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REVIEW

APPLICATIONS OF MOLECULAR DISTILLATION TECHNIQUE IN FOOD PRODUCTS

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ABSTRACT

There are several separation techniques -including conventional distillation- for extracting heat sensitive compounds from food products. However, some compounds may have high boiling points at which other compounds might be adversely affected. Vacuum application is also needed for such kinds of foods. Molecular distillation is an advanced vacuum distillation method performed by short-path evaporators. Distance between evaporator and condenser is extremely reduced which results in minimized pressure drop. Heat sensitive material meets heat for a short time under high vacuum, thus low or no decomposition occurs. This review aims to discuss the basics and uses of molecular distillation in foods.

- Keywords: molecular distillation, purification, separation, short-path, vacuum -

INTRODUCTION

Distillation is a simple physical separation process of liquid mixtures based on differences in boiling points of components in the mixture. Very first usage of distillation dates back to 1st century (FORBES, 1970). Further experiments leaded to new knowledge that is known as fundamentals of distillation now. In 1830, Aeneas Coffey - an Irish inventor - patented his distillation column (GAISER *et al.*, 2002). Coffey's column (a.k.a "continuous still", "patent still" or "Coffey still") achieved to reach higher concentrations of alcohol. In 20th century, some special distillation equipments and techniques were produced in correlation with increasing innovations in petrochemical industry. Especially in chemical and food research, demand for extracting compounds with high purity leaded to development of computer aided systems. Today, several distillation techniques are present for various purposes. Appropriate distillation method should be chosen depending on properties of liquid mixture and distillation equipment. Some of the specific distillation methods could be listed as:

- Repeated evaporation-condensation cycles, known as fractional distillation.
- Steam distillation of heat sensitive materials (Harwood and Moody, 1989)
- Vacuum distillation of heat sensitive materials under reduced pressure.
- Reactive distillation
- Azeotropic distillation
- Extractive distillation
- Catalytic distillation
- Molecular distillation, an advanced vacuum distillation method.

General information on molecular distillation

Pure substances have certain vapor pressure values related to vaporization temperature. These vapor pressure-temperature data are plotted to a P-T diagram, which is called "phase diagram". Fig. 1 demonstrates a sample phase diagram of any pure substance.

As seen in Fig. 1, vaporization temperature (or boiling point) decreases when the ambient pressure is reduced along the vaporization curve. This principle is the basis of vacuum distillation. Distillation of compounds, which may be decomposed at high boiling points and/or may be air-sensitive can be possible with vacuum distillation. Typically, there are two types of vacuum distillation:

- Simple vacuum distillation: applied when higher vacuum levels are not needed. Ex: rotary evaporators, Perkin triangle.
- High vacuum distillation: applied when higher vacuum levels are needed for separation. Purity of distillate is higher than those of other distillation techniques. Ex: thin film evaporators (TFE) and short-path distillation equipment (SPD).

According to SHI *et al.* (2007), distillation method can be called as molecular distillation if the distance between evaporator and condenser reaches to mean free path of a vapor molecule. Lei *et al.* (2005) described the mean free path, $< \lambda >$, with the following equation:

$$< \lambda > = \frac{RT}{\sqrt{2}\pi d^2 N_A P}$$

where d (m) is the diameter of molecule, N_A is Avogadro constant (6.023×10^{23} mol^{-1}), P (Pa) is pressure, R is universal gas constant and T (K) is temperature. LUTIŠAN and CVENGROŠ (1995) defined molecular distillation as "the safest method to separate and purify thermally unstable compounds". SHI *et al.* (2007) pointed out that risk of thermal decomposition could be reduced with low temperature; as well oxidation could be prevented with air removal by vacuum. In addition, DE MORAES *et al.* (2006) drew one's attention to advantages of molecular distillation (e.g. avoiding toxicity, protect environment) that other chemical agent-based techniques do not have. LUTIŠAN and CVENGROŠ (1995) defined the main features of molecular distillation as; short time of exposure to heat, low evaporating temperature and a characteristic mass transfer. According to MARTINELLO *et al.* (2007), "small distance between evaporator and condenser" can also be defined as a feature of molecular distillation.

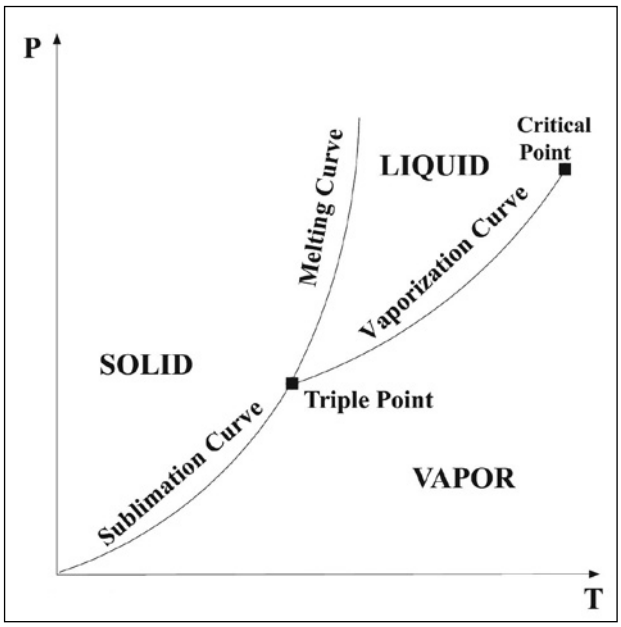

Fig. 1 - Sample phase diagram of any pure substance.

HIGH VACUUM AND MOLECULAR DISTILLATION EQUIPMENT

There are typically two types of evaporators used in high vacuum distillation, i.e. thin film evaporators (TFE) and short-path evaporators (SPE). These equipments have similar designs with few differences. In both evaporators, feed is agitated with a rotor-wiper system and high vacuum is produced by vacuum pumps. In TFE, operating pressure can be reduced to 1-100 mbar (UIC GmbH, 2014) and there is no other unit between vacuum and condenser (PILODIST, 2014). Fig. 2 shows an illustration of a TFE.

In SPE, condenser is placed in the centre of evaporator unit, so distance between boiling and condensation surface is extremely reduced and pressure drop is minimized. The operating pressure can be reduced up to 0.001 mbar. Distillation performed by a short-path evaporator is also called as "molecular distillation" (Buss-SMS-Canzler GmbH, 2014a; Buss-SMS-Canzler GmbH, 2014b; PILODIST, 2014; TECHNOFORCE, 2014). Fig. 3 shows an illustration of a SPE.

There are many parameters that can affect distillation yield and molecular evaporation rate. Molecular evaporation rate, k_i, can be calculated by Langmuir-Knudsen equation (ROSSI *et al.*, 2011):

$$k_i = \frac{P_{v_i}(T^S)}{\sqrt{2\pi R M_i T^S}}$$

where T^S is evaporation temperature, R is universal gas constant, M_i is molecular weight of evaporating component and P_{v_i} is vapor pressure of component. XU *et al.* (2002) describes the most important parameters of molecular distillation as evaporator temperature, flow rate, vacuum and wiper speed. Flow rate has an important effect on the contact time of the molecules with hot surface during evaporation. Higher flow rates reduce the residence times of molecules being vaporized. Wiper speed affects film thickness and viscosity. Feed becomes highly turbulent with intensive agitation, which leads to high heat transfer coefficients (Buss-SMS-Canzler GmbH, 2014c).

MOLECULAR DISTILLATION IN FOOD PROCESSING: SOME EXAMPLES OF RECENT STUDIES

Molecular distillation has many application areas in food industry. Some of these applications can be summarized as but not limited to: concentration of ω-3 fatty acids, distillation of monoglycerides from di- and triglycerides, concentration of tocopherols and tocotrienols (Buss-SMS-Canzler GmbH, 2014d), fractionation of squalene (SUN *et al.*, 1997), recovery of carotenoids (BATISTELLA and WOLF-MACIEL, 1998). As distillation is a separation process, studies about molecular distillation generally focus on either removal of undesired compounds or concentration of valuable compounds.

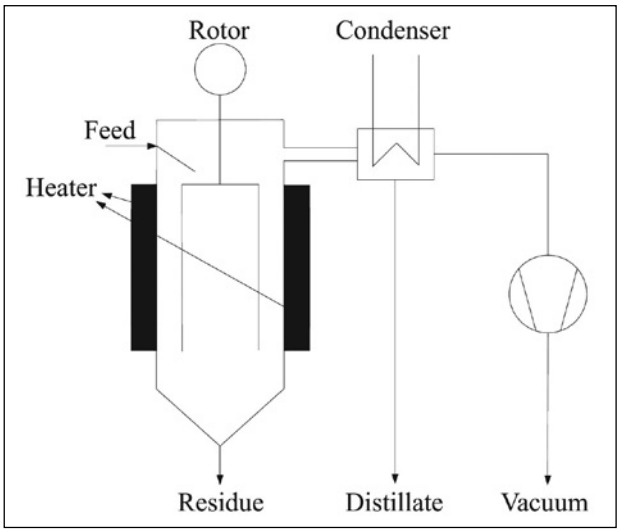

Fig. 2 - Illustration of a TFE unit.

Removal of undesired compounds

In a study about removal of cholesterol from butter and lard by using molecular distillation (LANZANI *et al.*, 1994), researchers reported that cholesterol content of lard was reduced from 988 ppm to 105 ppm in the residue after 2 hours of distillation under 10^{-4} torr pressure and 250°C evaporator temperature.

Molecular distillation can also be used for physical deacidification. MARTINS *et al.* (2006) separated free fatty acids (FFA) from vegetable oil deodorizer distillate. They achieved to reduce

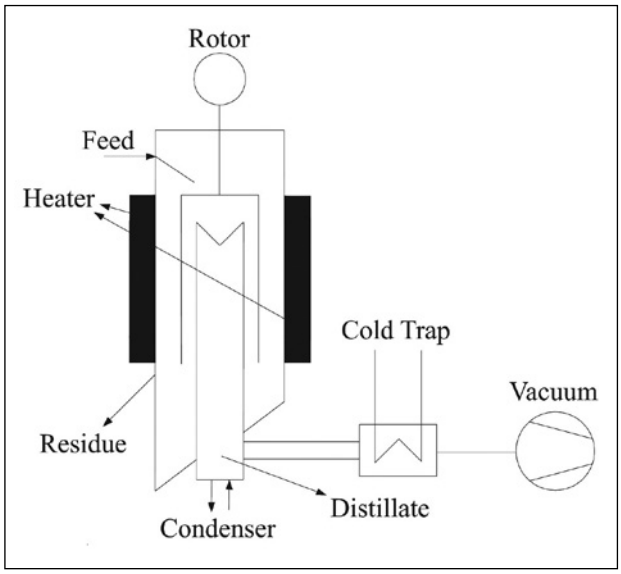

Fig. 3 - Illustration of a SPD unit.

FFA content to 6.4% from initial FFA content of raw material with 57.8% at 160°C evaporator temperature, under 10^{-6} bar pressure and 10.4 g min^{-1} feed flow rate. They also noted that concentration of tocopherol in residue stream was found 18.3%, while initial tocopherol concentration was 8.97%. FFA elimination was 96.16% and tocopherol recovery was found 81.23%.

WANG *et al.* (2010) aimed to separate FFAs and diacylglycerols (DAG) from enzimatically hydrolyzed soybean oil. They achieved to increase the removal of FFAs from 88.8% to 99.44% by increasing evaporator temperature from 125°C to 160°C, under 0.5-1.0 Pa process pressure, 200 mL h^{-1} feed rate and 300 rpm wiper speed.

OLLI *et al.* (2013) studied removal of organic pollutants in fish oils. Their SPD system, which has an evaporator temperature of approx. 220°C and operating pressure below 0.03 mbar, achieved to remove total amount of chlorinated pesticides (some of them are DDT and HCH) from 215.07 ng g^{-1} to 21.95 ng g^{-1}, corresponding to 89% reduction.

According to MEYER *et al.* (2011), total pesticide traces in rapeseed deodorizer distillate were dropped below 0.05 mg kg^{-1} from an initial content of 0.968 mg kg^{-1} by achieving more than 94.8% reduction. SPD evaporator temperature was set to 110°C, feed flow rate was 200 mL h^{-1} and pressures were between 0.006 and 0.01 mbar. Researchers stated that it would be a mistake to affirm that all types of pesticides were removed by using SPD according to this reduction data, because many different types of pesticides might be present before distillation and analysis of effects on specific compounds has to be performed.

Concentration and/or fractionation of compounds

BATISTELLA and WOLF-MACIEL (1998) studied the recovery of carotenoids from palm oil by using a molecular distillator and after a set of distillation trials, they achieved to increase carotene concentration to 19500 ppm from an initial feed concentration of 600 ppm under $9x10^{-5}$ torr pressure and 170°C evaporator temperature.

SUN *et al.* (1997) fractionated squalene from alkali-refined amaranth seed oil and their highest recovery of squalene was 67.8% with SPD conditions of 100 mtorr pressure and 180°C distillation temperature.

Campos *et al.* (2003) fractionated milk fat by SPD and recorded distillate yields (w/w) as a function of temperature. They observed that distillate yield was 0.3% at 125°C process temperature; however a 42.7% recovery was observed when process temperature was increased to 250°C, which meant a significant and positive effect of temperature on process efficiency.

SPD was performed on lemongrass essential oil by TOVAR *et al.* (2011) and researchers reported that they were able to increase citral concentration in distillate stream from 17.658 mg mL^{-1} to 33.576 mg mL^{-1} when evaporator temperature was increased from 60°C to 120°C with a feed flow rate of 1.5 mL min^{-1} and pressure of 5 Pa.

Mono and diglyceride (MDG) concentration and production are also possible with molecular distillation. FREGOLENTE *et al.* (2010) produced partial glycerides from soybean oil by using molecular distillation. Concentration of monoglyceride (MG) in distillate stream increased with elevated evaporator temperature. At 250°C with 10 mL min^{-1} feed flow rate, MG concentration was increased from initial feed value of 12.75% to 80.00% in distillate stream under 24 Pa operating pressure. They also pointed that lower flow rate increased recovery of MG, because molecules contacted with hot evaporator surface for a longer period of time. Recovery for any component is defined with following equation:

$$Recovery(\%) = 100\, x \frac{Distillate}{Feed}$$

ZHANG *et al.* (2013) studied effects of evaporation temperature, feeding rate, feeding temperature and wiper speed on concentration of ω-3 fatty acids by molecular distillation and optimized these parameters with response surface methodology (RSM). Researchers reported the optimum conditions as 110.4°C evaporator temperature, 78.7 mL h^{-1} feeding rate, 350 rpm wiper speed, 10 Pa operating pressure and 80°C feed temperature.

CONCLUSIONS

Separation techniques such as extraction, evaporation, distillation etc. are accepted as unit operations in food industry. Vacuum distillation is frequently used both in chemical and food industries; however simple vacuum distillation might not be capable of separation of heat-sensitive materials from food products. In that case, molecular distillation (short-path distillation) should be used for separation of these materials. Molecular distillation has been used more in pharmaceutical, chemical and petrochemical applications, but nowadays importance of molecular distillation has increasingly been understood in food industry. Separation, concentration and purification of commercially valuable food constituents can be easily performed by molecular distillation; furthermore, healthier food products can be produced by removal of some health damaging compounds such as excess cholesterol, organic pollutants. Authors expect an increasing trend in usage of molecular distillation in food industry when taking all these applications into consideration.

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PAPER

ANTIBIOTIC SUSCEPTIBILITY OF POTENTIALLY PROBIOTIC *LACTOBACILLUS* STRAINS

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ABSTRACT

Susceptibility of 29 *Lactobacilli* to 13 antibiotics was assayed by paper disc diffusion method. Plasmids and gastrointestinal tolerance were detected. The relationship between plasmids and antibiotic resistance was discussed. The results showed that all of the strains were resistant to bacitracin, polymyxin B, kanamycin, and nalidixic acid. Many strains were relatively sensitive to chloramphenicol and tetracycline. Six strains contained plasmids and showed good gastrointestinal tolerance. β-lactam resistance gene *blr* was found in the plasmid of *L. plantarum* CICC 23180 by PCR. The study will be helpful to promote the safety evaluation and development of potentially probiotic lactic acid bacteria.

- Keywords: antibiotic resistance; *Lactobacillus*; gastrointestinal tolerance; plasmid; probiotic -

1. INTRODUCTION

Due to the claimed benefits, *Lactobacillus* bacteria are widely used in food, feed, medical and health related fields. Many lactic acid bacteria (LAB), such as *Streptococcus thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus*, have been used safely for a long history. They are agreed to be secure and do not have the possibility of pathogenic. Currently, new beneficial bacteria are being developed continuously and will enter the market. However, the security of these new strains has caused great concern. Evaluation of antibiotic sensitivity is an important part of safety assessment.

Now, overuse of antibiotics has become a serious social problem. This led to the emergence of a large number of antibiotic-resistant strains. Once the resistance-related factors are tranferred to other microorganisms, especially pathogens via food carrier, it will cause tremendous problems. The evolution of antibiotic-resistant foodborne pathogens has been widely reported (THRELFALL *et al.*, 2000; WALSH *et al.*, 2008; WHITE *et al.*, 2002). Moreover, the resistance and resistance-related genes of *Bifidobacterium*, *Lactobacillus* and *Pediococcus* strains to different antibiotics were studied systematically (HUMMEL *et al.*, 2007; HUYS *et al.*, 2004; MARIA *et al.*, 2007). The *tetM* gene transfer of tetracycline resistance in *Lactobacillus plantarum* among strains was reported by NIAMH *et al.* (2010).

In this study, 29 *Lactobacillus* strains isolated from the food environment with potentially probiotic effects (JIN *et al.*, 2009; LI *et al.*, 2009; LIU *et al.*, 2011; SUN *et al.*, 2009; ZHAO *et al.*, 2013) were used. These strains were assayed for susceptibility to 13 antibiotics by agar disc diffusion method. Furthermore, some strains with higher resistance were analysed for the presence of plasmids. Then, the tolerance of the plasmid-containing strains under simulated gastrointestinal conditions was investigated. By plasmid elimination and PCR, the relationship between the plasmid profiles and resistance patterns of the strains was explored. This will provide a reference for the safety evaluation method and also will be helpful to improve the evaluation system of probiotics.

2. MATERIALS AND METHODS

2.1 Bacterial strains and cultivation

29 *Lactobacillus* strains used in the study were listed in Table 1. *Lactobacillus* strains were cultured in MRS (De Man, Rogosa, and Sharpe) medium at 37°C for 18h under anaerobic condition.

Quality control strain recommended by Clinical and Laboratory Standards Institute (CLSI) in the antibiotic sensitivity test was *E. coli* ATCC25922 purchased from the Institute of Microbiology, Chinese Academy of Sciences. The *E. coli* ATCC25922 was activated and cultivated in LB medium (yeast extract 5 g/L, tryptone 10 g/L, NaCl 10 g/L) at 37°C.

2.2 Testing of antibiotic susceptibility

13 kinds of antibiotics paper discs were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Table 2), each piece with a diameter of 6.5 mm. The quality was fully complied with the WHO criteria.

Antibiotic susceptibility was semi-quantitatively determined with K-B method by antibiotic paper disc diffusion referring to the CLSI as described by CHARTERIS *et al.* (1998a).

Briefly, 1.0 mL *Lactobacillus* suspension (approximately 1.5×10^8 CFU/mL) was added to sterile petri dish with diameter of 90 mm, and then mixed with a 15 mL MH (Muller Hinton, MH) agar (beef extract powder 6g/L, casein ac-

Table 1 - Source of the tested strains for antibiotic susceptibility test.

Species	Source (original number)
Lactobacillus plantarum	CICC[a] 23124 (L11), CICC 23131 (B31), CICC 23135 (B37), CICC 22195 (C35), CICC 23166 (ZJ1), CICC 23138 (C8-1), CICC 23180 (CH8)
Lactobacillus rhamnosus	CICC 23119 (1132), CICC 22175 (LL), ATCC[b] 7469, CICC 22151 (LK-Mt), CICC 22173 (R11)
Lactobacillus salivarius	CICC 23182 (CH-10)
Lactobacillus acidophilus	CICC 22162 (CH-2)
Lactobacillus casei	CICC 23184 (Y5-2b)
Lactobacillus helveticus	CICC 22154 (LLB)
Lactobacillus pentosus	CICC 23116 (SN23), CICC 22161 (Lp-4), CICC 22160 (Lp-5), CICC 22159 (Lp-B), CICC 22156 (Ind-3), CICC 22157 (Lp-A)
Lactobacillus paralimentarius	CICC 22148 (412), CICC 22149 (413)
Lactobacillus delbrueckii	CICC 22153 (LB), CICC 22163 (LC)
Lactobacillus paracasei	CICC 22165 (5M1), CICC 22167 (5M7), CICC 23183 (D-400)

[a]CICC, China center of industrial culture collection. [b]ATCC, American type culture collection.

Table 2 - The content of antibiotic paper discs and criterion for judgement.

Antibiotics	Content/disc	inhibition zone diameter (mm)		
		R[c]	I	S
Vancomycin	30 μg	≤9	10-11	≥12
penicillin G	10 U	≤14	15-17	≥18
ampicillin	10 μg	≤21	22-28	≥29
Bacitracin	0.04 U	≤10	10-12	≥12
cephalothin	30 μg	≤14	15-17	≥18
streptomycin	10 μg	≤11	12-14	≥15
kanamycin	30 μg	≤13	14-17	≥18
tetracycline	30 μg	≤11	12-14	≥15
chloramphenicol	30 μg	≤12	13-17	≥18
gentamicin	10 μg	≤12	13-14	≥15
nalidixic acid	30 μg	≤13	14-18	≥19
multi-polymyxin B	300 μg	≤11	12-14	≥15
rifampicin	5 μg	≤16	17-19	≥20

[c]Note: R-Resistant; S-Susceptible; I-Intermediate.

ids hydrolysate 17.5 g/L, soluble starch, 1.5 g/L, agar 17 g/L, pH 7.3±0.1) until the medium solidified. The antibiotic paper discs were pasted closely onto the solidified medium with sterile tweezers after 5min at room temperature. Three discs were pasted in each dish. The distance was more than 24 mm of each disc center and more than 15 mm from disc edge to the inner edge of dish. Next, the dishes were placed at room temperature for 1.5 h and then incubated at 37°C. After 24 h, the inhibition zone diameter was measured around the antibiotic disc with vernier caliper and recorded. For one tested strain, each antibiotic disc was done 3 times. The inhibition zone diameter was averaged

Standard sensitive strain of *E. coli* ATCC25922 was used as the control. The operation was the same as the above.

The antibiotic susceptibility of the tested strains was evaluated according to the CLSI criteria (Table 2).

2.3 Plasmid DNA extraction

10 mL of *Lactobacillus* suspension cultured overnight was centrifugated at 10000 rpm for 5 min. Then the precipitation was suspended with 500 μL of lysozyme solution (10 mg/mL). The mixture was placed in a water bath for 45 min at 37°C. Then plasmid DNA of *Lactobacilli* strains was extracted and purified with DNA extraction and purification kit of Tiangen Biotech (Beijing) Co., LTD. Plasmid DNA was observed by agarose gel electrophoresis.

Antibiotic susceptibility and plasmid stability were tested after cultivated 30 generations at 37°C in MRS medium according to the above methods.

2.4 Gastrointestinal tolerability test

In order to explore the application safety in human, the gastrointestinal tolerability of those lactic acid bacteria containing the plasmids were tested.

For acid tolerance test, Lactobacillus cells were harvested by centrifugation at 6000 rpm for 15 min, washed twice with 0.01 mol/L PBS, pH 7.2 after cultured for 18 h at 37°C in MRS broth, and then suspended in 20 mL sterile saline (0.85%, w/v) adjusted to pH 2.5 with sterile hydrochloric acid.

For bile tolerance test, the modified method of LEE *et al.* (1999) was referred to test bile tolerance. The *Lactobacillus* cells were centrifuged (6000 rpm, 15 min) after cultivated for 18 h at 37°C in MRS broth and suspended in 20 mL sterile saline (0.85 %, w/v) supplemented with 0.3% (w/v) bile salts (taurocholate, Sigma) at pH 6.8.

For pepsin and trypsin tolerance test, *Lactobacillus* cells were centrifuged (6000 rpm, 15 min) after cultivated for 18 h at 37°C in MRS broth, then suspended in 20 mL sterile simulated gastric and pancretic juices. Fresh simulated gastric and pancreatic juices were prepared daily according to Charteris *et al* (1998b). Pepsin (Sigma) was added into the simulated gastric juice with a final concentration of 5 mg/mL. Then the pH was adjusted to 2.5 with sterile hydrochloric acid. Trypsin (Sigma) was added into the simulated pancreatic juices with a final concentration of 10 mg/mL. Then pH was adjusted to 8.0 with 0.1 M NaOH.

All of the tolerability detection, the initial bacterial counts were adjusted to about 10^8 CFU/mL and were checked by viable count determination on MRS agar. For the tolerance assay, the bacterial suspensions were incubated and counted at 37°C for 0,1,2,3,4,5,6 h, respectively.

All tests were repeated three times to estimate the standard error.

2.5 Detection of antibiotic resistance genes

Part of the antibiotic-resistant genes of those lactic acid bacteria containing both plasmids and high tolerance were investigated. The β-lactam resistance-related gene sequence of *blr*, *ECP-1569*, *nps-1* and the chloromycetin resistance-related gene sequence of *cmlA*, *cat*, *cmlA1* in plasmids were found in National Center for Biotechnology Information (NCBI). The primers were designed and synthesized by Beijing Sunbiotech Co., LTD (Table 3).

The PCR programmes were performed with the plasmid template of the tested strains according to the following procedures: initial heating at 94°C for 4 min was followed by 34 cycles of the following sequence: 94°C for 30 s, 72°C for 1 min, and 72°C for 1 min. Final extension took place at 72°C for 7 min.

The amplification products were separated

Table 3 - The primers of the resistance genes in the experiment.

Gene	Sequence of the primer	Annealing temperature	Fragment size
blr--up *blr*--down	5'-CGTCTTATTGAATTAACAGGTTGG -3' 5'-CACGAAGCCATGTTGTGTTC -3'	53°C	125 bp
ECP-1569--up *ECP-1569*--down	5'-CAATCAACAGAGATGTGGGCTG-3' 5'-GTACCGTAGTACTCTGTTCAGGTGG-3'	57°C	155 bp
nps-1--up *nps-1*--down	5'-TCATTCTTCTGGCCTGTAGC-3' 5'-GGCGATACCGCTCAGTTAC-3'	54°C	782 bp
cmlA--up *cmlA*--down	5'-CAAGGAGATGGTTTCGTGCG-3' 5'-CATGCCCAAACCTAGAAACGC-3'	56°C	551 bp
cat--up *cat*--down	5'-GGCATTTCAGTCAGTTGCTC-3' 5'-TGGAAGCCATCACAAACG-3'	55°C	530 bp
cmlA1--up *cmlA1*--down	5'-GCTGAAGCCAAGCTGAGAC-3' 5'-CTACGTTGTGGCGTCAATG-3'	56°C	492 bp

by conventional 1.0% (w/v) agarose gel electrophoresis (100V, 4°C) in TAE (tris-acetate-EDTA) buffer and visualised by ethidium bromide staining. The target fragment was recovered and sequenced by TaKaRa Biotechnology (Dalian, China) Co., Ltd. The resistance-related gene of plasmid was determined by comparison with the known fragment.

3. RESULTS AND DISCUSSION

3.1 Antibiotic susceptibility

Antibiotic susceptibility of the tested strains was evaluated according to the anti-microbial drug sensitivity standard of CLSI criteria. The sensitivity of the tested *Lactobacillus* to 13 kinds of antibiotics was shown in Table 4. The tested 29 strains were generally resisitant to multi-polymyxin B, bacitracin, kanamycin, nalidixic acid, and were mostly sensitive to chloramphenicol and tetracycline. The same species of *Lactobacillus* generally had similar resistance patterns. But there was species specificity such as the different antibiotic sensitivity in *L. plantarum*, *L. rhamnosus*, and *L. pentosus*. Moreover, the antibiotic-resistant level of different strains is also different.

Antibiotic resistance of the foodborne lactic acid bacteria had heen reported in the 1980s. The researchers generally believed that the resistance was a result of the long evolution and it was generally endogenous resistance and obtained resistance (Zeng *et al.*, 2004). So, the resistant lactic acid bacteria of natural or isolated from human intestinal can indirectly reflect the habitat of used antibiotic.

It can be seen from Table 5, the 29 strains showed different patterns of resistance to 13 kinds of antibiotics. To bacitracin, polymyxin B, kanamycin and nalidixic acid, the resistance rate of the 29 tested strains was 100%. To β-lactam and aminoglycosides, the resistance percentage was 20.7%-37.9% and 86.2%-100%, respectively. All of the 29 strains were mostly sensitive to chloramphenicol and tetracycline.

Among of the tested antibiotics, the nalidixic acid and polymyxin B can inhibite DNA synthesis and interfer cell membrane formation, respectively. The resistance of lactobacillus to these kinds of antibiotics may be due to the thicker cell wall of Gram-positive bacteria. While the tested strains showed different sensitivity to the antibiotics, such as streptomycin, kanamycin, tetracycline, chloramphenicol, gentamicin with protein synthesis inhibitition effect. Most lactobacillus strains showed resistance to those antibiotics against gram-negative bacteria, for example, streptomycin, gentamicin, kanamycin. This was consistent with report of Zhang *et al* (2007).

3.2 Plasmid DNA extraction of antibiotics-resistant lactobacillus strains

16 CICC strains with relatively strong antibiotic resistance were screened for plasmid extraction. As can be seen from Fig. 1, among these strains, only CICC 23180, 22161, 22175, 22157, 23124, and 22154 contained plasmids.

L. plantarum CICC 23180 showed 6 plasmid DNA bands and there is one band greater than 23 kb. *L. pentosus* CICC 22157 showed two plasmid DNA bands of 10 kb and 5 kb, respectively. *L. rhamnosus* CICC 22175 and *L. plantarum* CICC 23124 contained respectively 2 and 4 of plasmid DNA bands and both of the two strains contained a 10 kb plasmid. *L. helveticus* CICC

Table 4 - The sensitivity results of 29 *Lactobacillus* strains to 13 antibiotics.

	L plantarum							*L pentosus*							*L rhamnosus*				*L salivarius*	*L acidophilus*	*L casei*	*L.helveticus*	*L paracasei*			*L delbrueckii*		*L paralimentarius*	
	CICC 23166	CICC 23131	CICC 23180	CICC 23135	CICC 23124	CICC 23138	CICC 22195	CICC 22161	CICC 22157	CICC 22160	CICC 22159	CICC 22156	CICC 23116	CICC 22175	CICC 23119	ATCC 7469	CICC 22151	CICC 22173	CICC 23182	CICC 22162	CICC 23184	CICC 22154	CICC 22165	CICC 22167	CICC 23183	CICC 22153	CICC 22163	CICC 22148	CICC 22149
Vancomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	S	S	R	R
penicillin G	R	R	S	R	R	R	R	S	R	—	R	S	S	—	R	R	—	—	S	S	S	S	S	S	S	S	S	S	R
cephalothin	R	R	R	R	S	S	S	—	—	—	—	S	S	R	S	—	—	—	—	—	S	S	S	S	S	S	S	—	—
Bacitracin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
ampicillin	R	—	—	—	—	—	—	—	—	R	—	R	—	R	—	—	R	—	R	—	—	R	—	—	R	—	—	R	R
Multipolymysin B	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
streptomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	—	R	R
kanamycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
tetracycline	R	—	S	R	S	—	—	—	S	S	S	S	—	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
chloramphenicol	—	S	R	S	S	S	S	R	S	—	—	—	—	R	S	—	—	—	—	S	—	—	S	S	S	S	S	—	S
gentamicin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	—	R	R
nalidixic acid	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
rifampicin	R	R	R	—	R	R	S	R	—	R	—	S	S	—	S	S	S	S	R	R	S	S	S	S	S	S	S	S	R

Table 5 - The percentage of the antibiotic resistance of 29 *Lactobacillus* strains.

Antibiotics	Quantity of resistant strains	Percentage of resistance (%)
vancomycin	26	89.7
penicillin G	11	37.9
cephalothin	6	20.7
bacitracin	29	100
ampicillin	10	34.5
multi-polymyxin B	29	100
streptomycin	27	93.1
kanamycin	29	100
tetracycline	3	10.3
chloramphenicol	3	10.3
gentamicin	25	86.2
nalidixic acid	29	100
rifampicin	10	34.5

22154 showed only one plasmid DNA band of about 10 kb.

Lactic acid bacteria generally contain plasmids. The plasmid size was usually 1.9 kb-84.8 kb. Most of the plasmid was less than 20 kb (WANG and LEE, 1997). In the culture process from generation to generation, many plasmids might disappear from the bacterial cell, but most of the plasmids were stable. In the study, the plasmids of the above six strains and the antibiotic susceptivity showed no changes after cultivated 30 generations.

3.3 Gastrointestinal tolerability

Resistance to gastrointestinal stress is very important for one strain to play the potential probiotic function (GUGLIELMOTTI *et al.*, 2007). If the strains have a high tolerance to gastrointestinal stress, it will have the chance to survive and play the probiotic effects in the gastrointestinal environment.

The tolerance of the selected six strains to low

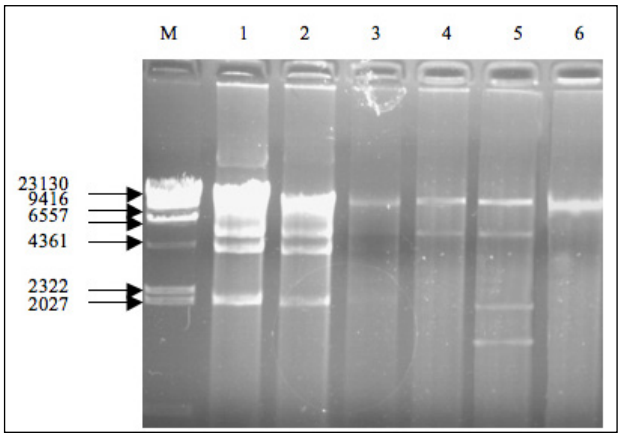

Fig. 1 - The plasmids in *Lactobacillus* (1.CICC 23180, 2.CICC 22161, 3.CICC 22175, 4.CICC 22157, 5.CICC 23124, 6.CICC 22154. M. λHindIII marker).

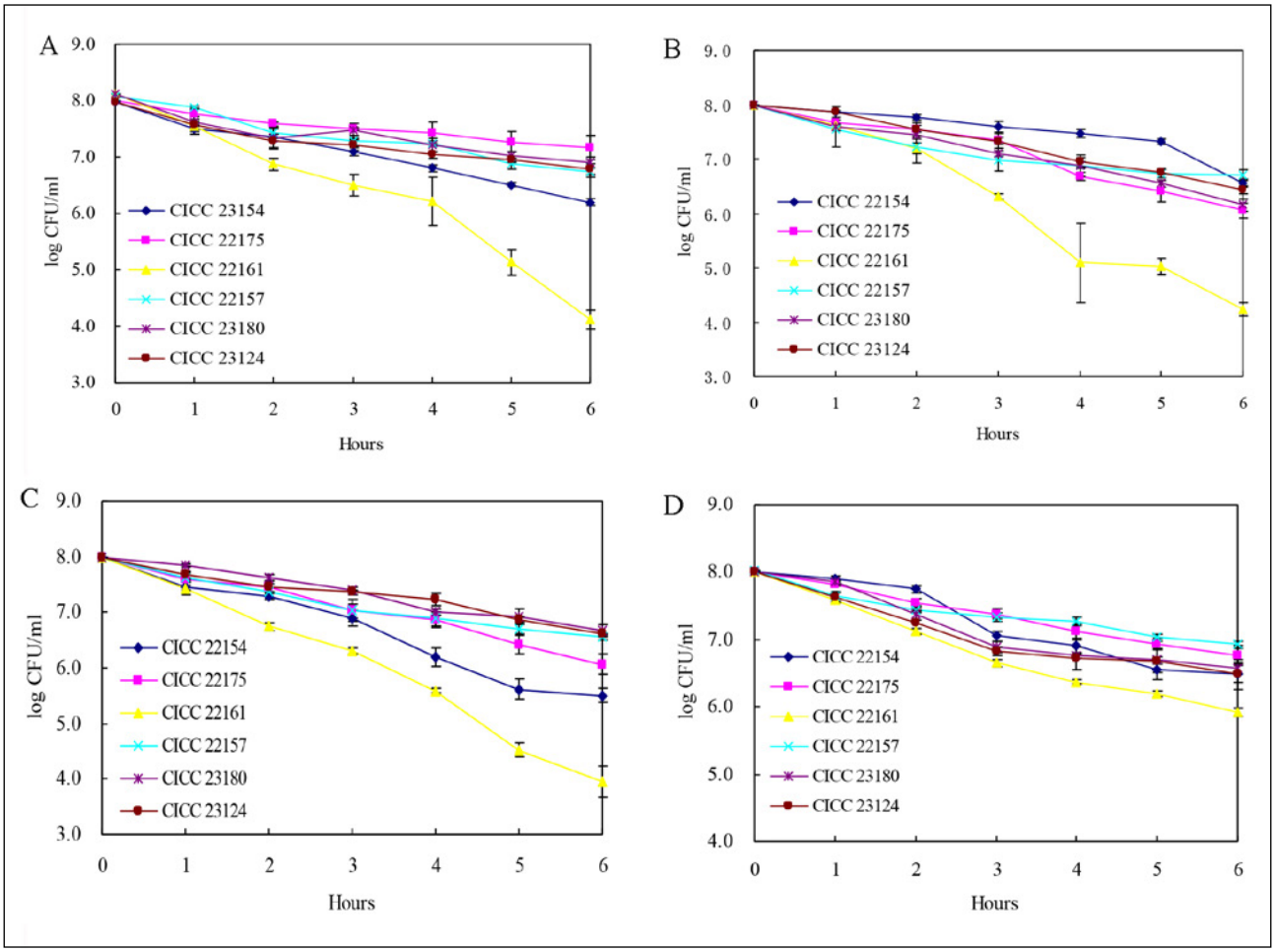

Fig. 2 - The viable counts of strains [C]CICC 22154, 22175, 22161, 22157, 23180 and 23124 in the gastrointestinal environment after 6 hs at 37°C.
A: pH 2.5; B: 3 mg/mL bile; C: 5mg/mL pepsin; D: 10 mg/mL trypsin.

pH, bile salt, pepsin and trypsin is presented in Fig. 2. As shown in Fig. 2A, the viable counts of *L. pentosus* CICC 22161 strain reduced to below 10^6 CFU/mL after 4 h and 1.32×10^4 CFU/mL after 6 h. However, the viable numbers of *L. helveticus* CICC 22154, *L. pentosus* CICC 22157, *L. plantarum* CICC 23124, 23180 and *L. rhamnosus* CICC 22175 were still more than 10^6 CFU/mL after 6 h in the gastric acid of pH 2.5. Thus, these five strains showed higher tolerance in acid environment.

For bile tolerance, except the *L. pentosus* CICC 22161, the viable counts of the other five strains were still more than 10^6 CFU/mL after 6 h in the medium containing bile salt (Fig. 2B). However, the viable cells of *L. pentosus* CICC 22161 had decreased to 2.0×10^6 CFU/mL within 3 h. And it declined to only 1.8×10^4 after 6 h.

For pepsin tolerance, among of six strains, the viable cells of *L. pentosus* CICC 22161 and *L. helveticus* CICC 22154 decreased significantly in 6 h and it is less than 10^4 CFU/mL and 10^6 CFU/mL after 6 h exposure to 5 mg/mL pepsin solution (pH 2.5), respectively (Fig. 2C).

For trypsin tolerance, as can be seen in Fig. 2D, the viable counts of the tested six strains still remained at 10^6 CFU/mL or more after 6 h exposure to 10 mg/mL trypsin solution (pH 8.0).

3.4 Detection of Resistance genes

According to the above results, except strain *L. pentosus* CICC 22161 and *L. helveticus* CICC 22154, the tested strains may be able to survive in the simulated gastrointestinal environment. However, if the above strains contain antibiotics-resistant plasmids, there is the possibility of resistance transfer to other bacteria, especially pathogenic bacteria. It will be a potential hazard to human health and be a serious social problem. So, the plasmid-determined resistant gene should be checked firstly before subsequent utilization.

After 0.02% SDS combined with heat treatment of the four strains (CICC 22175, 22157, 23124, 23180), only the plasmids of *L. plantarum* CICC 23180 were removed and the resistance to cephalothin and chloromycetin disappeared simultaneously (unpublished results). So, the primers of β-lactam resistance-relat-

ed genes including *blr*, *ECP-1569* and *nps-1* as well as chloromycetin resistance-related genes including *cmlA*, *cat* and *cmlA1* were designed. The plasmid-determined resistant genes of *L. plantarum* CICC 23180 were detected by PCR. As shown in Fig. 3, the plasmid of *L. plantarum* CICC 23180 contained β - lactam resistance gene *blr*, excluding other resistant genes. *blr* gene encodes beta-lactamase, which can hydrolyze β-lactam ring and then make the β-lactam antibiotic inactivation. This is probably the main reason of the bacteria resistant to β-lactam antibiotics. In the present study, the successful amplification of *blr* gene in *L. plantarum* CICC 23180 indicated that its cefalotin

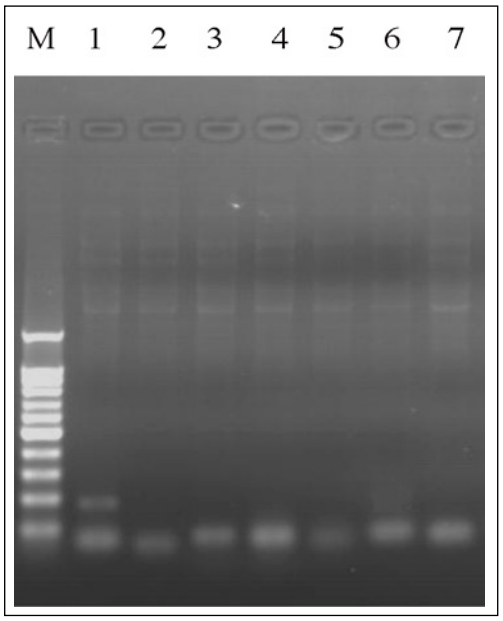

Fig. 3 - The PCR result in the genome and plasmid of CICC 23180.
M. Marker; 1. blr; 2. ECP-1569; 3. nps; 4. cmlA; 5. cat; 6. cmlA1; 7.control.

resistance may be due to the effect of the beta-lactamase to β-lactam antibiotics.

While in the study, the genes of *cat*, *cmlA* and *cmlA1* were not detected in the plasmids of *L. plantarum* CICC 23180. However, *L. plantarum* CICC 23180 strain was resistant to chloramphenicol. At the same time, plasmid elimination and *Escherichia coli* transformant test showed that chloramphenicol resistance-related genes should be present in plasmid DNA of *L. plantarum* CICC 23180 (unpublished results). Therefore, the plasmid of the CICC 23180 strain may contain other genes encoding chloramphenicol resistance.

In recent years, more studies have been done on antibiotic resistance of probiotics. It was shown that the antibiotic resistance was variable, species-dependent and related to the product types. And studies have shown that more genes associated with antibiotic resistance are located in plasmids and transposons (DOUCET *et al.*, 1992; MAYYA *et al.*, 2011). But unlike the chromosome DNA, both plasmids and transposons can provide the possibility of transferability for resistance genes between bacteria. PIER *et al.* (2003) proved the high transferability of plasmid pCF10 that encodes tetracycline resistance from *Enterococcus faecalis* OG1rf to *Enterococcus faecalis* BF3098c during cheese and sausage fermentation. JOANNA *et al.* (2008) reported the transferability of erythromycin resistant plasmid (pAMβ1) from *Lactococcus lactis* SH4174 to *Lactococcus lactis* Bu2-60. A similar study also indicated that the transferability of tetracycline resistance in *E. italicus* LMG 22195 from fermented milk (MIRIAM *et al.*, 2010).

So, the assessment of antibiotic resistant of potentially probiotic lactic acid bacteria used in food industry, especially the resistance-related genes and the transferability are very necessary. We can also say that, exploring the probiotic property and safety of lactic acid bacteria are equally important.

4. CONCLUSIONS

The tested 29 strains of potential probiotic *lactobacillus* showed different resistance to antibiotics. Those resistant strains containing both plasmids and high tolerance to gastrointestinal condition may cause food safety problems. So these strains need to be re-assessed carefully. The study found that the plasmid of *L. plantarum* CICC 23180 exactly carried the cephalothin-related gene *blr*. However, the transferibility of the resistance-related gene remains to be further studied. This study provides a reference in investigating the relationships between antibiotic resistance spectrum and the plasmids and evaluating the safety of probiotics.

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PAPER

EFFECT OF AGEING TIME IN VACUUM PACKAGE ON VEAL LONGISSIMUS DORSI AND BICEPS FEMORIS PHYSICAL AND SENSORY TRAITS

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ABSTRACT

Study evaluated the effects of vacuum ageing (2, 4, 6, 8, 10, 12, 16 days) on veal loin (*longissimus dorsi;* LD) and silverside (*biceps femoris;* BF) physical and sensory characteristics. Ageing did not affect cooking loss, increased LD pH and L*, a* and b* in both muscles. Shear force (SF) decreased until day 6 in LD and day 10 in BF. Aroma, flavor and taste were not affected, while texture traits were improved. SF was negative correlated with tenderness and juiciness and positive correlated with BF fibrousness and stringy sensation. Ageing improved texture properties without altering other sensory traits.

