by RZ fining at all the concentrations used and were similar to the values obtained after treatments with G1 and G3, around 0.62 and 1.2 for hue and color, respectively (data not shown). These data are in accord with those observed in a previous study on wine fining with plant proteins (LEFEBVRE *et al.*, 1999). The wine used in these fining experiments was quite young and most of the color was due to free anthocyanins.

DISCUSSION

After alcoholic fermentation, wine contains many particles in suspension. Since the density of these particles is close to that of wine, spontaneous clarification, due to physical sedimentation, would probably take a very long time and would be insufficient to make the wine stable. Therefore, it is necessary to fine the wine by adding proteins that interact with substances that are responsible for instability and cause their precipitation (MARCHAL *et al.*, 2002).

In this paper, the wine fining ability of corn zeins was studied. These proteins are particularly rich in glutamic acid (21-26%), leucine (20%), proline (10%) and alanine (10%) (SHUKLA and CHERYAN, 2001). The high proportion of non-polar amino acids makes zeins relatively hydrophobic (KOJIMA *et al.*, 1997)

Table 3 - Proanthocyanidin quantification (mg/L) after fining treatment. Means of three determinations. Standard Deviation <0.4%. C: centrifuged (control) wine.

Treatment	0 g/hL	5 g/hL	10 g/hL	15 g/hL
RZ		1,654	1,592	1,530
G1		1,777	1,702	1,674
G3		1,527	1,509	1,447
C	1,895			

and this property could determine the effectiveness of these plant proteins in fining red wine, as previously shown for animal gelatin (SARNI-MANCHADO *et al.*, 1999). The data here reported indicate that only the zeins prepared in the presence of a reducing agent were effective in fining wine. Native zeins occur as a heterogeneous protein mixture that also contains disulfide-linked aggregates. Under reducing conditions, the disulfide-bonds in the aggregates are broken which induces the release of protein monomers with molecular weights of 17 kDa (corresponding to β–zein), 22 (α-zein) and 27 kDa (γ-zein) (ESEN, 1987). Since wine fining ability of proteins seems to depend mainly on their molecular weight and conformation (MAURY *et al.*, 2003), the good fining effectiveness of RZ could be due to the presence of proteins with a relatively low molecular weight (<32 kDa), which could be obtained by treating corn flour with a reducing agent. In contrast, the low fining ability of the native zein (non-reduced) may be due to the inaccessibility of the phenol-binding sites, which are buried inside the disulfide bond-stabilized protein structure. Therefore, the reduction of disulfide bonds by opening the protein structure would improve the binding site accessibility by favoring polyphenol-protein interactions through hydrophobic interaction and hydrogen bonds.

The best results in reducing turbidity were obtained with 10 g/hL of RZ; the turbidity values were similar to those measured in the wine treated with G1 at the same concentration. The decrease in anthocyanin concentration after RZ treatment was also similar to that observed with G1. These results were also confirmed for zeins, indicating that their behavior is similar to that of other protein fining agents (SARNI-MANCHADO *et al.*, 1999). The hue and color of RZ-treated wine was unaffected by the fining treatment as was previously observed using other plant proteins (LEFEBVRE *et al.*, 1999).

In summary, corn zeins extracted under reducing conditions show a good fining action in term of decreasing turbidity, removing phenolic compounds and preserving red wine color. The clarifying ability of zeins is similar to that of animal gelatin so this plant protein could be a good substitute for animal proteins in wine-making. To complete this study, more compositional and sensory analyses are needed to evaluate what impact the use of RZ as fining agent has on wine aroma.

Due to the chemical-physical properties of corn zeins they have many industrial applications such as in the production of fiber, adhesives, coatings, ceramics, inks, cosmetics, textiles, chewing gum and degradable plastics (SHUKLA and CHERYAN, 2001). The use of corn zeins as a wine-fining agent could be another application for these proteins. In the present study, RZ was extracted from the residue remaining after the water/salt-soluble components were removed from maize flour. The direct extraction of zeins from a commercial corn gluten sample gave exactly the same SDS-PAGE protein pattern as the zeins extracted from maize flour. Therefore corn gluten, the residual by-product produced in very large quantities by the corn processing (starch) industry, could probably be used to produce a low-cost fining agent that could be successfully employed in wine making.

Finally, zeins are designated as "Generally Recognized As Safe" (GRAS) by the American Federal Drug Administration and, unlike wheat gluten, are safe for consumers suffering from celiac disease. The risk for allergic patients is also rather low, since allergies to corn seem to be rarer than those to other protein materials of plant and animal origin that are used or have been proposed for use as wine-fining agents (MILLS and BREITENEDER, 2005).

REFERENCES

AOAC. 1984. "Official Methods of Analysis", 14th ed. Association of Official Analytical Chemists. Arlington. VA.

Boulton R., Singleton V., Bisson L. and Kunkee R. 1996. "Principles and Practices of Winemaking". Chapman & Hall, New York.

Cao N.G., Xu Q., Ni J. and Chen L.F. 1996. Enzymatic hydrolysis of corn starch after extraction of corn oil with ethanol. Appl. Biochem. Biotechnol. 57/58: 39.

EC Regulation, 2005. N° 2165, Official Journal of the European Union. Bruxelles, Belgium.

Dombrink-Kurtzman A. and Bietz J.A. 1993. Zein composition in hard and soft endosperm of maize. Cereal Chem. 70: 105.

Esen A. 1987. A proposed nomenclature for the alcohol-soluble proteins (zeins) of maize (*Zea mays* L.). J. Cereal Sci. 5: 117.

FSANZ (Food Standards Australia New Zealand). 2004. Final assessment report. February, 2004. Application A482-Plant proteins as wine processing aids. Canberra.

Glories Y. 1984. La Couleur des Vins Rouges. 2a Partie. Mésure Origine et Interprétation. Connaissance de la Vigne et du Vin. 18: 252.

Hach C.C., Brayton S.V. and Kopelove A.B. 1985. A powerful Kjeldahl nitrogen method using peroxymonosulfuric acid. J. Agric. Food Chem. 33: 1117.

Kojima M., Hanna M.A. and Gennadios A. 1997. Water solubility and macromolecular properties of corn meal extrudates as affected by epichlorohydrin. Cereal Chem. 74: 526.

Laemmli I.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 227: 680.

