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PAPER

STUDIES ON PREPARATION OF MIXED TOFFEE FROM AONLA AND GINGER

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ABSTRACT

Studies were conducted to develop a technology for the preparation of mixed toffee from aonla pulp and ginger extract and to evaluate the changes in the quality of prepared toffees during storage for 90 days under both ambient and refrigerated conditions. Among the various blends of aonla pulp and ginger extract evaluated, toffee prepared from an 80:20 w/w (aonla pulp: ginger extract) blend was found to be superior to other blends in terms of yield, organoleptic properties and nutritional quality. The cost of toffee prepared from an 80:20 (aonla pulp:ginger extract) blend was Rs. 70.78/kg. Storage studies of toffee packed in 200 gauge polyethylene bags indicated that the content of TSS, reducing and total sugars increased as the duration of the storage period increased, whereas the moisture and acidity content decreased. The rate of reaction was relatively higher at the ambient temperature than at the refrigerated temperature. Although the sensory quality of the toffees also decreased more rapidly during 90 days of storage under ambient conditions than under refrigeration, the toffees were found to be acceptable even after 90 days under either condition.

- Keywords: aonla, ginger, toffee, mixed toffee -

INTRODUCTION

Aonla is one of the richest sources of vitamin C and of polyphenols, and these polyphenols are considered to have a high medicinal value. As a result, the fruit has acquired an important therapeutic role in the Ayurvedic and Unani systems of medicine (MEHTA and RATHORE, 1979). It contains 20 times more vitamin C than citrus (GOYAL *et al.*, 2008). Ascorbic acid has several uses in food processing. It acts as a preservative to prevent enzymatic browning during processing and is also an antioxidant. Moreover, ascorbic acid promotes both clarity and the preservation of taste and flavour (CHAUHAN *et al.*, 1998). The fruit is valued, e.g., for its antiscorbutic, diuretic, laxative and cooling properties (RADHA and MATHEW, 2007). It is seldom consumed as fresh/raw fruit because it is astringent in taste. Aonla has substantial potential for value addition because its consumption in fresh form is extremely low due to its highly acidic and astringent taste (TONDON and KUMAR, 2005). It can be processed to yield a variety of products, e.g., juice, preserves, murraba, pickle, concentrates, squash, syrup and dehydrated amla (KALRA, 1988).

Ginger is widely used in foods, beverages, confectionery and medicines. It is the most effective flavouring agent known and is used in confectionery, ginger beer, ginger champagnes and beverages. Ginger is also used as preserved ginger and candied ginger and as a carminative and digestive stimulant. Ginger is valued for its manifold medicinal properties and is useful in gastritis, dyspepsia and flatulence and in colds and coughs as an expectorant (ARYA, 2003). Toffee is a confectionery product. It is reported that pulpy fruits, e.g., mango, guava, papaya, fig, chikku, jackfruit or aonla, can be employed for the preparation of fruit toffee. Such fruit toffees are naturally very nutritious, as they contain most of the constituents of the fruit from which they are made (JAIN *et al.*, 1958). Spices may also be used in toffee preparation to give good flavour and increase the shelf life of the product. However, very little work has been conducted on mixed toffees.

This study was conducted to prepare mixed toffees by combining aonla pulp with ginger extract and to evaluate the storage stability of the product. This toffee blend provides good nutrition as well as several medicinal benefits to the consumer. Aonla is a good source of ascorbic acid, and ginger helps to prevent colds and coughs.

MATERIALS AND METHODS

Plant material

Fully matured aonla (NA-7) fruits and ginger (local) rhizomes were obtained for this project. The aonla fruits were obtained from the All India Coordinated Research Project on Dry Land Fruit Crops of the Department of Horticulture, Mahatma Phule Krishi Vidyapeeth, Rahuri, and the ginger rhizomes were obtained from a local market.

Chemicals and additives

All chemicals used in this investigation were of analytical grade. Cane sugar, hydrogenated fat, salt and skim milk powder were obtained from a local market and used as ingredients for the preparation of mixed toffee from aonla and ginger.

Packaging materials

Butter paper, metal-coated polythene wrappers and LDPE or polythene 200 gauge bags were obtained from a local market.

Extraction of pulp

Fully mature aonla fruits with a firm texture and uniform in size were blanched and used for the experiment. The fruits were processed for extraction of pulp with a home-scale pulping machine to obtain a fine pulp. The ginger rhizomes were washed in clean water and passed through the home-scale pulping machine to obtain a fine pulp with the addition of water (1:1; w/w), and ginger extract was obtained by straining the resulting pulp through muslin cloth.

Standardisation of toffee recipe

Aonla-ginger mixed toffees were first prepared from 11 blends involving different levels of pulp and ginger extract. The other ingredients, such as sugar, hydrogenated fat, skim milk powder and salt were kept constant (Table 1). The preferable level of pulp and extract was finalised based on the sensory evaluation of the toffees by a semi-trained panel of ten judges using a 9-point hedonic scale (AMERINE *et al.*, 1965).

Preparation of toffee

Following standardisation, four types of toffees were prepared using the optimum ratios of aonla pulp: ginger extract: 100:00 (control), 85:15, 80:20 and 75:25 w/w. The other ingredients, such as 750 g sugar, 50 g butter fat, 50 g skim milk powder and 2 g salt per kg pulp, were kept constant. The homogenised pulps were placed in a stainless steel container and mixed well with the other ingredients (e.g., sugar, butter fat and skim milk powder) according to the selected treatment protocol. The mixture was heated until the TSS content reached 80°Brix. Salt was dissolved in a small quantity of water, mixed with the above mixture and again heated until the TSS of the contents reached 82-83°Brix. The heated mass was spread thinly on a stainless steel plate that had

Table 1 - Various blends of aonla pulp and ginger extract for the preparation of mixed toffee.

Aonla pulp (%)	Ginger extract (%)	Organoleptic overall acceptability*	Rank/Remark	Ranking for further study
100	00	7.15	Control	T1
95	05	7.20	Not selected	-
90	10	7.23	Not selected	-
85	15	8.25	Selected for further study	T2
80	20	8.85	Selected for further study	T3
75	25	8.43	Selected for further study	T4
70	30	7.23	Not selected	-
65	35	6.66	Not selected	-
60	40	6.34	Not selected	-
55	45	5.65	Not selected	-
50	50	5.46	Not selected	-

Other ingredients, such as sugar: 750 g; fat: 50 g; skim milk powder: 50 g; and salt: 2 g were kept constant for all blends. Four replications.
*Nine-point hedonic scale; 10 semi-trained judges were used for sensory evaluation.

previously been smeared with fat, resulting in a sheet 1 to 2 cm in thickness. This sheet was allowed to cool and set for two to three hours, and the solid sheet was then cut into cubes measuring 1.5 to 2.5 cm on a side with a stainless steel knife (PARPIA, 1967).

Chemical analysis of toffee

The toffee was chemically analysed for moisture, TSS, acidity, reducing sugar and total sugar content according to the standard methods of A.O.A.C. (1990).

Sensory evaluation of toffee

The sensory evaluation of aonla-ginger mixed toffee was conducted according to the standard procedure (AMERINE *et al.,* 1965) on a nine-point hedonic scale. The mean score obtained from a minimum of 10 semi-trained judges for each quality parameter, namely, colour and appearance, texture, taste, flavour and overall acceptability, was recorded.

Packaging and storage of toffees

The prepared toffees were wrapped in metal-coated polyethylene wrappers. Four replications were used. The wrapped toffees were packed in plastic bags (200 gauge) and stored at the ambient temperature (27°±2°C) as well as under refrigeration (10°±2°C) for up to 90 days. The stored toffees were evaluated for chemical composition, sensory properties and microbial quality at intervals of 30 days.

Microbial quality of toffees

Microbial counts were recorded using a standard plate count (SPC). Each colony was counted. Tryptone dextrose yeast extract agar was used as the growth medium, and petri dishes were incubated at 37°±5°C for 48 h to count bacterial colonies. The colonies were counted with a magnifying lens. The total count was recorded, and pinpoint colonies were likewise noted.

Statistical analysis

The data were analysed according to a factorial completely randomised design (FCRD) with four replications for statistical significance, as specified by PANSE and SUKHATME (1967).

RESULTS AND DISCUSSION

The recovery of aonla pulp was found to be 975 g/kg of fruit without straining, and the recovery of ginger extract was found to be 820 g/kg of rhizome. KOHINKAR *et al.* (2012) have reported 99% recovery of fig pulp and 65% recovery of guava pulp. PAWAR *et al.* (1992) have reported that fig fruits consist of 84% skin and 16% seeds. KHANDEKAR *et al.* (2005) have reported a fig pulp recovery value of 995.50 g/kg of fruit.

The toffee prepared from 80:20 aonla pulp:ginger extract and 750 g sugar, 50 g butter fat, 50 g skim milk powder and 2 g salt/kg of pulp was found to be superior in colour and appearance, texture, taste, flavour and overall acceptability to those prepared from other blends (Table 1).

The yield of aonla-ginger mixed toffee ranged from 1.124 to 1.240 kg/kg of pulp (Table 2). It has been reported that the yield of fig toffees ranged from 1.218 to 1.220 kg/kg of pulp (KHANDEKAR *et al.,* 2005). Additionally, the yield of guava toffees has been reported as 1.410 to 1.360 kg/kg of pulp (JAIN *et al.*, 1958). It has been reported that the yield of custard apple toffee increased to 1.35 kg/kg of pulp with an increase in the sugar level (DHUMAL *et al.,* 1996). The 165 yield of tamarind, 166 mango, and papaya blended toffees has been reported as 1.196 to 1.210 kg/kg of pulp (NALE *et al.,* 2007; KAUSHAL *et al.*, 2001; KERAWALA and SIDDAPPA 1963a, 1963b).

Table 2 - Yield and chemical composition of aonla-ginger mixed toffee.

Treatment (Aonla:Ginger)	Yield (kg/kg of pulp)	Moisture (%)	TSS (°Brix)	Acidity (%)	Reducing sugars (%)	Total sugars (%)	Ascorbic acid (mg/100 g)
T1 = 100:00	1.240	8.60	82.40	0.47	35.71	55.18	145.90
T2 = 85:15	1.180	8.43	83.90	0.43	33.81	52.07	128.05
T3 = 80:20	1.148	8.49	84.00	0.40	33.77	52.04	116.44
T4 = 75:25	1.124	8.38	84.35	0.39	34.23	51.60	107.42
Mean	1.173	8.48	83.66	0.42	34.13	52.72	124.45
S.E. ±	0.043	0.020	0.024	0.002	0.020	0.022	0.024
CD at 5% (n=4)	0.130	0.058	0.070	0.006	0.057	0.064	0.068

The moisture content of aonla-ginger mixed toffee ranged from 8.4 to 8.6%. Significant differences in the moisture content of toffee have been found. It has been reported that the moisture content of guava toffees ranged from 8.3 to 8.5% (JAIN *et al.*, 1958). The moisture content of fig toffees has been found to range from 8.4 to 8.5% (KHANDEKAR *et al.,* 2005).

The Total Soluble Solids (TSS) content of aonla-ginger mixed toffee ranged from 82.4 to 84.4°Brix. The 80:20 blend had a higher TSS content than the 85:15 blend and the control but a lower TSS content than the 75:25 blend. The TSS content was found to increase with increases in the level of ginger extract. The TSS content of the blends differed significantly. The TSS content of fig toffee has been found to range from 82.5 to 83.7°Brix (KHANDEKAR *et al.,* 2005). The TSS content of guava fruit toffee has been found to range from 82.1 to 82.4°Brix. The TSS content of custard apple toffee has been found to range from 82.4 to 82.8°Brix (DHUMAL *et al.*, 1996). The TSS content of tamarind, mango and papaya blended toffee has been found to range from 84.2 to 84.8°Brix (NALE *et al.,* 2007; SIDDAPPA and KERAWALA, 1963a, 1963b, 1963c).

The titratable acidity of aonla-ginger mixed toffee ranged from 0.39 to 0.47%. The control had 0.47% acidity, whereas the 85:15, 80:20 and 75:25 blends had 0.43, 0.40 and 0.39% acidity, respectively.

The reducing sugar content of aonla-ginger mixed toffee ranged from 33.8 to 35.7%. The 80:20 blend showed the lowest content of reducing sugar (33.8%) of any blend tested. It is possible that the observed variation in the reducing sugar content of the fresh toffee was due to differences in the level of pulp and ginger extract. The reducing sugar content of aonla-ginger mixed toffee showed significant differences among blends. The reducing sugar content of fresh fig toffee has been reported to range from 36.3 to 39.1% (KHANDEKAR *et al.,* 2005). The reducing sugar content of guava fruit toffee has been reported to range from 40.9 to 41.3%.

The total sugar content of aonla-ginger mixed toffee ranged from 51.6 to 55.2% (Table 2). The total sugar content of fig toffee has been reported to range from 73.6 to 75.8% (KHANDEKAR *et al.,* 2005). Similarly, guava toffee has been reported to contain from 75.1 to 77.2% total sugar. Other reports have shown the total sugar content of mango toffees to be 67.3% (KERAWALA and SIDDAPPA, 1963a, 1963b), that of custard apple toffee to range from 72.2 to 78.9% (DHUMAL *et al.,* 1996) and that of tamarind, mango and papaya blended toffee to range from 55.7 to 60.1% (ARUNA *et al.*, 2000; NALE *et al.,* 2007).

The ascorbic acid content of aonla-ginger mixed toffee ranged from 107.4 to 145.9 mg/100 g. As the percentage of ginger extract increased, the ascorbic acid content decreased.

The score for colour and appearance was 8.2, 8.7, 8.6 and 8.4 for the 100:00, 85:15, 80:20 and 75:25 blends, respectively (Table 3). The score for colour and appearance of the control

Table 3 - Sensory score of aonla-ginger mixed toffee.

Treatment (Aonla:Ginger)	Sensory score*				
	Colour and appearance	Texture	Flavour	Taste	Overall acceptability
T1 = 100:00	8.25	8.22	8.20	8.00	8.20
T2 = 85:15	8.68	8.66	8.65	8.30	8.35
T3 = 80:20	8.65	8.78	8.67	8.60	8.50
T4 = 75:25	8.35	8.57	8.50	8.33	8.29
Mean	8.49	8.56	8.51	8.31	8.34
S.E. ±	0.022	0.025	0.022	0.019	0.021
CD at 5% (n=10)	0.063	0.072	0.062	0.054	0.060

*Nine-point hedonic scale; 10 semi-trained judges were used for sensory evaluation.

was less than the score of the 85:15 blend. The scores for colour and appearance of the 80:20 and 75:25 blends were also greater than that of the control but were less than that of the 85:15 blend. It is possible that the white colour of the ginger extract improved the colour of the toffee in comparison with that of the control.

The texture score for aonla-ginger mixed toffee ranged from 8.2 to 8.8. The 80:20 blend received the highest score (8.6), whereas the control received the lowest score (8.2).

The flavour score for aonla-ginger mixed toffee ranged from 8.2 to 8.8. The flavour scores differed significantly among blends. The 80:20 blend received the highest flavour score (8.8), whereas the control received the lowest flavour score (8.2). It is possible that the increase in the flavour score was due to the increase in the level of ginger extract. The fully mature ginger rhizome had an extremely strong flavour. This characteristic contributed to the flavour of the mixed toffee. The strong ginger flavour was the principal reason for the high flavour score received by the 80:20 blend.

The taste score for aonla-ginger mixed toffee ranged from 8.0 to 8.6. It is possible that the high taste scores resulted from higher levels of ginger extract. The taste score for papaya toffee has been reported to range from 8.1 to 8.4 (DIWATE *et al.,* 2004). The taste score for tamarind, mango and papaya blended toffee has been reported to range from 8.0 to 8.8 (NANE *et al.,* 2007).

The overall acceptability of the tested blends differed significantly. The 80:20 blend received the highest overall acceptability score (8.5), followed by the 85:15 blend (8.3). The control received the lowest overall acceptability score (8.2). The high scores received by the 80:20 and 85:15 blends might be a result of the superior colour and appearance, texture, flavour and taste of these toffees.

The moisture content of the toffee blends decreased significantly during storage, and the magnitude of this decrease varied among blends. The smallest moisture loss was found for the 85:15 blend, a decrease from 8.3 to 7.7% under ambient conditions and from 8.3 to 7.9% under refrigeration. These results might reflect the temperature difference between the storage conditions. The mean TSS content of the four aonla-ginger mixed toffees increased from 84.0 to 85.9°Brix under ambient conditions and from 83.2 to 85.3°Brix under refrigeration (Table 4). The TSS content of all tested blends increased significantly during storage. The increase in TSS content during storage might reflect a decrease in moisture content during storage (KOHINKAR *et al.*, 2012; KHANDEKAR *et al.,* 2005). Under ambient conditions, the 75:25 blend showed a decrease to the smallest observed post-storage value of acidity, from 0.39 to 0.37%, followed by the 80:20 blend, from 0.40 to 0.38%, and the 75:25 blend, from 0.43 to 0.40%. Under refrigeration, the acidity decreased only for the 80:20 blend, from 0.40 to 0.38%, and for the 80:20 blend, from 0.39 to 0.38%. The rate of decrease in the acidity percentage was greater in ambient storage than in refrigerated storage. At the ambient temperature, the maximum increase in the reducing sugar content was observed for the 80:20 blend, from 33.7 to 34.5%. Under refrigeration, the maximum increase in the reducing sugar content was also observed for the 80:20 blend, from 33.7 to 34.0%. The rate of increase of the reducing sugar content was greater at the ambient temperature than under refrigeration. The increase in the reducing sugar content during storage was due to the hydrolysis of non-reducing sugars. At the ambient temperature, the maximum increase in the total sugar content was observed for the 85:15 blend, from 52.1 to 53.1%. A similar trend was observed under refrigeration. The increase in the total sugar content of the mixed toffee might be due to the loss of moisture under both storage conditions. Increases in total sugar content during storage have been reported in banana toffee (from 73.7 to 74.1%), in sapota toffee (from 73.8 to 74.1%), in guava toffee (from 76.1 to 76.5%), and in fig toffee (from 74.8 to 75.1%) (KHANDEKAR *et al.,* 2005). A similar trend in the content of ascorbic acid was observed in all studied toffee samples.

Table 4 - Effect of storage period on chemical composition of aonla-ginger mixed toffee after three months storage.

Treatment (Aonla:Ginger)	Moisture (%)		TSS (%)		Acidity (%)		Reducing sugars (%)		Total sugars (%)		Ascorbic acid (mg/100 g)		Standard plate count (log cfu/g)	
	A	R	A	R	A	R	A	R	A	R	A	R	A	R
T1 = 100:00	8.02	8.28	84.00	83.20	0.445	0.450	36.48	36.25	55.92	55.81	144.09	144.00	9.5	8.5
T2 = 85:15	7.90	8.17	85.10	84.85	0.402	0.407	34.75	34.59	53.18	53.10	120.82	126.68	8.5	6.5
T3 = 80:20	7.84	8.00	85.90	85.14	0.382	0.386	34.52	34.25	52.53	52.43	115.51	115.35	6.5	5.5
T4 = 75:25	7.79	7.94	85.50	85.30	0.370	0.375	34.25	34.02	52.00	51.90	106.26	106.13	6.5	4.5
Mean	7.89	8.10	85.13	84.62	0.400	0.405	35.00	34.78	53.41	53.31	121.67	123.04	7.7	6.2
C. D. at 5% (n=4)	NS	NS	0.140	0.120	0.085	0.086	0.120	0.113	0.064	0.130	0.142	0.137	NS	NS

A- Ambient (27°±2°C); R - Refrigerated (10°±2°C).

Table 5 - Sensory quality of mixed toffees of aonla : ginger after 3 months storage.

Treatment (Aonla:Ginger)	Colour and appearance		Flavour		Texture		Taste		Overall acceptability		Ranks	
	A	R	A	R	A	R	A	R	A	R	A	R
T1 = 100:00	7.66	7.70	7.90	8.00	7.71	7.80	7.69	7.75	7.87	8.00	4	4
T2 = 85:15	8.16	8.20	8.22	8.30	7.80	7.90	8.02	8.08	8.10	8.10	2	2
T3 = 80:20	8.33	8.38	8.38	8.40	8.30	8.38	8.18	8.30	8.27	8.33	1	1
T4 = 75:25	8.00	8.06	8.25	8.28	8.16	8.22	8.00	8.02	8.00	8.08	3	3
Mean	8.04	8.09	8.19	8.25	7.99	8.08	7.97	8.04	8.06	8.13	-	-
C. D. at 5% (n=10)	0.102	0.106	0.059	0.062	0.071	0.072	0.054	0.061	0.060	0.065	-	-

A - Ambient (27°±2°C); R - Refrigerated (10°±2°C). Nine-point hedonic scale; 10 semi-trained judges were used for sensory evaluation.

Changes in the sensory properties of aonla-ginger mixed toffee during storage

Colour and appearance: A gradual decrease in the colour and appearance score from 8.2 to 7.7, 8.7 to 8.2, 8.6 to 8.3 and 8.4 to 8.0 for the control, the 85:15 blend, the 80:20 blend and 75:25 blend, respectively, was observed by the end of storage at the ambient temperature, whereas mixed toffee stored under refrigeration showed decreases from 8.2 to 7.7, 8.7 to 8.2, 8.6 to 8.4 and 8.4 to 8.1 for the control, the 85:15 blend, the 80:20 blend and the 75:25 blend (Table 5). Refrigerated storage yielded a better colour than ambient-temperature storage. The reason for this result might be that the temperature, as well as the environment, affected the colour and appearance of the product.

Texture: A gradual decrease in the texture score from 8.2 to 7.7, 8.7 to 7.8, 8.8 to 8.3 and 8.6 to 8.2 for the control, the 85:15 blend, the 80:20 blend and the 75:25 blend, respectively, occurred during storage at the ambient temperature. A similar trend was observed under refrigeration.

Flavour: The flavour score decreased significantly during storage. The flavour score decreased more rapidly in ambient-temperature storage than in refrigerated storage. The principal reason for this finding is the temperature difference between the storage conditions.

Taste: The taste score decreased from 8.0 to 7.7, 8.3 to 8.0, 8.6 to 8.2 and 8.3 to 8.0 for the control, the 85:15 blend, the 80:20 blend and the 75:25 blend, respectively during storage at the ambient temperature. The taste score decreased from 8.0 to 7.7, 8.3 to 8.1, 8.6 to 8.3 and 8.3 to 8.0 for the control, the 85:15 blend, the 80:20 blend and the 75:25 blend, respectively, under refrigeration. The taste score decreased significantly during storage. The rate of decrease of the taste score was greater at the ambient temperature than under refrigeration. This effect is a result of the temperature difference between the storage conditions.

Overall acceptability: The overall acceptability score decreased gradually from 8.2 to 7.9, 8.4 to 8.1, 8.5 to 8.3 and 8.3 to 8.0 for the control, the 85:15 blend, the 80:20 blend and the 75:25 blend, respectively, during storage at the ambient temperature. A similar trend was observed under refrigeration. The overall acceptability score decreased significantly during storage. A statistical analysis showed that the blend and storage period had significant effects on overall acceptability, but the interaction was not statistically significant. The overall acceptability of the 80:20 blend after storage was greater than that of the other blends under both the ambient and refrigerated conditions. The basis for this result might be the superior scores for colour and appearance, texture and taste for the 80:20 blend. It has been found that the overall acceptability score decreased after storage in banana toffee (from 8.7 to 8.3), in sapota toffee (from 8.6 to 8.4), in guava toffee (from 7.4 to 7.9), in fig toffee (from 8.6 to 8.1) (KHANDEKAR *et al.,* 2005) and in tamarind-mango blended toffee (from 8.4 to 7.1) (NALE *et al.,* 2007). The results of the present study are consistent with the results reported in the literature.

Microbial quality: The results of this study showed that the standard plate count was directly proportional to the moisture content of the toffee. Although the refrigerated toffee had a higher moisture content, the low temperature prevented microbes from attacking the toffee. The acceptability of the product by the panel members after three months of storage confirms that the minimum changes that might have occurred due to microbes were within the safe limit for human consumption (HARRIGON and MCCANCE, 1967). The 80:20 blend received the highest acceptance rating, followed by the 85:15 blend, the 75:25 blend and the control.

CONCLUSIONS

The results of the present study show that toffee of superior quality can be prepared from aonla pulp and ginger extract using 80% aonla

pulp, 20% ginger extract, 750 g sugar, 50 g skim milk powder, 50 g fat and 2 g common salt per kg pulp. Toffee can be stored in good condition longer than 90 days at the ambient temperature and under refrigeration.

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PAPER

EFFECT OF REDUCED DRY SALTING ON THE CHARACTERISTICS OF PDO PECORINO ROMANO CHEESE

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ABSTRACT

Pecorino Romano protected designation of origin (PDO) cheese was subjected to traditional (TS) and reduced (RS) dry salting methods. TS cheese was analyzed from 2 to 581 d of ripening and RS cheese was analyzed from 2 to 258 d of ripening. RS cheese had a significantly lower salt to moisture (S/M) ratio than TS cheese (4.48 *vs* 7.20%; 9.04 *vs* 12.09% in the inner and outer sections, respectively). A significant difference in S/M was obtained between the inner and outer sections of TS cheese. RS cheese had higher moisture content than TS cheese (41.02 *vs* 38.91%; 39.80 *vs* 37.29% in the inner and outer sections, respectively). RS cheese had higher pH 4.6 soluble N/total N ratios and total and individual amino acid contents. However, proteolysis was not significantly different between RS and TS. In TS and RS, total amino acid content was significantly higher in the inner than in the outer sections. Total free fatty acid content was higher in RS than in TS. Lipolysis was not significantly different between RS and TS.

- Keywords: Pecorino Romano, reduced dry-salting, proteolysis, lipolysis -

INTRODUCTION

There is an increased demand for reduced sodium foods, including cheese. Salt, which has a preservative effect, affects many characteristics of cheese including its composition, microflora, enzymatic activity, ripening rate, texture, flavor, and quality (GUINEE and FOX, 2004). Altogether, these factors make it very difficult to reduce the salt content of cheese without adversely affecting its quality.

Proteolysis is the most complex biochemical reaction during cheese ripening or maturation (MCSWEENEY, 2004). Proteolysis affects cheese texture and flavor through the release of amino acids that are precursors of important volatile flavor compounds (MCSWEENEY, 2004). Salt affects proteolysis by affecting casein hydration and aggregation, water and enzymatic activities, and starter and non-starter bacterial growth (GUINEE and FOX, 2004).

Lipolysis is one of the major biochemical reactions during cheese ripening. Free fatty acids (FFAs) released during lipolysis affect cheese flavor (WOO *et al.*, 1984) either directly by contributing to flavors and taste (WOO and LINDSAY, 1984; ADDIS *et al.*, 2005a; ADDIS *et al.*, 2005b; PIRISI *et al.*, 2007) or indirectly by forming methyl ketones, alkanes, lactones, and esters (URBACH, 1991). It has been reported that lipolysis is negatively affected by salt (THAKUR *et al.*, 1975; LINDSAY *et al.*, 1982; REDDY and MARTH, 1993).

Pecorino Romano is a protected designation of origin (PDO) cheese. It is a hard cheese made from whole sheep milk in Sardinia, Latium, and Tuscany. The minimum length of ripening of Pecorino Romano is 5 months for table cheese and 8 months for grated cheese. Its weight varies from 20 to 35 kg. Pecorino Romano made with lamb rennet paste has a particular flavor (ADDIS *et al.*, 2005a).

According to PDO specifications, salting methods can be dry (i.e., traditional process), wet (i.e., brine), or a combination of wet and dry methods (i.e., cheese is immersed in brine and salt is applied on the surface). Among the Italian cheeses, Pecorino Romano contains the highest salt content (>5.0%); however, the salt content of Pecorino Romano is currently lower than that reported in the past (MARCIALIS *et al.*, 1968; SALVADORI DEL PRATO *et al.*, 1993; NIEDDU *et al.*, 2010). The objective of this study was to assess proteolysis and lipolysis in Pecorino Romano PDO cheese subjected to traditional and reduced dry salting methods.

MATERIALS AND METHODS

Sample preparation

Pecorino Romano cheese was manufactured in a commercial cheese plant according to PDO specifications (Gazzetta Ufficiale Repubblica Italiana, 2009). During the cheese manufacturing process, sheep milk was heat-treated, inoculated with starter cultures, and coagulated with lamb rennet paste (38°-40°C). The resulting curd was heated at 45°-48°C and pressed to allow whey drainage. Pecorino Romano cheese from the same vat was marked after molding. Dry salting of cheese was performed with sodium chloride crystals of approximately 0.3 cm in size. Traditionally-salted cheese (TS) was subjected to four dry salt surface applications during the first two months of ripening. Reduced-salt cheese (RS) was subjected to two dry salt surface applications during the first month of ripening. The interval between the different salt applications varied; several salt surface applications were performed at the beginning of ripening. Dry salting was performed at 8°-10°C and 95% RH. Following the complete absorption of salt, TS and RS were packaged under vacuum at156 d and 35 d, respectively, and stored at 10°C and 95% RH. The used film for vacuum packaging was characterized by controlled permeability (oxygen barrier and carbon dioxide permeable). TS was sampled after 2, 9, 35, 65, 156, 258, 397, and 581 d of ripening; RS was sampled after 2, 9, 35, 65, 156, and 258 d of ripening.

RS and TS samples had a height of 28 cm (ripened cheese) to 32 cm (fresh cheese) and a diameter of 33 cm (ripened cheese) to 36 cm (fresh cheese). The cheese samples, which consisted of 1/6 of the whole cheese, were obtained from diametrically and diagonally opposite sides. The samples were then divided into two sections: an inner section (i.e., 9 cm from the inner section toward the rind and 7 cm toward the plate of the cheese) and an outer section (i.e., the remaining portion). The weight of the inner section was approximately 12.5% of the total weight of the whole cheese. Prior to chemical analyses, the cheese samples were ground.

Chemical analyses

The inner and outer cheese sections were submitted to biochemical analyses. The chemical analyses, which were conducted in duplicate, consisted of measuring pH (IDF, 1989), a_w (AOAC, 1995), moisture (IDF, 1986), total nitrogen (FIL-IDF, 1986), soluble nitrogen (FIL-IDF, 1991), fat (FIL-IDF, 2001), salt (IDF, 1988), ash (AOAC, 2000), free amino acids (FAAs) by HPLC (RESMINI *et al.*, 1985), and free fatty acids (FFAs) by capillary gas chromatographic method (DE JONG and BADINGS, 1990). FFAs were expressed as mmol/kg to assess each individual FFA independent of its molecular weight.

Statistical analyses

The GLM procedure (SAS software, SAS Institute Inc. ,Cary, NC, USA) was used to analyze the data. The following model was used,

$$Yijkl=\mu+Ai\times Bj+Ck\times Bj+Eijkl,$$

where Yijkl is the qualitative characteristics of the cheese, Ai is the fixed effect of the sampling point of the cheese (i=1: inner; i=2: outer), Bj is the fixed effect of dry salting applications (j=1: two salting applications; j=2: four salting applications), Ck is the fixed effect of ripening days (k=1 at 65 d; k=2 at 156 d; k=3 at 258 d; k=4 at 397 d; and k=5 at 581 d), and Eijkl is the residual error.

RESULTS AND DISCUSSION

The analytical results of the inner and outer sections of TS and RS are shown in Table 1. Salt was expressed as salt content and as salt per moisture ratio (S/M). Compared to TS, RS had lower S/M in the inner and outer sections (4.48 *vs* 7.20% and 9.04 *vs* 12.09%, respectively). The outer section of TS and RS had higher S/M than the inner section. Significant differences were obtained between the inner and outer sections of TS.

The moisture content of TS and RS is shown in Table 1. Compared to TS, RS had higher moisture in the inner and outer sections (41.02 *vs* 38.91% and 39.80 *vs* 37.29%, respectively); significant differences were obtained only between the outer sections. The inner section of TS and RS had higher moisture content than the outer section.

The ash content of TS and RS is shown in Table 1. Compared to RS, TS had higher ash in the outer and inner sections (7.16 *vs* 5.42% and 6.14 *vs* 4.42%, respectively). Significant differences were obtained between the inner and outer sections of TS and RS. Table 2 shows the salt content, S/M, and moisture content before the first and second salt applications; at the end of salting, which coincided with the complete salt removal at 35 d of ripening for RS and at 156 d of ripening for TS; at 258 d of ripening for RS; and at 581 d of ripening for TS. At the end of salting, the amount of salt in the inner section of RS was negligible compared to that present in the outer section (0.34 *vs* 9.38 S/M). In TS, S/M in the inner section was more than one third of the S/M in the outer section (5.62 *vs* 14.88 S/M). Therefore, the amount of salt in the inner section was dependent on the salt amount added and on salting time.

The moisture content in the inner section of RS decreased from 43.40% at the beginning of salting to 42.44% at the end of salting (Table 2). In the outer section, moisture content decreased from 45.74 to 38.60%. In TS, the differences were more important; moisture content decreased from 43.40 to 38.33% in the inner section and from 45.74 to 35.79% in the outer section. The trend obtained for moisture content was opposite to that obtained for salt content.

At 258 d of ripening, S/M in the inner section of RS was 80% of that present in the outer section (6.42 *vs* 8.06%, respectively). An S/M equilibrium between the inner and outer sections of TS was reached after 581 d of ripening (11.72 *vs* 11.89%, respectively). The moisture equilibrium between the inner and outer sections was reached at 258 d of ripening in RS (39.35 *vs* 39.30%, respectively) and at 397 d of ripening in TS (36.35 *vs* 36.22%, respectively).

The S/M results obtained suggest that the amount of salt absorbed was highly affected by the amount of salt applied. In the wet salting method, the amount of salt absorbed increases with salting time and brine concentration (GUINEE and FOX, 2004). Similar salt uptake mechanisms occur in dry and wet salting methods; however, at the beginning of dry salting, crystal salts need to be fully dissolved on the surface before slowly diffusing inside the cheese (GUINEE, 2004).

During salting, a reduced salt gradient from the surface to the inner section of the cheese sample is accompanied by a decreased moisture gradient from the center to the surface of the cheese sample, which results in moisture loss and salt incorporation. It has been report-

Table 1 - Composition of the inner and outer sections of cheese subjected to traditional (TS) and reduced (RS) dry salting methods.

	RS		TS	
	Inner section	Outer section	Inner section	Outer section
Salt (%)	1.84 B	3.60 ab	2.80 b	4.48 A
Salt in moisture (%)	4.48 B	9.04 ab	7.20 b	12.09 A
Moisture (%)	41.02 A	39.80 a	38.91 ab	37.29 b
Protein (%)	24.87	24.60	24.64	24.76
pH 4.6 soluble N/tot. N (%)	19.87	15.90	14.77	11.09
Fat (%)	27.88 b	28.78 ab	29.79 a	28.77 ab
Ash (%)	4.42 c	6.14 ab	5.42 bc	7.16 A
A_w	0.93	0.92	0.92	0.90
pH	5.54 b	5.67 a	5.51 B	5.66 A

a,b,c: $p<0.05$; A,B,C: $p<0.01$.

ed there is an inverse relationship between salt and moisture in cheese (GUINEE and FOX, 2004).

The diffusion of salt during ripening is a slow process. The salt is concentrated in the outer section of cheese and migrates toward the inner section. S/M equilibrium is affected by salt gradients and ripening conditions. However, few studies have focused on these factors (MORRIS *et al.*, 1985; GUINEE and FOX, 2004).

It is difficult to compare Pecorino Romano with other hard cheeses due to differences in cheese manufacturing conditions, salting methods, cheese characteristics, and shape. However, Parmigiano Reggiano cheese (40 kg) had similar S/M in the inner and outer sections after 11 months of ripening (RESMINI *et al.*, 1974). In Cheddar cheese (9.5 kg), S/M had not reached equilibrium after 24-25 weeks (SUTHERLAND, 1977; MORRIS *et al.*, 1985). Parmigiano Reggiano cheese is generally salted by the wet method and Cheddar is salted by the dry method.

The effects of dry and wet salt on cheese surface are very similar. In both salting methods, there is a rapid loss of moisture near the surface. However, in Pecorino Romano, the impact of dry salt for a long time, several weeks, on cheese surface, the loss of moisture causes considerable contraction of cheese structure and reduces porosity, which impairs moisture movement out of the cheese and salt movement into the cheese and decreases the rate of salt uptake (GODINHO and FOX, 1981a; GUINEE and FOX, 1983; MELILLI *et al.*, 2003).

The main factors affecting moisture content in cheese are salt content and dehydration during cheese ripening (HARDY, 1990). However, the moisture content of Pecorino Romano is dependent on the vacuum packaging. It is possible that the moisture content was higher in this study than in Pecorino Romano cheese that was not vacuum packaged. SALARI *et al.* (2011) reported that sheep cheese that was vacuum packaged had higher moisture content than non-vacuum packaged cheese (SALARI *et al.*, 2011).

Salt content of foods is of great interest to nutritionists and consumers. The difference in salt content between RS and TS at 258 and 581 d of ripening, respectively, was 1.2% (3.17 *vs* 4.34%). The lower salt content in RS results in a sodium content of approximately 0.5 g per 100 g of cheese. The salt content previously reported in Pecorino Romano was 6.71% (MARCIALIS *et al.*, 1968), 5.59-5.79% (SALVADORI DEL PRATO *et al.*, 1993), and 5.67% (NIEDDU *et al.*, 2010). These data are indicative of the gradual reduction in salt content of Pecorino Romano cheese during the last years.

The trend of the ash content was the same as that obtained for the salt content that is the higher component of ash.

The water activity (a_w) values during ripening are shown in Table 1. In this study, a_w was higher in the inner than in the outer section of RS and TS (0.93 *vs* 0.92; 0.92 *vs* 0.90, respectively). Salting was the main factor that contributed to low a_w values. Furthermore, dehydration and low molecular weight compounds reduce a_w values (HARDY, 1990). The a_w value obtained in the outer section of TS (0.90) is not common; however, the a_w value obtained in the outer section of RS cheese (0.92) is comparable with that reported in whole loaf of Parmesan (0.92), Provolone (0.91), and Roquefort (0.91) (GUINEE and FOX, 2004).

The pH values of TS and RS are shown in Table 1. The pH values were significantly higher in the outer section of TS and RS (5.66 and 5.67, respectively) than in the inner section (5.51 and 5.54, respectively). According to MARCIALIS *et al.* (1968), the pH values of Pecorino Romano at 6-7 month of ripening were 5.3; however, cheese manufacturing, salting, and ripening conditions are likely changed in the recent years. GUINEE and FOX (1984) reported a higher pH in the outer section than in the inner section of Romano type cheese. It is possible that different pH gradients from the inner to the outer section (5.35 in the inner section and 5.48 in the outer section at 2 d of ripening) may be attributed to different temperature gradients. During this time, microbial growth increases as a result of slow cooling in the inner section and contributes to a reduction in pH as a result of lactic acid formation from residual lactose.

The protein content of TS and RS (Table 1) were similar (24.64 *vs* 24.87% and 24.76 *vs* 24.60%, in the inner and outer sections, respectively). The protein content of whole cheese of 22 and 26% was reported by SALVADORI DEL PRATO *et al.* (1993) and NIEDDU *et al.* (2010).

The pH 4.6 soluble N/total N ratios of TS and RS are shown in Table 1. The pH 4.6 soluble N/total N ratios in RS were 19.87% in the inner section and 15.90% in the outer section, which were higher than the corresponding sections in TS (14.77 and 11.09%, respectively). There were no significant differences in pH 4.6 soluble N/total N ratios between RS and TS. The mean values in the inner section were slightly higher than those in the outer section of RS and TS.

An inverse relationship between casein degradation (assessed by pH 4.6 soluble N/total N ratio) and salt concentration in cheese was observed in different cheeses (GUINEE and FOX, 2004). AL-OTAIBI and WILBEY (2005) reported a significant effect of S/M on soluble N of white-salted cheese. In Cheddar cheese salted with 0.9-2.3% white salt, soluble N was negatively affected by salt content (MØLLER *et al.*, 2013). GUINEE and FOX (1984) reported a reduction in pH 4.6 soluble N in the outer section of Romano-type cheese. This reduction was probably due to lower moisture and higher salt contents in the outer section compared to the inner section (GUINEE and FOX, 1983).

A higher soluble N content has been reported

Table 2 - Composition of the inner and outer sections of different samples of cheese subjected to traditional (TS) and reduced (RS) dry salting methods.

Sampling phase	first salt application		second salt application		end of salting of RS		end salting of TS		last sampling of RS		last sampling of TS	
Days of ripening	2		9		35		156		258		581	
	Outer	inner	outer	inner	outer	inner	outer	inner	outer	inner	outer	inner
Salt (%)	0.00±0.00	0.00±0.00	1.19±0.06	0.14±0.04	3.62±0.01	0.14±0.04	5.32±0.06	2.15±0.01	3.17±0.01	2.53±0.02	4.34±0.02	4.35±0.00
Salt in moisture (%)	0.00±0.00	0.00±0.00	2.77±0.14	0.33±0.09	9.38±0.02	0.34±0.10	14.88±0.18	5.62±0.0	27.80±0.01	6.15±0.04	11.89±0.05	11.72±0.00
Moisture (%)	45.74±0.08	43.40±0.05	42.78±0.04	42.51±0.13	38.60±0.11	42.44±0.03	35.79±0.03	38.33±0.05	39.30±0.04	39.35±0.01	36.50±0.01	37.08±0.01

in the inner than in the outer sections of sheep and cow cheeses such as Pecorino Umbro (GOBETTI *et al.*, 1997) and PDO Ragusano (FALLICO *et al.*, 2004; MELILLI *et al.*, 2004). The pH 4.6 soluble N/total N ratio of Pecorino Romano reported in other studies was 18-28% at 6 months of ripening (PETTINAU and BOTTAZZI, 1971) and 29-33% at 10 months of ripening (SALVADORI DEL PRATO, 1993). In this study, the soluble N/total N ratio in RS cheese after 258 d of ripening was 18.33 and 20.38% in the outer and inner sections, respectively. The soluble N/total N ratio in TS was 19.92% in the outer section and 26.40% in the inner section after 581 d of ripening.

The SN/TN ratio in our study was lower than that previously reported for Pecorino Romano. However, in those studies, the cheese samples were not vacuum-packaged and were subject to different ripening conditions. GUINEE and FOX (1984) reported that in Romano-type cheese, the proteolytic activity from surface microflora increased during the second part of the ripening process. In our study, vacuum packaging might not have favored the growth of surface microflora.

Table 3 shows the individual and total free amino acid (TFAA) content in TS and RS. RS had a higher content of amino acids than TS. Amino acids were ordered according to their individual amount in the outer section of RS. There were no significant differences in TFAAs and individual amino acids between TS and RS, with the exception of ornithine, which was significantly different between the outer sections of RS and TS (37.8 *vs* 15.5 mg/100 g, respectively). These results were consistent with those obtained for the pH 4.6 soluble N/total N ratio. In white-salted cheese, TFAA content was not significantly affected by salt concentrations (AL-OTAIBI *et al.*, 2005). On the other hand, Cheddar cheese had a lower TFAA content with decreasing salt concentration (MØLLER *et al.*, 2013). The salt content of RS is in any case very high, so it is difficult to find significant differences from TS.

The inner sections of TS and RS had significantly higher TFAA content than the outer section (3229.1 *vs* 1782.3 mg/100 g and 3712.5 *vs* 2347.1 mg/100 g, respectively). The individual amino acid content was significantly higher in the inner than in the outer sections. Contrary to the findings obtained in Pecorino Romano, the different layers of PDO Ragusano cheese were not significantly different in free amino acid content (FALLICO *et al.*, 2004).

The reduced dry salting method in this study did not affect soluble N or individual amino acid contents in Pecorino Romano. The different contents of TFAA in the inner and outer sections were probably due to the different effects of salt and moisture on proteolysis (FALLICO *et al.*, 2004).

The main FAAs in the inner and outer sections were Lys, Glu, Leu, Val, and Gln. Ragusano cheese contains mostly Lys, Glu, Leu, followed by Pro and Val (FALLICO *et al.*, 2004). Lysine, which was the most abundant FAA (207.1-411.9 mg/100 g), is not susceptible to degradation during cheese ripening (PELLEGRINO and HOGENBOOM, 2007). Its content in TS after 581

Table 3 - TFAAs content (mg/100 g) in the inner and outer sections of cheese subjected to traditional (TS) and reduced (RS) dry salting methods.