- Keywords: meat quality, postmortem ageing, sensory panel, tenderness, veal calves -

INTRODUCTION

Tenderness is one of the main factors affecting consumer's preference (REICKS *et al*., 2011). Since in EU veal calves are slaughtered at no more than 8 months of age (EU Regulation 1234/2007) consumers expect a tender meat from them. Therefore, ensuring a tender product is a critical aspect for veal producers and retailers, because tenderness is closely related to consumer's satisfaction and they are also willing to pay more for tender meat (DRANSFIELD *et al*., 1998; FEUZ *et al*., 2004; ALFNES *et al*., 2005). Post mortem ageing improves meat tenderness due to the proteolysis of myofibrillar, structural and connective proteins starting from the onset of post-mortem phase (KEMP *et al*., 2010; NISHIMURA, 2010; OUALI *et al*., 2013). Nowadays meat cuts are extensively stored vacuum packaged, a practice that does not significantly affect veal aroma, color, appearance, flavor and texture traits when compared to traditional bone-in carcass ageing (NGAPO and GARIÉPY, 2006). In light of the different degrees of tenderness and tenderization rates among muscles (RHEE *et al*., 2004), this technique can allow to maximize tenderization through the different duration of postmortem ageing, based on specific muscle or commercial cut characteristics. Since veal is not commonly aged in commercial practice, it is necessary to evaluate the effects of long term chilling storage not only on meat tenderness, but also on physical and sensory properties that can affect it. For example, color is an important aspect for veal quality, so preserving veal appearance is essential. However, in some studies prolonged ageing has led to development of off-flavor in beef (SPANIER, 1997). The aim of this study was to evaluate the effect of postmortem ageing time in vacuum package at refrigeration temperature on physical and sensory parameters of veal loin, *(m. longissimus dorsi; LD*) and silverside (*m. biceps femoris; BF*), frozen after ageing and then thawed before quality evaluation, in order to simulate a typical consumer habit (JEREMIAH, 1996). The first cut was selected due to its economic significance, while the second one because its recognition as a less tender hindquarter beef cut when cooked with dry-heat cooking methods (SULLIVAN and CALKINS, 2011), failing thus consumers' expectation for tenderness.

MATERIAL AND METHODS

Two (2) days post mortem, 8 right loin (LD muscle from 6th rib to the 6th lumbar vertebrae) and silverside (BF muscle) whole primal cuts were collected from the carcasses of 8 male milk-fed Holstein veal calves. Calves were similar in age (231±16 d) and sourced from the same farm, being fed the same diet and slaughtered on the same day. Cold carcass weight (163.50±15 kg), conformation (SEUROP conformation score: R), fatness score (European fatness score 1-5: 2) and serum lactate (54.52±1.32 mg/dL) were similar. The serum lactate was determined using blood samples collected during exsanguination by the Central Laboratory of the Veterinary Hospital of the University of Milan using a commercial kit (Sentinel Diagnostics, Milan, Italy). This evaluation was aimed to assess differences in individual animal stress level, which can impair meat tenderness (GRUBER *et al*., 2010). After collection, each muscle was divided in 8 subsamples and each of them was then vacuum packaged. Subsamples were assigned to one of the seven different postmortem ageing treatments: 2, 4, 6, 8, 10, 12 and 16 days randomized, while the remaining one was used to determine chemical composition. Subsamples distribution between treatments was done ensuring that each portion of the muscle was equally represented in every ageing time, as reported by MANDELL *et al*. (2001). All subamples were kept at 0°C until the end of the established ageing period before being frozen at -20°C, as done by CAMPO *et al*. (2000) and MANDELL *et al*. (2001). Prior to measurement, subsamples were thawed for 24 hours at 4°C and from each subsample a 1.50 cm steak was removed for sensory evaluation, while the remaining part was used for physical and chemical analysis.

Physical and chemical analysis

Chemical composition (dry matter, ether extract, crude proteins and ash) was determined, according to AOAC (2000), on designated samples trimmed from the external fat and connective tissue, and homogenized for 30 seconds. On each subsample subjected to different aging time, a fresh cut surface was created removing a slice perpendicular to the fiber axis and, after blooming for 60 minutes in a dark room at 4°C, its color was assessed by a CR-300 Chroma Meter device (Minolta Camera, Co., Osaka, Japan) calibrated on the CIE L*a*b* (CIE, 1976) color space (Calibration Plate 21533131 Y 93.4 x 0.3456 y 0.3321, Minolta Cameras). The Chroma Meter had an 8-mm measuring area, was set in D-65 lighting, and an average of 10 repetitions was recorded as the value for each sample. pH was measured with a portable pH-meter (HI 98150, HANNA Instruments Inc., Woonsocket, RI, USA) on a homogenate prepared by grinding the slice removed to create a fresh cut surface and mixing it with deionized water. Cooking loss was determined, as described by HONIKEL (1988), as the weight lost after cooking in water bath, until core temperature attained 75°C (monitored with a temperature meter Hanna Instruments HI98840) and 24 hours of storage at 4°C. Before being weighed after cooking, samples were blotted dried. The difference between pre- and post-cooking weights was used to calculate the percentage lost during cooking (cooking loss). After cooking loss deter-

mination, from 2.5 cm thick cooked samples six cylindrical cores (1.27 cm in diameter), parallel to fiber orientation, were obtained and used for shear force (SF) evaluation with a Warner-Bratzler shear force texture analyzer (model 4466; Instron Corp., Canton, MA). Peak force (kg/cm^2) was recorded for each core and the average of six values per sample was used for the statistical analysis.

Sensory analysis

Steaks of BF and LD samples were cut to 1.5 cm thick and cooked for 60 seconds at the greatest power (200°C) on double-plated grills, before being cut into 1.5 cm cubes. Core temperature was monitored with a thermocouple (Pentronic AB,198 Gunnebobruk, Sweden) and it was not allowed to exceed 68°C. Sensory evaluation was performed by 10 expert and trained judges (UNI EN ISO 13299:2010), confident with meat sensory evaluation, on each sample aged for 2, 4, 8, 10 and 16 days. Three cubes per samples were presented on white plastic plates to each panelist, which during training and sampling, had access to unlimited water and unsalted crackers and each sample was identified by 3-digit codes. Judges were trained in two tasting session with the aim to allow them to find and familiarized with sensory descriptors relative to veal aroma, taste, flavor and texture. At each judge was asked to evaluate the intensity of each attribute by assigning a score between 1 (absence of the sensation) and 9 (extremely intense sensation). Descriptors (Table 1) includes the main beef sensory parameters and some of the defects that could affect vacuum packaged aged meat.

Statistical analysis

Statistical analysis was performed using SAS® 9.3 (SAS Institute Inc., 2012 Cary NC) software. Data from the physical analysis was analyzed by one-way ANOVA, considering post mortem ageing time as the main effect. Data from sensory profile were analyzed by three-way ANOVA considering the effects of judge, replications and ageing time and their interactions. Least square means were compared according to F test, with the level of significance set at P≤0.05. Pearson correlation analysis was also performed to evaluate the relationship between SF and sensory texture characteristics.

Table 2 - Average chemical composition of the muscles sampled for the trial (least square means±SD).

Trait	*Longissimus dorsi*	*Biceps femoris*
Moisture, g kg^{-1}	751.72±5.12	754.91±3.32
Ash, g kg^{-1}	12.34±1.04	11.72±0.65
Crude Protein, g kg^{-1}	211.50±3.22	212.24±3.96
Ether extract, g kg^{-1}	24.52±3.24	21.20±2.47

RESULTS AND CONCLUSIONS

Physical and chemical characteristics

The average chemical composition of LD and BF muscles is summarized in Table 2. Results are consistent with data reported for lean veal meat in some national food composition databases (Denmark: National Food Institute, 2009; USA: United States Department of Agriculture, 2011). Although a significant effect (P=0.05) of ageing time on LD pH was found (Table 3), it increase only from 2 to 8 days of ageing, while no significant differences were evident since the day 4 of ageing. Regarding BF, its pH was not affected by ageing time. This last data is consistent with other studies that found no differences in veal pH during 7 (REVILLA *et al.*, 2006) or 14 days of ageing (OLIETE *et al.*, 2006), and the review by

Table 1 - Descriptors, definitions and standards for sensory analysis.

Attribute		Definition
Aroma	Veal	Aroma associated with cooked veal loin
	Metallic	Aroma associated with blood or rare meat
	Off flavor	Aroma associated with meat at the end of shelf life
Taste	Salty	Salty taste
	Sweet	Sweet taste
Flavor	Veal	Flavor associated with cooked veal loin
	Metallic	Flavor associated with blood or rare meat
	Off flavor	Flavor associated with meat at the end of shelf life
Texture	Tender	The force needed to masticate the meat ready for swallowing (chewing 5 times)
	Fibrous	Presence of fibers during swallowing
	Juicy	The degree of juice released while chewing the meat
	Stingy	Production of a large quantity of saliva for swallowing

NGAPO and GARYÉPI (2006), that suggests post-mortem ageing did not increase veal pH. However, although the reported slight differences in LD pH values across post mortem times, it fell within the normal range (5.40-5.70) for both muscles. Cooking loss (Table 3) was not affected by ageing time in either muscle. Findings are in agreement with other works (KLONT *et al*. 2000; MANDELL *et al*. 2001), even if is difficult to make a comparison with the previous study due to the different cooking methods and endpoint temperatures to between studies. However, for both muscles, results of the present study are intermediate between the cooking loss values reported by the previous authors (19.1-38.2%). Regarding color parameters (Table 3), ageing increased lightness (L*), redness (a*) and yellowness (b*) in both muscles (P≤0.01). This concurs with MANDELL *et al*. (2001), which suggested color parameters tended to increase only during the first week of ageing, before becoming stable. INSAUSTI *et al*. (1999) also found L* to increase during vacuum storage in *longissimus dorsi* of young cattle. Lightness increasing can be attributed, as reported by KLONT *et al*. (2000), to the increasing of meat light scatter properties due to post mortem protein denaturation and degradation. OLIETE *et al*. (2006) found an increasing of a* and b* measured 1 hour after blooming in vacuum packaged veal and young cattle *longissimus dorsi*. These studies attributed the increasing in redness to the faster blooming of aged meat. Indeed, the more meat is aged the faster it blooms because of the reduced activity of enzyme that compete for oxygen with Mb. The rising of yellowness was, instead, attributed the increasing of metmyoglobin formation during storage time. A lightness increasing can exert a positive effect on veal appearance, while an improvement of redness can represent a negative factor. Indeed, in several studies on veal carcasses, a decreasing in lightness and an increasing in redness moving from lightest to darkest veal was reported, while b* was not related to color score (DENOYELLE and BERNY, 1999; HULSEGGE *et al*. 2001; LAGODA *et al*. 2002; VANDONI and SGOIFO ROSSI, 2009). The magnitude of L*, a* and b* increasing, higher than those found in the study of MANDELL *et al*. (2001), could be promoted by the combination of freezing and thawing and blooming time, this latter not applied by MANDELL *et al*. (2001), that can have exacerbated the impact of ageing on

Table 3 - Effect of ageing time on veal LD and BF physical traits (least square means±SEM).

	Ageing time							
	2 d	**4 d**	**6 d**	**8 d**	**10 d**	**12 d**	**16 d**	**p**
				pH				
LD	5.54±0.04 a	5.62±0.04 ab	5.63±0.04 ab	5.70±0.04 b	5.68±0.04 b	5.71±0.04 b	5.70±0.04 b	0.05
BF	5.60±0.04	5.59±0.04	5.63±0.04	5.63±0.04	5.68±0.04	5.68±0.04	5.67±0.04	NS
				cooking loss, %				
LD	25.41±0.53	26.16±0.53	25.72±0.53	25.96±0.53	25.98±0.53	25.79±0.53	25.66±0.53	NS
BF	28.81±0.11	28.55±0.11	29.87±0.11	28.81±0.11	29.85±0.11	27.62±0.11	28.77±0.11	NS
				L*				
LD	48.20±0.68 a	50.32±0.47 b	51.00±0.54 bc	52.91±0.93 cd	52.80±0.90 cd	52.49±0.86 cd	53.08±0.90 d	≤0.01
BF	48.51±0.53 a	50.46±0.43 b	50.71±0.52 b	52.74±0.75 c	53.04±0.55 c	53.12±0.56 c	53.88±0.55 c	≤0.01
				a*				
LD	9.71±0.47a	10.27±0.34 a	12.82±0.40b	12.49±0.68 b	12.52±0.66 b	12.57±0.63 b	12.53±0.66 b	≤0.01
BF	10.85±0.25 a	11.27±0.20 a	13.70±0.25b	14.86±0.34 b	14.87±0.27 b	14.11±0.25 b	14.03±0.26 b	≤0.01
				b*				
LD	9.86±0.25 a	10.52±0.17 b	11.97±0.20c	12.22±0.34 c	12.41±0.33 c	12.73±0.31 c	12.87±0.33 c	≤0.01
BF	10.58±0.25 a	11.23±0.20 b	12.46±0.25c	13.62±0.34 d	13.65±0.27 d	13.39±0.25 d	13.22±0.26 d	≤0.01
				SF, kg				
LD	2.89±0.15 a	2.59±0.11 ab	2.42±0.16 bc	2.21±0.15 c	2.12±0.18 c	2.09±0.21 c	2.05±0.17 c	≤0.01
BF	2.89±0.13 a	2.73±0.09 ab	2.65±0.13 ab	2.45±0.13 bc	2.22±0.11 cd	2.19±0.11 cd	1.96±0.11 d	≤0.01

a,b,c,d in the same row indicates significant differences between the different ageing times.

meat color stability. Indeed, freezing and thawing promote myoglobin denaturation, increase susceptibility to oxidation and reduce the activity of metmyoglobin reducing enzymes. These effects, coupled with the loss of NADH (cofactor of these enzymes) in the exudate, reduce meat color and oxidative stability as reviewed by LEYGONIE *et al*. (2012).

Post mortem ageing of LD reduced SF (P≤0.01), but there were no further improvements in tenderness after 8 days of ageing (Table 3). These findings are consistent with MANDEL *et al*. (2001), who reported decreases in SF for LD and semimembranosus muscles comparing veal aged for 2 days with veal aged for at least 7 days, but the same study found no differences in SF for ageing periods beyond 7 days. Furthermore, REVILLA *et al*. (2006) also found a reduction in SF loin during 7 days of ageing. Eight days of ageing was needed to significantly decrease SF values for 2 days aged BF; there was no further improvement in SF values until BF was aged for 16 days. This slower tenderization rate of BF compared to LD agrees with data relative to this muscle collected from lean beef carcasses graded as USDA quality grade Select (GRUBER *et al*., 2006).

Sensory analysis

The F values of ageing time for aroma, taste, flavor and texture parameters of LD and BF sensory profile are reported in Table 4 and Table 5, respectively. Results indicated that postmortem ageing time affected (P ≤0.01) sensory texture of both LD and BF. In particular, postmortem ageing improved LD sensory tenderness (P≤0.01). Tenderness was higher at day 4 days in comparison with day 2, and at day 16 in comparison to day 4 (Tab. 4). Increasing ageing from 2 to 4 days also improved juiciness (P≤0.01) and reduced stringy sensation (P≤0.01). Improvements in eating quality associated with ageing were also perceived for fibrousness, with significant reduction (P≤0.01), starting from the 8th days post mortem. These results are consistent with Mandell *et al*. (2001), which found an increase in perceived LD tenderness comparing samples aged 2 days with the average of the values recorded for samples aged 7 and 14 days, while no significant difference was detected increasing ageing period from 7 to 14 days. BF sensory analysis (Table 5), showed that perceived tenderness significantly increased with ageing (P≤0.01) from days 2 and 4 to day 8, and also between 8 to 16 days post mortem. Juiciness was improved (P≤0.01) from 2 to 8 days of ageing, but no further. Fibrousness was also reduced (P≤0.01) from 2 to 8 days and from 8 to 16 days of ageing and stringy sensation decreased (P≤0.01) from 2 to 4 days and from 4 to 10 days of ageing, but no further. The improvement of perceived tenderness and juiciness, as well as reductions in fibrousness and stringy rankings, are common when sensory panels evaluate the effects of postmortem ageing on beef palatability attributes (JEREMIAH and GIBSON, 2003; MILLER *et al*. 1997 and

Table 4 - Effect of ageing time on LD sensory profile (least square means).

Descriptors	F value	2 d	4 d	8 d	10 d	16 d	SEM	p ageing time
				Aroma				
Veal	1.47	6.98	6.77	6.62	6.92	6.65	0.20	N.S.
Metallic	1.32	4.18	4.29	4.59	4.17	4.87	0.32	N.S.
Off flavor	3.02	2.33	2.15	2.52	2.37	2.87	0.22	N.S.
				Taste				
Sweet	1.14	4.67	5.27	5.07	5.20	5.38	0.32	N.S.
Salty	1.47	3.81	4.32	3.83	3.68	4.22	0.30	N.S.
				Flavor				
Veal	0.89	6.62	6.82	6.50	6.82	6.95	0.24	N.S.
Metallic	1.56	4.12	4.61	4.34	3.92	4.77	0.32	N.S.
Off flavor	1.64	2.97	2.21	2.96	2.88	3.08	0.31	N.S.
				Texture				
Tender	16.21	4.92 a	6.35 b	6.77 bc	6.88 bc	6.94 c	0.23	≤0.01
Juicy	10.82	4.75 a	5.79 b	5.95 b	6.34 b	6.33 b	0.26	≤0.01
Fibrous	4.94	5.05 a	3.95 b	3.87 b	3.70 b	3.57 b	0.30	≤0.01
Stringy	3.89	4.68 a	3.96 ab	3.62 b	3.32 b	3.34 b	0.29	≤0.01

a,b,c in the same row indicates significant differences between the different ageing times.

Table 5 - Effect of ageing time on BF sensory profile (least square means).

Descriptors	F value	2 d	4 d	8 d	10 d	16 d	SEM	p ageing time
				Aroma				
Veal	1.00	6.69	6.60	6.80	7.04	6.93	0.18	N.S.
Metallic	1.50	4.15	4.43	4.31	3.81	3.71	0.29	N.S.
Off flavor	0.19	2.31	2.40	2.42	2.35	2.26	0.19	N.S.
				Taste				
Sweet	0.74	5.00	5.12	5.34	5.41	5.31	0.27	N.S.
Salty	2.27	3.73	3.84	4.33	3.88	4.33	0.28	N.S.
				Flavor				
Veal	1.47	6.38	6.48	6.67	6.94	6.52	0.20	N.S.
Metallic	2.47	4.04	4.78	4.44	4.25	4.36	0.29	N.S.
Off flavor	1.54	3.04	3.00	2.58	3.27	2.29	0.22	N.S.
				Texture				
Tender	15.43	3.80 a	4.52 a	5.65 b	6.38 bc	6.64 c	0.31	≤0.01
Juicy	5.83	4.27 a	4.57 ab	5.13 b	5.80 b	5.80 b	0.29	≤0.01
Fibrous	9.88	6.58 a	5.34 b	4.78 bc	4.15 cd	3.76 d	0.33	≤0.01
Stringy	21.37	6.64 a	5.81 ab	5.00 bc	4.41 cd	3.39 d	0.30	≤0.01

a,b,c,d in the same row indicates significant differences between the different ageing times.

CAMPO *et al*. 1999). In the latest study a multivariate approach (Principal Component Analysis) was used to differentiate aged from unaged meat. This indicated that aged meat was characterized by tenderness and juiciness sensation, while unaged meat was characterized by fibrousness and residue (similar to stringy sensation) ones. In the present study post mortem ageing did not affect aroma, flavor and taste for both muscle. This disagrees with MANDELL *et al*. (2001), where meat flavor was improved by ageing veal more than 7 days. There was a low incidence for the panel detecting undesirable palatability attributes such as metallic aroma and flavor and off flavor. This concurs with JEREMIAH and GIBSON (2003), that found low levels of off flavor and metallic aroma/flavor attributes in beef, regardless of post mortem ageing time. Furthermore, the same authors, did not find differences in off or metallic aroma and salt and metallic flavor prolonging ageing time until 28 days. The lack of effect of ageing time on negative sensory descriptors is an important outcome, as some past studies have reported increases in undesirable flavor and aroma defects for beef after prolonged ageing (SPANIER *et al*. 1997 and MONSÓN *et al*. 2005).

Correlation between SF and texture sensory traits

Based on Pearson correlation coefficients to examine the relationship of sensory texture characteristics and SF for both muscles, there was a negative relationship between SF and sensory tenderness (r=-0.67; P≤0.01 and r=-0.83; P≤0.001 for LD and BF respectively) and juiciness (r=-0.53; P≤0.05 and r=-0.72; P≤0.01 for LD and BF respectively). The negative correlation between SF and sensory tenderness is in agreement to the findings of SHACKELFORD *et al*. (1999) in beef and MONTEIRO *et al*. (2013) in veal. Positive correlations were found between SF and fibrousness (r= 0.78; P≤0.01) as well as SF and stringy sensation (r= 0.78; P≤0.01) for BF. These findings agrees with the positive correlation between fibrousness and SF found by Caine *et al*. (2003) and the negative correlation between SF and juiciness found by MONTEIRO *et al*. (2013). The lack of relationship between SF with stringy and fibrousness rankings for LD muscle in respect to BF muscle could be explained by the lower collagen content of LD relative to BF (RHEE *et al*. 2004). Indeed, fibrousness and stringy sensation were lower in LD muscle and the lower detectability could have been at the basis of the lack of significant interaction.

Our results indicate that postmortem ageing under vacuum conditions improved the instrumental and sensory tenderness rankings for veal *m. longissimus dorsi* and *m. biceps femoris*, without any negative effects on the main meat sensory traits such as aroma, flavor, taste and juiciness measured after frozen storage and thawing. Ageing, coupled with freezing and thawing, have, however, reduced oxidative stability in both muscles,

without affecting other veal technological properties as cooking loss and pH. There were different postmortem tenderization trends for each muscle evaluated in the study. The improvements in LD tenderness and related sensory traits occurred mainly during the first week of postmortem ageing, while in BF postmortem ageing effects were also evident until the tenth day. At these experimental conditions, a minimum period of 4 days for LD muscle and 8 days for BF muscle was necessary to obtain a perceivable tenderizing effect. A prolonged ageing, for at least one week for veal LD and two weeks for veal BF can be applied for frozen veal, mainly destined for ho.re.ca market, in which product appearance is a secondary trait, while tenderness is the primary goal. Vacuum ageing could be also apply for fresh veal market, considering indeed its potentially lower impact than that emerged in this study on oxidative stability, as veal will not undergone to freezing and thawing process before being prepared for retail exposition.

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PAPER

QUALITY CHARACTERISTICS OF CHICKEN BURGERS ENRICHED WITH VEGETABLE OILS, INULIN AND WHEAT FIBER

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ABSTRACT

The aim of the study was to modify the composition of chicken burgers in terms of nutritional value by substitution of 20% of pork jowl with a mixture of rapeseed oil and linseed oil, and addition of inulin (1%) or wheat fiber (3%). Substitution of pork jowl with vegetable oils resulted in significant increase in polyunsaturated fatty acids, and rosemary extract retarded the oxidation process of lipids. Addition of wheat fiber was helpful in maintaining the thermal processing yield and texture of burgers. Microbiological quality of vacuum packed burgers subjected to 21-day storage at +4°C±1 and -20°C±1 was satisfactory.

- Keywords: chicken burger, inulin, quality, vegetable oil, wheat fiber -

INTRODUCTION

Despite the constant dissemination of knowledge in the field of proper nutrition, consumers do not always consider it when choosing foods. Results of research over a composition of a daily diet of the average Polish consumer indicated, among others, that the consumption structure of fatty acids and the level of intake of fiber were not consistent with nutritional recommendations (DYBKOWSKA *et al.*, 2004; RADZYMIŃSKA *et al.*, 2005). Therefore, in recent years scientists and manufacturers have taken actions towards reformulation of various food products aimed at improving their nutritional value (WASZKOWIAK *et. al.*, 2001; KOWALSKI and PYRCZ, 2009).

Since meat products provide considerable amounts of fat to the diet (GIVENS *et al.*, 2006), practical strategies of modifying their nutritional value include enrichment with polyunsaturated fatty acids (PUFA) (JIMÉNEZ-COLMENERO, 2007; PYRCZ *et al.*, 2007; VALENCIA *et al.* 2006; ÖZVURAL and VURAL, 2008). Fatty acid (FA) composition of meat products may be changed by introducing of vegetable or fish oil into the composition of formula or by replacing some animal fatty raw material with vegetable oil. However, the substitution of the animal fatty raw material with vegetable oil may have negative effect on the technological quality and sensory desirability of the product, among others, the increase of thermal loss, the acceleration of fatty acid oxidation process (NITSCH, 2007; ANDRÉS *et al.*, 2009; DECKER and PARK, 2010). In order to prevent adverse changes in quality of meat products prepared with vegetable oil, addition of other ingredients of natural origin may be applied. Potential deterioration of structure or sensory attributes of such products could be avoided by using both vegetable oil and fiber preparation (VURAL *et al.*, 2004; JAVIDIPOUR *et al.*, 2005). The effective method for retardation of the FA oxidation of meat products enriched with unsaturated fatty acids is the addition of antioxidants of natural origin, such as plant extracts (GEORGANTELIS *et al.*, 2007; FORELL *et al.*, 2010).

Recently, ready-to-eat meat products have grown in popularity with Polish consumers (STANGIERSKI and KIJOWSKI, 2002; GÓRSKA-WARSEWICZ, 2007). Therefore, the main objective of the present study was to develop a popular in Poland ready-to-eat meat product, which is chicken burger, with improved nutritional value. Launching such a product into market would facilitate composing a quotidian diet without necessity of changing eating habits or giving up favourite meals. This work includes determination of the effect of 20% substitution of pork jowl with a mixture of vegetable oils (rapeseed oil and linseed oil in mass ratio 7 to 3) and addition of inulin (1%) or wheat fiber (3%) on physical, chemical, and microbiological of chicken burgers.

MATERIALS AND METHODS

Materials

Raw materials: chilled chicken thigh meat and pork jowl, were collected from the local meat processing plant (Karczew near Warsaw, Poland). Pork jowl (about 10 kg) was purchased once, then coarse ground in a laboratory grinder Mesko WN60 (Mesko, Skarżysko-Kamienna, Poland) equipped with a plate with three kidney-shaped orifices. The ground jowl was divided into four lots, which were vacuum packed and stored at -20°C±1 until further use. Chicken meat (about 4 kg) was purchased prior to the each replication of experiment.

Fiber preparations were obtained from the manufacturers: inulin Orafti® HPX from Beneo-Orafti Ltd. (Tienen, Belgium) and wheat fiber Vitacel WF400® from J. Rettenmeier & Söhne GmbH + Co. (Rosenberg, Germany). Cold pressed unrefined vegetable oils: rapeseed oil and linseed oil, and spices were obtained from the local supermarket.

About 24 h prior to the production of chicken burgers, inulin gel was prepared: 1 part of inulin powder was dissolved in 3 parts of water using an electric blender Braun Multiquick 7 (Braun GmbH, Kronberg, Germany). The solution was heated to boiling. Heating was continued until a clear solution was obtained. The inulin solution was chilled at the room temperature for 60 min, then placed in a laboratory refrigerator (4°C±1).

A mixture of vegetable oils was used in the production process of burgers in the form of an emulsion with soy protein. The emulsion was prepared directly before the onset of production of burgers. Rapeseed oil and linseed oil were used in a mass ratio 7 to 3, to prepare the mixture of oils. Soy protein isolate SPI 733 (Solae ™, St. Louis, MO, USA) was rehydrated (1 part of dry preparation: 4 parts of water) using water provided in the composition of formula. To obtain the emulsion the mixture of oils was mixed with hydrated soy protein using the electric blender (Braun Multiquick 7) at low speed. The mass ratio of oils, rapeseed and linseed oil, was adopted on the basis of literature data on the nutritional properties of oils and the applicability of them as ingredients in meat preparations, as well as own calculation (KUNACHOWICZ *et al.*, 2005; MIŃKOWSKI *et al.*, 2010). The calculation suggested that the content of polyunsaturated fatty acids (PUFA) in chicken burgers, as a result of modification of the recipe composition, should not be less than 1.5 g per 100 g of product.

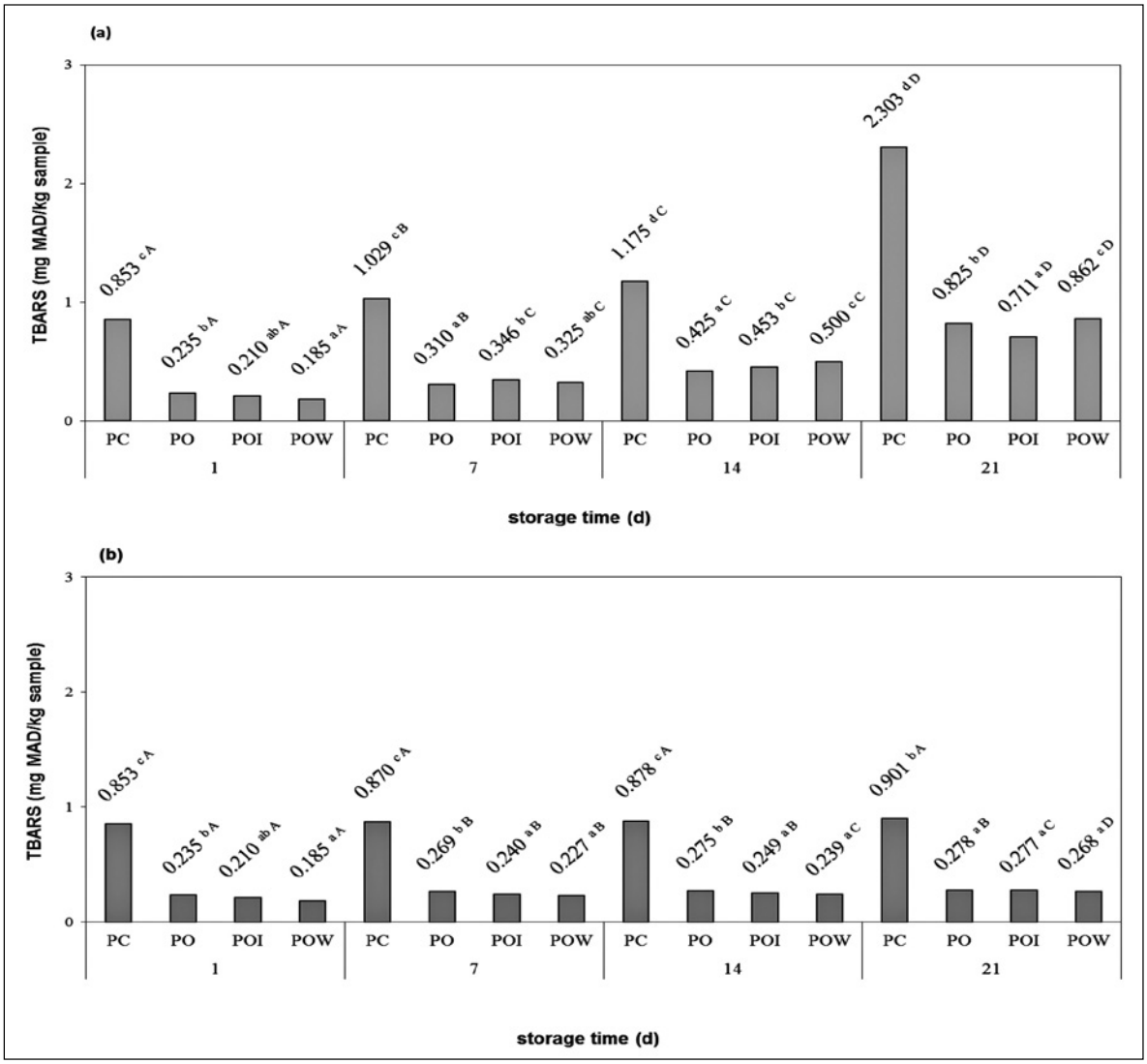

Fig. 1 - Thiobarbituric acid reactive substances (TBARS) values of chicken burgers formulated with different combinations of pork jowl, vegetable oils and dietary fiber preparations, during 21 days of storage at +4°C±1 (a) and at -20°C±1 (b).
For product description see Table 1.
[a-c]Means in the same figure (a, b) without a common lowercase letter differ significantly ($p < 0.05$) – influence of recipe composition of burgers (product formula) on TBARS value of burgers stored in different periods.
[A-D]Means in the same figure (a, b) without a common lowercase letter differ significantly ($p < 0.05$) – influence of storage time on TBARS value of burgers of each formula.

Table 1 - Composition of chicken burgers containing different combinations of pork jowl, vegetable oils, and dietary fiber preparation.

Ingredient	Product formula[a]			
	PC	PO	POI	POW
Chicken thigh meat (%)	85.0	85.0	85.0	85.0
Pork jowl (%)	15.0	12.0	12.0	12.0
Mixture of rapeseed and linseed oil (%)	-	3.0	3.0	3.0
Total raw materials (%)	100.0	100.0	100.0	100.0
Water[b] (%)	10.0	10.0	10.0	10.0
Sodium chloride[c] (%)	1.8	1.8	1.8	1.8
Soy protein isolate[c] (%)	1.5	1.5	1.5	1.5
Black pepper[c] (%)	0.3	0.3	0.3	0.3
Rosemary extract[c] (%)	-	0.03	0.03	0.03
Inulin[c] (%)	-	-	1.0	-
Wheat fiber[c] (%)	-	-	-	3.0

[a]Product formula: PC - control burgers; PO, - burgers formulated with substitution of 20% of pork jowl by mixture of vegetable oils; POI - burgers formulated with substitution of 20% of pork jowl by mixture of vegetable oils, and added inulin; POW - burgers formulated with substitution of 20% of pork jowl by mixture of vegetable oils, and added wheat fiber. [b]In relation to the mass of chicken meat and pork jowl (total raw materials). [c]In relation to the mass of chicken meat, pork jowl and water.

Chicken burger preparation

Four formulas of chicken burgers with different combinations of pork jowl, vegetable oils, and dietary fiber preparation (PC, PO, POI, POW) were prepared (Table 1). The level of substitution of pork jowl with the mixture of vegetable oils and the addition level of inulin or wheat fiber were adopted on the basis of previous studies results (CEGIEŁKA and PĘCZKOWSKA, 2008; CEGIEŁKA, 2011).

Before the production of burgers, pork jowl was thawed (4°C±1, 12 h). Chicken meat and pork jowl were ground using a laboratory grinder Mesko WN60 equipped with a plate having 5 mm diameter orifices. Meat batters were prepared in laboratory mixers Kenwood KM 070 (Kenwood Ltd., Havant, England). After mixing of chicken meat with NaCl (about 5 min) fatty raw materials were added: pork jowl only (PC) or pork jowl and emulsion of oils with soy protein isolate (PO, POI, POW). Rosemary extract Flavour Guard P GIN:601331 (Chr. Hansen A/S, Hørsholm, Denmark) was added to batters containing oils. After the next 5 min, other ingredients were added: black pepper, hydrated soy protein isolate (PC), and – depending on the product formula – inulin gel (POI) or wheat fiber (POW). Mixing was continued until a homogenous distribution of all the ingredients was obtained (about 10 min).

Burgers (100 g±1) were formed using a hamburger mould (about 10.0 cm diameter and 1.0 cm high) and placed in laboratory refrigerator (-28°C±2) for 30 min, in order to maintain the shape. Burgers were cooked in a commercial electric grill (Unox S.p.A., Vigodarzere-Padova, Italy) preheated to reach the temperature of 200°C. Cooking was continued until the internal temperature of burger reached 72°C. The temperature of burgers was monitored using a portable skewer digital thermometer HI 98804 (Hanna Instruments, Woonsocket, RI, USA). The burgers were then cooled at room temperature (about 30 min) over absorbent paper.

After cooling, chicken burgers of each formula were divided into two lots: the first one was left in the refrigerator at 4°C±1 until next day (about 24 h), and the second one was devoted to storage research.

The procedure was replicated four times.

Storage conditions

Before the storage chicken burgers of each formula were vacuum packed in bags in lots of four and then stored at +4°C±1 and -20°C±1 for a maximum of 21 days.

Yield after thermal processing

Yield after thermal processing of chicken burgers was determined by weight, after cooking and chilling the products to about 4°C, in relation to the weight of raw burgers.

Chemical analysis

Chemical analyzes were carried out on cooked and chilled (4°C±1, 24 h) chicken burgers.

Content of moisture, protein, total fat, salt, and ash was determined using analytical techniques according to AOAC (1990). All analyzes were done in 2 replications.

Analysis of texture

Measurements of texture were conducted on cooked and chilled (4°C±1, 24 h) chicken burgers. The measurements were taken using the universal testing machine Zwicki 1120 (Zwick GmbH & Co., Ulm, Germany) equipped with the Warner-Bratzler blade. Shear force (N), the maximum value of the force registered during movement of the blade through the sample, was estimated at the speed of cross-head of 50 mm/min. Burger samples were prepared by cutting the products into cuboid-shaped pieces (9 mm high, 30 mm wide and 90 mm long). Five replicates were measured from five burger samples of each formula.

Fatty acid composition

Fatty acid (FA) composition was determined in cooked and chilled (4°C±1, 24 h) chicken burgers and in chicken burgers stored at +4°C±1 and -20°C±1 for 21 days.

To determine the contents of FA the lipid extracts of the burgers were analyzed by gas chromatography. Procedure proposed by FOLCH *et al.* (1957) was used for lipid extraction from the sample. Fatty acid methyl esters (FAME) were obtained according to method of MORRISON and SMITH (1964). Chromatographic analyzes of FAME were performed using an Agilent 7890A GC System gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a split-spiltless injector and a flame ionization detector, using a fused silica capillary column Rt®-2330 (0.25 mm internal diameter and 105 m long; Restek Corp., Bellefonte, PA, USA). The mobile phase consisted of helium at a flow of 1.2 mL/min. The FAMEs were identified by comparing their retention times with FAMEs of the reference standards (Supleco 37 Component Fame Mix; Sigma-Aldrich, St. Louis, MO, USA). Quantification of FA was done by determining the surface areas of their peaks. All analyzes were done in 2 duplicates.

Lipid oxidation

Lipid oxidation was assessed in cooked and chilled (4°C±1, 24 h) chicken burgers and in chicken burgers stored at +4°C±1 and -20°C±1

for 7, 14 and 21 days. The 2-thiobarbituric acid (TBA) test was carried out in each sample in duplicate. Thiobarbituric acid reactive substances (TBARS) values were determined by an extraction method according to the procedure of SHAHIDI (1990). A constant coefficient of 2.34 was employed for converting the absorbance units to TBARS values, which were expressed as mg malondialdehyde/kg sample (mg MAD/kg).

Microbiological analysis

Microbiological analyzes were carried out in cooked and chilled (4°C±1, 24 h) chicken burgers and in chicken burgers stored at +4°C±1 and -20°C±1 for 7, 14 and 21 days.

The analyzes were conducted in Analytical Center of Warsaw University of Life Sciences - SGGW (Warsaw, Poland) in conditions accordant to requirements of PN-EN ISO 7218:2008 standard (PCS, 2008). The microbiological culture media were prepared according to PKN-CEN ISO/TS 11133-1:2009 standard (PCS, 2009). The preparation of test samples for microbiological analyzes, initial suspension and decimal dilutions was carried out according to PN-EN ISO 6887-2:2005 (PCS, 2005b). For quantitative analyzes, 10 g of the sample from central part of burger was collected. Next, the first decimal dilution was performed by dosing physiological solution with peptone according to PN-EN ISO 6887-1:2000 (PCS, 2000). Determination of total bacteria count (TBC) was conducted according to PN-EN ISO 4833:2004+Ap1:2005 standard (PCS, 2005a) using PCA culture medium (Plate Count Agar) of Bio-Rad company (Bio-Rad Laboratories, Inc., Herkules, CA, USA). Determination of coliform bacteria was conducted according to PN-ISO 8432:2007 standard (PCS, 2007) using VRBL medium (Violet Red Bile Lactose Agar; Bio-Rad) and BGBBL (Bile Green Brilliant Lactose Broth; Bio-Rad). The presence of *Salmonella ssp.* in 25 g of product was determined according to PN-EN ISO 6579:2003 standard (PCS, 2003) using MKTTn selective media (Müller-Kauffman's medium with tetrathionate and novobiocin), RVS (medium acc. to Rappaport-Vassilliads with soya), XLD (xylose lysine deoxycholate) and Hektoen of Bio-Rad Company. The colonies typical for *Salmonella ssp.* and suspicious colonies were confirmed using API 20E biochemical tests of bioMérieux Company (bioMérieux Sp. z o.o., Warsaw, Poland).

Statistical analyses

Microbiological data was analyzed using Statistica 6.0 (StatSoft Inc., Tulsa, Okla., U.S.A.). All the other data was analyzed using Statgraphics Plus 4.1. (STSC Inc., Rocville, MD, U.S.A.) by means of the one-way ANOVA test. Differences between burger formulas were tested by the Tukey HSD test. Pearson's correlation coefficients (r) were calculated to determine the linear correlation between chosen quality attributes of chicken burgers.

RESULTS AND DISCUSSION

Yield after thermal processing, chemical composition and texture

Yield after thermal processing of chicken burgers ranged from 82.0 to 88.4% and was not affected ($p > 0.05$) by applied modifications of the composition of formula (Table 2).

The results obtained in this study are in agreement with those obtained by ANDRÉS *et al.* (2009) who showed that an introduction of squid oil into the composition of formula of frankfurters instead of beef tallow did not affect thermal loss of the product. PYRCZ *et al.* (2007), LÓPEZ-LÓPEZ *et al.* (2009), and YOUSSEF and BARBUT (2011) proved, in turn, that thermal loss of scalded sausages increased as the result of replacement of some animal fat with vegetable oil. Decrease in processing yield of meat products enriched with

Table 2 - Processing yield, chemical composition, and shear force of chicken burgers formulated with different combinations of pork jowl, vegetable oils, and dietary fiber preparation.

Characteristic	Product formula[1]			
	PC	PO	POI	POW
Processing yield (%)	88.4±3.9[a]	84.5±3.6[a]	82.0±2.8[a]	87.7±4.6[a]
Moisture (%)	62.3±0.2[a]	62.8±0.7[a]	62.3±0.1[a]	62.6±0.6[a]
Protein (%)	18.0±0.5[a]	18.4±0.6[a]	18.7±0.1[a]	18.3±0.1[a]
Fat (%)	14.5±0.6[a]	13.3±1.7[a]	13.0±1.3[a]	13.7±0.8[a]
Chlorides (%)	2.3±0.1[a]	2.3±0.1[a]	2.2±0.1[a]	2.3±0.1[a]
Ash (%)	2.7±0.1[a]	2.7±0.2[a]	2.7±0.2[a]	2.8±0.2[a]
Shear force (N)	31.1±2.6[b]	23.7±1.6[a]	21.9±2.5[a]	29.9±1.1[b]

[1]Product formula: see Table 1.
[a, b]Means within a raw without a common lowercase letter differ significantly ($p < 0.05$).

oil may be counteracted - like in this study - by an application of oil in form of an emulsion with hydrated vegetable protein (YOUSSEF and BARBUT, 2011) or combined addition of oil and fiber preparation (VURAL *et al.*, 2004; JAVIDIPOUR *et al.*, 2005).

Chemical composition of chicken burgers formulated with different combinations of pork jowl, vegetable oils, and dietary fiber preparation is shown in Table 2. The content of any of the analyzed chemical component of burgers was not differentiated significantly ($p > 0.05$) by the applied modifications the composition of formula. Slightly lower fat content in burgers prepared with a contribution of vegetable oils (PO, POI, POW), when compared to control product (PC), could have been caused by poorer oil maintenance in protein matrix of the product, and as a consequence its loss during thermal treatment.

The results obtained in this study are in agreement with those presented by KAYAARDI and GÖK (2003), MUGUERZA *et al.* (2003), PELSER *et al.* (2007) and CÁCERES *et al.* (2008) who also showed that replacement of some animal fatty raw material with oil did not exert any influence on the chemical composition of scalded sausages and raw fermented sausages. In contrast, GARMIENE *et al.* (2007), and LÓPEZ-LÓPEZ *et al.* (2009) found in studies on frankfurters and scalded sausages, respectively, that substitution of some animal fatty raw material with oil resulted in a significant increase in water content and decrease in protein content in these meat products.

Enrichment of ready-to-eat meat products with wheat fiber: beef burgers (CEGIEŁKA and BONDERSKI, 2010) and poultry burgers (CEGIEŁKA and PĘCZKOWSKA, 2008), did not differentiate the chemical composition of these products when compared to their counterparts prepared without the fiber. It was also shown that the application of inulin did not affect the chemical composition of turkey meat balls (ERGÖNÜL *et al.*, 2009).

Mean values of shear force measured in chicken burgers ranged from 21.9 N to 31.1 N (Table 2). Measurements of shear force of chicken burgers revealed that the texture of products was impacted ($p < 0.05$) by the applied modifications the composition of formula (Table 2). It was found that substitution of 20% of pork jowl with vegetable oils (PO) or application of both oils and inulin (POI) resulted in a significant ($p < 0.05$) decrease of shear force when compared to the control product (PC). The product enriched with oils and wheat fiber (POW) was characterized by a comparable ($p > 0.05$) shear force to the control product (PC).