Landry J., Delhaye S. and Damerval C. 2000. Improved method for isolating and quantitating α-amino nitrogen as nonprotein, true protein, salt-soluble proteins, zeins and true glutelins in maize endosperm. Cereal Chem. 77: 620.

Lefebvre S., Maury C., Poinsaut P., Gerland C., Gazzola M. and Sacillotto R. 1999. Le collage de vins: influence du poids moleculaire des gelatines et premiers essais de colles d'origine vegetale. Revue des Œnologues. 94: 37.

Marchal R., Marchal-Delahaut L., Lallement A. and Jeandet P. 2002. Wheat gluten used as a clarifying agent of red wines. J. Agric. Food Chem. 50: 177.

Maury C., Sarni-Manchado P., Lefebvre S., Cheynier V. and Moutounet M. 2003. Influence of fining with plant proteins on proanthocyanidin composition of red wines. Am. J. Enol. Vitic. 54: 105.

Mills E.N.C. and Breiteneder H. 2005. Food allergy and its relevance to industrial food proteins. Biotechnol. Advances. 23: 409.

OIV. 1990. Office Internationale de la Vigne et du Vin. Recueil des Méthodes Internationales d'Analyse des Vins et des Moûts, Paris.

Pasini G., Simonato B., Curioni A., Vincenti S., Cristaudo A., Cantucci B., Dal Belin Peruffo A. and Giannattasio M. 2002. IgE-mediated allergy to corn: a 50 kDa protein, belonging to the Reduced Soluble Proteins, is a major allergen. Allergy. 57: 98.

Paulis J.W. 1981. Disulfide structures of zein proteins from corn endosperm. Cereal Chem. 58: 542.

Pompei C., Peri C. and Montedoro G. 1971. Le dosage des leucoanthocyanes dans les vins blancs. Ann. Technol. Agric. 20: 21.

Ribéreau-Gayon P. and Stonestreet E. 1965. Le Dosage des Anthocyanines dans le Vin Rouge. Bulletin de la Societe Chimique de France: 2469, Paris.

Sarni-Manchado P., Deleris A., Avallone S., Cheynier V. and Moutounet M. 1999. Analysis and characterization of wine condensed tannins precipitated by proteins used as fining agent in enology. Am. J. Enol. Vitic. 50: 81.

Shewry P.R. and Tatham A.S. 1990. The prolamin storage proteins of cereal seeds: structure and evolution. Biochem. J. 267: 1.

Shukla R. and Cheryan M. 2001. Zein: the industrial protein from corn. Ind. Crops and Prod. 13: 171.

Siebert K.J., Carrasco A. and Lynn P. 1996. Formation of protein-polyphenol haze in beverages. J. Agric. Food Chem. 44: 1997.

Versari A., Barbanti D., Potentini G., Parpinello G.P. and Galassi S. 1999. Preliminary study of the interaction of gelatin red wine. Ital. J. Food Sci. 11: 231.

Vitale A., Smaniotto E., Longhi R. and Galante E. 1982. Reduced soluble proteins associated with maize endosperm protein bodies. J. Exp. Bot. 33: 439.

Wilson C.M., Shewry P.R. and Miflin B.J. 1981. Maize endosperm proteins compared by sodium dodecyl sulphate gel electrophoresis and isoelectric focusing. Cereal Chem. 58: 275.

Yokosuka K. and Singleton V.L. 1995. Interactive precipitation between phenolic fractions and peptides in wine-like model solution: turbidity, particle size and residual content as influenced by pH, temperature and peptide concentration. Am. J. Enol. Vitic. 46: 329.

Revised paper received March 12, 2008 Accepted May 26, 2008

SHORT COMMUNICATION

COBALT, LEAD AND NICKEL LEVELS IN HONEY PRODUCED IN KAHRAMANMARAŞ, TURKEY

I LIVELLI DI COBALTO, PIOMBO E NICHEL IN MIELI PRODOTTI A KAHRAMANMARAŞ, IN TURCHIA

O. ERDOĞRUL and M. EZER[1]
Department of Food Engineering, Faculty of Agriculture,
University of Kahramanmaraş Sütçü İmam, Kahramanmaraş, Turkey
[1]Department of Chemistry, Faculty of Sciences and Arts,
University of Kahramanmaraş Sütçü İmam, Kahramanmaraş, Turkey
*Corresponding author: Tel. +90 344 2191575, Fax +90 344 2191526,
e-mail: oerdogrul@ksu.edu.tr

ABSTRACT

The cobalt (Co), lead (Pb), and nickel (Ni) levels were determined in twenty-one honey samples from the Kahramanmaraş region in Turkey by graphite furnace atomic absorption spectrometry (GFAAS). The mean Co, Ni and Pb values were 0.02±0.004, 0.13±0.01, and 0.23±0.01 mg kg^{-1}, respectively. Although the samples were not free of the heavy metals, they were within the permitted levels.

RIASSUNTO

Sono stati determinati, tramite spettrofotometria ad assorbimento atomico con fornetto di grafite (GFAAS), i livelli di cobalto (Co), piombo (Pb) e Nichel (Ni) in ventuno campioni di miele proveniente da Kahramanmaraş, in Turchia. I valori medi di Co, Ni e Pb erano rispettivamente 0,02±0,004, 0,13±0,01 e 0,23±0,01 mg kg^{-1}. Sebbene i campioni non fossero trovati privi di metalli pesanti, i loro valori rientravano nei livelli permessi.

- Key words: Environmental contamination, graphite furnace atomic absorption spectrometry, heavy metals, honey -

INTRODUCTION

Honey is a very important energy food and is used as an ingredient in hundreds of manufactured foods, particularly cereal-based products, for sweetness, color, flavor, caramelization and viscosity. Turkey is an important honey-producing country because it is very suitable for apiculture in terms of the flowers. Turkey produces about 74,000 tons of honey per year (DİE, 2003). Honey possesses valuable nutritional, health and prophylactic properties that are based on its chemical composition. As a foodstuff used for health purposes, honey must be free of any harmful substances. Honey may contain minerals and heavy metals as a result of air pollution. It is a good indicator and monitor of the chemical constituents of the plants. Honeybees are continuously exposed to contaminants that are present and widespread throughout the areas surrounding the apiary for the duration of their foraging activity (i.e. from spring to fall). Honeybees and their products can supply a suitable amount of biological material that can be easily sampled and analyzed throughout the year (CRANE, 1975). The foraging activity associated with an apiary generally extends over a surface area of approximately 7 km^2. Due to the large surface area worked by the honeybees and the presence of biological materials in their products, they are suitable bioindicators of chemical pollution. Therefore it is important to determine the chemical composition and quality of the honey. A number of researchers (RODRIGUEZ *et al.*, 2005; RASHED and SOLTAN, 2004; DEVILLERS *et al.*, 2002; YILMAZ and YAVUZ, 1999) have examined the physical and chemical composition of honey.