	RS		TS	
	Inner	Outer	Inner	Outer
Lys	411.9 a	266.3 ab	376.0 a	207.1 b
Glu	452.3 A	235.2 B	397.2 A	196.9 B
Leu	350.6 A	221.0 bc	307.3 ab	158.9 C
Val	280.8 A	178.2 bc	246.6 ab	129.4 C
Gln	297.0	147.5	248.3	143.2
Ile	238.9 a	144.6 ab	218.6 a	106.7 b
Asn	192.8 A	126.7 bc	165.9 ab	96.0 C
Phe	189.6 A	122.5 ab	168.8 a	91.8 B
Ser	179.6 a	107.9 ab	130.4 a	66.8 b
Gaba	88.0	101.7	64.9	52.0
Ala	117.7 a	80.0 ab	103.9 a	64.0 b
Tyr	108.0 a	76.5 ab	85.7 ab	54.1 b
Asp	108.1 A	67.4 bc	94.1 ab	50.0 C
Met	103.2 a	65.9 ab	93.6 A	51.1 B
Arg	92.2	55.1	101.4	83.9
Thr	85.3 A	52.1 bc	71.3 ab	35.1 C
Orn	44.6 A	37.8 a	30.3 a	15.5 B
Gly	54.3 A	37.2 bc	44.9 ab	27.2 C
His	55.2 ab	31.3 b	64.0 a	36.6 b
TFFAs	3712.5 A	2347.1 bc	3229.1 ab	1782.3 C

TFFAs = Total Free Amino Acids.
a,b,c: $p<0.05$; A,B,C: $p<0.01$.

Table 4 - TFFAs content (mmol/kg) in the inner and outer sections of cheese subjected to traditional (TS) and reduced (RS) dry salting methods.

	RS		TS	
	Inner	Outer	Inner	Outer
C4:0	7.08	5.88	4.98	4.16
iC5:0	0.06 b	0.16 A	0.04 b	0.08 b
iC6:0	2.08	0.42	0.55	0.96
C6:0	0.73	1.73	1.33	0.81
C8:0	1.17	1.14	0.87	0.90
C10:0	2.51	2.47	1.90	1.98
SCFFAs	13.64	11.79	9.67	8.89
C11:0	0.04	0.03	0.03	0.03
C12:0	1.22	1.16	0.92	0.98
C14:0	1.47 ab	1.67 a	1.24 b	1.47 ab
C15:0	0.14	0.16	0.12	0.14
C16:0	2.42 ab	2.90 a	2.13 b	2.46 ab
C16:1	0.16 ab	0.17 a	0.13 b	0.16 ab
MCFFAs	5.44 ab	6.09 a	4.56 b	5.24 ab
C17:0	0.04	0.04	0.03	0.04
C18:0	0.61 b	0.86 a	0.58 b	0.67 ab
C18:1	2.29 ab	2.90 a	1.93 b	2.44 ab
C18:2	0.26 a	0.23 ab	0.19 b	0.25 a
C18:3	0.23	0.23	0.17	0.21
LCFFAs	3.43 ab	4.26 A	2.90 B	3.61 ab
TFFAs	22.51	22.14	17.13	17.75

SCFFAs = short chain free fatty acids.
MCFFAs = medium chain free fatty acids.
LCFFAs = long chain free fatty acids.
TFFAs = total free fatty acids.
a,b,c: p<0.05; A,B,C: p<0.01.

d of ripening (383.2 mg/100 g in the inner section and 326.4 mg/100 g in the outer section) was approximately one-third of the amount detected in Parmigiano Reggiano (1009.1 mg/100 g) (UPADHYAY *et al.*, 2004).

Glu ranged from 196.9 to 452.3 mg/100 g. These concentrations were approximately one-third of the Glu content in Parmigiano Reggiano cheese (1448.9 mg/100 g) (UPADHYAY *et al.*, 2004) and slightly lower than the Glu content in Ragusano cheese (300-700 mg/100 g) (FALLICO *et al.*, 2004). Because of its flavor-enhancing properties (KRAUSE *et al.*, 1997), Glu may contribute to the development of flavors in Pecorino Romano.

The content of GABA in Pecorino Romano ranged from 51.9 to 101.7 mg/100 g. Italian cheese varieties generally have a GABA content of 0.03-39.1 mg/100 g (SIRAGUSA *et al.*, 2007). GABA, a non-protein amino acid, has several roles. Besides functioning as a neurotransmitter, GABA has hypotensive, diuretic, and sedative effects (JAKOBS *et al.*, 1993; WONG *et al.*, 2003).

Table 1 shows the fat content of TS and RS. The inner section of TS had significantly higher fat content than the inner section of RS (29.79 *vs* 27.88%). The fat content of the outer sections of TS (28.77%) and RS (28.78%) was similar.

In Cheddar cheese, high salt content corresponds to high fat content due to the loss of moisture during salting (GUINEE and FOX, 2004). The inner section of RS had low fat content and high moisture content. Therefore, the "concentration effect" was more pronounced in the outer section of RS and in both sections of TS.

Table 4 shows the individual and TFFA content in TS and RS during ripening. The TFFA content was higher in RS than in TS: 22.51 *vs* 17.13 mmol/kg and 22.14 *vs* 17.75 mmol/kg, in the inner and outer section, respectively. The inner and outer sections of RS and TS had similar TFFA content.

The short chain free fatty acids (SCFFAs) were the most abundant FFAs in TS and RS. Similarly to TFFAs, higher SCFFA content was obtained in cheeses with lower salt content (13.64 *vs* 9.67 mmol/kg and 11.79 *vs* 8.89 mmol/kg, in the inner and outer section, respectively), but the difference was not significant.

Butyric acid was the most abundant SCFFA. Higher butyric acid content was present in RS than in TS: 7.08 *vs* 4.98 mmol/kg and 5.88 *vs* 4.16 mmol/kg in the inner and outer section, respectively. However, the results were not significantly different.

The trend of the medium chain free fatty acids (MCFFAs) (5.44 *vs* 4.56 mmol/kg and 6.09 *vs* 5.24 mmol/kg in the inner and outer section of RS and TS, respectively) and long chain free fatty acids (LCFFAs) (3.43 *vs* 2.90 mmol/kg and 4.26 *vs* 3.61 mmol/kg in the inner and outer section of RS and TS, respectively) was the same as that obtained for TFFAs. MCFFAs and LCFFAs were higher in RS than in TS.

There is little evidence on the effect of salt on lipolysis in hard-type cheeses. Cheddar cheese has higher lipolytic rate in unsalted than in the salted types (THAKUR *et al.*, 1975; LINDSAY *et al.*, 1982; REDDY and MARTH, 1993). In blue cheese, lipolysis is delayed in the presence of high salt concentrations (GODINHO and FOX, 1981b).

The lower salt content did not significantly affect the FFA profile of Pecorino Romano. However, considering the importance of lipolysis on the sensorial characteristics of Pecorino Romano, it would be interesting to establish whether low salt content affects the sensorial characteristics of cheese.

CONCLUSIONS

Reduced dry salting resulted in a reduction in S/M in the inner and outer sections of cheese. The results revealed that the amount of salt absorbed is affected by the number of times the salt is applied. The S/M equilibrium between the inner and outer sections was reached at 581 d of ripening in TS. The diffusion of salt during ripening is a slow process. Dry salt results in moisture reduction, contraction of cheese structure, and decreased porosity, which reduces moisture

movement out of the cheese and salt movement into the cheese.

Reduced dry salting increased moisture content in the inner and outer sections of Pecorino Romano cheese; there were significant differences in the moisture content between the outer sections of RS and TS. Vacuum packaging could have affected moisture content.

RS had higher proteolytic rates than TS. An inverse relationship between casein degradation and salt concentration in cheese has been reported, which might explain the results obtained in this study. There was a significant difference in the TFAA content between the inner and outer sections probably due to the different effects of salt and moisture on proteolysis (FALLICO *et al.*, 2004).

Lipolysis was higher in RS than in TS. Similar to proteolysis, this behavior was probably due to the inhibitory effect of salt on lipolysis.

In conclusion, reduced dry salt applications in Pecorino Romano did not significantly affect salt content, proteolysis, or lipolysis. The sodium content of cheese with reduced salting was decreased by 0.5 g per 100 g of cheese.

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PAPER

EFFECT OF GUM ARABIC AND GUM GHATTI ON THE STABILITY OF BEVERAGE EMULSIONS

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ABSTRACT

The aim of this study was to evaluate the effect of gums: arabic and ghatti on the stability of beverage emulsions. The stability was determined based on the characteristics of particle size of dispersed phase and by measuring changes in the intensity of backscattered light. Oil droplet distributions were measured by laser-light scattering. The highest stability was found in the emulsions stabilised by 10% of gum arabic or by 5% of gum ghatti. The addition of the emulsifier may be reduced to 3% of emulsion mass, without decreasing emulsion stability, by applying a mixture (1:1 v/v) of these gums.

- Keywords: beverage emulsion, gum arabic, gum ghatti -

INTRODUCTION

Beverage emulsions are oil-in-water emulsions that are normally prepared as a concentrate and then diluted in a sugar solution in order to produce the finished beverage (BUFFO *et al.*, 2002). The emulsion in its both concentrated and diluted form ought to be characterised by high stability (TAN and WU HOLMES, 1988).

Emulsion instability results from physical processes, *i.e.* flocculation, coalescence, Ostwald ripening and gravity separation. The rate of these changes can be measured by determining the size and distribution of oil droplets in the emulsion (McCLEMENTS and COUPLAND, 1996; MIRHOSSEINI *et al.*, 2008). The Stokes' law states that the velocity at which a droplet moves is proportional to the square of its radius. The stability of emulsion to gravity separation can therefore be enhanced by reducing the size of the droplets (CHANAMAI and McCLEMENTS, 2000; HUANG *et al.*, 2001).

In soft drinks the beverage emulsion may provide flavour, colour and suitable cloudy appearance (REINECCIUS, 1994). A typical composition includes flavour oils (often essential oils) and weighting agents in the oil phase as well as water, hydrocolloid, citric acid, preservatives, colorants and a sweetener in the water phase (BUFFO and REINECCIUS, 2000; CHANAMAI and McCLEMENTS, 2001). In beverage emulsions hydrocolloids serve as emulsifiers and stabilizers. They stabilised emulsions through viscosity effects, steric hindrance and electrostatic interactions. The most common hydrocolloid stabilizers include xanthan, gum arabic, modified starches, pectin and carrageenan (BUFFO *et al.*, 2001).

Gum arabic, the dried exude from certain species of the acacia tree, is one of most widely used biopolymer on an industrial scale. It is deemed exquisite in many of its properties including the ability to form stable emulsions over a wide pH range and in the presence of electrolytes (DICKINSON, 2003; JAYME *et al.,* 1999; SANCHEZ *et al.*, 2002; TIPVARAKARNKOON *et al.*, 2010; DJORDJEVIC *et al.*, 2008). Gum arabic consists of at least 3 high molecular weight biopolymer fractions. The surface-active fraction of branched arabinogalactan blocks attached to a polypeptide backbone (RANDELL *et al.*, 1988; CHANAMAI and McCLEMENTS, 2001; AOKI *et al.*, 2007). The hydrophobic polypeptide chain adsorbs on the oil/water interface, while the hydrophilic arabinogalactan blocks extend into the solution, thus assuring stability against droplet aggregation through steric and electrostatic repulsion (JAYME *et al.* 1999; CHANAMAI and McCLEMENTS, 2001). Ghatti gum is also a plant exudates, the main species is *Anogeissus latifolia* (DESHMUKH *et al.*, 2012; MIRHOSSEINI and AMID, 2012). Ghatti gum is a non-starch polysaccharide. The polysaccharide of gum ghatti has an extremely complex structure, its hydrolysis results in the production of: Ara, Gal, Man, Xyl, and GlcA (ratio: 48:29:10:5:10 M) and less than 1% Rha. Recent investigations have revealed its complete molecular structure, which proved that it has two fractions – gelling and soluble (KANG *et al.*, 2011a, KANG *et al.* 2011b, KANG *et al.* 2011c). As a result of its two fractions ghatti gum exhibits gelling surface-active as well as emulsifying properties, even better than these of gum Arabic (DESHMUKH *et al.*, 2012). Ghatti gum is not digested in the stomach and small intestine of humans and is fermented in the large intestine by *Bacteroides longum* (MARONPOT, 2013). It is classified as *generally recognized as safe* (GRAS) (DESHMUKH *et al.*, 2012).

The purpose of this study was to investigate the effect of gum arabic, gum ghatti and their mixture on the stability of model beverage emulsions.

MATERIALS AND METHODS

Materials

Gum arabic samples (Valgum and Valspray A) and rosin esters (Valrosin) were provided by the Valmar, France. Ghatti gum was obtained from Hortimex, Poland. Essential citrus oil was purchased from JAR, Poland. Sodium benzoate and citric acid food grade were from Orffa Food Eastern Europe. Distilled water was used to prepare solutions and emulsions.

Emulsion preparation

Emulsion concentrates were prepared according to the following formula: essential oil - 10% (w/w), weighting agent (rosin esters) - 10% (w/w), emulsifier (gums) - 10 or 5 or 3 or 1.5% (w/w), sodium benzoate - 0.1% (w/w), citric acid and distilled water up to 100% (w/w). The mixture of Valgum and Valspray was added to the emulsions at a ratio of 1:1w/w. The emulsifiers were dispersed for half an hour with an RW 20 DZW mixer by Janke & Kunkle, Germany, in water at 40°C, in which the sodium benzoate had been previously dissolved. The water phase was stored for 24 hours to hydrate the emulsifier. Pre-emulsion was prepared by adding together the water and oil phases (i.e., the hydrocolloid solution and the essential oil with a weighting agent) and stirring with an RW 20 DZM mixer for 15 min with the velocity 1700 rpm. At this point, the pH value of the premixes was adjusted to 4 with 2 M citric acid. A fine emulsion was achieved by subjecting the premixes to a two-stage homogenization with an APV-1000 homogenizer by APV, Denmark,

at 55 MPa at the first stage and 18 MPa at the second stage.

Particle size determination

Mean particle size and particle size distribution of beverage emulsions were determined in the range of 0.05 – 1000 μm by the laser light scattering method using a Mastersizer (Malvern Instruments Ltd., Malvern, UK), equipped with an He-Ne laser (λ = 633 nm). The volume size distribution is calculated from the intensity of light diffracted at each angle using the Mie theory. A refractive index ratio of 1.529 was used by the instrument to calculate the particle size distributions. The samples of emulsion were diluted at 1:200 with distilled water in a diffractometer cell, under stirring. The emulsion was measured the next day after being prepared. Each sample was analysed three times and data are presented as average values.

The average droplet size was characterised by mean diameters related to the volume $D_{[4,3]}$ defined respectively by:

$$D_{[4,3]} = \Sigma_i n_i d_i^4 / \Sigma_i n_i d_i^3,$$

where n_i is the number of droplets with diameter d_i.

Emulsion stability evaluation by turbidity measurement

Turbidity measurement was applied to determine emulsion stability (KAUFMAN and GARTI, 1984). It consisted in the measurement of absorbance of emulsion samples diluted at 1 to 1,000. The absorbance was measured at 400 and 800 nm, using a Helios β spectrophotometer (Unicam). The size index (R) was determined from the ratio of absorbance values at 800 and 400 nm.

Emulsion stability measurement by the backscattering light method

The stability of emulsions was determined using Turbiscan (Turbiscan Lab., Formulaction) by measuring the backscattering of monochromatic light (λ = 880 nm) from the emulsion as a function of its height. Emulsions were placed into flat-bottomed cylindrical glass tubes (40 mm height, 16 mm internal diameter) and stored at 37°±0.5°C for two weeks. The backscattering of light from emulsions was then measured as a function of height every other day for 2 weeks. The results are presented as backscattering versus height.

Statistical analysis

Data were analyzed using Statgraphics Plus 5.1. software (STSC Inc., Rockville, MD, USA). One-way analysis of variance (ANOVA) was performed. Significant differences between features were verified on the basis of Tukey HSD test at a significance level of $p \leq 0.05$.

RESULTS

Effect of the type and amount of hydrocolloid on the dispersion degree of beverage emulsions

The destabilisation processes in beverage emulsions may be slowed down by among other things, obtaining a proper dispersion degree (HORNE and HERMAR, 1998). Emulsion stability is expected to be higher when the droplet size is smaller. An emulsion containing weighting agents and an acceptable emulsifying constituent will typically not separate if the average particle size of the emulsion is below 1 μm (BUFFO and REINECCIUS, 2000).

Fig. 1a presents cumulative distribution of particles in the emulsions stabilised by gum arabic. Most of particles (over 93%) with diameters below 1 μm were found in the samples of emulsion stabilised by 10% addition of gum arabic. The lower gum concentration (5% and 3%) resulted in a reduced number of particles with diameters below 1 μm to 84 and 50%, respectively.

Reducing the concentration of gum arabic caused an increase in the mean size of oil droplets. In the emulsion with 10% addition of gum arabic, the value of $D_{[4,3]}$ diameter was 0.57 μm, whereas in the emulsion with 3% of gum arabic it was almost twofold higher and reached 1.08 μm. Emulsifier concentration, which ensures a stable emulsion, should provide a complete coverage of the oil surface (ONSAARAD *et al.*, 2006). Gum arabic is used typically in high concentrations, i.e. 15-25% of the emulsion (LEROUX *et al.*, 2003).

The results of particle size index R measurement confirmed the significant effect of gum arabic concentration on the dispersion degree of emulsions. The index R was increasing with a decreasing content of gum arabic in the beverage emulsions (Table 1).

Different observations were made in the case of oil droplets size distribution in the emulsions stabilised by gum ghatti. In the emulsion with 10% addition of gum ghatti, only 68% of the particles had diameters under 1 μm. The decrease in emulsifier concentration to 5%, and further to 1.5%, resulted in 99% of the particles having diameters below 1 μm (Fig. 1b). It indicates that the lower dose of gum ghatti improves the dispersion degree in beverage emulsions. This effect was probably related to reduced viscosity of the water phase. The viscosity of the water phase of the emulsion containing 10% of gum ghatti was undoubtedly too high and prevented the formation of a proper dispersion of the emulsion at the adopted parameters of homogenisation. This effect was not observed when us-

Table 1 - The particle size of the dispersed phase in beverage emulsions.

Addition of emulsifier (%)	Size index (R)		Droplet size (μm)		
	Immediately after production	After12 weeks	$D_{[4,3]}$	$D_{[V\ 0.5]}$	$D_{[V\ 0.9]}$
Gum arabic					
10	0.40^{d}	0.44^{c}	0.57	0.50	0.96
5	0.50^{e}	0.55^{d}	0.77	0.73	1.15
3	0.58^{e}	0.64^{c}	1.08	1.06	1.50
Gum ghatti					
10	0.38^{cd}	0.42^{c}	1.13	0.69	2.12
5	0.21^{a}	0.23^{a}	0.43	0.39	0.67
3	0.26^{b}	0.31^{b}	0.45	0.42	0.67
1.5	0.25^{b}	0.53^{d}	0.44	0.40	0.69
Gum arabic + Gum ghatti					
5 (1:1)	0.21^{a}	0.24^{a}	0.43	0.40	0.66
3 (1:1)	0.34^{c}	0.36^{b}	0.48	0.45	0.74
3 (1:2)	0.25^{b}	0.25^{a}	0.46	0.43	0.69

Different letters in the same column indicate significant differences (P<0.05).

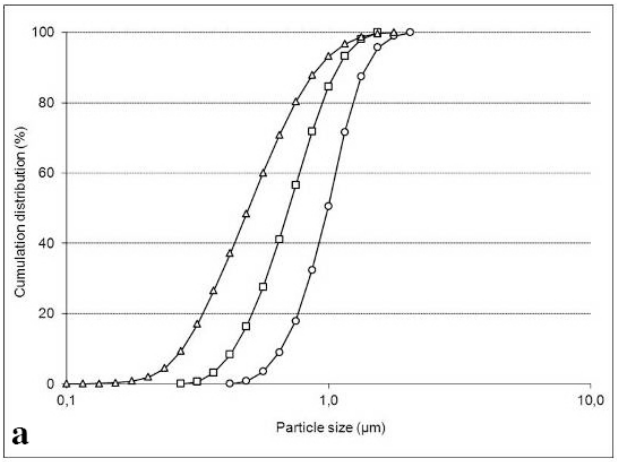


Fig. 1a - The cumulative distribution of the dispersed phase particles in beverage emulsions with arabic gum in different concentration (△ -10%; □ -5%; ○ - 3%).

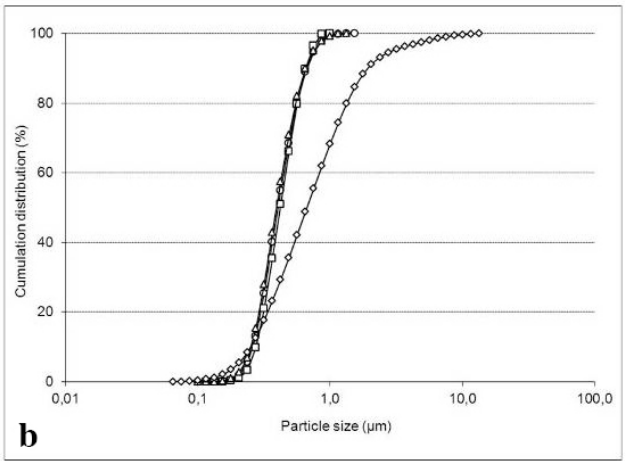


Fig. 1b - The cumulative distribution of the dispersed phase particles in beverage emulsions with ghatti gum in different concentration (○ -1,5%; □ -3%; △ - 5%; ◇ - 10%).

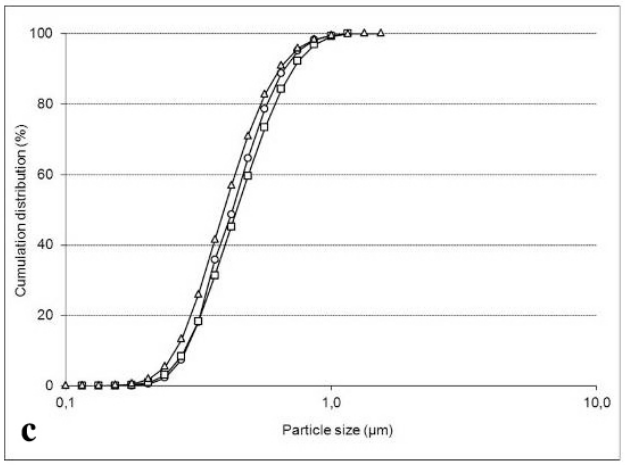


Fig. 1c - The cumulative distribution of the dispersed phase particles in beverage emulsions with mixture of arabic and ghatti gums in different concentration (○ -3% (1:2); □ -3%; △ - 5%)

ing gum arabic because this hydrocolloid forms a solution with much lower viscosity than gum ghatti does in the same concentration.

The course run of curves in Fig. 1c was almost similar. Irrespective of the dose of a gum mixture addition, over 99% of particles of the dispersed phase of the examined emulsions had diameters lesser than 1 μm. Likewise, the mean size of oil droplets, $D_{[4,3]}$, was contained in a very narrow interval of 0.43-0.48 μm. A decrease in the dose of the arabic and ghatti gums (1:1, w/w) mixture from 5 to 3% resulted in an increased value of the size index R. However, the value of this index in the emulsion with 3% addition of the gum mixture (0.34 immediately after obtaining) was so small that it did not indicate the possibility of emulsion stability deterioration as a result of decreasing the amount of the emulsifier.

Effect of the type and amount of hydrocolloid on emulsion stability monitored by measuring the backscattering light

The examination of emulsion stability by backscattering light method was based on exposing the samples of emulsions to the action of infrared light with a wavelength of 880 nm. As a result, curves were obtained that showed transmission and backscattering light level in the function of height of the test tube with the emulsion. The study was carried out for 2 weeks, during which samples of emulsions were stored at 37°±0.5°C, in order to accelerate possible processes leading to emulsion break down. The curves of successive measurements showing the percentage distribution of transmission and backscattering light for stable products should overlap, while the curves of unstable products have a diverse course.

Fig. 2a shows the course run of the curves of backscattering light level for an emulsion with a 3% addition of gum arabic. The analysis of the data in Fig. 2a showed that the curves on the left side of the graph, between 0 and 10 mm of the height of the tube, did not meet. This was caused by a decrease in the backscattering level at the bottom of the emulsion, resulting from a decreased droplet concentration. This was characteristic of the beginning of the creaming process. Lesser changes were observed in the emulsion with a 5% addition of gum arabic. On the other hand, the curves plotted for the emulsion with 10% of gum arabic covered each other perfectly. Similarly, no changes were noted in the course of the curves plotted for the emulsion stabilised by 5% of gum ghatti. For this emulsion the backscattering of light was fairly constant. For the emulsion containing 3% of gum ghatti (Fig. 2b) slight deflections of the curves were observed in the first part of the graph. This could indicate that the processes leading to emulsion destabilization, *i.e.* gravity separation and/or flocculation, had already begun.

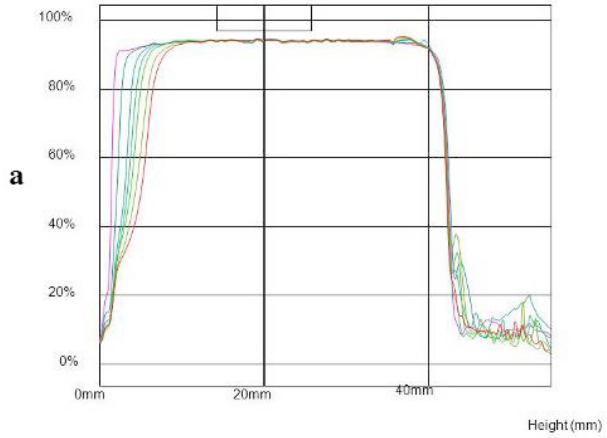


Fig. 2a - Profile of backscattering of the beverage emulsions with 3% addition of gum arabic (the purple line (first from the left) is the first measurement, and the red line is the last measurement (last from the left).

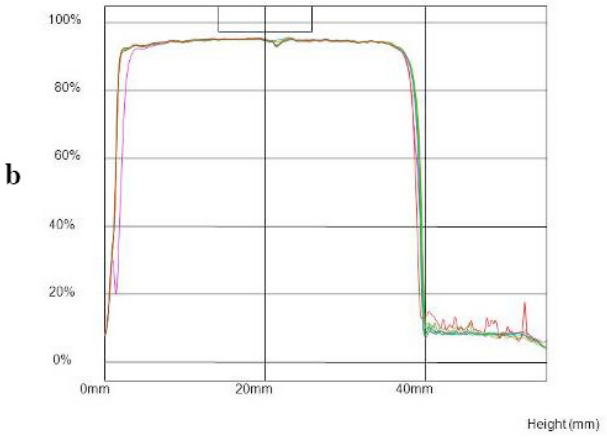


Fig. 2b - Profile of backscattering of the beverage emulsions with 3% addition of gum ghatti (the purple line (first from the left) is the first measurement, and the red line is the last measurement (last from the left).

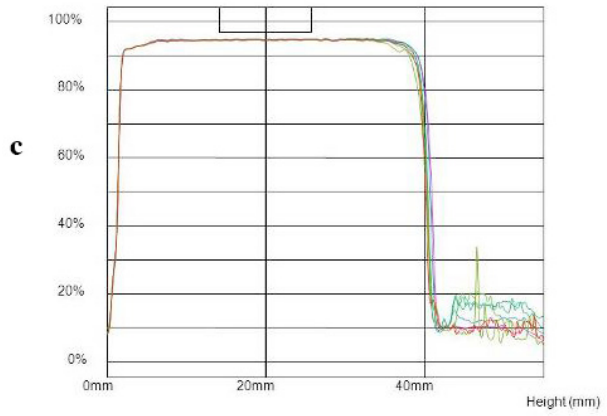


Fig. 2c - Profile of backscattering of the beverage emulsions with 3% addition of a mixture of arabic and ghatti gum (1:1) (the purple line (first from the left) is the first measurement, and the red line is the last measurement (last from the left).

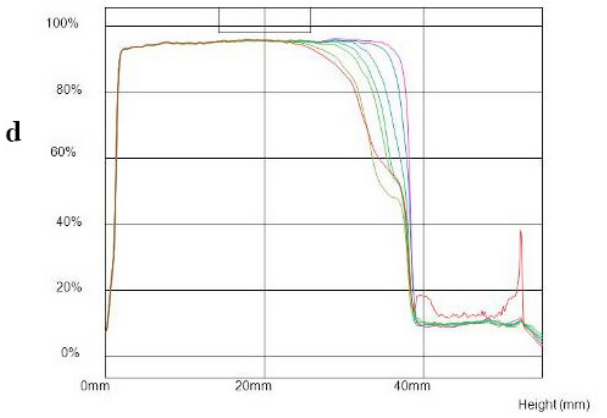


Fig. 2d - Profile of backscattering of the beverage emulsions with 3% addition of a mixture of arabic and ghatti gum (1:2) (the purple line (first from the left) is the first measurement, and the red line is the last measurement (last from the left).

For emulsions with the addition of a mixture of arabic and ghatti gums (1:1, w/w) no noticeable disturbances were noted in the course of curves illustrating the stability of the examined sample (Fig. 2c). However, analysing the course of the curves showing changes of backscattering light level (Fig. 2d), a relatively low stability was determined for the emulsion with arabic and ghatti gums addition in 1:2 (w/w) proportion. The curves showing the percentage distribution of backscattering light clearly differed from each other at the top, which was certainly caused by the flocculation and/or sedimentation phenomenon.

Amongst the examined samples of beverage emulsions the highest stability was found in emulsions stabilised by 10% of gum arabic or by 5% of gum ghatti. The addition of the emulsifier can be reduced to 3% of emulsion mass, without decreasing its stability, by applying a mixture of these gums (1:1, w/w).

CONCLUSIONS

Both gum arabic as well as gum ghatti form stable emulsions. In order to obtain emulsions with the same stability it is necessary to use double amount of gum arabic in relation to gum ghatti. Emulsions with high stability can be produced by applying a mixture of these gums. Emulsions with higher stability were formed using a mixture of gum arabic and gum ghatti in the ratio of 1:1 (w/w), as opposed to a mixture prepared in the ratio of 1:2 (w/w). The synergetic effect of gum arabic and gum ghatti enables reducing emulsifier addition without decreasing emulsion stability.

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PAPER

EFFECTS OF OZONIZED FLAKE ICE ON SENSORY AND MICROBIOLOGICAL QUALITY OF *PAGELLUS ERYTHRINUS*

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ABSTRACT

To investigate the efficacy of ozone combined with both water and ice on the quality loss of *Pagellus erithrynus* during a 16-day chilling period, a total of 72 samples were collected and analysed. Sensory and microbiological analyses (skin and muscle) were carried out after 0, 3, 5, 7, 10, 12, 14 and 16 days. Collected samples were stored under flake ice (control batch), pre-treated with ozonized water (water ozone batch) and stored under ozonized flake ice (ozone batch). The highest freshness category up to 5 days and the best antimicrobial success (lower than 5 Log CFU per cm^2/g up to 16 days) were found in the ozone batch. The use of ozonized flake ice might represent a useful tool to enhance the shelf-life of fresh *Pagellus erithrynus*.

- Keywords: ozone, *Pagellus erithrynus*, shelf-life -

INTRODUCTION

Microorganisms have a critical rule in marine fish spoilage limiting the shelf-life of fresh fish. As well known, bacterial activity has a deep impact on sensory features (KOBATAKE *et al.*, 1992; ASAKAWA *et al.*, 1998; GENNARI *et al.*, 1999) and leads to a degradation of extractive nitrogen, amino acids, fats and sugars, and to a develop of ammonia-like off-flavors (GRAM *et al.*, 2002) due to the presence of chemical molecules, such as trimethylamine (TMA), ammonia and hydrogen sulphide. The nitrogen substances are then decomposed by proteolytic bacteria (*Achromobacter*, *Pseudomonas*, *Micrococcus*, *Bacillus*, *Alteromonas putrefaciens*) until the liberation of amino acids (LISTON, 1980). The subsequent demolition of the substrate amino acid leads to the appearance of foul smelling volatile compounds, including amines, ammonia, short chain fatty acids, mercaptans and hydrogen sulfide (MAKARIOS-LAHAM and LEE, 1993). Spoilage is also linked to several factors like handling technique (unhygienic handling), fish species (moisture and fat content), storage condition (OLAFSDÖTTIR *et al.*, 1997) and starts immediately after the caught of fish (ABABOUCH *et al.*, 1991). Thus, reducing the growth of many spoilage microorganisms would enhance the quality of fishery products and subsequently increase their shelf-life. Several authors have published their findings on refrigeration systems based on the use of ozone to inhibit spoilage and preserve freshness. Different fish species have been examined after pre-treatment with ozonized water (KOTTERS *et al.*, 1997) and ozonized slurry ice (CAMPOS *et al.*, 2005; AUBOURG *et al.*, 2009; ALVAREZ *et al.*, 2009). Ozone (O_3) has a strong oxidizing effect (lower only to fluorine) on a broad antimicrobial, antiviral and antifungal spectrum (GUZEL-SEYDIMA *et al.*, 2004). O_3 has already been recognized as a valid method GRAS (Generally Recognized As Safe) and it can be used as antimicrobial agent in both aqueous and gaseous phase in the treatment, storage and processing of food including beef and poultry (USDA, 2002).

The aim of the present study was to investigate the efficacy of ozone combined with both water and flake ice on the quality loss of *Pagellus erythrinus* (or common pandora fish) during chilled storage; this fish is a commercially appreciated species of *Sparidae* family from the Mediterranean and the Black Sea, and the eastern coast of the Atlantic Ocean (Angola to Norway) (FISCHER *et al.*, 1987). Sensory and microbiological analyses were carried out to evaluate the quality changes during a 16-day shelf life period.

MATERIALS AND METHODS

A total of 72 samples (*Pagellus erithrynus*) were collected at the local fishery market three hours after the caught neither headed nor gutted. Collected samples were divided in three batches, one of which used as control (CB, control batch). In the CB, chilling was guaranteed by covering fishery products with a thin plastic film with flake ice on the top. The second batch (WOB, water ozone batch) was firstly washed with ozonized water (3 mg/L) for 3 min and then chilled by keeping samples under a thin plastic film with flake ice on the top. In the last batch (OB, ozone batch) chilling was done by covering samples with ozonized ice (3mg/L). Treated water and ice were obtained by injection of ozone using a prototype (OXITECH S.r.l., Italy). In both cases (ozonized ice and flake ice), the fish/ice ratio was 1:1. During the experimental time the ice was renewed repeatly. On fixed days (0, 3, 5, 7, 10, 12, 14 and 16), three samples per each batch were taken and transported cooled to the laboratory where sensory and bacteriological analyses were carried out within 3 hours. Sensory analyses were done by a panel of five untrained panelists up to sixteen days. A quality index classifying samples in freshness categories, highest quality (E) to unacceptable (C), was attributed to each sample in accordance with parameters listed in Table 1 (Council Regulation 2406/96/ EC). To evaluate the ozone efficiency on the microbiological contamination of fishery products two aliquots were collected from each sample (skin surface and muscle). The skin surface was processed by using the double wet/dry swabbing technique over a 5 cm^2 area delimited by a sterile template. Briefly, the wet swab was rubbed vertically, horizontally, then diagonally across the template surface (20 sec). Swabbing was then repeated with a dry swab. Swabs were placed into a sterile stomacher bag and homogenized in 10 mL of 0.1% peptone water (Oxoid Ltd., Hampshire, UK) for 60 sec. The second aliquot (5 g of muscles) was aseptically cut off using a sterile blade and then placed in a sterile stomacher bag. Muscles were homogenized in 45 mL of 0.1% peptone water (Oxoid Ltd.) for 60 sec. Microbiological analyses were done by culture after a dilution step. For enumeration of total bacterial count a subset (0.1 mL) from each dilution was inoculated onto Plate Count Agar (Oxoid Ltd.) and incubated at 30°C for 3 days (TBC 30°C) and at 5°C for 10 days (TBC 5°C). Enumeration of proteolytic bacteria was estimated by plating 0.1 mL from each dilution onto casein-agar medium (PHAFF *et al.*, 1994), as described by BEN-GIGIREY *et al.* (2000). Microbiological counts were expressed as Log CFU per cm^2/g of the average values of three independent determinations. One-way ANOVA with Tukey post tests was performed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. A confidence interval at the 95% level ($P < 0.05$) was considered to explore significance of differences among microbiological parameters throughout storage for each refrigeration system.

RESULTS AND DISCUSSION

Results of sensory analyses are reported in Table 2. A score decrease was observed gradually. The appearances of skin mucus and eyes limited firstly the fish acceptability in all batches. CB showed good quality until day 3 (E and A categories) and acceptable until day 10. WOB retained a good quality until day 5 and acceptable until day 14. The best results were found in OB, that showed good quality until day 7 and acceptable until day 16 highlighting the effectiveness of ozonized flake ice to keep freshness. Sensory results in this study are in agreement with previous studies on the application of ozone to extend the shelf life of different fish species as rockfish (KOETTERS *et al.*, 1997), catfish fillets (KIM *et al.*, 2000) and *Pagellus bogaraveo* where the highest sensory quality was assessed up to 9 days after treatment with flow ozonized ice (ALVAREZ *et al.*, 2009). Microbiological results regarding skin aliquots are reported in Table 3. Regarding TBC 30°C, statistically significant differences ($P < 0.05$) were evidenced on day 5 until day 16 between OB and CB. On the contrary, differences were not evi-

Table 1 - Freshness categories according to Council Regulation 2406/96/ EC.

Attribute	Highest quality (E)	Good quality (A)	Fair quality (B)	Unacceptable (C)
Skin	Bright pigmentation, Bright, shining, Iridescent	Loss of lustre and shine; duller colours; less difference between dorsal and ventral surfaces	Dull, lustreless, insipid colours; Skin creased when fish curved	Very dull pigmentation; Skin coming away from flesh
Skin mucus	Aqueous, Transparent	Slightly cloudy	Milky	Yellowish grey, Opaque mucus
Consistency of flesh	Very firm, rigid	Fairly rigid, firm	Slightly soft	Soft (flaccid)
Gill covers	Silvery	Silvery, slightly Red or brown	Brownish and extensive Seepage of blood from vessels	Yellowish
Eye	Convex, bulging; Blueblack bright pupil, transparent 'eyelid'	Convex and slightly sunken; dark pupil; Slightly opalescent Cornea	Flat; blurred pupil; blood seepage grey Around the eye	Concave in the centre; pupil; milky corne
Gills	Uniformly dark Red to purple. No Mucus	Less bright colour, paler at edges. Transparent Mucus	Becoming thick Discoloured, opaque Mucus	Yellowish; milky mucus
Smell of gills	Fresh, seaweed; Pungent; iodine	No smell or seaweed. Neutral smell	Slightly sulphureous fatty Smell, rancid bacon Cuttings or rotten fruit	Rotten sour

Table 2 - Comparative sensory evaluation of *Pagellus erithrynus* samples under conventional chilling (CB, Control batch), chilling after pre-treatment with ozonized water (WOB, Water Ozone Batch), flake ozone ice chilling (OB, ozone batch) during a 16-day storing time.

	CB	WOB	OB	CB	WOB	OB	CB	WOB	OB	CB	WOB	OB	CB	WOB	OB	CB	WOB	OB	CB	WOB	OB	CB	WOB	OB
DAYS	0			3			5			7			10			12			14			16		
SKIN	E	E	E	A	E	E	A	A	E	A	A	A	A	A	A	B	A	A	B	B	B	C	B	B
SKIN MUCUS	E	E	E	A	E	E	B	A	E	B	B	A	C	B	A	C	B	B	C	C	B	C	C	C
CONSISTENCY OF FLESH	E	E	E	A	E	E	B	A	A	B	A	A	B	A	A	B	B	B	C	B	B	C	C	B
GILL COVERS	E	E	E	A	E	E	A	A	A	B	A	A	B	A	A	B	A	A	C	B	B	C	C	B
EYE	E	E	E	A	E	E	B	A	A	B	A	A	B	B	B	B	B	B	C	C	B	C	C	C
GILLS	E	E	E	A	A	A	A	A	A	A	A	A	B	A	A	B	B	B	C	B	B	C	B	C
SMELL OF GILLS	E	E	E	A	E	E	B	A	A	B	A	A	B	B	B	B	B	B	C	B	B	C	B	C

Table 3 - Comparative microbial evaluation of skin *Pagellus erithrynus* samples under conventional chilling (CB, Control batch), chilling after pre-treatment with ozonized water (WOB, Water Ozone Batch), flake ozone ice chilling (OB, ozone batch) during a 16-day storing time.

	Total bacterial count 30°C			Total bacterial count 5°C			Proteolytic bacteria		
Day	CB	WOB	OB	CB	WOB	OB	CB	WOB	OB
0	1,50(0,23)^{a}	1,11(0,21)^{a}	1,20(0,29)^{a}	1,16(0,22)^{a}	1,18(0,32 ^{a}	1,20(0,33)^{a}	1,10(0,11)^{a}	1,20(0,12)^{a}	1,00(0,23)^{a}
3	1,73(0,29)^{a}	1,43(0,12)^{a}	1,31(0,27)^{a}	2,00(0,23)^{a}	2,10(0,24)^{a}	1,50(0,27)^{b}	1,84(0,13)^{a}	2,00(0,22)^{a}	1,63(0,29)^{a}
5	2,02(0,34)^{a}	2,20(0,23)^{a}	1,51(0,33)^{b}	2,86(0,34)^{a}	2,49(0,22)^{a}	1,71(0,34)^{b}	2,24(0,23)^{a}	2,50(0,21)^{a}	1,75(0,35)^{b}
7	2,51(0,26)^{a}	2,82(0,43)^{a}	1,81(0,51)^{b}	2,97(0,13)^{a}	2,64(0,23)^{a}	2,45(0,44)^{b}	2,89(0,33)^{a}	2,31(0,33)^{a}	1,90(0,23)^{b}
10	2,93(0,25)^{a}	2,35(0,25)^{a}	1,99(0,78)^{b}	4,56(0,13)^{a}	4,35(0,19)^{a}	3,78(0,29)^{b}	3,30(0,23)^{a}	3,05(0,35)^{a}	2,92(0.38)^{a}
12	3,19(0,24)^{a}	3,28(0,45)^{a}	2,52(0,45)^{b}	4,02(0,22)^{a}	4,19(0,34)^{a}	3,93(0,65)^{a}	3,50(0,32)^{a}	3,80(0,32)^{a}	3,20(0,41)^{a}
14	4,20(0,33)^{a}	4,11(0,31)^{a}	3,12(0,45)^{b}	4,60(0,24)^{a}	4,54(0,33)^{a}	4,01(0,54)^{b}	4,48(0,34)^{a}	4,08(0,44)^{a}	3,51(0,28)^{b}
16	4,37(0,11)^{a}	4,06(0,19)^{a}	3,48(0,43)^{b}	5,67(0,12)^{a}	5,60(0,38)^{a}	4,53(0,33)^{b}	4,97(0,28)^{a}	4,93(0,51)^{a}	3,98(0,32)^{b}

Microbiological counts were expressed as Log CFU per cm^2/g^{-1} of the average values of three independent determinations. Values within a row without a common superscript are significantly different at P<0.05.

Table 4 - Comparative microbial evaluation of muscle *Pagellus erithrynus* samples under conventional chilling (CB, Control batch), chilling after pre-treatment with ozonized water (WOB, Water Ozone Batch), flake ozone ice chilling (OB, ozone batch) during a 16-day storing time.