In contrast to the results of this study, instrumental measurements of texture of scalded sausages showed that substitution of some animal fat with vegetable oil significantly decreased hardness of these products (AMBROSIADIS *et al.*, 1996; PYRCZ *et al.*, 2007; ÖZVURAL and VURAL, 2008). However, in studies on raw sausages it was reported that the deterioration of texture of sausages prepared with oil could be counteracted by addition of dietary fiber preparation (VURAL *et al.*, 2004; JAVIDIPOUR *et al.*, 2005).

Some literature findings suggest that dietary fiber preparations could help to obtain the desired texture of ready-to-eat meat products. It was found that the addition of wheat fiber increased the shear force of poultry burgers (CEGIEŁKA and PĘCZKOWSKA, 2008) and beef burgers (CEGIEŁKA and BONDERSKI, 2010). In other studies ERGÖNÜL *et al.* (2009) showed that inulin addition did not affect significantly the instrumental hardness of turkey meat balls. The above mentioned products, however, did not contain vegetable oil in the composition of formula.

Fatty acid composition

The share of main FA in the overall FA pool of chicken burgers is shown in Tables 3, 4 and 5. The results obtained showed that substitution of 20% of pork jowl with a mixture of vegetable oils did not totally changed fatty acid profile of chicken burgers, but improved nutritional value of them in terms of the share of saturated and polyunsaturated fatty acids (SFA and PUFA).

Products enriched with vegetable oils, irrespectively of an addition of fiber preparation (PO, POI, POW), contained significantly ($p < 0.05$) less saturated fatty acids (SFA) than the control product (PC; Table 3). In burgers of all the formulas, palmitic acid (C16:0) and stearic acid (C18:0) were present in the highest amounts among SFA, and their contents were significantly ($p < 0.05$) higher in the PC product when compared to burgers prepared with vegetable oils. Introduction of a mixture of vegetable oils into the composition of formula of chicken burgers did not significantly ($p > 0.05$) increase the share of monounsaturated fatty acids (MUFA) in the overall FA pool (Table 4), but PO, POI, and POW products contained significantly ($p < 0.05$) more PUFA, including nutritionally valuable PUFA *n-3*, when compared to the PC product (Table 5). Among MUFA, oleic acid (C18:1 *n-9*) was predominant is the products of all the formulas. In burgers prepared with oils, significantly lower ($p < 0.05$) amounts of myristoleic (C14:1) and elaidic acid (C18:1*t*) were found when compared to the PC product.

The content of polyunsaturated fatty acids (PUFA) in chicken burgers with oils (PO, POI, POW) was higher than 2.5 g per 100 g of product. Irrespectively of the burger formula, the highest share in PUFA pool had linoleic acid (LA; C18:2 *n-6*). The LA content in burgers was not significantly ($p > 0.05$) differentiated by application of vegetable oils. Chicken burgers of all the formulas contained relatively high amounts of linolenic (C18:3 *n-3*), arachidonic (C20:4 *n-6*)

Table 3 - SFA of chicken burgers (g/100 g total FA) formulated with different combinations of pork jowl, vegetable oils, and dietary fiber preparations, with different storage conditions, during 21 days of storage.

FA/FA group	Storage conditions	Product formula[1]			
		PC	PO	POI	POW
Capric C10:0	+4°C±1, 24 h	0.081^{aA}	0.070^{aA}	0.046^{aA}	0.062^{aA}
	+4°C±1, 21 d	0.083^{aA}	0.073^{aA}	0.044^{aA}	0.060^{aA}
	-20°C±1, 21d	0.079^{aA}	0.066^{aA}	0.041^{aA}	0.076^{aA}
Lauric C12:0	+4°C±1, 24 h	0.648^{aA}	0.562^{aA}	0.522^{aA}	0.512^{aA}
	+4°C±1, 21 d	0.643^{aA}	0.545^{aA}	0.512^{aA}	0.511^{aA}
	-20°C±1, 21d	0.643^{aA}	0.531^{aA}	0.476^{aA}	0.516^{aA}
Myristic C14:0	+4°C±1, 24 h	1.466^{bA}	1.212^{aA}	1.185^{aA}	1.152^{aA}
	+4°C±1, 21 d	1.367^{abA}	1.218^{abA}	1.188^{aA}	1.154^{aA}
	-20°C±1, 21d	1.469^{bA}	1.206^{abA}	1.163^{aA}	1.158^{aA}
Palmitic C16:0	+4°C±1, 24 h	21.576^{bA}	18.350^{aA}	18.114^{aA}	17.638^{aA}
	+4°C±1, 21 d	21.740^{bA}	18.550^{aA}	18.244^{aA}	17.868^{aA}
	-20°C±1, 21d	21.733^{bA}	18.579^{aA}	18.392^{aA}	17.915^{aA}
Stearic C18:0	+4°C±1, 24 h	8.633^{bA}	7.352^{aA}	7.351^{aA}	7.023^{aA}
	+4°C±1, 21 d	8.672^{bA}	7.395^{aA}	7.334^{aA}	7.112^{aA}
	-20°C±1, 21d	8.656^{bA}	7.537^{aA}	7.604^{aA}	7.256^{aA}
Arachidic C20:0	+4°C±1, 24 h	0.130^{aA}	0.179^{bA}	0.179^{bA}	0.193^{bA}
	+4°C±1, 21 d	0.129^{aA}	0.180^{bA}	0.177^{bA}	0.184^{bA}
	-20°C±1, 21d	0.129^{aA}	0.181^{bA}	0.182^{bA}	0.195^{bA}
Behenic C22:0	+4°C±1, 24 h	ND[2]	0.073^{aA}	0.076^{aA}	0.085^{aA}
	+4°C±1, 21 d	ND[2]	0.069^{aA}	0.076^{aA}	0.081^{aA}
	-20°C±1, 21d	ND[2]	0.073^{aA}	0.079^{aA}	0.085^{aA}
SFA	+4°C±1, 24 h	32.944^{bA}	28.142^{aA}	27.827^{aA}	27.007^{aA}
	+4°C±1, 21 d	33.128^{bA}	28.378^{aA}	27.930^{aA}	27.315^{aA}
	-20°C±1, 21d	33.120^{bA}	28.518^{aA}	28.294^{aA}	27.533^{aA}

[1]Product formula: see Table 1. [2]ND - not detected (the content of the FA was lower than 0.05 g/100 g of total FA). ^{abc}Means within a row without a common lowercase letter differ significantly ($p < 0.05$) – influence of product formula on FA content in burgers stored in different conditions. ^{A}Means within a column with a common uppercase letter do not differ significantly ($p < 0.05$) – influence of storage conditions on FA content in burgers of different formula.

Table 4 - MUFA of chicken burgers (g/100 g total FA) formulated with different combinations of pork jowl, vegetable oils, and dietary fiber preparations, with different storage conditions, during 21 days of storage.

FA/FA group	Storage conditions	Product formula[1]			
		PC	PO	POI	POW
Myrictoleic C14:1	+4°C±1, 24 h	0.133^{bA}	0.114^{aA}	0.113^{aA}	0.115^{aA}
	+4°C±1, 21 d	0.132^{bA}	0.108^{aA}	0.112^{aA}	0.114^{aA}
	-20°C±1, 21d	0.135^{bA}	0.108^{aA}	0.112^{aA}	0.113^{aA}
Palmitoleic C16:1	+4°C±1, 24 h	3.456^{aA}	2.874^{aA}	2.824^{aA}	2.876^{aA}
	+4°C±1, 21 d	4.438^{bcA}	2.860^{aA}	2.800^{aA}	2.853^{aA}
	-20°C±1, 21d	3.446^{cA}	2.821^{aA}	2.870^{abA}	2.847^{aA}
Elaidic C18:1t	+4°C±1, 24 h	0.372^{bA}	0.282^{abA}	0.288^{abA}	0.271^{aA}
	+4°C±1, 21 d	0.542^{bA}	0.311^{aA}	0.325^{aA}	0.305^{aA}
	-20°C±1, 21d	0.455^{abA}	0.306^{aA}	0.317^{aA}	0.306^{aA}
Oleic C18:1 (*n-9)*	+4°C±1, 24 h	40.237^{abA}	40.837^{abA}	39.712^{aA}	42.830^{bA}
	+4°C±1, 21 d	40.429^{aA}	41.096^{aA}	39.810^{aA}	40.911^{aA}
	-20°C±1, 21d	40.313^{aA}	40.953^{aA}	39.499^{aA}	40.779^{aA}
Eicosanoic C20:1	+4°C±1, 24 h	0.734^{aA}	0.770^{aA}	0.744^{aA}	0.773^{aA}
	+4°C±1, 21 d	0.731^{aA}	0.774^{aA}	0.741^{aA}	0.779^{aA}
	-20°C±1, 21d	0.731^{aA}	0.778^{aA}	0.747^{aA}	0.767^{aA}
Eruic C22:1	+4°C±1, 24 h	0.054^{aA}	0.084^{aA}	0.055^{aA}	0.061^{aA}
	+4°C±1, 21 d	0.045^{bA}	0.018^{aA}	0.017^{aA}	0.017^{aA}
	-20°C±1, 21d	0.038^{bA}	0.019^{aA}	0.039^{bA}	0.019^{aA}
MUFA	+4°C±1, 24 h	45.959^{aA}	45.960^{aA}	44.720^{aA}	47.926^{aA}
	+4°C±1, 21 d	48.297^{aA}	48.234^{aA}	46.749^{aA}	48.066^{aA}
	-20°C±1, 21d	48.143^{aA}	48.113^{aA}	46.544^{aA}	47.705^{aA}

[1]Product formula: see Table 1. [2]ND - not detected (the content of the FA was lower than 0.05 g/100 g of total FA). ^{abc}Means within a row without a common lowercase letter differ significantly ($p < 0.05$) – influence of product formula on FA content in burgers stored in different conditions. ^{A}Means within a column with a common uppercase letter do not differ significantly ($p < 0.05$) – influence of storage conditions on FA content in burgers of different formula.

Table 5 - PUFA of chicken burgers (g/100 g total FA) formulated with different combinations of pork jowl, vegetable oils, and dietary fiber preparations, with different storage conditions, during 21 days of storage.

FA/FA group	Storage conditions	Product formula[1]			
		PC	PO	POI	POW
Linoleic C18:2 *(n-6)*	+4°C±1, 24 h	13.341^{aA}	14.939^{aA}	15.508^{aA}	15.555^{aA}
	+4°C±1, 21 d	13.054^{aA}	14.730^{aA}	15.433^{aA}	15.400^{aA}
	-20°C±1, 21d	13.274^{aA}	14.700^{aA}	15.403^{aA}	15.515^{aA}
γ- Linolenic C18:3 *(n-6)*	+4°C±1, 24 h	0.081^{aA}	0.072^{aA}	0.072^{aA}	0.068^{aA}
	+4°C±1, 21 d	0.077^{aA}	0.071^{aA}	0.072^{aA}	0.068^{aA}
	-20°C±1, 21d	0.080^{aA}	0.070^{aA}	0.069^{aA}	0.071^{aA}
Linolenic C18:3 *(n-3)*	+4°C±1, 24 h	1.631^{aA}	5.353^{bA}	6.410^{bA}	6.092^{bA}
	+4°C±1, 21 d	1.599^{aA}	5.248^{bA}	6.480^{bA}	6.932^{bA}
	-20°C±1, 21d	1.587^{aA}	5.211^{bA}	6.362^{bA}	6.015^{bA}
Eicosadienoic C20:2 *(n-6)*	+4°C±1, 24 h	0.327^{aA}	0.291^{aA}	0.275^{aA}	0.280^{aA}
	+4°C±1, 21 d	0.324^{aA}	0.290^{aA}	0.271^{aA}	0.280^{aA}
	-20°C±1, 21d	0.324^{aA}	0.296^{aA}	0.289^{aA}	0.285^{aA}
Eicosatrienoic C20:3 *(n-6)*	+4°C±1, 24 h	0.110^{aA}	0.103^{aA}	0.101^{aA}	0.098^{aA}
	+4°C±1, 21 d	0.112^{aA}	0.099^{aA}	0.096^{aA}	0.098^{aA}
	-20°C±1, 21d	0.114^{aA}	0.104^{aA}	0.104^{aA}	0.101^{aA}
Eicosatrienoic C20:3 *(n-3)*	+4°C±1, 24 h	0.158^{aA}	0.155^{aA}	0.134^{aA}	0.140^{aA}
	+4°C±1, 21 d	0.140^{aA}	0.115^{aA}	0.107^{aA}	0.110^{aA}
	-20°C±1, 21d	0.124^{aA}	0.115^{aA}	0.106^{aA}	0.107^{aA}
Arachidonic C20:4 *(n-6)*	+4°C±1, 24 h	0.198^{aA}	0.212^{aA}	0.202^{aA}	0.195^{aA}
	+4°C±1, 21 d	0.196^{aA}	0.173^{aA}	0.179^{aA}	0.172^{aA}
	-20°C±1, 21d	0.216^{aA}	0.226^{aA}	0.218^{aA}	0.179^{aA}
Eicosapentaenoic (EPA) C20:5 *(n-3)*	+4°C±1, 24 h	ND[2]	ND[2]	ND[2]	ND[2]
	+4°C±1, 21 d	ND[2]	ND[2]	ND[2]	ND[2]
	-20°C±1, 21d	ND[2]	ND[2]	ND[2]	ND[2]
Docosahexaenoic (DHA) C22:6 *(n-3)*	+4°C±1, 24 h	ND[2]	ND[2]	ND[2]	ND[2]
	+4°C±1, 21 d	ND[2]	ND[2]	ND[2]	ND[2]
	-20°C±1, 21d	ND[2]	ND[2]	ND[2]	ND[2]
PUFAs	+4°C±1, 24 h	15.862^{aA}	21.125^{bA}	22.701^{bA}	22.427^{bA}
	+4°C±1, 21 d	15.501^{aA}	20.726^{bcA}	22.639^{cA}	22.058^{cA}
	-20°C±1, 21d	15.720^{aA}	20.722^{bcA}	22.569^{cA}	22.271^{cA}

[1]Product formula: see Table 1. [2]ND - not detected (the content of the FA was lower than 0.05 g/100 g of total FA). [abc]Means within a row without a common lowercase letter differ significantly ($p < 0.05$) – influence of product formula on FA content in burgers stored in different conditions. [A]Means within a column with a common uppercase letter do not differ significantly ($p < 0.05$) – influence of storage conditions on FA content in burgers of different formula.

and eicosatrienoic acid (C20:3 *n-3*). The significant ($p < 0.05$) increase in the share of PUFA in overall FA pool in PO, POI, and POW products - when compared to PC product - was mainly determined by an increased content of linolenic acid. The presence of valuable nutritionally long-chain PUFA *n-3* acids: eicosapentaenoic (EPA) and docosahexaenoic acid (DHA), was not observed in the products prepared with oils. This was possibly due to the fact that the share of vegetable oils in the recipe composition of burgers was relatively low.

The ratio of PUFA to SFA and the ratio of PUFA *n-6* to PUFA *n-3* are often used in nutritional characteristics of lipids in food. The values of these ratios for control burgers (PC) were 0.48 and 7.91, respectively (Table 6). The introduction of mixture of vegetable oils into the formula composition of burgers resulted in significant ($p < 0.05$) changes in the value of both ratios. For the PO, POI, and POW burgers the ratios of PUFA to SFA ranged from 0.75 to 0.83, and the ratios of PUFA *n-6* to PUFA *n-3* varied between 2.47 and 2.84. The significant ($p < 0.05$) increase in the PUFA to SFA ratio, and decrease in the PUFA *n-6* to PUFA *n-3* ratio in burgers formulated with oils - when compared to the control product - was the positive effect indicating improvement of the nutritional value of fat in these products.

Regardless of the temperature of 21-day storage no significant ($p > 0.05$) changes in the content of any FA were found in any of the burgers.

The results obtained confirm the thesis put forward by JIMÉNEZ-COLMENERO (2007), who - based on the literature data - reported that substitution of some animal fatty raw material with oil was an effective method of improvement of FA composition in a wide range of meat products. Usefulness of linseed oil and rapeseed oil in improvement to nutritional value of lipids in meat products, expressed by increased contribution of UFA and PUFA *n-3,* was confirmed by GARMIENE *et al.* (2007), MAKAŁA and JERZEWSKA (2008) in scalded sausages, and by PELSER *et al.* (2007) in fermented sausages. It has been also found that the FA composition of meat products may be modified by the application of olive oil (KAYAARDI and GÖK, 2003), soybean oil (MUGUERZA *et al.*, 2001), or mixture of vegetable oils (ÖZVURAL and VURAL, 2008; LOPÉZ-LOPÉZ *et al.*, 2009). Mixture of oils was also used for an improvement in nutritional value of lipids in ready-to-eat meat products: beef burgers (FORELL *et al.*, 2010) and pork patties (LEE *et al.*, 2006).

Lipid oxidation

Changes in TBARS value in chicken burgers subjected to storage at the temperature of +4°C±1 and -20°C±1 are presented in Figs. 1a and 2b, respectively. The highest TBARS values were observed in control burgers (PC), irrespectively of the storage temperature and time. Significantly lower ($p < 0.05$) TBARS values were observed in burgers prepared with vegetable oils (PO, POI, POW), which meant inhibition of the oxidation process of lipids when compared to the PC product. It should be noted that enrichment of burgers with oils was accompanied by addition of rosemary extract, which was intended to protect FA against oxidation.

The results obtained confirmed that, irrespectively of product formula, freezing was better method of storage than refrigerating. Although lowering the temperature of the storage from +4 to -20 degrees did not stop completely the oxidation process of FA in burgers, it was inhibited significantly.

When compared to the results presented by other authors (FERNÁNDEZ-LÓPEZ *et al.*, 2005; PIETRZAK and MYRON, 2008; FORELL *et al.*, 2010), the TBARS values in chicken burgers were relatively low, both after manufacturing (24 h) and 21 days of storage.

Incorporation of oil into the formula composition of meat product may influence the oxidative stability of lipids in the product. According to KAYAARDI and GÖK (2003), the adverse changes of lipids in beef sausage were caused by the partial replacement of beef tallow with olive oil. In turn, MUGUERZA *et al.* (2003), and PELSER *et al.* (2007) reported that replacement of some animal fatty raw material with vegetable oil in fermented sausages did not intensify the adverse changes in lipids, such as oxidation and hydrolysis. MAKAŁA and JERZEWSKA (2008) also found that the quality of frankfurters enriched with linseed oil, in terms of lipids oxidative changes, was satisfactory even after 8-week of refrigerating storage.

In order to extend the storage stability of chicken burgers with enriched oils, an anti-

Table 6 - Proportions of PUFA : SFA and PUFA *n-6* : PUFA *n-3* in chicken burgers formulated with different combinations of pork jowl, vegetable oils, and dietary fiber preparations, with different storage conditions, during 21 days of storage.

FA group	Storage conditions	Product formula[1]			
		PC	PO	POI	POW
PUFA *n-6*	+4°C±1, 24 h	14.056^{aA}	15.617^{aA}	16.157^{aA}	16.196^{aA}
	+4°C±1, 21 d	13.762^{aA}	15.363^{aA}	16.052^{aA}	16.018^{aA}
	-20°C±1, 21 d	14.010^{aA}	15.397^{aA}	16.082^{aA}	16.160^{aA}
PUFA *n-3*	+4°C±1, 24 h	1.806^{aA}	5.508^{bA}	6.544^{bA}	6.231^{bA}
	+4°C±1, 21 d	1.739^{aA}	5.364^{bA}	6.587^{bA}	6.041^{bA}
	-20°C±1, 21 d	1.711^{aA}	5.362^{bA}	6.487^{bA}	6.122^{bA}
PUFA : SFA	+4°C±1, 24 h	0.48^{aA}	0.75^{bA}	0.82^{bA}	0.83^{bA}
	+4°C±1, 21 d	0.47^{aA}	0.73^{bcA}	0.81^{cA}	0.81^{cA}
	-20°C±1, 21 d	0.48^{abA}	0.73^{bcA}	0.80^{cA}	0.81^{cA}
PUFA *n-6* : PUFA *n-3*	+4°C±1, 24 h	7.91^{bA}	2.84^{aA}	2.47^{47aA}	2.60^{aA}
	+4°C±1, 21 d	7.91^{bA}	2.86^{aA}	2.44^{aA}	2.65^{aA}
	-20°C±1, 21 d	8.19^{bA}	2.89^{aA}	2.48^{aA}	2.64^{aA}

[1]Product formula: see Table 1. ^{abc}Means within a row without a common lowercase letter differ significantly ($p < 0.05$) – influence of product formula on FA content in burgers stored in different conditions. ^{A}Means within a column with a common uppercase letter do not differ significantly ($p < 0.05$) – influence of storage conditions on FA content in burgers of different formula.

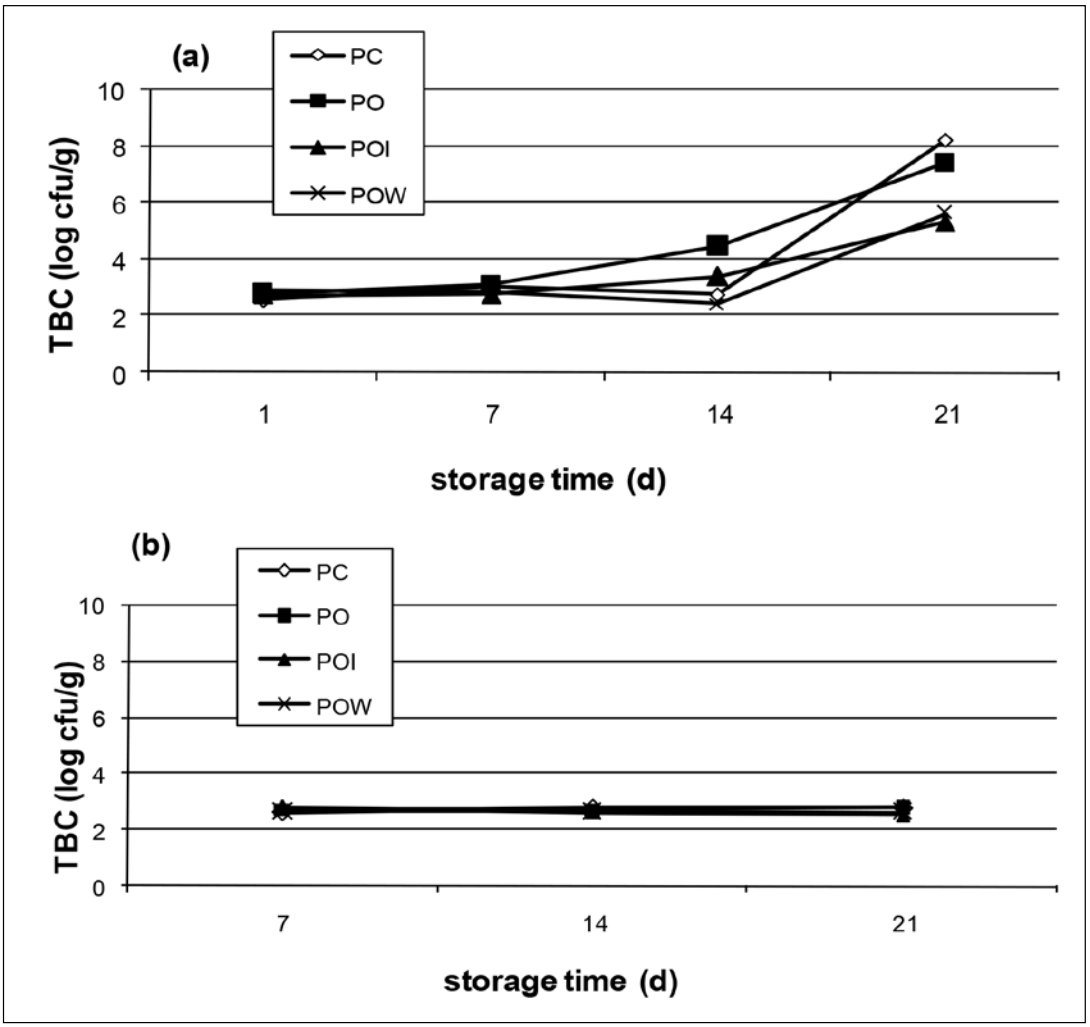

Fig. 2 - Total bacteria count of chicken burgers formulated with different combinations of pork jowl, vegetable oils and dietary fiber preparations, during 21 days of storage in refrigerator (a) or freezer (b).
For product description see Table 1.

oxidant additive of natural origin, which was rosemary extract, was used. The effectiveness of this component in the inhibition of lipid oxidation had already been confirmed in studies on ready-to-eat meat products (NISSEN *et al.*, 2004; FERNÁNDEZ-LÓPEZ *et al.*, 2005; GEORGANTELIS *et al.*, 2007; FORELL *et al.*, 2010; KONG *et al.*, 2010).

Microbiological analysis

The changes in TBC in chicken burgers stored at the temperature of +4°C±1 and -20°C±1 are shown in Figs. 2a and 2b, respectively. It was found that 24 h after preparing, TBC in burgers was as follows: 2.54 log cfu/g for PC product, 2.73 log cfu/g for POI product, 2.78 log cfu/g for PO product, and 2.88 log cfu/g for POW product, and was not significantly ($p < 0.05$) differentiated by the applied modifications of the composition of formula.

After the 21-day storage at +4°C±1, the TBC increased to the level of: 5.32 log cfu/g for POI product, 5.61 log cfu/g for POW product, 7.40 log cfu/g for PO product, and 8.21 log cfu/g for PC product. The increase of TBC during the whole period of storage was statistically significant ($p < 0.05$) only in the PC and PO product.

After the 21-day storage at -20°C±1 the TBC increased to the level of: 2.54 log cfu/g for POI product, 2.65 log cfu/g for POW product, 2.79 log cfu/g for PO product, and 2.80 log cfu/g for PC product. The TBC of any of the frozen products was not significantly ($p < 0.05$) differentiated during the whole period of storage. For chicken burgers of each formula the TBC was significantly ($p < 0.05$) higher in the refrigerated product than in the frozen one after 21 days of storage (results not showed).

The presence of *Salmonella ssp.* was not found in chicken burgers, and the number of coliform bacteria was lower than 10 cfu/g during the whole storage period, regardless of the product formula and storage conditions (temperature and time).

The results obtained proved that the microbiological quality of chicken burgers of all the four formulas fulfilled the requirements mentioned

in EC REGULATION (2007) with respect to *Salmonella ssp*. Despite the fact that the regulation does not require determination of coliform bacteria nor total bacteria count in ready-to-eat meat products from poultry meat, it should be noticed that they may influence both health safety and shelf-life of these products.

The results obtained are in agreement with these obtained ANDRÉS *et al.* (2009) who showed that microbiological quality of poultry frankfurters containing squid oil instead of beef tallow was not significantly differentiated when compared to the control product. Similarly, LOPÉZ-LOPÉZ *et al.* (2009), on the basis of determination of total bacteria count and lactic bacteria count, found that microbiological quality of pork frankfurters enriched with olive or algae oil did not differ significantly during storage. TBC in frankfurters after manufacturing ranged - depending on product formula - from 2.64 to 4.18 log cfu/g, and was comparable to the results obtained in the present study.

CONCLUSIONS

After summarizing the results of this study it was found that 20% substitution of pork jowl with a mixture of vegetable oils in the composition of formula of chicken burgers resulted in an improvement in nutritional quality in terms of FA composition. Chicken burgers enriched with oils contained significantly less SFA and more PUFA, including nutritionally valuable PUFA *n-3*, than the control product, what means the improvement in nutritional value of lipids in these products. The oxidation process of lipids in products containing vegetable oils could be retarded significantly by the addition of 0.03% of rosemary extract. The results of measurements of the shear force of burgers indicated that addition of 3% of wheat fiber to product prepared with the mixture of vegetable oils as the 20% substitute of pork jowl counteracted the changes in texture. Microbiological quality of vacuum-packed burgers subjected to 21-day storage at the temperature of +4°C±1 and -20°C±1 was satisfactory.

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PAPER

MANAGEMENT OF MALOLACTIC FERMENTATION AND INFLUENCE ON CHEMICAL COMPOSITION OF AGLIANICO RED WINES

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ABSTRACT

A study has been carried out to determine the effects of lactic acid bacteria inoculation time on the fundamental components, procyanidins and biogenic amines content of Aglianico wines produced in Apulia region. Three different malolactic fermentation (MLF) techniques were compared: the co-inoculation, the sequential inoculation, and the traditional technique (spontaneous MLF). In the co-inoculation technique there was a delayed start and a late finish of the alcoholic fermentation. The colour intensity of the wine obtained with a spontaneous MLF was higher both at racking and after 12 months. Significant changes in content of flavan-3-ols were found in wines made with different MLF managements. The levels of catechin monomers ((+)-catechin, (-)-epicatechin, (-)-epicatechin-O-gallate) and procyanidin oligomers (B1-B4, and trimer C1) were lower in the co-inoculation wine. In the wine produced with a spontaneous MLF, the content of biogenic amines was significantly higher compared to the other two wines.

- Keywords: anthocyanins, biogenic amines, co-inoculation, procyanidin, sequential inoculation, wine -

INTRODUCTION

The most important microbial activity that is responsible for the conversion of must into wine is the alcoholic fermentation (AF), which is carried out by Saccharomyces yeasts. The malolactic fermentation (MLF) typically follows alcoholic fermentation and is carried out by indigenous lactic acid bacteria (LAB) or induced by inoculation with selected bacterial starters. It is a decarboxylation of L-malic acid, a dicarboxylic acid, with formation of a monocarboxylic acid, the L-lactic acid and carbon dioxide, which is catalyzed by malolactic enzymes which are NADP dependent and require divalent cations such as manganese or magnesium ions (VINCENZINI *et al.*, 2005). The MLF causes a significant evolution of wine and produces remarkable changes in its phenolic composition and sensorial characteristics (COSTELLO *et al.*, 2012; CABRITA *et al.*, 2008; LÒPEZ *et al.*, 2011; SURIANO *et al.*, 2012). In addition to the reduction of the acidity of the wine, MLF increases the aromatic complexity and smoothness (VERSARI *et al.*, 1999; COSTANTINI *et al.*, 2009; LOPEZ *et al.*, 2011). Generally the MLF is favoured in red wines, in *novello* wines, in white wines aged in barrique, or in some sparkling base wines (CAVAZZA *et al.*, 2003). On the other hand, this fermentation produces a small amount of acetic acid and sometimes may also generate unpleasant odours, bitter-tasting compounds or substances that may be dangerous to consumers' health, such as biogenic amines or precursors of ethyl carbamate (LONVAUD-FUNEL, 1999). It has been verified by analysis that the concentration of biogenic amines in wine at the end AF is always quite low, while increases after MLF (GAFNER, 2005). Moreover, it was found that wines which undergo spontaneous MLF often have higher biogenic amine concentration than those in which the MLF is conducted by select malolactic bacteria (CERRUTI *et al.*, 1987; MASQUÈ *et al.*, 2008). Biogenic amines are synthesized by microorganism through decarboxylation of amino acids. Between the main biogenic amines in wines there are tyramine, histamine, putrescine, cadaverine and phenylethylamine, synthesized by the decarboxylation of the amino acids tyrosine, histidine, ornithine, lysine and phenylalanine. These compound can cause adverse physiological reactions in susceptible individuals. Histamine can cause headaches, allergies, diarrhoea, palpitations and vomiting (STOCKLEY, 2004; BODMER *et al.*, 1999), while tyramine is strongly vasoconstrictive (SILLA-SANTOS, 1996). These effects may be enhanced by alcohol, which prevents the organism's detoxifying mechanisms from working properly and by the presence of other amines such as putrescine and cadaverine (LANDETE *et al.*, 2005), both associated with poor sanitary quality of grapes (LEITAO *et al.*, 2005) and responsible for major sensory defects in wines (LEHTONEN, 1996). Usually, the LAB used for MLF belong to the Oenococcus oeni species, anyway, it is possible to also find other bacteria of the Lactobacillus, Leuconostoc and Pediococcus species (DICKS *et al.*, 1995). However, even for the most resistant bacteria the conditions found in wine are close to the limits of survival, so that the transformation of 4-5 g/L of malic acid may requires even 15-20 days (CAVAZZA *et al.*, 2003). Several times, this process may take several months, may occur in some barrels and tanks but not in others and may be responsible for the occurrence of problems related to indigenous LAB species carrying out the MLF (LONVAUD-FUNEL, 2001) which may cause a range of undesirable changes to wine sensory properties, altered wine colour, and may even lead to the generation of biogenic amines (DAVIS *et al.*, 1985). Such a long time can be especially critical for those wines (such as *novello* wines) that must be processed and placed on the market in a short time, and moreover could be a risk since in the season in which the MLF takes place there may be sudden temperature drops which may determine an arrest of the process until the next spring. There are advantages subsequent to an early and fast MLF such as: a more efficient utilization of fermentation tank in the busy postharvest period, thus a decrease of energetic costs resulting in optimization of the winemaking process; moreover it is possible a decrease of the microbiological risks reducing the growth of undesired microorganism and also allows an early commercialization of wines (JUSSIER *et al.* 2006). It is therefore of fundamental importance a correct management of MLF. In this paper the influence of inoculation of lactic bacteria on changes occurring on the polyphenolic characteristics, colour, biogenic amines and proanthocyanidin in Aglianico red wines was investigated by comparing the techniques of co-inoculation and sequential inoculation to a spontaneous MLF.

MATERIALS AND METHODS

Experimental design and winemaking

This research was conducted during the 2012 harvest on Aglianico grape variety, grown in a vineyard trained on espalier training system with Guyot pruning and cultivated according to the principles of organic viticulture. Concerning the different possibilities of MLF management, in the Le.Vin.Sud Company of Cerignola (Foggia, Southern Italy) were carried out three experimental tests in order to evaluate the influence of the timing of lactic bacteria inoculation, comparing the technique of co-inoculation (inoculation of bacteria 24 hours after the yeast

inoculation), sequential inoculation (at the end of the AF) and the traditional technique without inoculation of any LAB, i.e. a spontaneous MLF, which was favoured by acting on certain oenological practices, as further explained. The Aglianico grapes were first destemmed and crushed, subsequently the mass of must and pomace was mixed, homogeneized and introduced in three different steel tank. From each steel tank, 3 x 100 Kg (in triplicate) of must with pomace was utilized for each of the three winemaking techniques adopted, with the aim to determine the repeatability of the differences among the compared treatments. The different batches of must and pomace were subjected to the following winemaking protocols:

- Co-inoculation or simultaneous inoculation of LAB (SIM). After crushing and destemming of grapes 40 mg/L of SO_2 was added. After two hours, *Saccharomyces cerevisiae* strain Lalvin R7 (Lallemand Inc, Castel D'Avezzano-Verona – Italia) previously hydrated in water for 15 min at 38 °C was inoculated in the must (20 g/hL, about 6 x 10^6 cfu/mL.). After 24 hours a lactic bacterial culture of *Lactobacillus plantarum* V22™ (Lallemand Inc, Verona-Italy) was inoculated. The inoculation rate was 1g/hL (2 x 10^7 cfu/mL) must/wine prior re-hydrated in chlorine free water at 20°C for 15 min. The alcoholic fermentation took place under controlled temperature by cooling the mass if the temperature exceeded the threshold of 26°C.

- Sequential inoculation post alcoholic fermentation of LAB (PAF). The only difference from the previous protocol was the time of addition of the bacteria. The lactic bacteria were added at racking, which was performed at the end of the alcoholic fermentation (10 day pomace contact). The doses of yeast and bacteria employed were the same. After the inoculation of bacteria, at a dosage of 20 g/hL Opti'Malo Plus bacterial nutrient (Lallemand Inc, Verona –Italy) were added at wine in according to the manufacturers instructions.

- Spontaneous MLF (Control). This MLF process was used as a comparison test for the others processes. After crushing and destemming of grapes were added about 40 mg/L of SO2, and then 20 g/L of previously hydrated *Saccharomyces cerevisiae* (Lalvin R7) yeast were inoculated. Also for this thesis, at racking/post alcoholic fermentation were added 20 g/hL of Opti'Malo Plus bacterial nutrient.

All the vinification were carried out at 26°C ± 1. During the fermentative pomace contact period (10 days in all vinifications) the cap was pumping over three times a day and the temperature and must density were recorded. At the end of this period, all wines were pressed at 2 bars, racked with no added sulphur dioxide for encourage MLF (in Control and PAF) and stored at 25°C. After MLF, the wines were racked again and 20 mg/L sulphur dioxide was added. The wines were cold stabilised (-4°C) for 1 month and then bottled without filtration. All analyses were made in triplicate at racking and after 6 months in the bottle (12 months after racking).

AF was monitored by ethanol production and sugar depletion. MLF was monitored by l-malic acid degradation and l-lactic acid production. AF and MLF were considered complete when residual sugars were less than 2.5 g/L and l-malic acid was less than 0.12 g/L.

Wine composition

Total acidity, volatile acidity, reducing sugars, pH, total SO_2, alcohol and total dry extract were all determined on wine according to EEC regulation 2676/90.

Chemicals and reference compounds

Standards, including trans-caffeoyl-tartaric acid, trans-p-coumaroyl-tartaric acid, caffeic acid, ferulic acid, p-coumaric acid, quercetin, myricetin, kaempferol, were supplied by Sigma Aldrich. While standard of (+)-catechin, (-)-epicatechin, procyanidin B1, procyanidin B2, epigallocatechin, epigallocatechin gallate were supplied by Extrasynthese. The purities of the standards were all over 95%. All the solvents (methanol, acetonitrile, ethyl acetate) were HPLC grade. All the solutions were obtained with distilled deionised water using Carlo Erba reagents.

Spectrophotometric analysis

Phenolic compounds were determined by spectrophotometric methods (DI STEFANO *et al.*, 1989; Di Stefano *et al.*, 1997) using a UV/VIS Mod Lambda 25 double beam Spectrophotometer (Perkin Elmer S.p.A.). The total anthocyanins index was expressed as malvidin 3-glucoside and calculate by the following expression: E_{maxvis} x 16.17 x d (d=dilutions). The monomeric anthocyanins after separation and absorption on a C18 Set Pak cartridge were eluted with 5 ml of acetonitrile and then diluted with hydrochloric ethanol and calculated by: E_{maxvis} x 16.17 x d (d=dilutions). The total polyphenols index expressed as (+)-catechin was measured by: E1cm, 75 0nm x 186.5 x d (d=dilutions). The total flavonoids index was expressed as (+)-catechin and calculate with the graphic method of DI STEFANO (1989). The flavanols reactive to vanillin (flavonols vanillin assay) were expressed as (+)-catechin = ΔE x 290.8 x d (ΔE=absorbance difference between tests with and without vanillin; d=dilution). The proanthocyanidin content was determined after hot acid hydrolysis (Bate-Smith reaction) using a ferrous salt ($FeSO_4$) as catalyst and expressed as cyanidin chloride. Colour intensity and hue were estimated by measuring absorbance at 420, 520 and 620 nm according to EU Regulation 1990.

HPLC analysis

The fixed acids of wine (tartaric, malic, lactic, citric and shikimic acids) were determined by an HPLC isocratic elution (HPLC 1100 series Agilent technologies) with a Phenomenex Synergi 4u Hydro-RP 80A (250x4.60 mm, 4 micron) with guard column, a mobile phase of phosphoric acid 10^{-3} M, 0.7 mL/min flow rate, 25°C and a UV detector set at 210 nm (CANE, 1990).

For flavans determination, the wine was separated into two fractions containing, respectively, individual catechins and oligomeric proanthocyanidins, using a C18 1g Sep-Pak cartridge as described by SUN *et al.* (1999). About 5 ml of wine was adjusted to pH 7 and then filtered through a Sep-Pak cartridge preconditioned with H_2O. Elution was carried on with 10 mL of H_2O to eliminate phenolic acids. After drying the cartridges with N_2, elution was carried out with 15mL of ethyl acetate to elute catechins and oligomeric proanthocyanidins (F I + F II). Each fraction was evaporated to dryness and dissolved in methanol, followed by HPLC analysis. A Thermo ODS RP-C18 Hypersil 200x2.1 (5 μm) column with a guard column was used for flavans analysis. Two ml of each extracted fraction were filtered on a 0.45 μm nylon membrane and immediately inject according to Squadrito's method (2007). Separation was carried out at 30°C, the flow rate was 0.25 mL/min and the injection volume 10 μL. The detection was set at 280 nm, using phosphoric acid 10^{-3} M (solvent A) and acetonitrile (solvent B). The gradient elution program was: from 91 to 86% A in ten minutes; from 86 to 82% A in ten minutes; from 82 to 60% A in ten minutes; from 60 to 40% A in five minutes; from 40 to 91% A in five minutes; equilibration time of five minutes. The peaks identification was performed comparing the retention times and absorption spectra of pure compounds (supplied from Extrasynthese) and were found analogues to values reported in the literature (BAOSHAN *et al.*, 1998; RICARDO *et al.*, 1991).

The determination of biogenic amines (BA) in wine was carried out by HPLC/FLD. A Hewlett-Packard (Agilent Technologies Palo Alto, CA, USA) 1100 series HPLC instrument was used, with a fluorescence detector set at excitation and emission wavelengths of 340 and 450 nm, respectively. The samples were subjected to an automatic pre-column derivatization procedure using o-phthalaldehyde (OPA Reagent, Agilent Technologies, Palo Alto, CA, USA). All separations were performed on a 200 x 4.6 mm, 5-μm Alltima C18 column (Alltech, Deerfield, IL, USA), protected by a 7.5x4.6 mm guard cartridge of the same type. Samples were injected into the column after being filtered through a 0.2 mm RC filter (Schleicher and Schuell, Keen, NH, USA). The two eluents used as mobile phases were sodium acetate 50 mM (pH 7.2)/THF (96:4) v/v (eluent A) and methanol (eluent B). The elution gradient programme followed the method described by NICOLINI (2003). From a stock solution of 200 mg/L containing agmatine, cadaverine, phenylethylamine, histamine, putrescine, and tyramine (standards purchased by Sigma-Aldrich) in methanol, four diluted solution were prepared and injected: 2.5, 5.0, 10.0, 20.0 mg/L. Quantification of the BA was performed with an internal standard of 10mM of norvaline solution.

Statistical analysis

Multivariate statistical analysis was performed using R Statistical Software (R Core Team (2013), R Foundation for Statistical Computing, Vienna, Austria). Chemical analyses were repeated three times for each sample and the data are presented as mean ± SD. The one way analysis of variance (ANOVA), and Duncan multiple comparison test to measure variation between treatments at a probability level of $p<0.05$ were performed.

RESULTS AND DISCUSSION

Wines composition

The musts collected from the steel tanks had the following chemical/physical characteristics: Control (spontaneous MLF) 210 g/L of reducing sugars, pH 3.30 and total acidity 6.40 g/L; SIM: 205 g/L of reducing sugars, pH 3.27 and total acidity 6.52 g/L; PAF: 214 g/L of reducing sugars, pH 3.35 and total acidity 6.24 g/L. The winemaking process began on the 12th of October with the crushing and destemming of grapes and the yeasts inoculation for all the three experimental processes. The kinetics of AF and malic acid degradation are reported in Figs. 1 and 2 respectively. In Table 1 it is reported the time required for the AF and the MFL for each winemaking. The duration of the fermentation process was identical for the PAF and the Control (both 8 days), while it was longer for the SIM (about 10 days). However, all alcoholic fermentations were regular and complete. LAB in the SIM were able to perform MLF in 23-24 days from the beginning of winemaking. The wine obtained by sequential inoculation (PAF) carried out the degradation of malic acid in 40-41 days from the beginning of the winemaking. Instead, the wine underwent a spontaneous MLF, despite the absence of added LAB, has finished the MLF after 57 days from the beginning of the vinification. Therefore, the wine obtained by the SIM technique has finished the MLF 33-34 days before of Control wine. This data is important since time is a key factor from an economic, techni-

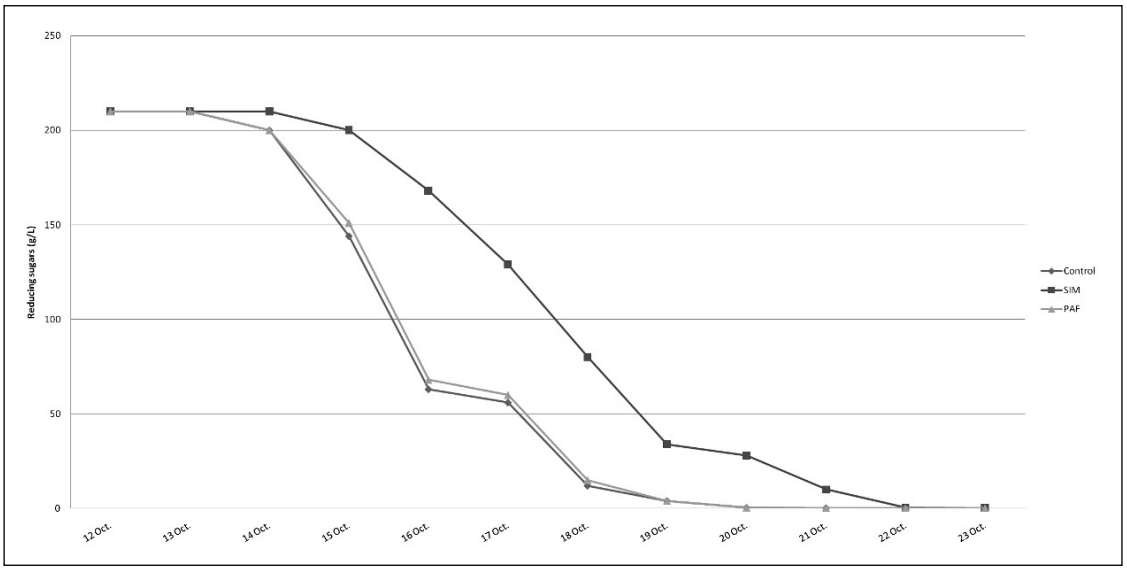

Fig. 1 - Kinetics of alcoholic fermentation.