Graphite furnace atomic absorption spectrometry (GFAAS) is a suitable technique for determining trace elements in food and biological and environmental samples because it is fast, requires minimal sample preparation, can be automated and is sensitive (HUANG *et al.*, 2000; TÜZEN *et al.*, 1998). However, the detection of trace metals in honey samples by GFAAS is difficult because the complicated matrix greatly affects the analytical results. Therefore, different chemical modifiers are used to stabilize the analyte. $NH_4H_2PO_4$ is used as a chemical modifier to form stable lead phosphate and remove halides which allows higher pyrolysis temperatures to be used (SCHLEMMER and RADZİCK, 1999). Overall chemical modifications, stabilized temperature platform furnace (STPF) conditions (SLAVIN *et al.*,1983; TAHVONEN and KUMPULAINEN, 1994) and powerful background correction procedures have been used to minimize background absorption signals and interference effects prior to the atomization stage.

The following investigation is focused on three heavy metals: cobalt, lead and nickel. Reliable, reference background levels were determined. They will be very useful for constant monitoring and to prevent future problems due to the emission of heavy metals in the environment.

MATERIAL AND METHODS

Honey samples and sampling area

Twenty-one natural liquid honey samples (100 g) were randomly collected from various beekeepers in Kahramanmaraş, Turkey in February-March 2004. The honey samples (100 g) were stored in glass jars at room temperature and in the dark until analyzed. All the samples examined were unprocessed honeys of random (mixed) floral type.

Analytical determinations

Ash contents were determined by heating 10 g of honey first at 100°C until the moisture decreased and then

Table 1 - Temperature programs used for Pb, Co and Ni with GFAAS.

Steps	Pb	Ni	Co
Dry1 (ramp/hold)	110°C (1/30)	110°C (1/30)	110°C (1/30)
Dry2 (ramp/hold)	130°C (15/30)	130°C (15/30)	130°C (15/30)
Ash (ramp/hold)	500°C (10/30)	1,100°C (10/20)	1,400°C (10/20)
Atomize(ramp/hold)	1,600°C (0/5)	2,300°C (0/5)	2,400°C (0/5)
Clean (ramp/hold)	2,450°C (1/3)	2,450°C (1/3)	2,500°C (1/5)

at 500°C until constant weight. An infra-red lamp was used to prevent foaming. Three metals, cobalt (Co), lead (Pb) and nickel (Ni) were selected and measured using a Perkin Elmer atomic absorption spectrometer (AA800, Waltham, MA, USA). Solutions containing the analytes were obtained by dissolving ash in a 10 mL mixture (1:1) of perchloric acid (60%, Merck Darmstadt, Germany) and nitric acid (65%, Merck Darmstadt, Germany). The ash containing samples were covered with watch glasses and left overnight. The ash of the honey samples was measured and filtered through Whatman N. 41 filter paper. Co, Ni and Pb were determined directly in the ash solution after a 1/5 dilution with distilled water. The following wavelengths were used for the metals: Nickel 232.0 nm, cobalt 242.5 nm and lead 283.3 nm. The analytical procedures have been described in detail in the analytical methods for atomic absorption spectrophotometry (PERKIN-ELMER, 1982).

A Perkin-Elmer model AA800 atomic absorption spectrometer equipped with a THGA graphite furnace and AS-100 furnace auto-sampler was used for the investigation. Background correction was performed by means of the longitudinal Zeeman effect. Platform atomization from transversely heated, pyrolytically coated graphite tubes was used. During the analyses, the internal argon flow rate through the graphite tube was 300 mL min^{-1} and the gas flow was interrupted during atomization. No modifications were made to the recommended temperature program for Ni and Co but the ashing temperature and holding time for Pb were modified (Table 1).

Matrix modifiers were added, 50 μg $NH_4H_2PO_4$ + 15 μg $Mg(NO_3)_2$ for Pb and 15 μg $Mg(NO_3)_2$ for Co. Each GFAAS analysis needed 20 μL of sample solution, and 5 μL of the matrix modifier was added, if necessary. Working solutions of each element, 100 μg L^{-1}, were prepared daily by appropriate dilution from a stock solution (1,000 mg L^{-1}; Custom-Grade Standards, Inorganic Ventures Inc., Lakewood, NJ, U.S.). Calibration solutions for each element were prepared from working solutions by the furnace auto sampler. In order to validate calibration during the measurements a calibration standard was read after every ten measurements and a method for adding the standard to a honey sample was successfully applied (with 95% or better recovery). Calibration standards were read three times and a blank (18 MΩ de-ionized water) was read ten times (ten consecutive injections). The samples (honey and method blank) were read twice (two consecutive injections) and the mean values and the relative standard deviations were

Table 2 - Ni, Pb and Co levels in Kahramanmaraş honey samples (mg kg^{-1}).

Metal	Mean	Range	Standard deviation
Ni	0.13	0.030-0.370	0.01
Pb	0.23	0.100-0.510	0.01
Co	0.02	0.004-0.035	0.004

computed. The final concentrations of analytes (in mg kg^{-1}) were calculated relative to the initial sample mass and corrected for any dilutions.

RESULTS AND CONCLUSIONS

The 3σ limit of detections for Pb, Co, and Ni were 0.08, 0.2 and 0.2 μg kg^{-1}, respectively (n=10). The Pb, Co and Ni contents in the 21 honey samples, together with the corresponding standard deviations and coefficients of variation are shown in Table 2. The mean Co, Ni and Pb values were 0.02±0.004, 0.13±0.01, and 0.23±0.01 mg kg^{-1}, respectively, with the Co level being the lowest. This value is much lower than that reported by YILMAZ and YAVUZ *et al.* (1999), but is in agreement with the value reported by SEVIMLI *et al.* (1992), and TERRAB *et al.* (2005). It is important to note that only a few honey samples had any detectable amounts of Co.