	Total bacterial count 30°C			Total bacterial count 5°C			Proteolytic bacteria		
Day	CB	WOB	OB	CB	WOB	OB	CB	WOB	OB
0	0,93(0,51)^{a}	0,90(0,35)^{a}	1,09(0,23)^{a}	2,00(0,18)^{a}	2,50(0,21)^{a}	2,20(0,24)^{a}	1,00(0,32)^{a}	1,00(0,33)^{a}	0,90(0,23)^{a}
3	2,27(0,43)^{a}	1,90(0,39)^{a}	1,82(0,43)^{a}	3,50(0,19)^{a}	2,70(0,27)^{b}	2,10(0,35)^{b}	1,73(0,23)^{a}	1,80(0,25)^{a}	1,31(0,19)^{b}
5	2,51(0,43)^{a}	2,70(0,41)^{a}	2,19(0,38)^{a}	3,11(0,27)^{a}	2,92(0,29)^{a}	2,80(0,24)^{a}	2,85(0,19)^{a}	3,10(0,25)^{a}	2,10(0,11)^{b}
7	3,81(0,37)^{a}	3,30(0,45)^{a}	2,77(0,28)^{b}	4,03(0,24)^{a}	3,21(0,33)^{b}	3,00(0,27)^{b}	2,86(0,18)^{a}	2,81(0,28)^{a}	2,33(0,19)^{b}
10	3,88(0,25)^{a}	3,30(0,35)^{ab}	2,96(0,56)^{b}	4,18(0,34)^{a}	3,79(0,32)^{ab}	3,40(0,43)^{b}	3,26(0,27)^{a}	3,20(0,33)^{a}	2,90(0,27)^{a}
12	4,17(0,34)^{a}	3,77(0,23)^{ab}	3,59(0,36)^{b}	5,08(0,31)^{a}	4,48(0,65)^{b}	4,00(0,37)^{b}	4,20(0,33)^{a}	4,04(0,41)^{a}	3,80(0,26)^{a}
14	4,59(0,28)^{a}	4,28(0,23)^{ab}	3,9(0,34)^{b}	5,70(0,56)^{a}	6,29(0,56)^{a}	4,43(0,36)^{b}	4,62(0,32)^{a}	4,04(0,22)^{ab}	3,90(0,21)^{b}
16	4,46(0,19)^{a}	4,98(0,32)^{a}	4,18(0,19)^{b}	6,52(0,67)^{a}	6,32(0,76)^{a}	4,91(0,28)^{b}	4,99(0,21)^{a}	5,02(0,23)^{a}	5,00(0,22)^{a}

Microbiological counts were expressed as Log CFU per cm^2/g^{-1} of the average values of three independent determinations. Values within a row without a common superscript are significantly different at P<0.05.

denced between WOB and CB. The average difference determined for CB and OB up day 16 was 0,56 Log units. In OB the microbial count was below 2 Log CFU/cm^2 up to 10 day and did not reach concentrations of 4 Log CFU/cm^2 after 16 days of storage. The microbial load in all the batches was below 6 Log CFU/cm^2 up to 16 days, value considered necessary to induce fish spoilage (GRAM and HUSS, 1996). With regard to TBC 5°C, statistically significant differences ($P < 0.05$) were evinced between OB and CB starting from day 3 up to 16 (day 12 excepted). Significant differences were not proved between WOB and CB. In both batches microbial load was 2 Log CFU/cm^2 at day 3 with a significant increase at day 10. The average difference between OB and CB was 0,59 Log units. Regarding proteolytic bacteria statistically significant differences ($P < 0.05$) were evinced between OB and CB at days 5,7,14,16. Differences between WOB and CB were not observed. The average difference between OB and CB was 0,55 Log units. With regard to muscle aliquots, comparative results are reported in Table 4. In TBC 30°C significant differences were found between CB and OB from day 7 to the end, but no differences were found between WOB and CB samples; the average difference between OB and CB was 0,41 Log units. In TBC 5°C significant differences ($P < 0.05$) were observed between CB and OB from day 3 to day 16 (day 5 excepted) and between WOB and CB at days 3,7 and 12. The average difference between CB and OB was 1,03 Log units and between WOB and CB was 0.23 Log units. Concerning proteolytic bacteria significant differences ($P < 0.05$) were evidenced between CB and OB at days 3, 5 , 7 and 14, but not evidenced between CB and WOB: the average difference between CB and OB was 0,40 Log units.

To the best of our knowledge, this is the first study providing evidence of the antimicrobial efficacy of ozonized flake ice. The batch treated with ozonized flake ice (OB) showed the highest freshness category (up to 5 days) and the best antimicrobial success. In agreement with recent

studies (AUBOURG *et al.*, 2006; AUBOURG *et al.*, 2009; CAMPOS *et al.*, 2005; LU et al., 2012) the ozone activity was confirmed by reducing fish spoilage bacteria both in muscle and skin aliquots during storing time. ALVAREZ *et al.* (2009) by using a combination of flow ice and ozone found an average reduction of 0.56, 0.46 and 0.46 Log units respectively for total aerobes and psychrotrophes and proteolytic bacteria in *Pagellus bogavero* muscle. On the other hand, in our study a lower reduction (0.41 and 0.4 Log units) for TBC 30 and 5°C and a grater reduction (1.03 Log units) for proteolytic was demonstrated. Based on our data, an ozone concentration of 3 mg/L might positively affect sensory quality and be effective in slowing down microbial activity when used in combination with flake ice. Differently, the use of the same concentration in water pre-treatment slightly influenced sensory features and did not significantly affect microbial contamination.

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PAPER

CHEESE MAKING USING PIG RENNET AND CALF RENNET: MICROORGANISMS AND VOLATILE COMPOUNDS IN FARINDOLA EWE CHEESE

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ABSTRACT

Pecorino cheese, a traditional local product of Farindola, is a unique cheese made using pig rennet in Italy. In this study the evolution of bacterial flora and volatile substances at different ripening times of pig rennet and calf rennet cheese were investigated, taking into consideration the "Production Regulation". The results showed interesting differences between the two types of cheese as a function of volatile substance and microorganism evolution. Gas-Chromatographic analysis showed the particular volatile substances profile of cheeses made with pig rennet. Linear Discriminant Analysis (LDA) was applied to classify the cheese samples according to different rennets and treatments.

- Keywords: Pig rennet, calf rennet, microorganism ewe cheese, Farindola ewe cheese -

INTRODUCTION

The functionality and the origin of rennet are considered important factors in the making of cheese (BALCONES *et al.*, 1996) and in the production of cheese flavors (URBACH, 1997).

Farindola ewe cheese is a traditional product made using pig rennet and its production takes place in Farindola (Abruzzo) and in some areas of the country in the provinces of Pescara and Teramo. This product comes from an old tradition dating from the Roman period which is called "Vestini cheese". In the scientific literature it seems to be the only known cheese product that is made using pig rennet. The use of pig rennet became obsolete in industrial cheese production because it is not very stable and operates at a pH range that is narrower than that of calf rennet; a standardization to obtain constant enzyme activity in pig rennet is more difficult than in calf rennet.

Farindola ewe cheese is a niche product, the preparation of which is regulated by a "Production regulation", as has been described in previous studies (DI GIACOMO *et al.*, 2009; DI GIACOMO *et al.*, 2014). In these works a comparative study between cheeses made with pig rennet and calf rennet as a function of maturation, amino acids, fatty acids, vitamins, cholesterol evolution and the qualitative determination of some volatile substances was carried out. In addition, a sensory panel of expert tasters showed differences between the two types of cheese: the ewe cheese made with calf rennet is consistently more spicy ("hot" flavor) and more bitter than the ewe cheese made with pig rennet, whereas this latter is always sweeter and never bitter.

One of the most important parameters that effects the judgment of tasters is the flavor of cheese, which depends on the different cheese varieties and on the correct balance and concentration of a wide range of taste and aromatic compounds. Furthermore, the use of raw and unpasteurized milk, increases the flavour notes (GRAPPIN and BEUVIER, 1997) greatly thanks to the different microorganisms naturally present (MARILLEY and CASEY, 2004; GARDE *et al.*, 2002).

This study represents an advanced research of Farindola ewe cheese with the aim of observing the evolution of volatile compounds and the relationships with the microbial flora. In fact, only lower molecular weight compounds contribute significantly to cheese flavor. An important group of low weight molecular compounds are the volatile compounds (SABLE´ and COTTENCEAU, 1999). The biochemical pathway for the production of flavour compounds in cheese during their ripening is reviewed by MCSWEENET and SOUSA (2000).

MATERIALS AND METHODS

Cheese making

Fresh ewe milk, coming from ewes that produce less than 1 litre/day in about 100 milking days, was kept cool (10°-12°C). A small quantity of milk was curdled at 31°-33°C with pig rennet and another quantity was processed under the same conditions with calf rennet. The setting time varied from 40 to 60 minutes; after the curdle was broken into granules of 0.5 to 2 cm, it was placed into straw forms to harden; it was then dry salted with coarse salt on both sides – one side of the cheese was salted one day, and the other side the next day. Then the salt was washed off. The period of ripening varied from a minimum of three months to a maximum of nine months and each cheese form weighed between 1 and 2 kg.

Samples and Parameters

1) 2 kg forms were made. They are generally commercialized after 3-6 months, but even up to 1 year; they are ripened at a temperature between 10° and 14°C; 2) Time: the samples were analyzed at 3, 6 and 9 months; 3) the surface of some cheese forms were treated with extra virgin olive oil and vinegar (this treatment is included in the "Production Regulation"), some other were left untreated[1].

Microorganism analysis

The growing substrate, the growing temperature and the aerobic/anaerobic conditions are reported in the following scheme.

Microorganism substrate	Growing	Growing temperature	Oxygen condition	Method references
Cocci	M17	37°C	anaerobic conditions	ISO 7889/2003
Lactobacillus spp.	MRS	37°C	anaerobic conditions	ISO 7889/2003
Ethero-fermentative mesophilic lactobacilli	FH	30 °C	anaerobic conditions	Isolini *et al*., 1990
Citrate fermenting bacteria	MRS-C	30°C	anaerobic conditions	Bottazzi *et al*., 1971
Leuconostoc spp.	MSE	21°C	aerobic conditions	Mayeux *et al*., 1962
Yeasts	YGC	25°C	aerobic conditions	ISO 6611/1981
Moulds	YGC	25°C	aerobic conditions	ISO 6611/1981
Enterococcus spp.	KAA	42°C	aerobic conditions	Mossel *et al*., 1978
Propionicbacterium spp.	PAL	30°C	anaerobic conditions	Thierry and Madec 1995
Lipolytic bacterium	TBA	30°C	aerobic conditions	Fryer *et al*., 1967
Proteolytic bacteria	AL	30°C	aerobic conditions	Internal method of CRA-FLC[2]

[1] From this point on, for the sake of brevity, the words "treated" or "untreated" will be used.
[2] The Fodder and Dairy Productions Research Centre (CRA-FLC) of Lodi.

Volatile compounds analysis

The time evolution of 31 volatile compounds was determined using gas chromatography – mass spectrometry; the extraction methodology of volatile compounds was discussed extensively in an earlier study regarding ewe dairy products (POVOLO *et al.*, 2007). This study can be summarized as follows: a divinylbenzene/carboxen/polydimethylsiloxane, 50/30 μm, 2-cm-long fiber was used to collect volatile fractions by SPME. 6 g of grated cheese, roughly cut into small pieces shortly before the analysis, was weighed in a crimp-top vial. Cheese samples were allowed to equilibrate to 45°C in a thermostatic bath for 5 min. The extraction of volatile compounds from pasture was performed in duplicate, maintaining the sample at room temperature, and exposing the fiber to the headspace for 15 min. The gas chromatographic analysis of the volatile compounds adsorbed on the SPME fiber was carried out with a CP-WAX 52CB capillary column. A mass spectrometer was used.

Statistical analysis

Linear Discriminant Analysis (LDA) was applied to separate the analyzed cheese samples according to the type of rennet and treatment in order to evaluate the sample differentiation and classification of the data expressed as discriminant scores. All data obtained were analyzed statistically using the multivariate statistical approach through the use of SPSS 8.0 statistical software. This methodology was applied to separate the cheese samples based on the presence of volatile compounds and microorganisms.

LDA has been extensively discussed by several authors (ANDERSON 1984; LEBART *et al.*, 1984; MARDIA *et al.*, 1993).

RESULTS AND DISCUSSION

Microorganism evolution

The microorganism evolution, during the ripening is reported in Table 1. The values are expressed in CFU/g of cheese. Most of the microorganisms show a typical decreasing trend as a function of the ripening. As an example, the trend of lactobacilli, *Enterococcus spp.*, yeasts and moulds is shown in Figs. 1-4.

At starting time, the most important microbiological difference between the cheese made with pig rennet and the cheese made with calf rennet is the content of mesophilic lactobacilli (1.4×10^7CFU/gin cheese made using pig rennet compared; 9.6×10^6 CFU/g in cheese made using calf rennet), the yeast content (2.7×10^5CFU/g in cheese made using pig rennet; 2.9×10^6 CFU/g in cheese made using calf rennet), the mould

Table 1 - Evolution of microorganism (CFU/g) during the ripening (PR= pig rennet, CR= calf rennet, TPR = treated pig rennet, UPR= untreated pig rennet, TCR = treated calf rennet, UCR = untreated calf rennet).
The results obtained from 3 observations, for each compound.

	Starting time		3 months				6 months				9 months			
Microorganisms	PR	CR	TPR	UPR	TCR	UCR	TPR	UPR	TCR	UCR	TPR	TCR	UCR	
cocci	2.4E+08	4.1E+08	2.4E+07	2.1E+07	2.9E+07	2.8E+07	5.6E+06	3.2E+06	5.4E+06	8.4E+06	2.0E+06	4.9E+06	3.6E+06	
lactobacilli	1.5E+07	2.7E+07	4.0E+07	2.7E+07	1.6E+07	2.0E+07	1.9E+06	1.3E+06	4.4E+06	3.6E+06	2.5E+05	4.5E+05	2.4E+05	
mesophilic	1.4E+07	9.6E+06	8.3E+07	3.1E+07	2.1E+07	2.5E+07	4.5E+06	1.2E+06	2.3E+06	9.1E+05	1.5E+05	5.8E+05	2.5E+05	
lactobacilli citrate fermentating bact.	3.4E+07	1.7E+07	2.7E+06	8.6E+06	4.8E+05	7.5E+06	2.9E+04	1.4E+04	5.0E+03	5.0E+03	7.1E+03	3.0E+03	5.5E+01	
leuconostoc	2.3E+06	4.0E+06	9.1E+05	8.0E+05	4.4E+06	3.4E+05	5.0E+03	5.0E+03	5.0E+03	5.0E+03	1.5E+02	2.5E+02	5.0E+01	
yeast	2.7E+05	2.9E+06	1.0E+03	3.0E+04	1.4E+03	2.0E+03	1.9E+05	2.3E+05	2.7E+03	5.9E+03	2.3E+04	3.9E+03	2.0E+02	
mould	3.0E+03	8.5E+04	2.5E+02	3.5E+04	5.0E+01	1.0E+03	5.0E+01	5.0E+01	5.0E+01	5.0E+01	3.5E+02	2.8E+03	5.0E+01	
enterococci	5.0E+06	6.0E+06	1.9E+06	2.0E+06	2.0E+06	1.5E+06	3.8E+05	1.9E+05	6.7E+05	6.0E+05	1.2E+05	3.7E+05	4.4E+05	
propionic bacteria	4.0E+02	1.6E+03	5.0E+01	5.0E+01	5.0E+01	5.0E+01	5.0E+01	5.0E+01	5.0E+01	5.0E+01	1.7E+03	2.0E+04	1.1E+03	
beta lipolytic bacteria	1.7E+06	2.1E+06	1.3E+06	2.6E+06	2.3E+06	3.1E+06	5.0E+03	2.7E+04	5.0E+03	5.0E+03	7.9E+05	3.5E+05	5.5E+05	

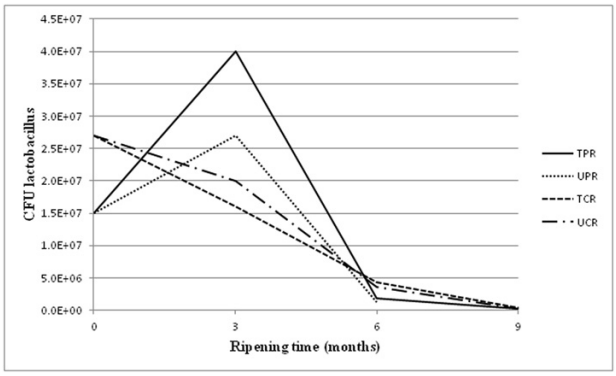


Fig. 1 - Lactobacilli evolution at different times of ripening.

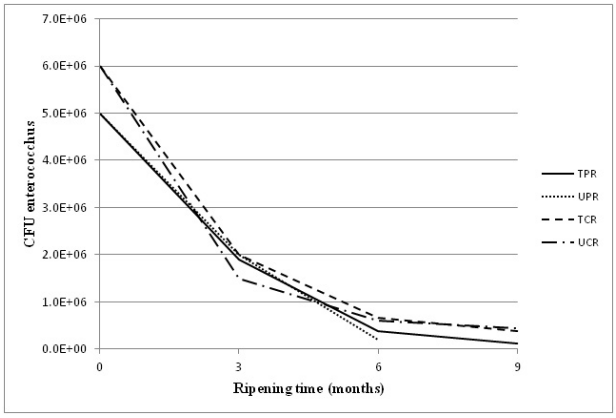


Fig. 2 - Enterococci evolution at different times of ripening.

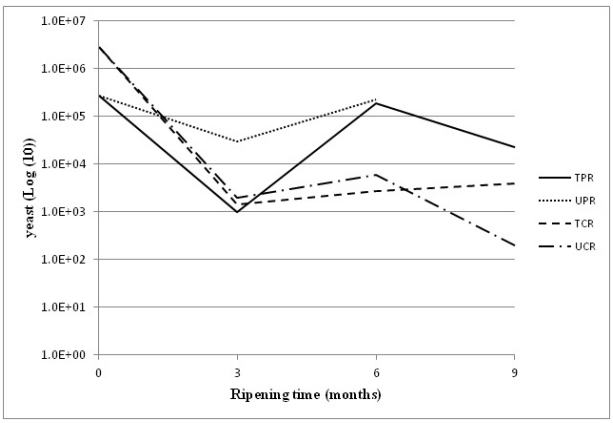


Fig. 3 - Yeasts evolution at different times of ripening.

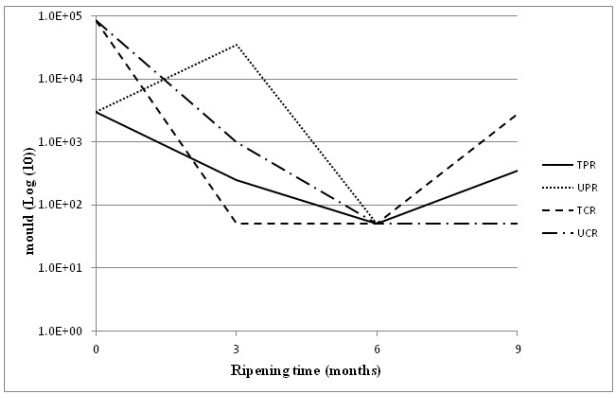


Fig. 4 - Moulds evolution at different times of ripening.

content (2.0×10^3CFU/g in cheese made using pig rennet; 8.5×10^4 CFU/g in cheese made using calf rennet) and propionic bacteria content (4.0×10^2CFU/g in cheese made using pig rennet; 1.6×10^3 CFU/g in cheese made using calf rennet).

In the third month of ripening, the cheese made using treated pig rennet has a lactobacilli content of 4.0×10^7 CFU/g, while the cheese made using untreated pig rennet has a content of 2.7×10^7 CFU/g; in the cheeses made using calf rennet the content of lactobacilli is lower: 1.6×10^7 CFU/g in treated cheese and 2.0×10^7 CFU/g in the untreated cheese respectively. It's interesting to observe that, at this time of ripening, the content of mould in cheese made using untreated pig rennet (3.5×10^4 CFU/g) is greater than in other samples (2.5×10^2 CFU/g in cheese made using treated pig rennet; 5.0×10^1CFU/g in cheese made using treated calf rennet; 1.0×10^3 CFU/g in cheese made using untreated calf rennet).

At six months, the trend of lactobacilli changes completely with regards to the type of rennet. At this time, the lactobacilli content in cheese made with pig rennet decreased: 1.91×10^6 CFU/g in the treated cheese and 1.3×10^6 CFU/g in the untreated cheese; on the contrary, cheese made using calf rennet at this point in the ripening shows an increase in the lactobacilli content (4.4×10^6 CFU/g in the treated cheese; 3.6×10^6 CFU/g in the untreated cheese).

At the same time, the yeast content is greater in the cheeses made using pig rennet (1.9×10^5 in the treated cheese and 2.3×10^5 CFU/g in the untreated cheese) than in cheeses made using calf rennet (2.7×10^3 CFU/g in the treated cheese and 5.9×10^3 CFU/g of cheese in the untreated cheese).

As regards the evolution of mold, all four types of cheese show the same mold content after six months of ripening.

The citrate fermenting bacteria show the major differences between the samples at six months: a content of 7×10^6CFU/g in cheese made using treated pig rennet, 3.4×10^6CFU/g in cheese made using untreated pig rennet while the sample made using untreated calf rennet contains only 5.5×10^1CFU/g.

The cheese made using untreated pig rennet has a higher content (2.7×10^4 CFU/g) of beta lipolityc bacteria at six months, compared with the other cheese sample (5×10^3).

However, these differences observed are not statistically significant at p value less than 0.05.

Quantification and evolution of volatile substances

The volatile compound evolution during the ripening is reported in Table 2. The evolution of volatiles showed a similar trend both in cheeses made with calf rennet and in cheese made

Table 2 - Evolution of volatile compounds (μg/g of cheese dry matter) during the ripening (TPR = treated pig rennet, UPR= untreated pig rennet, TCR = treated calf rennet, UCR = untreated calf rennet).
The results were obtained from 3 observations, for each compound; the standard deviation ranged from 0.1 to 305.31 of nonanone.

	Three months				Six months				Nine months		
Substances	**TPR**	**UPR**	**TCR**	**UCR**	**TPR**	**UPR**	**TCR**	**UCR**	**TPR**	**TCR**	**UCR**
3-octene	22.1	18.4	24.3	22.5	22.7	33.5	53.4	50.0	29.6	33.9	31.2
toluene	17.0	11.3	11.7	8.9	43.9	31.2	58.4	50.9	40.1	52.6	38.3
hexane	0.6	0.5	0.7	0.8	0.8	0.8	0.8	0.6	0.1	0.1	0.1
heptane	1.1	0.9	1.3	1.3	0.5	0.1	1.2	1.1	0.1	0.1	0.1
acetic acid	575.0	439.1	300.5	310.6	1295.7	1289.6	838.3	717.8	827.1	409.7	422.4
propionic acid	12.3	9.0	8.1	7.7	45.2	34.0	31.5	24.5	119.6	12.4	13.4
butyric acid	507.8	425.2	384.9	385.5	3628.4	2606.3	2059.4	2182.2	2756.8	1296.1	1564.0
isobutyric acid	33.2	49.7	38.6	51.3	85.2	73.2	63.1	99.1	57.7	36.1	31.1
pentanoic acid	6.7	4.0	4.4	4.4	54.8	38.4	34.4	26.8	43.7	17.5	17.0
isopentanoic acid	58.5	70.3	57.9	112.4	258.7	207.8	156.5	188.2	152.4	64.4	89.4
hexanoic acid	209.6	162.2	198.9	184.2	2949.8	2226.3	1705.8	1667.1	2322.8	1040.0	1300.6
3-metil 1 - butanol	330.4	299.9	354.5	340.3	227.0	345.8	492.5	481.9	179.0	305.1	268.7
ethanol	187.4	93.4	179.8	147.7	168.1	112.8	277.6	288.1	91.1	196.9	179.4
2-propanol	13.5	0.1	8.1	5.0	55.9	16.9	32.2	22.9	37.7	28.4	14.9
1 - butanol	12.7	5.2	11.4	10.7	14.4	12.0	33.6	13.6	14.3	26.6	13.7
2-butanol	1463.1	1297.4	541.2	672.1	1646.2	1391.0	577.0	423.2	1075.2	554.0	789.5
1-pentanol	0.1	0.1	0.1	0.1	7.7	9.9	10.7	12.7	7.1	9.5	7.0
2-pentanol	158.0	65.3	77.1	64.9	843.4	500.6	277.7	182.0	386.0	241.6	219.1
acetoin	14.9	33.3	24.0	25.5	16.2	33.0	60.3	57.0	18.0	80.3	70.2
2-propanone	43.1	19.9	111.7	65.4	64.1	65.5	317.3	339.4	49.8	169.3	60.9
methyl ethyl ketone	972.9	2636.9	1618.7	1764.2	1143.6	2044.0	1796.1	1648.4	1080.8	1136.7	1406.0
methyl propyl ketone	204.8	258.4	410.6	419.9	874.1	960.4	1477.2	1466.6	541.5	1037.0	830.5
2-heptanone	149.8	156.9	242.0	202.5	2747.5	2337.3	2736.9	2550.6	1832.8	1877.1	1513.9
2-nonanone	32.2	22.8	61.9	35.6	2014.2	1443.6	1470.8	2276.5	1229.8	794.9	928.2
diacetyl	16.7	12.4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
benzaldehyde	1.1	1.7	1.4	1.3	4.8	8.9	11.4	4.0	9.2	21.5	10.2
ethyl butyrate	115.8	73.1	84.3	69.3	329.1	239.7	418.8	425.5	393.4	523.5	498.0
acetic acid ethyl ester	148.5	98.6	27.8	32.9	134.3	40.7	50.7	48.8	71.9	83.9	51.8
dimethyl-sulfide	3.0	3.2	2.7	2.4	1.7	2.0	2.2	2.5	1.0	0.9	0.8
dimethyl-sulphone	2.9	3.0	2.6	2.8	10.5	13.6	10.4	7.6	8.8	4.6	4.8
limonene	0.6	0.7	0.7	0.6	0.1	0.1	0.1	0.1	1.8	0.1	0.1

with pig rennet. The total volatile compound concentration increases up to six months and then slightly decreases in the next months. The cheeses made with pig rennet are richer in volatile substances, with the exception of some compounds.

At three months of ripening, in the cheese made using treated pig rennet, the volatile substance with the greatest concentration is 2 – butanol (1,463.1 μg/g); it is also present at high levels in cheese made using untreated pig rennet (1,297.4 μg/g) whereas it is present in lower levels in the cheese made using calf rennet (541.2 and 672.1 μg/g for treated and untreated cheese, respectively). The volatile substance, which is most abundant in cheeses, is methyl ethyl ketone (2,636.9 μg/g in untreated pig rennet cheese; 1,618.7 μg/g in treated calf rennet cheese; 1,764.2 μg/g in untreated calf rennet cheese), while the cheese made using treated pig rennet is poorer in this substances (972.9 μg/g of cheese).

The level of acetic acid in cheeses made using pig rennet (575.0 μg/g in treated and 439.1 μg/g in untreated) is higher than cheeses made using calf rennet (300.5 μg/g in treated and 310.6 μg/g in untreated). The content of butyric acid is more than 300 μg/g in all types of cheeses.

After six months of ripening, the volatile compounds profile changes. In the cheese made using pig rennet, butyric acid is the volatile substance present in the highest quantity (3,628.4 μg/g in treated and 2,606.3 μg/g in untreated), a higher level than that found in cheeses made using calf rennet (2,059.4 μg/g in treated and 2,182.2 μg/g in untreated cheese). 2 – heptanone is present at high levels both in cheese made using calf rennet (2,736.9 μg/g in treated and 2,550.6 μg/g in untreated cheese) and in cheese made using pig rennet (2,747.5 μg/g in treated and 2,337.3 μg/g in untreated cheese). Another important substance is hexanoic acid, which is present in higher levels in cheeses made using pig rennet (more than 2,000 μg/g) in comparison with cheeses made using calf rennet. Other volatile substances present in quantities of more than 1,000 μg/g in cheeses made with

treated pig rennet are 2 – butanol, methyl ethyl ketone and 2 – nonanone.

The profile of volatile substances differs depending on the type of rennet, time of aging and treatment. The best quality of Farindola Cheese is that made using pig rennet and this is confirmed by the different chemical profiles of volatile substances.

Correlations between volatile compounds and microorganisms

Tables 3 and 4 show the most significant correlations (Pearson) between some volatile substances and microorganisms, at 3 and 6 months of ripening ($p < 0.01$) for pig rennet and calf rennet cheeses respectively. As can be observed from the correlation (Tables 3 and 4), the evolution of microorganisms and volatile substances is clearly different in the cheeses made with the two types of rennet. In particular, some bacteria are correlated differently with volatile substances as in the following examples that are reported:

a) the mesophilic bacteria are negatively correlated with some substances in cheeses made with calf rennet while they are not correlated in those made with pig rennet; b) *Enterococcus spp.* are correlated with some substances of cheeses made with pig rennet, while they are not correlated in the cheeses made with calf rennet; c) coccus show a positive correlation with limonene in cheeses made with calf rennet and they show a strong positive correlation with diacetyl in cheeses made with pig rennet; d) the lactobacilli showed a strong positive correlation with diacetyl in cheeses made with pig rennet, while there is a strong negative correlation with ethanol in cheeses made with calf rennet.

Statistical analysis

A significant Wilks Λvalue was obtained when the cheese samples were classified as a function of the rennets, using the microorganism evolution data set. In this case, one discriminant function was estimated, since the number of groups in this sample was 2, and 2–1 is the maximum allowable number of eigenvalues for the matrix $W^{-1}B$. The first discriminant eigenvalue (9.430) had a Wilks Λ value close to zero (0.096). The distribution of data expressed as discriminant scores along the first eigenvector is presented in Fig. 5. The two sample classes, corresponding to cheese samples with pig rennet and calf rennet, respectively, were clearly distinct. In this case,

Table 3 - Pearson correlation between volatile substances and micro-organism at 3 and 6 months of ripening of pig rennet cheese (p-level < 0.01).

Substances	Cocci	Lactobacilli	Leuconostoc	Yeast	Enterococci	Beta lipolytic bacteria
acetic acid					–0.993	
1-pentanol	–0.99			0.994	–0.994	
2-propanone						–0.999
methyl propyl ketone	–0.998	–0.995	0.997	–0.995		
2-heptanone			–0.989			
diacetyl	0.992	0.997				
dimethyl - sulphone				0.99		
limonene					0.993	

Table 4 - Pearson correlation between volatile substances and micro-organism at 3 and 6 months of ripening of calf rennet cheese (p-level < 0.01).

Substances	Cocci	Lactobacilli	Mesophilic lactobacilli	Yeast	Beta lipolytic
3-octene	–0.997				
toluene	–0.997				
acetic acid	–0.997				
butyric acid			–0.991		
isobutyric acid				0.992	
pentanoic acid	–0.994				
hexanoic acid	–0.996	–0.99			
3-metil 1 - butanol	–0.994				
ethanol		–1			–0.998
acetoin	–1				
2-propanone			–1		
methyl propyl ketone	–0.996				
ethyl butyrate	–0.992				
limonene	0.991				

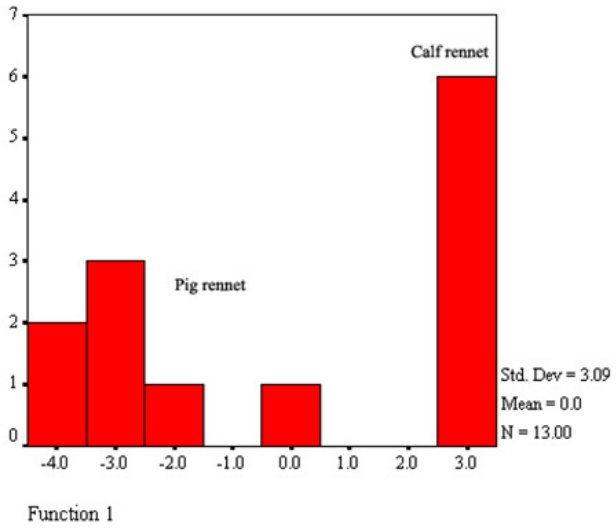


Fig. 5 - The distribution of data regarding the micro-organism evolution, related to cheese samples as function of the two rennets, expressed as discriminant scores along the first eigenvector. 100.0% of original grouped cases correctly classified.

all cheese samples were correctly assigned to the group they belong to. Furthermore the overall classification success was 100.0%.

According to Wilks Λ value another distribution was quite significant. In fact, if the data set regarding the volatile substance content is analysed, the results obtained are the following. In this case, one discriminant function was estimated, since the number of groups in this sample was 2, and 2–1 is the maximum allowable number of eigenvalues for the matrix $W^{-1}B$. The first discriminant eigenvalue (108.743) had a Wilks Λ value close to zero (0.009). The distribution of data regarding the volatile substances expressed as discriminant scores along the first eigenvector is presented in Fig. 6. In this representation of all volatile substances, the two sample classes, corresponding to cheese samples as function of the two rennets, respectively, were distinct and the overall classification success was 100.0%.

A significant Wilks Λvalue was obtained when the data set regarding the volatile substances was classified as a function of treatments. In this case, one discriminant function was estimated; the first discriminant eigenvalue (20.227) had a Wilks Λ value close to zero (0.047). The distribution of data regarding the volatile substances as a function of treatment expressed as discriminant scores along the first eigenvector is presented in Fig. 7. In this representation of data regarding the volatile compounds, the two sample classes, corresponding to cheese samples as function of the treatments, respectively, were clearly distinct. In fact the overall classification success was 100.0%.

A significant Wilks Λvalue was obtained when the cheese samples were classified as a function of the rennets and of the treatment, using a volatile substance data set. In this case, 3 discriminant functions were estimated, since the number of groups in this sample was 4, and 4–1 is the maximum allowable number of eigenvalues for the matrix $W^{-1}B$. The first discriminant eigenvalue (27.979) had a Wilks Λ value close to zero (0.005). The distribution of data expressed as discriminant scores along the first two eigenvectors is presented in Fig. 8. In this representation of all data, the four sample classes, corresponding to cheese samples with treated pig rennet (1), untreated pig rennet (2), treated calf rennet (3) and untreated calf rennet (4), respec-

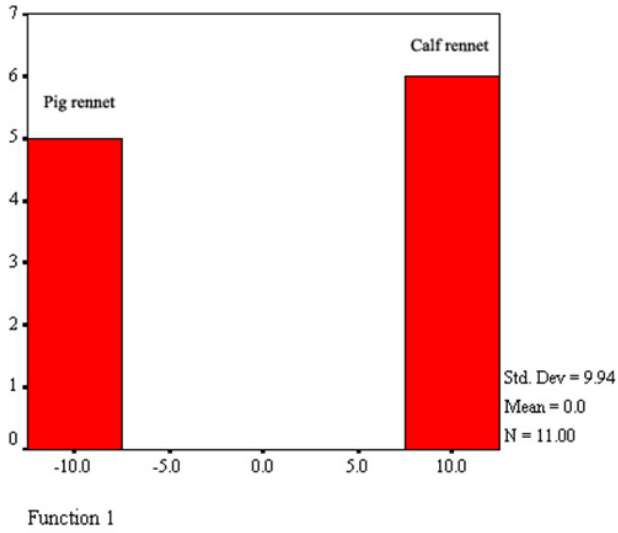


Fig. 6 - The distribution of data regarding the volatile substances, related to cheese samples as function of the two rennets, expressed as discriminant scores along the first eigenvector. 100.0% of original grouped cases correctly classified.

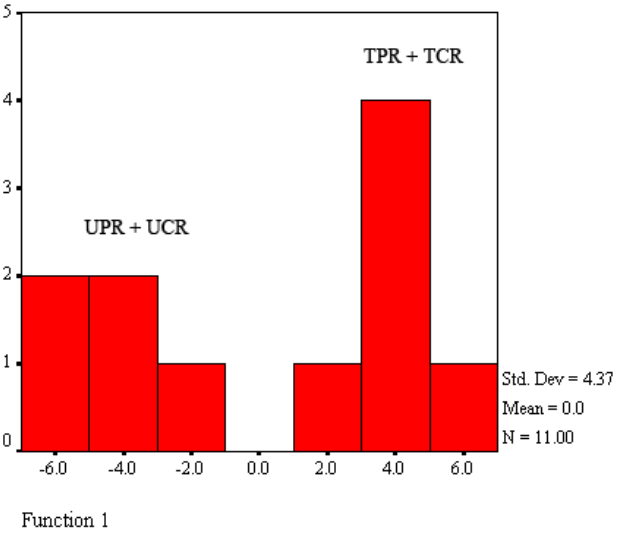


Fig. 7 - The distribution of data regarding the volatile substances content, related to cheese samples as function of the treatments, expressed as discriminant scores along the first eigenvector. 100.0% of original grouped cases correctly classified.

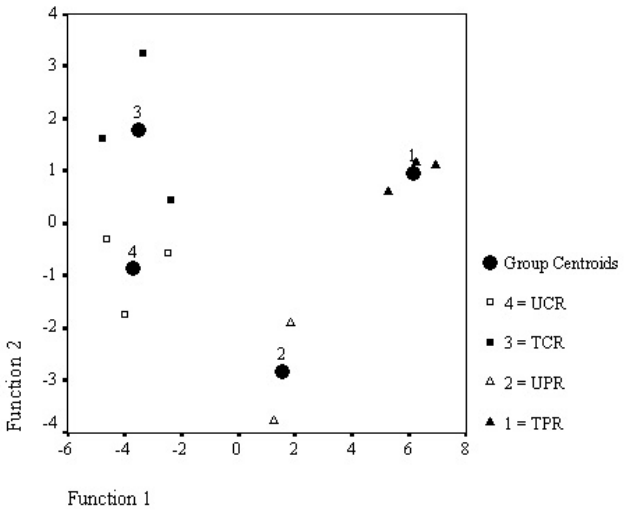


Fig. 8 - The distribution of data regarding the volatile substances, related to cheese samples as function of rennets and treatments, expressed as discriminant scores along the first two eigenvectors. 100.0% of original grouped cases correctly classified.

tively, were clearly distinct. It may also be noted that the centroids of each group fall into different quadrants.

In this case, all cheese samples were correctly assigned to the group they belong to. Furthermore the overall classification success was 100.0%.

Panel test

Fig. 9 shows sensorial differences analyzed by expert tasters between cheese made with calf rennet and pig rennet (sweeter, less bitter and less spicy) in the same production condition (DI GIACOMO *et al.*, 2009, DI GIACOMO *et al.*, 2013).

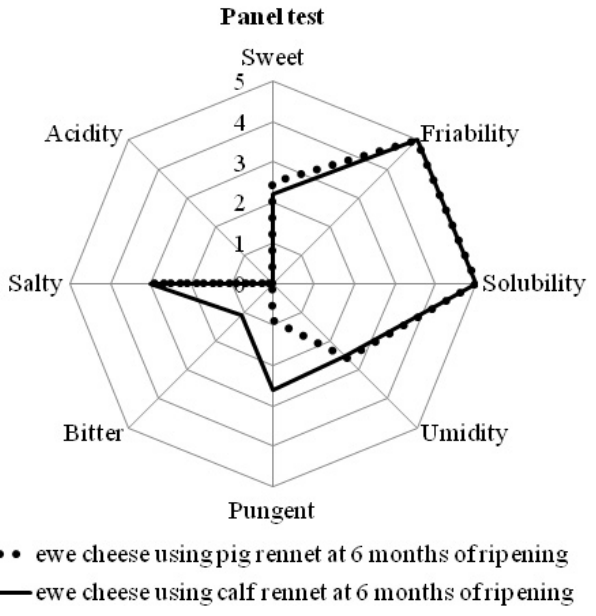


Fig. 9 - Sensorial analysis relates to a sample obtained with pig rennet "treated" with a time of aging of 6 months and to a sample obtained with calf rennet "treated" with a time of aging of 6 months.

CONCLUSIONS

It can be observed that there is a different microorganism evolution depending on the type of rennet, especially as regards lactobacilli, *Enterococcus* spp. and yeast. It follows that the volatile compounds profile is different between the cheese made with calf rennet and the cheese made with pig rennet. Pearson correlations between micro – organisms and volatile compounds show differences as a function of type of rennet.

These differences influence the aromatic profile, as confirmed in a study carried out by a panel of experts who judged the Farindola ewe cheese sweeter, less bitter and less spicy than ewe cheese made with calf rennet in the same production conditions. Probably consumers prefer Farindola ewe cheese for these reasons.

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PAPER

DETERMINATION OF SHELF LIFE OF FISH BALL MARINATED AFTER FRYING PROCESS

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ABSTRACT

The shelf life of fish ball marinated after frying process was investigated in the present study. The fish ball was stored at ±4°C. Total Volatile Basic Nitrogen (TVB-N), Thiobarbituric Acid Reactive Substances (TBAR), Trimethyl-Amine Nitrogen (TMA-N) and pH values of fish balls were 13.66 mg/100 g, 5.68 mg MA/kg, 5.63 mg/100 g and 3.42 at the end of the storage period (on day 150), respectively. The microbiological analysis results did not exceed the limit values. According to sensory evaluation results, the shelf life of balls was determined to be 135 days at ±4°C.

- Keywords: anchovy, fish ball, fried, marination, shelf life -

INTRODUCTION

Seafood may spoil sooner in contrast to other meat products due to higher water amount and lower connective tissue. (VARLIK *et al.*, 2004). Under normal refrigerated storage conditions, the shelf life of these products is limited by enzymatic and microbiological spoilage (ASHIE *et al.*, 1996). Microorganisms are the major cause of spoilage of most seafood products. However, only a few members of the microbial community like the specific spoilage organisms, give rise to the offensive off-flavours associated with seafood spoilage (GRAM and DALGAARD, 2002).

A great number of seafood processing techniques have been applied to slow down that spoilage. These processing technologies have been increased through developing knowledge. On the other hand, while that increase is being achieved, traditional methods have not been given up completely; in fact, these methods are still being used and developed (VARLIK *et al.*, 2004).

Marination is one of the oldest processing methods that is used for preservation of fish and other seafood (GIUFFRIDA *et al.*, 2007). Generally, marinated fish is ready to eat food that is not heat- processed (GRAM and HUSS, 1996). Marination is the process of ripening fish and making it edible by treating with vinegar or organic acids and salt without using the heating process. Products after ripening process are packaged with brine, sauce, cream, mayonaisse or oil and served for consumption. The fresh, frozen and salted fish or fish parts may be used in marination technology. Fishes mainly used in marination are herring, anchovy, sardine, snakefish, trout, mackerel and silverside. Also, shellfish like mussel and shrimp may be processed as marinade. The best marinade product is made as a result of using fatty fish species like anchovy and herring. The marinated products can be split into 3 groups as cold, cooked and fried (GOKOGLU, 2002; BAYGAR *et al.*, 2000).

1. Cold: The fresh material is being ripened in solution containing acetic acid and salt. There is no heat treatment.

2. Cooked: The fish are immersed into solution containing acetic acid and salt at 85°C. Thus, most of the bacterias are killed and enzymes are inactivated.

3. Fried: In material that is fried in acetic acid and salt solution before packaging, most of the bacteria are killed and enzymes are denaturated (CLUCAS and WARD, 1991; KILINC and CAKLI, 2004).

Fried marinade are products that are obtained by utilization of fried fish or fish products as marinade. For making fried marinade, fish is fried in vegetable oil previously and then immersed into solution (ERSAN, 1961; VARLIK *et al.*, 1993). Fresh and frozen fish or fish parts are also fried and coated with brine or sauces. Herring, snakefish, river snakefish, whiting, codfish species, mediterranean sand smelt and some types of flatfishes are used to make fried marinade. The temperatures of frying in oil must be between 160° and 180°C. The frying time depends on temperature of oil, thickness and water content of the flesh of fish. The time of frying process is between 5-12 min. Rising of fried fish onto oil surface during frying process in fry-pan occurs as a result of their losing water and absorption of oil due to their specific weight. The fried fish must be packaged after it was cooled. The rate of fish:coating solution is approximately 2:1. This rate depends on absorption of solution by fried fish. The fish lose approximately 20% of their water during frying process. This loss is compensated from coating solution. The contents of acetic acid and salt of solution are 2-3.5% and 3-5%, respectively. But these rates can be changed depending on water content of product and seasonal changes (MEYER, 1965).

In this study, the fish ball produced with anchovy (*Engraulis encrasicolus*) was fried in oil and then marinated. It was aimed to determine shelf life of the fish ball marinated after frying.

MATERIALS AND METHODS

Materials

Anchovies (*Engraulis encrasicolus*, L.1758) were purchased from a fisherman in Sinop. In total, 10 kg of fresh anchovy with an average lenght of 9±1 cm were used. Fish were headed, gutted and washed.

Preparation of fish meat balls

The anchovies were boiled for 5 min and then minced with a blender after the bones were removed. The mixture was kneaded after addition of 0.58% semolina, 0.60% crumb, 0.83% egg, 1% parsley, 1% onion, 0.10% garlic, 0.08% salt, 0.03% black pepper, 0.03% cummin, 0.03% red pepper, 0.03% thyme and 0.03% ginger. Small pieces were taken apart from fish ball mix and given ball shape by hand. Then, they were fried in sunflower seed oil.

Marination process

The fried meat balls were put into the solution containing 7% salt and 1.5% vinegar after they were cooled. Besides, parsley, mustard seed, garlic and white pepper were added into the solution and jar caps were closed. Then, they were stored at 4°C for 150 days.

Chemical analysis

pH analysis was carried out with the instrument Werkstatten 82362 Weilheim, Germany, according to CURRAN *et al.* (1980). Total vola-

tile basic nitrogen (TVB-N) was determined according to method of Lucke and Geidel modified by ANTONACOPOULOS (1989). Thiobarbituric Acid Reactive Substances (TBAR) was determined according to TARLADGIS *et al.* (1960). TMA (mg TMA-N/100 g) analysis was carried out according to the method proposed by BOLAND and PAIGE (1971).