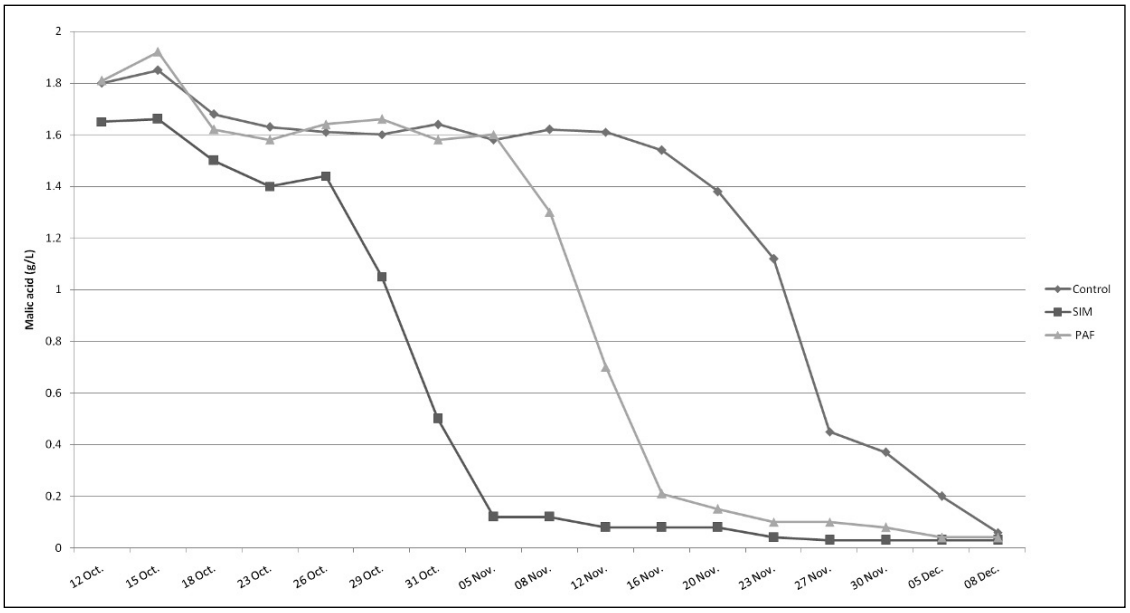

Fig. 2 - Time course of malic acid degradation from the start of alcoholic fermentation.

cal and practical point of view, for a good organizational management of the winery. Table 2 shows the results of the chemical/physical analysis of wines at the end of alcoholic fermentation (racking) and 12 months after racking. Differences were observed in the alcohol content, acidic profile, pH and total acidity. The SIM wine after alcoholic fermentation showed obvious signs of the beginning of MLF. Indeed, the malic acid content (1.44

Table 1 - Time required to complete AF and MLF.

Treatment	Time for AF (days)	Time for malic acid degradation (days after bacterial inoculation) †	Vinification time AF + MLF (days) ‡
Co-inoculation (SIM)	10±0	23±1	24±1
Sequential inoculation (PAF)	8±0	33±4	41±4
Spontaneous MLF (Control)	8±0	NA	57±4

† MLF was considered complete when malic acid concentration was below 0.12 g/L.
‡Vinification time is the time from destemming/crushing to completion of AF and MLF.

Table 2 - Wines composition after AF (racking) and after MLF (12 months after racking).

		At racking			12 months after racking		
		Control	SIM	PAF	Control	SIM	PAF
Alcohol (Vol. %)	x	12.39 ab	12.06 b	12.48 a	12.40 a	12.10 b	12.42 a
	s	0.08	0.07	0.08	0.12	0.08	0.09
Residual sugars (g/L)	x	2.40 a	2.40 a	2.40 a	2.35 b	2.42 a	2.45 a
	s	0.18	0.15	0.20	0.22	0.25	0.20
Total dry extract (g/L)	x	30.50 a	29.40 b	30.50 a	30.20 a	29.80 b	30.40 a
	s	2.50	1.80	2.10	2.08	2.18	2.10
pH	x	3.35 b	3.53 a	3.36 b	3.45 b	3.61 a	3.44 b
	s	0.02	0.03	0.02	0.03	0.02	0.02
Total acidity (g/L)	x	7.65 a	6.15 b	7.50 ab	5.63 a	5.03 c	5.10 b
	s	0.38	0.42	0.45	0.35	0.40	0.39
Volatile acidity (mg/L)	x	0.54 b	0.60 a	0.56 b	0.55 b	0.60 a	0.50 c
	s	0.03	0.02	0.03	0.07	0.08	0.06
Total SO2 (mg/L	x	24.05 a	22.10 b	22.04 b	32.10 b	58.10 a	30.10 c
	s	4.10	3.80	3.50	2.90	3.50	2.80
Tartaric acid	x	3.04 b	2.90 c	3.20 a	2.91 a	2.86 ab	2.62 b
	s	0.28	0.22	0.18	0.4	0.38	0.25
L-malic acid (g/L)	x	1.61 ab	1.44 b	1.64 a	0.12 a	0.05 b	0.06 b
	s	0.09	0.10	0.12	0.01	0.00	0.00
L-lactic acid (g/L)	x	0.10 b	0.60 a	0.12 b	1.45 b	1.52 a	1.41 b
	s	0.00	0.01	0.00	0.08	0.06	0.05
Citric acid (g/L)	x	0.25 b	0.27 ab	0.28 a	0.23 b	0.30 a	0.25 ab
	s	0.02	0.02	0.04	0.01	0.02	0.02

x, mean of three replicates; s, standard deviation.
Mean values followed by the same letter in a row are not significantly different at the 0.05 level of significance.

g/L) was less than Control (1.61 g/L) and PAF (1.64 g/L) wines. In SIM wine the partial transformation of malic acid has produced a certain amount of lactic acid already at racking. Moreover, SIM wine showed a lower total acidity and a higher pH compared to Control and PAF wines. This was mainly due to the transformation of a diprotic acid (malic acid) with two acidic functional groups into a monoprotic acid (lactic acid) with only one acidic functional group, with a corresponding decrease in acidity and an increase of pH. It was observed a difference in the alcohol content of wines, in particular the SIM wine showed the lowest alcohol content. Probably, since the sugar content of the must subjected to the SIM process was slightly smaller thus less alcohol was produced. Another possible explanation is linked to the lactic acid bacteria that could have used part of the reducing sugars, in addition to malic acid, as nutrients for their metabolism. This not only may have influenced the alcohol content of wine, but furthermore had furnished an increased energy for the cellular development of the bacteria resulting in the production of more volatile compounds and greater amounts of acetic acid, as it was found in SIM wine. Indeed, the volatile acidity expressed as acetic acid was slightly higher in the SIM wine than in the other two wines. Both possibilities may have contributed to the lower alcoholic content, as it is confirmed by the findings of some other authors which have observed a delay of alcoholic fermentation and a use of the sugars of must by LAB (LAFON-LAFOURCADE *et al.*, 1983). After one year of storage, the SIM wine still has a lower acid strength, represented by a higher pH and a lower total acidity compared to the other wines. The content of sulfur dioxide, in order to favour the MLF especially in the Control, has been deliberately kept low. There were no significant differences in respect of tartaric acid and citric acid.

Polyphenolic composition and wine colour

Table 3 shows the polyphenolic composition and chromatic characteristics of wines after alcoholic fermentation and 12 months after racking. The effects of different MLF starts showed a marked change in the content of polyphenols in SIM wine already at the end of the AF. Indeed, the index of total polyphenols, the total flavonoids, the total and monomeric anthocyanins contents are higher in the SIM wine, with variations ranging from 5 to 17%, than in the other wines. The differences in tannins (proanthocyanidins and flavans reacting with vanillin) content between wines were not significant. After 12 months from racking, all the wines had finished the MLF thus a natural reduction of the polyphenolic compounds (total flavonoids, flavans, and an-

Table 3 - Phenolic composition and chromatic characteristics of Aglianico wines after AF (racking) and after MLF (12 months after racking).

		At racking			12 months after racking		
		Control	SIM	PAF	Control	SIM	PAF
Total phenols (mg/L)	x	1812 b	2062 a	1831 b	1674 b	1793 a	1652 ab
	s	66	66	37	49	52	41
Total flavonoids (mg/L)	x	1982 c	2388 a	2008 b	1466 b	1585 a	1583 a
	s	73	74	45	42	44	36
Vanillin index (mg/L) V	x	1026 a	1013 b	1017 b	829 b	935 a	659 c
	s	38	39	23	19	33	21
Proanthocyanidins (mg/L) L	x	2489 a	2490 a	2398 b	2135 a	1693 b	1533 c
	s	71	77	59	67	55	38
Total anthocyanins (mg/L)	x	321 ab	338 a	315 b	223 b	242 a	169 c
	s	15	16	19	12	12	15
Monomeric anthocyanins (mg/L)	x	232 ab	251 a	227 b	90 b	110 a	92 ab
	s	12	9	12	4	6	8
D.O. 420 nm (P.O. 1cm)	x	3.49 a	2.82 c	3.06 b	2.84 a	2.54 b	2.47 ab
	s	0.07	0.07	0.06	0.06	0.05	0.05
D.O. 520 nm (P.O. 1cm)	x	7.16 a	5.17 c	6.23 b	4.03 a	3.69 b	3.39 c
	s	0.20	0.16	0.18	0.10	0.01	0.01
D.O. 620 nm (P.O. 1cm)	x	1.06 a	0.85 c	0.94 b	0.75 c	0.78 b	0.99 a
	s	0.03	0.02	0.03	0.02	0.03	0.04
Colour intensity (P.O. 1cm)	x	11.71 a	8.84 c	10.23 b	7.62 a	7.01 b	6.85 c
	s	0.20	0.10	0.14	0.10	0.12	0.08
Tint (E420/E520)	x	0.49	0.54	0.49	0.70	0.69	0.73
V/L index	x	0.41	0.41	0.44	0.39	0.55	0.43

x, mean of three replicates; s, standard deviation.
Mean values followed by the same letter in a row are not significantly different at the 0.05 level of significance.

thocyanins) was observed. Anyway, the total anthocyanins were still slightly higher in the SIM wine compared to Control and considerably higher compared to PAF wine. From the other side, the content of proanthocyanidins (high molecular weight tannins) was significantly higher in the Control than in the other wines. Therefore, an early MLF as for the SIM and PAF processes, causes greater losses over time of higher molecular weight tannins (proanthocyanidins). The increase in pH observed at racking for SIM wine has promoted the polymerization processes of high molecular weight tannins leading to a partial precipitation and thus their removal by pouring operations. Some authors (CAROLINE and EVELINE, 2011; COSTELLO *et al.*, 2012) in tests of management of MLF have observed similar effects in respect of these compounds. It is well known that MLF can reduce the colour intensity in red wines due to numerous factors associated with the MLF (BURNS *et al.*, 2013). Indeed, the Aglianico wines obtained with the SIM process, already at the end of alcoholic fermentation (Table 3), showed a colour intensity of 8.84 which was significantly lower than PAF and Control wines. Usually, this index is positively correlated to the anthocyanin content, in this case, considered that in SIM wine there was an increase in pH mostly due to the partial MLF, despite a slightly higher anthocyanin content respect to the other wines, a change in the balance of the pH-dependent anthocyanin pigments has determined a certain loss of the red colour. The colour reduction may also be due to the precipitation of the free anthocyanins molecules with polysaccharides and potassium bitartrate. Twelve months after racking, the differences in colour intensity, although reduced, remained quite significant and the Control wine still showed the highest value. Also the 420 nm and 520 nm absorbances were higher in the Control wine, while the hue did not show significant differences between wines.

The composition of monomeric catechins and oligomeric procyanidins is shown in Table 4. A common feature to all wines is the predominance of the (+)-catechin among all monomeric flavanols. Among dimeric procyanidins B2 and B4 are present in greater quantities. The trimeric procyanidins were detected in all wines but in small quantities. At racking, the SIM wine differed from the other two wines because of the lowest content of almost all the flavan-3-ols, with the exception of a few gallic acid esters such as epicatechin gallate, epigallocatechin gallate and procyanidin B2 gallate. Less difference were found between the Control and PAF. After one year from racking, all wines showed a reduction in the content of (+)-catechin, (-)-epicatechin, epigallocatechin, procyanidin B1, B3 and B4, while there was a general increase of procyanidin B2, gallic acid esters and trimeric procy-

Table 4 - Concentration (mg/L) of monomeric catechins and oligomeric procyanidins in Aglianico wines.

		At racking			12 months after racking		
		Control	SIM	PAF	Control	SIM	PAF
(+)-Catechin	x	40.36 ab	36.7 c	41.34 a	35.06 b	28.73 c	39.68 a
	s	1.74	1.83	1.88	1.25	1.44	1.32
(–)-Epicatechin	x	25.61 b	19.24 c	27.22 a	21.98 b	16.96 c	24.59 a
	s	1.21	0.71	1.29	1.12	1.09	1.15
Procyanidin B1	x	17.60 a	13.30 b	17.42 ab	12.09 a	7.76 c	10.39 b
	s	0.45	0.80	0.73	0.71	0.74	0.83
Procyanidin B2	x	37.40 a	29.60 b	36.40 ab	39.06 b	33.18 c	40.95 a
	s	1.75	1.67	1.98	1.44	1.56	1.22
Procyanidin B3	x	10.80 a	5.81 b	10.30 ab	7.52 b	4.95 c	8.84 a
	s	0.78	0.46	1.04	0.34	0.46	0.27
Procyanidin B4	x	30.73 b	27.55 c	32.60 a	28.26 b	24.77 c	32.59 a
	s	1.75	1.85	1.78	1.88	1.36	1.33
Procyanidin B2 gallate	x	22.28 c	23.10 b	25.20 a	32.36 a	26.33 c	27.39 b
	s	0.97	0.85	1.04	0.98	1.44	1.65
Epicatechin gallate	x	3.74 b	4.30 a	3.31 c	5.73 b	4.55 c	7.12 a
	s	0.24	0.88	0.37	0.54	0.74	0.67
Gallocatechin	x	5.21 a	3.40 c	4.30 b	5.62 a	2.48 c	5.04 b
	s	0.24	0.23	0.27	0.38	0.55	0.29
Epigallocatechin	x	4.33 ab	2.41 b	4.73 a	3.99 a	3.08 b	3.48 ab
	s	0.37	0.46	0.63	0.74	0.74	0.74
Epigallocatechin gallate	x	1.35 c	3.30 a	2.37 b	1.24 c	4.45 a	4.01 b
	s	0.08	0.08	0.04	0.04	0.03	0.04
Trimer T2	x	6.53 c	6.95 b	8.64 a	7.67 b	8.44 a	8.22 ab
	s	0.72	0.83	6.78	0.34	0.46	0.48
Trimer C1	x	7.33 a	5.39 b	7.11 a	8.17 b	6.70 c	9.28 a
	s	0.77	0.71	0.84	0.66	0.53	0.79

x, mean of three replicates; s, standard deviation.
Mean values followed by the same letter in a row are not significantly different at the 0.05 level of significance.

anidins C1 and T2. The SIM wine confirmed a lower content of almost all forms of monomeric catechins and oligomeric procyanidins, while the PAF showed concentrations that were even higher than Control wine. Also in this case, a faster MLF in SIM and PAF from the early stages of racking had caused a lower acidic strength, resulting in a loss of these compounds.

Biogenic amines composition

Table 5 shows the concentrations of biogenic amines in Aglianico wines. The average concentration of total amines at racking differs slightly between thesis submitted at different management of MLF, ranging from 9.73 mg/L in SIM wine to 10.38 mg/L in Control wine. Af-

Table 5 - Concentration (mg/L) of biogenic amines in Aglianico wines.

		At racking			12 months after racking		
		Control	SIM	PAF	Control	SIM	PAF
Histamine	x	2.78 a	2.44 c	2.65 b	3.53 a	0.24 ab	0.20 b
	s	0.74	0.22	0.39	0.92	0.02	0.02
Agmatine	x	1.45 b	1.54 a	1.57 a	1.56 c	2.41 b	2.93 a
	s	0.46	0.38	0.40	0.55	0.63	0.74
Putrescine	x	3.74 a	3.32 b	3.66 ab	10.51 a	8.48 c	9.54 b
	s	0.82	0.67	0.74	1.86	1.59	1.48
Tyramine	x	0.70 b	0.72 ab	0.74 a	0.76 b	1.40 a	0.60 c
	s	0.08	0.09	0.06	0.09	0.10	0.03
Cadaverine	x	1.42 ab	1.35 b	1.44 a	1.60 a	1.59 a	1.16 b
	s	0.36	0.42	0.40	0.39	0.28	0.29
Phenylethylamine	x	0.29 ab	0.36 a	0.27 b	0.42 b	0.60 a	0.58 ab
	s	0.05	0.06	0.06	0.06	0.07	0.06
Total biogenic amines	x	10.38	9.73	10.33	18.38	14.72	15.01

x, mean of three replicates; s, standard deviation.
Mean values followed by the same letter in a row are not significantly different at the 0.05 level of significance.

ter 12 months of storage, when wines had already reached a chemical/physical and biological stabilization, it was observed an increase in the BA content. The differences found between the different winemaking protocols were significant. The average content of total biogenic amines in the wine obtained from spontaneous MLF (18.38 mg/L) was higher than co-inoculation (14.72 mg/L) and sequential inoculation (15.01 mg/L) wines. In percentage the increase was 77.0% in Control, 51.3% in SIM and 45.3% in PAF wine. This higher percentage in the Control wine could be ascribed to a release of amino acids as a consequence of yeast lysis during AF and to the proliferation of LABs with carboxylase activity during spontaneous MLF. Putrescine was the most abundant amine, with Control showing the highest amount at racking (3.74 mg/L before MFL) and also 12 months after racking (10.51 mg/L). Histamine, which is thought to be the cause of various adverse reactions to wines (TAYLOR *et al.*, 1989; WANTKE *et al.*, 1996), after 12 months increased its concentration in the wine produced with spontaneous MLF (Control) wine and vice versa decreased in SIM and PAF wines. Indeed, after 12 months histamine was almost absent in wines subjected to inoculation of LAB (0.24 mg/L in SIM and 0.20 mg/L in PAF), while the Control wine showed an higher value (3.53 mg/L) which was anyway very similar to values reported by other authors (IZQUIERDO-CAÑAS *et al.*, 2008; PRAMATEFTAKI *et al.*, 2006). Probably the histidine decarboxylase activity was present and active in wine produced with spontaneous MLF for the whole period of aging, instead was absent or not active in wines subjected to inoculation of LAB.

CONCLUSION

The simultaneous inoculation with yeast and bacteria (SIM) has reduced the duration of MLF of about 33 days compared to the wine obtained without the addition of any LAB (Control). The simultaneous inoculation already at the end of the AF showed evident signs of the onset of MLF. After 12 months from racking, there was a weakening of differences in phenolic compounds content, but the wine underwent a spontaneous MLF (Control) remained more colourful and more rich in high molecular weight tannins. Concerning the content of monomeric catechins and oligomeric procyanidins it was observed the predominance of (+)-catechin among the monomeric flavanols and the procyanidin B2 among the dimeric procyanidins. These compounds were lower in the co-inoculation wine compared to the other two wines. The different MLF managements led to a different evolution in the content of biogenic amines. The simultaneous (SIM) and the sequential (PAF) inoculation of lactic acid bacteria for the MLF led to a significant reduction in almost all the amines investigated with respect to the Control (spontaneous MLF). After 12 months from racking, the average total content of biogenic amines was lower in the wine underwent sequential inoculation compared to the co-inoculation.

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PAPER

QUALITY OF AUTOCHTHONOUS SICILIAN PLUMS

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ABSTRACT

Thirty four plum local varieties and accessions obtained from different growing area of the Sicilian island were analyzed for their qualitative and nutraceutical properties and three commercial cultivar were used as references. These properties included the fruit fresh weight (g), the pulp firmness (FFF), the total soluble solids (TSS), the titratable acidity (TA), the total anthocyanins, the phenolics content and the antioxidant activity.

This preliminary study showed significantly differeces among the plums; Zuccarato giallo and Prunu Niuru presented TSS higher than the commercial cultivars (24.9 and 21.6 °Brix respectively) and interesting data obtained on the nutraceutical compounds values suggested these local cultivars as sources of polyphenols (Zuccarato giallo with 663 mg GA/100 gFW) and natural antioxidants (Pruno Regina with 47.46 Fe^{2+}/100 gFW). The characterization of these plums could represent also an important resource for the international activity in the genetic improving and the collection of the more interesting quality traits could be useful for improving the *Prunus* database actually in use.

- Keywords: agrobiodiversity, plum, quality, nutraceutical compounds -

1. INTRODUCTION

The varietal diversity is one of the agricultural biodiversity (HEYWOOD, 1999) and the management by farmers at the local community level is one of the factors involved to preserve it. The International Treaty on Plant Genetic Resources for Food and Agriculture approved in 2001 has assigned to all joined Nations the duty to take specific local actions in terms of preservation of genetic resources, particularly those that are directly related with the food and agriculture, ie. agrobiodiversity. Since that, many national initiatives have been set up aiming at the recovery and characterization of the genetic autochthonous resources as well as the enhancement in both nutritional and nutraceuticals traits.

The need to maintain, to protect and to manage agobiodiversity is increasing; the identification of the composition of locally cultivated food as sources of nutraceutical compounds is essential to promote a more food-base approach to nutrition and health (SCOONES, 1992). Plums are the most taxonomically diverse of stone fruits (DAS *et al.*, 2011) and the varietal diversity is strongly related to the high percentage of self-incompatibility that led over the centuries to various cross-pollinations with recombinations of characters (SOTTILE *et al.*, 2010a). The management in situ is one of the commonly recommended germplasm conservation approaches (MAXTED *et al.*, 2010) and in Sicily plum fruits were cultivated since the sixteenth and seveteenth centuries as reported in literature (CUPANI, 1696; NICOSIA, 1735) due the propitious pedoclimatic conditions of the territory for their development and genetic diversification (IMPALLARI *et al.*, 2010). The most representative areas for the diffusion of plum trees are described by these authors as the Palermo and Trapani Province which today have an important rule to improve and to diffuse the cultivation of the specie by using its natural and favorable climatic conditions. The characterization and identification of plum varieties usually is performed on morphological data (SOTTILE *et al.*, 2010b), phenototic traits (HORVATH *et al.*, 2011) and more recently on some molecular markers (GREGOR *et al.*, 1994; ORTIZ *et al.*, 1997; GHARB *et al.*, 2014), but the study of the nutraceutical compounds (SOTTILE *et al.*, 2010b), represent today an important tool to improve the collected data and to describe better the varietal diversity (VASANTHA RUPASINGHE *et al.*, 2006; DÍAZ-MULA *et al.*, 2009). The replacement of local cultivars with the new one introduced by genetic improvement programs, due to a higher productivity, as well as resistance or tolerance to pests and diseases, or to abiotic stress, has caused a strong genetic erosion of the indigenous fruit tree species germplasm (IMPALLARI *et al.*, 2010). As a consequence of the globalization process, the homologation involved the fruit consumption and deeply contributed in the loss of the unique taste of these fruits.

Many studies have showed that the locally available cultivars, varieties and wild underutilized ecotypes; JABLONSKA-RYS *et al.*, 2009; PETRUCCELLI *et al.*, 2013;) are in many cases more rich in nutrients than similar commercially foods, confirming the old ecotypes as genetic resources of fruit nutritional traits.

Plums have the potential to contribute greatly to human nutrition because of their richness in fiber and antioxidants (STACEWICZ-SAPUNTZAKIS *et al.*, 2001; SOTTILE *et al.*, 2010b). In many cases the genetic resources with a higher relevance in terms of nutraceutical facts are related to old varieties and reported with a high risk of erosion. To limit the loss of biodiversity and to adopt collaborative conservation strategies it is necessary to improve the knowledge of the genetic resources and their horticultural aspects. The reduction in the genetic variability is increasing from the past century so as established by international approaches to biodiversity preservation protocols, each Country is responsible for its own genetic resources (DAS *et al.*, 2011). No detailed study concerning physical and nutritional properties of old Sicilian plum ecotypes have been performed up to now, so the aim of this study was to determine some qualitative and nutritional traits of plum fruits belonging to some local cultivars identified in the Sicily island.

2. MATERIALS AND METHODS

2.1 Plant material and collection of data

One hundred forty-three georeferenced plum cultivars and accessions were identified in the Sicilian island from existing bibliography and a territorial investigation but in this preliminary study only thirty-seven varieties are considered. In the Table 1 the total 37 local cultivars and accessions of plums used for the qualitative and nutraceutical analysis on fruits are reported. These included 34 plum trees from different locations in the Sicily region and 3 commercial varieties respectively of *Prunus domestica* L. (cv. Stanley) and *Prunus salicina* Lindl. (cv. Shiro and cv. Angeleno), as references. The investigated area is located both in the West and in the Eastern part of Sicily. For each local cultivars and accession 30 fruits randomly collected from the entire production were used.

The plants of the Sicilian germplasm were maintained on site in their natural habitat where they are routinely grown by local farmers. In all cases minimal cultural techniques have been applied, without any fruit thinning. All cultivars produce primarily on spurs and pruning is not commonly carried out. An integrated pest management approach is ordinary adopted by

Table 1 - List of cultivars and accessions plums collected from the local germplasm of Sicily and sampling locations.

	Authoctonous cultivars and accession name	Location*
1	69SUS005P	Messina (ME)
2	Lazzarino	Palermo (PA)
3	Sanacore Tardivo	Palermo (PA)
4	Zuccarino Rosa	Messina (ME)
5	Prunu Nucidda	Messina (ME)
6	Cuore di Bue	Catania (CT)
7	Pruno Regina	Catania (CT)
8	107SUS009E	Trapani (TP)
9	107SUS008B	Trapani (TP)
10	107SUS007B	Trapani (TP)
11	Rapparinu Russu	Trapani (TP)
12	Sanacore	Palermo (PA)
13	Prunu Niuru	Catania (CT)
14	Ariddo di Core	Palermo (PA)
15	Occhio di Bue	Catania (CT)
16	Ranco' Nero	Catania (CT)
17	Don Ciccino	Catania (CT)
18	Cugghiuni di Mulu	Catania (CT)
19	Papale	Catania (CT)
20	71SUS028B	Catania (CT)
21	President	Catania (CT)
22	Pruna di S. Antonio	Messina (ME)
23	Susine Nere	Messina (ME)
24	Nivuru Purmintia	Messina (ME)
25	Nivuru	Messina (ME)
26	Pruna i Sceccu	Messina (ME)
27	Santu Vitu	Messina (ME)
28	66SUS052P	Messina (ME)
29	Prunu Ciraseddu	Catania (CT)
30	Prugnolo rosso	Messina (ME)
31	Pruno Rosa	Palermo (PA)
32	Primintio	Palermo (PA)
33	Prunu Nivuru Codulusu	Trapani (TP)
34	Zuccarato Giallo	Messina (ME)
35	Shiro	Palermo (PA)
36	Stanley	Palermo (PA)
37	Angeleno	Palermo (PA)

*Data on geographical position (latitude and longitude) are available for all plums.

growers by following the regional governmental rules for plum.

The fruits were picked by hand at the ripe stage (Table 2). The fruits damaged were removed, were graded for color and size uniformity and they were immediately transported to the pomological laboratory for analysis.

2.2 Fruit quality traits

The weight was obtained measuring individually 30 fruits per each local cultivars and accessions. The data were expressed as the mean ± SE. Fruit weight (g) was performed using an electronic balance (SE622, WVR, USA) with an accuracy of 0.01 g.

The fresh fruit firmness (FFF) was measured using an Effegi hand-held penetrometer (Turoni, Italy) with a 5-mm-diameter plunger in accordance with standard industry practice. The skin of the fruits was partially removed before measuring. Two measurements (30 fruits) were made on opposite sides of the central zone of the fruits and then averaged to yield a mean value for the fruit. The measurements were reported in kg force (kgf) cm^{-2}.

After the firmness measurements for the total soluble solids (TSS) and the titratable acidity (TA) determination, the same fruits were completely hand-peeled and skin and pulp were cut in small pieces to obtain homogeneous samples. For TSS and TA determination 10 g of pulp samples were squeezed using a commercial blender and the extracted juice was later sieved and centrifuged at 8,000 × g for 20 min (Sigma 3-18 K, Osterode and Harz, Germany). An aliquot of this supernatant was used to determine TSS with a digital pocket refractometer Atago PAL-1 (Atago Co. Ltd., Japan) calibrated at 20°C to 0% with distilled water, and expressed as per-

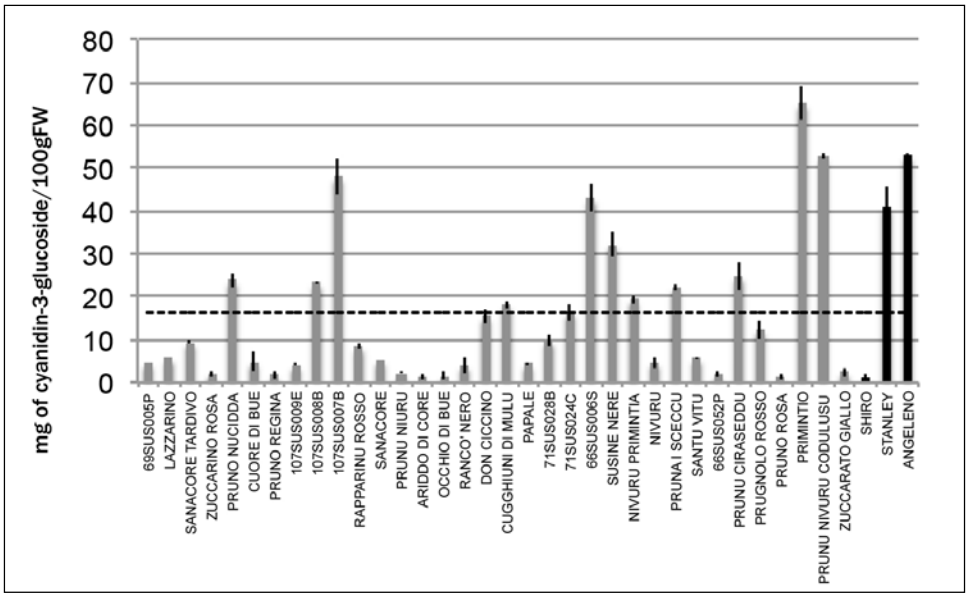

Fig. 1 - Total anthocyanins of local Sicilian plums

Table 2 - Harvesting dates for the authochtonous Sicilian plums.

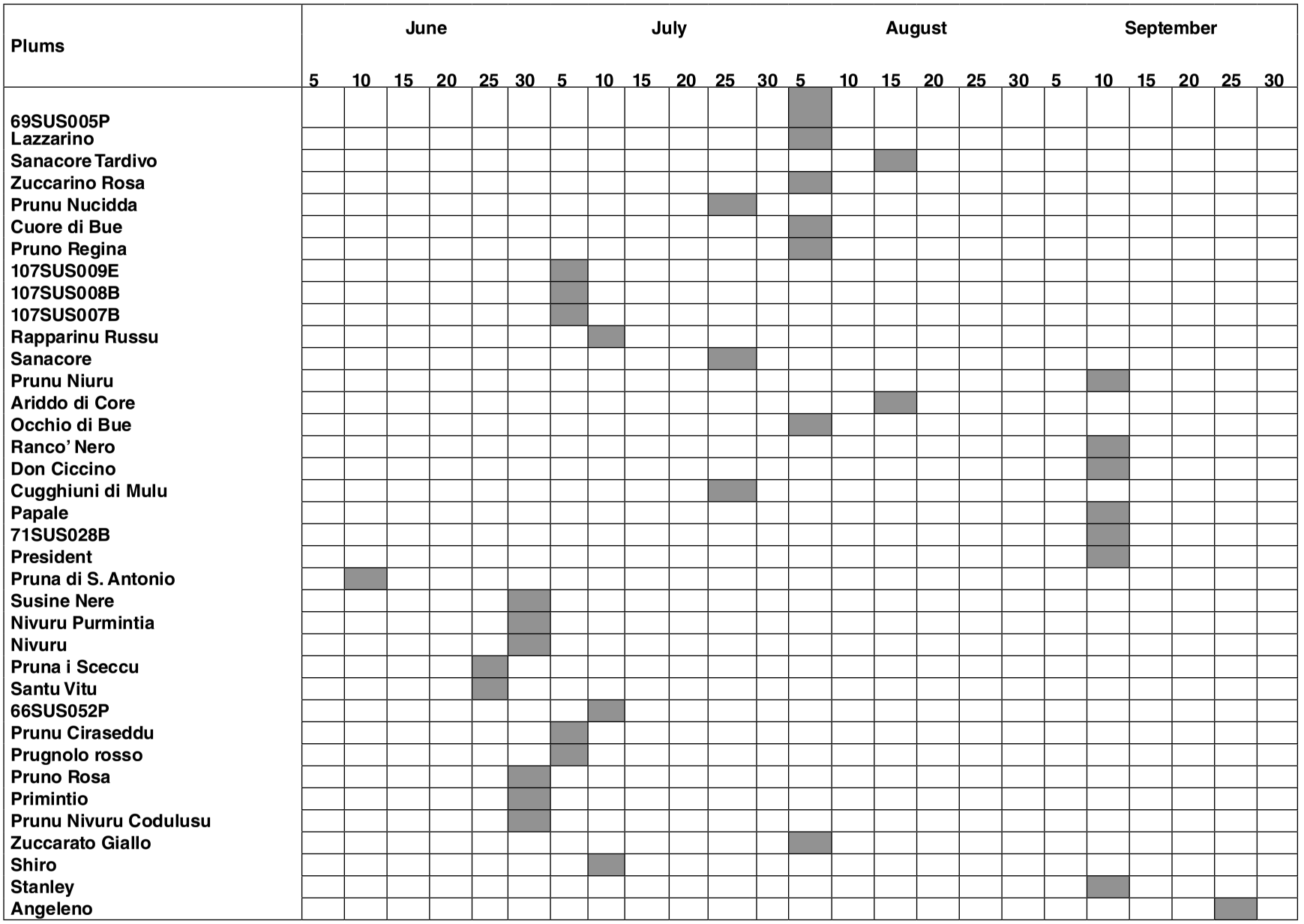

Table 3 - Fruit quality traits of 37 authochtonous Sicilian plums.

	Fruit weight (g)		FFF kgf cm^{-2}		TSS °Brix		TA g malic acid L^{-1}		MI
Plums									
69SUS005P	34.20±4.51	G-I	0.88±0.21	D-I	19.6±0.21	B-E	7.76±1.74	G-O	2.5
LAZZARINO	16.77±2.90	O-S	0.58±0.26	L-R	16.5±1.47	F-I	6.28±0.41	I-Q	2.6
SANACORE TARDIVO	28.98±7.13	L-M	0.80±0.22	G-N	15.3±0.06	H-N	7.72±0.26	G-P	2.0
ZUCCARINO ROSA	13.67±1.99	R-U	0.31±0.12	S-U	15.1±0.70	H-N	9.02±0.14	F-L	1.7
PRUNO NUCIDDA	12.06±4.32	S-U	1.14±0.36	D	21.2±0.28	B-C	9.00±0.13	F-L	2.4
CUORE DI BUE	83.79±9.02	A	0.76±0.21	H-O	13.3±0.21	L-P	12.21±1.22	C-E	1.1
PRUNO REGINA	33.62±3.58	H-I	0.76±0.22	H-O	18.4±0.21	C-G	7.95±0.09	G-N	2.3
107SUS009E	21.07±7.06	N-O	0.63±0.20	I-Q	11.5±0.21	O-Q	16.56±0.57	B	0.7
107SUS008B	14.26±2.44	Q-U	0.40±0.12	Q-T	12.8±0.00	M-P	11.97±1.02	C-E	1.1
107SUS007B	14.93±3.43	P-U	0.40±0.11	Q-T	15.5±0.42	G-N	10.56±0.15	E-H	1.5
RAPPARINU ROSSO	13.75±2.25	Q-U	0.58±0.21	L-R	15.7±1.91	G-N	4.27±0.75	M-Q	3.7
SANACORE	19.26±5.86	O-Q	0.93±0.30	D-H	16.0±1.06	F-L	7.98±0.43	G-M	2.0
PRUNU NIURU	26.36±4.44	L-N	0.69±0.15	G-P	21.6±1.49	B	4.93±0.25	M-Q	4.4
ARIDDO DI CORE	16.75±3.19	O-S	1.07±0.35	D-F	20.1±2.03	B-D	6.13±0.76	I-Q	3.3
OCCHIO DI BUE	41.76±8.28	E-F	0.55±0.10	N-S	15.6±0.28	G-N	4.86±0.08	M-Q	3.2
RANCO' NERO	44.13±5.39	E	0.60±0.10	L-R	16.6±0.07	E-I	7.29±0.09	H-P	2.3
DON CICCINO	37.22±5.92	F-H	0.47±0.06	P-S	19.1±0.28	B-F	10.12±0.21	E-I	1.9
CUGGHIUNI DI MULU	61.53±8.92	C	0.56±0.13	M-S	14.6±0.06	I-O	8.24±0.39	G-M	1.8
PAPALE	45.19±6.46	D-E	0.57±0.17	M-R	15.4±0.10	G-N	9.60±1.17	E-I	1.6
71SUS028B	49.99±8.77	D	0.55±0.07	N-S	16.7±0.15	E-I	7.67±0.24	G-P	2.2
71SUS024C	66.52±12.77	B-C	0.87±0.27	E-I	13.7±0.06	I-P	12.78±0.33	C-E	1.1
66SUS006S	16.16±4.37	O-T	0.65±0.17	I-Q	14.8±0.07	H-N	9.43±0.91	E-I	1.6
SUSINE NERE	9.48±1.69	U	0.52±0.05	O-S	14.2±0.07	I-O	11.83±0.99	C-E	1.2
NIVURU PRIMINTIA	40.70±7.23	E-F	0.82±0.17	F-M	14.5±0.96	I-O	10.84±0.26	E-G	1.3
NIVURU	15.21±2.91	P-T	0.69±0.26	H-P	14.9±0.07	H-N	9.65±1.26	E-I	1.5
PRUNA I SCECCU	39.79±7.30	E-G	0.83±0.31	F-L	11.0±0.28	P-Q	22.62±0.59	A	0.5
SANTU VITU	20.41±2.48	O-P	0.52±0.25	O-S	14.0±3.02	I-P	4.45±0.45	P-Q	3.1
66SUS052P	65.64±10.75	B-C	1.04±0.64	D-G	12.8±0.35	N-Q	14.56±0.47	B-C	0.9
PRUNU CIRASEDDU	14.21±2.05	Q-U	0.35±0.04	R-U	10.1±0.14	Q	3.60±0.18	Q	2.8
PRUGNOLO ROSSO	18.45±2.07	O-R	1.10±0.27	D-E	17.7±0.78	D-H	6.76±0.42	I-P	2.6
PRUNO ROSA	17.90±3.32	O-R	0.55±0.26	N-S	16.2±0.74	F-L	8.66±1.07	G-L	1.9
PRIMINTIO	21.09±5.33	N-O	0.46±0.17	P-S	15.7±1.44	G-N	10.94±2.15	D-G	1.4
PRUNU NIVURU CODULUSU	10.78±1.68	TU	0.12±0.04	U	16.7±0.07	E-I	14.23±0.40	B-D	1.2
ZUCCARATO GIALLO	13.57±2.89	R-U	0.16±0.05	T-U	25.0±0.35	A	5.86±0.27	L-Q	4.3
SHIRO	36.62±5.16	F-H	1.57±0.09	C	15.0±0.40	G-M	10.63±0.81	E-H	1.4
STANLEY	40.10±3.35	E-F	2.46±0.10	B	15.9±0.36	G-M	5.23±0.20	L-Q	3.0
ANGELENO	67.70±9.80	B	4.33±0.39	A	21.2±1.28	B-C	4.64±0.61	P-Q	4.6

Data are means ± SD. Values with the same letter at the column level are not statistically different with the Tukey's test (0.05).

centage (°Brix). TA was determined in 1 mL of the above supernatant diluted in 25 mL of distilled water by titration with 0.1 N NaOH up to pH 8.1, using an automatic titration device (484 Titrino plus, Metrohm, Switzerland) and results expressed as grams of malic acid L^{-1}. Three replicates per measurement were used and the data reported are the mean ± SE.

The TSS:TA ratio was calculated for individual fruit from the TSS and TA results and it expressed the maturity index (MI).

2.3 Total anthocyanins, phenolic content and antioxidant activity

To determine the total anthocyanin content, the total phenolic content and the total antioxidant capacity, fruit extract was obtained using 10 g of fruit added to 25 ml of extraction buffer (500 ml methanol, 23.8 ml deionized water and 1.4 ml hydrochloric acid 37%). After 1 h in the dark at room temperature, the samples were thoroughly homogenized for a few minutes with an ultra turrax (IKA, Staufen, Germany) and centrifuged for 15 min at 3,000 rpm. The clear supernatant fluid was collected and stored at -20 °C until analysis.

The total anthocyanin content was quantified according to the pH differential method of CHENG and BREEN (1991). Anthocyanins were estimated by their difference of absorbance at 510 and 700 nm in a buffer at pH 1.0 and pH 4.5, where $A_{tot} = (A_{515} - A_{700})$ pH 1.0 - $(A_{515} - A_{700})$ pH 4.5. The results are expressed as milligrams of cyanidin-3-glucoside (C3G) equivalent per 100 g of fresh weight (fw).

The total phenolics content was measured using a Folin-Ciocalteu reagent with gallic acid

as a standard at 765 nm following the method of SLINKARD and SINGLETON (1977). The results are expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (fw).

The antioxidant activity was determined using the FRAP (Ferric Reducing Antioxidant Power) assay, according to BENZIE and STRAIN (1996) method modified by PELLEGRINI *et al.* (2003). The antioxidant capacity of the dilute fruits extract was determined by its ability to reduce ferric iron to ferrous iron in a solution of 2,4,6-tris(2-pyridyl)-s-tri-azine (TPTZ) prepared in sodium acetate at pH 3.6. The reduction of iron in the TPTZ–ferric chloride solution (FRAP reagent) results in the formation of a blue-coloured product (ferrous tripyridyltriazine complex), the absorbance of which was read spectrophotometrically at 595 nm 4 min after the addition of appropriately diluted fruits extracts or antioxidant standards to the FRAP reagent. The results were expressed as mmol Fe^{2+} equivalents per kilogram of fresh fruits.

All of these analyses were performed using a UV-Vis spectrophotometer 1600-VWR. Three replicates per measurement were used.

2.4 Statistical analysis

The data obtained were treated with one-way analysis of variance (ANOVA) using SPSS for Windows version 20.0 and the means were separated using the Tuckey test ($P \leq 0.05$).

3. RESULTS

3.1. Quality parameters

In the Table 3 the qualitative traits studied for the different plums are reported.

As already reported, fruit size is an important quality parameter to evaluate the economic value for the consumption of fresh fruits (PETRUCCELLI *et al.*, 2013) and to determine the category of the ranges of marketability of many fruits. It is affected by a number of variables, including source–sink relationship (SNELGAR *et al.*, 1998), water availability (INTRIGLIOLO and CASTEL, 2006) as well as temperature and growing conditions in general. In our study a great variability on fruit weight has been reported among the different plums suggesting a different fruit surface/volume (EIFERT *et al.*, 2006), a fruit size distribution into different classes and a different correlation to physical-chemical parameters, such TSS and TA. Fruits with greater weight would have a greater proportion of edible flesh (FRANCO-MORA *et al.*, 2009). The fresh weight of all plums analysed revealed a mean value of 30.91 g ranging from 83.79 to 9.48 g; the maximum value was observed for the Cuore di Bue cultivar while the lowest was observed for the Susine Nere plums. Less than half of the plums (sixteen) showed higher values than the mean value. The Don Ciccino cultivar was the only one to show the weight (37.22 g) similar to the values of the commercial cv. Shiro (36.62 g) while Nivuru Primintia and Occhio di Bue with respectively 40.70 and 41.76 g showed similar weight to the commercial cv. Stanley (40.10 g). All plums showed statistically differences from the commercial cv. Angeleno which weight was of 67.70 g.

Flesh firmness is a key quality parameter, since it is directly related to fruit ripeness, and is often a good indicator of shelf-life potential (VALERO *et al.*, 2007). The highest pulp firmness was observed for all the three commercial cultivars, the cv. Angeleno with 4.33 (kgf) cm^{-2} was the higher value followed by the cv. Stanley with 2.46 (kgf) cm^{-2} and the cv. Shiro with 1.57 (kgf) cm^{-2}. All autochthonous plums (34) showed statistically differences from these last and only 11% of them showed FFF value major than 1(kgf) cm^{-2}. The mean value was of 0.82 (kgf) cm^{-2}, the lowest value was reached by the Prunu Nivuru Codulusu probably due to the low weight (10.78 g), while the highest was observed for the Pruno Nucidda (1.14) (kgf) cm^{-2}.

The physical-chemical parameters, such TSS and TA, strongly influence the consumer preference for stone fruit quality and the aromatic profile for the plums consumption (CRISOSTO *et al.*, 2007).

The Zuccarato giallo and the Prunu Niuru showed the highest TSS content (25.0 and 21.6 °Brix, respectively) while the lowest TSS value (10.1 °Brix) was scored by the Prunu Ciraseddu cultivar. Observing the Table 2 there is a tendency for late season plums to have higher TSS than early season plums.