The mean Pb content was 0.23 mg kg^{-1} which is much higher than that reported by TERRAB *et al.* (2005) for avacado honeys produced in Spain. The mean Ni content was 0.13 mg kg^{-1}, which is much lower than that reported by TERRAB *et al.* (2005).

The literature data regarding the Pb, Co and Ni contents in different kinds of honey samples (LATORRE *et al.*, 1999; YILMAZ and YAVUZ, 1999; BULDINI *et al.*, 2001; CONTI and BOTRE, 2001; PRZYBYŁOWSKI and WILCZYŃSKA, 2001; RASHED and SOLTAN, 2004; ERBILIR and ERDOGRUL, 2005; TERRAB *et al.*, 2005; IOANNIDOU *et al.*, 2005) are illustrated in Table 3. In general, the results re-

Table 3 - Summary of literature data on the Ni, Pb and Co contents of different origin.

Element	Mean value	Site	Reference
Ni	<0.05 ppm	Galician honey, Spain	LATORRE *et al.*, 1999
	nd*	South-East Anatolia	YILMAZ and YAVUZ, 1999
	nd	K.maraş, Turkey	ERBILIR and ERDOGRUL, 2005
	0.33 ppm	Spain	TERRAB *et al.*, 2005
	nd	Greece	IOANNIDOU *et al.*, 2005
	400 ppb	Ashok, India	BULDINI *et al.*, 2001
	400 ppb	Bologna, Italy	BULDINI *et al.*, 2001
	1.25 ppm	Egypt (sesame honey)	RASHED and SOLTAN, 2004
Pb	3.3 ppb	Rome	CONTI and BOTRE, 2001
	0.08 ppm	Spain	TERRAB *et al.*, 2005
	0.048 ppm	Pomerian	PRZYBYŁOWSKI and WILCZYŃSKA, 2001
	710 ppb	Ashok, India	BULDINI *et al.*, 2001
	620 ppb	Bologna, Italy	BULDINI *et al.*, 2001
	6.3 ppm	Egypt (sesame honey)	RASHED and SOLTAN, 2004
Co	<0.05 ppm	Galician honey-Spain	LATORRE *et al.*, 1999
	1 ppm	South-East Anatolia	YILMAZ and YAVUZ, 1999
	0.02 ppm	Spain	TERRAB *et al.*, 2005
	0.071 ppm	Greece	IOANNIDOU *et al.*, 2005
	<50 ppb	Ashok, India	BULDINI *et al.*, 2001
	<50 ppb	Bologna, Italy	BULDINI *et al.*, 2001
	1.75 ppm	Egypt (sesame honey)	RASHED and SOLTAN, 2004

* nd: not detected.

ported in the present study are similar to those reported in the literature. Only a few samples had Ni and Co values that were lower than LOD. The Ni concentration was higher than in our first study (ERBİLİR and ERDOĞRUL, 2005) in which flame atomic absorption was used to analyze the trace element. This difference could be due to the different techniques used. No Ni was detected in the honey samples when the flame AAS technique was used. Therefore graphite furnace AAS was used to analyze the Ni content in the honey samples.

The three elements determined in the honey samples were present in concentrations of less than 1 mg kg^{-1}. It is important to note that low levels of Pb, Ni and Co are appropriate when testing environmental contamination (PRZYBYLOWSKI and WILCZSKA, 2001); the results indicate that there is a low level of contamination in the atmosphere and water in the area where the honey was produced.

REFERENCES

Buldini P.L., Cavalli S., Mevoli A. and Sharma J.L. 2001. Ion chromatographic and voltammetric determination of heavy and transition metals in honey. Food Chem. 73: 487.

Conti M.E. and Botrè F. 2001. Honeybees and their products as potential bioindicators of heavy metals contamination. Environ. Monit. Assess. 69: 267.

Crane E. 1975. "Honey: A Comprehensive Survey". Heinemann, London.

Devillers J., Dore J.C., Marenco M., Poirier-Duchene F., Galand N. and Viel C. 2002. Chemometrical analysis of 18 metallic and nonmetallic elements found in honey sold in France. J. Agr. Food Chem. 50: 5998.

DİE 2003. Devlet İstatistik Enstitüsü, Ankara, Turkey (in Turkish).

Erbilir F. and Erdoğrul Ö. 2005. Determination of heavy metals in honey in Kahramanmaraş city, Turkey. Environ. Monit. Assess. 109: 181.

Huang Y.L., Chuang I.C., Pan C.H., Hsiech C., Shi T.S., and Lin T.H. 2000. Determination of chromium in whole blood and urine by graphite furnace AAS. Atom. Spectrosc. 21: 10.

Ioannidou M.D., Zachariadis G.A Anthemidis A.N. and Stratis J.A. 2005. Direct determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry. Talanta 65: 92.

Latorre M.J., Peńa R., Pita C., Botana A., García S. and Herrero C. 1999. Chemometric classification of honeys according to their type. II. Metal content data. Food Chem. 66: 263.

Perkin-Elmer 1982. Analytical methods for atomic absorption spectrophotometry. Perkin Elmer Manual. Perkin-Elmer Corp. USA.

Przybylowski P. and Wilczska A. 2001. Honey as an environmental marker. Food Chem. 74: 289.

Rashed M.N. and Soltan M.E. 2004. Major and trace elements in different types of Egyptian mono floral and non-floral bee honeys. J. Food Comp. Anal. 17: 725.

Rodriguez J.C., Barciela J., Herrero C., Garcia S. and Pena R. 2005. Direct and combined methods for the determination of chromium, copper, and nickel in honey by electrothermal atomic absorption spectroscopy. J. Agr. Food Chem. 53: 6616.

Slavin W., Carnrick G.R., Amnning D.C. and Pruszkowska E. 1983. Recent experiences with the stabilized temperature platform furnace and Zeeman background correction. Atom. Spectrosc. 4: 69.

Schlemmer G., and Radzick B. 1999. Analytical gpahite furnace atomic absorption spectrometry. Birkhäuser. Basel.

Sevimli H., Bayülgen N. and Varınlıoğlu A. 1992. Determination of trace elements in honey by INAA in Turkey. J. Radionanal. Nuclear Chem. Lett. 165: 319.