Microbiological analysis

Microbiological analyses were made according to Baumgart (19). 10 g of fish sample was taken, transferred into 90 mL sterile Physiological Saline Solution (0.85%) and then homogenized in a homogenizer (IKA Yellow Line DI 25 Basic). From the 10^{-1} dilution, other decimal dilutions were prepared and inoculated. Plate Count Agar was used as medium for total mesophilic aerobic bacteria and psychrophilic bacteria counts, petri dishes were incubated at 28°C for 3 days and 4°±1°C for 10 days, respectively. For total yeast-mold count, Potato Dextrose Agar was used as medium and petri dishes were incubated at 28°C for 3 days. To count coliform bacteria, Violet Red Bile Agar was used as medium and petri dishes were incubated at 35°C for 24 h. Results were given as log cfu/g. (ROGER *et al.*, 1987; GOKTAN, 1990; VARLIK *et al.*, 1993; GOKALP *et al.*, 1999).

Sensory analysis

Sensory analyses were made according to NEUMAN *et al.* (1983). 10 g of fish sample was served to each panelist to evaluate the sensory attributes (appearance, odor, texture, flavor) of the sample. The samples were presented in separate plates to every panelist. According to the scoring test, a total score of sensory attributes of 20 was indicating excellent quality. Scores between 18.2 and 19.9 indicated "very good" quality, scores between 15.2 and 18.1 indicated "good" quality and scores between 11.2 and 15.1 indicated "middle" quality; scores between 7.2 and 11.1 indicated the limit of acceptability, and scores between 4.0 and 7.1 indicated spoiled samples.

Statistical analysis

The Minitab 15 (Minitab Inc. USA) program was used to search for significant differences among mean values of different results. Differences between means were analyzed by one - way analysis of variance (ANOVA). The results are presented as mean ± SE. The P value ($P < 0.05$) was used to determine significant differences.

RESULTS AND DISCUSSION

TVB-N values of the fish ball marinated after frying are shown in Fig. 1. The TVB-N content of fresh material was determined as 7.6 mg/100 g. The initial TVB-N content of fish ball marinated after frying was 5.6±0.00 mg/100 g. This value increased to 13.66±0.08 mg/100 g at the end of the storage period of 150 days. It was observed that TVB-N values increased significantly ($P<0.05$) during storage of the fish ball.

There have been limited number of studies related with the fish ball marinated after frying. However, there have been great number of studies about the marinades without frying (AKSU *et al.*, 1997; DOKUZLU, 2000; GOKOGLU *et al.*, 2004; OZDEN and BAYGAR, 2003; DUYAR and EKE, 2009; OZOGUL *et al.* 2009; GUNSEN *et al.*, 2011).

GOKOGLU *et al.* (2004) reported that TVB-N values of sardine marinade samples containing 2 and 4% acetic acid were 28.9 and 23.32 mg/100 g at the end of the storage period of 150 days, respectively. In another report TVB-N value in anchovy marinated with acetic acid of 4% and stored at 4°C increased from 9.8 to 14 mg/100 g during the storage of 8 months (DOKUZLU, 2000). DUYAR and EKE (2009) stated that TVB-N values of anchovy and bonito marinades were 17.63 and 18.67 mg/100 g at the end of the storage period of 170 days, respectively. These values were higher than our TVB-N finding (13.66 mg/100 g). Using different fish types and different rates of salt and acetic acid, application of heating process (frying) that has lethal effects in bacteria can be considered as the possible reasons of this situation. In addition, probability of solubilization in the marination solution of volatile amines might be effective to diminish TVB-N value. VARLIK *et al.* (2000) marinated fried anchovy meat ball similarly to our study and stored at 4°C. TVB-N val-

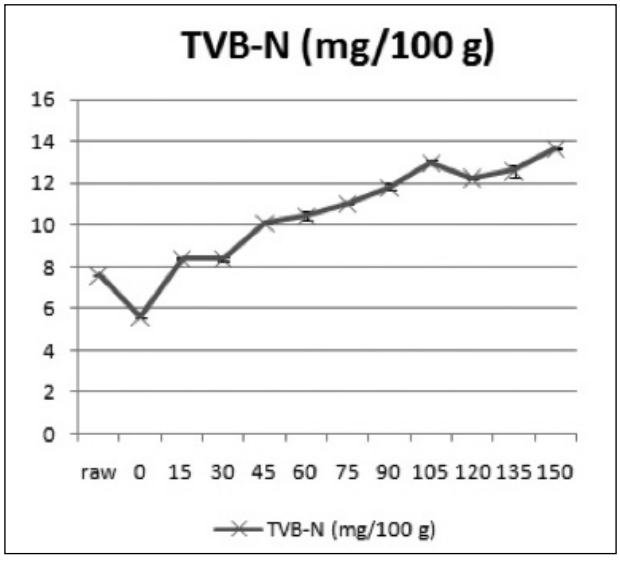


Fig. 1 - TVB-N values of raw material and the fishball marinated after frying stored at 4°C.
Markers are shown as mean ± standard error of triplicates.

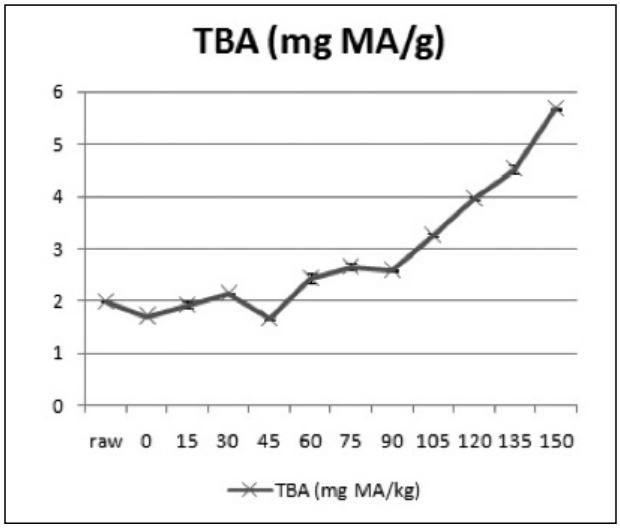


Fig. 2 - TBA values of raw material and the fishball marinated after frying stored at 4°C.
Markers are shown as mean ± standard error of triplicates.

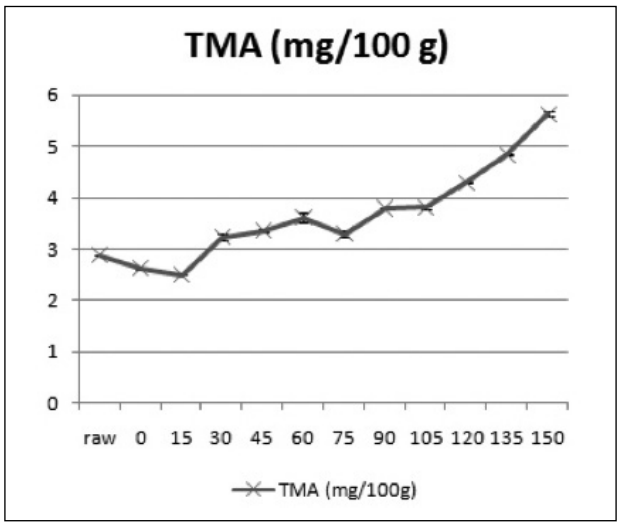


Fig. 3 - TMA values of raw material and the fishball marinated after frying stored at 4°C.
Markers are shown as mean ± standard error of triplicates.

ue of the product was reported as 10.45 mg/100 g at the end of the storage period of 150 days. This value was similar to the our finding (13.66 mg/100 g). Accordingly, it could be said that the frying process that was applied before marination was effective to diminish TVB-N value of marinated products.

One of the most important chemicals in fish muscle is trimethylamine N-oxide. Trimethylamine N-oxide is found in seafish mostly. TMA is produced by the decomposition of trimethylamine N-oxide caused by bacterial spoilage and enzymatic activity (SCHORMULLER, 1968; KIETZMANN, 1969). It was stated by FAO (1986) that, the TMA-N value is approximately 1 mg/100 g for fresh seafood and it is over 8 mg/100 g for spoiled samples. Besides, according to the TMA-N values, the seafood is classified as 'good' up to 4 mg/100 g; 'marketable' up to 10 mg/100 g; 'spoiled' if bigger than 12 mg/100 g (KUNDAKCI, 1989).

TMA values of the fish ball marinated after frying are shown in Fig. 3. The TMA content of the fresh material was 2.88 mg/100 g. In the present study, initial TMA-N value of the fish ball was found as 2.62 mg/100 g. At the end of the storage period of 150 days, it increased to 5.63 mg/100 g. In the study investigating the TMA-N content of the anchovy ball marinated after frying, it was stated that the initial TMA-N value increased from 1.85 to 2.85 mg/100 g at the end of the storage period of 150 days (VARLIK *et al.*, 2000). In our study, the TMA-N value was 2.88 mg/100 g initially and it was observed as 5.63 mg/100 g at the end of the storage. It was reported that the TMA content changes depending on season, catching area, species, type muscle and processing techniques (HERBARD *et al.*, 1982). It was thought that this difference was resulted from high initial TMA content and processing techniques such as immersing into different marination solution.

When compared with only marinated (without frying) samples, it was seen that our TMA values were higher than other studies. GOKOGLU *et al.* (2004) reported that TMA values of the sardine marinade samples containing 2 and 4% acetic acid were 1.67 and 0.86 mg/100 g at the end of the storage period of 150 days, respectively (DUYAR and EKE, 2009).

TMA values of the anchovy and sardine marinades were reported as 0.50 and 0.80 mg/100 g at the end of the storage period of 170 days by DUYAR and EKE (2009), respectively. These values are quite lower than our findings. It was thought that this situation was resulted from high TMA contents of the anchovies which were our raw material.

TBAR values of the fish ball marinated after frying are shown in Fig. 2. TBAR value of the fresh material was determined as 1.98 mg MA/kg. The initial TBAR value of the fish ball increased from 1.71 to 5.68 MA/kg at the end of the storage period of 150 days. It was reported that, TBAR value that is used to determine rancidity in lipid is less than 3 mg MA/kg in a very good material and it must not be bigger than 5 mg MA/kg in a good material. Rancidity began if it exceeded 4 mg MA/kg and acceptability limit value is 7-8 mg MA/kG (CURRAN, 1980; VARLIK *et al.*, 1993). Therefore it may be said that, the rancidity in the fish ball began on day 135. Similar to our study, DUYAR and EKE (2009) stated that the anchovy marinade which has 10.93 MA/kg of TBAR value at day 100 exceeded the acceptability limit values at day 130. There haven't been any studies investigating TBAR value of the fish ball marinated after frying. But there are studies related with marinades without frying (CADUN *et al.*, 2005;

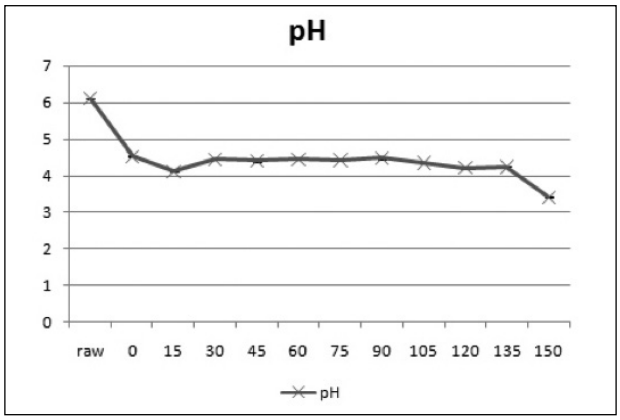


Fig. 4 - pHvalues of raw material and the fishball marinated after frying stored at 4°C.
Markers are shown as mean ± standard error of triplicates.

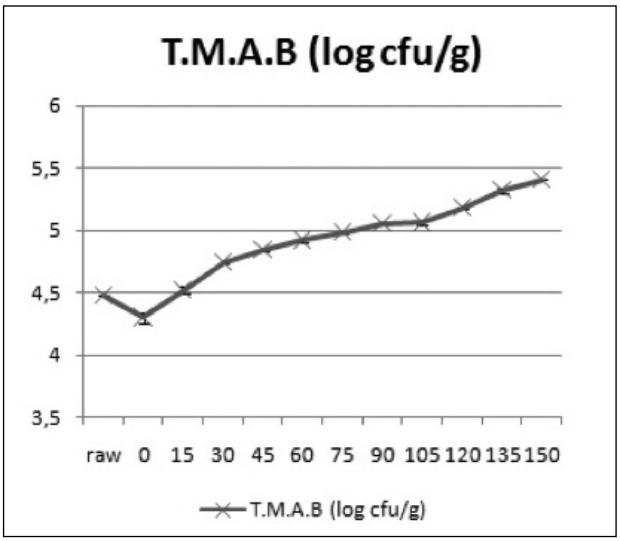


Fig. 5 - T. M. A. B (Total Mesophilic Aerobic Bacteria) values of raw material and the fishball marinated after frying stored at 4°C.
Markers are shown as mean ± standard error of triplicates.

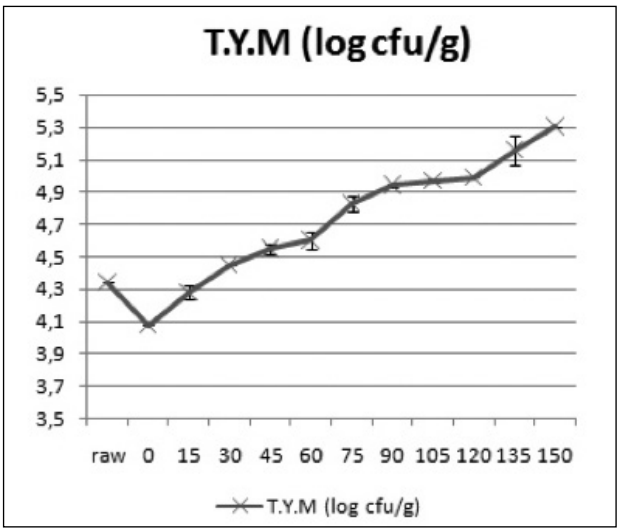


Fig. 6 - T.Y.M (Total Yeast Mold) values of raw material and the fishball marinated after frying stored at 4°C.
Markers are shown as mean ± standard error of triplicates.

DUYAR and EKE, 2009; BİLGİN *et al.*, 2011; GUNSEN *et al.*, 2011; SIMAT *et al.*, 2011).

pH values of the fish ball marinated after frying are shown in Fig. 4.The pH value of the fresh material was determined as 6.1. The initial pH value of the fish ball that was 4.53 decreased to 3.42 at the end of the storage period of 150 days.

Microbiological quality of the fresh material and the fish ball marinated after frying is shown in Fig. 5, 6 and 7. The total mesophilic aerobic bacteria count of the fresh material that was 4.48 log cfu/g increased to 5.41 log cfu/g for the fish ball marinated after frying at the end of the storage period of 150 days. Total yeast-mold count decreased along with the marination of the fresh anchovy; however it increased progressively throughout the storage.

Generally, *Pseudomonas*, *Achromobacter* and *Flavobacterias* are existing in newly caught fishes. Most of them have proteolitic properties. These microorganisms are sensitive to acid, so they are not existing in the marinades. Because development of these microorganisms is inhibited at about pH of 4.5. *Betabacterium buchneri* group of microorganisms are existing dominantly in the spoiled marinades. Beta-bacterias are isolated not only from cold marinades, but also from fried marinades and some special products with the cold marinade.

Because free aminoacids are in small amount, this situation does not occur too often in the fried marinades. The beta-bacterias and gamma-aminobutyric acid that is one of the reduced products in decarboxylation of aminoacids bind acetic acid and increase the pH value. The molds play important role only in the marinades kept in open cases (MEYER, 1965; KILINC and CAKLI, 2004). The total mesophilic bacteria of the fresh anchovy was determined as 4.8 log cfu/g by GUNSEN *et al.* (2011). There are not any studies

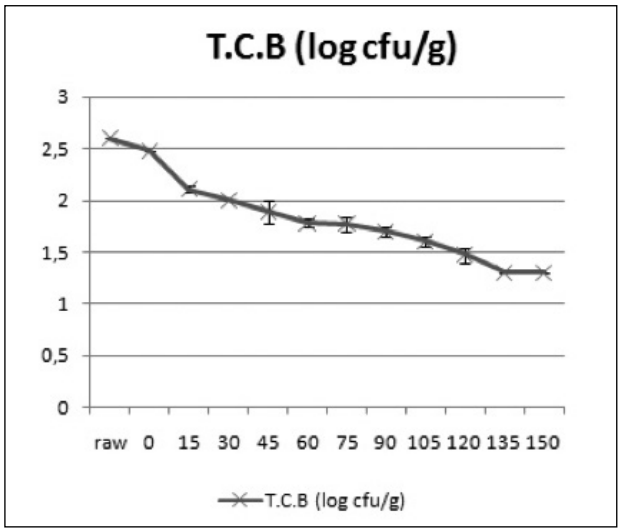


Fig. 7 - T.C.B (Total Coliform Bacteria) values of raw material and the fishball marinated after frying stored at 4°C.

Table 1 - Sensory analysis results of the fishball marinated after frying stored at 4°C.

Fishball marinated after frying	Storage (days)										
	0	15	30	45	60	75	90	105	120	135	150
Appearance	5±0.00^{a}	5±0.00^{a}	4.8±0.20^{ab}	4.4±0.24^{bc}	4.2±0.20^{c}	4.6±0.24^{abc}	4±0.00^{cd}	3.6±0.24^{d}	3±0.32^{e}	2.8±0.20^{e}	1±0.00^{f}
Odor	5±0.00^{a}	4.6±0.24^{abde}	4.2±0.20^{bcde}	4±0.32^{cde}	4.2±0.20^{de}	4.2±0.20^{de}	4±0.00^{e}	3.4±0.24^{f}	2.8±0.20^{g}	2.6±0.24^{g}	1.4±0.24^{h}
Flavor	5±0.00^{a}	5±0.00^{a}	4.8±0.20^{a}	4.4±0.24^{ab}	4±0.32^{bc}	4±0.00^{bc}	3.6±0.24^{cd}	3.4±0.24^{de}	3±0.00^{e}	2.4±0.24^{f}	1.2±0.20^{g}
Texture	5±0.00^{a}	4.6±0.26^{b}	4.2±0.22^{bc}	3.8±0.22^{cd}	3.6±0.22^{de}	3.2±0.22^{ef}	3±0.00^{fg}	2.6±0.22^{g}	2.2±0.22^{hi}	1.8±0.22^{i}	1±0.00^{j}
Overall acceptability	20±0.00^{a}	19.2±0.50^{ab}	18±0.82^{bc}	16.6±1.02^{cd}	16±0.94^{d}	16±0.66^{d}	14.6±0.24^{de}	13±0.94^{e}	11±0.74^{f}	9.6±0.90^{f}	4.6±0.44^{g}
Quality	Very Good	Very Good	Good	Good	Good	Good	Middle	Middle	Middle	Acceptable	Spoiled

investigating the microbiological quality of the fish ball marinated after frying. However several studies of the marinades without frying are existing (OLGUNOGLU, 2007; KURT KAYA, 2009; OZOGUL *et al.*, 2009; PELIN CAN and ARSLAN, 2011).

Changes in the sensory quality and overall acceptability of the fish ball marinated after frying are shown in Table 1. Considering overall acceptability results of sensory analysis of the fish ball, it was seen that the fish ball having score of 9.6 indicated acceptable quality on day 135 of the storage period. And then the fish ball having score of 4.6 was evaluated as spoiled. Even though chemical and microbiological quality criteria did not exceed the acceptability limit values at the end of the storage periods of 150 days, the product spoiled in terms of sensory properties. Flavor and aroma development in the marinade is resulted from the degradation of protein and lipid with mutual effects of the acetic acid and salt with enzymes. (McLAY, 2001).

The shelf life of anchovy ball marinated in solution containing 6% salt and 1% vinegar after frying was determined as 120 days by VARLIK *et al.* (2000). This result was similar to our study. In addition, there are several studies investigating the shelf life of the marinades without frying (AKSU *et al.* 1997; DOKUZLU, 1997; OZDEN and BAYGAR, 2003; DUYAR and EKE, 2009).

CONCLUSIONS

The shelf life of the fish ball marinated after frying was determined as 135 days. Even though the acceptability limit values were not exceeded according to chemical and microbiological analysis results, the product spoiled in terms of sensory properties. As a consequence, it might be suggested that the fish ball must not be consumed if it is stored over 4 months.

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PAPER

ASSESSMENT OF DIETARY EXPOSURE TO 5-HYDROXYMETHYLFURFURAL FROM TRADITIONAL IRANIAN FLAT BREADS

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ABSTRACT

The aim of this study was to determine the contents and bioaccessibility of hydroxymethylfurfural (HMF) in traditional Iranian flat breads. Four abundant flat breads were analyzed for HMF content and physicochemical parameters. Bioaccessibility levels were determined using an *in vitro* digestive model. The mean HMF values were 48.5±8.09 and 40.4±6.89 in pre- and post-digestive samples, respectively, implying 83.0% bioaccessibility. Daily intake of HMF from these food items was 11.7 mg. A correlation was found between HMF and carbohydrate content of bread (r=-0.5, $p<0.05$). It is concluded that HMF levels remain high even after *in vitro* digestion.

- Keywords: reducing sugars, HPLC, bioaccessibility, *in vitro* digestion -

INTRODUCTION

The process of preparing bakery products such as bread, cookies, and biscuits consists of baking dough at a temperature of 200°C or higher which induces several chemical reactions (HIDALGO and BRANDOLINI, 2011b). One of the most common reactions which occurs during the manufacturing of bakery products is the Maillard reaction whose substrates include free amino acids and reducing carbohydrates in addition to having intermediate moisture and pH 4-7 (VAN BOEKEL, 2006). Caramelization is another reaction which is promoted through the direct degradation of carbohydrates and requires other conditions such as a temperature above 120°C and pH<3 or pH>9 (RAMÍREZ-JIMÉNEZ *et al.*, 2001).

5-hydroxymethylfurfural (HMF) is formed during the caramelization process and is also an intermediate product of the Maillard reaction (RAMÍREZ-JIMÉNEZ et al., 2000). It has been detected in honey (WHITE Jr, 1980), dried fruits and coffee (MURKOVIC and PICHLER, 2006), milk (MORALES *et al.*, 1997), breakfast cereals (GARCIA-VILLANOVA *et al.*, 1993), pasta (RESMINI *et al.*, 1993), breads (FERNÁNDEZ-ARTIGAS *et al.*, 1999), and other bakery products (HIDALGO and BRANDOLINI, 2011b). It is a browning indicator of bread (RAMIREZ-JIMENEZ *et al.*, 2000) and has a positive correlation with length and cooking temperature during the baking process (PURLIS, 2010).

HMF is a bioactive compound known to induce preneoplastic cell injuries in rat entrocytes (ZHANG *et al.*, 1993), hepatocellular adenoma in female mice (TOXICOLOGY, 2008), and skin papillomas in mice (SURH *et al.*, 1994). This chemical is a substrate for sulfotransferase and can be converted to sulfoxymethylfurfural, which is an active metabolite of HMF. The latter has the capacity to bind DNA and may exert mutagenic effects (DURLING *et al.*, 2009); however, in vitro studies on the genotoxicity and mutagenicity of HMF have given controversial results, and there is not enough evidence to prove its toxicity (DURLING *et al.*, 2009; JANZOWSKI *et al.*, 2000; SEVERIN *et al.*, 2010). Therefore, it is necessary to estimate the amount of dietary exposure to HMF from different kinds of diets and make some modifications in the processing and storage conditions of food items if their intake surpasses the threshold of concern.

As for many other food ingredients, there is limited data regarding the bioaccessibility of HMF from different kinds of bakery products. Previous studies have shown that the in vitro digestion of breakfast cereals causes a decline in the soluble fraction of HMF levels (DELGADO-ANDRADE *et al.*, 2008; RUFIÁN-HENARES and DELGADO-ANDRADE, 2009).

As in many developing countries, recent national data on Iranian household food consumption patterns shows that grains, including bread, rice, pasta, and other cereals, supply 52% of the energy requirements of the country (SHOCKRAVI *et al.*, 2012). Traditional flat breads named lavash, taftoon, sangak, and barbari are the main sources of cereals consumed in Iran. These breads are generally produced from soft white wheat flour with a higher extraction rate (GOCMEN *et al.*, 2009). In Iran, their preparation (FARIDI *et al.*, 1983) is associated with long and direct heating in conventional clay ovens. Therefore, it seems reasonable that this process may augment the amount of HMF produced. The aim of this research was to determine HMF contents, as one heat-damage index, in the most-consumed Iranian breads and its bioaccessibility and to look for any correlation between HMF content and physicochemical parameters.

MATERIALS AND METHODS

Materials

Traditional breads, including lavash, taftoon, sangak and barbari which are consumed mostly in Iran, were obtained from randomly selected local bakery shops in Tabriz, East Azerbayjan Province, Iran. 5-HMF, pepsin, and pancreatin were purchased from Sigma-Aldrich (St. Louis, Missouri, USA).

Sampling

Five loaves of each type of bread (lavash, taftoon, sangak, and barbari) were randomly selected among 100 breads baked from the same batch to diminish the effect of confounders such as distance from heat source and timing of baking. The breads were made from wheat flour. The characteristics of the flours used for the different types of bread are shown in Table 1. The breads were cooled and frozen to maintain their physicochemical properties in a stable condition until further analysis. They were thawed, ground, powdered, and then homogenized 4-5 hours before analysis.

Measurement of physicochemical properties

The physicochemical properties of the selected breads, including moisture, ash, pH, proteins, total carbohydrates, and fiber, were measured following standard AOAC methods (AOAC, 1998). Moisture percentage was calculated after an air-oven drying at 130°±3°C for 1 h after the temperature reached the adjusted amount (AOAC, Method 925.10). The ash content was determined by incinerating the breads in a furnace at 550°C until gray ash resulted (AOAC, Method 923.03). The pH of the samples was measured after mixing 10 g of each sample with 100 mL deionized water (AOAC, Method 943.02) in a MP 225 pH meter with glass electrode (Met-

Table 1 - Characteristics of flours used for different type of breads.

Variable	Lavash	Taftoon	Sangak	Barbari
Crude protein (%)	11.8	12	12	11.6
Gluten (%)	28.5	26	26.5	28.7
Ash (%)	0.8-1.1	0.8-1.1	1.1-1.5	0.7-0.8
Dough development time of dough (min)	5	4	6	3
Extensibility of dough (mm)	140	152	149	160

tler-Toledo GmbH, Schwerzenbach, Switzerland). The Micro-Kjeldahl method was applied to determine the percentage of nitrogen, which was then multiplied by factor 5.7 to obtain the percentage of proteins (AOAC, Method 950.36). The fiber content of the breads was determined by digesting the samples with H_2SO_4 and NaOH solutions (AOAC, Method 962.09). The total carbohydrate content of the breads was measured by difference.

HMF analyses

The HMF content of the samples was measured before and after digestion as described by DELGADO-ANDRADE *et al.* (2008). Briefly, 1 g of powdered sample was transferred into a 10 mL centrifuge tube and suspended in 10 mL of deionized water. After shaking for 1 minute, the solution was clarified with 0.5 mL each of 15% potassium ferrocyanide (w/v) and 30% zinc acetate (w/v). The mixture was centrifuged at 3500 g for 10 minutes at 4°C. The supernatants were then collected, and the same procedure was repeated twice more with distilled water to enhance the extractions.

The determination of HMF was carried out by HPLC on filtered (0.45 μm) solution. The HPLC system used in this study consisted of a CE-1100 pump, a CE-1100 ultraviolet- visible detector (Cecil instruments, Cambridge, England, United Kingdom) with a 20 μL injection loop chromatograph, and a Spherisorb S5 ODS1 (250 mm × 4.6 mm id) column (SUGELABOR, Madrid, Spain). The mobile phase was a mixture of acetonitrile and methanol in water (4% v/v and 6% v/v, respectively). The flow rate was adjusted on 0.8 mL/min, and the UV detector was set at 284 nm. HMF in different concentrations, within the range of 5-100 mg/liter, was used for calibration.

In *vitro* digestion of breads

In order to determine the bioaccessibility of HMF from bread after digestion, the gastrointestinal digestion process was performed using the method suggested by Delgado-Andrade (DELGADO-ANDRADE *et al.*, 2008). The model consisted of two serial stages: a peptic digestion, and a subsequent intestinal digestion. Briefly, one gram of each sample was diluted in 10 mL double distilled water. The pH of the mixture was adjusted to 2 with HCL 6mol/liter. Then, a pepsin solution made with HCl 0.1 mol/liter at a proportion of 0.05 g of pepsin per gram of samples was added and incubated at 37°C on a rocking platform shaker for 2 h. The pH was then raised to 6 using $NaHCO_3$ 1 mol/liter and 2.5 mL of pancreatic solution containing 50 mL of 0.1 mol/liter $NaHCO_3$. The final pH of the mixture was adjusted to 7.5 with $NaHCO_3$ 1 mol/liter. The solutions were incubated for 2 h at 37°C on a rocking platform shaker. After *in vitro* digestion, the flasks were incubated in a polyethylenglycol bath at 100°C for 4 minutes to inactivate the enzymes. They were then immediately cooled using an ice bath. Finally, the solutions were centrifuged at 3,200 g for 60 minutes and were used to measure the bioaccessible HMF contents of the bread.

Statistical analysis

The statistical significance of data was tested. Means of physicochemical parameters and HMF levels of the four types of traditional breads were compared by one-way analysis of variance (ANOVA), followed by the Tueky's test. Pairwise comparisons were conducted before and after digestion for each of the traditional breads using paired sample t-tests. The Pearson correlation analysis was applied to determine any correlation between physicochemical parameters and HMF content. A P-value of less than 0.05 was considered significant. All data was checked for distribution. Analyses were performed using SPSS software, version 16 (SPSS, Chicago, Illinois, USA).

RESULTS

The results of physicochemical analyses (moisture, ash, pH, carbohydrate, protein, and fiber) of breads including lavash, taftoon, sangak and barbari are shown in Table 2. Results indicated that the moisture content of breads ranged from (17.0 ± 3.30)% for lavash to (29.0 ± 3.60)% for barbari. The ash content of the breads measured between (0.4 ± 0.03)% for barbari and (1.5± 0.20)% for sangak. The highest level of pH was 6.7± 0.05, and the lowest was 5.8 ± 0.20 belonging to taftoon and barbari, respectively. The

Table 2 - Chemical properties of the selected breads.

Variable	Barbari	Sangak	Taftoon	Lavash	Test results
Moisture [%]	29.0±3.60^{bc}	28.8±2.60^{b}	22.0±2.80^{a}	17.0±3.30^{a}	P < 0.01
Ash [%]	0.4±0.03^{b}	1.5±0.20^{c}	0.6±0.20^{b}	1.0±0.10^{a}	P < 0.01
pH [%]	5.8±0.20^{c}	6.0±0.03^{b}	6.7±0.05^{a}	6.6±0.04^{a}	P < 0.01
Carbohydrate [%]	22.0±1.32^{c}	23.3±1.00^{c}	31.2±3.10^{b}	37.6±5.24^{a}	P < 0.01
Protein [%]	8.2±0.50^{b}	8.2±0.50^{b}	9.1±0.50^{a}	8.4±0.20^{ab}	P < 0.05
Fiber [%]	0.3± 0.03^{b}	0.1±0.02^{a}	0.1±0.01^{a}	0.1±0.01^{a}	P < 0.01

Values are expressed as mean ± SD. In each raw, mean value with different superscript letters are significantly different (P < 0.05) using ANOVA.

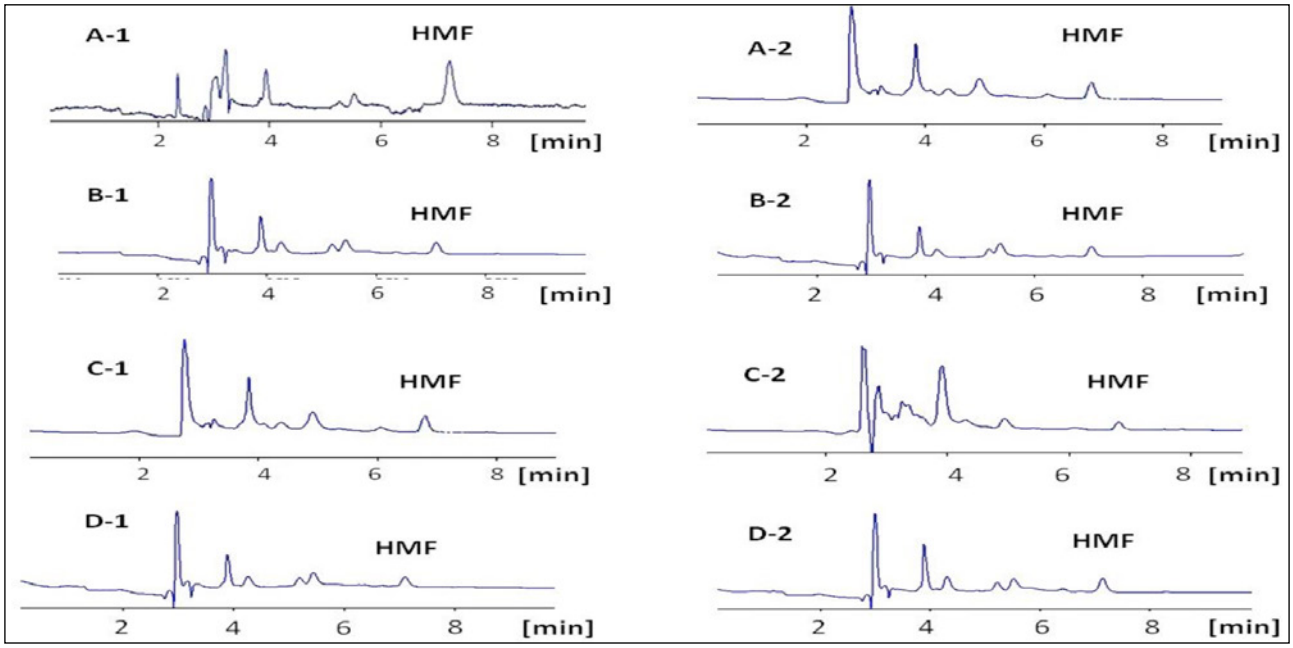


Fig. 1 - HPLC chromatograms of Hydroxymethylfurfural (HMF) in different bread samples before and after digestion. A-1; Lavash before digestive process, A-2; Lavash after digestive process.B-1; Taftoon before digestive process. B-2; Taftoon after digestive process. C-1; Barbari before digestive process.C-2; Barbari after digestive process. D-1; Sangak before digestive process. D-2; Sangak after digestive process.

highest and lowest amounts of total carbohydrates were (37.6± 5.24)% in lavash and (22.0 ± 1.32)% in barbari. It should be mentioned that lavash contained more carbohydrates, but less moisture. Barbari was significantly (p<0.05) higher in fiber and lower in ash, pH, and carbohydrates. There were no significant differences in moisture or fiber content between lavash and taftoon. There was no statistically significant difference in the carbohydrate contents of sangak and barbari. Protein content was significantly different only between taftoon and sangak as well as taftoon and barbari (p<0.05).

Fig. 1 shows the HPLC chromatograms for the HMF before and after *in vitro* digestion. The same method and chromatographic conditions were applied to all the different samples. The retention time was 7 minutes for the appearance of the peak of HMF.

Pre- and post-digestive contents of HMF are represented in Fig. 2. The HMF content of san-

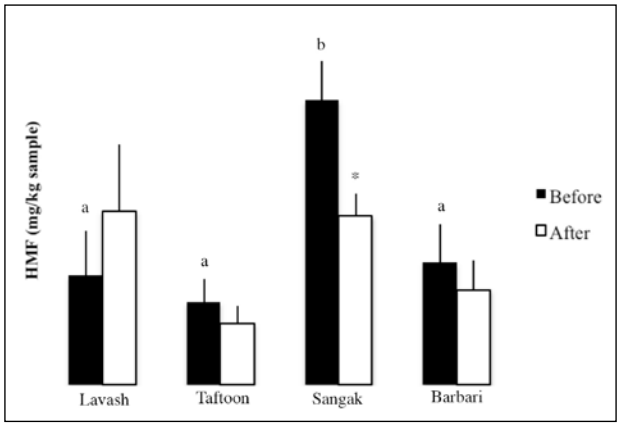


Fig. 2 - Hydroxymethylfurfural (HMF) contents of tested bread samples. Black and white bars represent mean (SEM) of HMF before and after in vitro digestion, respectively. Different letters represent statistical significance using ANOVA, followed by the Tueky's test. A single asterisk indicates p less than 0.05 by paired t-test.

gak was significantly higher than that of lavash, taftoon, and barbari before digestion; however, there was no statistically significant difference between the groups after digestion. A decrease in HMF content after the digestive process was observed for taftoon, sangak, and barbari (26.1, 40.8, and 22.3%, respectively), which was statistically significant different only for sangak ($p<$ 0.05). The HMF content in lavash increased 1.6-fold after digestion.

Estimated mean HMF intake from this food item based on the consumption of Iranian flat breads was 11.7 mg per person per day. Exposure to this compound from lavash is the highest. Barbari, taftoon and sangak account for the rest of the HMF intake (Table 3).

Correlation analyses between the chemical properties of breads and HMF content before the digestive process indicated that there was a moderate reverse relationship between the HMF and carbohydrate contents of the breads (r= -0.5, $p<0.05$); there was no statistically significant correlation between the HMF content of the breads and protein.

Table 3 - Estimation of dietary exposure to HMF based on consumption of Iranian flat breads.

Estimated mean HMF intake [mg/d]	Bread intake [g/d]	Type of bread
4.8	135.0	Lavash
2.1	79.0	Taftoon
2.0	21.8	Sangak
2.8	70.9	Barbari
11.7	306.7	Total

Intakes are expressed per person and day.

DISCUSSION

This cross-sectional study was performed to evaluate the HMF content of Iranian flat breads, the bioaccessibility of HMF from breads, and to estimate HMF intake based on consumption of the breads. An *in vitro* gastrointestinal (GI) digestive model was applied to examine the effect of GI tract enzymes on HMF degradation or absorption.

In the samples analyzed, the mean content of HMF was 48.5 mg/kg, and the range was 2.2 - 136.2 mg/kg. This broad range of HMF content (2.2-87.7 mg/kg) has been observed in some food items such as coffee, bakery products, and bread (ARRIBAS-LORENZO and MORALES, 2010; PURLIS, 2010; RAMÍREZ-JIMÉNEZ *et al.*, 2000). Similarly, the HMF contents found by others in cereal products including breakfast cereals (Rufián-Henares *et al.*, 2006), pasta (Resmini *et al.*, 1993), and cookies (AMEUR *et al.*, 2006) were highly variable. It seems that elements of processing such as temperature, duration, water activity, and/or composition of the cereals might be responsible for these variations.

The breads used in the present study were ones most abundantly consumed all over Iran. There was a significant difference in HMF content between different breads made from wheat flour; sangak was observed to have the highest HMF content, followed by barbari, lavash, and taftoon, respectively, before *in vitro* digestion. This variation in HMF contents disagrees with some other reports. RUFIÁN-HENARES *et al.* (2006) reported no differences between the HMF contents of breakfast cereals prepared from maize, wheat, and rice. These findings must be attributed to a difference in flour type or in the heating process used to prepare the cereals.

There was no statistically significant correlation between the protein content of bread and HMF production in our study. This is in agreement with the findings of Hidalgo *et al.* (HIDALGO and BRANDOLINI, 2011a), who reported that the einkorn, which had a higher content of protein than either durum or wheat breads, had lower heat damage. Furthermore, Rufián-Henares (RUFIÁN-HENARES *et al.*, 2009) found no statistically significant differences in the HMF contents of different toasted flours with various protein levels. Our data suggests that carbohydrates might be more important than proteins for heat damage development. It is well known that HMF, as a common Maillard reaction product, is formed through reactions between reducing sugars and amino acids during the heating of food. It can also be produced through the acid-catalysed dehydration of carbohydrates in which protein and amino acids have no role (HUSØY *et al.*, 2008).

In the present study, we found a negative correlation between the carbohydrate content of the breads and HMF production. RUFIÁN-HENARES *et al.* found that, despite a relatively lower content of total reducing sugars, wheat flour had a higher HMF content compared to corn flour (RUFIÁN-HENARES *et al.*, 2009). It has been reported that the amount of HMF of any particular food item appears to be related to its chemical characteristics (ROCHA *et al.*, 2004). Earlier studies have shown that yeast and enzymatic activity during mixing and dough leavening induce starch hydrolyzation and, subsequently, the formation of reducing carbohydrates which participate in the start of the Maillard reaction (AHMED OMER, 2012; Rufián-Henares *et al.*, 2008). Therefore, we speculate that a variation in the degree of fermentation determines substrate content for the Maillard reaction and caramelization and contributes to HMF production along with the proteic activity and physicochemical characteristics of the examined breads.

In the case of HMF, a mean content of 40.4 mg/kg was found for all the *in vitro* digested breads in soluble fraction. As expected, there was a reduction in the HMF content of digest-

ed breads, and the mean of 17.0% decline was observed. Similarly, in recent studies, the HMF content showed a marked decrease in soluble fraction after the digestion of breakfast cereals (DELGADO-ANDRADE *et al.*, 2008; RUFIÁN-HENARES and DELGADO-ANDRADE, 2009). It should be mentioned that this did not hold true for lavash. The slight but unremarkable increase in HMF content of the lavash samples warrants further study.

Bread is one of the most important cereal products in Iranian dietary patterns, and it provides the majority of the energy requirements in this area. Among the various kinds of breads, traditional ones account for 96.1% of the country's bread consumption (Ministry of Industry, Mine and Trade, 2013). The estimated intake of HMF based on consumption of flat bread is a mean of 11.7 mg per person per day, which exceeds the threshold of concern (1600 μg per person per day) established by a scientific panel for food additives, flavoring, processing aids, and material contact with food. Similarly, in a recent study done in Spain, dietary intake of HMF from coffee was above the threshold of concern (8.6 mg/d). These authors investigated the dietary exposure to HMF from coffee, because of its high consumption rate compared with other food items in addition to the high level of HMF found in coffee (ARRIBAS-LORENZO and MORALES, 2010). In another study, the dietary intake of HMF from Norwegian foods was estimated on the basis of 24 h dietary recall. A maximum intake of 37 mg a day was observed; however, for most of the participants, the estimated HMF intake was lower than the measured total excretion of 5-hydroxymethyl-2-furoic acid, a metabolite of HMF, in urine. In this study, instant coffee had the highest HMF levels (3060 mg/kg). Other food items that contained high levels of HMF were prunes (237 mg/kg), dark beer (13.3 mg/kg), canned peaches (5.8 mg/kg), and raisins (5 mg/kg) (HUSØY *et al.*, 2008). It should be noted that dried fruits, one of the main sources of HMF, are consumed in large amounts by the Iranian population. It seems reasonable that the overall HMF intake among Iranians would be higher considering all dietary items consumed in Iran.

CONCLUSION

In conclusion, the estimated mean HMF intake from breads was above the threshold of concern. Although the *in vitro* digestion process caused a significant decline in the HMF content of the breads, its bioaccessibility remained higher than the recommended upper limit. Further studies are needed to reveal other sources for HMF exposure with consideration given to the insoluble portion of the compound. Furthermore, our results warrant future studies to estimate the in vivo bioaccessibility and toxicodynamics of HMF.

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PAPER

OXIDATION COMPOUNDS IN EXTRA VIRGIN OLIVE OILS, FRESH OR STORED, AFTER FRYING

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ABSTRACT

The influence of the storage time on the levels of thermo-oxidation compounds of extra virgin olive oil (EVOO) during frying was evaluated. To this aim, two extra virgin olive oils (*cv* Bosana, EVOO1 and *cv* Semidana, EVOO2) fresh and after storage, were used, for deep-frying of potatoes. EVOO stored for a longer time led to a significant increase of the TAGP and ox-TAG content in both the residual fried oils and the oil extracted from potato chips, with respect to the levels observed when frying was performed with oils stored for a shorter time.

- Keywords: extra virgin olive oils, frying process, oil storage, oxidative degradation, polar compounds, triacylglycerol oligopolymers -

INTRODUCTION

Deep fat frying is a widely used cooking method in commercial food processing (BASEM *et al.*, 2010). In spite of the positive modifications occurring in fried products in terms of sensory characteristics (golden brown color, desirable flavor and texture), several physical and chemical changes affect the oxidative and hydrolytic degradation of oils and fats when brought to high temperatures in the presence of air and moisture (DOBARGANES and MARQUEZ RUIZ, 2006; SEBEDIO and JUANEDA, 2006). During frying, the modifications that occur in oils and fats depend on several factors, such as the length and temperature of the process, the number of cycles (PEDRESCHI *et al.,* 2008; ALADEDUNYE and PRZYBYLSKI, 2009; ROMANI *et al.,* 2008, ROMANI *et al.,* 2009; SÁNCHEZ-GIMENO *et al.,* 2006), the product to oil ratio and the consequent decrease of the initial oil temperature after foods were immersed (FISELIER *et al.,* 2006; ROMANI *et al.*, 2009; GÄKMEN *et al.*, 2006) and the type of oil used.