Less than half of the plums (43%) showed the TSS content greater than the mean value (16.6°Brix). The 69SUS005P, Pruno Nucidda, Pruno Regina, Prunu Niuru, Ariddo di Core and Don Ciccino authoctonous plums showed similar values to the commercial cv. Angeleno (21.2 °Brix) and no statistically significant differences were observed between these fruits. The 66% of the plums showed value not statistically different from the commercial cv. Shiro and Stanley which scored 15.0 and 16.0 °Brix respectively. The TSS values measured in the local cultivars and accession are quite high when compared to the value find in the literature; in fact TSS between 14% and 16% (WESTWOOD, 1978) or 10 and 15% (DÍAZ-MULA *et al.*, 2009) could suggest edible fruit ready for consumption.

The TA mean value was of 9.10 g malic acid L^{-1} and the 58% of the local plums showed TA values inferior to the average. The lowest acidity was for the Prunu Ciraseddu (3.60 g malic acid L^{-1}) while the highest value was for the Pruna i Sceccu (22.62 g malic acid L^{-1}).

The variations observed in TSS and TA affected the values of the maturity index (MI). Great

differences were observed among values which ranged from a minimum of 0.5 in the case of the Pruna i Sceccu to a maximum of 4.6 for the commercial cv. Angeleno. The Prunu Niuru and the Zuccarato giallo with the MI of 4.4 and 4.3 showed TSS/TA ratio similar to the cv. Angeleno while Primintio (1.4) and Santu Vitu (3.0) were similar to the cv. Shiro and the cv. Stanley respectively.

3.2 Total anthocyanins, phenolic content, and antioxidant activity

The evaluation of the total anthocyanins content (Fig. 1), in general, has shown that this component does not assume, in percentage terms, a high importance in the context of polyphenolic compounds (Fig. 4). The Primintio accession had significantly more total anthocyanins content (65.22 mg of cyanidin-3-glucoside /100 g FW) than other fruits, although it is only 34.5% of the total polyphenols (Fig. 4). The lowest value was for Ariddo di Core (1.22 mg of cyanidin-3-glucoside/100 g FW) which content was similar to the commercial cv. Shiro and the fraction measured on the total content of the total polyphenols was of 0.3 %. Although the anthocyanin fraction mean value was of the 5.8% of the total polyphenol content it was observed that the absolute values detected in some local cultivars and accessions such as Primintio, 107SUS007B and Pruna di S. Antonio were higher than values previously reported for other varieties (TOMÁS-BARBERÁN *et al.*, 2001; CEVALLOS-CASALS *et al.*, 2006; USENIK *et al.*, 2009).

Polyphenols represent the largest group of water-soluble phytochemicals. They have been known to be chemotaxonomic markers for classification purposes in plum fruits (TREUTTER *et al.*, 2012) and their content could contribute strongly to the antioxidant activity in fresh fruits. Generally the polyphenol composition is related to the cultural practices and abiotic factors such as the outside air temperature and the rainfall rate (SALGADO *et al.*, 2008; MILETIC *et al.*, 2012). Strong variations in the total polyphenol content were observed among the plums of the study (Fig. 2) whose mean value was of 301.67 mg GA/100 g FW. The minimum value of 104.87 mg GA/100 g FW was observed for the Sanacore Tardivo while the maximum value of 663.99 mg GA/100 g FW was observed for the Zuccarato giallo. The total phenolic content in the commercial cultivars used as references was lower than that reported by CEVALLOS-CAVALS *et al.*, 2006 (298 to 563 mg/100 g FW) for *Prunus salicina* cv. Shiro (191.17 mg GA/100 g FW) and cv. Angeleno (242.01 mg GA/100 g FW) while according to LOS *et al.*, (2000) it was in the range for *Prunus domestica* (160-300 mg/100 g) (cv. Stanley 211.23 mg GA/100 g FW). The 58% of the local plums included a total polyphenol content major than cv. Angeleno. Previous studies showed as the averages of the total phenolic content of plums were significantly higher than the content in other fruits such as apples (LEE AND SMITH, 2000; PROTEGGENTE *et al.*, 2002) and our data confirmed that.

The total antioxidant capacity of fresh fruits (Fig. 3), expressed as mmol Fe^{2+} per kg of fresh fruits ranged from a maximum value of 47.46 measured for Pruno Regina and a minimum value of 4.14 for the Rapparinu Russu accessions. No statistically significantly differences were ob-

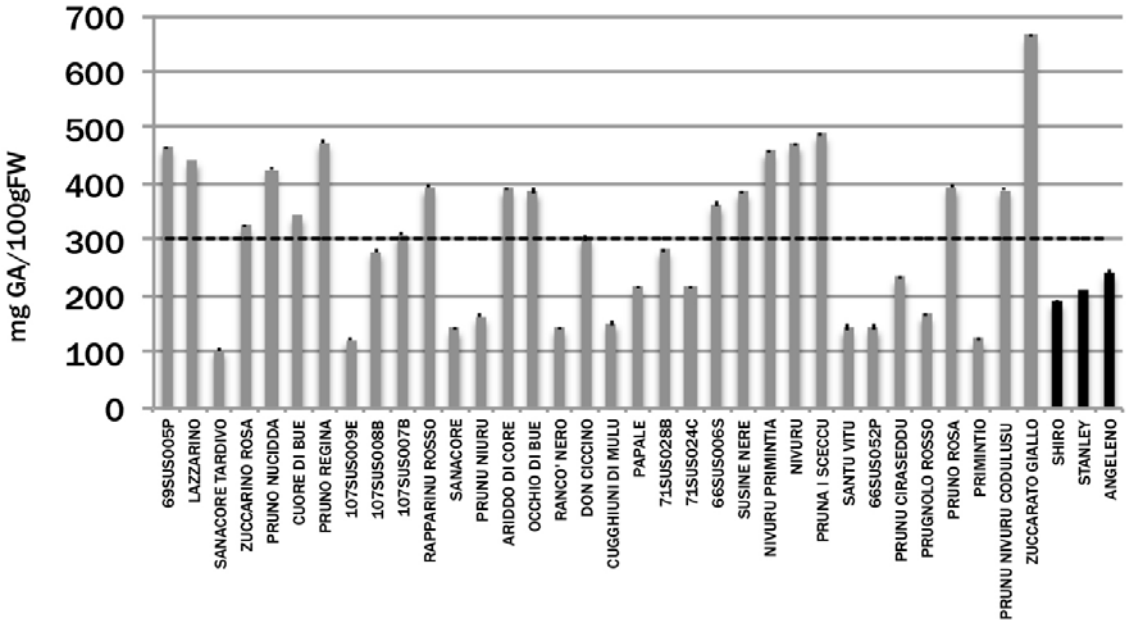

Fig. 2 - Total phenolics content of local Sicilian plums

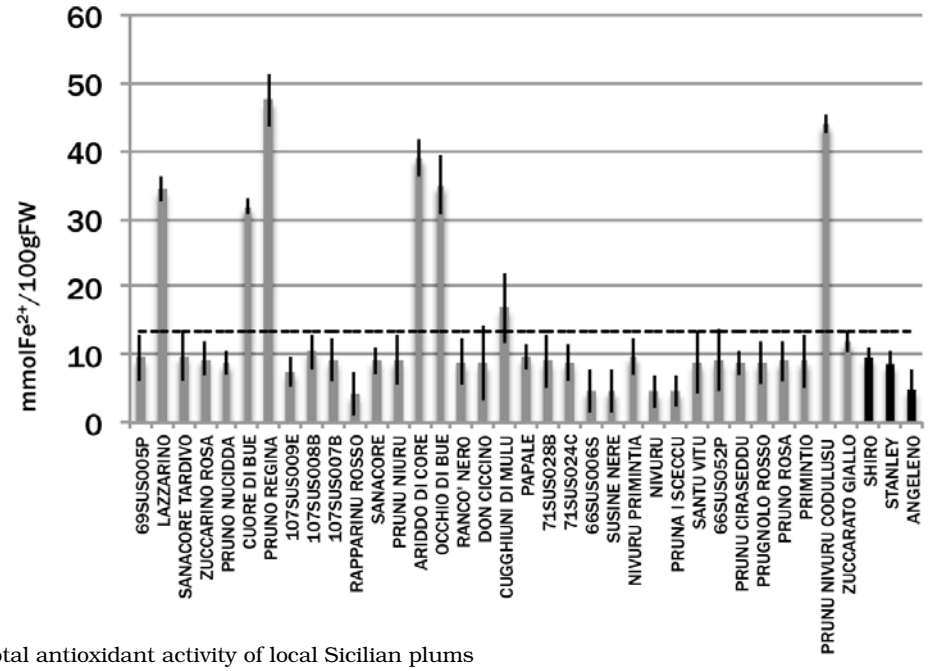

Fig. 3 - Total antioxidant activity of local Sicilian plums

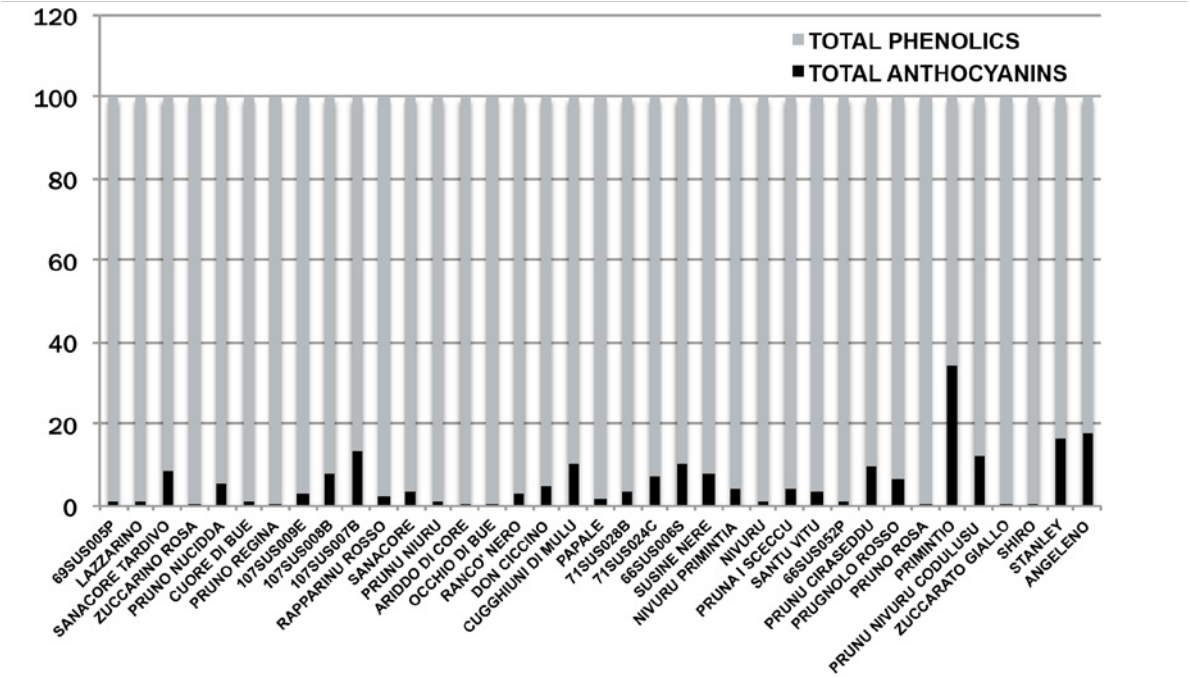

Fig. 4 - Total anthocyanins and phenolics distribution (%) in authochtonous Sicilian plums.

served among the cv. Shiro, Stanley and Angeleno which values ranged between 4.86 and 9.59 mmol Fe^{2+}/100 g FW and the 20% of the fruits showed values major than the average (13.45 mmol Fe^{2+}/100 g FW). As described by FRANKEL and MAYER (2000), the measure of fruit antioxidant capacity is influenced by the analytical method used and this could represent a limit for the evaluation. According to MILETIC *et al.*, (2012), fruits containing the highest total phenols do not necessarily exhibit the highest antioxidant capacity. In fact the highest value observed for the Pruno Regina accession is related to the relative high phenolic content but the same correlation wasn't observed for the Zuccarato giallo which corresponded the highest phenolic content (663.99 mg GA/100 g FW) but the low total antioxidant activity of 11.86 mmol Fe^{2+}/100 g FW.

Comparing the total antioxidant activity of the studied plums to the total FRAP of other fruits reported in previous work (GUO *et al.*, 2003) it is interesting to observe the high values find that could suggest interesting uptake of plums for the human diet.

4. CONCLUSIONS

This study provides important data for qualitative and nutraceutical properties of the fruits.

The results obtained by this preliminary study reveals that the authocthonous Sicilan plums contains important amounts of anthocyanins and phenols; often their concentrations is higher than found not only in the commercial cultivars but also in fruits that are reported in literature to have high content of nutraceutical compounds. Considerable and significantly different among the plums were observed and the environmental conditions and the activity of propagation and exchange of genetic material results of the local farmers could be both responsible for the differentiation of the germplasm collected in the investigated areas of the Sicilian territory.

The maintaining of local genetic materials is important for the biodiversity and the action of localization and characterization of old fruits cultivar and accessions is fundamental to improve the management of the European *Prunus* Database for Plum (EPDP) making them available for research and genetic improvement.

The knowledge of the qualitative traits of fruits represent a good opportunity not only for the health advantages but also for the general consumption; in fact the enhancement and the safeguarding of these fruits can be thought as an opportunity of new marketing channel but more studies need to be undertaken about the evolution of the qualitative and nutraceutical compounds during storage to answer the consumer's demands and expectations.

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PAPER

DIFFERENT PACKAGING METHODS EFFECTS ON SENSORY QUALITY AND CHEMICAL CRITERIA OF MARINATED SHAD (*ALOSA IMMACULATA*, B., 1838)

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ABSTRACT

In this study the sensory and chemical parameters of marinated shad (*Alosa immaculata*, Bennett, 1838) were determined. Fish were marinated with different package methods (in brine, oil and vacuum packed) and stored at 4±1°C. During the storage period, diffusion of proximate composition, acetic acid and sodium chloride into the fish fillets were determined. At the end of 7 months storage period, TVB-N were 8.05, 16.81 and 17.56 (mg/100 g), TMA were 2.28, 2.53 and 2.73 (mg/100 g), TBA were 7.08, 7.13 and 6.05 (mg malonaldehyde/kg) and pH were 4.42, 4.72 and 4.77 for brine, oil and vacuum packed samples, respectively. Throughout the storage period, effect of different package methods on TVB-N, TMA, TBA, pH, aw, acetic acid, sodium chloride and sensory issues were significant ($p<0.05$).

- Keywords: chemical-sensory quality, marinating, shad, shelf life -

INTRODUCTION

Fish is really healthy food and is known as the only food having a life-sustaining balanced ratio of protein, fat, carbohydrates, vitamins and minerals, which are essential to maintain good health. In addition, because of having low fat content, cholesterol and calories, fish meat is preferable for consumers (KORAL, 2006).

Shad is a close relative to sardines has an average length of 30-33 cm. shad are a Black Sea fish, but it can be found in the Marmara, Aegean and the Mediterranean seas. Shad live close to the coast as herd by shoal, they enter in the river to spawn at reproduction time in the spring. Today, shad is also heavily fished in the United States and around. According to Turkish Statistical Institute-TSI (2013), 1699 tons shad were caught in Turkey at 2012. Turkish consumers generally prefer as fresh, salted or smoked shad.

Marinating is traditionally a fish preservation method. Meat tenderization and flavoring are consequences of the marinating process. Sodium chloride, polyphosphates, sugars spices and sauces are considered important ingredients of marinades and they improve meat tenderness and flavor (SINDELAR *et al.,* 2003; SUDERMAN, 1993). The purpose of bringing rigidity to the marinated product, enriching the meat flavor and preserving the meat longer (CHEN, 1982; RESURRECCIÓN, 2003). The immersion of meat in the marinating solution is done not only in Turkey.

The physical characteristics of fish change in acid and salt in several days. Muscle tissue softens; skin and bones can be removed easily. Marinate generally contains 4-5% vinegar and 7-10% salt. Acetic acid leads to breakdown of structural proteins and enables the tearing of muscle membrane (MEYER, 1965; ERDEM *et al.,* 2005). Muscle tissue, which has been softened during initial days by the joint effect of acid and salt, lost 15-20% of raw material weight at the end of the process (ALPARSLAN *et al.,* 2013).

The aim of the present study is to determine the sensory and chemical changes of marinated and brine, oil and vacuum packed shad fillets. Therefore, the use of shad as an alternative to the other fish species used in marinade production will be investigated.

MATERIALS AND METHODS

A total of 186 pontic shad (*Alosa immaculata)* samples with an average weight of 191.53±33.63 g and average length of 28.71±1.58 cm were purchased from a local fish market in Trabzon, Turkey. They were stored in a thermally insulated container, brought to the laboratory and stored at –30°C with the purpose of eliminating the risk of parasites until the marinating process.

Marination process

Before marinating process, fishes were cleaned at the head, and the integral organs, after that fillet and washed with clean water. Approximately 36 kg fillets were immersed into the following different marinating solutions for 48 h (1:2 fish:solution ratio) in a refrigerator. After two days of storage in refrigerator, the fillets were dipped in 4.5% acetic acid, 0.2% citric acid and 10% salt marination solutions, the marinated fishes were divided into tree groups (brine, sunflower oil and vacuum packaging). 70 grams of marinated shad fillets were packed into plastic box of 250 mL capacity with 150 mL brine, 150 mL sunflower oil, vacuum packaged and than were stored at 4±1°C for 7 months. Proximate, chemical quality and sensory analyses were performed in triplicate on days 1, 30, 90, 150, and 210th.

Chemical analysis

Samples were homogenized and subjected to moisture and ash analyses using AOAC (1990) methods. Crude protein content was calculated by converting the nitrogen content according to Kjeldahl's method (AOAC 1990), and lipid content was determined according to the method of the BLIGH and DYER (1959). Thiobarbituric acid (TBA) amounts were determined using the method of TARLADGIS *et al.,* (1960), expressed as mg malonaldehyde/kg sample using a conversion factor of 7.8. The pH was determined from homogenates of minced samples in distilled water in a ratio of 1:10 (w/v) by using a digital pH meter (Hanna, Germany) (CURRAN *et al.,* 1980). Total volatile basic nitrogen (TVB-N) was determined on steam distillation using the Kjeldahl distillation apparatus and until red color was titrated, for clarification (ANTONOCOPOULUS 1973). The method of AOAC (1990) was used for trimethylamine nitrogen (TMA-N) analysis.

The water activity determination Aqualab 3 TE (0.100 to 1.000 ± 0.003), were measured by U.S. brand equipment. Salt content was measured using the method proposed by KARL (1994). 20 g fish were homogenized for 5 minutes with 100 mL distilled water, and than 150 mL distilled water was added and it was filtered. To this solution was added 2.5 mL of 10% K_2CrO_4. Until red color was titrated with $AgNO_3$ 0.1 N and according to the following formula amount of salt (%) was calculated:

$$\text{NaCl (\%)} = \text{A} \times 0.00585 \times 100 \times 500 / \text{Amount of sample (g)} \times 50$$

A: $AgNO_3$ consumption (mL)

For the sensory evaluation of the marinated products five panelists were used. Sensory analysis to assess appearance, odor, flavor and texture criteria were used, and analyises results,

Table 1 - The proximate composition in percentage of fresh and different marinating process of shad (*Alosa immaculata,)* fillets during storage.

Storage days	Protein (%)	Lipid (%)	Dry Matter (%)	Ash (%)
Fresh fish	17.41±0.51[a]	18.98±0.66[a]	39.15±0.15[a]	2.17±0.16[a]
Brine	18.83±0.28[b]	20.14±0.11[c]	47.11±0.16[b]	10.28±0.04[c]
Oil Marinated	18.06±0.33[b]	20.30±0.19[c]	47.05±0.70[b]	8.68±0.11[c]
Vacuum Marinated	18.16±0.15[b]	20.21±0.33[c]	47.05±0.02[b]	8.12±0.07[b]

Data are expressed as means±standard deviation.

which were scored on a scale of 1 to 9. In the scoring system points from 9 to 7 indicates "very good", from 4.1 to 6.9 indicates "good", 4 indicates "expendability" (4 is the rejection line), and from 1 to 3.9 indicates unacceptability (VARLIK *et al.,* 1993).

Statistical analysis

The statistical analysis was performed using Minitab Release 13.20 (Minitab Inc., State College, PA, USA). Differences were analyzed by one-way analysis of variance and Tukey's test. In all statistical tests, $P<0.05$ was considered as statistically different (SUMBULOGLU and SUMBULOGLU, 2000).

RESULTS AND DISCUSSION

The proximate composition of the shad fillet in each stage of the process is shown in Table 1. These results are wet fish sample and marinated shad. Protein, lipid, dry matter and ash of fresh shad fillets were 17.41%, 18.98%, 39.15% and 2.17%, respectively. At the end of the storage, all of these values have increased. Maximum protein value was brining sample (18.83%) while maximum values at the lipid and dry matter were 20.30% and 47.11% at Oil marinated and brining, respectively. GUNER *et al.* (1998), and BORAN and KARACAM (2011) reported 22.42% and 19.80% for protein and 15.91% and 9.34% lipid in fresh shad fillets, respectively. In another experiment, at the end of the brining stage the protein and lipid content were 18.32 g.100 g_{-1} and 3.20 g.100 g_{-1} of fish fillets (YEANNES and CASALES 2008). The results of proximate analysis of fresh shad fillets were in agreement with our study.

TVB-N of raw material was 14.01(mg/100 g) (Table 2). At the end of 7 months storage period for marinated shad that were packaged differently in brine, oil and vacuum packed, TVB-N values were 8.05, 16.81 and 17.56 (mg/100 g), respectively. Statistically difference was found between the brine group with the other groups on TVB-N value ($P<0.05$). ÖZDEN and ERKAN (2006) reported that TVB-N in fresh fish and marinated trout were 7.35 mg/100 g and 6.78 mg/100 g, respectively. TVB-N values increased to 12.08 mg/100 g and 11.98 mg/ 100 g at the end of storage in vacuum and oil packed samples, respectively. Similar results obtained in marinated fish packaged in vacuum and stored in refrigerator at a different time (AKSU *et al.,* 1997, METİN *et al.*, 2000 and ARIK *et al.,* 2001).

TBA was 0.99 malonaldehyde/kg at the beginning of the this study (Table 2). This value increased to 7.08 in the brine, 7.13 in the oil and 6.05 mg malonaldehyde/kg in the vacuum packaged group on the last day (210^{th} day) of storage. Statistically difference was found between the vacuum group with the other groups on TBA value ($P<0.05$). TBA value is an important indicator and is excessively used to determine the level of lipid oxidation in fish (SALLAM 2007; CADUN *et al.*, 2008; TURHAN *et al.*, 2009). VARLIK *et al.* (1993) reported that a consumable limit was between 7-8 mg malonaldehyde/ kg TBA in sea fish.

ÖZDEN and ERKAN (2006) reported 0.45 mg malonaldehyde/kg TBA values for fresh rainbow trout and 2.8 mg malonaldehyde/ kg TBA values for marinated fish after 90 days, while 9.5 mg malonaldehyde/kg for vacuum and 10.26 mg malonaldehyde/kg for oil packaged marinated trout fillets were determined.

Water activity (a_w) of the raw material changed from 0.98 to 0.99, indicating that, it is the most suitable period for microbial growth (Table 2). The water activities (a_w) at the marination were 0.93-0.94 between each group. The findings regarding to the a_w value are in compliance with BORGSTROM'S (1968) with YEANNES and CASALES (2008).

In this study, pH level was 4.42, 4.72 and 4.77 in the brine, oil and vacuum packaged samples at 210 days, respectively and there were no significant differences between groups ($p>0.05$). The pH of fresh raw fish was initially approximately 6.30 and then changed during the maturation process to 4.29 after 90 days (ÖZDEN and ERKAN 2006). In another study, pH values in anchovy marinated with 2% and 4% acetic acid increased was changed from 4.25 to 4.53 (AKSU *et al.,* 1997).

The sodium chloride content of the fillet remained stable during the marinating stage while the salt level becomes richer in the brin-

ing stage and the salt concentration in the marinating solution becomes lower. According to KOLAKOWSKI and BEDNARCZYK (2002), the acetic acid in the marinating solution caused the decrease in the water content of fillet. Sodium chloride in fresh fish muscle was 0.27%, while at the end of the study, it was reached to 6.32%, 4.39% and 4.51% for brine, oil and vacuum package groups, respectively. According to DUERR and DYER (1952), FENNEMA (1977) and HONIBEL (1989), myosin denaturation, as measured by salt solubility, occurs at a definite salt concentration, about 8 to 10% sodium chloride in the tissue.

MEYER (1965) stated that the amount of the acid in high quality marine products should be between 2-3% in fish tissue at the end of the ripening period. In this study the acid amount changed between 1.20-1.66 during the trial.

The sensory scores of marinated shad fillets indicated a good quality of the storage period (Table 3). There were significant differences between brine, oil and vacuum packaged shad marinades ($p<0.05$) for sensory value. These results are in agreement with the findings of ÖZDEN and BAYGAR (2003) for marinated chub mackerel, horse mackerel, sardine and anchovy packaged in jars with vegetable oil and vacuum packed in polyethylene bags, then stored at 4±1°C. In addition to these studies, YEANNES and CASALES (2008) reported that there were not quality changes on sensory analysis of marinated anchovy (*Engraulis anchoita*) throughout storage time.

Many studies showed that good quality marinated fish is between 3 and 6 months (ERKAN *et al.,* 2000; VARLIK *et al.,* 2000; ÖZDEN and BAYGAR 2003; KILINC and ÇAKLI 2004; GÖKOGLU *et al.,* 2004, ERDEM *et al.,* 2005; KABA *et al.,* 2013).

CONCLUSIONS

In this study, the effects of brine, oil and vacuum packing on chemical and sensory changes in marinated shad stored at 4°C were investigated. A quality assessment was performed by monitoring sensory quality, total volatile basic nitrogen and thiobarbituric acid, pH, aw, and salinity count. The results of this study indicate that the shelf life of brine and oil packed marinated shad fillets had a shelf life of 210 days. Accord-

Table 2 - Effects of brine, oil and vacuum packets on chemical changes of marinated shad (*Alosa immaculata*) fillets during refrigerated storage.

			Storage Days			
		After marinated	30	90	150	210
TVB-N mg/100 g	Brine	14.01±0.00^{a}	8.40±0.0.23^{bA}	5.80±0.00^{deC}	5.95±0.0.49^{cdC}	8.05±0.49^{bC}
	Oil	14.01±0.00^{a}	8.55±0.89^{aA}	12.01±0.00^{bB}	13.61±0.00^{cB}	16.81±0.00^{aB}
	Vacuum	14.01±0.00^{a}	8.86±0.50 ^{aA}	14.16±0.49^{bcA}	15.66±0.66^{cdA}	17.56±0.00^{eA}
TMA-N mg/100g	Brine	0.63±0.01^{aB}	0.70±0.01^{aB}	0.73±0.01^{bC}	1.06±0.04^{cC}	2.28±0.04^{eA}
	Oil	0.63±0.01^{aB}	0.69±0.01^{aB}	0.88±0.01^{abC}	1.94±0.04^{cA}	2.53±0.03^{dA}
	Vacuum	0.63±0.01^{aB}	0.93±0.03^{cA}	1.22±0.03^{dA}	1.48±0.02^{eB}	2.73±0.04^{fB}
TBA mg malonaldehyde /kg	Brine	0.99±0.01^{a}	1.82±0.06^{bcA}	3.21±0.36^{dA}	5.36±0.18^{eA}	7.08±0.06^{fA}
	Oil	0.99±0.01^{a}	1.92±0.06^{bcA}	3.66±0.15^{dA}	5.56±0.18^{eA}	7.13±0.06^{fA}
	Vacuum	0.99±0.01^{a}	1.28±0.04^{aA}	2.32±0.12^{cbB}	3.75±0.21^{dB}	6.05±0.35^{eB}
a_w	Brine	0.99±0.001^{a}	0.94±0.001^{cB}	0.93±0.001^{dB}	0.93±0.001^{eB}	0.93±0.001^{cB}
	Oil	0.98±0.001^{a}	0.95±0.001^{deA}	0.94±0.001^{eA}	0.94±0.001^{fA}	0.94±0.001^{fA}
	Vacuum	0.99±0.001^{a}	0.95±0.001^{bA}	0.94±0.001^{dA}	0.94±0.001^{cdA}	0.94±0.001^{bB}
pH	Brine	6.42±0.02^{a}	4.25±0.01^{cdB}	4.31±0.03^{bcdA}	4.33±0.04^{bcdB}	4.42±0.03^{bB}
	Oil	6.42±0.02^{a}	4.37±0.01^{fgA}	4.53±0.04^{deA}	4.65±0.01^{bcA}	4.72±0.01^{bA}
	Vacuum	6.42±0.02^{a}	4.39±0.01^{fgA}	4.49±0.01^{deA}	4.59±0.01^{cA}	4.77±0.01^{bA}
Acidity	Brine	0.15±0.00^{a}	1.28±0.00^{cA}	1.58±0.11^{dA}	1.65±0.00^{dB}	1.66±0.01^{dA}
	Oil	0.15±0.00^{a}	1.20±0.11^{cA}	1.38±0.11 ^{cdB}	1.53±0.11^{cdA}	1.65±0.00^{dA}
	Vacuum	0.15±0.00^{a}	1.35±0.00^{cB}	1.48±0.11 ^{dC}	1.53±0.11^{dA}	1.65±0.00^{eA}
Sodium chloride	Brine	0.27±0.12^{a}	6.29±0.29^{cA}	6.79±0.16^{cA}	6.00±0.37^{cA}	6.32±0.00^{cA}
	Oil	0.27±0.12^{a}	4.77±0.21^{bB}	4.92±0.50^{bB}	4.77±0.04^{bB}	4.39±0.08^{bB}
	Vacuum	0.27±0.12^{a}	4.75±0.42^{bB}	4.47±0.04^{bB}	4.65±0.37^{bB}	4.51±0.04^{bB}

Data are expressed as means±standard deviation.
a,b,c: Differences between groups expressed with different letters in the same column are important ($p<0,05$).
A,B,C: Differences between groups expressed with different letters in the same line are important ($p<0,05$).

Table 3 - Effects of different package methods on sensory property of marinated shad (*Alosa immaculata*) fillets during storage time.

			Storage Days			
		After marinated	30	90	150	210
Appearance	Brine	9.55±0.38^{aA}	9.00±0.38^{aA}	7.05±0.69^{bA}	6.40±0.98^{cA}	5.00±0.32^{dA}
	Oil	9.57±0.41^{aA}	8.50±0.55^{aB}	7.13±0.52^{bA}	6.04±0.39^{cA}	4.53±0.52^{dB}
	Vacuum	9.65±0.35^{aA}	930±0.61^{aC}	7.47±0.41^{aB}	5.81±0.32^{dA}	4.20±0.32^{dB}
Odor	Brine	9.25±0.25^{aA}	8.58±0.49^{abA}	7.42±1.02^{bcA}	5.92±0.92^{cA}	4.28±0.35^{dA}
	Oil	9.52±0.61^{aA}	8.60±0.41^{abA}	7.08±0.92^{bA}	6.25±0.76^{cA}	4.57±0.75^{dB}
	Vacuum	9.61±0.41^{aA}	8.70±0.32^{abA}	7.83±0.41^{cdA}	5.97±0.26^{dA}	3.91±0.29^{aC}
Flavor	Brine	9.63±0.05^{aA}	8.75±0.35^{abA}	7.25±0.15^{cA}	5.90±0.10^{dA}	4.13±0.18^{eA}
	Oil	9.63±0.05^{aA}	8.50±0.20^{bB}	7.25±0.20^{cA}	6.00±0.15^{dA}	4.50±0.06^{eB}
	Vacuum	9.63±0.05^{aA}	9.25±0.09^{abC}	7.75±0.18^{bA}	4.75±0.15^{cB}	3.50±0.00^{eC}
Texture	Brine	9.85±0.39^{aA}	8.58±0.41^{bA}	7.08±0.44^{cA}	6.00±0.33^{dA}	4.20±0.13^{eA}
	Oil	9.88±0.37^{aA}	8.79±0.20^{bA}	7.67±0.21^{cAB}	5.58±0.27^{dA}	4.40±0.15^{eA}
	Vacuum	9.93±0.23^{aA}	9.71±0.26^{aB}	7.93±0.24^{bB}	5.33±0.15^{cB}	4.00±0.21^{dB}

a,b,c Differences between groups expressed with different letters in the same column are important (p<0,05).
A,B,C Differences between groups expressed with different letters in the same line are important (p<0,05).

ing to this study, fishbones of the shad which has a large number of them melted as a result of the marination process.

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PAPER

PREVALENCE AND ANTIBIOTIC RESISTANCE OF FOOD BORNE BACTERIAL CONTAMINATION IN SOME EGYPTIAN FOOD

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ABSTRACT

This study was undertaken to investigate the prevalence and antibiotic resistance of food borne bacterial contamination in some Egyptian food. Total viable bacteria and total coliform bacteria were isolated from different sources of food; carbohydrates (bread, flour and basbousa), vegetables (outer and inner tissues of potato and outer and inner tissues of cucumber) and proteins (minced meat, cheese and milk). The study resulted in maximum value of total viable bacteria found in outer tissue of potato $68X10^4 \pm 1.0$, while the minimum value found in inner tissues of potato and cucumber. The study resulted in total coliform was maximum value in minced meat $6.4X10^3 \pm 0.3$. Basbousa and inner tissue of potato and cucumber were free from coliforms. The ability of isolates to producing proteolytic enzymes was tested, we found that 326 isolate (63.92%) from all isolates had this ability, thus we selected most 2 potent proteolytic isolates. The two isolates were identified as *Bacillus cereus* and *Escherichia coli.* The identification confirmed by microlog 34.20 system and 16SrRNA for two isolates and the same result was founded. Sensitivity tested for the most potent proteolytic species to 12 of the most commonly used antibiotics in the Egyptian pharmacy. The results showed that all species were sensitive to most of antibiotics, except *B. cereus* which was strongly susceptible to azteronam and ceftazidim. The data showed that raw meat, cooked food products, and raw milk were most commonly contaminated with foodborne pathogens and many pathogens were resistant to different antibiotics. The study provided useful information for assessment of the possible risk posed to consumers, which has significant public health impact.

- Keywords: foodborne pathogens, *Bacillus cereus*, *Escherichia coli*, proteolytic enzymes, 16SrRNA -

INTRODUCTION

It has been estimated that as many as 30% of people in industrialized countries suffer from a food borne disease each year and in 2000 at least two million people died from diarrheal disease worldwide (WHO, 2002a). Foods are not only of nutritional value to those who consume them but often are ideal culture media for microbial growth, chemical reactions that cause offensive and sensory changes in foods are mediated by bacteria that use food as carbon and energy source. Some of the major bacterial genera which cause food born infection and intoxication (PUNDIR and JAIN, 2011). Contamination of food can affect a large number of populations. About 2.5 million people die every year from water born diseases. More than 40% of total population of Indonesia & 60% in Thailand suffered from gastroenteritis per year. A total of 32.7% outbreak was involved with restaurant catering bakery products (NAZIR and ISLAM, 2007).

Fruits and vegetables carry microbial flora while passing from the farm to the table. The produce is exposed to potential microbial contamination at every step including cultivation, harvesting, transporting packing, storage and selling to the final consumer (FDA, 2000). Sources of environmental microbial contamination include raw materials, processing equipment, manufacturing activities, sanitation and maintenance practices, workers, waste, animal and insect pests, and microbial growth niches embedded in equipment and in structural components of the building. The survival and growth of microorganisms in a food-processing environment may lead to contamination of the finished product that may, in turn, result in a reduction of microbiological safety and quality. Most food plants have locations that can promote the growth of pathogens and spoilage microorganisms that may be transferred directly on to product or carried into additional niches. The origins of these growth habitats are mainly unhygienic design, construction, and maintenance and repair activities that prevent easy cleaning and disinfection. The presence of water and nutrients (food product) is required to form a microbial growth niche and the chemical composition of the food and conditions of water activity, pH, temperature, etc., will select the "normal" organisms that can grow there. Microbial growth niches may be established when water is used to clean dry processing environments not designed for wet cleaning and not all points in the equipment are promptly and completely dried (JAY, 1996, TESFAYE *et al.*, 2011). VANDERZANT and SPLITTSTOESSER, (1992) demonstrated that the microbial growth on equipment for processing perishable foods is governed mainly by the ecology of the food, the process, packing room temperature, presence of food residue on the equipment, and efficacy of cleaning and disinfection. Recontamination of a biocidaly treated food may increase the risk of foodborne illness if the food is not heated to destroy pathogens before consumption. Perishable foods that do not receive a biocidal treatment in the final container may be decontaminated by spoilage microorganisms before packing (VANDERZANT and SPLITTSTOESSER, 1992, HAILESELASSIE *et al.*, 2013).

The research project will deal with investigate the prevalence and antibiotic resistance of *Bacillus cereus*, *Escherichia coli* contamination in some Egyptian food.

2. MATERIALS AND METHODS

2.1. Food samples

All food samples were collected from Mansoura city, Egypt. They collected from different shops, super market, groceries and butchers. Ten samples were taken from each of bread, flour, basbousa, potatoes, cucumber, minced meat, cheese and milk.

2.2. Preparation of samples

Twenty five grams of each of the following samples bread, flour, basbousa, outer tissue and inner tissue of potatoes and cucumber, minced meat, cheese and ten ml of raw milk (unboiled/unpasteurized) was homogenized in 225 ml sterile physiological saline solution (0.85% NaCl) in 500 ml conical flask using a plender for 1-2 minutes, then decimal dilutions were prepared.

2.3. Isolation of bacteria

One ml of appropriate dilution was inoculated on both of Nutrient agar medium and MacConkey agar medium; the plates were incubated aerobically for 24h at37°C. Total viable bacteria (T.V.B) were enumerated on Nutrient agar medium using pour plate technique. Total coliform (T.C) bacteria were counted on MacConkey agar medium by using pour plate technique also. The plates were incubated aerobically for 24h at 37°C.

2.4. Purification

After the incubation period (24h), the growing colonies were enumerated for counting. After counting a sterile wire loop was used to pick the isolate from the plate and was streaked on freshly prepared nutrient agar medium then inoculated for 24h at 37°C in order to get pure culture. The growing colonies were purified and examined by using cultured morphological appearance and Gram reaction.

2.5. Proteolytic assay

Proteolytic activity was carried out according to Casein - Pholine method (RAMALAKSHMI *et al.*, 2012). Culture media was Centrifugated at 7200 rpm for 10 min and supernatant was used as enzyme source. However, 1% casein (in 0.1 M phosphate buffer and pH 7.0) was used as substrate. 1 mL each of enzyme and substrate was incubated at 50°C for 60 min. The reaction was terminated by adding 3 mL of Trichloroacetic acid (TCA). One unit of protease activity was defined as the increase of 0.1 unit optic density at 1 h incubation period. Then it was centrifuged at 5000 rpm for 15 min. From this, 0.5 mL of supernatant was taken, to this 2.5 ml of 0.5M sodium carbonate was added, mixed well and incubated for 20 min. Then it was added with 0.5 ml of folin phenol reagent and the absorbance was read at 660 nm using Spectrophotometer. The amount of protease produced was estimated and expressed in microgram of tyrosine released under standard assay conditions. Based on the tyrosine released the protease activity was expressed in microgram of tyrosine released by 1 mL of enzyme in 30 minutes at 300C on tyrosine equivalent.

2.6. Identification of bacterial isolates

2.6.1. Conventional methods

The appearance of cultures, cell morphological characteristics and physiological characteristics of the purified selected identified isolates were studied. Media and reagent were prepared according to standard and procedures as described by (MACFADDIN, 1980). The identification was carried out by traditional characters and biochemical tests for isolates according to (Krieg *et al.,* 1994) and confirmed out by biolog micolog 34.20 system for most potent proteolytic bacterial species. Characterization of the most potent isolates were completed and confirmed by Biolog Microlog 34.20 system at the Unit of Identification of Microorganisms and Biological Control Unit of Agriculture Reasearch Center, Giza, Egypt.

2.6.2. Molecular method

The polymerase chain reaction (PCR) methods based on 16S rRNA gene for identification of isolates were used. Genomic DNA was extracted and purified by using Qiagen kit (Qiagen Company). The purity was assessed from the A260/A280 ratios: Cultures of bacteria were streaked on tryptic soy agar medium and incubated at 37°C for 24 h. A single colony of each pathogen was grown in (LB) broth medium in Erlenmeyer flask and incubated at 37°C for 24 h. Culture was harvested by centrifugation at 4°C for 10 min, DNA was extracted from pellets according Qiagen kit instructions. Full length 16S rRNA (1500 bp) were amplified from isolates by PCR using universal forward primer 518F (5'–CCAGCAGCCGCGGTAATACG-3') and 800R (5'-TACCAGGGTATCTAATCC-3'). Optimum conditions (denaturation 94-1 min, annealing 63-45 s and extension 72-2 min, 35 cycles). Amplified 16S rRNA was purified from 0.8% melting point agarose gel. Bands obtained from PCR product were eluted and purify by (Qiagen elution kit) PCR instructions, DNA band desired was excised from ethidium bromide stained agarose gel with a razor blade, transferred to Ependorf tube. DNA was sequenced directly using specific primer with concentration 20 pmol Macrogen Sequencing Company, Korea.

2.7. Antibiotic susceptibility test

2.7.1. Antibiotic disks

Antimicrobial susceptibility profile of identified bacterial species, *Bacillus cereus* and *E.coli* against different antibiotics ampicillin, aztreonam, cefadroxil, ceftazidime, chloramphenicol, ciprofloxacin, erythromycin, imipenem, neomycin, norfloxacin, streptomycin and vancomycin were studied. The antibiotic discs used in this research were purchased from Oxoid LtD., England.

2.7.2. Disc diffusion agar method

Antibiotic susceptibility test for the bacterial isolates was carried out by disc diffusion technique according to BAURE *et al.*, (1966). The technique was done by inoculation of pure colonies of the tested organism into 5 mL of sterile nutrient broth and incubation at 37°C for 24h. Then 0.1ml of bacterial suspension (0.5 McFarland turbidity) was spreading by sterile swabs on nutrient agar plates. Duplicate plates were prepared for the strain. Antibiotic discs were applied to the surface of plates at constant distances. The plates were incubated at 37°C for 24h. At the end of incubation period zones of inhibition were measured as (mm). The entire diameter of the zone was measured including the diameter of the disc. The end point of the reading was taken as complete inhibition of the growth to the naked eye. Our (+++) or (++++) indicate high inhibitory effect (large diameter of clearing zone) and (-) indicate no inhibitory effect (good growth).

2.8. Statistical analysis

The variations between experiments were estimated by standard deviations, and statistical significance of changes was estimated by student's t-test. Only the probability $P \leq 5\%$ was regarded as indicative of statistical significance.

Table 1 - Counts of total viable bacteria (T.V.B) and total coliform (T.C.B) & Log10 cfu/ ml for samples of food collected from Al-Mansoura city, Egypt.

T.C.B	T.V.B		Types of Food	
	Counts (cfu/ml)	Log 10 (cfu/ml)	Counts	Log 10
I-Carbohydrates				
Bread	$40.5x10^3 \pm 0.5$	4.61	$5.3x10^3 \pm 0.4$	3.72
Flour	$35.3x10^3 \pm 0.9$	4.55	$5.4x10^3 \pm 0.4$	3.73
Basbousa	$1.1x10^3 \pm 0.1$	3.03	0.0	0.0
II-Vegetables				
Potato				
Outer tissue	$68x10^4 \pm 1.0$	5.83	$3.2x10^3 \pm 0.3$	3.51
Inner tissues	$0.1x10^3 \pm 0.1$	1.98	0.0	0.0
Cucumber				
Outer tissue	$52.7x10^4 \pm 2.5$	5.72	$1.8x10^3 \pm 0.2$	3.26
Inner tissues	$0.1x10^3 \pm 0.0$	2.0	0.0	0.0
III-Proteins				
Minced meat	$28x10^3 \pm 3.5$	4.44	$6.4x10^3 \pm 0.3$	3.80
Cheese	$42.7x10^4 \pm 2.3$	5.63	$2.1x10^3 \pm 0.2$	3.32
Milk	$39.7x10^4 \pm 0.6$	5.60	$2.33x10^3 \pm 2.5$	3.37

All values are the means of triplicate plate.