Tahvonen R. and Kumpulainen J. 1994. Determination of lead at low concentration in food samples by electrothermal atomic absorption spectrometry. JAAS. 9: 1427.

Terrab A., Recamales A.F., Gonzalez-miret M.L. and Heredia F.J. 2005. Contribution to the study of avocado honeys by their contents using inductively coupled plasma optical emission spectrometry. Food Chem. 92: 305.

Tüzen M., Özdemir M., and Demirbaş A. 1998. Heavy metal bioaccumulation by cultivated *Agaricus bisporus* from artificially enriched substrates. Z. Lebens.Unter. Forsch. 206: 417.

Yılmaz H. and Yavuz Ö. 1999. Content of some trace metals in honey from south-eastern Anatolia. Food Chem. 65: 475.

Revised paper received May 13, 2008 Accepted June 24, 2008

ERRATA CORRIGE

In the paper "Traditional Italian Tomato (*Lycopersicon esculentum* Mill.) Cultivars and Their Commercial Homologues: Differences in Volatile Composition", by M.T. Lisanti, P. Piombino, A. Genovese, R. Pessina and L. Moio, published in Vol. 20, N. 3, 2008, Table 1 on pages 338, 339, 340, referring to two different groups of samples (San Marzano, Ranco F1, Corbarino, Faino F1 and Vesuviano, Principe Borghese, Sorrento Cuore di Bue), was misprinted.

IJFS apologizes for the error. The table should appear as follows:

Table 1 - Volatile compounds detected in the tomato cultivars.

peak no.	compound °	concentration µg I.S./ 100g §							
		San Marzano		Ranco F1		Corbarino		Faino F1	
	Aldehydes								
3	**hexanal** (a, b, c, d, e, f)	15.87 ± 3.232		9.77 ± 3.082		16.55 ± 2.012		9.02 ± 0.348	*
4	**(E)-2-pentenal** (d, e)	3.82 ± 0.140		2.39 ± 0.500	*	3.50 ± 0.771		1.74 ± 0.290	
7	heptanal	0.34 ± 0.002		0.57 ± 0.177		0.42 ± 0.103		0.23 ± 0.078	
8	**(E)-2-hexenal** (a, b, d, e)	0.75 ± 0.052		0.59 ± 0.009	*	1.04 ± 0.092		0.68 ± 0.092	*
14	**(E)-2-heptenal** (d)	3.26 ± 1.014		1.84 ± 0.342		1.44 ± 0.338		1.47 ± 0.310	
19+20+21	**(Z) - 3-hexen-1-ol** (a, b, d) + (E,E)-2,4-hexadienal+ NI	0.47 ± 0.087		0.49 ± 0.042		0.73 ± 0.239		0.49 ± 0.118	
24	(E)-2-octenal	2.54 ± 1.020		2.37 ± 0.434		1.78 ± 0.662		1.90 ± 0.533	
29	(E,E)-2,4-heptadienal	0.24 ± 0.060		0.17 ± 0.042		0.30 ± 0.082		0.06 ± 0.033	*
31	**benzaldehyde** (d)	0.21 ± 0.011		0.15 ± 0.043		0.17 ± 0.032		0.11 ± 0.028	
35	(E,E)-2,4-octadienal	0.22 ± 0.040		0.21 ± 0.052		0.14 ± 0.026		0.20 ± 0.057	
37	**β-cyclocitral** (d)	0.11 ± 0.013		0.08 ± 0.018		0.14 ± 0.032		0.07 ± 0.017	
38	2-hydroxybenzaldehyde	0.07 ± 0.004		nd		tr		nd	
40	**(Z)-citral (neral)** (d)	0.02 ± 0.003		0.03 ± 0.011		0.06 ± 0.012		0.03 ± 0.004	
41	**(E)-citral (geranial)** (d)	0.10 ± 0.006		0.09 ± 0.020		0.22 ± 0.059		0.05 ± 0.001	*
44	**(E,E)-2,4-decadienal** (g)	0.08 ± 0.016		0.07 ± 0.010		0.07 ± 0.017		0.04 ± 0.014	
	Total aldehydes	28.08 ± 0.915		18.79 ± 0.849		26.55 ± 0.615		16.09 ± 0.210	
	Ketones								
1	**1-penten-3-one** (b, d, f, e)	18.83 ± 1.902		10.84 ± 3.288		12.83 ± 3.034		5.84 ± 1.094	
13	**1-octen-3-one** (f)	0.34 ± 0.012		0.60 ± 0.163		0.19 ± 0.004		ne	
16	**6-methyl-5-hepten-2-one** (b, c, d)	36.24 ± 3.978		25.07 ± 4.765		55.35 ± 13.123		27.36 ± 2.065	
36	6-methyl-3,5-heptadien-2-one	0.21 ± 0.015		0.15 ± 0.012	*	0.40 ± 0.088		0.18 ± 0.000	*
46	**geranylacetone** (d)	0.06 ± 0.001		0.07 ± 0.013		0.04 ± 0.006		0.03 ± 0.001	
50	**β-ionone** (b, d, g)	0.03 ± 0.001		0.02 ± 0.003	*	0.02 ± 0.001		tr	
	Total ketones	55.70 ± 1.800		36.75 ± 2.365		68.81 ± 5.499		33.40 ± 1.168	
	Alcohols								
5	1-butanol	0.46 ± 0.155		ne		nd		nd	
6	**1-penten-3-ol** (d)	0.81 ± 0.157		0.34 ± 0.076	*	0.50 ± 0.092		0.29 ± 0.006	
9	**2+3-methyl-1-butanol** (b, e)	0.99 ± 0.052		0.40 ± 0.125		2.78 ± 0.163		1.24 ± 0.042	*
12	**1-pentanol** (d)	3.31 ± 0.670		1.72 ± 0.431	*	2.01 ± 0.418		1.57 ± 0.019	
15	(Z)-2-penten-1-ol	nd		nd		nd		nd	
17	**1-hexanol** (a, d)	0.25 ± 0.061		0.08 ± 0.021		0.36 ± 0.021		0.23 ± 0.047	*
28	**6-methyl-5-hepten-2-ol** (d)	0.45 ± 0.225		0.20 ± 0.013		0.36 ± 0.106		0.57 ± 0.057	
30	2-ethyl-1-hexanol	nd		nd		nd		nd	
48	benzyl alcohol	0.05 ± 0.001		0.04 ± 0.006	*	0.06 ± 0.012		0.06 ± 0.019	
49	**2-phenylethyl alcohol** (e, d, c)	0.11 ± 0.030		0.14 ± 0.038		0.10 ± 0.043		0.05 ± 0.001	
	Total alcohols	6.44 ± 0.264		2.93 ± 0.173		6.16 ± 0.179		4.00 ± 0.034	