Regarding the type of oil used during the frying process, numerous studies highlighted the convenience of extra virgin olive oil to cook and fry foods. In particular, PERSSON *et al.* (2003) verified frying beefburgers with extra virgin olive oil reduced the formation of heterocyclic amines when compared to refined olive and seed oils. The high resistance of extra virgin olive oil against the thermo-oxidative phenomena with respect to other types of oils, has also been highlighted elsewhere (SÁNCHEZ-GIMENO *et al.,* 2008; KATRAGADDA *et al.,* 2010). Furthermore, the type of oil used influenced the sensorial characteristics of the fried food. In particular, some researches highlighted the benefits of using oils rich in mono-unsaturated fatty acids during the frying process because oils with high levels of linolenic acid rapidly deteriorate during the process and develop rancid and fishy flavors (DOBARGANES *et al.*, 1993; WARNER AND KNOWLTIN, 1997).

Extra virgin olive oil, obtained from the fruit of the olive tree solely by mechanical or other physical means and that is not subjected to any chemical change, is not a standard quality foodstuff, since great differences can be observed in the chemical parameters of different extra virgin olive oils. Moreover, the oil storage time and conditions also significantly affect the chemical composition of extra virgin olive oil, with particular regards to minor components and the evolution of oxidative degradation (PSOMIADOU and TSIMIDOU, 2002; MORELLÒ *et al.*, 2004; CAPONIO *et al.*, 2005; DEL CARO *et al.*, 2006).

The studies already carried out suggest using extra virgin olive oil for deep-frying, but no information is yet available, to the best of our knowledge, on the behavior of extra virgin olive oil during the frying process with regards to the storage time. The purpose of this investigation was to assess the influence of the storage time of extra virgin olive oils obtained from different cultivars on the levels of thermo-oxidation compounds during the frying process.

MATERIALS AND METHODS

2.1. Sampling

The experimental investigation was carried out on two extra virgin olive oils obtained from two Sardinian cultivars from the Oristano area (*cv* Bosana, EVOO1 and *cv* Semidana, EVOO2), which were mechanically harvested at an optimal ripeness degree and extracted with a "continuous method" that included: discrushing procedure at 1450 rpm, malaxation at 28°C for 20 min, oil separation through a 3-phase variable dynamic pressure (VDP) decanter (Alfa Laval, Monza, Italy), and filtration.

Immediately after extraction, the oils were transferred into green 250 mL glass bottles and hermetically sealed. Twenty-four bottles of oil were taken from each batch, and subdivided into 4 lots. One lot was immediately analyzed and submitted to frying as described below (T0). The oils of the other three lots were analyzed and subjected to frying after 6 (T6), 12 (T12) and 18 (T18) months of storage in the dark and at room temperature. Two bottles were used, from each group, to conduct the analytical characterization of the oils before the frying process and four bottles (1L) were used for the frying process.

2.2. Oil frying operations

A 0.5 L domestic fryer was used for frying. Two independent frying processes, each employing 500 mL of oil, were consecutively carried out on each oil. The oil was placed in the fryer and heated at 180°±5°C. Fresh potatoes cut into sticks were introduced into hot oil and fried for 4.5 min. A 1:4 (w/v) food-oil ratio was used. For each frying process, three aliquots of potatoes were consecutively fried, each time letting the fryer reach a temperature of 180°±5°C before the subsequent frying procedure (about 2 minutes). Preliminary trials showed the absence of significant differences in the level of the oxidative and hydrolytic degradation, among the three aliquots. So, at the end of the frying process, the three aliquots of fried potatoes were grouped and immediately subjected to oil extraction (yielding fried potato oil, FPo) while the residual frying oil (Fo) was filtered onto anhydrous sodium sulphate.

2.3. Oil extraction from fried potatoes

The extraction of the FPo was performed according to the method described by TABEE *et*

al. (2009) with slight modifications. In particular, the fried potatoes were placed in a beaker and a mixture of petroleum ether and diethyl ether (90:10, v/v) was added with a ratio sample-solvent mixture of 1:3.5 (w/v). The fried potatoes were homogenized with an Ultra-Turrax Omni Mixer 17106 (DuPont Instruments, Newtown, CT) at 24,000 rpm for 1.5 min. The homogenate was washed with 30 mL of a mixture of petroleum ether:diethyl ether (90:10, v/v). After 30 min of maceration, the solvent was separated by filtration through anhydrous sodium sulphate and evaporated under vacuum in a rotary evaporator at 40°C.

2.4. Analytical determinations

The determinations of free fatty acids (FFA), peroxide values (PV), UV spectrophotometric constants and fatty acids composition were carried out according to the EC Regulation no. 2568/91 (Official Journal of the European Communities, 1991) and subsequent amendments and integrations.

The phenolic compounds were extracted, purified, and determined according to the method described in a previous paper (BAIANO *et al.,* 2009), using the Folin-Ciocalteau reagent and measuring the absorption at 750 nm. The results obtained were expressed as mg of gallic acid per kg of oil.

The concentration of total carotenoids was calculated by measuring absorption at 449 nm of 0.25 g of oil dissolved in 5 mL *n*-hexane; using a calibration curve (y = 0.0532x; R^2 0.9986) previously obtained by measuring the absorption of solutions of β-carotene having a known concentration. For all the spectrophotometric determinations a Beckman UV-VIS model DU Series 60 (Beckman Instruments, Inc. Fullerton, CA, USA) was used.

The polar compounds (PC) were separated from the oils by silica gel column chromatography according to the AOAC method no. 982.27 (2003). After elution of the non-polar components with 150 mL of petroleum ether-diethyl ether (87:13, v/v), the polar compounds were recovered with 150 mL of diethyl ether. The efficacy of separation was checked by thin layer chromatography as recommended by the same method. The polar compounds recovered in tetrahydrofuran (THF) were then analyzed by means of high-performance size exclusion chromatography (HPSEC) using THF as eluent at a flow rate of 1 mL/min. The HPSEC system consisted of a series 200 pump (Perkin-Elmer, Norwalk, CT, USA) with a Rheodyne injector, a 50 μL loop, a PL-gel guard column (Perkin-Elmer, Beaconsfield, UK) of 5 cm length × 7.5 mm i.d., and a series of two PL-gel column (Perkin-Elmer, Beaconsfield, UK) of 30 cm length × 7.5 mm i.d. each. The columns were packed with highly cross-linked styrene-divinylbenzene copolymer with particles of 5 μm and a pore diameter of 500 Å. The detector was a series 200A refractive index (Perkin-Elmer, Norwalk, CT, USA). Peaks on the chromatograms were identified and quantified as reported in previous papers (GOMES and CAPONIO, 1999; CAPONIO *et al.,* 2003).

2.5. Statistical analyses

All the determinations were carried out in triplicate. Analysis of variance (ANOVA) followed by a Tukey HSD test for multiple comparisons were carried out on the experimental data with the XLStat Software (Addinsoft, New York, NY, USA).

RESULTS AND DISCUSSION

Table 1 reports the mean values, the standard deviations and the results of the statistical analyses of the analytical parameters determined for a preliminary characterization of the two extra virgin olive oils immediately after being extracted from olives (T0, fresh oils). The FFA, PV and the UV spectrophotometric indices of the oils were below the limits of the EVOO reported by the current regulations (Official Journal of the European Union, 2011). However, the extra virgin olive oil from Bosana *cv* (EVOO1) showed higher values for all these parameters with respect to those observed in the oil the Semidana *cv* (EVOO2), showing significant differences with regards to

Table 1 - Mean values, standard deviations and results of the statistical analysis of the analytical parameters determined on the oils immediately after their extraction.

Parameters	EVOO1		EVOO2		*p*-values
	Meanvalue	SD	Meanvalue	SD	
FFA (%)	0.57	0.06	0.48	0.06	0.064
PV (meqO_2/kg)	13.7	3.2	10.9	2.9	0.254
K_{232}	1.84	0.09	1.59	0.03	**0.002**
K_{270}	0.14	0.00	0.12	0.01	**0.036**
Δk	0.01	0.00	0.00	0.00	**< 0.001**
Total carotenoids (mg/kg)	6.76	0.65	14.09	1.05	**0.012**
Total phenols (mg/kg)	239	10	236	6	0.972
FATTY ACID COMPOSITION (%)					
$C_{16:0}$	17.3	0.46	16.8	0.43	0.371
$C_{16:1}$	1.1	0.02	0.9	0.16	0.287
$C_{17:0}$	0.1	0.00	0.2	0.01	**0.005**
$C_{17:1}$	0.2	0.03	0.5	0.11	**0.046**
$C_{18:0}$	1.7	0.11	1.8	0.20	0.648
$C_{18:1}$	61.3	0.68	63.0	0.45	0.097
$C_{18:2}$	16.8	0.42	15.2	0.15	**0.037**
$C_{18:3}$	0.8	0.04	0.9	0.04	0.185
$C_{20:0}$	0.4	0.01	0.3	0.03	0.326
$C_{20:1}$	0.4	0.03	0.4	0.05	0.247

FFA, free fatty acids; PV, peroxide value; EVOO1, extra virgin olive oil from Bosana *cv*; EVOO2, extra virgin olive oil from Semidana *cv*.

Table 2 - Mean percent values and the standard deviations of the total polar compounds and of the single classes constituting them of the non-fried oils (NFo), of the residual fried oils (Fo) and of the oils extracted from fried potatoes (FPo) for two examined extra virgin olive oils at different storage times.

Parameters		Storage time											
		T0			T6			T12			T18		
		NFo	Fo	FPo	NFo	Fo	FPo	NFo	Fo	FPo	NFo	Fo	FPo
TAGP	EVOO1	0.03±0.01	0.40±0.07	0.27±0.02	0.03±0.00	0.42±0.06	0.28±0.06	0.06±0.01	0.42±0.05	0.30±0.05	0.06±0.01	0.56±0.05	0.43±0.07
	EVOO2	0.00±0.00	0.24±0.04	0.17±0.02	0.01±0.00	0.31±0.04	0.23±0.05	0.03±0.01	0.29±0.05	0.22±0.03	0.04±0.00	0.32±0.04	0.23±0.03
ox-TAG	EVOO1	0.83±0.07	0.95±0.10	0.93±0.10	0.93±0.05	1.00±0.03	0.88±0.07	0.85±0.06	0.96±0.07	0.87±0.07	1.11±0.04	1.10±0.05	1.02±0.04
	EVOO2	0.53±0.08	0.65±0.09	0.63±0.13	0.73±0.10	1.01±0.05	0.85±0.15	0.73±0.03	0.72±0.06	0.73±0.03	0.86±0.14	0.88±0.11	0.84±0.10
DAG	EVOO1	2.86±0.11	2.71±0.12	2.50±0.06	2.92±0.07	2.77±0.17	2.65±0.20	2.80±0.03	2.90±0.05	2.74±0.13	2.96±0.12	3.18±0.20	3.07±0.17
	EVOO2	2.18±0.16	1.98±0.17	2.04±0.12	2.20±0.15	2.41±0.12	2.25±0.15	2.23±0.13	2.32±0.12	2.25±0.08	2.63±0.10	2.47±0.26	2.43±0.09
FFA	EVOO1	0.57±0.06	0.56±0.05	0.68±0.13	0.56±0.03	0.77±0.03	0.73±0.09	0.58±0.03	0.59±0.11	0.53±0.13	0.56±0.01	0.56±0.05	0.61±0.08
	EVOO2	0.48±0.06	0.52±0.03	0.65±0.19	0.51±0.00	0.60±0.07	0.55±0.06	0.49±0.04	0.51±0.14	0.55±0.17	0.48±0.01	0.56±0.09	0.53±0.07
PC	EVOO1	4.84±0.25	5.14±0.32	4.88±0.11	4.97±0.10	5.48±0.24	4.99±0.41	4.70±0.06	5.36±0.11	4.89±0.25	5.19±0.21	5.96±0.26	5.68±0.39
	EVOO2	3.59±0.38	3.76±0.38	3.85±0.49	3.84±0.11	4.73±0.20	4.24±0.33	3.85±0.31	4.20±0.45	4.11±0.27	4.43±0.29	4.44±0.61	4.43±0.19

TAGP, triacylglycerol oligopolymers; ox-TAG, oxidized triacylglycerol; DAG, diacylglycerols; FFA, free fatty acids; PC, total polar compounds; T0, fresh extra virgin olive oil; T6, extra virgin olive oils stored for 6 months; T12, extra virgin olive oils stored for 12 months; T18, extra virgin olive oils stored for 18 months; EVOO1, extra virgin olive oil of *cv Bosana*; EVOO2, extra virgin olive oil of *cv Semidana*.

the K_{232}, K_{270} and ΔK. Differences between the extra virgin olive oils were also observed for the carotenoid content, with significantly higher values in EVOO2 than in EVOO1. The higher content of carotenoids in the Semidana *cv* than in the Bosana *cv* was already found by FADDA *et al.* (2012), but with much lower values. The carotenoid content of the oil extracted from the Bosana *cv* was similar to those reported in DEL CARO *et al.* (2006) for extra virgin olive oils from whole and de-stoned fruit. The two extra virgin olive oils under investigation showed similar fatty acids composition and polyphenol contents. Moreover, as reported in Table 2, EVOO1 showed higher values of PC, triacylglycerol oligopolymers (TAGP, products of secondary oxidation), oxidized triacylglycerols (ox-TAG, including hydroperoxides and triacylglycerols having greater polarity than that of unaltered ones) and diacylglycerols (DAG, deriving from the hydrolytic degradation of triacylglycerols) than EVOO2. The differences observed in the chemical characteristics of the two types of extra virgin olive oils, were also considered as an independent variable in the statistical elaboration (Type of Oil "O" variable). Table 2 reports the mean values and the standard deviations of the total PC and of the single PC classes in non-fried oils (NFo), Fo and FPo for EVOO1 and EVOO2 at different storage times. The results of the statistical analysis (three-way ANOVA with first order interaction) are reported in Table 3. The *frying process (F)* and the *storage time of fresh oils (T)* considering both the oils, and the *Types of oil* were considered as independent variables.

The data of NFo at different storage times, reported in Table 2, are indicative of the evolution of the oxidative and hydrolytic degradation of fresh oils during storage. In particular, an increase of the TAGP and ox-TAG was found during storage with respect to fresh oil, in accordance with the results of a previous research carried out by BILANCIA *et al.* (2007). With regards to the hydrolytic degradation indices, the DAG content increased with respect to T0, whereas the FFA content was unchanged. During storage, moreover, a significant decrease in polyphenols and carotenoids content was observed (data not shown), confirming the existing literature about the evolution of the antioxidant compounds during storage (CAPONIO *et al.*, 2005; DEL CARO *et al.*, 2006; BILANCIA *et al.*, 2007; ESTI *et al.*, 2009).

As shown in Table 3, all the analytical parameters examined were significantly influenced by the *frying process* variable. When compared to the NFo, the frying process showed a significant increase of the TAGP content in FPo and Fo and of the ox-TAG content, in significant extent only in Fo. Moreover, significantly lower values of TAGP and ox-TAG were found in FPo than in Fo, probably due to the decrease of the oil temperature when the oil and potatoes come in contact. The protective effect of the water addition during frying process on the oil quality was already reported in a previous study carried out by DANA *et al.* (2003). Moreover, DELCURATOLO *et al.* (2008) – when assessing the changes in the oxidative state of the extra virgin olive oil used to bake Italian focaccias depending of different toppings – highlighted lower oxidative levels in the oil extracted from focaccias topped with potatoes than in those extracted from focaccias topped with other ingredients characterized by minor water contents. Such difference is due to the evaporation of the water contained in potatoes, which had the effect of stemming a

Table 3 - Results of the statistical analysis (three-way ANOVA with first order interactions) of the total polar compounds and of the single classes constituting them considering the frying process (*F*, NFovsRFovsFPo), types of oil (*O*, EVOO1 *vs* EVOO2) and the storage time (*T*, T0 *vs* T6 *vs* T12 *vs* T18) as independent variables.

Parameters	Variables														
	p-values						Frying process (F)			Storage time (T)				Type of oil (O)	
	Model	F	T	O	F*T	F*O	NFo	FPo	Fo	T0	T6	T12	T18	EVOO1	EVOO2
TAGP	< 0.001	< 0.001	< 0.001	< 0.001	0.050	< 0.001	C	B	A	C	BC	B	A	A	B
ox-TAG	< 0.001	< 0.001	< 0.001	< 0.001	0.043	0.391	B	B	A	C	B	C	A	A	B
DAG	< 0.001	0.008	< 0.001	< 0.001	0.183	0.375	A	A	B	C	B	B	A	A	B
FFA	0.001	0.004	0.010	< 0.001	0.052	0.982	B	A	B	AB	A	B	B	A	B
PC	< 0.001	< 0.001	< 0.001	< 0.001	0.454	0.242	C	B	A	C	B	BC	A	A	B

TAGP, triacylglycerol oligopolymers; ox-TAG, oxidized triacylglycerol; DAG, diacylglycerols; FFA, free fatty acids; PC, total polar compounds; *F*, *frying process* variable; *T*, *storage time* variable; *O*, *types of oil* variable; NFo, non-fried oils; Fo, residual fried oils; FPo, oils extracted from fried potatoes; T0, fresh extra virgin olive oil; T6, extra virgin olive oils stored for 6 months; T12, extra virgin olive oils stored for 12 months; T18, extra virgin olive oils stored for 18 months; EVOO1, extra virgin olive oil from Bosana*cv*.; EVOO2, extra virgin olive oil from Semidana *cv*.

rise in the temperature of the focaccia. With regards to the hydrolytic degradation, an increase of the FFA content with respect to the NFo was observed during the frying process both in FPo and in Fo (significantly only for the first), as already verified by other authors (TABEE *et al.*, 2009; CASAL *et al.*, 2010). The DAG content, instead, decreased during the frying process, significantly only for Fo, probably due to the thermo-oxidation of DAG (ALADEDUNYE and PRZYBYLSKI, 2009). As a consequence of the evolution during frying of the single classes of compounds, also the PC content significantly increased during the process, both in FPo and Fo (Table 3). The highest observed level of PC, found in the fried oil obtained from EVOO1 and stored for 18 months (5.96%), was much lower than the limit of 25% established for frying fats and oils for human consumption by most current regulations (FIRESTON, 2007). This confirms what was already reported by other authors (SÁNCHEZ-GIMENO *et al.*, 2008; KATRAGADDA *et al.*, 2010) about the opportunity to use extra virgin olive oils in frying process of foods.

The storage time of the extra virgin olive oil significantly affected the evolution of the oxidative degradation both in Fo and FPo. In fact, the use of long-term stored extra virgin olive oils in the frying operations determined marked increases of the TAGP content (significantly at T12) and of the ox-TAG content (significantly at T6 and T18) in fried oils. Moreover, for the TAGP content a significant first order interaction *F*T* was observed both for FPo and Fo (Fig. 1A and 1B). This indicates a different triacylglycerol polymerization rate during the frying process depending on the storage time of the oils used. In particular, when extra virgin olive oils stored for 18 months were used for frying, the triacylglycerol polymerization occurred with greater intensity. This is due to a higher content of TAGP and ox-TAG in extra virgin olive oils that are stored

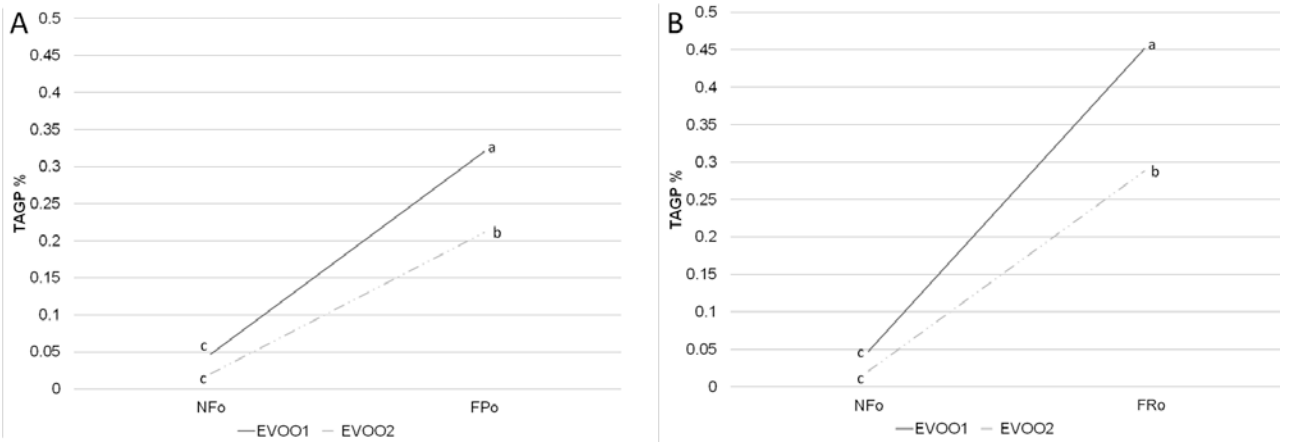


Fig. 1 - Evolution of the triacylglycerol oligopolymers (TAGP) content of two different extra virgin olive oils during the frying process: oils extracted from fried potatoes (A) and residual fried oils (B). (NFo, not fried oils; Fo, residual fried oils; FPo, oils extracted from fried potatoes; EVOO1, extra virgin olive oil from Bosana *cv.*; EVOO2, extra virgin olive oil from Semidana *cv*.

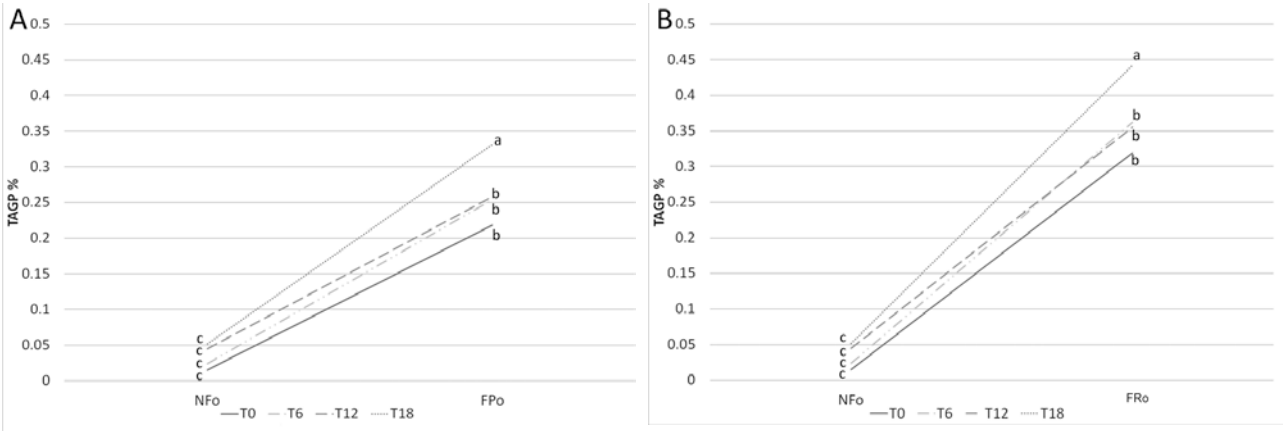


Fig. 2 - Evolution of the triacylglycerol oligopolymers (TAGP) content during the frying process in oils extracted from fried potatoes (A) and residual fried oils (B) with different time of storage times. (NFo, not fried oils; Fo, residual fried oils; FPo, oils extracted from fried potatoes; T0, fresh extra virgin olive oil; T6, extra virgin olive oils stored for 6 months; T12, extra virgin olive oils stored for 12 months; T18, extra virgin olive oils stored for 18 months).

for 18 months with respect to those stored for a shorter time period (Table 2) but also a lower carotenoid and polyphenol content (data not shown). Indeed, previous studies highlighted the pro-oxidant effect of the polar compounds and of the single classes of substances constituting them (GOMES *et al.*, 2011), particularly when frying (SÁNCHEZ-GIMENO *et al.*, 2008; TABEE *et al.*, 2009; CASAL *et al.*, 2010) and the effectiveness of antioxidants such as polyphenol in reducing the oxidation rate in the oil (NAZ *et al.*, 2004). The use of stored extra virgin olive oils when frying also led to significant increases in the DAG content, while the FFA content significantly decreased. For these parameters, the results obtained for the fried oils reflected the trend observed in the non-fried oils during storage. Indeed, no significant first order interaction between the variables was observed.

Considering the influence of the type of oil, the frying conditions here applied did not level the initial quality characteristics of the oil used, as the different quality of the fresh extra virgin olive oils was still evident after the frying process in both the residual fried oils and the oils extracted from the potato chips. Moreover, a significant first order interaction *F*O* was observed for the TAGP content. As shown in Fig. 2A and 2B, the evolution of the triacylglycerol polymerization process during frying, was more evident in the oils of lower quality due to the aforesaid pro-oxidant activity of TAGP and ox-TAG.

CONCLUSIONS

The age of the extra virgin olive oils used for frying operations significantly influenced the formation of thermo-oxidation compounds. In particular, the use in the frying operations of extra virgin olive oils stored for a longer period of time led to a significant increase of the TAGP and ox-TAG content both in the residual fried oils and in the ones extracted from potato chips with respect to the levels observed when frying with oils that were stored for a shorter amount of time. The frying conditions applied in this experiment, similar to domestic frying, did not lead therefore to a leveling of the quality characteristics of the used oil, since the different quality of the fresh extra virgin olive oils utilized was still evident after the frying process in both the residual fried oils and in the oils extracted from the potato chips.

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PAPER

DEVELOPMENT OF MAILLARD REACTION IN PASTA DRIED BY MICROWAVES

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ABSTRACT

The aim of this research was to evaluate the development of Maillard reaction in pasta dried by microwaves and hot air at different processing conditions.

The Maillard reaction was more developed in samples dried by microwaves than in those treated by hot air as resulted from decrease of pH values (5.77 vs. 6.07) and increase of absorption at 294 nm (1.67 vs. 1.57) and 420 nm (0.77 vs. 0.11). Even if no remarkable colour change in samples differently dried was observed, the highest absorbance values indicate a greater potential degradation of product treated by microwaves during storage.

- Keywords: microwaves drying, Maillard reaction, dried pasta, colour measurements, spectrophotometric analyses -

INTRODUCTION

Drying is one of the oldest known methods for food preservation with minimal damaging effect on quality (PHOTON *et al.,* 2001).

Microwave technology is a relatively new and exciting field and the literature concerning this technology is rapidly growing. It is an efficient way to supply energy; heat is generated directly inside the product by the friction of the solvent molecules upon themselves, so there is no external heat transfer resistance. This is distinctive from conventional conductive drying methods in which energy is supplied, at the surface of the product, and then penetrates inside by thermal diffusion (LOMBRAÑA *et al.,* 2001).

The first researches carried out in the 70s have highlighted the shortness of microwaves drying system. In fact, MAURER *et al.* (1971) obtained pasta-drying speeds 20 times greater using microwaves when compared to conventional drying. ALTAN and MASKAN (2005) studied the effect of conventional and microwave drying on the quality parameters of cooked and uncooked macaroni. Macaroni samples were dried by conventional hot air or microwave alone and hot air followed by microwave drying methods. Microwave drying (70 and 210 W) or hot air combined with microwave drying (70 and 210 W) resulted in substantial shortening of the treatment time, but starch was not completely gelatinized. GOKSU *et al.* (2005) studied microwave assisted fluidized bed drying of macaroni beads using a household microwave oven. They found that the increase in microwave power and air temperature significantly reduced (at 50%) the drying time of the macaroni beads when compared with fluidized bed only. BERTELI and MARSAIOLI (2005) evaluated the efficiency of air drying of Penne type short cut pasta with the assistance of microwave energy and observed that the average drying time was reduced by more than ten times when compared to conventional air drying, without negatively affecting the appearance of the final product. The greatest benefits of drying pasta products using microwaves are the reduction of drying time and the maintenance of product quality.

It is known that among the numerous reactions, that take place during drying of pasta, Maillard reaction has a determining role for the quality and safety of this kind of product. In fact, an uncontrolled Maillard reaction reduces the nutritional value of wheat protein by lowering the level of nutritionally available lysine and causing excessive browning and off flavours. Literature relating to development of Maillard reaction and its effect on nutritional value and organoleptic quality of dried pasta through hot air at different moisture and temperature conditions is wide (FEILLET *et al.*, 2000). Nevertheless, studies about this reaction in pasta dried through microwaves are scanty. Recent studies on microwaves dehydration on this field focused on lipid oxidation of filled pasta and cooking quality of spaghetti dried by microwaves (DE PILLI *et al.*, 2008, 2009). In particular, DE PILLI *et al.* (2008) found that the development of Maillard reaction in pasta sheets at drastic conditions protected lipid fraction by oxidation. A deeply investigation should be advisable on this subject since the highest temperature reached during microwaves drying treatment.

For this purpose, the aim of this research was to evaluate and to compare the development of Maillard reaction in pasta made up of durum wheat semolina and water, dried at different process conditions through microwaves and hot air.

MATERIALS AND METHODS

Raw material

Pasta samples were made up of durum wheat semolina (Divella S.p.A., Rutigliano, Bari, Italy) purchased on local market and running water. The chemical characteristics of semolina are the following: moisture % (13.60 ± 0.10), ash % (0.78 ± 0.10), protein % (12.00 ± 0.10). Tap water was characterized by pH of 7.69 ± 0.10, hardness (°F) 25.10 ± 1.50, total dissolved solids dried at 180°C 645.00 ± 38.50 mg/L and chloride content 54.60 ± 0.40 mg/L.

Moisture content, ash and protein of semolina were determined according to the AACC methods (2003).

Preparation of samples

Dough was manually prepared with semolina and water (2:1 w/w) (DE PILLI *et al.* 2008). The sheets with a thickness of 1 mm of dough were made using a pasta machine Imperia mod. SP 150 (IPS, Torino, Italy) with a manual thickness-selector at six positions. Square shape (1 cm x 1 cm) of sheet of dough was obtained using an inox aluminum mould (Raviolamp 36 Imperia Trading S.r.L., Torino, Italy). Almost three repetitions of dough were carried out for each drying processing condition.

Samples were dried using a climatic room Binder mod. KBF 240 (Tuttlingen, Germany) according to the following processing parameters:

- temperature range 55-91°C ± 1.0;
- dryer air flow 100 m^3/h;
- drying time range 5400-78600 s.

For each drying cycle the temperature and relative humidity (80% RH) were kept constant until cooling started; then they were lowered to 30°C and 60% RH, respectively (ZWEIFEL *et al.*, 2000).

A Sfornatutto DeLonghi mod. Combi&Functions Convenction oven (DeLonghi Italia, s.p.a., Treviso, Italy) with a maximum power of 850 Watt was used to dry samples by microwave.

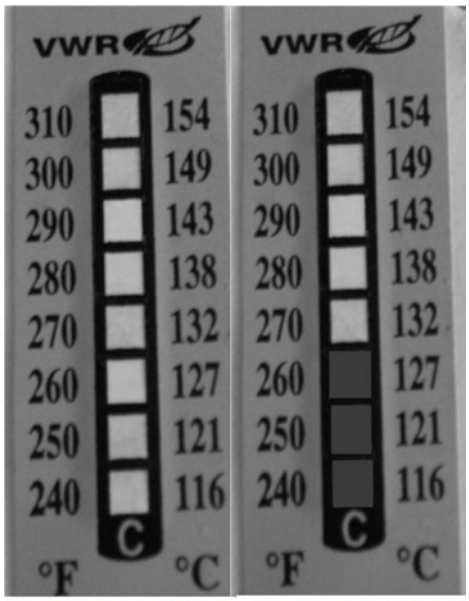


Fig. 1 - Graduated stripes of paper sensitive to heat, before and after thermal treatment, used to detect the maximum temperature reached in pasta dried by microwaves.

Table 1 - Experimental plan of pasta dried at different temperature by hot air with moisture content of 7% (dry basis).

Sample	Drying Temperature (°C)	Treatment Time (s)
Low temperature (LT)	55	3600
Medium temperature (MT)	70	3300
High temperature (HT)	85	1260
Very high temperature (VHT)	91	480

To detect the maximum temperature reached in pasta dried by microwaves, graduated stripes of paper (Fig. 1) (VWR International mod. MELB1762/MELB1763, Milan, Italy) sensitive to heat were put into two squares of pasta that were placed in different positions of rotating plate of microwave oven to evaluate the heterogeneity of electromagnetic field distribution. Ten repetitions of temperature measurement for each power percentages were carried out.

Moisture %

Moisture content was determined according to the AACC methods (2003) placing samples in an oven ISCO Model NSV 9090 (Italy) at 105°C until constant weight. This analysis was replicated at least three times.

Experimental design

In this research, pasta was dried at four different temperatures or power percentages. The values of drying temperature and power percentages were chosen to simulate a drying treatment at low, medium, high and very high temperature. The treatment times were chosen so that pasta samples reached a moisture of 14%. The values of moisture were determined through drying curves obtained taking a sample at regular periods of time.

Table 1 reported experimental plan of pasta dried at different temperature by hot air with moisture content of 14% (dry basis). Table 2 showed the maximum temperature reached into samples during drying, the treatment times and the microwave power percentages expressed as amount of irradiation absorbed by pasta per time unit i.e. Watt*irradiation times (seconds)/sample amount (g).

Analyses

Pasta samples were finely grinded by a mill (Waring Commercial mod. 38BL40, Torrington, Connecticut, USA).

The analytical indexes considered to check the development of Maillard reaction were: L*, a* and b* values; pH; absorption at the wavelengths of 294 and 420 nm. At least three measurements for each sample were carried out.

Colour measurement

The colour of pasta was evaluated by a tristimulus colorimeter (Chromameter-2 Reflectance, Minolta, Osaka, Japan) equipped with a CR 300 measuring head.

Colour was expressed as L*, a* and b* calibrated to a standard white tile (L*=91.7, a*=-1.16, b*=1.06). L* corresponds to lightness, a* repre-

Table 2 - Experimental plan of pasta dried at different power percentage by microwaves with moisture content of 7% (dry basis).

Sample	Power (%)	Pasta Temperature (°C)	Absorbed Irradiation (Watt*s/g)[1]	Treatment Time (s)
Low power (LP)	45 [390 W]	110	1529	98
Medium power (MP)	50 [425 W]	127	1615	95
High power (HP)	60 [510 W]	138	1224	60
Very high power (VHP)	70 [595 W]	149	1309	55

[1]Watt*irradiation times (s)/sample amount (g).

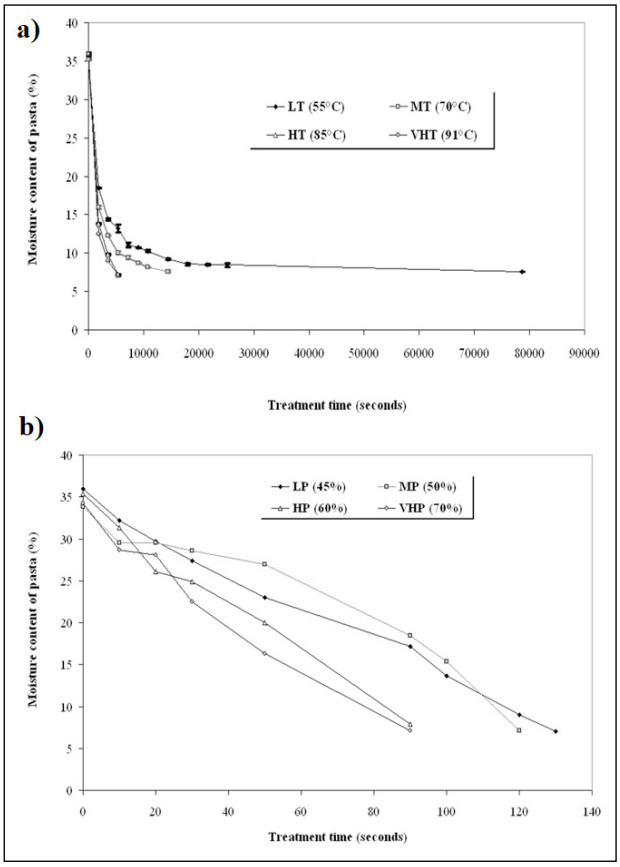


Fig. 2 - Drying curve of pasta dried by hot air (a) and microwaves system (b).

sents red (+) - green (-) and b* refers to yellow (+) - blue (-). Ten measurements were conducted for each treatment using 5 square sheets of pasta per one measurement. Chroma and Hue angle were suggested to be more practical measures of colour (MCGUIRE, 1992).

Hue angle (h) was calculated as arctan b*/a* and chroma was determined as $[(a^*)^2 + (b^*)^2]^{0.5}$.

Absorbance at 294 and 420 nm

The spectrophotometric analyses were performed on 10 g of powdered pasta previously mixed with distilled water (50 mL) through a laboratory blender for 15 min (Warning Commercial, Torrington, CT, USA). The homogenate was centrifuged (ALC mod. 423R, Milan, Italy) for 10 min at 5,000 rpm and 24°C and the liquid phase was filtered through Whatman # 1 paper. The absorbance values at 294 and 420 nm were measured using a UV/Vis spectrophotometer (Beckman DU 640, Fullerton, CA, USA).

Statistical analysis

The variance analysis (ANOVA) was carried out on results obtained from Maillard indexes. They were elaborated by software StatSoft ver. 6.0 (Statsoft, Tulsa, USA). The means of these results were compared by the Fisher's test.

Standard deviation and kinetic constants were calculated by Excel OFFICE XP Microsoft Corporation software (USA). The kinetic constants were estimated taking in account the linear regression of early stage of drying curves.

RESULTS

Fig. 2a shows the drying curves of samples dried nearly 7 % by hot air at different temperatures. As expected, the increase of treatment temperatures determined a decrease of drying times, which were included between 5,400 and 78,600 s, because of increase of drying speed. In fact, kinetic constant values increased with increasing of drying temperature (Table 3). Nevertheless, pasta dried to 85° and 91°C did not involve a remarkable difference of kinetic constant values (Table 3).

Drying curves of samples dried by microwaves nearly 7% at different power percentages were reported in Fig. 2b. The range of treatment time was included between 90 and 130 s. Also in this case, the increase of power percentage involved a decrease of treatment times even if more moderate than process carried out by hot air (Table 4), as evidenced by kinetic constant values that doubled increase during microwaves treatments while raised nearly six times during hot air drying (Tables 3 and 4).

From the comparison of processing times required to dry samples by hot air and microwaves, at the highest temperature or power percentages, a huge difference emerged. In fact, the time necessary to dry samples by hot air was 5,400 s versus 90 s required by microwave treatment.

Table 3 - Kinetic constant values and correlation coefficients relating to drying curve of pasta dried at different temperatures by hot hair.

Drying temperature (°C)	k	r
55	-0.35	-0.94*
70	-0.64	-1.00*
85	-0.98	-0.94*
91	-2.09	-1.00*

*Significant level (p-level) < 0.05.

Table 4 - Kinetic constant values and correlation coefficients relating to drying curve of pasta dried at different power percentages by microwaves.

Power (%)	k	r
45 [390 W]	-0.20	-0.98*
50 [425 W]	-0.17	-0.98*
60 [510 W]	-0.32	-0.98*
70 [595 W]	-0.35	-0.99*

*Significant level (p-level) < 0.05

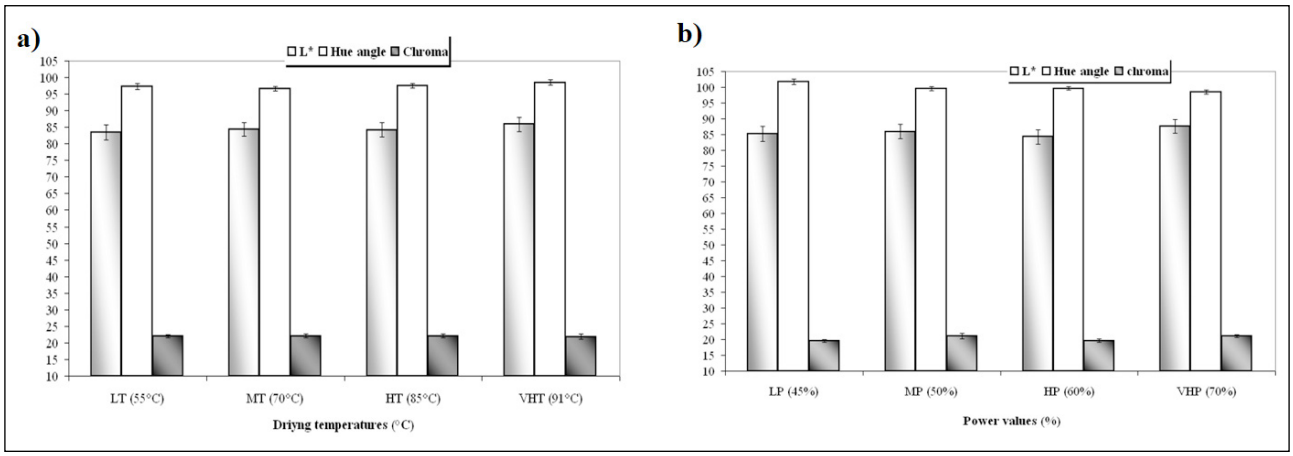


Fig. 3 - Colour attributes of pasta dried at different intensity of thermal treatments by hot air (a) and microwaves (b).

Pasta colour is an essential factor in assessing pasta quality, in particular, during drying processing. Figs. 3a and 3b show the colour parameters of pasta samples dried by hot and microwaves as a function of drying temperature or power percentages. The ANOVA's results showed that values of L* did not change significantly as a function of type and intensity of drying treatments (Table 5). Yet, hue angle and chroma values resulted significantly different as a function of drying system type (Table 5). In particular, samples dried by microwaves showed values of hue angle higher than pasta dried by hot air while chroma results showed an opposite behaviour (Figs. 3a and 3b).

It is important to remember that hue angle

Table 5 - Variance analysis of Maillard reaction index results obtained for samples dried by hot air (ha) and microwave (m).

Maillard reaction indexes	Effect	Sum square	Degree of freedom	Mean square	F	P
L*	Intercept	173930.8	1	173930.8	36154.35	0.01*
	Intensity of drying treatment	24.3	3	8.1	1.68	0.21[a]
	Type of drying system	18.0	5	3.6	0.75	0.60[a]
	Error	72.2	15	4.8		
Hue angle	Intercept	233570.8	1	233570.8	145147.6	0.01*
	Intensity of drying treatment	6.7	3	2.2	1.4	0.28[a]
	Type of drying system	35.0	5	7.0	4.4	0.01*
	Error	24.1	15	1.6		
Chrome	Intercept	10788.75	1	10788.75	22783.96	0.01*
	Intensity of drying treatment	3.28	3	1.09	2.31	0.12[a]
	Type of drying system	18.91	5	3.78	7.99	0.01*
	Error	7.10	15	0.47		
pH	Intercept	842.78	1	842.78	103110.0	0.01*
	Intensity of drying treatment	0.02	3	0.01	0.9	0.48[a]
	Type of drying system	0.64	5	0.13	15.7	0.01*
	Error	0.12	15	0.01		
Absorbance 294 nm	Intercept	60.95	1	60.95	14907.47	0.01*
	Intensity of drying treatment	0.02	3	0.01	1.34	0.30[a]
	Type of drying system	0.10	5	0.02	4.94	0.01*
	Error	0.06	15	0.00		
Absorbance 420 nm	Intercept	4.69	1	4.69	44.15	0.01*
	Intensity of drying treatment	1.37	3	0.46	4.28	0.02*
	Type of drying system	2.59	5	0.52	4.88	0.01*
	Error	1.59	15	0.11		

[a]Not significant differences level < 95%;
*Significant differences at 95% level.