3. RESULTS

3.1. Isolation of total viable bacteria from different types of food

All growing isolates were enumerated, collected, purified and tabulated. All growing isolates were collected from investigated types of food. Table 1 includes the isolates numbers and sources of collected isolates. Total viable bacterial counts (TVB) and total coliform bacterial count (TCB) of three main groups of food were tabulated in Table 4. In carbohydrates, the highest count 40.5x103cfu/gm was recorded in bread. The total viable bacterial count in flour was $35.3x10^3$cfu/gm. The lowest count $1.1x10^3$ cfu/gm was recorded in Basbousa. Both Fig. 1 and Table 4 show these results. The count of total coliform bacteria (TCB) was $5.4x10^3$ and $5.3x10^3$ cfu/gm in flour and bread respectively. This group of bacteria (TCB) was not recorded in Basbousa (Table 1). The counts of T.V.B were $68x10^4$ and $52.7x10^4$ cfu /ml in the epidermis of both of potato and Cucumber respectively. While the count was reduced to a lowest count $1.0x10^2$ cfu /mL of the inner tissues of both potato and Cucumber. It

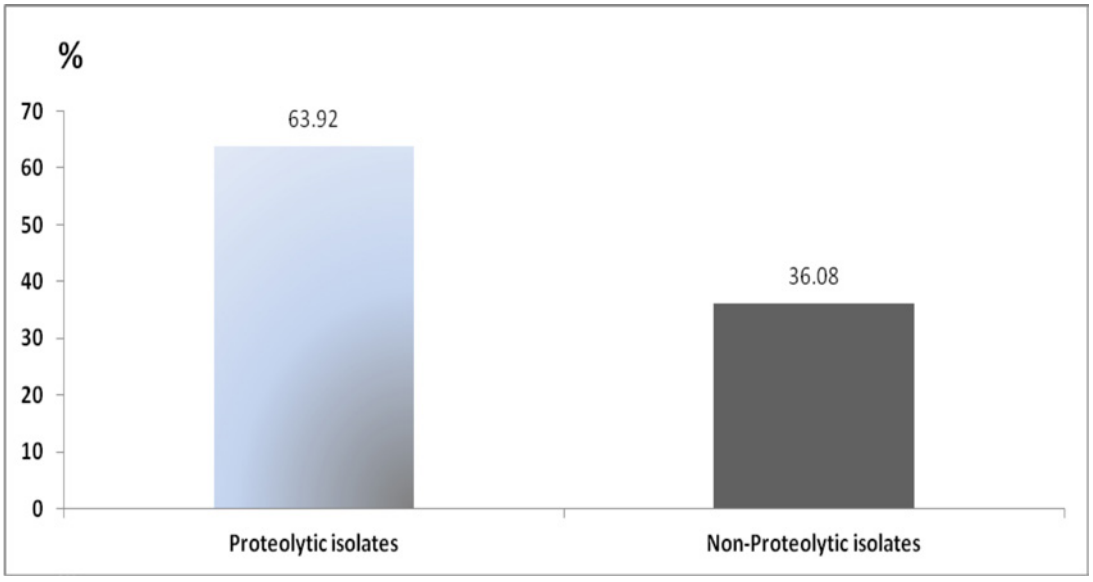

Fig. 1 - The percentage of proteolytic and non-proteolytic isolates from different kinds of food is

is important to notice that TCB was not recorded in inner tissues of potato and Cucumber. The counts of TCB were $3.2x10^3$ and $1.8x10^3$ cfu/mL from the outer tissue of potato and Cucumber respectively. The total viable bacterial count in milk was $39.7x10^4$cfu/mL while the TCB was $2.33x10^3$ cfu/mL. From Table (1) we notice that the highest count of total viable bacteria was $42.7x10^4$ in cheese. And also the lowest count of TCB was $2.1x10^3$cfu/mL in cheese. From Table 4, the count of T.V.B was $28x10^3$ cfu/mL, while the count of TCB was $6.4x10^3$ cfu/mL.

3.2. Screening test for detection of most potent proteolytic bacterial species

Fig. 1 showed the potency of proteolytic activities of all purified isolates. The proteolytic isolates were 326 isolates (63.92%), while the non-proteolytic isolates were 184 isolates (36.08%). It also shows that the largest clearing zone was 21 mm in case of isolates 62 and 412 (Fig. 2). This indicates that these isolates were the most potent of proteolytic activity.

3.3. Presumptive and confirmation identification

The cultural study, morphological appearance, Gram reaction and physiological characteristics of four most potent proteolytic isolates were studied according to KRIEG *et al.* 1994. These results were tabulated in Table 2. The presumptive identification of the two most potent proteolytic activities. Isolate numbers 62 and 410 were identified as *Bacillus cereus* and *Escherichia coli* respectively. The most potent bacterial isolates were confirmed by using Biolog Microlog 34.20 system for identification. 16S rRNA gene bands which were detect by specific primer at 1500 bp. The 16S rRNA sequences for two isolates were blasted with genebank sequence

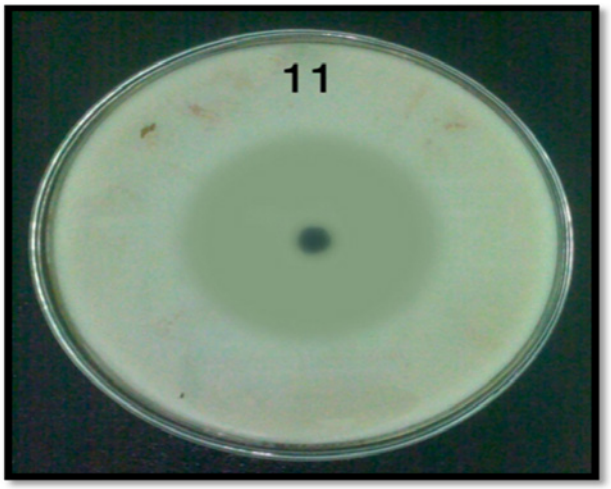

Fig. 2 - Example of proteolytic activity of isolated species.

Table 2 - Morphological and biochemical features of strains 62 and 412.

Character and biochemical tests Code of Isolate	62	412
Growth	Aerobic or Facultative anaerobic	Facultative anaerobic
Morphology of colony	Colony smooth, convex, circular and creamy in color	Colony smooth, convex, circular and creamy in color
Gram stain	+	-
Cell shape	Straight rods, arranged in paires	Straight rods, arranged singly or in pairs
Motility	Motile	Motile
Flagella arrangement	Peritrichous	Peritrichous
Oxidase		--
Gelatine hydrolysis	+	-
D-Glucose, acid production	+	+
D-Glucose, gas production	-	+
Nitrate reduction	+	+
Catalase production	+	+
Oxidation- fermentation	F	F
Voges-Proskaure	NT	-
Arginine dihydrolase	NT	-
Acid production:		
L-Arabinose	-	+
Lactose	NT	+
Maltose	NT	+
Trehalose	NT	+
D-Mannitol	-	+
D-Xylose	-	+
Indole production	NT	+
Methyle red	NT	+
Citrate (Simmons)	NT	-
H_2S production	NT	-

NT= not tested for this isolate because of results of another biochemical tests, F= fermentative.

Table 3 - BLAST analysis of 16S rRNA sequences of the representative isolates.

Isolate	Closest Validly Described Species		Identities		
	Description	Accession number	Match	Total	% Similarity
62	*Bacillus cereus* KM007-1	KF055368	481	485	99
412	*Escherichia coli* Xuzhou21	CP001925	1465	1469	99

database (Table 3) and found closet to the same isolates identified by conventional methods.

3.4. Antibiotic susceptibility test of different antibiotics

The antibiotic susceptibility test was obtained on the bacterial isolates by using 12 different antibiotics by disc diffusion method (Table 4). *Bacillus cereus was* resistant (non susceptible) to Aztreonam and ceftazidime. *Escherichia coli* (gram negative bacteria) were resistant (non susceptible) to vancomycin. While gram positive organisms *Bacillus cereus* was non susceptible (resistant) to vancomycine. *Bacillus cereus* was resistant to ampicillin, while *Escherichia coli* was susceptible (sensitive) to ampicillin. *Bacillus cereus* was resistant to ampicillin while *Escherichia coli* was non susceptible (sensitive) to ampicillin. The studied bacterial species both gram positive and gram negative were sensetive to 8 antibiotics (chloramphenicol, cephadroxil, erythromycin, norfloxacin, imipenem, neomycin, ciproloxacin and streptomycin). *Escherichia coli* was sensitive to three more (ampicillin, aztreonam and ceftazidime) i.e. it was sensitive to 11 antibiotics, thus *Escherichia coli* was the most sensitive bacterial species. *Bacillus cereus* was sensitive to one more vancomycin and ampicillin respectively i.e. each of them was sensitive to 9 antibiotics. The smallest inhibition zone was 8mm which was recorded in *Escherichia coli* due to the effect of erythromycin and ceftazidime respectively.

Table 4 - Antimicrobial susceptibility profile of studied microorganisms against different antibiotics expressed as diameter of clearing zones.

Antibiotics (Conc.)		*Bacillus cereus*	*Escherichia coli*
Ampicillin	**AM10**	0	10
Aztreonam	**ATM30**	0	17
Cefadroxil	**CFR30**	22	16
Ceftazidime	**CAZ30**	0	8
Chloramphenicol	**C30**	23	24
Ciprofloxacin	**CIP5**	20	20
Erythromycin	**E15**	23	13
Imipenem	**IPM10**	37	25
Neomycin	**N30**	20	17
Norfloxacin	**NOR10**	21	20
Streptomycin	**S10**	20	15
Vancomycin	**VA30**	17	0

4. DISCUSSION

The bacterial count is considered an index of quality that gives an idea about the hygienic measures during processing and helps in the determination of keeping quality of the product (ABERLE *et al.*, 2001). This work will emphasize on the counts and characteristics of bacterial genera that is considered to be important in healthy foods giving an attention to their classification and identification. The main scope of this work is to count the different bacterial species found in the different food sources and study the antibiotic susceptibility patterns of these bacterial species which are isolated from Al-Mansoura city, Egypt. Bacterial counts of foods includes T.V.B and T.C.B are similar with the results of PRADNYA and SONALI (2008) who found the counts of T.V.B and T.C.B were in range 9-10 log cfu/mL for local open market in India. All isolated bacterial species are common components of the bacterial flora of mammals, birds, insects' reptiles and are commonly found in soil, on plants, water and foods as normal flora (GILMORE *et al.*, 2013).

These groups of bacteria were isolated by PRADNYA and SONOLI, 2008; NAZIR and ISLAM, 2007; EASA, 2010; OLUFEMI and AKINYERA, 2011; KUDJAWU *et al.*, 2011. Presence of *Escherichia coli* and T.C.B in food usually indicates lack of hygiena in handling and post process contamination, therefore *Escherichia coli* & T.C.B enumeration are used as food quality parameter (GONZALZ *et al.*, 2003). The present study was initiated by collection of food samples. All isolates were selected and purified and initial morphologically identified (cocci 46.7% and rods 53.3%) and (Gram's stain as gram positive rods & cocci 60.3% and Gram negative rods 39.7%). For some species, the range is wide and the growth occurs in a variety of substrates (as a true for coliform bacteria) but for others (e.g. many of pathogens) can grow in limited kinds of substrates. Thus, the bacteria found in food differ according to their ability of utilization of energy. The foods that are most often involved in Staphylococcal food poisoning differ widely from one country to another (BENNETT and LANCETTE, 1995). The present study was concerned with isolation of the bacterial content of different food samples, which collected from open markets in Al-Mansoura city, Egypt. All isolates were selected, purified and initial morphological-

ly identified by shape and gram stain as 46.7% cocci and 53.3% rods and gram positive cocci and rods 60.3% and gram negative rods 39.7%.

16S rRNA gene sequencing will continue to be the gold standard for the identification of bacteria, and the automation of the technique could enable it to be used routinely in clinical microbiology laboratories, as a replacement of the traditional phenotypic tests. Modern technologies have made it possible to construct a high density of oligonucleotide arrays on a chip with oligonucleotides representing the 16S rRNA gene sequence of various bacteria. Such a design will facilitate automation of the annealing and detection of the PCR products of 16S rRNA gene amplification, and hence routine identification of most clinical isolates will be possible. The use of 16S rRNA gene sequencing has several advantages. First, the turnaround time is short. Because amplification of the 16SrRNA gene takes only four to six hours, and the annealing and detection of PCR products takes only another few hours, theoretically the identification can be completed within one day. Second, it can be used for slow growing bacteria, unlike most commercially available kits that are based on phenotypic tests that require the detection of growth of the organism in the presence of certain specific substrates, and hence the slow growing bacteria are usually "unidentified" when the growth control shows a negative result. Third, the problem of "unidentifiable strains" will be overcome and there would be minimal misidentification – the identification of a clinical strain is clearly defined by the number of base differences between it and the existing species. Fourth, oligonucleotides representing all bacterial species, including those rarely encountered clinically, can be included in the array, making it easy to identify the rare species. Lastly, such a technique will be applicable not only to pyogenic bacteria, but also to other organisms such as mycobacteria (EL-HADEDY and ABU EL-NOUR, 2012).

Antibiotic resistance (Gram positive and Gram negative bacterial species) from food sources are important and serious problem in clinical field (EL-AIDY, 2007). The antibiotic sensitivity against bacteria is assayed by disc-diffusion method and in our study as well (SELIM, 2011; SELIM *et al.*, 2012; 2013)). In this study, the antibiotics susceptibility patterns of potent proteolytic bacterial species (*Bacillus cereus* and *Escherichia coli*) against 12 different antibiotics were investigated. Antibiotics include ampicillin (AM), aztreonam (ATM), cefadroxil (CFR), ceftazidime (CAZ), chloramphenicol (C), ciprofloxacin (CIP), erythromycin (E), imipenem (IPM), neomycin (N), norfloxacin (NOR), streptomycin (S) and vancomycin (VA). In this study *Bacillus cereus* was resistant to 3 antibiotics (ampicillin, azactam and cerazdime). Beta-lactam antibiotics also bind to inhibit the action of other cytoplasmic proteins that had a role in peptidoglycan synthesis and turn over (ABIGAL and DIXE, 1994). Transpeptidation reactions that cross links the peptide side chain of polysaccharide peptidoglycan back bone. Transpeptidase and other proteins were called "penicillin binding protein". The net result of beta- lactam binding to this protein was to stimulate endogenous enzyme that degrade peptidoglycan (ABIGAL and DIXE, 1994). The inhibitory effect of vancomycin was similar to the effect of Beta-lactam antibiotic.

Our results showed that *Bacillus cereus* and *Escherichia coli* were sensitive to cefadroxil, chloramphenicol, ciprofloxacine, erythromycin, imipenem, neomycin, norfloxacin and streptomycin. Neomycin, aminoglycoside antibiotic; erythromycin, as macrolid antibiotic have the ability to bind to the 50S or 30S ribosomal subunit (inhibit protein synthesis). Also, inhibitory effect of ciprofloxacin and norfloxacin, as quinolones antibiotic, may be due to having the ability to inhibit bacteria by interfering with their ability to make DNA with diverse targets DNA gyrase, this inhibition effect leads to preventation of multiply of bacteria. The present results showed that all tested *Escherichia coli* strains were resistant to vancomycin antibiotic, while Srinivasona *et al.*, (2007) who found that all tested *Escherichia coli* strains were resistant to two or more antimicrobial used in veterinary medicine. *Bacillus cereus* was resistant to aztreonam and ceftazidime.

Antibiotic resistance can be categorized in three types: natural or intrinsic resistance; mutational resistance and extrachromosomal or aquired resistance. The resistance of isolates to beta-lactam antibiotic may be due to drug inactivation: i.e. AmpC cephalosporinase (beta lactamase enzyme that open the Beta-lactam (ring) as an intrinsic resistance. Target site modification (i.e. change in PBPs- penicillin binding proteins-) as mutational resistance represented in drug inactivation (ABIGAIL and DIXIE, 1994). Moreover the resistance of isolates to aminoglycoside antibiotic and erythromycin macrolides antibiotics may be due to inaccessibility of the target as an intrinsic resistance, reduced permeability or uptake as mutational resistance and aquired resistance represented in drug activation (DIAB *et al.*, 2002; 2004). The resistance of isolates to furadantine antibiotic may be due to chromosomal or plasmide mediated and inhibition of nitrofuran reductase. Also the resistance of isolates to fluoroquinolones antibiotics may be due to reduced permeability or uptake as mutational resistance (FANGE *et al.*, 2009). The mode of action of ciprofloxacin and norfloxacine as a quinolones antibiotic as accumulated with the explanation of FANGE *et al.*, (2009).

In our present study, four studied bacterial species which isolated from food sources were investigated to all selected antibiotics. Most of the selected antibiotics represent the following classes ; beta-lactam, aminoglycoside, macrolide and quinlones. Total bacterial counts is con-

sidered an index of quality which gives an idea about the higienic measures during processing and help in the determination of keeping quality of the product (ABERLE *et al.*, 2001). In this study, the identified bacterial species were *Bacillus cereus* (13.72%) and *Escherichia coli* (18.3). These results had a strong support of many researches. These bacterial species are common components of the microbial flora community. Generally, the methods of production, transportation, handling and sale of food entirely unhygienic and entirely depend on the traditional system, such system could pose favorable environment for bacterial contamination. The existences of these bacterial species which isolated in different food sources. These results are agreement with FDA, 2000. Moreover the studied food sources are considered as reservoir for some pathogenic bacteria (MANGES *et al.*, 2006).

The *Bacillus* species are of the soil origin and may contaminate bread through the raw material and bakery requirements used. *Bacillus cereus* is widely distributed in the environment and is found almost everywhere including, dust, water and decaying matter. The microbiotas in dried traditional vegetables sold in open market in parts of Ghana are dominant by aerobic mesophilic bacteria including mainly Bacillus, lactic acid bacteria, coliform and moulds. Many studies provided evidence that *Escherichia coli* is a frequently occurring organism in milk (SOOMRO *et al.*, 2002), this evidence agrees with the obtained results. Presence of *Escherichia coli* and T.C in food usually indicates lack of hygiene in handling, storing food and production inadequate storage and post process contamination. Therefore *Escherichia coli* and T.C enumeration are used as food quality parameter (GANZALEZ *et al.*, 2003). *Escherichia coli* was isolated from the samples and also has been detected in many studies

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PAPER

BREAD QUALITY SUBSTITUTED BY POTATO STARCH INSTEAD OF WHEAT FLOUR

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ABSTRACT

Wheat bread constitutes the most regularly consumed food in the World, the international market for wheat undergoes strong pressure and prices are unceasingly increasing. The aim of this study is to substitute wheat flour by potato starch in bread preparation. Mixtures flours were characterized for composition, damaged starch, and Alveograph properties. According to the results of alveograph parameters, they decrease with the rate of incorporation of potato starch. This decrease can be corrected by adding vital gluten. The results of physicochemical analysis showed a decrease in protein levels, an increase in moisture content (about 2%) and carbohydrates levels due to the composition of potato starch. However, sensory analysis ($p \leq 0.05$) showed that the addition 80% of potato starch leads to bread with better characteristics: taste, colour and odour, based on that, it is highly advisable as an ingredient in the standard preparation of wheat bread.

- Keywords: bread, flour, potato starch, wheat -

INTRODUCTION

Bread is an important component of the Algerian diet, while wheat production in the country is insufficient. Therefore, substantial quantities of this cereal must be imported every year. Making bread by partial substitution of wheat is not a new idea and it is worthwhile to reveal some of the efforts made in the past to make bread from local materials, such as cereal flour or root starches. Potato occupies the fourth place in the World list of food crops, after wheat, rice and corn, with an annual World production of approximately 300 million Mg (CIP, 2008). The country now produces enough potatoes and its price is also within affordable limits of average people. So potato appears to be one of the most promising substitutes in bread making in order to help reduce dependence on wheat flour.

Potato starch is an important raw material in the food industry because its properties and their proportions vary according to the environment and genotypes of potato (VASANTHAN *et al.*, 1999; KAUR *et al.*, 2002; ZAIDUL *et al.*, 2007). Potato starch is largely used in food and non-food fields (paper, cardboard, textiles, mining, drilling, adhesives, etc.). Originally, it was produced for baking by adding it to cereal flour (ROUSSEL *et al.*, 1996; SINGH *et al.*, 2003). The addition of modest amounts of potato starch helps preserve the freshness of bread and it also confers a distinctive character and a pleasant flavor (YANEZ *et al.*, 1981; WILLARD and HIX, 1987).

The aim of this work is to evaluate the possibility of substituting wheat flour for high percentages of potato starch in bread making process and to evaluate the physical, chemical, nutritional and sensory properties of the produced bread.

MATERIAL AND METHODS

Raw material

Algerian wheat flour was obtained from NEKHLA mill, Algeria. Ingredients like sugar, salt, instant active dry yeast and shortening were purchased from the local market, while Potato starch was obtained from Michel Come, Rambouillet, France.

Methods

Physical and chemical composition

Moisture content

The moisture content of samples was determined according to the AACC Official Methods 46-30, where a sample of 5 g is weighed and placed in a moisture dish. The sample is warmed to 130°C in an air oven during 2 hours; then the residue is cooled to room temperature and weighed (AFNOR, 1991).

Ash content

It was determined according to the AACC Official Methods 08-01 (AACC, 1995). Where a sample of 3-5 g is weighed and placed in an ash cup, then the sample is heated at 900°C in an ash oven until complete combustion of the organic matter, and the residue is cooled to room temperature and then weighed (AACC, 1995).

Protein content

Protein content is determined by the Kjeldahl distillation method (by analyzing total nitrogen contents). Two grams of dry sample are weighed and placed with hot concentrated sulfuric acid. The ammonia liberated from the resulting ammonium sulphate, after adding sodium hydroxide was distilled into 1 M boric acid then titrated with 0.1 M HCl. The nitrogen value estimated was multiplied by 5.7 (protein factor) to obtain the value of crud protein. This is expressed as the percentage of dry sample mass (AACC, 1995).

Fat content

According to UGRINOVITS *et al.* (2004) the crude fats were determined by the Soxhlet method. They are extracted from 10 g of each sample using a Soxhlet apparatus with low boiling point petroleum ether (40-60°C) as solvent. A rotary-evaporator was used to evaporate the solvent after each extraction.

Falling Number Test

The level of enzyme activity was measured by the Falling Number Test (standard method AACC 56-81B), and this is to evaluate the α-amylase activity of the flour by measuring the consistency of the gelatinized starch.

Seven grams of the sample is weighed and combined with 25 mL of distilled water in a glass falling number tube with a stirrer and shaken to form a slurry. Then, it is placed in the falling number instrument (AACC, 1995).

Alveograph characteristics

An amount of 250 g of the sample with salty solution was mixed in the alveograph mixer. After 8 minutes of kneading, the passage was opened and the extraction began. The dough patty was cut as soon as it arrived at a mark on the extraction plate. The dough patty was rolled and cut with the cutter and then was placed in the oven of the dough pieces at 25.5°C. After 28 minutes, each dough patty was inflated with air and its individual characteristics (P, L, W) were measured (AACC, 1995).

Table 1- Bread mix formula.

Formula	
Wheat flour	500 g
Salt	10 g
Sugar	5 g
Yeast	10 g
Water	300 mL
Dough improver	0.1 g

W: The work of the deformation energy (baking strength);
L: The length of the curve (Extensibility);
P: Maximum height (Tenacity);
P / L: Ratio curve configuration.

Bread making

The conventional straight-dough method for pan bread was performed according to the procedure developed by AACC. The formula used to make bread is given in Table 1. The level of substitution of wheat flour by potato starch was 80%.

To make bread, the dry ingredients were manually mixed and then added to a mix containing water. The components were thoroughly kneaded with the mixer for 5 min at low speed. The mixing speed was then changed to high speed for 5 min. The dough was divided into pieces of 100 g, rounded by hand and allowed to relax for 25 min. The dough was moulded then panned and fermented for 90 min at 30°C in a fermentation cabin.

Gas retention during fermentation was evaluated using an indicator of growth containing 25 g of dough which was subjected to fermentation in the same conditions as the dough.

The bread was baked at 220°C/20 min in an electric oven. Subsequently, the baked bread samples were then depanned and cooled to evaluate their external and internal properties by a 1h at room temperature, packed in polyethylene bags used for further analyses.

Bread evaluation

Loaves were organoleptically evaluated for their external and internal properties by a jury of twenty tasters. The method of 5 point score (in a hedonistic qualification scale) was used (AMERINE *et al.*, 1973). The panel members were asked to score for crust colour, crumb colour, texture, flavour and overall acceptability.

Statistical analysis

In this study, all experiments were performed in triplicate. Statistical analysis was performed using XLSTAT program to compare the results. The level of significance was considered at $p \leq 0.05$.

RESULTS AND DISCUSSION

Physical and chemical composition

The result of proximate composition analysis of wheat flour and potato starch is as shown in Table 2. The wheat flour protein content used in this study was about 10%, this result is similar to that reported by LINDAHL and ELIASSON (1992). According to UGRINOVITS *et al.* (2004) the strength of the flour is partially determined by its wet gluten content.

The wet gluten content of potato starch is about 1.72% where as wet gluten content of wheat flour is 30.08%, which is a normal level, potato starch contained lower proteins (trace) and higher carbohydrate than wheat flour. However this value of wet gluten, added to the vital gluten in the mixture allows compensating for the deficit of protein potato starch (32.77%).

The results of baking test show that potato starch alone is not enough to produce bread, and the same result was found for the mixture with high level. It might be due to the value of gluten that is lower. Therefore, it is necessary to add a percentage of vital gluten.

However, the value of the falling number of the

Table 2 - Physical and chemical characteristics of mixtures.

Parameter\Product	100%	100% S.P	20% F 80% S.P	
			Wh.G	G
Moisture (%)	15.80±0.026	17.80±0.035	17.60±0.011	16.21±0.015
Mineral (Ash) (%)	0.53±0.025	0.18±0.02	0.19±0.0152	0.27±0.01
Wet Gluten (%)	30.10±0.155	/	1.72±0.092	32.77±0.196
Falling number (s)	312	220	181	126
Protein content (%)	10	Trace	/	/
Fat content (%)	0.9	Trace	/	/

F: Wheat Flour; S.P: Potato Starch; Wh.G: Without Gluten; G: With Gluten.

Table 3 - Rheological characteristics of the flours (with and without addition of vital gluten).

Rheological characteristics		Rate of incorporation of the potato starch		
		0%	80% without gluten	80% with gluten
Alveographic measurements	P (mm)	81	20	99
	G (cm)	19.1	06.7	14.8
	P/L	01.09	06.68	02.25
	W (10^{-4} J)	210	10	193

mix is lower (220) than that of wheat flour and also lower than the optimal standards for bread which is 200 to 300 seconds (GODON and LOISEL, 1997). This might be due to the decreased resistance of potato starch to enzymatic degradation. Also, the fact that the gelatinization temperature is lower than that of wheat flour can be considered as another reason.

Alveograph characteristics

The results of Alveograph Test, summarized in Table 3, make it possible to predict baker quality of flour. This test is an interesting practice which is very appreciated by professionals of the second transformation, due to the fact that it reflects through alveographic parameters measured the ability of flour to be managed according to its baking strength for a specific purpose (ROUSSEL and CHIRON, 2002).

The Alveograph parameters of wheat flour and mixtures (with 80% of potato starch) showed (Table 3), that overpressure (P), a measure of dough tenacity, which is an indicator of gas retention by the dough as indicated by WANG *et al.* (2002), varied from 58 to 150 mm.

The measure of Alveograph dough extensibility (L), ranged from 45 to 116 mm. The values for curve configuration ratio, indicating the configuration ratio of the Alveograph curve, varied from 1.09 to 2.25. The index of swelling (G) varied from 6.9 to 19.10 cm and the baking strength representing the energy necessary to inflate the dough bubble to the point of rupture ranged from 10 to 210*10^{-4} J. These differences of results are due to the addition of gluten which controls these parameters, where the P value increases to 99mm H_2O. It is higher than the limit of 80 mm.

These results show that the composite potato starch and wheat flour lead to dough which is less resistant to deformation and low extensibility for rate incorporation of 80%.

Mixed baking test

In order to know the influence of the substitution of wheat flour by potato starch (20/80), several tests were carried out in the laboratory and other tests at the bakery with the assistance of a French expert in bakery (J. PRODHOMME).

The experimental baking studies showed that the concentration of 80% potato starch with the addition of gluten did not affect the handling of dough except for some defects of extensibility during shaping. This similarity in results is due to the role of the added gluten; the essential element for baking (especially during kneading and shaping), which plays a very significant role in increasing the uptake of water and the resistance of the dough. A significant criterion observed during almost all stages of baking, is the stickiness of the dough.

In a similar vein, the properties of gas retention within the composite dough are followed by measurement of the volume of the dough during fermentation using the indicator of growth containing 25g of dough subjected to fermentation under the same conditions as the loaves of bread. The results obtained are highly significant ($p \leq 0.05$), they show that the pastes incorporate up to 80 % of potato starch, which experience less raising during fermentation, but remain comparable with those obtained with 100 % of wheat flour (Fig. 1).

External and internal aspects of breads obtained are shown in Figs. 2 and 3. The incorporation of potato starch at levels of 80% gives breads with optimal characteristics.

The breads resulting from the potato starch have a good appearance, presenting regular and smooth crusts similar to the breads resulting from wheat flour.

As for the coloration of the crust, bread with potato starch presents a less dark coloring compared to bread with wheat flour. DUPIN *et al.* (1992) and BOYACIOGLU and D'APPOLONIA (1994) showed that the dark coloration of bread is influenced by the rise of the rate of both damaged starch and total sugars present in flour, which were highest in starch potato.

Concerning the appearance of the crumb related to (Fig. 2), bread has aired cells, badly dispersed and not homogeneous. That can be explained by the irregular distribution or the incorporation of α-amylases.

As for the appearance of crumbs (Fig. 3), bread has aired cells, poorly dispersed and not homogeneous. Again, that can be explained by the bad distribution or the incorporation of α-amylases.

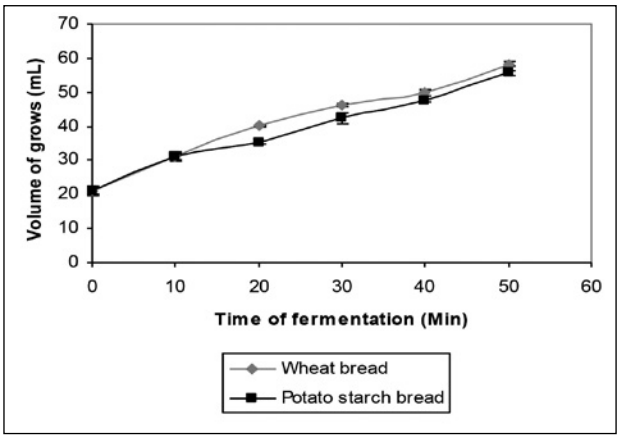

Fig. 1 - Influence incorporation of potato starch on the profiles of gas retention.

Fig. 2 - Appearance of the crust of potato starch bread (80%).

Fig. 3 - Appearance of the crumb of potato starch bread (80%).

Bread evaluation

The approximate composition of bread made from potato starch and wheat flour is shown in Table 4.

The control bread obtained shows a protein rate of 10.5 %, a rate 60.4% of sugar of and 0.9 % of fat. These values are similar to the values given by CABROL (2006).

However, bread prepared containing potato starch 80% present a reduction in proteins (6.87%) and fat (0.37%). This result might be due to the composition of potato starch that, in fact, is rich in sugar and low in fat and proteins.

The sensory quality statistics reveal that potato starch influences the crumb of bread (Fig. 4). Bread with potato starch 80% acquires a very white coloration, and therefore receives the highest score compared to control bread. Meanwhile, the texture of bread is more developed than that of the control bread.

However, we observed that there is significant difference ($p \leq 0.05$) in the P value therefore stating the sensory characteristics of potato starch bread 80% are not affected.

CONCLUSIONS

The aim of this study was to analyze samples of bread at 80% of potato starch and compared with control bread produced from wheat flour under the same conditions for their nutritional, physicochemical and sensory characteristics. The formulation of our bread was made as follows: potato starch, wheat flour, gluten, yeast, salt and a dough improver. The results show that the loaves can be prepared by potato starch even at high percentage (80%) and gluten. Breads obtained by this formula were nutritionally, physically, chemically and at the

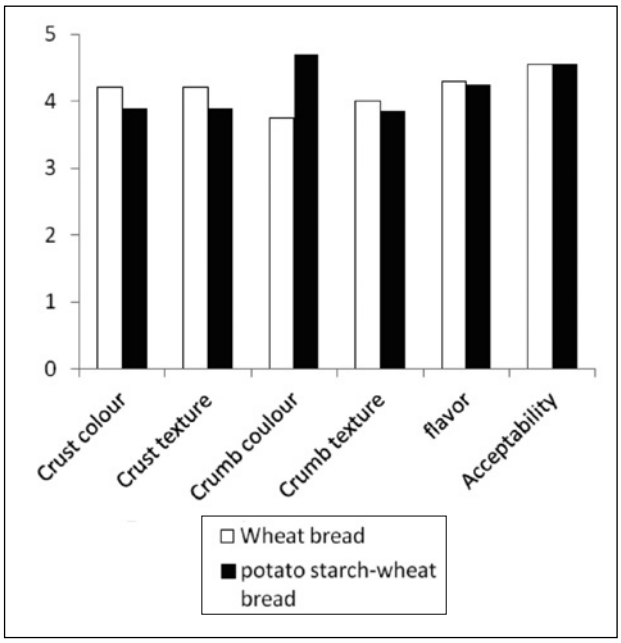

Fig. 4 - Evaluation of some characters of quality of the potato starch bread 80% and wheat bread prepared in bakery.

Table 4 - Nutritional composition of breads.

Products	Humidity %	Glucids (%)	Protein (%)	Fat (%)	Fibre (%)
Wheat bread	28.20	60.4	10.5	0.9	-
Potato starch bread	31.33	61.36	6.87	0.37	0.07

sensorial level comparable to the control bread. A high percentage of consumers said they saw no difference.

These results support the partial substitution of wheat flour by potato starch in wheat-based food products to minimize costs. Future studies are needed to investigate the pasting properties of mixtures of wheat flour and potato starch (by RVA), to determine the interaction between wheat flour and potato starch and also to interpret their rheological properties by differential scanning calorimeter and rheometer.

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PAPER

THE EFFECT OF COOKING AND STORAGE ON FLORFENICOL AND FLORFENICOL AMINE RESIDUES IN EGGS

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ABSTRACT

The aim of this study was to evaluate the effects of storage conditions (room temperature, refrigerator) and cooking methods (frying, boiling) on florfenicol (FF) and florfenicol amine (FFA) residue levels in eggs. Without any significant difference between storage conditions at 20˚C and +4˚C, residue levels decreased within days, but were still present on day 28. Frying and boiling for 1 and 5 min yielded similar results to the storage conditions just described; there was a significant decrease in residue levels, but still not enough for decomposing. These findings indicate that FF and FFA residues are heat-labile.

- Keywords: cooking, egg, florfenicol, florfenicol amine, residue, storage -

INTRODUCTION

When veterinary drugs are administered to farm animals, either therapeutically or to promote growth, residues remain in their meat, milk or eggs if proper precautions are not followed (BOTSOGLOU and FLETOURIS, 2001). Antibiotics play an important role among such drugs. In addition to their positive effects, they can also cause health problems, including drug hypersensitivity (PAIGE *et al.*, 1999; DONOGHUE, 2003). Antibiotics not only threaten food safety, but also cause the development of some resistant bacterial strains from among sensitive bacteria even when used at moderate doses for long periods of time (PAIGE *et al.*, 1999; FILAZI *et al.*, 2005).

Testing for drug residues are ordinarily performed on raw products. Almost no edible animal products or byproducts are consumed raw, but require some type of processing or cooking, such as frying, boiling, or roasting, before consumption. These processes can cause denaturation of proteins, elevation of temperature, loss of water and fat, and pH variations that can eventually result in alteration to the concentration, chemical nature, chemical reactions, and solubility of drug residues in a particular food item. Many drugs are chemically unstable to varying degrees, and therefore may undergo degradation during storage, cooking or processing in consumable foods (BOTSOGLOU and FLETOURIS, 2001).

In general, the temperatures achieved during cooking are assumed to degrade antibiotic residues in food; however, ordinary cooking procedures are unreliable for degrading or inactivating several commonly used veterinary drugs. Earlier studies have indicated that sulfamethazine (ROSE *et al.*, 1995; PAPAPANAGIOTOU *et al.*, 2005) chloramphenicol (BOTSOGLOU and FLETOURIS, 2001), streptomycin (INGLIS and KATZ, 1978; O'BRIEN *et al.*, 1980), neomycin (KATZ and LEVIN, 1978), gentamicin (SIRELI *et al.*, 2006), fluoroquinolones (BAYDAN *et al.*, 2000a, b; BAYDAN *et al.*, 2002), penicillin G (NOUWS and ZIV, 1976; BOISON *et al.*, 1992), nitrofurantoin (COOPER and KENNEDY, 2007), oxacillin, clindamycin, novobiocin, trimethoprim, vancomycin, and azlocillin are heat-stable (TRAUB and LEONHARD, 1995), whereas oxytetracycline (KITTS *et al.*, 1992) and amphenicols (FRANJE *et al.*, 2010) heat-labile. On the other hand, several β-lactams including ampicillin and amoxicillin are partially heat-labile (TRAUB and LEONHARD, 1995). Antibiotics of the same class were reported to vary in heat stability according to the type of matrix and heating treatment involved (KITTS *et al.*, 1992; ROSE *et al.*, 1996; FRANJE *et al.*, 2010). As such, the effect of different matrices on the stability of every veterinary drug should be investigated.

Although most edible animal products are consumed after cooking or some type of processing, for the licensing of veterinary drugs research concerning the effects of storage and cooking of the drugs on different matrices are lacking. Most data on drug residues in edible animal products and government regulation concern raw products. It is therefore essential to determine the effect of processing on all veterinary drugs when assessing human exposure to drug residues in animal food products (IBRAHIM and MOATS, 1994; MOATS, 1988; MOATS 1999; BOTSOGLOU and FLETOURIS, 2001).

Florfenicol (FF) is a wide-spectrum, synthetic antibacterial that is structurally related to D(–) threo-chloramphenicol; however, FF differs from chloramphenicol in that FF contains a *p*-methyl sulfonyl group instead of a *p*-nitro group and it contains a fluorine atom instead of a hydroxyl group in the terminal primary alcohol group (EMEA, 1999). FF has not been approved for use in laying hens; however it is used in cattle, swine, poultry, and fish (EMEA, 2000).

FF is metabolized into florfenicol amine (FFA), florfenicol oxamic acid, florfenicol alcohol, and mono-chloroflorfenicol in animals. FFA is the longest-lived metabolite in the bovine liver; therefore, FFA can be used as a marker for the calculation of withdrawal time (ANADON *et al.*, 2008; XIE *et al.*, 2011).

In light of the apparent advantages over chloramfenicol and its availability as an additive, the potential for off label use of FF is high. Due to its broad spectrum antibacterial activity, ready availability and low cost, it remains a possibility that FF residues will continue to be found in such animal food products as eggs. For example, Xie *et al.* (2011) analyzed 50 egg samples obtained from a local supermarket in China and reported 19 ppb of FF and 36 ppb of FFA in only 1 egg. FILAZI *et al.* (2014) reported that the concentration of FF and FFA in eggs were 0.1%, 0.08% respectively regardless of the route of administration.

Data on the heat stability of FF is essential for food safety; however, the literature contains few data regarding its heat-stability during cooking. Under environmental conditions FF is stable at 25 °C, yet photodegradation occurs at varying rates in water under various lighting conditions (GE *et al*, 2009). FF was shown to rapidly degrade to FFA in the deep sediment of marine environments via biodegradation (HEKTOEN *et al*, 1995). A few studies on the residue of FF and FFA in eggs have been published (XIE *et al.* 2011; FILAZI *et al.*, 2014); but the data are insufficient. FRANJE *et al.* (2010) reported that amphenicols exhibit differential behavior in terms of heat-induced degradation in solutions and protein matrices. Although the level of amphenicol degradation in soybean sauce and meat was high, heating may generate product with antimicrobial activity; therefore, heating amphenicol residues in food cannot always be considered safe. FF is the most commonly used veterinary antimicrobial agent in Turkey, particularly so due to its illegally use in laying hens. Nonetheless,

few studies have examined FF residue levels in eggs. An earlier study reported that FF and FFA were detected in the eggs of hens administered with FF (FILAZI *et al.*, 2014)

Chicken eggs are widely used in the preparation of many types of food, including many baked goods. Some of the most common preparation methods include fried in oil, hard-boiled, soft-boiled and omelets. Data regarding FF and its main metabolites in cooked and stored eggs are lacking. As such, the aim of the present study was to determine the effects of different storage conditions (room temperature and refrigeration) on FF residue levels in eggs stored up to 28 days and to determine the effect the different cooking methods (frying and boiling) on FF and FFA residue levels.

MATERIALS AND METHODS

Animals

The study protocol was approved by the Ankara University Ethics Committee (2007-15-45). The study included 50 ISA Brown laying hens aged 48 weeks and weighing 1.9-2.4 kg. The hens were housed individually in fiber cages (30x35x45 cm), in a ventilated room maintained at 20°C under 14 h day light condition. The hens received standard commercial layer mash (120 g/d) and water ad libitum. The hens were fed for 1 week and their eggs were collected for preliminary analysis to determine if they were analyte-free.

Trials

A veterinary drug containing 300 mg of FF in 1 mL was used (Mediflor 30% Oral Solution, Medicavet Company, Turkey) for the clinical trials. FF was administered at a dose of 20 mg/kg/day via gavage for 3 days to the 50 laying hens, and then their eggs were collected daily thereafter. The effect of storage procedures on the residues was determined on the first day using 44 eggs. The effects of cooking procedures were determined on the second day using 32 eggs. In all, 20 of the eggs collected on the first day were kept at 4°C in a refrigerator, and 20 were kept at 15-20°C (room temperature). In addition, 4 eggs were analyzed on day 4, 7, 14, 21, 28 of storage to determine FF and FFA residue levels. Lastly, 4 uncooked eggs collected on day 1 were analyzed as a control group; of the eggs collected on day 2, 8 uncooked, 8 fried in oil, 8 undercooked (1 min in boiling water) and 8 overcooked (5 min in boiling water) were then analyzed.

Sample preparation and analysis

FF and FFA were extracted from homogenized eggs via phosphate buffer (pH:7) and ethyl acetate. Following purification, the samples underwent high-performance liquid chromatography (HPLC) using a photodiode array detector (PDA) and C18 column; the method was validated according to ICH guidelines, as described elsewhere (FILAZI *et al.*, 2014). According this method, limits of detection and of quantitation values were 1.94 and 6.45 ppb for FF, respectively, and 0.48 and 1.58 ppb for FFA, respectively. Relative standard deviation values of intra-day and inter-day variation below 11% also confirmed the usefulness of the method for analysing FF and FFA in eggs.

STATISTICAL ANALYSIS

Variance analysis was performed with all data and a multiple range test was used to determine the differences between groups. All analyses were performed using SPSS v. 17.0 for Windows.

Results and Conclusion

The effects of different storage temperatures and durations on FF and FFA residue levels in

Table 1 - Mean±SD* concentration (in ppb) of florfenicol and florfenicol amine residues in eggs stored at room temperature (15-20°C) and in a refrigerator (+4°C).

Days	Florfenicol		Florfenicol amine	
(n=4)	Room temperature (15-20°C)	Refrigerator (+4°C)	Room temperature (15-20°C)	Refrigerator (+4°C)
0	290.65±11.02^{a}	290.65±11.02^{a}	91.79±6.77^{a}	91.79±6.77^{a}
4	151.24±10.69^{b}	167.43±8.18^{b}	58.26±5.98^{b}	58.61±5.85^{b}
7	79.65±9.43^{cx}	105.10±4.25^{cy}	28.95±5.03^{c}	35.40±2.33^{c}
14	68.23±8.74^{dx}	87.84±5.01^{dy}	20.52±3.92^{d}	24.37±1.20^{d}
21	29.43±4.91^{ex}	61.82±2.11^{ey}	10.42±1.54^{e}	8.54±1.04^{e}
28	18.57±3.48^{f}	22.14±0.03^{f}	6.74±0.79^{f}	7.06±1.21^{f}

*SD: Standard Deviation.
abcdef: Differences between values with different letters in the same columns are significant (P<0.05).
xy: Differences between values with different letters in the same rows are significant (P<0.05).

Table 2 - Mean±SD*, quantity of florfenicol and florfenicol amine residues (in ppb) after different cooking methods.

Residues (n=8)	Raw	Fried	Undercooked (1 minute)	Overcooked (5 minutes)
Florfenicol	265.45±13.67[a]	56.51±9.68[b]	35.67±4.57[c]	5.68±1.17[d]
Florfenicol amine	110.31±12.73[a]	19.77±4.71[b]	10.20±1.72[c]	4.57±0.92[d]

*SD: Standard Deviation.
abcd: Differences between values with different letters in the same row are significant (P<0.05).

eggs are shown in Table 1. Both FF and FFA amine residue levels in eggs were observed on day 28, though their levels had decreased significantly (P<0.05). HEKTOEN *et al.* (1995) reported that FF rapidly depurated in the sediment of marine environments and that its metabolite (FFA) was isolated from the sediment. This finding suggests that FF is degraded to FFA in the sediment via metabolization or leaching; however, the present study FF residues in eggs following storage for 28 day at room temperature and in a refrigerator were observed. FF residue levels in eggs were higher than FFA residue levels in the present study, which indicates that the in vitro degradation of FF might occur at a very low level or that it differs from its biological degradation. Further research would be required to understand the effect of storing on the FF and FFA residues in the eggs.

FRANJE *et al.* (2010) studied the heat stability of amphenicols in chicken meat and reported that 5-min heating of amphenicols in water in a microwave oven generated a comparable percentage of degradation as did boiling in a water bath for 30 min 1 h; FF produced thiamphenicol (TAP) as a product of its breakdown, but not FFA. It was reported that although a higher level of degradation of amphenicols was observed in soybean sauce, heating treatment might still generate product with antimicrobial activity (FF to TAP) and as such, heating amphenicol residues in food cannot always be safe.