Table 1 (continued).

peak no.	compound °	concentration µg I.S./ 100g §				
		San Marzano	Ranco F1	Corbarino	Faino F1	
	Esters					
2	ethyl 3-methylbutyrate	0.31 ± 0.011	ne	0.77 ± 0.101	0.32 ± 0.022	**
11	ethyl hexanoate	nd	ne	ne	nd	
18	methyl octanoate	nd	nd	nd	nd	
25	ethyl octanoate	nd	nd	nd	nd	
42	**methyl salicylate** (d)	3.84 ± 1.271	3.18 ± 0.599	1.61 ± 0.420	1.66 ± 0.671	
43	ethyl salicylate	3.08 ± 1.530	2.57 ± 1.266	1.21 ± 0.138	0.60 ± 0.116	*
	Total esters	7.23 ± 1.148	5.75 ± 0.990	3.58 ± 0.262	2.58 ± 0.393	
	Acids					
26	acetic acid	0.05 ± 0.007	0.03 ± 0.004	0.06 ± 0.011	0.11 ± 0.032	
39	isovaleric acid	nd	ne	tr	nd	
45	hexanoic acid	0.02 ± 0.006	0.03 ± 0.008	0.06 ± 0.020	0.03 ± 0.011	
	Total acids	0.07 ± 0.007	0.05 ± 0.006	0.11 ± 0.016	0.14 ± 0.024	
	Phenols					
47	guaiacol	1.11 ± 0.208	0.70 ± 0.123	0.40 ± 0.192	0.28 ± 0.066	
51	phenol	0.19 ± 0.025	0.16 ± 0.038	0.16 ± 0.030	0.15 ± 0.025	
	Total phenols	1.31 ± 0.148	0.87 ± 0.091	0.56 ± 0.137	0.44 ± 0.050	
	Sulphur compounds					
22	2-isobutylthiazole	1.16 ± 0.609	3.44 ± 0.738	6.68 ± 1.213	18.8 ± 3.685	*
32	3-methylisothiazole	nd	0.05 ± 0.004	0.04 ± 0.009	0.05 ± 0.003	
34	dimethylsulfoxide	nd	nd	0.07 ± 0.059	nd	
	Total sulphur compounds	1.16 ± 0.609	3.49 ± 0.522	6.80 ± 0.701	18.87 ± 2.605	
	Oxygen-containing compounds					
10	2-pentylfuran	0.37 ± 0.037	0.36 ± 0.054	0.31 ± 0.080	0.38 ± 0.056	
27	furfural	nd	nd	nd	0.18 ± 0.061	
33	5-methylfurfural	nd	0.06 ± 0.005	nd	0.07 ± 0.026	
	Total oxygen-containing comporids	0.37 ± 0.037	0.42 ± 0.039	0.31 ± 0.080	0.63 ± 0.050	
	Unsaturated hydrocarbons					
23	3-ethyl-2-methyl-1,3-hexadiene †	nd	nd	nd	nd	

Table 1 (continued).

peak no.	compound °	Vesuviano	P.Borghese	Sorrento	C. di Bue
	Aldehydes				
3	**hexanal** (a, b, c, d, e, f)	10.01 ± 2.414	17.03 ± 1.570 *	6.58 ± 1.907	12.27 ± 3.250
4	**(E)-2-pentenal** (d, e)	2.71 ± 0.378	4.97 ± 0.819 *	4.61 ± 0.051	4.17 ± 1.135
7	heptanal	ne	0.52 ± 0.070	0.13 ± 0.069	ne
8	**(E)-2-hexenal** (a, b, d, e)	0.76 ± 0.214	1.39 ± 0.199 *	1.11 ± 0.285	1.42 ± 0.598
14	**(E)-2-heptenal** (d)	1.10 ± 0.271	1.22 ± 0.434	2.63 ± 0.088	1.98 ± 0.131 **
19+20+21	**(Z)-3-hexen-1-ol** (a, b, d) + (E,E)-2,4-hexadienal+ NI	0.37 ± 0.130	0.49 ± 0.111	0.39 ± 0.147	0.38 ± 0.038
24	(E)-2-octenal	1.08 ± 0.211	1.45 ± 0.262	0.75 ± 0.037	1.58 ± 0.258 *
29	(E,E)-2,4-heptadienal	0.22 ± 0.039	0.30 ± 0.094	0.11 ± 0.026	0.35 ± 0.087 *
31	**benzaldehyde** (d)	0.14 ± 0.020	0.22 ± 0.057	0.11 ± 0.032	0.27 ± 0.069
35	(E,E)-2,4-octadienal	0.12 ± 0.029	tr	0.03 ± 0.012	0.20 ± 0.058 *
37	**β-cyclocitral** (d)	0.08 ± 0.016	0.09 ± 0.016	0.08 ± 0.029	0.10 ± 0.023
38	2-hydroxybenzaldehyde	0.05 ± 0.010	nd	tr	nd
40	**(Z)-citral (neral)** (d)	0.03 ± 0.002	tr	0.03 ± 0.001	nd
41	**(E)-citral (geranial)** (d)	0.09 ± 0.026	0.05 ± 0.004	0.10 ± 0.001	tr
44	**(E,E)-2,4-decadienal** (g)	0.03 ± 0.004	nd	0.06 ± 0.018	ne
	Total aldehydes	16.81 ± 0.663	27.71 ± 0.561	16.70 ± 0.518	22.73 ± 1.110
	Ketones				
1	**1-penten-3-one** (b, d, f, e)	13.56 ± 2.438	20.60 ± 3.946	23.37 ± 0.702	19.74 ± 4.765
13	**1-octen-3-one** (f)	ne	0.61 ± 0.065	0.46 ± 0.019	1.08 ± 0.218 **
16	**6-methyl-5-hepten-2-one** (b, c, d)	27.37 ± 7.085	51.48 ± 13.50	25.18 ± 4.676	20.82 ± 6.128
36	6-methyl-3,5-heptadien-2-one	0.23 ± 0.045	tr	0.14 ± 0.044	0.11 ± 0.029
46	**geranylacetone** (d)	0.05 ± 0.010	nd	0.01 ± 0.006	0.03 ± 0.008
50	**β-ionone** (b, d, g)	nd	nd	nd	nd
	Total ketones	41.22 ± 3.746	72.69 ± 8.121	49.16 ± 2.115	41.79 ± 3.473
	Alcohols				
5	1-butanol	nd	0.66 ± 0.137	nd	ne
6	**1-penten-3-ol** (d)	0.49 ± 0.029	0.96 ± 0.085 **	0.52 ± 0.046	1.42 ± 0.350
9	**2+3-methyl-1-butanol** (b, e)	2.88 ± 0.816	2.25 ± 0.177	3.06 ± 0.647	2.58 ± 0.633
12	**1-pentanol** (d)	1.63 ± 0.146	2.87 ± 0.411 *	1.47 ± 0.354	3.29 ± 0.830
15	(Z)-2-penten-1-ol	nd	nd	nd	0.68 ± 0.111
17	**1-hexanol** (a, d)	0.33 ± 0.110	0.36 ± 0.126	0.12 ± 0.034	0.31 ± 0.057
28	**6-methyl-5-hepten-2-ol** (d)	0.24 ± 0.017	0.22 ± 0.045	0.27 ± 0.040	0.36 ± 0.001
30	2-ethyl-1-hexanol	nd	nd	0.18 ± 0.048	0.20 ± 0.046
48	benzyl alcohol	0.03 ± 0.010	0.04 ± 0.006	0.05 ± 0.018	0.05 ± 0.009
49	**2-phenylethyl alcohol** (e, d, c)	tr	0.08 ± 0.000	0.18 ± 0.042	0.05 ± 0.011 *
	Total alcohols	5.61 ± 0.342	7.44 ± 0.175	5.85 ± 0.263	8.95 ± 0.370