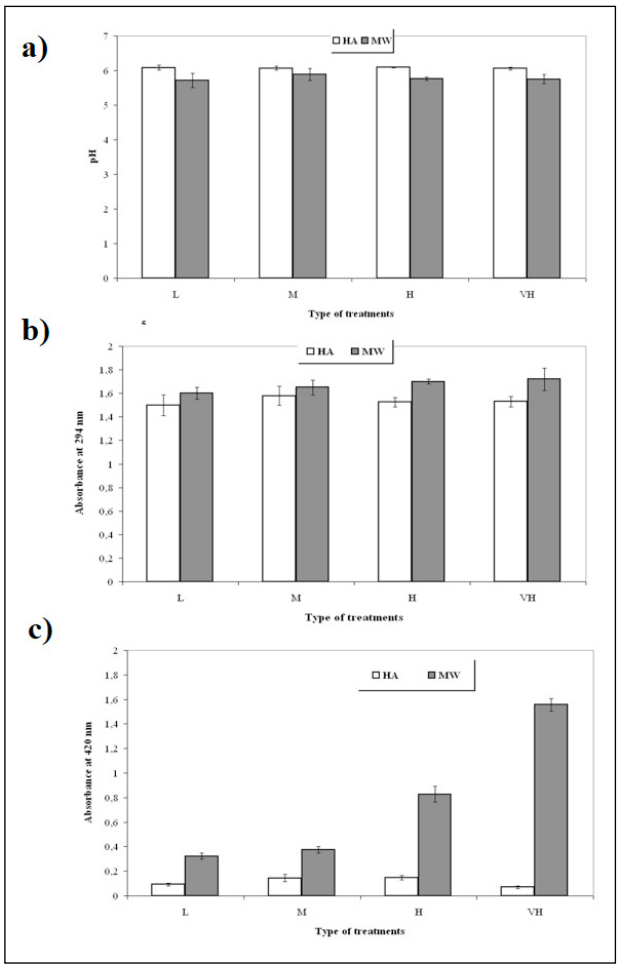


Fig. 4 - pH values of samples dried at different intensity of thermal treatments by hot air and microwaves (a), absorbance values at 294 nm (b) and at 420 nm (c) of samples dried at different intensity of thermal treatments by hot air and microwaves.

represents the full spectrum of colour and exists from 0° to 360°. If a* is negative and b* is positive, the colour is a combination of green and yellow, the hue angle value will be between 90° and 180° (YANCEY and KROPF, 2008). Moreover, high values of hue angle involved a decrease of yellow as regards to green component. Also, chroma is an index of purity of colour, then low values of this index means a loss of pure red colour and, consequently, a degradation of carotenoid pigments (WIRIYA *et al.*, 2009). It is possible to assume a degradation of carotenoid in pasta dried by microwaves. FRATIANNI *et al.* (2010) monitored the degradation of carotenoids in orange juice during microwave heating at different time/temperature conditions. They observed that compounds with provitamin A activity (β-carotene, α-carotene and β-cryptoxanthin) and lutein were unstable at higher temperatures (>75°C). In our research, pasta dried by microwaves reached temperature ≥ 110°C (Table 2) while samples dried by hot air reached a maximum temperature of 91°C. The highest temperatures reached in pasta dried by microwaves, for each considered power percentage, probably favoured a degradation of carotenoids (high values of hue angle and low values of chroma) causing a loss of quality and nutritional characteristics of dried pasta by microwaves compared to samples dried by hot air (Fig. 3b).

Results of colour parameters seem to indicate that Maillard reaction did not occur during drying processing for both types of treatments. In fact, samples did not showed a decrease of L* and hue angle values and an increasing of chroma, as expected when the Maillard reaction takes place (CAPUANO *et al.*, 2009).

According to colour results, pH values did not show a significant difference between samples dried at different temperatures or power percentages (Fig. 4a and Table 5), while a significant difference was observed between samples dried by hot air and microwaves (Table 5). In particular, pasta dried by microwaves involved values of pH lower than samples dried by hot air for all considered processing conditions (Fig. 4a). The pH value is a good index of Maillard reaction. MORALES and JIMENEZ-PEREZ (2001), BRANDS and VAN BOEKEL (2002) and BENJAKUL *et al.* (2005) found that, during the Maillard reaction, the pH frequently decreases as the heating time increases because of the formation of organic acids, such as formic and acetic acid. OGURA *et al.* (2000) reported that formation of the acid form of glycine, or glycyl-glycine, was due to the lowering of pH, which occurred, because oxygen evolution occurs in further positive polarisation. HUBER and WÄCHTERSHÄUSER (1998) reported that the lowering of pH can be explained by the formation of acids from CO during the heating process of peptides. In addition, some authors have reported that, during the reaction of peptides with carbonyl compounds, peptide bond breaking occurs through an anionic reaction (CHUYEN *et al.*, 1973). However, the concentration of the anionic form is not a limiting factor (REYNOLS, 1969) for pH values above the isoelectric point of the amino compound (5.57 for glycine, 5.59 for diglycine and 5.58 for triglycine). Besides, it is expected that the molecule size could play a role in the smaller reactivity of peptides as compared to glycine (BUERA *et al.*, 1987). For this reason, the pH values of pasta samples dried through both systems showed a slight change in pH values (5.72-6.09).

Absorbance results at 294 nm did not show a significant difference between samples dried at different processing conditions for both drying system, while a significant difference between pasta dried by hot air and microwaves was observed (Fig. 4b and Table 5). In particular, pasta dried by microwaves showed values of absorbance at 294 nm greater than sample dried by hot air (Fig. 4b). These results suggested the formation of uncoloured compounds, which could be the precursors of the Maillard reaction (AJANDOUZ *et al.*, 2001).

Higher values of absorbance at 420 nm for samples dried by microwaves compared to samples treated by hot air were observed (Fig. 4c). Moreover, an increase of absorbance with increasing of treatment intensity was observed for samples dried by microwaves (Fig. 4c). In fact, ANOVA of absorbance at 420 nm results showed a significant effect of type and intensity of used drying system (Table 5). Nevertheless, the short treatment times of microwaves drying did not involve a remarkable development of Maillard reaction as deeply to modify the colour characteristics of pasta measured by colorimeter.

CONCLUSION

In the considered processing conditions, despite short treatment time, Maillard reaction developed in samples dried by microwaves more then pasta dried by hot air. Nevertheless, the intensity of Maillard reaction did not involve a change in colour characteristics of pasta even if a loss of colour saturation was observed. This could be due to degradation of carotenoids, which could involve a loss of nutritional values. Moreover, the highest absorbance values indicate a greater potential degradation of product treated by microwaves during storage. For this reason, it is very important monitoring temperature inside product during drying processing when a microwaves system was used. A possible prosecution of this research could be related to the study of the development of Maillard reaction during shelf-life of pasta dried by microwaves.

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PAPER

HARVESTING AND FIELD PACKING OF TREE-RIPENED PEACH FRUITS, CRITICAL EVALUATION

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ABSTRACT

To satisfy consumer demand for riper fruits, a system for field packing stone fruits was set up based on an experimental electrical traction platform. Tree-ripened peaches and nectarines were sorted and field packed in plastic clamshell containers and delivered directly to retail outlets. Productivity and fruit characteristics such as size, flesh firmness and soluble solids concentration were evaluated. While the observed working capacity was comparable to that of conventional harvesting systems, the fruit characteristics (size and flesh firmness) did not always meet the required consumer-oriented higher quality standards.

- Keywords: consumer acceptance, field packing, flesh firmness, *Prunus persica*, ripeness, soluble solids concentration -

INTRODUCTION

In a standard supply chain, after harvesting, fresh fruits are transported to packing houses where they are sorted, packed and sometimes refrigerated before marketing (SHEWFELT and PRUSSIA, 2009). The sorting and packing processes are usually performed automatically by machines which play a major role in the occurrence of skin damage and product down-grading (RAGNI and BERARDINELLI, 2001). According to the European Commission Regulation (EC, 1221/2008) on marketing standards, there should be no mechanical injuries with the possible consequent degenerative processes on fresh fruits belonging to the "extra" categories (tolerance of 10%).

These quality standards are met by harvesting at an early stage of maturity as higher values of flesh firmness reduce the probability of damage (BERARDINELLI *et al.*, 2006), but this practice precludes the achievement of ideal ripeness (ROBERTSON *et al.*, 1992).

The determination of stone fruit maturity depends on the combination of parameters such as ground color, firmness and size (CRISOSTO, 1994). Fruit flesh firmness and soluble solids concentration (SSC) are the main indices related to quality as perceived by the consumer. The SSC at the same acidity level, is related to the perception of fruit sweetness intensity; values higher than 9.5% identify peaches with an acceptable taste for quality fruit production (ALAVOINE *et al.*, 1988). Flesh firmness is one of the best indicators of ripeness and a predictor of shelf life (CRISOSTO, 1994). Even if it is not possible to define universally accepted values of flesh firmness which identify the fruit maturity level, CRISOSTO (2002) reported that fruit are considered "ready to buy" at 26.5-35.3 N and "ready to eat" at 8.8-13.2 N and DI MICELI *et al.* (2010) indicated that values higher than 53.9 N always resulted in unacceptable fruit. However, when the product has little handling or is sorted and packed directly in the field the reference range that provides improved organoleptic characteristics can reach lower values of firmness (CRISOSTO and VALERO, 2008).

Several researches have shown that the intensity and gradation of skin color, fruit size, together with eating quality (flavor, taste and texture), influence consumer acceptance and therefore sales (BRUHN, 1991a, b; HARKER, 2001; GARITTA *et al.*, 2008) and that very often flavor components never reach levels that can provide an acceptable or good flavor (BASSI and SELLI, 1990; CRISOSTO *et al.*, 2001). More generally consumer dissatisfaction is mainly related to the lack of ripeness (HERRERO-LANGREO *et al.*, 2012) and especially to flesh hardness and lack of flavor (CRISOSTO *et al.*, 2006; IGLESIAS and ECHEVERRÍA, 2009). This dissatisfaction is reflected directly on consumption. For example, CLARETON (2000) reports that as many as 80% of consumers in France are dissatisfied with peach quality. The reduction in peach consumption observed in Italy in recent years was also mostly attributed to the low product quality which was not appreciated by consumers because harvested when unripe or characterized by a heterogeneous maturity level (DELLA CARA, 2005). The quality of peaches can be improved if the fruits are harvested at a later stage of physiological maturity. In fact, early harvesting compromises quality and disappoints consumer expectations since peach flavors are only produced on the plant at the advanced maturity stage (ROBERTSON *et al.*, 1992). Consumer demand for a ripe product contrasts with the current methods of harvesting in big containers such as field bins or crates, which are not suitable for tree-ripened fruits that are to be delivered directly to retail outlets.

To improve the organoleptic quality of fresh fruits and consumer satisfaction some post-harvest processes such as sorting and packing could be done directly in the field during the harvest, which would allow more mature fruit to be picked. It is evident that this "freshly picked fruit" product has a limited shelf life, requires specific packaging and should be delivered directly to retail outlets through a suitable supply chain. Field packing systems for vegetables and fruits are gaining importance mainly due to the lower cost compared to packinghouse facilities and fewer injuries to the product because of reduced handling (KADER, 2002; CRISOSTO and VALERO, 2008). Few studies have considered the efficiency of field packing systems of fruits in terms of consumer-oriented quality improvement. A type of fruit called "Tree Ripe" was evaluated on the U.S. market that are usually harvested when more mature and packed on small, labor intensive hand packing lines, because they cannot withstand the rigors of typical commercial packing lines (MITCHELL *et al.*, 1989).

SHEWFELT *et al.* (1989) verified that harvesting of more mature peaches with packing in the orchard results in a quality superior to packinghouse peaches even if losses for field packed peaches were higher than packinghouse fruits, probably due to a lack of careful grading by the pickers in the orchard. However, the authors do not describe the field packing method considered.

In many typical areas for the production of stone fruits in Italy, it has become customary to set up the harvester for the housing of single-layer wooden boxes to field pack fruits and obtain more mature produce that is easy to sell. Preliminary economic analysis were carried out to evaluate the efficiency of these harvesting systems (VANNINI, 1999). Studies have also shown that high-quality stone fruits may obtain higher prices than lower quality fruits harvested beyond

the minimum maturity stage (BRUHN, 1991a; JORDAN *et al.*, 1990; PARKER *et al.*, 1990).

Based on the concept of providing the consumer with optimum quality, the Growers Cooperative Terremerse (Ravenna, Italy) and a large-scale retail trade company Coop Italia (Bologna, Italy), as part of the project "Appena Colta" (*freshly picked*), involved some local farms in marketing fruits with a high level of ripeness. Firmness values in the range 14.7-34.3 N were considered suitable for stone fruits for immediate consumption without further handling (MAZZINI *et al.*, 2007). In collaboration with the Cooperative Terremerse, this paper assesses a system for harvesting stone fruits with a high level of ripeness using an experimental electrical traction platform set up for field harvesting, sorting and packing peaches and nectarines.

MATERIALS AND METHODS

The harvesting, sorting and packing operations were carried out by a self propelled experimental field packing system. The vehicle was a platform with electrical traction constituted by two components (Fig. 1). The first was a self-propelled working platform manually operated by a shaft. Manual sorting and packing were done on this component. These operations were performed by two pickers, one on either side of the platform, who separately picked, sorted and packed according to three size classes: AAA (80 and over but under 90 mm), AA (73 and over but under 80 mm) and A (67 and over but under 73 mm) (EC, 1221/2008). The second component was a farm cart used to transport the empty packaging and the packaged product.

The field testing was conducted on a farm in the province of Ravenna, northeast Italy, considering two cultivars of *Prunus persica* (L.), Batsch



Fig. 1 - Platform for sorting, harvesting and packing fruits. 1, self-moving working platform; 2, farm cart for transport.


Fig. 2 - Sorting and packaging operations.

Rome Star (peach) and Sweet Red (nectarine), and two growers highly experienced in the selection of freshly picked fruits. Both orchards were 4 years old, trained to delayed vase, and planted with a distance of 5.5 m between rows and 3.5 m between trees on a row. The agronomic management of the orchards was aimed at obtaining fruits with a high level of flavor. In particular, the nitrogen fertilization and pruning were done before the fruit stone hardening and when fruits were 8-10 mm in diameter, respectively. These were followed by green pruning.

The picking, sorting and packing processes were conducted with the vehicle in stationary mode by two pickers who assessed the maturity level on the basis of their experience. The pickers worked in the inter-row area on either side of the platform. After picking, they placed the fruits on a side support attached to the packaging area by a revolving arm. After reaching a weight of 2-3 kg (about 25 peaches), the fruits were sorted using a fixed metallic sizer and packed in different clamshell plastic containers (jardipack®, Groupe Guillin, Ornans, France) according to size class (Fig. 2). The boxes, containing 4 fruits each, were then placed in collapsible crates (0.60 x 0.37 m, CPR System, Bologna, Italy) (6 boxes per crate). The tasks that completed the field operations involved placing the empty packaging and the packaged product on the second component of the vehicle, then transferring the crates onto an open lorry.

To assess the productivity of the field harvesting, handling and packing processes, the following working parameters were measured:
time(s) required for vehicle relocation;
time(s) required for fruit picking and basket filling;
time(s) required for fruit sorting;
time(s) required for fruit packaging;
time(s) required for transferring crates from the second component onto the open lorry;
global working time(s), including the time required

for placing the empty packaging on the platform; mass (g) of the total harvested product.

Work quality assessment was conducted considering both cultivars, two pickers and three size classes (A, AA, AAA), the mass (g) and diameter (mm) were measured on samples of 12 randomly selected fruits. The flesh firmness (N) was determined using a fruit texture analyzer (Guss Manufacturing Ltd., Strand, South Africa) and soluble solids concentration SSC (%) was assessed using a digital refractometer (Atago, Model PR 101). The mean value of two measurements conducted on two different peeled sides of the same fruit was considered. Data were statistically analyzed using SPSS software (SPSS 13.0 for Windows, IBM SPSS Statistics). Statistical differences between means were tested by Analysis of Variance (ANOVA) according to Tukey's HSD ($P<0.05$). Levene's test ($P<0.05$) was used to test the homogeneity of variances.

RESULTS AND DISCUSSION

Working capacity

Sorting and packing phases required more time (52.2%) than that necessary for fruit picking and basket filling (35.8%). On the whole, the main operations (fruit picking, basket filling, sorting and packaging) required 88% of the total working time. The remaining 12% was equally distributed between the complementary processes: vehicle relocation (3.3%), packaging transfer (4.2%), other times (4.4%).

A mean working capacity of 87.8 kg×h^{-1} (396.5 fruits×h^{-1}) and 82.1 kg×h^{-1} (393.2 fruits×h^{-1}) was observed for Rome Star and Sweet Red cultivars, respectively.

Quality parameters

Average values, standard deviations, min and max values calculated for the mass (g) and diameter (mm) of the sampled fruits together with the percentage of fruits belonging to each class are summarized in Table 1. Most packed fruits were in size class AA (54% for "Rome Star" and 47% for "Sweet Red"), while classes A (17%) and AAA (9%) were the least represented for Rome Star and Sweet Red cultivars respectively. The correspondence between the effective diameter of packed fruit and that indicated on the packaging is shown in Table 2. For the size classes A and AA, about 10% of fruits differed in size from that indicated on the packaging, apart from "Rome Star" class A (19%). For size class AAA, the correspondence between the declared and effective classification was very low: 52% of "Rome Star" and 56% of "Sweet Red" fruits were erroneously packed. The pickers were inclined to overestimate the fruit size and wrongly pack more than 50% of fruits in class AAA when in fact they corresponded to class AA. In size class AAA about 52% of fruits of both cultivars had a smaller diameter (3 mm less) than that indicated. However, according to the European Regulation (1221/2008/EC), a maximum of 10% of fruits with a diameter less than that declared meets the criteria of tolerance. Analyzing the fruit size distribution within the same cultivar, picker and size class (Table 3), no significant dif-

Table 1 - Peach cultivar size attributes (mass and diameter) and percentage of fruits in each box size class.

Cultivar	Size class	Mass (g)				Diameter (mm)				N° of fruits (%)
		X	SD	Min	Max	X	SD	Min	Max	
Rome Star	A	180	16	154	240	71	2	66	79	17
	AA	217	15	190	254	76	2	68	80	54
	AAA	261	17	232	312	80	2	77	86	29
Sweet Red	A	182	13	154	206	69	2	66	72	47
	AA	225	13	196	250	75	2	72	78	44
	AAA	276	20	240	330	80	2	75	87	9

Table 2 - Percentage of fruits corresponding to the size class declared on the packaging.

Declared size	Cultivar	A			AA			AAA		
		Picker 1	Picker 2	Total	Picker 1	Picker 2	Total	Picker 1	Picker 2	Total
Corresponding	Rome Star	75	88	81	100	83	92	50	46	48
	Sweet Red	88	92	90	96	83	90	33	54	44
Overestimated	Rome Star	8	4	6	0	17	8	50	54	52
	Sweet Red	13	8	10	4	17	10	67	46	56
Underestimated	Rome Star	17	8	13	0	0	0	0	0	0
	Sweet Red	0	0	0	0	0	0	0	0	0

Table 3 - Fruit diameter (mm) by picker within different size classes.

Cultivar	Picker	A				AA				AAA			
		X	SD	Min	Max	X	SD	Min	Max	X	SD	Min	Max
Rome Star	1	71.4 a	2.6	66.8	78.8	76.0 a	1.5	73.5	78.7	80.4 a	2.0	77.7	85.7
	2	69.9 b	1.9	66.1	74.3	75.5 a	2.7	68.3	80.0	80.2 a	2.0	77.0	83.5
Sweet Red	1	69.2 a	1.5	66.0	71.5	74.3 a	1.3	72.5	77.0	79.6 a	2.2	74.5	84.0
	2	69.2 a	1.8	65.5	72.0	74.9 a	1.9	71.5	78.0	80.0 a	2.7	75.5	87.0

Different letters indicate significant differences within the same cultivar and size class according to Tukey's HSD, P<0.05.

Table 4 - Flesh firmness (N) within different size classes.

Cultivar	A				AA				AAA			
	X	SD	Min	Max	X	SD	Min	Max	X	SD	Min	Max
Rome Star	48.5 a	18.3	6.0	78.7	51.1 a	19.0	8.5	80.8	44.8 a	16.5	11.3	65.8
Sweet Red	43.7 a	18.3	8.3	71.8	34.0 b	14.2	4.8	51.3	18.1 b	11.0	4.5	40.6

Different letters indicate significant differences between cultivars according to Tukey's HSD, P<0.05.

Table 5 - Soluble solids concentration (%) within different size classes.

Cultivar	A				AA				AAA			
	X	SD	Min	Max	X	SD	Min	Max	X	SD	Min	Max
Rome Star	11.6 a	1.4	9.1	14.7	11.4 a	1.1	9.1	13.4	12.4 a	1.1	9.6	14.2
Sweet Red	13.3 b	1.0	11.4	14.9	13.6 b	0.8	12.1	15.2	14.0 b	0.8	12.4	15.5

Different letters indicate significant differences between cultivars according to Tukey's HSD, P<0.05.

Table 6 - Percentage of fruits within flesh firmness classes.

Cultivar	Size class	Flesh firmness classes (N)				
		0-14.7	14.7-34.3	34.3-53.9	53.9-73.6	>73.6
Rome Star	A	8	8	46	33	4
	AA	8	8	29	46	8
	AAA	4	21	38	38	0
	Total	7	13	38	39	4
Sweet Red	A	13	13	42	33	0
	AA	17	25	58	0	0
	AAA	50	38	13	0	0
	Total	26	25	38	11	0

ferences were observed apart from "Rome Star" class A. In this case, picker no.1 selected fruits with a significantly larger diameter.

Average values of fruit flesh firmness and soluble solids concentration are given in Tables 4 and 5. In general, "Rome Star" packed fruits showed significantly higher mean values in terms of flesh firmness (N) than the Sweet Red cultivar apart from class A. On the contrary, in terms of SSC (%), highest mean values were observed for "Sweet Red" packed fruits. For flesh firmness the range of variation appeared especially wide for class A (7.6-78.7 N for "Rome Star" and 8.3-71.8 N for "Sweet Red"). A lower range of variation was measured for class AAA (15.5 - 65.8 N for "Rome Star" and 6.4 - 40.6 N for "Sweet Red"). A wide range of variation was also measured for the soluble solids concentration, especially for "Rome Star" class A (9.1-14.7).

The percentages of fruits within flesh firmness and soluble solids concentration classes are shown in Tables 6 and 7 respectively. For the flesh firmness 13% of "Rome Star" and 25% of "Sweet Red" fruits showed values corresponding to the recommended range (14.7-34.3 N). Most "Rome Star" packed fruits showed flesh firmness in the 34.3-53.9 N and 53.9-73.6 N classes and 7% of the packed product was overripe.

Table 7 - Percentage of fruits within soluble solids concentration classes.

Cultivar	Size class	Soluble solids concentration classes (%)				
		<10	10-11	11-12	12-13	>13
Rome Star	A	13	25	29	17	17
	AA	5	40	35	10	10
	AAA	4	8	25	38	25
	Total	7	24	30	21	17
Sweet Red	A	0	0	17	25	58
	AA	0	0	0	17	83
	AAA	0	0	0	8	92
	Total	0	0	6	17	78

For "Sweet Red" fruits, the 34.3-53.9 N class was over represented (38%) while 26% of the product was overripe.

For the soluble solids concentration, about 68% of the "Rome Star" cultivar fruits showed values higher than 11% and overall, the data showed a higher level of SSC related to the fruits of larger diameter. In fact, about 87.5% of the fruits in class AAA had an SSC higher than 11%. The percentage of fruits (7%) with values of SSC lower than 10% is in any case not negligible. For Sweet Red cultivar, no fruits had an SSC lower than 11% (class A) and 12% (classes AA and AAA) while 80% of fruit in classes AA and AAA had an SSC>13%.

Significant differences emerged between mean values calculated for the two pickers in terms of flesh firmness and SSC, apart from the flesh firmness for Sweet Red cultivar (Table 8).

CONCLUSIONS

The retail trade is paying increased attention to providing fruits of high quality to satisfy the consumer demand, especially in terms of a higher level of ripeness that is the primary criterion for the selection of fresh fruits by consumers (NICHOLS, 1993). The proposed system of field packing allows fruit handling and the time between harvesting and consumption to be reduced and is thus suitable for highly perishable tree-ripened fruits.

The average harvesting rates of 76.2 and 88.6 $kg \times h^{-1}$ per picker are comparable to those reported in literature for fruit harvest-aid (REID, 1976; VANNINI, 1999).

With regard to the ability of the pickers to correctly identify the fruit size and place them in clamshell boxes with the right diameter class, the results are not satisfactory and the correspondence between the declared and effective classification decreased passing from the first two classes (A and AA) to the third (AAA). In fact the pickers, even if skilled, were inclined to overestimate the fruit size, assigning a high percentage (> 50%) to the larger size class AAA, even if the mistake involved mainly fruits (52%) that were only 1 mm smaller than the minimum diameter of the class.

In terms of flesh firmness, harvested fruits did not show the required maturity characteristics. In fact, only 12% of "Rome Star" and 25% of "Sweet Red" packed fruits can be considered suitable for distribution as "freshly picked fruit". This is related to the fact that the maturity stage was assessed by a visual observation of the fruit color. These operations are undoubtedly subjective and depend on the experience and sensitivity of the picker (SLAUGHTER *et al.*, 2006). Furthermore, during the sorting process, the pickers also have to check for a wide range of potential defects making this a challenging inspection task (STUDMAN, 1998).

Since the evaluation of fruit ripeness based on skin background color has proved to be inadequate the field packing system should include rapid and non-destructive methods on the harvesting platform for an objective assessment of size and quality parameters (VALERO *et al.*, 2007). Examples of well-known techniques are those based on computer vision for the fruit size (LI *et al.*, 2011; MOREDA *et al.*, 2009) and VIS-NIR (visible-near infrared) spectroscopy for the internal quality parameters (BERARDINELLI *et al.*, 2010; SLAUGHTER, 1995; ZERBINI *et al.*, 2006; ZIOSI *et al.*, 2008).

Given that the manual sorting required a lot of time (23% of the total), the application on the harvesting platform of these evaluation systems of fruit size and ripeness could reduce sorting times and, at the same time, improve the quality of the packed product. In any case the field packing system has been shown to be lacking an expert crew leader to continuously monitor the orchard, de-

Table 8 - Average values of fruit flesh firmness and soluble solids concentration for the two pickers.

Cultivar	Picker	Flesh firmness (N)				Soluble solids concentration (%)			
		X	SD	Min	Max	X	SD	Min	Max
Rome Star	1	42.6 a	16.3	7.8	69.6	11.5 a	1.1	9.1	14.2
	2	53.7 b	18.2	5.9	80.4	12.1 b	1.4	9.1	14.7
Sweet Red	1	30.0 a	16.0	6.5	54.5	14.0 a	0.78	12.1	15.5
	2	33.8 a	20.0	4.5	71.8	13.3 b	0.98	11.4	15.2

Different letters indicate significant differences between pickers and within the same cultivar and parameter according to Tukey's HSD, P<0.05.

termine the optimum harvest maturity stage and oversee the sorting and packing by the field workers. The expert should be considered essential and integral to the field packing process (CRISOSTO and VALERO, 2008) and should be a skilled technician with a decision-making role who could be provided by the growers cooperative that acquires the fruits. So, with the integration of the proposed automated measurement systems, the supervision of an expert and eventually a penalty on the price paid to the producers who do not meet the quality standards, the system could be a good solution for field packing freshly picked peach fruits. Finally, given that the system could convey part of the valued added directly to the growers, the higher costs incurred for the improved sorting process may be balanced by increased revenue compared to that from traditional fruits.

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PAPER

VARIATIONS IN THE VOLATILE FRACTION OF BITTO CHEESE PRODUCED DURING HERD TRANSHUMANCE

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ABSTRACT

The volatile organic compounds (VOCs) in Bitto cheese were identified using dynamic headspace extraction and GC-MS techniques to ascertain the variability of VOC patterns in cheeses manufactured during transhumance in 2010 and 2011 (n=10 per year). Thirty-eight of the fifty-four VOCs identified in Bitto cheeses that had been ripened for 70 days were considered, and the most abundant identified compounds were 2-butanol, ethanol, 2-butanone, and butanoic acid. According to ANOVA, only ten compounds differentiated the cheeses according to their production year, thus accounting for a slight variability in the profile of the VOCs in Bitto cheeses produced under the same animal-milk-cheese chain.

- Keywords: Bitto, dynamic headspace gas chromatography-mass spectrometry, herd transhumance, volatile organic compounds, raw milk cheese -

INTRODUCTION

Bitto cheese is one of the most well-known Italian mountain cheeses, which has Protected Designation of Origin (PDO) status under the EU commission (COMMISSION REGULATION, 1996). Bitto cheese is manufactured in the Alpine area of Valtellina using raw whole milk from cows who graze pastures during summer transhumance, namely from June 1st until September 30th. After milk coagulation with calf rennet, the curd is cut to the size of rice grains, heated up to 48°-52°C and transferred from the vat into moulds that give the cheese its characteristic concave shape. After salting, the cheese undergoes a ripening period, which begins in rural small huts near the pasture and is completed in cellars in the valley for at least 70 days. As of 2012, approximately 70 factories manufactured Bitto cheese, resulting in an average production of 20000 wheels per year (CLAL ITALIAN DAIRY ECONOMIC CONSULTING, 2012).

Some mountain cheeses are not easily identifiable by their chemical characteristics. Indeed, the cumulated effects of herd management, cheese making practices and environmental conditions contribute to the variability of the characteristics of mountain cheeses produced from raw milk. This variability is somehow perceived as an added value for mountain cheeses. Nonetheless, the lack of reference compositional data concerning specific minor components, which can be peculiar to these cheeses, makes these products more difficult to be analytically differentiated from non-mountain cheeses. Volatile organic compounds (VOCs) in cheese are affected by both the cow's feeding and metabolic reactions promoted by microbiota present in the milk and in the environment where the cheese making and ripening took place (COLLINS *et al.*, 2003; SINGH *et al.*, 2003). For this reason, VOC patterns have been studied in mountain cheeses, most often with the aim to identify compounds that can be used for tracing the production area and manufacturing process, in addition to describing the cheese aroma (BERARD *et al.*, 2007; FAVARO *et al.*, 2005; INNOCENTE *et al.*, 2013). For these purposes, gas chromatography-mass spectrometry (GC-MS) is the method of choice for the analysing VOCs, in combination with different extraction techniques, such as simultaneous distillation extraction (SDE), static headspace (SHS), dynamic headspace (DHS) and purge and trap (P&T), and solid-phase micro-extraction (SPME) (MUSSINAN and MORELLO, 1998).

Little is known regarding the factors that influence the pattern of VOCs in Bitto PDO cheese. PANSERI *et al.* (2008) studied the profile of VOCs in commercial Bitto cheeses produced by different manufacturers, but no relationship was established with regards to transhumance. In a subsequent work, PANSERI *et al.* (2009) proposed only two VOCs in Bitto cheese, namely α-pinene and γ-terpinene, as tracers of the plant-animal-milk-cheese chain. POVOLO *et al.* (2011) analysed the volatile fractions of 39 samples of Bitto cheese (ripened for 70-85 d), but only with regards to comparing the patterns obtained by SPME-GC-MS and an Evolved Gas Analysis FT-IR. To our knowledge, no data are available concerning the variability of the profiles of VOCs in Bitto cheese produced from milk collected from the same herd during transhumance, least of all during successive years. In a previous work (DE NONI and BATTELLI, 2008), we studied the evolution of both fatty acid and terpenoid profiles in milk collected during summer transhumance of a herd, but only three samples of Bitto cheeses were analysed. In the present work, DHS-GC-MS was employed for studying the profile of VOCs in Bitto cheeses obtained from the same herd-milk-cheese chain during the entire summer transhumance periods of 2010 and 2011.

MATERIALS AND METHODS

Transhumance scheme

Thirty-two multiparous cows yielding 11-14 kg d^{-1} milk were part of the transhumance carried out from June 1st to September 30th, both in 2010 and 2011. During this period, transhumance developed in three different mountain locations, namely L1, L2 and L3, situated in the Bitto production area at 1400, 2100, and 2200 m altitude, respectively. In both years, the cows were allowed to feed in a natural pasture at each location for approximately 15-30 d and were moved to their subsequent locations for five consecutive periods (P1-P5) as detailed in Table 1. The grazing periods considered in the two years were not exactly the same because the cows were moved to different pastures according to the phenological development of the herbs.

Cheese manufacture

After a 10-day adaptation period, the milk was transformed by the same cheesemaker following the same procedure and according to specifications provided for Bitto cheese manufacturing, as briefly described by DE NONI and BATTELLI (2008). Two cheeses per period/location were produced on the same day and were then ripened in the same cellar (10°-15°C, 75-85% relative humidity) for 70 days. Overall, 20 cheeses were sampled for this study.

Cheese sampling, fat extraction and analysis of VOCs

For each period/location trial, two representative wedges (approximately 700 g) from oppo-

Table 1 - Timetable of transhumances.

	Period		Pasture Location (altitude, m a.s.l.)
	2010	*2011*	
P1	June 13-July 9	June 18-July 14	L1 (1400)
P2	July 10-August 11	July 15-August 15	L2 (2100)
P3	August 12-27	August 16-September 4	L3 (2200)
P4	August 28-September 27	September 5-26	L2 (2100)
P5	September 28-October 29	September 27-October 29	L1 (1400)

site sides of the two Bitto wheel were taken. After rind removal (10 mm), the wedges were combined and ground. Forty grams of the grated cheese was transferred into a 50 mL polycarbonate tube, and the fat was extracted as previously described by DE NONI and BATTELLI (2008). The VOCs were extracted from the fat by DHS extraction and then separated and identified by GC-MS according to DE NONI and BATTELLI (2008). The VOCs were identified by comparing retention times and spectra with those of pure standards (Sigma Aldrich, Milan, Italy) submitted to the same analytical conditions. Semi-quantitative evaluation of VOCs was performed by integrating the peak area of the characteristic ion (Q_{ion}) using the MS-Chemstation software (Agilent Technologies) and by expressing data in arbitrary units.

Data analysis

The dependent variables (abundances of the individual VOCs) were processed by analysis of variance (two-way ANOVA) using Minitab® software (Release 14, 2004; State College, PA, USA) and considering the year as the source of variation. The same package was used to perform the statistical analysis using Principal Component Analysis (PCA).

RESULTS AND CONCLUSIONS

The goal of this study was to ascertain the overall effect of a real transhumance scheme on Bitto VOCs while minimising variation by considering Bitto cheeses obtained from raw milk of the same herd, manufactured by the same cheesemaker, and ripened in the same cellar. Indeed, the work was not addressed to discriminate or to hierarchically order factors affecting profile of the VOCs of this mountain cheese made from raw milk.

Fifty-four VOCs were desorbed and identified by DHS-GC-MS, but only 38 compounds were considered in this study, including 2 aldehydes, 6 ketones, 9 alcohols, 8 acids, 4 esters, and 9 terpenes, with the other compounds present only at trace levels. The aromatic hydrocarbons mono- and dimethyl benzene were ignored because they are possible cheese contaminants (BOSSET *et al.*, 2000).

All the samples of Bitto were rich in alcohols, acids, and ketones (Table 2). This feature is common to other raw milk cheese varieties (BERARD *et al.*, 2007; INNOCENTE *et al.*, 2013) manufactured without the usage of starter cultures (BUCHIN *et al.*, 1998; ORTIGOSA *et al.*, 2001). Four major compounds, namely 2-butanol, ethanol, 2-butanone, and butanoic acid, dominated the overall pattern of VOCs in Bitto cheese. The VOC 2-butanone derives from the metabolism of citrate via 2,3-butanedione and 3-hydroxy-2-butanone (MCSWEENEY and SOUSA, 2000). As the cheese ripens, 2-butanone is continuously formed and reduced to 2-butanol. The progress of this pathway during maturation depends on the evolution of the microbial populations and the cheese environment, both of which can vary significantly from one cheese type to another. The intermediate ketone, 2,3-butanedione, was found to accumulate in Montasio cheese throughout ripening (INNOCENTE *et al.*, 2013), whereas 2-butanone accounted for approximately 30% of the total VOCs in the headspace of Cantal (ripened for 120 d), which is a French PDO cheese made from raw milk in the mountainous Auvergne region (DE FREITAS *et al.*, 2007).

Ethanol was also a dominating VOC in the profile of all Bitto samples considered in our work. This alcohol typically derives from the lactate metabolism pathway operated by non-starter lactic acid bacteria (NSLAB) (COLOMBO *et al.*, 2009; MORANDI *et al.*, 2011). Ethanol gives rise to the formation of several ethyl esters as a result of its esterification with short- and medium-chain fatty acids as promoted by microbial esterase (BUCHIN *et al.*, 1998; ORTIGOSA *et al.*, 2001). These ethyl esters in turn contribute to the flavour of cheese (MCSWEENEY, 2004). Indeed, esters of ethanoic, hexanoic, and octanoic acids were present in the volatile fraction of Bitto cheese, with ethyl hexanoate being the most abundant.

Butanoic acid was another compound prevailing in the headspace of these samples. In our study, its presence can be assumed as originating mainly from lipolysis because its ratio to the level of hexanoic acid is close to that in samples of raw milk fat (2:1).

Despite the different technique used by POVOLO *et al.* (2011) for extracting VOCs from cheese, they found the same four compounds (2-butanol, ethanol, 2-butanone, and butanoic acid) dominated the profile of VOCs in Bitto cheese. Overall, the relevance of the different classes of compounds was rather close to what was found in this study, and the differences may be attributed mainly to differences in the analytical techniques utilised. PANSERI *et al.* (2008) detected up to 84 VOCs in ripened Bitto cheeses (n=14). The main VOCs were 2-heptanone, 2-nonanone, ethyl acetate, ethyl butanoate, butanoic acid, and 2-butanol and, in some cases, large differences were found among cheeses obtained from different farms. However, the same authors recently reported a less complex pattern, i.e., among fifty-one VOCs, only 3 alcohols were identified, and 2-butanol was not detected (PANSERI *et al.*, 2009).

ANOVA was applied to all individual VOCs and showed differences ($P<0.05$) among the cheeses produced in different locations/periods only with respect to acetone, 2-methyl-1-propanol, 2-heptanol, butanoic acid, and δ-3-carene (Table 2).

On the contrary, the largest differences were found between cheeses produced in the two different years. In fact, n-pentanal ($P<0.001$), propanoic acid ($P<0.001$), and 2-methyl-1-propanol ($P<0.01$), significantly differentiated the cheeses produced in the two different years (Table 2). Seven other compounds, including ketones, alcohols, acids, and esters were also distinctive, but at a lower significance level ($P<0.05$). The aldehydes, namely n-pentanal ($P<0.001$) and n-hexanal ($P<0.05$), showed higher levels in cheeses produced in the first year than the second year (Table 2). Straight chain aldehydes resulting from the reduction of unsaturated fatty acids (SINGH *et al.*, 2003) are unstable and their amount in cheese depends on how rapidly they are either reduced to alcohols or oxidised to acids (DE FREITAS *et al.*, 2007). Those compounds were also found in Bitto cheese by PANSERI *et al.* (2008) but not by POVOLO *et al.* (2011). As already stated, these differences can be partially explained by the difference in the analytical techniques used by these authors.

Among the ketones, only the level of 3-hydroxy-2-butanone differed significantly ($P<0.05$) between cheeses produced in the two consecutive years (Table 2). Indeed, higher levels of this ketone were recorded in the cheeses produced in 2011 compared to 2010. PANSERI *et al.* (2008) found only one out of 14 Bitto samples contain 3-hydroxy-2-butanone, whereas this compound was not detected in the nine samples considered in their subsequent work (PANSERI *et al.*, 2009). The content of 2-butanol was significantly higher ($P<0.05$) in cheeses produced in the second year (Table 2). Similarly, the level of the secondary alcohol 2-heptanol significantly ($P<0.05$) differentiated the cheeses according to their production year.

As shown in Table 2, VOCs in Bitto cheese also included short chain fatty acids, such as butanoic, ethanoic, propanoic, and hexanoic acids, the level of which correlated with the activity of NSLAB in artisanal cheeses made from raw milk (MCSWEENEY, 2004).

Alpha-pinene, b-pinene, and Δ-3-carene were the most abundant terpenes found in Bitto (Table 2), confirming the findings of our previous study (DE NONI and BATTELLI, 2008). High levels of α-pinene proved to be a characteristic feature of other mountain cheeses made with raw milk, including "Fontina Valle d'Aosta" (BERARD *et al.*, 2007), Cantal (DE FREITAS *et al.*, 2007), and Ossolano (ZEPPA *et al.*, 2002). Based on the ANOVA, we found that none of the detected terpenes differentiated the cheese samples, neither with respect to the production year nor with respect to the various period/locations. The level of Δ-3-carene however was higher ($P>0.05$) in cheese produced with milk from cows grazing the highest elevation pastures (P3L3), without regard to the production year. This terpene represents one of the most abundant terpenes in Alpine Lovage (*Ligusticum mutellina*), which is a plant that grows in alpine pastures above 2000 m altitude (WILLEMS *et al.*, 2013; DE NONI and BATTELLI, 2008).

Overall, the differences discussed likely reflect perturbation in the microbial metabolism dependent on year-to-year variations in environmental conditions, including pasture vegetation, which are always higher in mountain locations with respect to those in lowlands. This source of variation is often disregarded in studies aimed at characterising the composition of VOCs in mountain cheeses and hence the samples usually considered are those produced in the same year in different farms. Nevertheless, this effect did not strongly affect the overall profile of the VOCs for the cheeses studied herein.

Further explanation of the data set for the VOCs was obtained using PCA, which combines all of the VOCs identified in the cheese from different periods and locations. The high variability of the data set accounts for a low percentage (20%) of the total variance explained by the first two components. Nevertheless, the cheese samples were located on the opposite sides of the plot with respect to the first component, namely the year of manufacturing (Fig. 1). The coefficients of the individual VOCs showed that, in addition to the molecules evidenced by the ANOVA, other alcohols (1-butanol, 1-pentanol, 3-methyl-1-butanol, and 3-methyl-2-buten-1-ol), ketones (2-butanone, 2-heptanone, and 2-nonanone) and ethyl lactate contributed, either positively or negatively, to the different positioning of the samples. These results strengthened the importance of considering the year-to-year variability, especially for studies regarding mountain cheeses produced during transhumance.

Additional differences were observed among the cheeses produced in 2010, particularly between samples from P3L3-10 and P2L2-10, both pro-

Table 2 - Effect of transhumance on VOCs composition of Bitto cheese samples. Data expressed as arbitrary units (means of triplicate experiments) of the peak area of Q_{ion} (in brackets).

Location[a]	L1		L2		L3		L2		L1		
Period[b]	P1-10	P1-11	P2-10	P2-11	P3-10	P3-11	P4-10	P4-11	P5-10	P5-11	Year-to-year difference[c]
aldehydes											
n-pentanal (44)	35	7	28	8	21	6	32	9	30	6	***
n-hexanal (56)	3	1	3	2	2	1	3	2	5	2	*
ketones											
acetone (58)	180^{A}	169^{A}	171^{A}	185^{A}	140^{AB}	163^{AB}	112^{B}	107^{B}	131^{B}	119^{B}	ns
2-butanone (72)	1105	802	803	831	961	678	824	729	1081	654	ns
2-pentanone (86)	23	16	28	43	42	27	24	21	39	29	ns
3-hydroxy-2-butanone (45)	25	45	31	43	35	37	33	49	46	57	*
2-heptanone (43)	77	112	62	142	86	115	70	146	92	87	ns
2-nonanone (58)	13	35	15	48	28	32	22	42	32	25	ns
alcohols											
ethanol (46)	2123	703	1870	682	1300	755	1580	788	991	801	*
2-methyl-1-propanol (43)	51^{AB}	35^{AB}	42^{AB}	38^{AB}	40^{AB}	21^{A}	55^{AB}	29^{AB}	63^{B}	44^{AB}	**
2-butanol (45)	5499	8994	6700	8524	5466	6951	5442	9647	8605	9256	*
1-butanol(56)	15	26	19	33	29	25	8	35	27	31	ns
3-methyl-2-butanol (45)	76	68	91	125	130	79	72	93	100	137	ns
3-methyl-1-butanol (55)	201	198	174	235	165	218	169	257	198	205	ns
3-methyl-2-buten-1-ol (71)	5	2	4	2	3	3	3	3	4	2	ns
1-pentanol (42)	7	24	4	19	5	8	6	7	2	5	ns
2-heptanol (45)	30^{A}	19^{A}	53^{A}	18^{A}	71^{B}	63^{B}	37^{A}	20^{A}	36^{A}	19^{A}	*
acids											
ethanoic acid (43)	430	261	345	248	312	354	415	257	452	362	ns
propanoic acid (74)	281	54	342	45	300	40	212	77	296	62	***
2-methyl propanoic acid (43)	29	55	39	37	35	32	48	44	31	33	ns
butanoic acid (60)	900^{B}	786^{AB}	798^{AB}	835^{AB}	654^{A}	645^{A}	893^{B}	884^{B}	702^{AB}	805^{AB}	ns
3-methyl butanoic acid (60)	117	159	172	137	157	129	128	173	106	149	ns
pentanoic acid (60)	11	7	9	8	8	8	6	8	8	11	ns
hexanoic acid (60)	319	245	241	316	268	282	246	246	262	289	ns
octanoic acid (60)	32	26	35	22	37	31	33	18	38	12	*
esters											
ethyl ethanoate (61)	50	22	37	33	11	41	41	49	38	39	ns
ethyl hexanoate (88)	91	85	69	71	72	128	115	106	126	118	ns
ethyl octanoate (88)	18	10	17	15	22	8	25	20	36	20	*
ethyl lactate (45)	19	2	17	5	8	8	17	5	6	6	ns
terpenes											
β-pinene (93)	22	109	339	14	331	168	150	143	22	94	ns
camphene (93)	1	1	18	1	12	11	11	12	2	2	ns
β-pinene (93)	14	17	241	19	79	63	75	69	2	43	ns
Δ3-carene (93)	1^{A}	4^{A}	30^{AB}	45^{AB}	100^{B}	215^{B}	39^{AB}	27^{AB}	6^{A}	1^{A}	ns
β-myrcene (93)	3	2	10	9	38	12	1	3	1	4	ns
limonene (93)	3	9	20	3	65	21	4	9	1	11	ns
1,8 cineol (93)	15	16	19	22	68	22	25	4	8	4	ns
p-cimene (119)	3	2	44	2	13	5	2	3	3	7	ns
allo-ocimene (121)	14	4	19	8	16	8	5	5	1	4	ns

The following abbreviations are used: a.s.l., above sea level.
[a]L1: 1400 m a.s.l.; L2: 2100 m a.s.l.; L3: 2200 m a.s.l.
[b]P1-10: June 13-July 9 2010; P1-11: June 18-July 14, 2011; P2-10: July 10-August 11, 2010; P2-11: July 15-August 15, 2011; P3-10: August 12-27, 2010; P3-11: August 16-September 4, 2011; P4-10: August 28-September 27, 2010; P4-11: September 5-26, 2011; P5-10: September 28-October 29, 2010; P5-11: September 27-October 29, 2011.
[c]Level of significance: ***, P< 0.001; **, P< 0.01; *P<0.05; ns = not significant.
[A-B]Values in the same row with different superscripts differ (P<0.05).

duced from milk obtained at the highest altitudes in the mid-summer, and cheeses produced either at the beginning (P1L1-10) or at the end (P4L2-10 and P5L1-10) of the transhumance period. These differences are mainly due with respect to the level of terpenes, proving this to be the VOC that correlated most with the altitude of the pasture from which the sample originated from (TORNAMBÉ *et al.*, 2006). In contrast, all cheeses produced in 2011 fell very close to each other, with the exception of P3L3-11, which was separated from the other cheeses by its terpenes content, confirming those compounds as markers for the altitude associated with the sample. The overall picture outlined by PCA led to the hypothesis that the different quality of the pastures of the two years could have mainly affected the pattern of the VOCs in the corresponding Bitto cheeses.