FF was reported to be hydrolytically stable and to have a hydrolysis half-life ≥ 1 year at 25°C in natural waters (HAYES *et al.*, 2003; POULIQUEN *et al.*, 2007; GE *et al.*, 2009). GE *et al.* (2009) performed photodegradation experiments on TAP and FF in aqueous solutions under irradiation from different light sources. They reported that under UV-Vis irradiation (λ>200 nm) photodegradation in seawater was fastest, followed by pure water and freshwater, whereas under solar or simulated sunlight (λ > 290 nm), photodegradation occurred only in freshwater. Under UV-Vis irradiation, Cl- (dominant sea water constituent) was observed to promote singlet oxygen formation and accelerated the photodegradation of phenicols, whereas phenicols did not photolyze under simulated solar irradiation, irrespective of the presence of Cl^-.

In contrast, HAYES *et al.* (2003) reported that FF was stable under a range of simulated field conditions, including various pipe materials and conditions of hard and soft and chlorinated or non-chlorinated water at low or high pH; therefore not only Cl^- but also some other minerals might effect the stability of FF.

The effects of different cooking procedures on FF and FFA residues in eggs observed in the present study are shown in Table 2. Even though, none of the cooking methods completely destroyed FF or FFA residues in eggs, there was a significant decrease in the level of detectable FF and FFA residues (P<0.05). Concentrations of both analytes were reduced by 78%-97% via frying and boiling. These findings suggest that FF and FFA heat labile in eggs, which indicates that both do not bind to proteins in eggs with high affinity. FRANJE *et al.* (2010) reported that amphenicol degradation was apparent following as little as 30 min of heating and that it was correlated with the length of heating, implying that as cooking time increased the degree of residual drug present in samples decreased; as such, it could be assumed that there was a strong correlation between the decrease in FF and FFA concentrations in observed eggs during different cooking methods and the duration of cooking (P<0.05, Table 2). SHAKILA *et al.* (2006) studied the stability of chloramphenicol (CHP) residues in white shrimp (*Penaeus indicus*) subjected to cooking (100 °C) for 10, 20 and 30 min as well as retorting (121°C) for 10 and 15 min, based on a microbial assay method using *Photobacterium leiognathi* as the test organism. They reported that the loss of CHP increased as temperature and duration of heating increased, where the drug could be completely destroyed. On the other hand BOTSOGLU and FLEUTORIS (2010) reported that CHP was quite stable under heating conditions when added to water or milk; after 2 h of boiling, it was decreased by ≤8%. These findings indicate that the heat stability of amphenicols is matrix dependent, where results from different matrices could not be attributed to eggs when interpreting.

Even though, FF is not approved for use in laying hens, its off label use for severe indications can result in antibiotic residues in eggs that both farmers and consumers should be informed about. As such, drug withdrawal periods should be extended prior to poultry slaughter

or egg distribution to avoid antimicrobial resistance. Thermal treatments may reduce the concentration of veterinary drug residues in foods and thereby might reduce the pharmacological and/or toxic effects of these compounds. (HSIEH *et al.*, 2011). In the current study, FF and FFA were observed to be heat labile in chicken eggs, the level of which depended on cooking method and duration.

The findings show that FF and FFA residue levels in eggs from treated laying hens were not completely eliminated via cooking or of up to 28 d; however, cooking did significantly decreased the level of the drug in eggs.

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The authors declare no competing financial interest.

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PAPER

KEFIRS MANUFACTURED FROM CAMEL (*CAMELUS DRAMEDARIUS*) MILK AND COW MILK: COMPARISON OF SOME CHEMICAL AND MICROBIAL PROPERTIES

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ABSTRACT

This study examined the production possibilities of kefir from fresh camel milk fermented with grain. The findings were then compared with kefir manufactured from cow's milk. Cow's milk was fermented with 2.5% grains. The 1% (v/w) glucose enriched camel's milk was fermented with 10% grains and left in an incubator at 25°C. Physical-chemical and sensorial analyses of the kefir samples were measured on day one (18 hours) of storage and microbiological analyses were measured on days one, three and five. Some physical-chemical parameters were found to be higher in camel milk and its kefir than in cow milk and its kefir, some were found to be close and some were found to be lower. Addition of 1% glucose and 10% grains to the camel milk affected the titration acidity and viscosity of kefir to significant levels. The kefir produced from camel milk was perceived as sourer, whereas its other properties were found to be close to those of cow milk. The cholesterol levels of camel milk and its kefir were detected to be higher when compared to those of cow milk and its kefir, but the cholesterol level decreased in both examples after the production of kefir. In terms of the composition of fatty acids, it was determined that SFA and the small, medium chain fatty acids ratio was low in camel milk and its kefir, but MUFA and the long chain fatty acids ratio was high. PUFA ratio was high in camel milk but low in its kefir. In microbiological analysis, yeast levels increased in kefir samples with the *Lactobacillus ssp.* strains, and the increase in the number of yeasts was higher than in the cow milk kefir. In kefir samples, *Lactobacillus ssp.* strains increased on day one and three of storage, but diminished after day three.

- Keywords: camel milk, kefir, grain, traditional method, chemical properties, microbial properties, flavor profile analysis -

INTRODUCTION

Kefir is a dairy product that has been produced for years in Eastern Europe and Mongolia, before spreading to Caucasia (GAWARE *et al.*, 2011). Kefir is produced by adding specific amounts of the kefir grain (traditional method) or the modified culture (industrial method) manufactured from this grain (POGAČIĆ *et al.*, 2013) into the milk of various animals. Ethyl alcohol and lactic acid fermentations are developed together during the product formation, thus causing it to taste somewhat acidic. Kefir grains are off-white and slightly yellowish, irregular in shape and with a circumference taken up by polysaccharide matrixes that (JIANZHONG *et al.*, 2009) compose 25% of the dry weight soluble in water (POGAČIĆ *et al.*, 2013), and a diameter of 0.3cm -2 cm (BESHKOVA *et al.*, 2002). Homofermentative lactobacilli make up 65-80% of the flora. In the grain flora, homofermentative and heterofermentative lactic acid streptococci make up 20%, and lactose-fermentative and non-fermentative yeasts make up a further 5%. The percentage of acetic acid bacteria (in production with grain) is relatively small (IRIGOYEN *et al.*, 2005). Species of the microorganisms in the grain, their proportion to each other and their numbers change according to the origin of the grain and conditions of use (FERREIRA *et al.*, 2010). Today, kefir is regarded as a fermented dairy beverage that is anti-bacterial and anti-inflammatory (LOPITZ-OTSOA *et al.*, 2006), anti-tumoral (SHIOMI *et al.*, 1982), anti-apoptotic (MATSUU *et al.*, 2003), anti-allergic (UMEDA *et al.*, 2005), anti-oxidant, and anti-mutagenic (LIU *et al.*, 2005). It also lowers systolic and diastolic blood pressure and bad cholesterol (AGERBAEK *et al.*, 1995), adjusts lactose dyspepsia (HERTZLER and CLANCY, 2003), and contains bioactive peptides, exopolysaccharides and their bacteriosis, and has a strong probiotic effect on human health (RATTRAY and O'CONNELL, 2011).

Camel milk differs from the milk of other ruminant animals in its composition and physiological properties. Camel milk is rich in long chain fatty acids, but contains low amounts of short chain fatty acids (GORBAN and IZZELDIN 1999). Vitamins A, B2, E, C and minerals Ca, Na, K, Zn, Mg and Fe are far more abundant in camel's milk than in cow's milk. Lactose intolerant people (EL-HATMI *et al.*, 2007) can consume camel milk. Due to camel milk not containing β-lactoglobulin and some casein derivatives, it is similar to human milk with its hypoallergenic (SHABO *et al.*, 2005), immunoglobulin content. It is anti-diabetic effective (HAMAD *et al.*, 2011) and because it contains more peptidoglycan recognition proteins (PGRP) and natural protective proteins than other ruminant milk, it has an antimicrobial and antiviral effect (EL-AGAMY *et al.*, 1992). Not to mention that camel milk is anti-carcinogenic and anti-hypertensive (HAMAD *et al.*, 2011) and renoprotective, it reinforces immunity, increases metabolism and muscle mass, is bone-forming and also has therapeutic effects on some diseases such as hepatitis B, autism (LAILA *et al.*, 2013) and tuberculosis (AGRAWAL *et al.*, 2004). Today, we know that yoghurt, probiotic yoghurt (ATTIA *et al.*, 2001; EL-AGAMY *et al.*, 1992), stabilizer augmented yoghurt (MULIOR *et al.*, 2013) and many mild cheeses whose clotting is poor due to enzymatic coagulation (MEHAIA, 1993; RAMET, 1987) can be produced from camel milk.

Our research utilized kefir that was manufactured from camel (*Camelus dramedarius)* milk (CaM). Kefir made from cow's milk (CoM) was used as the control sample. The physical-chemical properties in raw milks were analyzed, along with these properties in the cow milk kefir (CoMK) and camel milk kefir (CaMK) samples. Sensorial tests were conducted as of the eighteenth hour of day one and microbiological analyses were made on day one, three and five of storage.

MATERIALS AND METHODS

Camel milk, kefir grain

Camel (*Camelus dramedarius)* milk was procured from a native camel farm in Denizli Sarayköy (Turkey). Cow milk and kefir grain were procured from the Department of Dairy Technology Pilot Dairy Plant, Ege University Agricultural Faculty.

Kefir production

In this study, kefir was produced from camel and cow milk using the traditional (grain) method as shown in Fig. 1.

Physical-chemical analyses

In the raw milk and kefir samples, dry matter (Binder ED-53 Germany) and ash (Protherm PFL 110/6 Turkey) were calculated via a gravimeter, fat with the Gerber method, pH value of the titration acidity in terms of lactic acid with a SS-3 Zeromatic (Beckman Instruments Inc., California, USA) brand pH meter, protein with the Kjeldahl method (AOAC, 1990), lactose value with an Atago Polax x 2L (Japan) model polarimeter (HORWITZ, 1965), viscosity value with a Brookfield Digital Viscometer, MODEL DV-II+PRO (USA) model viscometer as cP [at speed 180 mPa, between 15.7% and 67.7% Torque].

Determining the fatty acid composition in samples and preparation of fat extraction and fatty acid methyl esters

Each homogenized sample was extracted using the Gerber method, thus fat was obtained

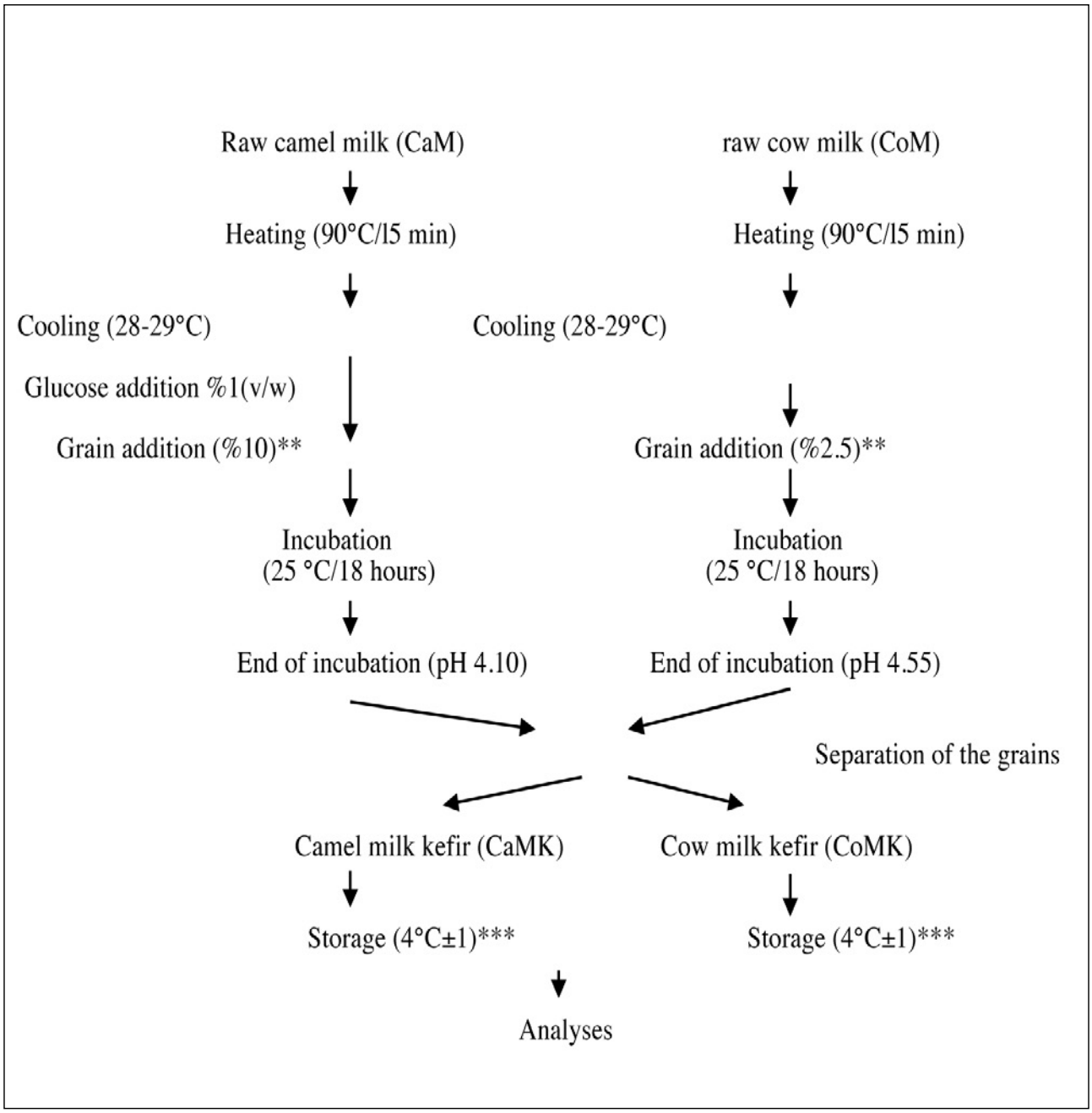

Fig. 1 - Kefir Production with the Traditional (Grain) Method.
** The glucose and grain ratio added to camel milk and cow milk was determined as a result of preliminary study.
***physical-chemical and sensorial analyses were measured at day one (18 hours) of storage and microbiological analyses were measured at days one, three and five.

(ISO 11870:2009 - IDF 152:2009) and fatty acid methyl esters were prepared pursuant to AOCS (2009), after which they were examined in the gas chromatograph (GC). [Chromatography is a Supelco SP-2380 fused silica capillary column (60 m 0.25 mm i.d., 0.2 mm film thickness; Supelco Inc., Bellefonte, PA, USA) and flame ionization detector Hewlett-Packard GC (model 6890). Injection volume was 1 μL. GC furnace temperature was set to reach 220°C from 100°C when 4°C/minute. Injector and detector temperature was 300°C, carrier gas was Helium and the flow rate was 1 mL/min].

Determining the cholesterol level in samples

In samples, the cholesterol level was analyzed according to the findings of OSSA *et al.*, (1995); and then examined by gas chromatography (GC). [Chromatography was a HP-5 silica capillary column (25 m 0.32 mm i.d., 0.52 mm film thickness; Hewlett-Packard, USA) and FID

(flame ionization detector) Hewlett-Packard GC (model 6890). Injection volume was 1µl. GC furnace temperature was set to 300°C, injector temperature to 280°C, colon temperature to 270°C for 15 minutes. Carrier gas was Helium and the flow rate was 1.5 mL/min.

Microbiological analyses

For counting *Lactobacillus* ssp., De Mann Rogosa Sharpe (MRS) Agara (Merck Darmstadt, Germany) (SHARPE *et al.*, 1966) was used. *Lactococcus* ssp. was cultured on M_{17} Agara (Merck Darmstadt, Germany) (TERZAGHI and SANDINE, 1975). For yeast, Glucose-Salt Agara (ANONYMOUS, 1990) plantation was done. Isolation and census of lactic acid bacteria were conducted according to IDF Standard 149 A (1997) and IDF Standard 163 (1992). Yeasts were incubated at 25°C for three to five days. After this period, colonies that had developed in petri dishes were counted as cfu/mL on days one, three and five of storage.

Sensory evaluation

The sensory evaluation was made by a panel of nine individuals who evaluated kefir samples in terms of consistency and flavor on a scale from 1 to 5. For ALTUĞ and ELMACI (2011), the method of Flavor Profile Analysis was utilized.

Statistics

Two different milk and two different kefir samples were analyzed in three parallels and two repetitions. SPSS statistics analysis software (IBM SPSS Statistics) was used. Data that gained importance were analyzed using the variance analysis ANOVA based on the Duncan multiple comparison test on a $p<0.01$ level.

RESULTS AND CONCLUSIONS

In the CaM sample, compared to the CoM sample, fat and lactose values were found to be higher; there was twice as much ash and similar pH and dry matter values. In addition, protein, titration acidity in terms of lactic acid (Table 1) and viscosity values were detected to be lower.

In the research, it was determined that results regarding the pH value 6.46 pH, fat (3.60%), protein (3.05%), lactose (6.22%), total dry matter (12.73%), ash (2.932%) and titratable acidity in terms of lactic acid (0.12%) of camel milk were within the boundaries found in the literature, and that the lactose and ash levels were higher in our studies (FAO, 1982; EL-AMIN and WILCOX, 1992). PH values of kefir samples were determined to correspond with the Turkish Food Codex (2009/25) and WSZOLEK *et al.* (2006). Lactic acid was detected to be higher in the CaMK sample (0.92%LA) than in the CoMK sample (0.81%LA), whereas for viscosity, it was vice versa. With the addition of 1% (v/w) glucose into the CaM sample, simulating the development of lactic acid bacteria, we aimed to increase the titration acidity and viscosity. To this end, the effect that the addition of 1% glucose (v/w) and 10% grain into the CaM sample has on titration acidity and viscosity was found to be significant ($p<0.01$), and lactic acid and viscosity were detected to have increased. However, viscosity in the CaMK sample was lower than the CoMK sample. In addition, the glucose and grain ratio added to camel milk was determined as a result of preliminary study. In that preliminary study, some portion of the CaM sample was inoculated with yeast grains in ratios of 2.5%, 5%, 7.5% and 10% without the addition of glucose, while the other portion of the sample had the same process with the addition of glucose. Afterwards, viscosity values and titration acidity were detected in kefir samples, and viscosity values are given in Tables 2 and 3. Grain being added into camel milk by 2.5% did not have a major effect, and since the 2.5% grain addition into the CoMK sample provided the desired viscosity value, other grain ratios were not tested. All in all, with the 1% glucose (v/w) and 10% grain addition into camel milk, a four times greater increase was reached in viscosity than in the one with just the grain addition, and the titration acidity diminished from

Table 1 - Physical-chemical properties of raw camel milk, raw cow milk and of kefirs produced from these milks.

Analyses	Milk samples		Kefir samples	
	CaM	CoM	CaMK	CoMK
Dry matter	12.73±0.12^{A}	12.80±0.09^{B}	11.10±0.02^{C}	10.70±0.03^{D}
Fat (%)	3.60±0.08^{A}	3.50±0.06^{B}	3.20±0.02^{C}	3.30±0.01^{D}
Titratable Acidity	0.127±0.02^{A}	0.132±0.02^{B}	0.92±0.01^{C}	0.81±0.02^{D}
pH	6.46±0.32^{A}	6.44±0.27^{B}	4.10±0.10^{C}	4.55±0.14^{D}
Protein (%)	3.05±0.03^{A}	3.21±0.03^{B}	2.82±0.03^{C}	3.09±0.03^{D}
Ash (%)	2.932±0.10^{A}	1.461±0.09^{B}	1.423±0.05^{C}	1.068±0.05^{D}
Lactose (%)	6.22±0.05^{A}	6.20±0.51^{B}	3.45±0.07^{C}	3.54±0.02^{D}

$^{A, B, C, D}$: The differences between the values in the same rows are statistically significant ($p < 0.01$).

Table 2 - Viscosity values of the kefir samples produced by injecting grain in certain amounts in CaM with/without glucose addition and the ones produced from CoM without glucose addition (cP).

Grain ratio (%)	CaMK (Gra) viscosity (cP)	CaMK (Gra+G) viscosity (cP)	CoMK (Gra) viscosity (cP)
2.5	5.21^{aA}	5.87^{aB}	111.475^{C}
5	5.46^{bA}	8.44^{bB}	NT
7.5	7.12^{cA}	18.81^{cB}	NT
10	9.28^{dA}	37.18^{dB}	NT

NT:Not-tested ; Gra: Grain ;G+Gra: Glucose +Grain

a, b, c, d, e: The differences between the values in the same column are statistically significant ($p < 0.01$).

A, B, C, D: The differences between the values in the same rows are statistically significant ($p < 0.01$).

Table 3 - Fatty acid compositions of kefir samples made from Camel/Cow milk (g/100g)

Name of Fatty Acid Methyl Ester and Formula of Molecule	(g/100g)			
	CoM	CoMK	CaM	CaMK
Butyric Acid Methyl Ester (C4:0)	0.064	0.043	ND	ND
Caproic Acid Methyl Ester (C6:0)	0.264	0.422	0.140	0.121
Caprylic Acid Methyl Ester (C8:0)	0.037	0.024	0.003	0.002
Capric Acid Methyl Ester (C10:0)	0.085	0.061	0.004	0.003
Undecanoic Acid Methyl Ester (C11:0)	0.007	0.009	ND	ND
Lauric Acid Methyl Ester (C12:0)	0.127	0.101	0.021	0.021
Tridecanoic Acid Methyl Ester (C13:0)	0.002	0.017	0.005	0.004
Myristic Acid Methyl Ester (C14:0)	0.386	0.303	0.321	0.309
Myristoleic Acid Methyl Ester (C14:1)	0.051	0.034	0.050	0.050
Pentadecanoic Acid Methyl Ester (C15:0)	0.021	0.013	0.029	0.029
cis-10- Pentadecanoic Acid Methyl Ester (C15:1)	0.012	0.036	0.013	0.010
Palmitic Acid Methyl Ester (C16:0)	0.901	0.740	1.049	0.967
Palmitoleic Acid Methyl Ester (C16:1)	0.063	0.027	0.292	0.268
Heptadecanoic (Margaric) Acid Methyl Ester (C17:0)	0.015	0.011	0.020	0.018
cis-10-Heptadecanoic Acid Methyl Ester (C17:1)	0.016	0.074	0.024	0.019
Stearic Acid Methyl Ester (C18:0)	0.430	0.361	0.507	0.457
Oleic Acid Methyl Ester (C18:1n9c)	0.840	0.581	0.843	0.715
Linoleic Acid Methyl Ester (C18:2 n6c)	0.088	0.072	0.107	0.099
γ-Linolenic Acid Methyl Ester (C18:3 n6)	0.034	0.177	0.053	0.036
Arachidic Acid Methyl Ester (C20:0)	0.028	0.017	0.028	0.023
cis-11- Eicosenoic Acid Methyl Ester (C20:1)	ND	ND	0.013	0.010
cis-11,14-Eicosadienoic Acid Methyl Ester (C20:2)	ND	ND	0.009	0.005
Arachidonic Acid Methyl Ester (C20:4n6)	0.012	0.067	0.028	0.017
Behenic Acid Methyl Ester (C22:0)	0.016	0.094	0.023	0.009
Other fatty acids	ND	0.017	0.017	0.007
Short-chain fatty acids (4-6C)	0.33	0.46	0.14	0.12
Medium-chain fatty acids (8-12C)	0.26	0.20	0.03	0.03
Long-chain fatty acids (>12C)	2.92	2.62	3.41	3.05
Saturated fatty acids (SFA)	2.38	2.22	2.15	1.96
Monounsaturated fatty acids (MUFA)	0.98	0.75	1.24	1.07
Polyunsaturated fatty acids (PUFA)	0.13	0.32	0.20	0.16

ND: Non-detected.

4.78 pH (in the sample with grain addition only) to 4.10 pH. Results correspond with the literature (LEWIS, 1986).

In this study, problems that might occur in fermentation were associated with low viscosity value obtained from the CaMK sample, lower serum protein content of camel milk than cow milk (FARAH, 1996), poor interaction between denature serum proteins of camel milk and κ-casein, lack of β-lactoglobulin from serum proteins and different derivatives of β-casein, low amounts of casein and its derivatives (LALEYE *et al.*, 2008) and the anti-bacterial effect of camel milk (HASHIM and KHALIL, 2004). Many factors (content of protein and denature serum proteins, casein ratio and its content, interactions between denature serum proteins and k-casein) may affect viscosity (PUVANENTHIRAN *et al.*, 2002). Based on the pH change, these factors might also affect the micelle surface area and size, micelle content and water binding capability in casein micelles (ANEMA and KLOSTMEYER, 1996; DALGLEISH and

LAW, 1988, 1989). The drop in pH causes the interaction between serum proteins and case micelles and the viscosity to increase (ANEMA *et al.*, 2004). However, in the current study it was determined that based on the drop in pH (4.10 pH) in the CaMK sample, the viscosity value was lower than in the CoMK samples. This is thought to result from the effect of one or more of the parameters given above (ANEMA and LI, 2003) Also, the camel milk is low in casein and serum proteins and the composition of these. It was found that the milk type in kefir samples has an important effect on titration acidity, pH and viscosity; the effect of titration acidity on viscosity is vital as well ($p<0.01$). Protein, lactose and fat in the CaMK sample were found to be higher than in the CoMK sample, whereas dry matter and ash were found to be lower. In general, effect of the milk type on dry matter, fat, protein and lactose was established as significant, as well as the effect of glucose addition into milk and grain ratio on titration acidity ($p<0.01$).

CaM and CoM, and fatty acid compositions of kefirs produced from these milks are given in Table 3. In CaM and CaMK samples, it was determined that the short (C4:0-C6:0) and medium (C8:0-C12:0) chain fatty acids ratio, as well as the saturated fatty acids (SFA) ratio were lower than in CoM and CoMK samples, but the long chain fatty acids ratio and the monounsaturated fatty acids (MUFA) ratio were higher. It was also established that the ratio of polyunsaturated fatty acids (PUFA) in the CaM sample was higher than the CoM sample; however, its ratio in the CaMK sample (0.16 g/100 g) was lower than in the CoMK sample (0.32 g/100 g). Thus, the conclusion: Camel milk and its kefir contain some fatty acids that affect our health positively in terms of fatty acid composition in higher amounts than cow milk and its kefir.

The camel milk fatty acid composition changes pursuant to the species and the diet of that specific camel, as well as its region and the climate of that region (CHILLIARD *et al.*, 2000; KONUSPAYEVA *et al.*, 2008). The results we obtained from this research were similar to the results of other researchers (AGRAWAL *et al.*, 2004; SHAMSIA, 2009).

Table 4 - Cholesterol levels of kefirs samples made from Camel/Cow milk (mg/100g).

Cholesterol levels (mg/100g)	
CoM	**CoMK**
14.60^{aA}	7.97^{aB}
CaM	**CaMK**
21.28^{bA}	18.24^{bB}

[a, b, c, d, e]: The differences between the values in the same column are statistically significant ($p < 0.01$).
[A, B, C, D]: The differences between the values in the same rows are statistically significant ($p < 0.01$).

In the CaM, CoM, CaMK and CoMK samples, cholesterol levels were different and an important relationship between the milk type and the cholesterol level was detected ($p<0.01$) (Table 4). Along with this, it was determined in the research that the cholesterol level decreased after production of kefir by using different milks, and the effect that kefir production has on the drop in cholesterol levels was regarded as significant ($p<0.01$). According to some researchers (GORBAN and IZZELDIN, 1999; GOUDJIL *et al.*, 2003; KONUSPAYEVA *et al.*, 2008; SIEBER, 2005), cholesterol level of kefir production was higher than cow milk, but it was also found to be lower according to some other researchers (ALABDULKARIM, 2012; AGRAWAL *et al.*, 2004).

Initially, in the grain, *Lactobacillus ssp.* strains were found to be as 1.93×10^7 cfu/mL, *Lactococcus ssp.* strains as 5.54×10^7 cfu/mL and yeast as 1.68×10^6 cfu/mL. In the study, *Lactobacillus ssp.* strains (Fig. 2a) and yeast levels (Fig. 2b) increased in both kefir samples throughout the storage process. In addition, the increase in the *Lactobacillus ssp.* strains in the CaMK sample was found to be higher. *Lactococcus ssp.* strains (Fig. 2c) were detected to have increased in kefir samples at day one and three of storage, but to have decreased after day three. Levels of *Lactobacillus ssp.* strains in kefir samples were close to one another at the inception of storage, but the one in the CaMK sample took the lead after day one of storage. *Lactobacillus ssp.* strains in the CaMK sample at days one, three and five of storage increased, in comparison with the starting level, respectively at levels of 0.99 cfu/mL, 1.71 cfu/ml and 2.59 cfu/mL. However, in the CoMK sample, it was respectively: 0.91 cfu/mL, 1.28 cfu/mL and 2.18 cfu/mL. *Lactococcus ssp.* strains in the CoMK sample at days one and three of storage increased, in comparison with the starting level, respectively at levels of 0.05 cfu/mL and 1.02 cfu/mL. However, this lessened in day five by 0.41 cfu/mL compared to day three of storage. Development of *Lactococcus ssp.* strains in the CaMK sample was the same as the CoMK sample at day one; however, its increase after day one of storage was lower than the one in the CoMK sample. In the CaMK sample, an increase respectively at levels 0.04 cfu/mL and 0.8 cfu/mL was detected in day one and three of storage in comparison with the starting level, whereas after day five, a decrease took place. This decrease was ten times more than the CoMK sample. Yeast level increased in both kefir samples throughout the storage process, but the one in the CoMK sample was approximately three times higher than the CaMK's. Generally, it was concluded that microorganism levels in CaMK and CoMK samples in storage were above the minimum values set forth by "Turkish Food Codex, Communiqué on Fermented Milks" (Turkish Food Codex, Communiqué no: 2009/25).

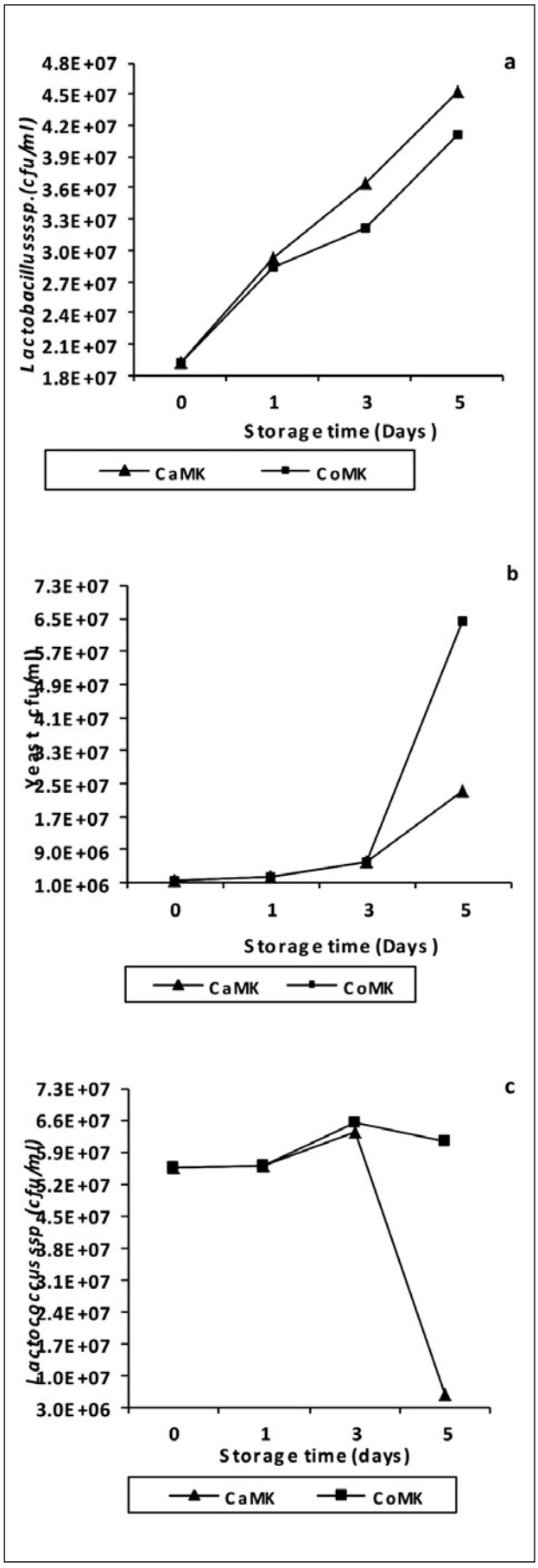

Fig. 2 - *Lactobacillus ssp.* (a), yeast (b) and *Lactococcus ssp.* (c) levels in kefirs produced from camel and cow milks.

Research helped discover an important relationship between the milk type and the storage period on microorganism levels. In addition, the effect that glucose has on microorganism development was established as significant ($p<0.01$). In the CaMK sample produced with the addition of 1% glucose, increase in the *Lactobacillus ssp.* strains was higher than in the CoMK sample, but this was vice versa for the increase in the yeast level. Progress of the increase (*Lactobacillus ssp.*, yeast), also the decrease (*Lactococcus ssp.*) in the microorganism levels in the CaMK sample after day three of storage show parallels with the CoMK sample (KOROLEVA *et al.*, 1978; KOROLEVA and BAVINA, 1978; ONER *et al.*, 2010). In the research, microbial flora in the CaMK sample went through a different development. This result corresponds with the literature data regarding other fermented dairy products of camel milk (ABU-TARBOUSH, 1996; ATTIA *et al.*, 2001; JUMAH *et al.*, 2001; ABDEL RAHMAN *et al.*, 2009). In addition, the research pinpointed that the usage of grain in producing kefir from camel milk was more effective (ABU-TARBOUSH, 1996; ABDEL RAHMAN *et al.*, 2009; MEHAIA, 1993).

In the flavor profile evaluation of kefir samples, panelists determined that sour, sweet, salty, bitter, fermented milk, cream, greasy, cheesy, sharp, gas, alcohol, metallic and burnt milk flavor densities were perceived differently between the CaMK and CoMK samples (Fig. 3). In the flavor profile evaluation, it was detected that the CaMK sample was sourer, cheesier and had a sharper aroma than the CoMK sample. Consistency and appearance in the CaMK sample were detected to be lower than the CoMK sample. In general, the CaMK sample was appreciated by the panelists and was defined as "sourer" than the CoMK sample.

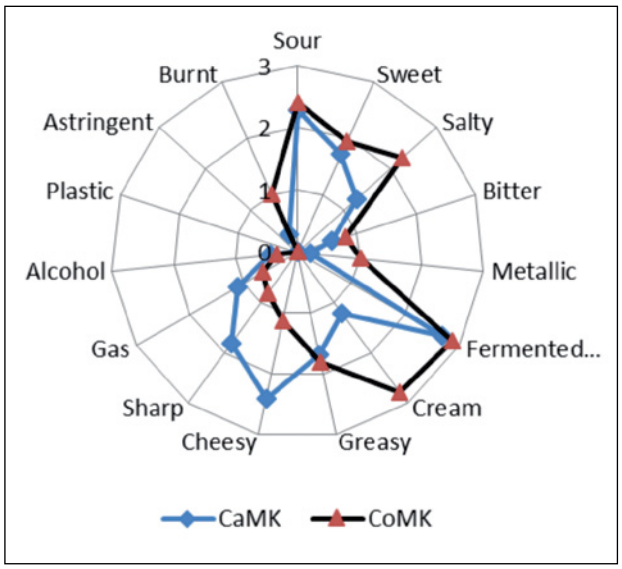

Fig. 3 - Flavor profile evaluation of the CaMK and CoMK samples.

CONCLUSIONS

In the current research, an increase was obtained in the viscosity value in the kefir produced by adding 1% (v/w) glucose and 10% grain into camel milk. Dry matter, ash and titratable acidity in the camel milk kefirs were higher than in cow milk kefirs; whereas fat, pH, protein and lactose values were lower. Cholesterol level of camel milk and its kefir product was found to be higher than that of cow milk and its kefir. Along with this, it was detected in this study that proportion of camel milk and its kefir to cow milk and its kefir in terms of SFA is low. However, it is high in terms of MUFA. The PUFA ratio of camel milk is high compared to cow milk. However, the PUFA ratio in the camel milk kefir was defined to be lower than the one in the cow milk kefir. Lastly, it was also confirmed in the study that some compounds, which have positive effects upon metabolism in camel milk and its kefir, have a higher impact than on the cow milk kefir's metabolism.

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PAPER

CHARACTERISTICS OF GELATIN FROM SWIM BLADDER OF YELLOWFIN TUNA (*THUNNUS ALBACORES*) AS INFLUENCED BY EXTRACTING TEMPERATURES

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ABSTRACT

Gelatin was extracted from the swim bladder of yellowfin tuna (*Thunnus albacores*) at different temperatures (60, 70 and 80°C) with the extraction yields of 35.6%, 41.1% and 47.3% (dry weight basis), respectively. The α-chains of gelatin decreased with increasing extraction temperatures. Similar amino acid compositions were noticeable among all gelatins, in which glycine constituted the major amino acid. Imino acids ranged from 169 to 172 residues/1,000 residues. The gel strength of gelatin extracted at lower temperature was higher than that of gelatins extracted at higher temperatures. Gelling and melting temperatures for swim bladder gelatin were 11.07-15.24 and 20.36-22.33°C, respectively. Higher gelling and melting points were observed for gelatin extracted at lower temperatures. Microstructure of gel of gelatin extracted at 60°C was finer with smaller voids, compared with others. FTIR spectra of obtained gelatins revealed the significant loss of molecular order of the triple-helix. Thus, extraction temperatures showed the direct impact on characteristics of gelatin from swim bladder.

- Keywords: gelatin, gel strength, extraction, temperature, swim bladder, yellowfin tuna -

INTRODUCTION

Gelatin is a fibrous protein obtained by partial denaturation or hydrolysis of collagen. Gelatin represents biopolymer with many applications in food, materials (for edible and biodegradable packaging), cosmetic, pharmaceutical and photographic industries (JELLOULI *et al.*, 2011). The source, type of collagen and processing conditions have the influence on the properties of the resulting gelatin (KITTIPHATTANABAWON *et al.*, 2010). Different types of gelatins have varying thermal and rheological properties such as gel strength, melting and gelling temperatures (BENJAKUL *et al.*, 2012). These properties are governed by several factors such as chain length or molecular weight distribution, amino acid composition and hydrophobicity, etc. (GÓMEZ-GUILLÉN *et al.*, 2002; NORZIAH *et al.*, 2009).

Commercial gelatins are produced mainly from porcine and bovine skins and bones by alkaline or acidic extraction (BENJAKUL *et al.*, 2009). However, both Judaism and Islam forbid the consumption of any pork-related products, while Hindus do not consume cow-related products. Additionally, bovine gelatin has a high risk for bovine spongiform encephalopathy (NAGARAJAN *et al.*, 2012). Furthermore, the need to exploit the fish processing byproducts has led to the production of gelatin as an alternative to mammalian counterpart (GÓMEZ-GUILLÉN *et al.*, 2011). Fish gelatin has been extracted mainly from fish skin such as seabass (SINTHUSAMRAN *et al.*, 2014), cobia (SILVA *et al.*, 2014) skipjack tuna, dog shark and rohu (SHYNI *et al.*, 2014) and unicorn leatherjacket (KAEWRUANG *et al.*, 2013), etc.

Among fish processing industries, canned tuna industry is economically important. Tuna including yellowfin, skipjack and tongol have been the important species for canning in Thailand with a large volume of raw materials used. Approximately two-thirds of the whole fish are utilized and the remainings involving the viscera, head, bone and swim bladder become the byproducts (KLOMKLAO *et al.*, 2004). Fish swim bladders can be used for production of "isinglass" (WEBER *et al.*, 2009). Recently, KAEWDANG *et al.*, (2014) reported that alkaline pretreatment was essential for gelatin extraction from yellowfin tuna swim bladder. However, no information on the effect of extracting temperature on characteristics and properties of gelatin has been reported. Therefore, the objectives of this investigation were to extract and characterize gelatin from the swim bladder of yellowfin tuna using different extraction temperatures.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals were of analytical grade. Sodium dodecyl sulphate (SDS), Coomassie blue R-250 and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were procured from Bio-Rad Laboratories (Hercules, CA, USA). High-molecular-weight markers were purchased from GE Healthcare UK Limited (Buckinghamshire, UK). Food grade bovine bone gelatin with the bloom strength of 150-250 g was obtained from Halagel (Thailand) Co., Ltd., (Bangkok, Thailand).

2.2. Collection and preparation of swim bladder

Swim bladders of yellowfin tuna (*Thunnus albacares*) were obtained from Tropical Canning Public Co., Ltd., Songkhla, Thailand. Swim bladders with the length of 8-12 cm were placed in polyethylene bags, inserted in ice using a sample/ice ratio of 1:2 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Songkhla. Upon arrival, swim bladders were washed with distilled water and cut into pieces with the length of approximately 2 cm. The prepared samples were then placed in polyethylene bag and frozen at -20°C. The samples were stored at -20°C until used. The storage time was not longer than 3 months. Prior to extraction, frozen swim bladders were thawed using running water until the temperature was 0-2°C

2.3. Extraction of gelatin from swim bladder

Prior to gelatin extraction, swim bladders were pretreated with alkaline solution as per the method of KAEWDANG *et al.* (2014). Prepared swim bladders were added with the mixed alkaline solution (Na_2CO_3:NaOH; 7:3) having the concentration of 4% (w/v) at a ratio of 1:10 (w/v). The mixture was stirred for 12 h at room temperature (28-30°C) using an overhead stirrer model W20.n (IKA®-Werke GmbH & CO.KG, Stanfen, Germany). The alkaline solution was changed every 6 h. The residues were washed with tap water until a neutral or faintly basic pH was obtained.

To extract gelatin, alkali pretreated samples were soaked in distilled water with different temperatures (60, 70 and 80°C) using a swim bladder/water ratio of 1:5 (w/v) in a temperature-controlled water bath (W350, Memmert, Schwabach, Germany) for 24 h with a continuous stirring at a speed of 150 rpm. The mixtures were then filtered using a Buchner funnel with a Whatman No. 4 filter paper (Whatman International, Ltd., Maidstone, England). The filtrates were freeze-dried using a freeze-dryer (CoolSafe 55, ScanLaf A/S, Lynge, Denmark). The dry gelatin extracted from swim bladder from yellowfin tuna at 60, 70 and 80 °C was referred to as 'G60', 'G70' and 'G80', respectively. All gelatin samples were weighed, calculated for extraction yield and subjected to analyses.

2.4. Analyses

2.4.1. Yield

Gelatin yield was calculated by the following equation.

$$\text{Yield (\%)} = \frac{\text{Weight of dry gelatin (g)} \times 100}{\text{Weight of initial dry swim bladder (g)}}$$

where the weight of dry swim bladder was calculated by subtracting moisture content determined by AOAC (2000) from the initial wet weight.

2.4.2. SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS–PAGE was performed by the method of LAEMMLI (1970). Samples were dissolved in 5% SDS solution. The mixtures were then heated at 85°C for 1 h using a temperature controlled water bath model W350 (Memmert, Schwabach, Germany). The mixtures were centrifuged at 8,500 g for 5 min using a microcentrifuge (MIKRO20, Hettich Zentrifugan, 170 Germany) to remove undissolved debris. Solubilized samples were mixed at 1:1 (v/v) ratio with the sample buffer (0.5 M Tris–HCl, pH 6.8, containing 5% SDS and 20% glycerol). Samples were loaded onto a polyacrylamide gel made of 7.5% separating gel and 4% stacking gel and subjected to electrophoresis at a constant current of 20 mA/gel. After electrophoresis, the gels were stained with 0.05% (w/v) Coomassie Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid for 30 min. Finally, they were destained with a mixture of 50% (v/v) methanol and 7.5% (v/v) acetic acid for 30 min and destained again with a mixture of 5% (v/v) methanol and 7.5% (v/v) acetic acid for 1 h. High-molecular-weight protein markers were used to estimate the molecular weight of proteins.

2.4.3. Amino acid analysis

Amino acid composition of gelatin samples was analyzed according to the method of NAGARAJAN *et al.* (2012) with a slight modification. The samples were hydrolyzed under reduced pressure in 4 M methanesulphonic acid containing 0.2% (v/v) 3-2(2-aminoethyl) indole at 115 °C for 24 h. The hydrolysates were neutralized with 3.5 M NaOH and diluted with 0.2 M citrate buffer (pH 2.2). An aliquot of 0.04 ml was applied to an amino acid analyzer (MLC-703; Atto Co., Tokyo, Japan).

2.4.4. Fourier transform infrared (FTIR) spectroscopic analysis

FTIR spectra of the gelatin samples were obtained using a FTIR spectrometer (EQUINOX 55, Bruker, Ettlingen, Germany) equipped with a deuterated *L*-alanine tri-glycine sulphate (DLATGS) detector. A horizontal attenuated total reflectance accessory (HATR) was mounted into the sample compartment. The internal reflection crystal (Pike Technologies, Madison, WI, USA), made of zinc selenide, had a 45° angle of incidence to the IR beam. Spectra were acquired at a resolution of 4 cm^{-1} and the measurement range was between 400 and 4,000 cm^{-1} (mid-IR region) at room temperature. Automatic signals were collected in 32 scans at a resolution of 4 cm^{-1} and were ratioed against a background spectrum recorded from the clean empty cell at 25°C. Analysis of spectral data was carried out using the OPUS 3.0 data collection software programme (Bruker, Ettlingen, Germany).