Table 1 (continued).

peak no.	compound °	Vesuviano	P.Borghese	Sorrento	C. di Bue
	Esters				
2	ethyl 3-methylbutyrate	0.52 ± 0.112	0.87 ± 0.002 *	1.11 ± 0.532	1.26 ± 0.183
11	ethyl hexanoate	ne	nd	nd	nd
18	methyl octanoate	nd	nd	nd	0.09 ± 0.015
25	ethyl octanoate	nd	nd	nd	0.21 ± 0.044
42	**methyl salicylate** (d)	0.66 ± 0.062	4.39 ± 0.573 *	2.83 ± 0.347	2.40 ± 1.337
43	ethyl salicylate	0.80 ± 0.244	2.49 ± 0.028 *	0.42 ± 0.122	1.66 ± 0.692
	Total esters	1.98 ± 0.159	7.75 ± 0.331	4.36 ± 0.373	5.62 ± 0.678
	Acids				
26	acetic acid	0.13 ± 0.021	0.15 ± 0.039	0.12 ± 0.046	0.12 ± 0.009
39	isovaleric acid	tr	0.03 ± 0.008	0.03 ± 0.008	0.02 ± 0.006
45	hexanoic acid	0.02 ± 0.007	0.04 ± 0.013	0.02 ± 0.003	0.04 ± 0.009 *
	Total acids	0.16 ± 0.015	0.23 ± 0.024	0.17 ± 0.027	0.18 ± 0.008
	Phenols				
47	guaiacol	0.21 ± 0.051	1.01 ± 0.119 **	0.60 ± 0.170	0.98 ± 0.018
51	phenol	0.13 ± 0.015	0.19 ± 0.009 *	0.13 ± 0.024	0.24 ± 0.019 **
	Total phenols	0.34 ± 0.038	1.20 ± 0.084	0.73 ± 0.121	1.22 ± 0.019
	Sulphur compounds				
22	2-isobutylthiazole	7.02 ± 2.234	8.02 ± 1.548	2.08 ± 0.864	1.96 ± 0.719
32	3-methylisothiazole	0.05 ± 0.004	nd	ne	0.04 ± 0.010
34	dimethylsulfoxide	nd	nd	nd	nd
	Total sulphur compounds	7.07 ± 1.580	8.02 ± 1.548	2.08 ± 0.864	1.99 ± 0.508
	Oxygen-containing compounds				
10	2-pentylfuran	0.10 ± 0.043	0.26 ± 0.027 *	0.19 ± 0.074	0.60 ± 0.117 *
27	furfural	ne	0.14 ± 0.013	nd	nd
33	5-methylfurfural	0.06 ± 0.015	0.11 ± 0.031	nd	0.07 ± 0.019
	Total oxygen-containing componds	0.16 ± 0.032	0.50 ± 0.025	0.19 ± 0.074	0.67 ± 0.084
	Unsaturated hydrocarbons				
23	3-ethyl-2-methyl-1,3-hexadiene [t]	nd	nd	nd	0.31 ± 0.063

Bold type indicates characteristic impact flavour or aroma compounds in tomato, as reported in the literature in brackets. References: a) RUIZ *et al.*, 2005; b) BUTTERY *et al.*, 1987; c) LANGLOIS *et al.*, 1996; d) STERN *et al.*, 1994; e) TANDON *et al.*, 2000; f) KRUMBEIN and AUERSWALD, 1998; g) BUTTERY *et al.*, 1971.

§ Means of triplicate samples. * Single asterisk indicates significant difference between the homologues at $p<0.05$. ** Double asterisks indicate significant difference between the homologues at $p<0.01$. ne=not evaluated. nd=not detected. tr=traces. t=compound tentatively identified by the comparison of mass spectral data with those of Wiley and NIST-98 libraries.

BOOKS

LYCOPENE: NUTRITIONAL, MEDICINAL AND THERAPEUTIC PROPERTIES

Authors: Victor R. Preedy: Department of Nutrition and Dietetics, King's College, London, UK; Ronald R. Watson: School of Medicine, University of Arizona, Tuscon, USA
Editors: Enfield, N.H.: Science Publishers
ISBN: 978-1-57808-538-5
August 2008
ca. 480 pages, hc
Price: £ 65.00

Tomatoes have become a dietary staple for humans in many parts of the world. The characteristic deep red color of the ripe tomato fruit and related products is mainly due to lycopene. Lycopene is the predominant carotenoid in tomatoes, followed by α-carotene, β-carotene, γ-carotene, and phytoene, as well as by several other minor carotenoids. Tomatoes and tomato-based foods have long been an important source of lycopene in the Western diet.