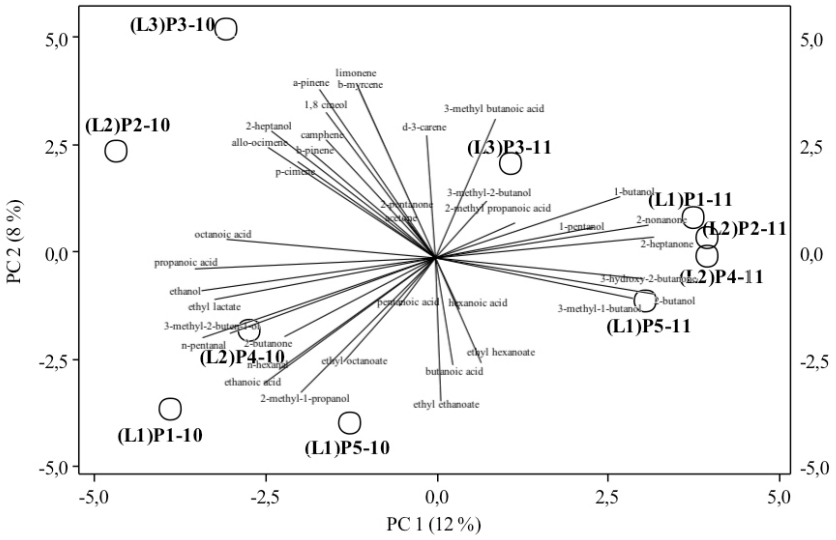


Fig. 1 - Biplot of the PCA of Bitto cheese samples for each variable (individual VOC) and the principal component scores for each sample (in bold). Letters refer to period/location and year of transhumance, respectively. L1: 1400 m a.s.l.; L2: 2100 m a.s.l.; L3: 2200 m a.s.l.. P1-10: June 13-July 9, 2010; P1-11: June 18-July 14, 2011; P2-10: July 10-August 11, 2010; P2-11: July 15-August 15, 2011; P3-10: August 12-27, 2010; P3-11: August 16-September 4, 2011; P4-10: August 28-September 27, 2010; P4-11: September 5-26, 2011; P5-10: September 28-October 29, 2010; P5-11: September 27-October 29, 2011.

For the first time, the variability in the profile of VOCs in Bitto cheese was assessed on samples produced during the entire transhumance. Data showed that few VOCs change in the cheese according to the period/location and the production year of transhumance. These changes are innate in artisanal mountain cheeses, but appear negligible if compared to variations in the profile of the VOCs previously reported for Bitto cheese not produced under the same animal-milk-cheese chain. It was revealed that the profile of VOCs in Bitto cheese is determined by specific traits relating to the mountain terroir, thus bringing additional insight into chemical characterisation of this Italian mountain cheese.

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PAPER

CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF PROTEIN IN HULLED DWARF OAT LINES AND THE EFFECT ON SERUM LIPID PROFILE IN RATS

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ABSTRACT

The aim of this study was to determine the chemical composition and protein quality in hulled oats (6 dwarf lines with the *Dw6* dwarf gene), and their hypocholesterolemic effects compared to the traditional hulled tall cultivar. The average levels of crude protein, crude fat and minerals were significantly greater and crude fiber level was lower in dwarf lines than in the traditional Krezus cultivar. The fatty acid profile, protein nutritive value and the effect on the level of lipids in the serum of rats of the grains were similar for dwarf and standard oats. The results of this study show that the grain of hulled dwarf oat may be a valuable component to the human diet.

- Keywords: dwarf oat, protein quality, fatty acids, cholesterol, β-glucan, rats -

INTRODUCTION

Oat (*Avena sativa* L.) has long been recognized as a natural ingredient of functional foods because they provide dietary fiber, protein, unsaturated lipids, vitamins, mineral components and antioxidants required for human health. Food can be regarded as functional if it has beneficial effects on target functions in the body, as a source of physical and mental well-being, contributing to the prevention and reduction of risk factors for several diseases, or enhancing certain physiological functions beyond adequate nutritional effects (JONES, 2002).

Hulled oat grains contain approximately 12% crude protein (MUSTAFA *et al.*, 1998). The amino acid composition of oat is superior to that of other cereals because its major storage protein is a globulin (KLOSE and ARENDT, 2012). Globulins have a higher concentration of lysine and other essential amino acids than do typical cereals storage proteins, prolamins.

Interest in increasing oat utilization for human consumption has been stimulated by the need for high soluble fiber in diet. The β-glucans are the primary components of oat soluble fiber responsible for its noted physiological effects. Some authors report that β-glucans viscosity in the GI tract is the most probable mechanism in which it decreases serum cholesterol levels, as well as improving postprandial glucose metabolism (WELCH, 1995; WOOD, 2007). Hulled oat grain contains 3-5% dry matter β-glucans (ZUTE *et al.*, 2011) and more lipids than other cereals.

Hulled oat grain contains more lipids than other cereals, ranging from 4.4-9.6% (PETERSON, 1998). Most oat fatty acids are unsaturated. The five fatty acids determined in various studies in oat grains [palmitic, C16:0 (range 13-26%), stearic, C18:0 (1-3%), oleic, C18:1 (22-47%), linoleic, C18:2 (25-52%), and linolenic, C18:3 (1-3%)], and together account for more than 95% of total fatty acids (ZHOU *et al.*, 1999).

Oat is a good source of antioxidants, phytic acid and various phenolic compounds (CHATURVEDI *et al.*, 2011). The polyphenols of oat exhibit anti-inflammatory, antiproliferative, and anti-itching activity, which may provide additional protection against coronary heart disease, colon cancer and skin irritation (MOHSEN, 2009).

The introduction of dwarf oat cultivars (*Avena sativa* L.) makes it possible to obtain higher yields of this cereal. Dwarf oat cultivars are less vulnerable to lodging, leading to increased grain quality (MÄKELÄ *et al.*, 2004; TANHUANPÄÄ *et al.*, 2006). The primary effects of the dwarfing genes on growth and yielding are particularly well demonstrated for wheat, while few publications concern the effect of the dwarfing gene *Dw6* on yield and quality of oat grain (MÄKELÄ *et al.*, 1996).

The objectives of this study were therefore to evaluate the chemical composition and nutritive value of proteins in hulled dwarf oat lines and the effect on serum lipid level in rats. Evaluation of these lines was made against a traditional hulled tall cultivar (standard).

MATERIALS AND METHODS

Oat samples were derived from the plot trials carried out in 2008-2010 on sandy clay soil type at the Lipnik Experimental Station (53°12'N; 14°27'E), Poland. The experiment was carried out on a light soil of the IVb botanical class, using 550 grains per m^2 and 90 kg $N \cdot ha^{-1}$. Dwarf hulled oat 6 lines – STH 114, STH 132, STH 5244, STH 6025, STH 6106, STH 6108 (with the *Dw6* dwarf gene introduced from the naked oat Badicoot cv.) were compared with the traditional tall oat cultivar Krezus. The grains were cleaned and rendered free of dust, then stored in tightly closed glass jars at room temperature until used.

The chemical compositions of all samples were determined by the following AOAC (2006) procedures: the dry matter result was found, by drying samples in an oven at 105°C until a constant weight was obtained; ether extract, by Soxhlet extraction with diethyl ether; crude ash, by incineration in a muffle furnace at 580°C for 8 h; crude protein (CP) (N×6.25), by the Kjeldahl method, crude fibre was determined using the Hennenberg-Stohmann method. Total carbohydrate was obtained by difference 100 – sum of moisture, crude protein, fat, ash and crude fibre. A flame photometer (Flapho-4) was used to determine calcium, potassium and sodium. After oven-drying of the samples, phosphorus content was determined by the vanadium-molybdenum colorimetric method (CAVELL, 1955). The fibre components were determined using the detergent method according to VAN SOEST *et al.* (1991) performed with a fibre analyzer ANCOM 220. Determining of the neutral detergent fibre (NDF) was carried out on an ash-free basis, including alpha-amylase and dodecylsulfat natrium salt (Merc 8.22050). Determining of acid detergent fibre (ADF) included hexadecyl-trimethyl-ammonium bromide (Merc 102342), while acid detergent lignin (ADL) was determined by hydrolysis of ADF sample in 72% sulphuric acid. Hemicellulose (HCEL) was calculated as a difference between NDF and ADF, while cellulose (CEL) – as a difference between ADF and ADL value. The content of β-glucans was determined enzymatically according to ICC (1998) using a Megazyme kit K-BGLU (Megazyme International Ireland Ltd., Bray, Ireland, 1998). All determinations were expressed on a dry matter basis.

The free fatty acid analysis was performed by gas chromatography using a VARIAN CP3800 camera. Separation was performed in a capillary column – CPWAX 52 CB (60 m×0.25 mm). The temperature of the injector and FID detec-

tor were 260°C. Helium was used as a carrier gas at a flow rate of 1.4 $cm^3 \cdot min^{-1}$.

Amino acids were determined using an AAA 400 automatic amino acid analyzer (INGOS, Czech Republic). Samples were subjected to acid hydrolysis in the presence of 6 M HCl at 105°C for 24 hours. Sulfur-containing amino acids were determined separately in 6 M HCl after oxidative hydrolysis (formic acid + hydrogen peroxide, 9:1 v/v, 20 h at 4°C). Tryptophan was determined according to the method described in AOAC (2006). The amino acid composition was expressed in g per16 g N.

The amino acids score (AAS) was calculated using the WHO/FAO/UNU (2007) reference pattern. The protein digestibility-corrected amino acid scores (PDCAAS) of the samples were calculated by multiplying the most limiting essential amino acid score by the true protein digestibility (TD). The PDCAAS scores were expressed in percentage terms.

In nitrogen balance studies were determined biological value (BV) and true digestibility (TD) of protein. The experiment was carried out, using 21 day-old Wistar male rats specific pathogen-free (SPF) in accordance with procedures described by EGGUM (1973), after the experimental protocol was approved, by the institutional animal ethics committee. The animals were randomly divided into four groups of ten animals, so that the difference between mean weights did not exceed 2.2 g. The rats were kept in individual stainless steel cages and maintained at 22°±3°C with a 12 h light/dark cycle. During experiment, the animals were fed with diets containing 10% protein prepared according to the method by EGGUM (1973) in dry matter: protein - 10% (I group: from Krezus cv., II group: from STH 5244 line, III group: from casein, IV – non-protein); sucrose – 10%; mineral-vitamins premix – 5.2 %; oil – 5%; cellulose - to about 4% including fiber of feed; starch – up to 100%.

N-balance studies were conducted for 10 days (3 days initial period and 7 days experimental period). Rats were assigned a restricted diet (10 g dry matter and 150 mg N/animal/day). Excreta (feces and urine) were collected quantitatively and pooled for each rat. Fecal samples were frozen and freeze-dried. Urine samples were adjusted to constant volume with 5% sulfuric acid.

After the experiment (14 hours after the last feeding) animals were anesthetized and blood samples were taken by cardiac puncture. The serum was obtained out by centrifuging the clotted blood at 1,400 × g for 10 minutes. The serum was stored in screw capped vials and kept in freezer at –20°C until further analysis.

In serum were determined: triacylglyceroles (TGC), with the enzymatic colorimetric technique of STEIN (1987); high density lipoprotein cholesterol (HDL_C), by elimination, a technique involving a HDL-dissolving detergent which blocks enzymatic activity of esterase and cholesterol oxidase

Table 1 - Chemical composition of hulled oat grains ($g \cdot kg^{-1}$ DM).

Item	Dry matter (g/kg)	Ash	Crude protein (N×6.25)	Crude fat	Crude fibre	Carbohydrates	Calcium	Phosphorus	Potassium	Sodium	Ca:P
Krezus – traditional tall hulled cv.	904.8	24.26	118.8	48.54	111.8	696.6	1.27	3.10	2.58	0.200	1:2.4
STH 114	902.7	28.65	128.1	49.90	83.9	709.5	1.29	3.25	1.72	0.206	1:2.5
STH 132	904.8	27.65	123.5	53.60	95.1	700.2	1.20	3.27	1.90	0.250	1:2.7
STH 5244	905.0	26.80	124.5	50.60	89.1	709.0	1.17	3.24	1.92	0.206	1:2.8
STH 6025	908.8	27.60	121.7	49.95	89.3	711.4	1.28	3.47	1.81	0.211	1:2.7
STH 6106	912.9	25.34	122.4	51.26	93.8	707.2	1.20	3.06	2.10	0.191	1:2.6
STH 6108	914.0	25.05	124.5	51.02	102.3	697.1	1.32	3.07	2.32	0.182	1:2.3
SEM	1.7	0.6	1.1	0.6	3.5	2.4	0.02	0.06	0.1	0.01	
The statistical results:											
$\bar{X}$ tall cultivar	904.8	24.26	118.8	48.54	111.8**	696.6	1.27	3.10	2.58**	0.200	1:2.4
$\bar{X}$ dwarf lines	908.0	26.85*	124.1**	51.06*	92.25	705.73	1.24	3.23	1.96	0.208	1:2.6

$\bar{X}$ means of lines with the Dw6 dwarfing gene. SEM Standard error of the mean. * $p \leq 0.05$; ** $p \leq 0.01$.

towards very low-density lipoprotein cholesterol (VLDL)- and low-density lipoprotein cholesterol (LDLC) (MORRISON, 1997). The analysis of serum was carried using COBAS Integra biochemical analyzer, Roche in a closed system. The analyser automatically calculated the LDL_C content.

All the measurements were made in triplicate. Results were expressed as mean values. The data was subjected to a one-way analysis of variance (ANOVA). The significance of difference between means was determined by Duncan's Multiple Range Test using Statistical computational software (Statistica PL, version 8.0).

RESULTS AND DISCUSSION

The composition of Krezus oat cultivar grains (Tables 1 and 2) in this study was comparable to the composition of hulled oat reported by GIVENS *et al.* (2004). Hulled dwarf oat lines, compared to the traditional tall Krezus cultivar, contained more crude protein ($p \leq 0.01$), ash and crude fat ($p \leq 0.05$), while less ($p \leq 0.01$) crude fiber (Table 1). TOBIASZ-SALACH *et al.* (2011) found a significantly higher content of crude protein and crude fat in some analyzed hulled dwarf oat lines than the traditional Krezus cultivar.

The average content of potassium in the dwarf lines was about 24% lower ($p \leq 0.01$) than in the Krezus cultivar. The content of other analyzed minerals were similar in all the investigated samples. Calcium (Ca) and phosphorus (P) are essential minerals for bones, but a proper proportion between them is also very important due to the close connection between their metabolisms. In this study, oat contained small amounts of Ca (1.24 and 1.27 $g \cdot kg^{-1}$ DM), and the content of P was 2.5-times higher than Ca. The resultant Ca:P ratio in evaluated oat grains (1:2.4-2.6) deviates from the recommended optimum ratio of Ca:P in the diet, at 1.3:1 (DRI, 1997).

Composition of dietary fiber is given in Table 2. Dwarf lines contained less neutral-detergent fiber (NDF) and acid-detergent fiber (ADF) (by 14.5 and 12.5% respectively, $p \leq 0.01$), cellulose (CEL), hemicellulose (HCEL) and acid detergent lignin (ADL) (respectively by: 16.5, 13.5 and 7.0%, $p \leq 0.05$) than the Krezus cultivar. The average content of β-glucans in the dwarf oat lines (29.24 $g \cdot kg^{-1}$ DM) was 6% higher (not significant) than the traditional tall Krezus cultivar. The main components of dietary fiber are non-starch polysaccharides, including cellulose, hemicellulose, β-glucans and pectins.

The oat grains may differ greatly in chemical composition due to genetic and environmental factors. The differences in chemical composition of oat grains of the present study may be attributed primarily to genetic background, since all tested sample oats were grown under the same environmental conditions.

KOEHLER and WIESER (2013) report that oat, as well as barley, has a high concentration of β-glucans (3.5-5% and 3-7%, respectively), whereas other cereals contain less than 2% β-glucans. Plant breeders are developing oat lines with high β-glucan concentrations that are particularly useful for food. For animal feed, however, low β-glucan concentration is desirable, particularly for young poultry.

Particular interest in oat fiber results from its capacity for rapid and significant reduction in total and LDL cholesterol levels (INGLETT and NEWMAN, 1994; TRUSWELL, 2002). These valuable properties are exhibited by β-glucans. In addition, short chain fatty acids (acetic acid, propionic acid and butyric acid) produced by fermentation of fiber in the colon may influence the production of glucose and its utilization by peripheral tissues (JENKINS *et al.*, 1995), and also may limit cholesterol synthesis (BRIDGES *et al.*, 1992). Dietary fiber also has a protective effect against a range of diseases including colorectal cancer and coronary heart disease (THEUWISSEN and MENSINK, 2008).

Table 2 - Components of dietary fibre of hulled oats grain ($g \cdot kg^{-1}$ DM).

Item	NDF	ADF	ADL	HCEL	CEL	ß-glucans
Krezus – traditional tall hulled cv.	326.4	160.6	26.91	133.7	165.8	27.62
STH 114	295.0	147.1	26.04	121.1	147.9	28.10
STH 132	273.6	144.0	26.62	117.4	129.6	27.90
STH 5244	261.8	139.1	24.70	114.4	122.7	30.15
STH 6025	269.9	138.1	24.98	113.1	131.8	28.00
STH 6106	297.8	136.2	24.31	111.9	161.6	30.26
STH 6108	275.5	138.5	23.15	115.4	137.0	31.03
SEM	8.4	3.2	0.5	2.8	6.3	0.5
The statistical results:						
Tall cultivar	326.4**	160.6**	26.91*	133.7*	165.8*	27.62
$\bar{X}$ dwarf lines	278.9	140.5	24.97	115.5	138.4	29.24

$\bar{X}$ means of lines with the Dw6 dwarfing gene. SEM, standard error of the mean. NDF neutral detergent fibre, ADF acid detergent fibre, ADL acid detergent lignin, HCEL hemicellulose, CEL cellulose. * $p \leq 0.05$;** $p \leq 0.01$.

Compared to the traditional Krezus cultivar, dwarf oat fat contained less SFAs (saturated fatty acids) ($p \leq 0.05$), including C16:0 ($p \leq 0.01$) and C18:0 ($p \leq 0.05$), but more C18:3 ($p \leq 0.01$) and slightly more other unsaturated fatty acids (Table 3). Lipids in the tested oat dwarf lines contained 75.5% unsaturated fatty acids (UFAs), compared to 73.3% in the Krezus tall cultivar. One of the main UFAs was oleic acid (37.2 and 36.1%), highly positively correlated with the total fat content (ZHOU *et al.*, 1998). Palmitic acid was the major SFA in the oat fat. A similar composition of the hulled oat fat was noted by GIVENS *et al.* (2004).

Oats are rich in polyunsaturated fatty acids (PUFA), including essential fatty acids in mammalian nutrition, such as linoleic acid (C18: 2, n-6) and linolenic acid (C18: 3, n-3). The fat in the analyzed hulled oat contained 36.93% PUFAs in the dwarf lines and 35.86% in the Krezus cultivar, including approximately 35% linoleic acid (C18: 2, n-6). The high content of these acids makes oat an attractive product for health-related purposes (ARO *et al.*, 2007). For example, linoleic acid decreases total cholesterol and LDL-cholesterol in humans (LUNN and THEOBALD, 2006).

Unsaturated fatty acids of oat are regarded as nutritionally important due to the high content of essential fatty acids and a high level of antioxidants (YOUNGS, 1986). In addition, the antioxidant properties of these compounds contribute to the increased stability and improved sensory properties of food (PETERSON, 2001).

Protein in dwarf lines contained significantly more ($p \leq 0.05$) methionine and threonine (by 7.8 and 7%, respectively), and the total content of other essential amino acids (EAAs) was slightly higher than in the traditional tall cultivar (Table 4). The content of sulfur-containing amino acids in the protein of the analyzed oat lines was high, in the range of 3.32-3.73 g per 16 g N, which makes oat a good complementary protein source to legumes (PED'O *et al.*, 1999).

Table 3 - Fatty acid composition of hulled oats grain (% of total fatty acids).

Item	C14:0	C16:0	C18:0	C20:0	C16:1	C18:1	C20:1	C22:1	C18:2	C18:3	SFA	UFA	MUFA	PUFA
Krezus – traditional tall hulled cv.	0.32	23.88	1.68	0.18	0.22	36.13	0.70	0.41	34.89	0.97	26.06	73.32	37.46	35.86
STH 114	0.39	22.75	1.12	0.19	0.28	37.01	0.73	0.56	35.53	1.25	24.45	75.36	38.58	36.78
STH 132	0.29	23.23	1.29	0.18	0.31	36.30	0.74	0.42	35.79	1.27	24.99	74.83	37.77	37.06
STH 5244	0.35	22.39	1.68	0.25	0.20	37.26	0.68	0.25	34.86	1.39	24.67	74.64	38.39	36.25
STH 6025	0.32	21.22	1.49	0.19	0.27	38.02	0.71	0.42	35.69	1.33	23.22	76.44	39.42	37.02
STH 6106	0.31	22.07	1.39	0.21	0.19	37.35	0.70	0.42	36.00	1.19	23.98	75.85	38.66	37.19
STH 6108	0.32	21.25	1.49	0.18	0.20	37.44	0.69	0.48	36.16	1.14	23.24	76.11	38.81	37.30
SEM	0.01	0.37	0.08	0.01	0.02	0.25	0.01	0.04	0.19	0.05	0.38	0.40	0.25	0.20
The statistical results:														
Tall cultivar	0.32	23.88*	1.68**	0.18	0.22	36.13	0.70	0.41	34.89	0.97	26.06*	73.32	37.46	35.86
$\bar{X}$ dwarf lines	0.33	22.15	1.41	0.20*	0.24	37.23	0.71	0.43	35.67	1.26**	24.09	75.54	38.61	36.93

$\bar{X}$ means of lines with the Dw6 dwarfing gene. SEM standard error of the mean. SFA saturated, UFA unsaturated, MUFA monounsaturated, PUFA polyunsaturated fatty acids. * $p \leq 0.05$;** $p \leq 0.01$.

Table 4 - Essential amino acid composition (g per 16 g N) and amino acid scoring (%) of protein of hulled oats grain.

Item	Lys	Met	Met+Cys	Thr	Ile	Trp	Val	Leu	His	Phe	Phe+Tyr	EAA	AAS_{Lys}
Krezus – traditional tall hulled cv.	3.31	1.29	3.39	3.09	2.77	1.12	4.40	6.78	2.22	4.63	7.02	31,88	57.1
STH 114	3.54	1.37	3.32	3.21	2.99	1.21	4.45	6.90	2.47	4.94	7.17	32.79	61.9
STH 132	3.44	1.31	3.45	3.39	2.86	1.19	4.66	6.95	2.19	4.79	7.23	33.17	59.3
STH 5244	3.45	1.57	3.73	3.27	3.03	1.08	4.61	7.18	2.52	4.99	6.99	33.34	59.5
STH 6025	3.58	1.40	3.38	3.41	2.82	1.30	4.72	6.98	2.31	4.82	7.09	33.28	63.4
STH 6106	3.42	1.31	3.52	3.39	2.88	1.22	4.79	7.02	2.53	4.68	6.99	33.23	59.0
STH 6108	3.37	1.34	3.62	3.29	3.02	1.07	4.98	7.21	2.51	4.51	7.01	33.27	58.1
SEM	0.05	0.03	0.05	0.04	0.03	0.03	0.07	0.05	0.05	0.06	0.04	0.45	0.52
The statistical results:													
Tall cultivar	3.31	1.29	3.39	3.09	2.77	1.12	4.40	6.78	2.22	4.63	7.02	31.88	57.1
$\bar{X}$ dwarf lines	3.44	1.39*	3.49	3.31*	2.94	1.18	4.72	7.06	2.40	4.80	7.10	33.24	60.2

$\bar{X}$ means of lines with the Dw6 dwarfing gene. SEM standard error of the mean. EAA essential amino acids. AAS_{Lys} was calculated based of the FAO/WHO/UNU reference pattern for requirements of the preschool-age children. * Significant at $p \leq 0.05$. Cys, Tyr are conditionally essential amino acids.

Table 5 - Indices of protein nutritive value of hulled oats grain determined in nitrogen balance experiment (%)

Item	BV	TD	PDCAAS
Krezus – traditional - tall hulled cv.	76	82	46.9
STH 5244 – dwarf line	78	84	49.9
SEM	1.01	0.7	0.6

BV Biological Value. TD True Digestibility. PDCAAS protein digestibility corrected amino acid-scoring. SEM standard error of the mean.

The content of amino acids in the oat protein was comparable to the value given by CARPENTER *et al.* (1989).

Comparisons with the WHO/FAO/UNU (2007) reference pattern for requirements of preschool-age children revealed that lysine was the most limited amino acid in oat grains, in the range from 57.1% (tall cultivar) to 60.2% (dwarf lines). Other authors also observed that lysine is the most limited amino acid in oat grains (ZARKADAS *et al.*, 1995; PED'O *et al.*, 1999).

Oat protein had a high and similar nutritional value (Table 5). The levels of biological value (BV), true digestibility (TD) and the protein digestibility-corrected amino acid scores (PDCAAS) of dwarf oat (STH 5244) were slightly higher than the traditional Krezus cultivar. The results for BV of protein were very similar to values (74.5-79.6%) reported by EGGUM and GULLORD (1983). PDCAAS values (49.9 and 46.6%) differ significantly from the TD of protein. PED'O *et al.* (1999) reports protein digestibility for Brazilian cultivars of oat at an average of 84.9%, while the average PDCAAS for the same cultivars was reported at 49%.

In the serum of rats receiving STH 5244 oat (dwarf line) and Krezus oat (traditional tall cultivar), levels of TCH, HDLc, LDLc and TGC were very similar (Table 6). Comparing the results obtained with the control group showed the beneficial effect of oat on the serum lipid profile. Animals in experimental groups receiving oat in their diet had lower concentrations of TCH and LDLc ($p \leq 0.05$) and TGC (by about 8.5%) compared with animals of the control group (casein). Hypocholesterolemic properties of oat have also been found in other studies in rats (MAKI *et al.*, 2003; PENG *et al.*, 2013), and were attributed mainly β-glucans. The hypocholesterolemic action of β-glucans was confirmed by KALRA and JOOD (2000).

CONCLUSIONS

The results show that the lines of hulled oat with introduced dwarfism gene *Dw6* do not differ in nutritional components, nutritional properties of protein, or hypocholesterolemic properties compared to the traditional tall cultivar, and even have slightly better properties. Due to the content of protein of high nutritional value, a high concentration of unsaturated fatty acids and dietary fiber, including β-glucans and a positive effect on serum lipid profile, dwarf oat grain and its products can be a valuable component of the human diet.

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Table 6 - Serum lipid profile of rats in the diet groups (mg/dL).

Item	TCH	HDL_c	LDL_c	TGC
Control (casein)	81.85[b]	46.71	18.53[b]	82.36
Krezus – traditional - tall hulled cv.	66.03[a]	39.37	16.21[a]	75.23
STH 5244 – dwarf line	65.25[a]	42.63	16.99[a]	76.12
SEM	1.4	1.1	1.1	1.6

TCH total cholesterol. HDL_c high density lipoprotein cholesterol. LDL_c low density lipoprotein cholesterol. TGC triacylglyceroles. SEM standard error of the mean. Different letters in the columns represent statistically significant differences ($p < 0.05$).

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PAPER

NUMERICAL ANALYSIS OF FLUID FLOW VELOCITY BETWEEN PLATES CHANNEL OF HEAT EXCHANGER BY DIFFERENT SURFACE CONFIGURATION IN REFERENCE TO THE EFFECTS OF CLEANING

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ABSTRACT

The work includes the analysis of the velocity distribution of fluid flow in the plate heat exchanger. The research was conducted on the basis of Finite Volume Method (FVM) of numerical computation using ANSYS CFX program. This method enabled making the description of the flow velocity distribution between plates of heat exchangers and drawing curves illustrating the flow velocity in each areas on plate. The space between a plates of heat exchanger of straight flow type constituted a computational model. Mathematical description of the researched process was made. The results of the research defined the influence of plate heat exchanger configurations on type of flow. It also pointed out the places on the plate in which insufficient flow can cause difficulties with cleaning. Numerical research was experimentally verified by determining the cleanliness of the plate's surface in certain areas after the process of cleaning. Obtained results of the research indicated that the areas hardest to clean in the plates are those opposite to the power supply. Such situation is caused due to slow fluid flow in the aforementioned areas.

- Keywords: CFD, plate heat exchanger, fluid flow, Clean-in-Place (CIP) method of cleaning -

INTRODUCTION

CIP (cleaning in place) method of cleaning is frequently used in many branches of food industry to sanitize devices and transmission installations. Cleaning takes place interiorly without disassembly. Due to the possibility of reusing detergents, CIP method is economical and eco-friendly, as it limits the sewage disposal (GILLHAM *et al.*, 2000; DRESCH *et al.*, 2001; STRUK-SOKOŁOWSKA, 2011.). The effectiveness of CIP depends on four major factors, namely: the duration of the process, the temperature in which it is conducted, the cleaning products used and mechanical interactions understood as the shearing stress. Many researchers shown that mechanical interactions are the crucial factor determining the final effect of cleaning (BLEL *et al.*, 2007; GRASSHOFF 1992; LELIEVRE *et al.*, 2002a; LELIEVRE *et al.*, 2003; JENSEN *et al.*, 2005). The degree to which a given device is cleaned depends on how easily its surface can be accessed; this is closely related to its construction. Many researchers prove that because of this fact, CIP process does not always bring satisfactory results (AUSTIN and BERGERON, 1995; LELIEVRE *et al.*, 2002b). The danger of insufficient cleaning is especially visible in the machines in which food contact surface is of complex geometry. The example of such apparatus is a plate heat exchanger which is widely used in food industry installations, particularly in milk processing industry.

In international literature one can find researches concerning milk sediment in pasteurized milk (TODOYA *et al.*, 1994; BANSAL and CHEN, 2005; AUGUSTIN *et al.*, 2007). Such studies focus on the descriptions of mechanisms governing the formation of milk sediment and its layering during the exploitation of the exchanger but they also indicate in which areas it is most visible. Depending on the type of exchanger, one can identify the areas in which fat and protein sediments are formed and in which they linger. It is closely related to the thermal conditions in a given device. The amount of fouling forming in plates of heat exchangers increases with moving away from the inlet to the heat exchangers. What is more, on some areas of plates the level of fouling is greater. GEORGIADIS *et al.*, (1998) and JUN and PURI (2006) indicate that high temperature is the answer to the whole process. Furthermore, in some of the areas the flow of milk is slow what prolongs the time which milk spends in high temperature. It results in an increased number of sediments which often scorch on the surface.

The cleanliness plates of heat exchanger decides not only about the safety of food production but also about the conditions in which they are exploited. Aggregated sediments negatively influence on heat exchange between flow factors, increase flow resistance and cause corrosion in the plates (GILLHAM *et al.*, 2000; MERHEB *et al.*, 2007; DIAKUN, 2013). Sediments are also undesirable accumulation of microorganisms and they are a source of microbiologic contamination. There is not much research concerning the conditions and effects of cleaning in plate heat exchangers. Much of the research work on this topic present very general problems of cleaning (CHRISTIAN and FRYER 2003; WILSON 2005). BEUF *et al.* (2003) focus on elaborating new construction materials. A MERCADÉ-PRIETO *et al.* (2005; 2007) deal with the influence of new chemical materials which are becoming more efficient when it comes to milk sediment. CHANGANI *et al.*, (1997) researched the influence of the alkali solutions, while FILLERY and MCQUILAN (2006) focused more on the acidic ones. BOYCE *et al.*, (2010) tested enzymatic substances being the mixture of enzymes, the surface-active substances, complexing and buffering compounds stabilizing pH of the solution. Such a great interest in the chemical aspect of the cleaning process leads to a lack of interest in other cleaning factors and the mechanisms of their mutual influence.

Plate heat exchangers are the object of research for many researchers. The studies focus mainly on the development of their optimal design to increase the thermal effectiveness. Following variables are being analyzed: the type and number of flow channels in the exchanger, the number of passes for both streams, the diameter and the location of inlet well against the surface of the plate and the order of giving the thermal factors. The authors investigate different plate profiles, their relationships positional in order to match optimal conditions of heat exchange and to minimize the cost of their exploitation (VLASOGIANNIS *et al.*, 2002; DAGDAS, 2007; ARSENYEVA *et al.*, 2011; PIEPIÓRKA-STEPUK and JAKUBOWSKI, 2013). They also define the pressure drop, kinetics of the process (DURMUS *et al.*, 2009), as well as the local coefficient of heat transfer (CIOFALO 2007). The research covers experiments, mathematical and physical modeling and numerical modeling. However, no research was conducted on the type of flow in plates channel of heat exchanger's in references to the clean conditions in CIP method cleaning. Flowing solution agents moistens and dissolves sediments on the plates' surfaces, leading to their separation and draining sediment from the exchanger. It depends on the conditions of the fluid flow between the plates and the accessibility of the cleaned surfaces. Thus, it is crucial to explore this subject. Experimental research of flow in the plate heat exchanger are difficult to implement therefore commonly used for this purpose are numerical research. They allow fast and cheap to analyze engineering problems.

Numerical simulations are based on the fi-

nite element method (FEM) or the finite volume method (FVM). These methods have numerical codes of a differential equation as a mathematical recording of basic laws of fluid dynamics and thermodynamics (ASENDRYCH *et al.*, 2013; SKOČILAS *et al.*, 2013). In plate heat exchanger modeling, the difficulty of using such methods lays in mapping of real algebraic geometry of the space between plates and in discretization of the model. Many studies include analysis of the limited fragment of the space between the plates. They mainly concern the type of flow (STASIEK *et al.*, 2004; LUAN *et al.*, 2008), friction factors (FERNANDES *et al.*, 2008) and heat transfer (STASIEK *et al.*, 2000). GRIJSPEERDT *et al.*, (2003) conducted a two-dimensional analysis of the fluid flow in the space between the plates, illustrating the influence of plate corrugation on the type of flow and heat exchange. In 2009, de BONIS and RUOCCO correlated temperature distribution with the places in which a milk fouling are created. The analysis concerning the flow of agents between the plates of a heat exchanger requires taking into account orientation of the corrugation. Corrugations are made in order to equally distribute liquid on the whole surface between the plates. No research was made on plate profiling. PÄÄKKÖNEN *et al.* (2007), as well as FREUND and KABELAC (2010) in their research present the results concerning minor fragments of spaces between the plates in the context of local conditions of heat exchange. JIN *et al.* (2008) and LIU and TSAI (2010) made an attempt of numerical research of entire plates of exchangers, while GALEAZZO *et al.* (2006) and SAMMETA *et al.* (2011) even concentrated on the whole packets. The analysis by the abovementioned researchers was conducted on geometrically simplified models with flat plates.

THE AIM OF THE RESEARCH

The aim of the research was to determine the conditions of the 3D flow between heat exchanger plates of different geometry by using the method of numerical analysis. On the basis of numerical analysis, the distribution of velocity field and streamlines in spaces between plates, formed by the plates of heat exchanger, was made. Characteristics of velocity were shown in three sections of a plate. The results of the research determined the influence of plate heat exchanger configurations on the type of flow and indicated the places on the plate in which insufficient flow can cause difficulties with cleaning. The results of numerical analysis referred to the experimental research and assessed cleanliness of particular areas of plate heat exchangers cleaned with CIP method.

MATERIAL AND METHODS

Numerical simulations

Computations were performed with the finite volume method (FVM) on which CFX program, being one of the modules for numerical computation, ANSYS, is based. CFX constitutes a set of tools and elements for computation, as well as analysis of fluid flow and accompanying phenomena. The program has a wide range of possibilities of declaring boundary conditions and material properties (KMIOTEK, 2008; ANSYS, 2009). The results of computations are showed graphically as a velocity maps and streamlines on the plate's surface and in three of its sections.

Computations were conducted on the basis of a single space between a plate heat exchanger of three surface configurations (Fig. 1):

Simplified model projecting flat plates (Fig. 1a);

Chevron plates (60° angle to the axis of symmetry), arranged symmetrically to each other (Fig. 1b);

Chevron plates inverted with respect to each other by180° (Fig. 1c).

Size and shape of model C plates were consistent with their position in a heat exchanger which, in the second stage of research, underwent the process of cleaning in flow. Characteristic size of the exchanger is shown in Table 1. A scheme of CIP station and circulation cleaning of a plate heat exchanger is presented by Fig. 2.

In each of the analyzed models the greatest distance between the plates was b = 0.008 m. A regular grid with the size of the element of 0.001 m was used for discretization of model A and B. Model C, due to complex geometry, was divided by the irregular grid with the size of the element up to 0.0001 m. As a result, a model with flat plates had 40 196 elements; model B - 61 254 elements and model C – 1 829 250 elements.

In order to perform numerical calculations, isothermal flow conditions were assumed. Water constituted the fluid of the viscosity and densi-

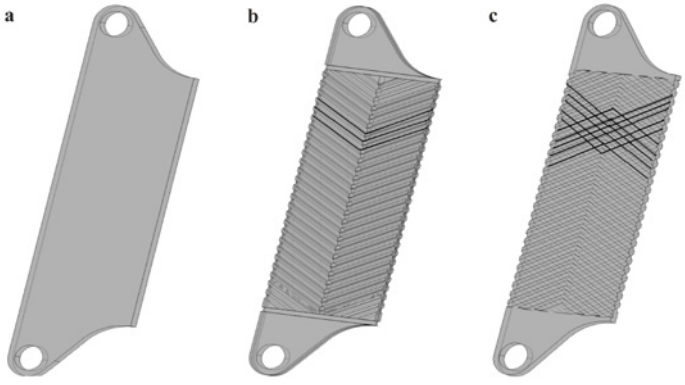


Fig. 1 - Models of plates used for numerical computation: a – Flat plates; b – Symmetrically arranged plates; c – Plates inverted with respect to each other.

Table 1 - Main geometrical characteristics of the plate heat exchanger used to the research.

Geometrical characteristics	Symbol	Unit	Value
Effective length of the plate	Lw	m	0,381
Effective width of the plate	Ls	m	0,11
Area of one plate	A= Lw× Ls	m^2	0,042
Area of heat transfer	Ac	m^2	0,46
The largest distance between plates	b	m	0,008
Mean distance between the plate	c = (b/2)	m	0,004
The surface area of the transverse flow	Pstr = cLs	m^2	0,0005
Wetted	Obc = 2c + 2Ls	m	0,23
Total number of plates in heat exchanger	Np	items	11
Number of channel in plate heat exchanger	Nc = (Np – 1)/2	items	5
Hydraulic diameter of the channel	dch	m	0,009
Corrugation angle	β	°	60
Type of plates	Straight - flow		

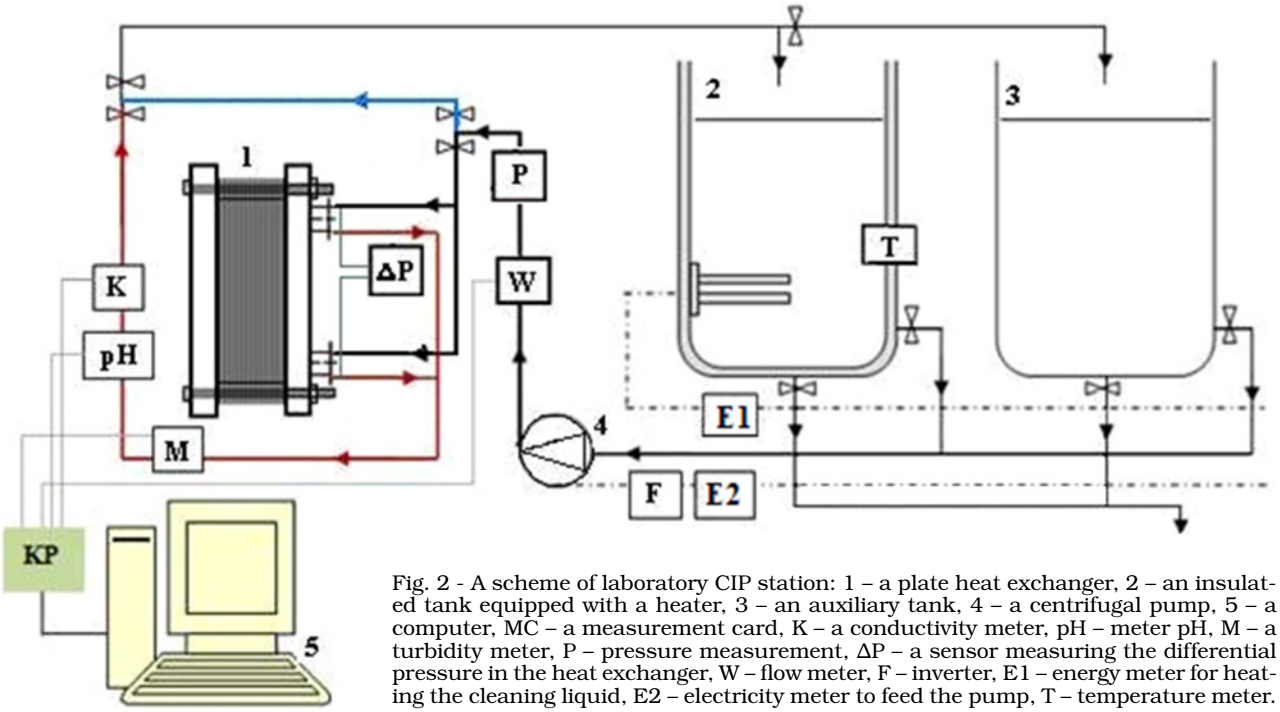


Fig. 2 - A scheme of laboratory CIP station: 1 – a plate heat exchanger, 2 – an insulated tank equipped with a heater, 3 – an auxiliary tank, 4 – a centrifugal pump, 5 – a computer, MC – a measurement card, K – a conductivity meter, pH – meter pH, M – a turbidity meter, P – pressure measurement, ΔP – a sensor measuring the differential pressure in the heat exchanger, W – flow meter, F – inverter, E1 – energy meter for heating the cleaning liquid, E2 – electricity meter to feed the pump, T – temperature meter.

ty, corresponding with water of the temperature T = 45°C. The flow conditions were established as time-invariant and the flow was described as stable. It was also assumed that the fluid flow is turbulent and the turbulence models include the standard definition of the description of the boundary layer. Thus, the κ - ε model was used for calculations. The boundary conditions in the form of zero velocity value on the walls and the edges of the plates were assumed for the calculations. The zero value of the kinetic energy and dissipation of turbulence in the first iteration were also used (Fig. 3). The surface roughness assumed at the level R_a = 0,4 μm.