There has been a growing interest in exploring the role of lycopene in the prevention of a variety of nutritional and health issues in humans, including some cancers and cardiovascular diseases. Recently, many case studies using cell cultures, animal models, and epidemiological investigations have shown a relationship between lycopene intake and a lowered risk of contracting some cancers and various chronic diseases. Increasingly, clinical evidence supports the role of lycopene as a nutrient with important health benefits, since it appears to provide protection against a broad range of epithelial cancers. The possibility that consumption of lycopene-rich foods may reduce the risk of such diseases has prompted numerous in-depth studies of the levels of lycopene in foods and of correlations between dietary lycopene and certain diseases. This monograph will serve as a reference for providing a better understanding of the role of lycopene in promoting health, and by encouraging a deeper understanding of approaches to a healthy diet and life.

TOMATOES AND TOMATO PRODUCTS: NUTRITIONAL, MEDICINAL AND THERAPEUTIC PROPERTIES

Authors: Victor R. Preedy: Department of Nutrition and Dietetics, King's College, London, UK; Ronald R. Watson: School of Medicine, University of Arizona, Tuscon, USA
Editors: Enfield, N.H.: Science Publishers
ISBN: 978-1-57808-534-7
May 2008
ca. 670 pages, hc
Price: £ 75.60

The tomato (*Lycopersicon esculentum*) is a vegetable crop of the solanaceae family that originated in the Andean region of South America. It increased in popularity and was rapidly expanded into large-scale cultivation during the last half-century. During this period, considerable progress was made in tomato breeding and use, such as advances in increased yields, improved quality, better handling and storage durability, improved pest resistance, expanded processing techniques, and the development of a plethora of new tomato-based products. Tomato production has been transformed into a large agricultural industry, and tomato is one of the most important commercially produced vegetables in the world.

Tomato quality is a function of several factors including the choice of cultivar, cultural practices, harvest time and method, storage, and handling procedures. The tomato processing industry has made tremendous advances, developing many forms of tomato-based foods, such as sauces, catsup (ketchup), puree, pastes, soups, juices and juice blends, and canned tomatoes either whole or in diced, sliced, quartered or stewed form. Tomatoes and tomato products are rich sources of vitamin C and A, lycopene, α-carotene, lutein, lectin, and a variety of phenolic compounds such as flavonoids and phenolic acids. They are rich in folates, potassium, fiber, and protein, but low in fat and calories, as well as being cholesterol-free.

NEWS

IDF WORLD DAIRY SUMMIT
Berlin, Germany
20-24 September, 2009

The International Dairy Federation (IDF) and the German National Committee of IDF are preparing stimulating conferences to cater for a wide range of interests in Berlin, 20-24 September 2009. As preparations for the conference gather pace, many senior representatives from dairy economy, politics and dairy science have already signed up for this leading event of the year.

Under the theme United Dairy World the IDF World Dairy Summit will assemble expertise from many leading experts from east and west. There will be plenty of time to get to know each other better and to discuss the achievements and the future of the global trade of dairy products.

Complementing the conferences and business sessions there will be a range of social events and technical tours designed to provide a memorable glimpse of Berlin. Many international fairs and other cultural highlights will take place at that time to celebrate the 20th anniversary of the re-united Germany. Before the start of the Summit, the international Berlin Marathon will take place: more than 40,000 participants from more than 100 nations will give the city an international flair. Hotel capacities will be exhausted.

To book your place at this exciting event, or for more information about the IDF World Dairy Summit 2009, please visit http://www.wds2009.com.

CONTRIBUTORS

Gratitude is expressed to the following entities for contributing to the realization of the Journal by being supporting subscribers for 2009.

Si ringraziano i seguenti Enti, Ditte ed Istituti per aver voluto contribuire fattivamente alla realizzazione della Rivista, sottoscrivendo un abbonamento sostenitore per il 2009.

ASSOCIATIONS

Associazione Italiana di Tecnologia Alimentare (A.I.T.A.) - Milano Fax +39-02-2365015
www.aita-nazionale.it

Società Italiana di Scienze e Tecnologie Alimentari (S.I.S.T.Al) - Perugia Fax +39-075-5857939
www.sistal.org

INDUSTRIES

Soremartec Italia srl - Alba Fax +39-0173-313966

Tecnoalimenti scpa - Milano Fax +39-02-67077405

RESEARCH INSTITUTES

Dipartimento di Scienze Tecnologie Agroalimentari (D.I.S.T.A.),
Facoltà di Agraria,
Università degli Studi della Tuscia, Viterbo Fax +39-0761-357498

Dipartimento di Ingegneria e Tecnologie Agro-Forestali,
Università di Palermo, Palermo Fax +39-091-484035

Dipartimento di Scienze degli Alimenti,
Università di Udine, Udine Fax +39-0432-558130

Dipartimento di Scienze e Tecnologie Agroalimentari e
Microbiologiche (DI.S.T.A.A.M.),
Università del Molise, Campobasso Fax +39-0874-404652

Dipartimento di Scienze e Tecnologie Alimentari
e Microbiologiche (DI.S.T.A.M.), Università di Milano, Milano Fax +39-02-50316601

Dipartimento di Valorizzazione e Protezione delle Risorse
Agroforestali (DI.VA.P.R.A.), Sezione Microbiologia
ed Industrie Agrarie, Università di Torino, Grugliasco Fax +39-011-6708549

ITALIAN JOURNAL OF FOOD SCIENCE
Rivista Italiana di Scienza degli Alimenti
DIRETTORE RESPONSABILE: Giovanni Chiriotti
AUTORIZZAZIONE: n. 3/89 in data 31/1/1989
del Tribunale di Perugia
Proprietà dell'Università di Perugia
TIPOGRAFIA Giuseppini - Pinerolo
Una copia € 6.00

ISSN 1120-1770 © 2009

CHIRIOTTI EDITORI sas - 10064 Pinerolo - Italy

VOLUME XXI IJFS No. 1, 2009

CONTENTS