The boundary conditions were assumed at the inlet and outlet of the plate in the form of mass flow rate of m_v = 0,272 kg×s^{-1}. Out of assumed values an analytical flow velocity between the plates, as well as the Reynolds number, in accordance with the method given by FERNANDES *et al.* (2008), were calculated. The calculated mean flow rate was u_{ch} = 0,55 m×s^{-1} and the Reynolds number accounts for $Re \approx$ 8200, which in the case of such a narrow slot indicates the turbulent nature of the flow. Flow characteristics were analyzed in three sections: the top, the middle and the bottom. The sections were designated as: section 1, section 2, section 3 (Fig. 3). On the basis of the numerical results areas where small velocity occurs were designated in plates.

Laboratory experimental

In experimental research plates of heat exchanger were fouled with milk by spraying it. Plates fouled with milk were heated for 2 minutes at 85°C. This operation was repeated three

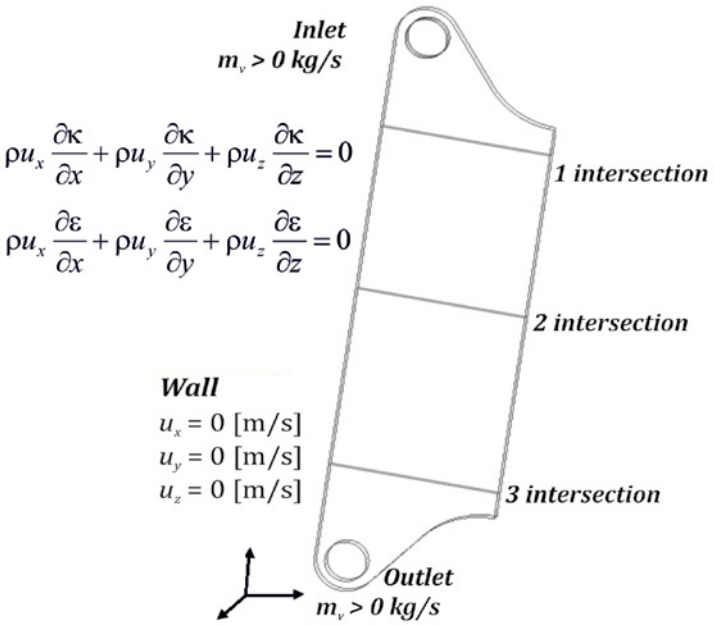


Fig. 3 - Boundary conditions for the analyzed models.

times, obtaining a difficult to remove fat-protein sediment. Then the plates were installed in the heat exchanger and subjected to a cleaning process in the flow. Cleaning was carried out at u_{ch} = 0,55 m×s^{-1}; t = 65 min; T = 45°C with pure water used as a cleaning agent. Cleanliness assessment was carried out for the first pair of plates in the exchanger. Sampling areas are described as: O_1; O_2; O_3; O_4; O_5 and shown in Fig. 9. Cleaning was determined on a scale of 0 – 10 with *Pro-Clean* swab test (PIEPIÓRKA 2012). A value of 0 signifies a dirty surface and 10 a completely clean one. The study was performed in eighteen repetitions.

CALCULATION

The models are described in a rectangular coordinate system x, y, z (in Cartesian coordinate system). Apart from the components of the gravity force and assuming, according to Reynold's hypothesis, that the instantaneous values of all the physical quantities characterizing the flow can be considered as the sum of the time averaged values, an average flow can be described by using equations of conservation of mass and momentum. The equation of conservation of energy was omitted, because isothermality of the process was assumed (JUN *et al.*, 2003; KAZIMIERSKI 2004; JUN and PURI, 2006; ZHANG, 2006; LUAN *et al.*, 2008).

Bernoulli's equation in divergence form

$$\frac{\partial \rho}{\partial t} + \rho \, div \, \vec{u} = 0 \qquad (1)$$

Under the assumption that the flowing agent is water of a constant temperature (incompressible fluid), a constant density ρ = *const.* was assumed in the model.

As a result, $\frac{\partial r}{\partial t} = 0$, therefore:

$$div \, \vec{u} = 0 \qquad (2)$$

By developing a devise, we get:

$$\frac{\partial u_x}{\partial x} + \frac{\partial u_y}{\partial y} + \frac{\partial u_z}{\partial z} = 0 \qquad (3)$$

Momentum balance equation (Navier - Stokes) as a vector for an incompressible fluid has the following form:

$$\frac{d\vec{u}}{dt} = \vec{F}_m - \frac{1}{\rho}\nabla P + \nu \nabla^2 \vec{u} \qquad (4)$$

When broken down by the above equation for rectangular components, the equation is presented as a system of equations:

$$\begin{cases} \frac{\partial u_x}{\partial t} + u_x \frac{\partial u_x}{\partial x} + u_y \frac{\partial u_x}{\partial y} + u_z \frac{\partial u_x}{\partial z} = -\frac{1}{\rho}\frac{\partial p}{\partial x} + \nu\left(\frac{\partial^2 u_x}{\partial x^2} + \frac{\partial^2 u_x}{\partial y^2} + \frac{\partial^2 u_x}{\partial z^2}\right) + \vec{F}_{mx}, \\ \frac{\partial u_y}{\partial t} + u_x \frac{\partial u_y}{\partial x} + u_y \frac{\partial u_y}{\partial y} + v_z \frac{\partial u_y}{\partial z} = -\frac{1}{\rho}\frac{\partial p}{\partial y} + \nu\left(\frac{\partial^2 u_y}{\partial x^2} + \frac{\partial^2 u_y}{\partial y^2} + \frac{\partial^2 u_y}{\partial z^2}\right) + \vec{F}_{my}, \\ \frac{\partial u_z}{\partial t} + u_x \frac{\partial u_z}{\partial x} + v_y \frac{\partial u_z}{\partial y} + u_z \frac{\partial u_z}{\partial z} = -\frac{1}{\rho}\frac{\partial p}{\partial z} + \nu\left(\frac{\partial^2 u_z}{\partial x^2} + \frac{\partial^2 u_z}{\partial y^2} + \frac{\partial^2 u_z}{\partial z^2}\right) + \vec{F}_{mz}. \end{cases} \qquad (5)$$

The above arrangement assumes that there are no mass forces F_m=0, and the flow is established $\frac{\partial u}{\partial t} = 0$. Given the above assumptions, the following equations were obtained:

$$\begin{aligned} u_x \frac{\partial u_x}{\partial x} + u_y \frac{\partial u_x}{\partial y} + u_z \frac{\partial u_x}{\partial z} &= \frac{1}{\rho}\frac{\partial p}{\partial x} + \nu\left(\frac{\partial^2 u_x}{\partial x^2} + \frac{\partial^2 u_x}{\partial y^2} + \frac{\partial^2 u_x}{\partial z^2}\right), \\ u_x \frac{\partial u_y}{\partial x} + u_y \frac{\partial u_y}{\partial y} + u_z \frac{\partial u_y}{\partial z} &= \frac{1}{\rho}\frac{\partial p}{\partial y} + \nu\left(\frac{\partial^2 u_y}{\partial x^2} + \frac{\partial^2 u_y}{\partial y^2} + \frac{\partial^2 u_y}{\partial z^2}\right), \\ u_x \frac{\partial u_z}{\partial x} + u_y \frac{\partial u_z}{\partial y} + u_z \frac{\partial u_z}{\partial z} &= \frac{1}{\rho}\frac{\partial p}{\partial z} + \nu\left(\frac{\partial^2 u_z}{\partial x^2} + \frac{\partial^2 u_z}{\partial y^2} + \frac{\partial^2 u_z}{\partial z^2}\right). \end{aligned} \qquad (6)$$

The turbulence equation

Classical turbulence modeling is based on the Reynold's concept, according to which each quantity describing turbulent flow is considered to be the sum of the volume averaged over time and fluctuating component. This value is a random function of time and space. Application of the above concept in the analysis of turbulent flows doubles the unknowns in the equations, transforming them into unequivocal solutions. Alternative solution in numerical analysis of turbulent flows is the use of turbulence models, including κ - ε model, adapted for a high level of flow turbulence in the channels with a large surface area

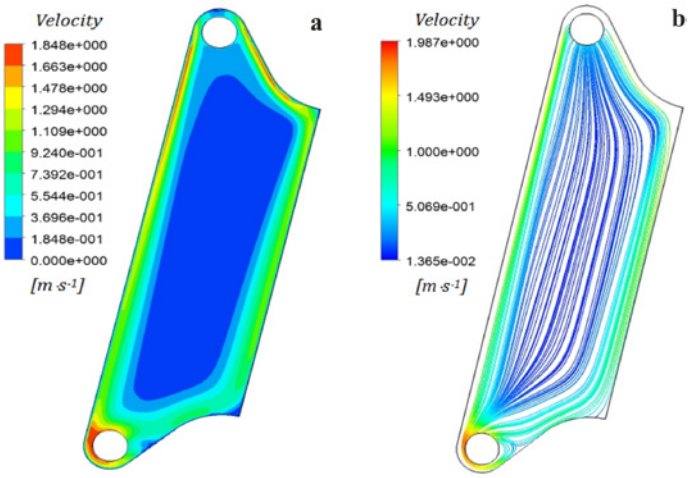


Fig. 4 - Model 1 – channel between flat plates: a – velocity field; b – streamlines.

relative to their volume. Thus, the turbulent kinetic energy is cascade-transferred from the large scale vortices to small scale vortices, where dissipation occurs. In the concept of this model, system of equations is closed (6) with two additional differential equations: the kinetic energy transport "κ" (8) and the dissipation rate of turbulent kinetic energy "ε" (9) (BOGUSŁAWSKI *et al.*, 2008).

The equation for kinetic energy *x*

$$\rho u_x \frac{\partial \kappa}{\partial x} + \rho u_y \frac{\partial \kappa}{\partial y} + \rho u_z \frac{\partial \kappa}{\partial z} = \frac{\partial}{\partial x}\left(\frac{\mu_e}{\sigma_\kappa}\frac{\partial \kappa}{\partial x}\right) + \frac{\partial}{\partial y}\left(\frac{\mu_e}{\sigma_\kappa}\frac{\partial \kappa}{\partial y}\right) + \frac{\partial}{\partial z}\left(\frac{\mu_e}{\sigma_\kappa}\frac{\partial \kappa}{\partial z}\right) + G - \rho\varepsilon \quad (7)$$

The equation for the dissipation rate ε

$$\rho u_x \frac{\partial \varepsilon}{\partial x} + \rho u_y \frac{\partial \varepsilon}{\partial y} + \rho u_z \frac{\partial \varepsilon}{\partial z} = \frac{\partial}{\partial x}\left(\frac{\mu_e}{\sigma_\varepsilon}\frac{\partial \varepsilon}{\partial x}\right) + \frac{\partial}{\partial y}\left(\frac{\mu_e}{\sigma_\varepsilon}\frac{\partial \varepsilon}{\partial y}\right) + \frac{\partial}{\partial z}\left(\frac{\mu_e}{\sigma_\varepsilon}\frac{\partial \varepsilon}{\partial z}\right) + C_1\frac{\varepsilon}{\kappa}G - C_2\rho\frac{\varepsilon^2}{\kappa} \quad (8)$$

In the above equations there are: energy dissipation for averaged motion,

$$G = \mu_t\left\{2\left[\left(\frac{\partial v_x}{\partial x}\right)^2 + \left(\frac{\partial v_y}{\partial y}\right)^2 + \left(\frac{\partial v_z}{\partial z}\right)^2\right] + \left(\frac{\partial v_x}{\partial x} + \frac{\partial v_y}{\partial y} + \frac{\partial v_z}{\partial z}\right)\right\} \quad (9)$$

$$\mu_t = \rho C_\mu \frac{\kappa^2}{\varepsilon} \quad (10)$$

effective viscosity, as a sum of a dynamic viscosity and turbulent viscosity determined by the equation,

$$\mu_e = \mu + \mu_t \quad (11)$$

and constant turbulence model C_μ = 0.09 and values of the coefficients σ_κ = 1.0; σ_κ = 1.3; C_1 = 1.44; C_2 = 1.92.

Mass conservation equations (3), the equation of conservation of momentum (6) and equation turbulence model adopted κ - ε (8, 9), are the mathematical models of the motion of fluid in the channel between the plates of the heat exchanger. These models are partial differential equations, in which the independent variables are the coordinates of the point considered in the x_i. On this basis it is possible to determine the distribution of the dependent variables such as the components of the velocity vector *u*, the pressure *p*, turbulent kinetic energy κ, and dissipation of turbulence ε (JUN and PURI, 2006; ZHANG *et al.*, 2006; PINSON *et al.*, 2007; LUAN *et al.*, 2008).

RESULTS AND DISCUSSION

The results of numerical simulations

The results of numerical calculations concerning the nature of the flow in the form of the distribution of velocity fields for each analyzed model of the space between the plates is shown in Figs. 4, 5, 6 and 7. It indicates that the greatest velocity occurs near the inlet and outlet holes. The maximum velocity of flow in these areas is substantially higher than the calculat-

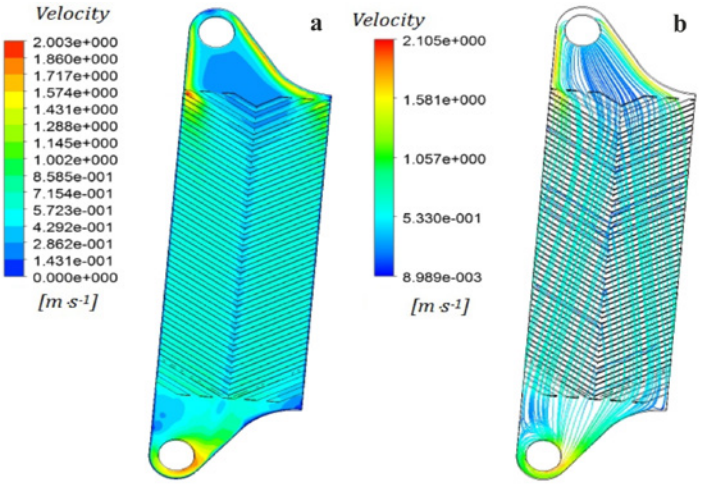


Fig. 5 - Model 2 – channel between symmetrical corrugated plates: a – velocity field; b – streamlines.

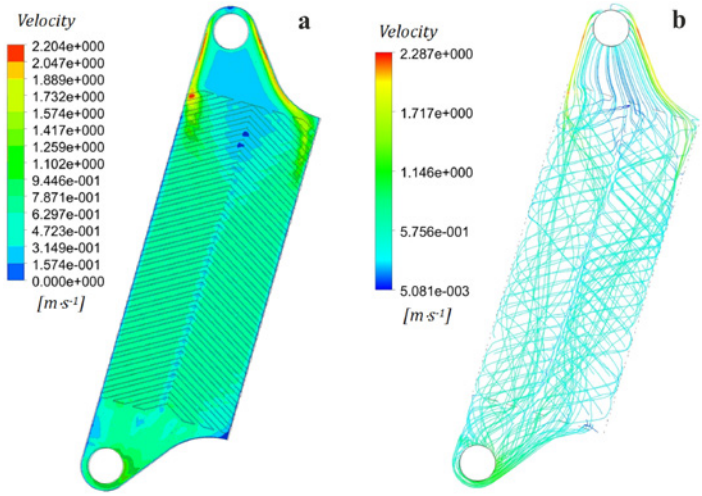


Fig. 6 - Model 3 - channel between corrugated plates inverted with respect to each other: a – velocity field; b – streamlines.

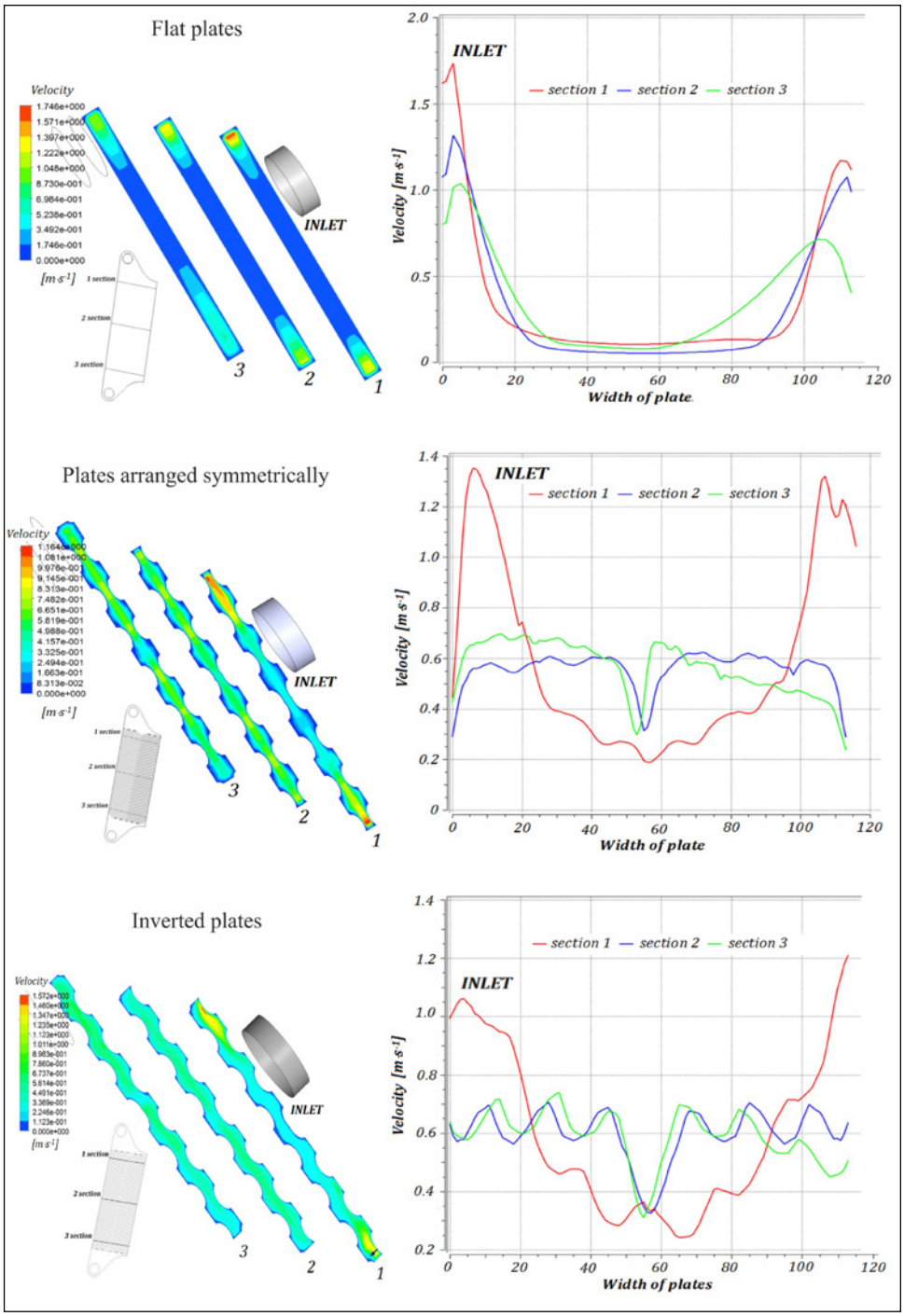


Fig. 7 - Distribution of velocity fields in different sections of the space between plates for the analyzed geometric models.

ed average. However, in the central part of the channel between the plates along its length, the flow velocity distribution is shaped differently depending on the configuration of the surface of evaluated plates.

Flow rates for plates with flat geometry (Fig. 4a) decrease with the distance to the axis of symmetry of the plate, where it reaches values close to zero. This indicates the importance of the use of corrugations which are a characteristic of plates in heat exchanger. The depth and the angle of corrugations are designed to homogenize the flow in the channel between the plates and whole volume of the heat exchanger. The lowest concentration of the streamlines occurs in central parts of the first channels. It results in low-velocity fluid flow in these regions (Fig. 4b). It can therefore be expected that the heat exchanger with plates of this type, in addition to low thermal efficiency, will have a lot of hard-to-remove milk fouling in the central zones of plate's surface.

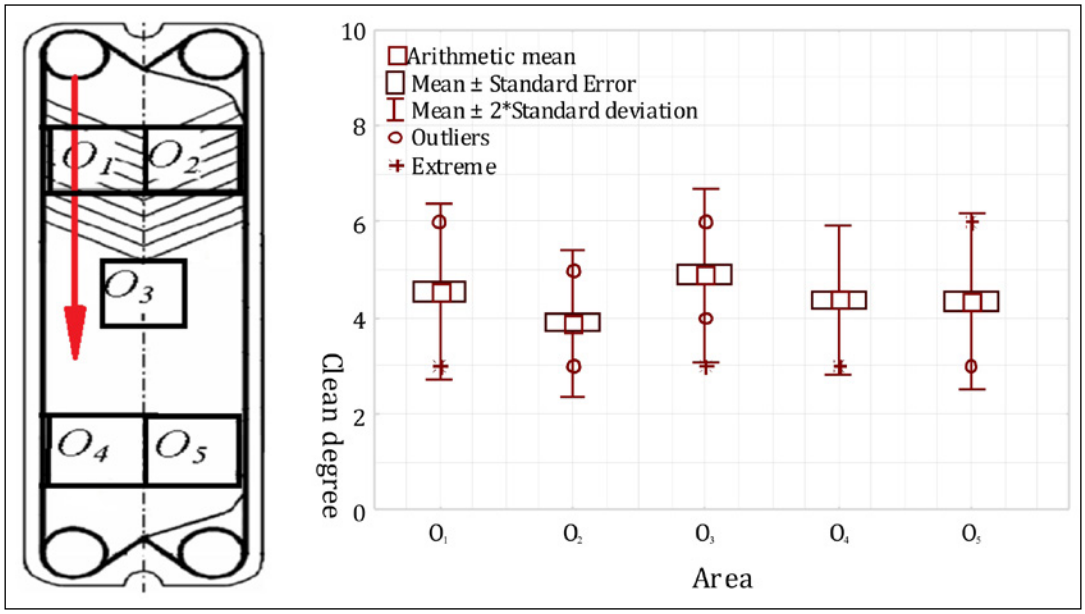


Fig. 8 - Assessment of selected areas on a plate: a – areas undergoing cleaning assessment; b - the degree of cleanliness of selected areas.

In the 3D model with corrugations arranged symmetrically to each other, the smallest value of the flow rate are below the inlet hole in the axis of the plate and in its lower corners Fig. 5a. The greatest condensation of streamlines is observed in the upper part of the plate, followed by dissolving and distancing from the axis of symmetry (Fig. 5b). One can see the places in which the fluid flows on the edges of the corrugations and is then drained outside the channel between the plates.

In the case of a 3D model with corrugations plates with inverted with respect to each other by 180°, velocity field distribution is similar to the symmetrical model, however, the flow velocity value is about 10% higher. Areas with the lowest flow values are the ones located below the inflow areas and in the lower corners of plates. The area in which flow conditions are most aligned is central and lower part of the plate – here the cleaning process should be most effective (Fig. 6a). The analysis of the streamline in the analyzed model indicates that the lines are scattered and not parallel as in the previous models. The flow is present in the whole capacity of the space between the plates, which is a crucial precondition in the process of cleaning.

Fig. 7 shows the velocity of water in three sections of the plates.

The resulting characteristics indicate a wide variation of flow conditions in the analyzed sections of the plates. In the case of flat plates, low flow velocity almost on the entire width of the plate along the analyzed section are clearly visible. In the rest of the models low flow velocity is present locally. In all the analyzed models, at the top of the plate are there are both top velocity (near the edges) and the lowest velocity (in the axis of symmetry of the plate). In the central and lower part of the plates the velocity reach approximate values. As far as flat plates are concerned, the flow velocity reach values close to u_{ch} = 0,2 m×s^{-1}, while in other models it was u_{ch} = 0,6 m×s^{-1}.

The results of laboratory experimental

On the basis of numerical research, areas in plates enabling to assess the cleanliness were chosen (Fig. 8a). The results as the arithmetic average and standard deviation are shown in Fig. 8b and Table 2.

It has been observed that the upper part of the plate (O_2) is harder to wash than the middle part and the lower areas (O_3; O_4; O_5), which were cleaned to similar extent. Areas of O_1 and O_2, are places located at the inflow - right and left upper corner of the plate in which, according to the results of the numerical research, values of the flow rate are the lowest. The area of O_2 received the worst marks in the assessment of cleanliness and thus, it is the area most difficult to clean. Given that in this place where fouling is also most accumulated (JUN and PURI, 2006), then the place in the plate heat exchang-

Table 2 - Results of statistical calculations for cleanliness each area on the plate.

	01	02	03	04	05
Arithmetic mean	4,55	3,89	4,89	4,39	4,33
Mean ± Standard Error	0,22	0,18	0,21	0,18	0,22
Mean ± 2*Standard deviation	1,84	1,52	1,80	1,53	1,81
Outliers	2(6)	4(5); 6(3)	5(6);4(4)	-	4(3)
Extreme	3	-	3	3	6

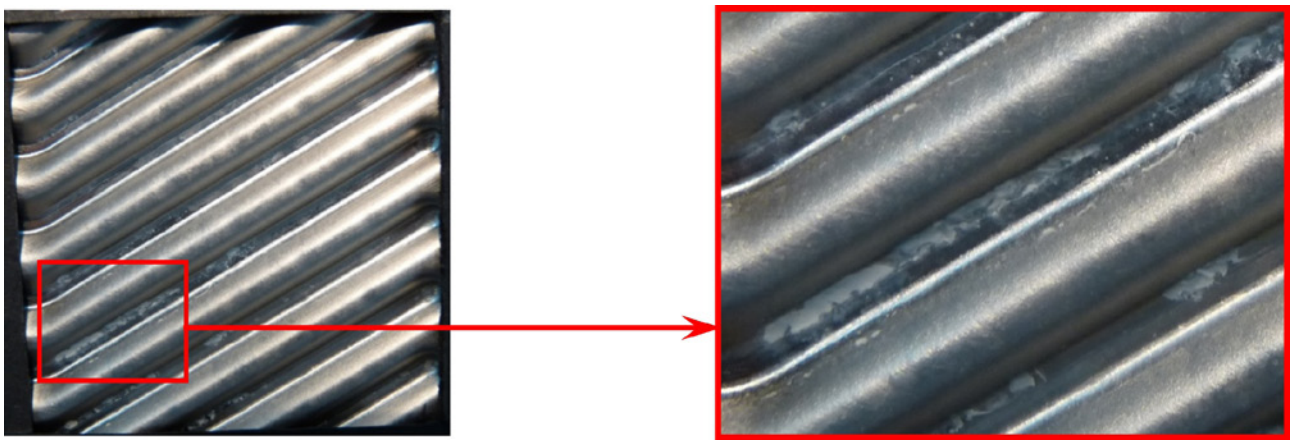


Fig. 9 - Residual fouling after the cleaning process in the flow, in sampling areas on a plate of a heat exchanger.

ers can be regarded as the most troublesome and dangerous for hygiene. To a smaller extent, but still better eluted, was the central area of the plate (O_3).

What is more, it was noted that the remaining milk fouling usually build up in the troughs of the plates (Fig. 9). This is due to the nature of the flow in this particular place, since the cross-section of the channel between the plates of the heat exchanger should be considered as a channel in which there are rapid dilations and contractions. The study shows that in places where there are dilations, there are areas with reduced flow rate. These places are impossible to clean. Therefore, it can be concluded that there are some unfavorable conditions for the cleaning process.

CONCLUSIONS

CFD calculations are an innovative tool used to solve engineering problems. In the conducted research, they allowed for information on fluid flow velocity distributions in the channel between the plates of heat exchanger with different surface shapes. The results indicate a wide variety of flow velocity. The velocity field and streamlines support the use of panels with corrugated surface. The design of the plate surface is the key issue in the product development of heat exchangers. Thus, the channels between the plates uniform distribution of the liquid.

The results of numerical research made it possible to locate the areas on the plates, which are of a reduced flow rate. These areas can be difficult to clean during the standard procedure of CIP cleaning, what has been proven in experimental research. Maximum flow velocity rate was obtained near the edges of the plates and in their lower areas, while the smallest rate could be observed in the part below the inlet. Velocity distribution in the channel between the plates of the heat exchanger are one of the crucial parameters of cleaning efficiency. Therefore, this numerical research indicates that different flow conditions in the channel can cause non-uniform conditions of CIP cleaning system.

It is important to continue the research in this field in order to simulate the flow conditions in combination with heat exchange. Such analysis would enable correct inference about any risks concerning the hygiene in such devices, both in terms of fouling formation, as well as with their removal during the cleaning process in the successive sections of the exchanger. Examining the nature of the flow in real conditions by using experimental methods is of equal importance. However, it is difficult to perform due to the specific design of plate heat exchangers, a narrow slot flow and the closed nature of their work. The method introduced in this paper gives a good basis for the further development of the fast and efficient modeling of heat exchangers.

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Nomenclature

C_μ; C_1; C_2; – model constants;
F_m – mass forces vector (N);
G – generation of the turbulence kinetic energy;
L_w – effective length of the plate (m);
L_s – effective width of the plate (m);
Re – Reynolds number = $\frac{\rho \cdot u_{ch} \cdot d_{ch}}{\mu}$;
T – temperature of fluid (°C);
b – the largest distance between plates (m);
d_{ch} – equivalent hydraulic diameter of the channel (m);
m_v – mean volumetric flow rate per channel ($m^3 \times s^{-1}$);
t – time (s);
u_x; u_y; u_z – flow velocity by spatial coordinates x, y, z;
u_{ch} – mean flow velocity per channel ($m \times s^{-1}$);

Greek symbols

β – corrugation angle (°);

ε - energy dissipation rate ($m^2 \times s^{-3}$);

ρ – fluid density ($kg \times m^{-3}$);

μ – coefficients of dynamic viscosity ($kg \times m^{-1} \times s^{-1}$);

μ_e – effective viscosity ($kg \times m^{-1} \times s^{-1}$);

μ_t – turbulent viscosity ($kg \times m^{-1} \times s^{-1}$);

ν – kinematic viscosity of the fluid ($m^2 \times s^{-1}$);

κ – kinetic energy ($m^2 \times s^{-2}$);

$\sigma_\kappa, \sigma_\varepsilon$ – coefficients of κ - ε model;

∇^2 – Laplacian operator;

Subscripts

ch – channel.

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SHORT COMMUNICATION

PROXIMATE ANALYSIS OF FISH DRIED WITH SOLAR DRIERS

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ABSTRACT

Nutritional composition of two fish species dried with solar driers were compared with other common methods of drying. For fish dried with solar driers, moisture content was 10.77-11.20 for *C. gariepinus* and 3.60-3.99 for *O. niloticus*; protein was 64.88-66.48 for *C. gariepinus* and 58.75-63.28 for *O. niloticus*; crude fibre was low in the two species <1.00%; fat was 8.19-8.96 for *C. gariepinus* and 6.80-7.82 for *O. niloticus*; carbohydrate was 4.68-5.68 for *C. gariepinus* and 11.03-17.00 for *O. niloticus*. The nutrient compositions of the species dried with the solar driers were high and compared well with other methods of open drying.

- Keywords: moisture, oven, protein, proximate analysis, solar driers, smoking kiln -

INTRODUCTION

Drying of fish is an age long practice for preserving fish for a fairly long time to prevent deterioration and spoilage in the quality of the product. It is also to reduce post harvest losses and make available the product in times of shortage, thereby ensuring cheap protein availability to people. Drying increases the shelf life, enhance the quality, provide ease of handling, further processing and sanitation (MUJUMDAR, 2007). Drying involves the application of heat to vaporize moisture or remove water vapour from a product (VISAVALE, 2012). The removal of water will stop or slow down the growth of microbes, oxidation of fat and autolytic activities in the fish with resultant reduction in weight and volume.

Various drying methods have evolved and in use over the years in sub-Saharan Africa. These include direct open sun drying, solar drying, smoking and smoke drying with the use of ovens. The use of one particular method depends on the product, availability of energy sources, cost, skill, and storage facilities. The advantages and disadvantages of each method have been summarized by BERKEL *et al.* (2004). However, a good drying method must be the one in which the nutritional quality of the fish is enhanced or minimally affected. OPARAKU and MGBENKA (2012) and HUDA *et al.* (2010) observed that different drying methods have different effects on the nutritional compositions of fish. TURKAN (2008) and WHITTLE (1997) also linked the availability of vital nutrients in fish to the methods of preservation and shelf life of the fish.

The use of solar driers most especially low-cost ones against the traditional open drying, smoking kiln and ovens is on the rise and becoming widely accepted in sub-Saharan Africa. It is therefore necessary to test for the nutritional qualities of the final dry products produced by these low cost solar driers. Proximate composition is an indicator of the nutritional value of food material and in fish species could vary depending on environment, age, sex, food and feeding habits, migration, methods of preservation, morphology, structure of the species, seasonality in abundance and catch. According to RODINGO (1998), the principal nutritional components of fish include water, protein, ash, lipids, fats and fibre. SESUGH *et al.* (2012) OGBONAYA (2009), OJIKUTU *et al.* (2009), EFFIONG and FAKUNLE (2012), OPARAKWU and MGBENKA (2012), RAHMAN *et al.* (2012), AKINWUMI *et al.* (2011) AHMED *et al.* (2011) TAO and LINCHUN (2008), OGBONAYA and IBRAHIM (2009), ADEBOWALE *et al.* (2008) have all analysed the nutritional and proximate compositions of various species of fish dried under different methods.

This study was carried out to evaluate the nutritional composition of two highly important commercial tropical African fish species *Clarias gariepinus* (African sharp tooth catfish) and *Oreochromis niloticus* (Nile tilapia) dried using low cost solar drier and comparing it with the common methods of open sun drying, smoking kiln and electric oven.

MATERIALS AND METHODS

Five different solar driers constructed from inexpensive and readily available materials were used for drying of the two fish species. The driers used were:

1. Plastic drier: This was constructed using a thermopile plastic material. It has a square size of 0.61 x 0.61 m. Inside the drier was placed a wooden stand having a dimension of 0.46 x 0.46 x 0.15 m (length, width and height). A 0.52 x 0.52 m wire mesh in which the fish species were placed was put on top of the stand (Fig. 1).
2. Aluminum drier: This was constructed from aluminum sheet. It has the same size, shape



Fig. 1 - Plastic drier with samples of the two fish species during drying.



Fig. 2 - From right to left, black stone inserted glass drier, aluminum drier and glass drier.



Fig. 3 - Mosquito net drier with samples of the two fish species during drying.



Fig. 4 - Open sun drying with samples of the two fish species during drying.

and dimensions with the plastic drier. Inside it was also placed a wooden stand and wire mesh of the same dimensions with that of the plastic. The drier was however coated both inside and outside with black paint (Fig. 2).

3. Glass drier: This was made of transparent glass. It has the same size, shape and dimensions with aluminum dryer. The drier also had a wooden stand wire mesh of the same dimensions with aluminum drier to hold the fish species during drying (Fig. 2).
4. Glass drier containing black stones: This is similar to the glass drier in every respect but with a black (igneous rock) stone placed in it (Fig. 2).
5. Mosquito net dryer: This was constructed by using plywood for the frame (edges). The drier was subsequently covered with mosquito net all around the wooden frame. The size, shape and dimensions of the drier were the same with the others. Inside it was also placed a wooden stand having a wire mesh on top. The dimensions of the wooden stand and the wire mesh were the same with the other driers (Fig. 3).

An unenclosed 0.61 x 0.61 m steel plate was used for open drying by exposure directly to the sun (Fig. 4). A smoking kiln constructed from steel drum with firewood as the source of energy and an electric oven were used in drying the fish samples (Fig. 5). Temperature of the electric oven was set at 120°C, dried for 30 min (OGBONAYA and IBRAHIM, 2009) in order to completely remove the water content from the fish species in



Fig. 5 - Smoking kiln.

no time. All the solar driers and the unenclosed drier were placed on top of a story building at the Physics laboratory of the University of Ilorin, Ilorin, Nigeria with no obstruction to sun rays and facing the direction of the prevailing mind.

Fresh samples *Clarias gariepinus* and *Oreochromis niloticus* used for this work were obtained from Kwara State Ministry of Agriculture, Ilorin, Nigeria. The fish samples were weighed and the total length measured. They were descaled (*O. niloticus* only) gutted and mob dried. The fish samples were then reweighed and measured before placing them in each drier and the control. Drying in the smoking kiln was done outdoor, while drying in the oven was done indoor in the laboratory.

Proximate analysis

Proximate analysis to determine the nutrient composition of the two fish species after drying in the different driers and the open sun drying was done according to AOAC (2005) and PEARSON (1981). The moisture, ash, crude fibre, crude protein, fat and carbohydrate contents of the dried fish samples were determined.

Measurement of temperature in the driers

Temperature measurement in all the driers was measured by using a laser sensor thermometer, while that of the electric oven was set at a value of 120°C in the oven.

Statistical analysis

SPSS (15) was used for the statistical analysis. The data were subjected to Analysis of Variance (ANOVA) with the Duncan New Multiple Range Test (DNMRT) used to examine the differences in the mean values and the significance put at $P<0.05$.

RESULTS

The results of the proximate analysis of the dried fish samples from the five different solar driers, open sun drying, smoking kiln and electric oven are presented in Table 1. Significant differences ($P<0.05$) were observed in the proximate composition between the two species and among the nutrients composition in the two species. The percentage moisture content in the solar driers and open sun-drying ranged from 10.26 to 11.42% for *C. gariepinus* and 3.68 to 4.00% in *O. niloticus*. The highest percentage protein was 66.48% in *C. gariepinus* and 63.28% in *O. niloticus*. Crude fibre content in the two species was low ranging from 0.50 to 0.81% in *C. gariepinus* and 0.89 to 1.00% in *O. niloticus*. Fat content of the two species was highest in black stone inserted glass drier with a percentage of 8.96% for *C. gariepinus* and 7.82% for *O. niloticus*. The percentage of carbohydrate was highest in *O. niloticus* than in *C. gariepinus* with the highest of 17.00% recorded from the glass drier, while the lowest of 4.68% was observed for *C. gariepinus* also from the glass drier. The highest percentage of ash was found in *C. gariepinus* recorded from the smoking kiln, while

Table 1 - Proximate composition of *C. gariepinus and O. niloticus* dried under low cost solar driers, open sun drying, smoking kiln and electric oven.

Driers	Fish samples	Moisture %	Ash %	Crude fibre %	Protein %	Fat %	Carbohydrates %
Plastic	*C. gariepinus*	11.20	9.67	0.53	64.88	8.19	5.53
	O. niloticus	3.99	12.84	0.98	58.92	6.89	16.38
Mosquito net	*C. gariepinus*	11.02	9.61	0.51	64.96	8.21	5.69
	O. niloticus	3.68	12.69	0.89	59.81	6.93	16.00
Glass	*C. gariepinus*	10.82	8.92	0.54	66.20	8.84	4.68
	O. niloticus	3.95	12.58	0.92	58.75	6.80	17.00
Aluminum	*C. gariepinus*	10.95	8.46	0.52	65.95	8.64	5.48
	O. niloticus	3.79	13.23	0.91	60.24	7.20	14.63
Glass with black stone	*C. gariepinus*	10.77	8.28	0.55	66.48	8.96	4.96
	O. niloticus	3.60	13.40	0.87	63.28	7.82	11.03
Open sun drying	*C. gariepinus*	10.84	8.92	0.54	65.88	8.52	5.30
	O. niloticus	3.75	12.58	0.96	60.15	7.15	15.41
Smoking kiln	*C. gariepinus*	11.42	9.69	0.50	64.41	8.20	5.78
	O. niloticus	4.00	13.03	0.95	59.26	7.10	15.66
Electric oven	*C. gariepinus*	10.26	8.96	0.81	64.62	8.10	7.25
	O. niloticus	3.82	13.42	1.00	60.15	7.01	14.60

electric oven recorded the highest percentage in *O. niloticus*.

The mean maximum and minimum temperatures in the low cost solar driers, open sun-drying, smoking kiln and electric oven showed that electric oven had the mean maximum and minimum temperatures at 120 °C, followed by smoking kiln with rage of 70° to 90°C. The temperatures in the black stone inserted glass drier ranged between 24.50° to 60.50°C while open sun drying had the least temperature range of 20.00° to 38.00°C among all the driers (Table 2). Significant differences ($P<0.05$) in the maximum and minimum temperatures was observed in all the solar driers and in the open sun drying, smoking kiln and the electric oven.

DISCUSSION

The ranges of the nutrient composition of the two species dried under the five low cost solar driers were in agreement with other workers such as ADAM-SULIEMAN and SIDAHMED (2012). The nutrient composition of the two fish samples, *C. gariepinus* and *O. niloticus* dried under the different low-cost solar driers were high and compared favourably well with traditional open sun drying, smoking kiln and electric oven. The low moisture contents recorded in the two species showed the effectiveness of the driers in evaporating water from the fish samples. Although electric oven evaporated more water from the fish in less time than the other driers but the cost of the oven and electricity used for operation beyond the reach of most people in sub-Saharan Africa. AKINWUMI *et al.* (2011) has also reported that electric oven produced the lowest moisture content in dried fish samples.

Consequently, the low moisture content increased the protein content in the fish species by coagulating the crude protein. According to STEFFENS (2006), protein forms the largest quantity of dry matter in fish. CLUCAS (1982) reported that a fish with moisture content reduced to 25% is well dried and if further reduced to 15% growth of mould will cease and shelf life increased. The higher moisture content in the *C. gariepinus* was as a result of the fish having higher water content that *O. niloticus*. This observation has also been reported by GALLAGHER *et al.* (1991). The same scenario was also found in the fat contents of the fish species. SESUGH *et al.* (2012) recorded a higher fat content in smoked *C. gariepinus* than in smoked *O. niloticus*. Higher ash content, crude fibre and carbohydrates were however recorded in *O. niloticus*. Substantial loss of moisture was responsible for the higher ash content in the fish species, most especially from smoking kiln and electric oven which produced the highest ash contents on the account of a higher heat source used in the two drying methods. The ash content in *O. niloticus* was similar to the values recorded by IPINMOROTI (2012) on *Tilapia zilli*.

A low fibre contents was recorded in all the solar driers which was not significantly different from what was obtain in the smoking kiln and electric oven. AKINNEYE *et al.* (2007), AKINWUMI *et al.* (2011) and OGBONNAYA (2009) have all recorded low fibre content in *Clarias gariepinus* and *O. niloticus* dried with electric oven, open sun drying and smoking kiln.

Clarias gariepinus could be described as a fatty fish even after drying because of its higher fat contents which was higher than that of *O. niloticus*. Low fat content in *O. niloticus* has been reported by OMOTOSHO and OLU (1995). Lower fat content which was observed in smoking kiln and electric oven was due to the high heat treatment of the two driers which tend to exude the fats from the species. OGBONNAYA (2009) had noticed low fat contents in fish dried with smoking kiln. The higher percentage of carbohydrates, low fat and low protein in *O. niloticus* than *C. gariepinus* could be explained in terms of the feeding habits of the species being a herbivorous one which forages on phytoplankton, while *C. gariepinus* is mainly a carnivorous species feeding on proteineous prey. The high fibre content in *O. niloticus* could also be related to the high carbohydrates in fish. *O. niloticus* is a lean fish with low fat content as compared to *C. gariepinus* even after drying.

Drying in these solar driers have shown to significantly reduce the moisture content, improve protein quality and increasing the shelve life of the two species. Glass drier with black stone was the most efficient of the solar driers due to its ability to evaporate large amount of water and high protein content. The drier was able to achieve this feat because of the black stone in it which absorb, retain and radiate heat to the fish samples. Although complete drying in these solar driers takes on the average a total of 10 days with relative effect of rainfall. BERKEL (2004) had reported that naturally fish needs about 3-10 days to dry. These solar driers were found

Table 2 - Mean maximum and minimum temperatures in the solar driers, open sun drying, smoking kiln and electric oven.

Driers	Maximum temperature (°C)	Minimum temperature (°C)
Plastic	50.0	22.0
Mosquito net	46.0	21.0
Glass	60.5	24.5
Aluminum	59.0	24.0
Glass with black stone	56.5	20.5
Open sun drying	38.0	20.0
Smoking kiln	90.0	70.0
Electric oven	120.0	120.0

to be faster and efficient and effective in drying the two species than the open sun drying. This is in agreement with CHAVAN *et al.* (2011) observation that solar drier are faster in drying than open sun-drying, while BERKEL (2004) showed that the quality of products dried with solar driers are higher than smoking.

CONCLUSION

The proximate analysis of the solar dried fish species showed that the nutrient composition especially the moisture contents of the species which was reduced significantly and the protein contents which was significantly higher to be good enough for consumption after drying. The nutritional qualities of the fish samples dried by these solar driers were high, hygienic, better, more preserved and acceptable.

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