2.4.5. Determination of gel strength

Gelatin gel was prepared by the method of KITTIPHATTANABAWON *et al.* (2010). Gelatin was dissolved in distilled water (60 °C) to obtain a final concentration of 6.67% (w/v). The solution was stirred until the gelatin was completely solubilized and then transferred to a cylindrical mold with 3 cm diameter and 2.5 cm height. The solution was incubated at the refrigerated temperature (4°C) for 18 h prior to analysis.

The gel strength was determined at 8-10°C using a texture analyzer (Stable Micro System, Surrey, UK) with a load cell of 5 kg and crosshead speed of 1 mm/s. A 1.27 cm diameter flat-faced cylindrical Teflon® plunger was used. The maximum force (grams), taken when the plunger had penetrated 4 mm into the gelatin gels, was recorded.

2.4.6. Determination of gelling and melting temperatures

Gelling and melting temperatures of gelatin samples were measured following the method of BORAN *et al.* (2010) using a controlled stress rheometer (RheoStress RS 75, HAAKE, Karlsruhe, Germany). The gelatin solution (6.67%, w/v) was prepared in the same manner as described previously. The solution was preheated at 35°C for 30 min. The measuring geometry included a 3.5 cm parallel plate and the gap was set at 1.0 mm. The measurement was performed at a scan rate of 0.5°C/min, frequency of 1 Hz, oscillating applied stress of 3 Pa during cooling from 35 to 5°C and heating from 5 to 35°C. The gelling and melting temperatures were calculated, where tan δ became 1 or δ was 45°.

2.4.7. Microstructure analysis of gelatin gel

The microstructure of gelatin gel was visualized using a scanning electron microscopy (SEM). Gelatin gels having a thickness of 2-3 mm were fixed with 2.5% (v/v) glutaraldehyde in 0.2

M phosphate buffer (pH 7.2) for 12 h. The samples were then rinsed with distilled water for 1 h and dehydrated in ethanol with a serial concentration of 25%, 50%, 70%, 80%, 90% and 100% (v/v). Dried samples were mounted on a bronze stub and sputter-coated with gold (Sputter coater SPI-Module, West Chester, PA, USA). The specimens were observed with a scanning electron microscope (JEOL JSM-5800 LV, Tokyo, Japan) at an acceleration voltage of 20 kV.

2.4.8. Determination of color of gelatin gel

The color of gelatin gels (6.67% w/v) was measured with a Hunter lab colorimeter (Color Flex, Hunter Lab Inc., Reston, VA, USA). *L**, *a** and *b** values indicating lightness/brightness, redness/greenness and yellowness/blueness, respectively, were recorded. The colorimeter was warmed for 10 min and calibrated with a white standard. The total difference in color (ΔE^*) was calculated according to the following equation. (GENNADIOS *et al.*, 1996):

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where ΔL^*, Δa^* and Δb^* are the differences between the corresponding color parameter of the sample and that of the white standard ($L^* = 93.6$, $a^* = -0.94$ and $b^* = 0.40$).

2.5. Statistical analysis

All experiments were run in triplicate, using three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out using a Duncan's multiple range test (STEEL and TORRIE, 1980). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Extraction yield

Yield of gelatin from the swim bladder of yellowfin tuna extracted at various temperatures was different. Increasing yield was obtained when the extraction temperatures increased ($P < 0.05$). Yield of 35.6%, 41.1% and 47.3% (on dry weight basis) was found for G60, G70, and G80, respectively. This result was in agreement with KAEWRUANG *et al.* (2013), DUAN *et al.* (2011) and KITTIPHATTANABAWON *et al.* (2010) who reported the increasing yield of gelatin as the extraction temperature increased with higher temperatures, the bondings stabilizing α-chains in the native mother collagen were destroyed to a higher extant. As a consequence, the triple helix structure became amorphous and could be extracted into the medium with ease, leading to the higher yield (SINTHUSAMRAN *et al.*, 2014). In addition, the higher energy applied could induce thermal hydrolysis of peptide chains, resulting in the formation of shorter peptides. As a result, those small peptides could be easily extracted into water. The yield and characteristics of gelatin are associated with the type of raw material and gelatin extraction process, including the pretreatment process and extraction temperatures. (NAGARAJAN *et al.*, 2012; KITTIPHATTANABAWON *et al.*, 2010; MONTERO and GÓMEZ-GUILLÉN, 2000).

3.2. Protein patterns

Protein patterns of gelatin from the swim bladder of yellowfin tuna extracted at different temperatures are shown in Fig. 1. The band intensity of α_1-chain and α_2-chain decreased with increasing extraction temperature. The decreases in α_1-chain band intensity were observed in G70 and G80, in comparison with that found in G60. Among all gelatin samples, G80 possessed the lowest α-chain band intensity. This might be caused by the degradation induced by the thermal process. Therefore, the extraction temperatures played a major role in protein components of resulting gelatin. KITTIPHATTANABAWON *et al.* (2010) reported that the gelatins extracted from the skins of brownbanded bamboo shark and blacktip shark with higher extraction temperature contained more peptides with the MW less than α-chain and the lower proportion of high MW (greater than β-chain) fractions, compared with those obtained from lower temperature extraction. Gelatins from splendid squid skin with higher extraction temperatures contained a lower band intensity of the α-chains than those obtained with lower extraction temperature (NAGA-

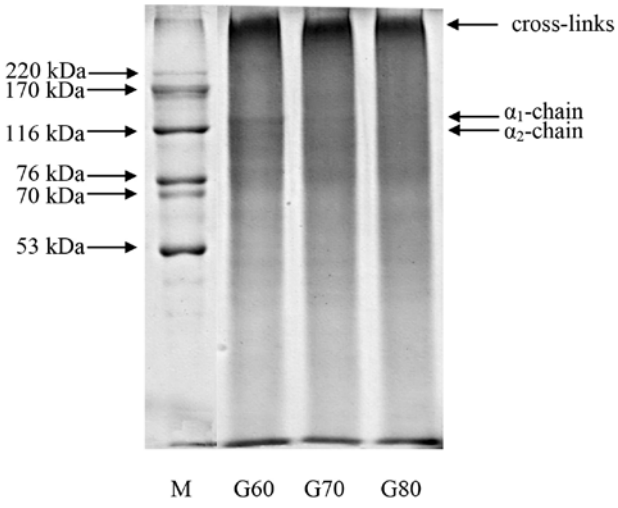

Fig. 1 - Protein patterns of gelatins from the swim bladder of yellowfin tuna extracted at different temperatures. M: high molecular weight markers. G60, G70 and G80 represent gelatin extracted from swim bladder at 60, 70 and 80°C, respectively.

RAJAN *et al.*, 2012). On the other hand, gelatin from skin of unicorn leatherjacket extracted at higher temperature (65-75°C) had α-chain retained at higher level than that extracted at lower temperature (KAEWRUANG *et al.*, 2013). This was due to the thermal inactivation of indigenous proteases in the skin of unicorn leatherjacket at high temperature. Generally, gelatins with a higher content of α-chains showed better functional properties including gelling, emulsifying and foaming properties (GÓMEZ-GUILLÉN *et al.*, 2002). In general, the formation of peptide fragments is associated with lower viscosity, low melting point, low setting point, high setting time, as well as decreased bloom strength of gelatin (MUYONGA *et al.*, 2004a). The results suggested that G70 and G80, which were extracted at higher temperatures, had the shorter chains as indicated by lower content of α-chain.

3.3. Amino acid composition

Amino acid compositions of gelatins from the swim bladder of yellowfin tuna extracted at different temperatures are shown in Table 1. Glycine was the predominant amino acid in all gelatin samples, ranging from 305 to 314 residues/1000 residues. This implied that gelatin obtained was derived from its mother collagen. Collagen consists of one-third glycine in its molecule (BALTI *et al.*, 2011). It was noted that G80 had the higher glycine content than G60 and G70. The higher glycine in G80 might be caused by free glycine, which was released to a high extent during extraction at high temperature. Alanine (121-122 residues/1000 residues) was found at high content. Alanine plays a role in viscoelastic property of gelatin (GIMÉNEZ *et al.*, 2005). Low contents of cysteine (1 residues/1000 residues), tyrosine (5-6 residues/1000 residues), histidine (7-8 residues/1000 residues) and hydroxylysine (10 residues/1000 residues) were observed in all gelatin samples. For imino acids, all gelatins contained proline and hydroxyproline contents of 95–99 and 72–74 residues/1000 residues, respectively. REGENSTEIN and ZHOU (2007) reported that glycine, alanine, proline and hydroxyproline are four of the most abundant amino acids in gelatin. The properties of gelatin are largely influenced by the amino acid composition and their molecular weight distribution (GÓMEZ-GUILLÉN *et al.*, 2009). When comparing the content of imino acids (proline and hydroxyproline), gelatin from swim bladder had the lower imino acid content than those from seabass skin (198-202 residues/1000 residues) (SINTHUSAMRAN *et al.*, 2014) and from carp skin (188-190 residues/1000 residues) (DUAN *et al.*, 2011). The imino acid content of fish collagens and gelatins correlates with the water temperature of their normal habitat (FOEGEDING *et al.*, 1996; REGENSTEIN and ZHOU, 2007). It has been known that imino acid content, especially hydroxyproline content, affected functional properties of gelatin, especially gelling property (AEWSIRI *et al.*, 2008; BENJAKUL *et al.*, 2009). Therefore, amino acid composition of gelatin from swim bladder was governed by extraction temperature.

Table 1 - Amino acid compositions of gelatins from the swim bladder of yellowfin tuna extracted at different temperatures.

Amino acids	Number of residues/1000 residues		
	G60	G70	G80
Alanine	121	121	122
Arginine	52	52	53
Aspartic acid/asparagine	49	48	46
Cysteine	1	1	1
Glutamic acid /glutamine	80	80	78
Glycine	307	305	314
Histidine	7	8	7
Isoleucine	14	14	13
Leucine	29	30	28
Lysine	26	26	26
Hydroxylysine	10	10	10
Methionine	17	16	16
Phenylalanine	16	16	16
Hydroxyproline	74	72	73
Proline	95	99	99
Serine	41	41	40
Threonine	30	30	30
Tyrosine	6	6	5
Total	1000	1000	1000
Imino acids	169	171	172

3.4. Fourier transform infrared (FTIR) spectroscopy

FTIR spectra of gelatins extracted using different temperatures are shown in Fig. 2. FTIR spectroscopy has been used as a well-established technique to monitor the functional groups and secondary structure of gelatin (KONG and YU,

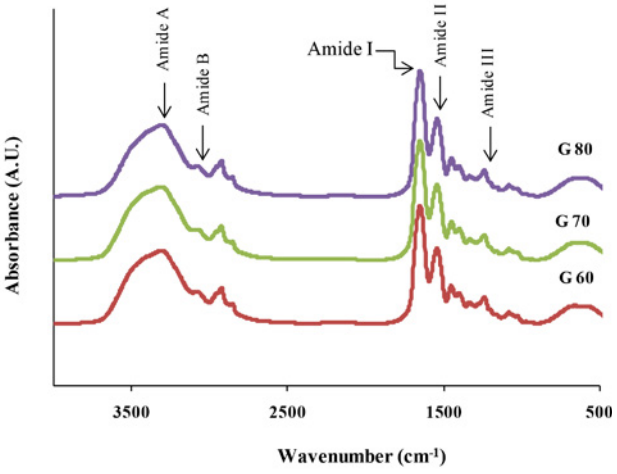

Fig. 2 - ATR-FTIR spectra of gelatins from the swim bladder of yellowfin tuna extracted at different temperatures (see Fig. 1 caption).

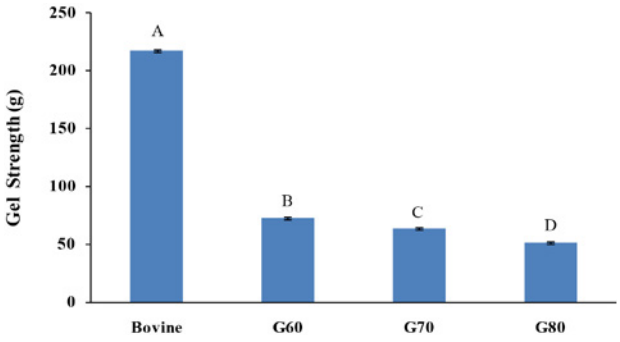

Fig. 3 - Gel strength of gelatin from the swim bladder of yellowfin tuna with different temperatures. Different uppercase letters on the bars denote significant differences (P< 0.05). Bars represent the standard deviations (n = 3). (see Fig. 1 caption).

2007). The absorption bands were situated in the amide region. The absorption in the amide-I region, owing to C=O stretching vibration, is probably the most useful for infrared spectroscopic analysis of the secondary structure of proteins (BENJAKUL *et al.*, 2009). It depends on the hydrogen bonding and the conformation of the protein structure (BENJAKUL *et al.*, 2009; URIARTE-MONTOYAETAL *et al.*, 2011). G60, G70 and G80 exhibited the amide-I bands at the wavenumbers of 1652.8, 1653.7 and 1652.9 cm^{-1}, respectively. The characteristic absorption bands of G60, G70 and G80 in amide-II region were noticeable at the wavenumbers of 1544.6, and 1545.5 and 1543.5 cm^{-1}, respectively. Amide-II arises from bending vibration of N-H groups and stretching vibrations of C-N groups. In addition, amide-III was detected at the wavenumbers of 1241.9, 1241.3 and 1240.8 cm^{-1} for G60, G70 and G80, respectively. The amide-III represents the combination peaks between C-N stretching vibrations and N-H deformation from amide linkages as well as absorptions arising from wagging vibrations from CH_2 groups from the glycine backbone and proline side-chains (JACKSON *et al.*, 1995). G80 had the lowest amplitude, whereas G60 exhibited the highest amplitude at amide-III region. This indicated that the greater disorder of molecular structure due to transformation of an α-helical to a random coil structure occurred at higher temperature. These changes were associated with loss of triple-helix state as a result of denaturation of collagen to gelatin (MUYONGA *et al.*, 2004b). The result reconfirmed the higher degradation of gelatin extracted at higher temperatures.

Amide-A band, arising from the stretching vibrations of the N-H group, appeared at 3338.3, 3339.1 and 3339.3 cm^{-1} for G60, G70 and G80, respectively. Amide-A represents NH-stretching coupled with hydrogen bonding. Normally, a free N-H stretching vibration is found in the range of 3400-3440 cm^{-1} (MUYONGA *et al.*, 2004b). When the N-H of a peptide is involved in a hydrogen bond, the position shifts to lower frequencies (DOYLE *et al.* 1975). In amide-A region, the lower wavenumber was found in G60, suggesting the hydrogen bonding involvement of N-H in α-chain. On the other hand, the lower wavenumber with the concomitantly higher amplitude of amide-A observed in G80 could be associated with the higher degradation of gelatin and higher free amino groups. The amide B was observed at 3082.1, 3080.9 and 3081.8 cm^{-1} for G60, G70 and G80, respectively. Amide B corresponds to asymmetric stretch vibration of =C-H as well as $-NH_3^+$. Thus, the secondary structure of gelatins obtained from the swim bladder of yellowfin tuna was affected to some degree by extraction temperature.

3.5. Gel strength

Gel strength of gelatin from the swim bladder of yellowfin tuna extracted at different temperatures is presented in Fig 3. G60, G70 and G80 had the gel strength of 72, 64 and 51 g, respectively. The difference in gel strength between the samples could be due to the differences in intrinsic characteristics, especially molecular weight distribution. Protein degradation resulted in the formation of peptides with shorter chain length, which might show the lower ability to from the junction zone or anneal each other. The longer chains in G60 could undergo aggregation to form gel network more effectively than G70 and G80. As a result, a stronger gel network could be formed as indicated by the higher gel strength. Bloom strength of commercial gelatins ranges from 100 to 300, but gelatins with bloom values of 250-260 are

Table 2 - Gelling and melting temperatures and gel color of gelatin from the swim bladder of yellowfin tuna extracted at different temperatures.

Samples	Melting point	Gelling point	Colour			
	(C°)	(C°)	*L**	*a**	*b**	Δ*E**
G60	22.33±0.42^{A}	15.24±0.27^{A}	27.98±0.57^{C}	-2.07±0.02^{C}	8.21±0.11^{C}	66.09±0.57^{A}
G70	22.05±0.45^{A}	14.86±0.24^{A}	42.79±0.47^{B}	-0.76±0.10^{B}	16.79±0.24^{B}	53.39±0.42^{B}
G80	20.36±0.27^{B}	11.07±0.58^{B}	45.79±0.78^{A}	-0.34±0.05^{A}	19.03±0.20^{A}	51.32±0.80^{C}

Mean ±SD (*n* = 3).
Different uppercase superscripts in the same column indicate significant differences (P < 0.05).

the most desirable (HOLZER, 1996). Different gel strength was reported for gelatin from skin of different species including splendid squid (85–132 g) (NAGARAJAN *et al.*, 2012), brownbanded bamboo shark and blacktip shark (206–214 g) (KITTIPHATTANABAWON *et al.*, 2010) and bigeye snapper (108 g) (BINSIA *et al.*, 2009).

3.6. Gelling and melting temperatures

The gelling temperatures of all the gelatin samples were in the range of 11.07-15.24°C (Table 2). Thermal transitions were monitored by changes in the phase angle (δ) of dissolved gelatins during cooling (35-5°C) and subsequent heating (5-35°C). It was found that G80 had the lowest gelling point (11.07°C) ($P < 0.05$), while no difference in gelling point were observed between G60 and G70 ($P > 0.05$). In general, fish gelatin is not able to form gel at room temperature (NORLAND, 1990). It has been known that imino acid content is an essential factor governing gelation of getatin (GILSENAN and ROSS-MURPHY, 2000). However, the similar amino acid content was observed among all samples (169-172 residues/1000 residues). The result indicated that the gelling temperature was affected by the extraction temperature, more likely related with varying chain length.

As a thermoreversible gel, gelatin gel starts melting when the temperature increases above a certain point, which is called the gel melting point (KARIM and BHAT, 2009). The melting temperatures of gelatin gel from swim bladder were in the range of 20.36-22.33°C. G80 had the lowest melting point (20.36°C) ($P < 0.05$). Nevertheless, G60 and G70 showed similar melting points ($P > 0.05$). Typical melting points for fish gelatins ranged from 11 to 28°C (KARIM and BHAT, 2009). GÓMEZ-GUILLÉN *et al.* (2002) reported that melting points of cod, hake, sole and megrim were 13.8, 14, 19.4 and 18.8°C, respectively. Melting points of red and black tilapia skin gelatins were 22.4 and 28.9°C, respectively (JAMILAH and HARVINDER, 2002). There was a relationship between melting point and molecular weight of gelatin. Low molecular weight gelatins melt at lower temperature than high molecular weight counterparts (GILSENAN and ROSS-MURPHY, 2000). The results suggested that lower melting point of G80 was attributed to the lower molecular weight of peptide chains. Temperature of the environment also affects the gelling and melting temperatures of gelatin (GUDMUNDSSON, 2002). Poorer gel strength of G80 (Fig. 3) was in accordance with lower gelling and melting points.

3.7. Microstructures of gelatin gels

The microstructures of gelatin gels from swim bladder with different extraction temperatures are illustrated in Fig. 4. In general, the conformation

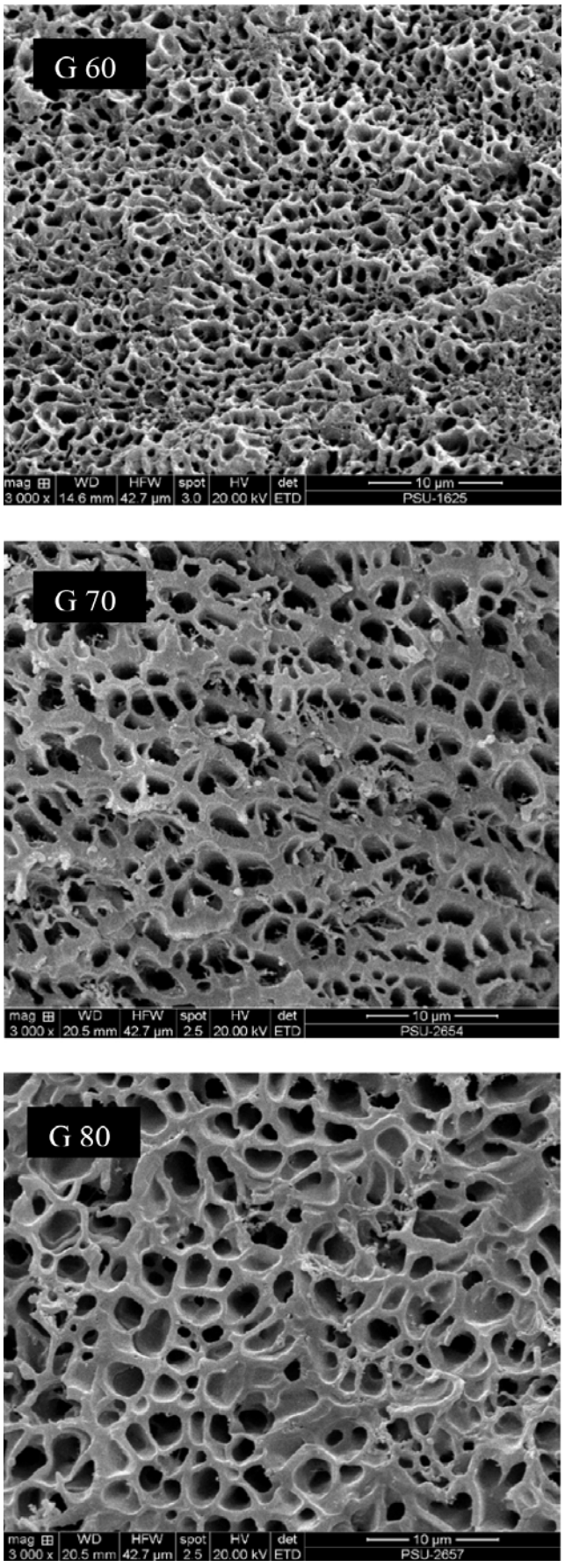

Fig. 4 - Microstructures of gelatin gel from the swim bladder of yellowfin tuna extracted at different temperatures. Magnification: 3000 (see Fig. 1 caption).

and chain length of the proteins in gel matrix directly contributed to the gel strength of gelatin (BENJAKUL *et al.*, 2009). Gelatin extracted at 60°C showed the finest gel network with small voids. Conversely, the coarser networks with the larger voids were found in gel of the gelatin extracted at higher temperatures. The fine gel structure of gelatin extracted at lower temperature was in accordance with the higher gel strength (Fig. 3). It has been known that the microstructure of the gel is related to the physical properties. The gelatin gel network was governed by the pretreatment conditions (YANG *et al.*, 2008) and gelatin concentration (YANG and WANG, 2009). Gelatin extracted at lower temperatures had the lower degradation, in which proteins with higher chain length were present. As a result, junction zones could be formed to a greater extent. This led to the high aggregation with a strong and ordered network. In the first stage of gel network formation, there is competition between intramolecular folding and intermolecular aggregate formation (YANG and WANG, 2009). For gelatin extracted at lower temperature, longer chains might undergo aggregation to a higher extent. Thus, the arrangement of peptides in the network during gelation as determined by chain length directly affected gel properties of gelatin.

3.8. Color

Color of the gelatin gel from swim bladder with different extraction temperatures expressed as *L**, *a** and *b** is shown in Table 2. Gel of gelatin extracted at lower temperatures (G60) showed the lower *L**-value (lightness) than others (G70 and G80) ($P < 0.05$). The higher redness (*a**-value) and yellowness (*b**-value) were found in the latters ($P < 0.05$). Generally, the increases in *L**, *a** and *b**-value of gelatin increased with increasing extraction temperatures. For yellowness (*b**-value), an increase was observed in all gelatin gels when the extraction temperatures increased ($P < 0.05$). This might be due to a non-enzymatic browning reaction taken place at the higher temperature, especially when the extraction time increased (AJANDOUZ and PUIGSERVER, 1999). Among all the gelatin samples, those extracted at a lower temperature (60°C) showed the highest total difference in the color value (ΔE^*) (66.09) with the lowest lightness (*L**-values). These results showed that the extraction temperatures had the impact on color of gelatin extracted from the swim bladder of yellowfin tuna.

4. CONCLUSION

Swim bladder from yellowfin tuna could be an alternative source of gelatin. Gelatin extracted at a higher temperature had the highest extraction yield, but possessed the poorer gel properties. Extraction conditions also affected the color of resulting gelatin. The appropriate extraction temperature for gelatin from swim bladder was 60 °C, providing the highest gel strength.

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PAPER

EFFECT OF THE ADDITION OF FRUIT JUICES ON GRAPE MUST FOR NATURAL BEVERAGE PRODUCTION

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ABSTRACT

The consumer attention for products with healthy properties is increased in time, and fruit juices, for their ease of consumption, can satisfy this demand providing them bioactive compounds. The grape juice has numerous health benefits demonstrated by several studies such as, among other, the antioxidant activities and the positive functions of their phenolic compounds. This work is aimed at blending grape and others fruits in a new fruit juice made only with natural ingredients of local production. The grape juice (cv Barbera) has substituted water and its percentage was fixed (70%). It was mixed with apple (cv Golden delicious), pear (cv Williams) and peach (cv Red Haven) juices to obtain 25 different prototypes. In each of these at least two fruit juices were present and added in a percentage variable from 0 to 25%, with a step of 5%. The objectives of this study were to check the feasibility of the mixing process and the evaluation of the samples overall pleasantness.

Other sensory aspects of samples were also evaluated by consumers with a JAR (just-about-right) structured scale. The results didn't reveal particular technological problems regarding the blending process. The Brix mean value of the samples was about 15.3, with a significant reduction compared to that of the grape juice (about 19). The pH mean value of the samples (3.44) was significantly higher than that of the grape juice (3.36). The titrable acidity and the antioxidant capacity mean value of the samples was, namely, 6.22 g L^{-1} and 535.18 mg L^{-1}. The penalty analysis of the liking test pointed out the importance of the persistence in mouth. The overall pleasantness was significantly ($p \leq 0,01$) positively correlated with the °Brix/acid ratio ($r=0.54$) and samples with the highest percentage of pear juice were generally preferred.

- Keywords: fruit, grape must, health, juice, sensory analysis -

INTRODUCTION

Fruit consumption has a positive impact on health (O'NEIL *et al.*, 2011) and, including also vegetables, five are their daily servings (FSA, 2010), though this advice is generally ignored (WOOTTON-BEARD and RYAN, 2011). In this regard, the "Dietary Guidelines for Americans" consider the 100% fruit juice as alternative to whole fruit (USDA, 2010). Indeed, fruit juices in general are deemed as one of the main sources of bioactive compounds for diet (RODRÍGUEZ-ROQUE *et al.*, 2014). Even if the link between weight and sweetened beverages, including fruit juice 100%, must to be taken into account, referring to these latters, there is no consistent association (O'NEIL *et al.*, 2011) and, actually, these have demonstrated to improve nutrient adequacy among children and adolescents of 2-18-year-olds (O'NEIL *et al.*, 2012). Also grape has proved to have numerous health benefits, such as antioxidant activity and the functions of flavonoid compounds (VISLOCKY and FERNANDEZ, 2010; WOOTTON-BEARD and RYAN, 2011). Grape-based products may prevent cardiovascular deseases, decrease oxidative stress and protect against atherosclerosis. Results from animal models suggest that especially purple grape juice more effectively improves blood lipids (VISLOCKY and FERNANDEZ, 2010). From a organoleptic and sensory perspective, grape juice is characterised by a high concentration of sugars and acids, a low pH and, generally, a very poor odour/aroma. Thus, grape juice has a high-energy value, which reduces the nutritional, while its high acidity and low odour/aroma intensity can reduce consumer preference. OJEDA *et al.* (2009) highlighted the too high sugar content of the pure grape juice and, for this reason, it is important to reach a right sugar/acids balance to develop appreciable grape juice. To reach this result it is necessary to use the optimal grape variety and/or mixing it with other fruit. The blending, indeed helps to improve flavour, taste, and nutritive value and it reduces the cost of production, improves storability and inhibits microbial growth (BHARDWAJ and PANDEY, 2011). As reported by BATES and MORRIS (2001), the reasons for producing blends are many and all attributable to adjust and improve acceptability. The aim of this work was to develop an innovative concept of fruit juice obtained by mixing grape with other fruit juices to reduce its sugar concentration, acidity and to improve its olfactory profile. The tested fruit juices (peach, pear and apple) were chosen based on their appreciation by consumers, low acidity and sugar content, high antioxidant activity and high odour/aroma intensity. The use of grape must would also help to reduce the wine surplus that, currently, amounts to, approximately, 30 million hectolitres world wide (RAMOS *et al.*, 2012; AYLWARD, 2012).

MATERIALS AND METHODS

Juice production

The grape juice (cv Barbera) was provided by Terre dei Santi (Castelnuovo Don Bosco, Asti, Italy), while the other fruit juices were provided by Valter Valle Farm (San Damiano d'Asti, Asti, Italy). The apple, pear and peach juices were obtained from the Golden delicious, Williams and Red Haven cultivars, respectively. For juice production, fruits were directly pressed, and the juice was filtered and stored at +1°C until use. Because the aim of this study was to develop a new grape-based juice, the percentage of grape juice was fixed (70%) and the other fruit juices were added in percentages from 0 to 25%, with a step of 5% (Table 1).

Table 1 - Experimental plan of blending.

Sample code	Barbera juice (%)	Fruit juices (%)		
		Pear	Peach	Apple
S-1	70	0	5	25
S-2	70	0	10	20
S-3	70	0	15	15
S-4	70	0	20	10
S-5	70	0	25	5
S-6	70	5	0	25
S-7	70	5	5	20
S-8	70	5	10	15
S-9	70	5	15	10
S-10	70	5	20	5
S-11	70	5	25	0
S-12	70	10	0	20
S-13	70	10	5	15
S-14	70	10	10	10
S-15	70	10	15	5
S-16	70	10	20	0
S-17	70	15	0	15
S-18	70	15	5	10
S-19	70	15	10	5
S-20	70	15	15	0
S-21	70	20	0	10
S-22	70	20	5	5
S-23	70	20	10	0
S-24	70	25	0	5
S-25	70	25	5	0

This ratio was defined taking into account that, generally, in a fruit juice, the fruit/water ratio is approximately 35:65 (FÜGEL *et al.*, 2005) and in this study water was replaced by grape juice. Because for each beverage at least two fruit juices must be present, a total of 25 mixed juices were obtained. The prototypes were then bottled, pasteurised (105°C, 25 min) and stored at ambient temperature.

Three replicates for each of the 25 recipes are been prepared.

Reagents

Folin-Ciocalteu reagent, sodium hydroxide, glucose, fructose, phosphoric acid, methanol, sulphuric acid, caesium chloride, tartaric, malic and citric acids were purchased from Sigma-Aldrich (Milano, Italy). Ultrapure water was obtained from a Milli-Q gradient A10 instrument (Millipore Corporation, Billerica, USA).

Analyses

Density, extract, pH, sulphur dioxide, titrable acidity, total sugars, glucose, fructose, ashes and potassium of grape must were determined in accordance with the Commission Regulation (EEC) No. 2676/90 of 17 September 1990, while tartaric, malic and citric acids were determined by HPLC (CANE, 1990). The polyphenolic composition of the grape must and fruit juices (total polyphenols, anthocyanin and flavonoid contents) was determined by spectrophotometry (DI STEFANO *et al.*, 1989). The glucose, fructose, total sugars, ashes, titrable acidity, pH, tartaric acid, malic acid, citric acid and potassium of the fruit juices and beverages were determined in accordance with Italian Standard Methods (DM 03/02/1989). The fruit juice antioxidant capacity, expressed as Vitamin-C Equivalent Amount or VEAC Index, was determined according to KIM *et al.* (2002). The colour was measured using a Konica Minolta spectrophotometer CM-5 (Minolta Corp, Osaka, Japan) in the CIELab colour system with a D65 illuminant. The parameters measured were L* (whiteness or brightness/darkness), a* (redness/greenness) and b* (yellowness/blueness). Each sample was evaluated in a 40-mL cuvette (1-cm thickness). All evaluations were performed in triplicate.

Liking test

As reported by MAMMASSE and SCHLICH (2014), literature recommend a range from 50 to 100 consumers in hedonic tests and generally no replication are needed. Taking into account this and the limited quantity of samples, the liking test was executed once by recruiting 50 consumers (22 males and 28 females, aged 26-65 years).

They have received an invitation and voluntarily have participated to the tests. All tests were conducted individually, and social interaction was not permitted. The test was performed inside an air-conditioned meeting room with white light. The temperature was approximately 21 °C, and the relative humidity was approximately 50%. Tests were performed from 11 a.m. over 5 days. For each session, five experimental beverage samples (approximately 30 mL each) were presented in a completely randomised and balanced order. The samples were offered to the consumers in coded plastic cups. Natural bottled water was provided to each participant for palate cleansing. To decrease fatigue, there was a 5 minutes break between each sample. During each break, the consumers rinsed their mouths with water. All beverages were evaluated for specific parameters by consumers on a Just-about-right (JAR) structured scale, and then the consumers were asked to express the overall pleasantness of each product.

For JAR evaluation, consumers rated the samples on a 5-point JAR scale (1 = much too low, 2 = a little too low, 3 = just about right-JAR, 4 = a little too much, and 5 = much too much) for five sensory parameters: colour, odour, aroma, sweet taste and persistence in the mouth. For the overall pleasantness evaluation, a segment of known length (100 mm), limited to the extremes of two adjectives of opposite meaning (bipolar scale) was used. Consumers were asked to mark the line that corresponded to their degree of overall pleasantness. The data were collected on a paper card. According to PAGÈS *et al.* (2014), the 5 JAR variables were reduced to 3 for data evaluation: "not enough" (by grouping the "much too low" and "a little too low" responses), "JAR" and "too much" (by grouping the "much too much" and "a little too much" responses). This grouping of variables leads to simpler analyses, and it allows for obtaining more stable results because non-JAR categories are associated with higher frequencies (PAGÈS *et al.*, 2014).

Statistical analysis

Compositional data and overall pleasantness were examined by one-way analysis of variance (ANOVA) with Tukey's test ($p \leq 0.05$) as a multiple range test with XLSTAT 2011 (Addinsoft SARL, California, USA) and then used for a Principal Component Analysis, also performed with XLSTAT 2011 (Addinsoft SARL, California, USA). The °Brix/acid ratio and the overall pleasantness were subjected to Pearson's test (r). The JAR data were subjected to a penalty analysis with XLSTAT-MX 2014.2.07 (Addinsoft SARL, California, USA).

RESULTS AND DISCUSSION

Compositional aspects

The compositions of grape and fruit juices used for beverage production are reported in Table 2, while the composition of the obtained beverages are reported in Tables 3 and 4.

As highlighted by MORALES-DE LA PENA *et al.* (2010), the overall quality of a fruit juice

Table 2 - Composition of grape and fruit juices used for beverages production. Data are expressed as mean ± SD.

	Fruit juices			Grape juice	
	Pear	Peach	Apple		
Glucose (g L^{-1})	19.08±0.2	39.78±0.2	20.41±0.2	Glucose (g L^{-1})	86.94 ±0.2
Fructose (g L^{-1})	75.43±0.4	41.59±0.5	61.94±0.2	Fructose (g L^{-1})	94.82 ±0.09
Ashes (g Kg^{-1})	2.6±0.03	4.3±0.02	2.7±0.02	Ashes (g L^{-1})	3.4 ±0.1
Potassium (mg Kg^{-1})	1720±0.2	3542±6	1540±0.4	Potassium (mg Kg^{-1})	1223±5
°Brix	13.5±0.4	11.5±0.3	11±0.2	°Brix	19±0.3
Total Acidity (g L^{-1})	4.3±0.2	4.85±0.3	4.13±0.1	Total Acidity (g L^{-1})	6.26±0.04
pH	3.73±0.03	3.79±0.01	3.76±0.01	pH	3.36±0.05
Tartaric acid (g L^{-1})	0.281±0.01	0.233±0.02	0.26±0.03	Tartaric acid (g L^{-1})	2.73±0.02
Malic acid (g L^{-1})	0.312±0.03	0.892±0.02	1.028±0.01	Malic acid (g L^{-1})	2.35±0.03
Citric acid (g L^{-1})	0.588±0.03	0.788±0.04	nd	Citric acid (g L^{-1})	0.1 ±0.01
Polyphenols (mg Kg^{-1})	126.7±5	81.9±4	96.5±4	Polyphenols (mg L^{-1})	446 ±6
				Density (g L^{-1})	1.07715 ±0.0004
				Extract (g L^{-1})	206.6±0.4
				Free Sulphur Dioxide (mg L^{-1})	nd
				Total Sulphur Dioxide (mg L^{-1})	11.2±0.4
				Anthocyanins (mg L^{-1})	228 ±1.74

(nd – not determined).

is evaluated by a few parameters such as soluble solids, pH and acidity. The grape juice displayed a total soluble solids content of 19 °Brix with approximately 170 g L^{-1} of sugars, while the peach, apple and pear juices exhibited 11.5, 11.0 and 13.5 °Brix, respectively. The mean value of °Brix for new beverages was approximately 15.3, with a significant reduction with respect to grape juice, approximately 19. The obtained value is similar to that of a fruit juice (GUNATHILAKE *et al.*, 2014) and ideal for the formulation of nutraceutical food beverages (SARAVANAN and ARADHYA, 2011a). The content of fructose in apple juice (approximately 62 g L^{-1}) was higher than that reported by WU *et al.* (2007) but lower than that reported by WILL *et al.* (2008) and MARKOWSKI *et al.* (2009). Additionally, the fructose content of pear juice (approximately 75 g L^{-1}) was higher than that reported by COLARIC *et al.* (2006). Acidity is one of the most important quality parameters for fruit juices (BHARDWAJ and PANDEY, 2011), as confirmed by AL BITTAR *et al.* (2013), who included this factor in the sensory analysis of an innovative grape juice enriched in polyphenols. Nevertheless, LIU *et al.* (2006) highlighted that: "high acidity has a negative influence on the palatability of table grapes, as well as the suitability for wines". The value of the total acidity, expressed as tartaric acid, of the grape juice used in this study (6.26 g L^{-1}) is comparable to that reported for juices made with different grape cultivars (MARSELLÉS-FONTANET *et al.*, 2013; LIU *et al.*, 2006; SOYER *et al.*, 2003). The main organic acid in grape is tartaric acid, which has a pK_1 of 3.04, followed by malic acid, which has a pK_1 of 3.40 (LIU *et al.*, 2006). The grape juice had a tartaric acid content of 2.73 g L^{-1}, similar to juice reported by LIU *et al.* (2006). The pH value also plays an important role in the preparation of beverages (BHARDWAJ and PANDEY, 2011). The blending process here studied is aimed to increase the pH value of grape must (3.36). Our obtained results indicated that the addition of fruit juice with pH values of 3.79 (peach), 3.76 (apple) and 3.73 (pear) increased the pH of grape juice so that it reached a mean value of 3.44 in the prepared beverages. In his research on the properties of fruit juices used for functional beverages, GUNATHILAKE *et al.* (2014) reported a pH of 3.60 for apple juice, while ANDRÉS *et al.* (2014) in their evaluation of the bioactive compounds in non-fermented beverages highlighted that the pH ranged between 3.20 and 4.01, in agreement with SAARELA *et al.* (2011). Typically, the pH values of fruit juices are below 4, or even 3, depending on the fruits used. The amount of organic acids in the fruit juices depended on the cultivar: apple displayed the highest amount of malic acid, with a content of 1.028 g L^{-1}, while pear juice had the highest citric acid content (0.588 g L^{-1}). AGUILAR-ROSAS *et al.* (2007) reported a malic acid content below 0.35 g L^{-1} for the same cultivar, whereas BURON-MOLES *et al.* (2014) reported a malic acid content of 1.4 g L^{-1}. For beverages, the most abundant organic acid was malic acid, with a mean content of 2.84 g L^{-1}, while the mean tartaric acid amount in these samples was 2.39 g L^{-1}. For this compound, the concentration was similar among all of the beverages because the same quantity of grape must was used and because the quantity of tartaric acid is very low for fruit juice. The highest values were

Table 3 - Composition of samples obtained by mixing grape juice and fruit juices of pear, peach and apple and results of Anova with Tukey's test. Data are expressed as mean ± SD. For samples code see Table 1. Values in each column having different letters are significantly different at p<0.05.

Sample code	Extract (g L^{-1})	°Brix	Glucose (g L^{-1})	Fructose (g L^{-1})	Acidity (g L^{-1})	pH	Organic acids (g L^{-1})			Ashes (g kg^{-1})	Potassium (mg kg^{-1})	°Brix/ acid ratio
							tartaric	malic	citric			
S-1	167.85±0.21	15.75±0.35 b	66.19±0.01 def	80.3±0.71 cd	6.00±0.00 j	3.44±0.01 fgh	2.52±0.00 ab	3.45±0.00 ab	0.55±0.03 cdefg	2.6±0.01 bc	1243±1.68 bcdefg	26.25
S-2	177.90±0.00	17.00±0.00 a	71.40±1.41 abcd	85.10±1.56 bc	6.15±0.00 h	3.45±0.01 cdefg	2.51±0.00 ab	3.32±0.09 ab	0.73±0.02 defg	2.7±0.02 bc	1298±4.15 abcdef	27.64
S-3	181.80±0.00	17.00±0.00 a	75.53±0.62 ab	87.44±0.55 ab	6.49± 0.06 ef	3.43±0.01 ghi	2.69±0.03 ab	3.54±0.05 a	0.94±0.04 abcde	2.8±0.01 bc	1385±9.72 abc	26.19
S-4	183.10±0.00	17.00±0.00 a	76.18±0.81 a	85.95±0.78 abc	6.75±0.00 c	3.45±0.00 defg	2.76±0.34 a	3.60±0.26 a	1.32±0.30 ab	3.1±0.01 abc	1424±2.64 ab	25.19
S-5	181.45±0.21	17.20±0.00 a	75.47±0.18 ab	83.01±0.09 bc	6.94±0.00 b	3.44±0.01 fgh	2.66±0.18 ab	3.47±0.20 ab	0.28±0.02 fg	3.2±0.01 ab	1464±2.51 a	24.78
S-6	176.25±0.21	16.80±0.00 a	68.45±0.64 cde	85.78±0.82 bc	7.20±0.00 a	3.43±0.00 ghi	2.47±0.01ab	3.22±0.02 abc	0.18±0.00 g	2.6±0.01 c	1161±5.66 cdefg	23.33
S-7	174.30±0.00	16.80±0.00 a	70.39±1.42 bcd	85.02±1.56 bc	6.45±0.00 f	3.45±0.01 defg	2.51±0.04 ab	3.21±0.06 abc	0.20±0.02 g	2.7±0.01 bc	1233±0.69 bcdefg	26.05
S-8	178.05±0.21	16.90±0.14 a	73.62±3.69 abc	87.18±2.48 ab	6.60±0.00 d	3.45±0.01 defg	2.37±0.18 ab	3.18±0.01 abcd	0.22±0.03 g	3.2±0.00 ab	1275±0.65 abcdef	25.61
S-9	180.50±0.00	16.8±0.00 a	73.33±1.82 abc	86.15±0.62 ab	6.51±0.03 e	3.43±0.01 ghi	2.64±0.05 ab	3.30±0.07 ab	1.18±0.00 ab	3.1±0.01 abc	1304±0.16 abcdef	25.81
S-10	181.70±0.14	17.00±0.00 a	75.70±0.82 ab	86.54±0.41 ab	6.64±0.00 d	3.40±0.00 j	2.47±0.13 ab	3.22±0.01 abc	0.27±0.03 fg	3.2±0.03 ab	1370±3.08 abcd	25.60
S-11	182.10±2.23	17.00±0.00 a	76.33±1.14 a	84.12±1.20 bc	6.75±0.00 c	3.40±0.00 j	2.60±0.00 ab	3.09±0.09 abcde	0.21±0.00 g	3.5±0.01 a	1328±5.83 abcde	25.19
S-12	148.60±0.00	14.65±0.21 c	51.46±0.10 g	75.87±0.57 de	5.55± 0.00 o	3.39±0.00 j	2.30±0.04 ab	2.87±0.04 bcdef	0.35±0.27 efg	2.6±0.01 c	1059±9.92 g	26.40
S-13	134.00±0.00	13.10±0.14 e	45.21±0.16 hi	68.31±0.29 fg	5.70± 0.00 m	3.48±0.01 abc	2.21±0.04 ab	2.60±0.06 cdefg	0.79±0.14 bcdefg	2.6±0.01 c	1156±9.72 efg	22.98
S-14	132.90±0.00	13.00±0.00 e	45.68±0.25 hi	66.86±0.44 g	5.63±0.00 n	3.50±0.00 a	2.38±0.09 ab	2.65±0.08 cdefg	0.93±0.07 abcde	2.8±0.01 bc	1157±1.59 efg	23.09
S-15	136.00±0.00	13.10±0.14 e	48.33±0.66 ghi	67.92±0.91 fg	5.93±0.00 k	3.48±0.01 abc	2.33±0.04 ab	2.62±0.07 cdefg	1.14±0.06 abc	3±0.00 abc	1222±3.08 bcdefg	22.09
S-16	138.50±0.14	13.20±0.00 e	50.76±0.04 gh	68.92±1.02 fg	6.00±0.00 j	3.48±0.00 ab	2.29±0.03 ab	2.59±0.03 cdefg	1.51±0.20 a	3.1±0.01 abc	1245±3.94 bcdefg	22.00
S-17	134.60±0.14	13.20±0.00 e	44.51±0.35 i	70.63±0.42 efg	5.55±0.00 o	3.46±0.00 bcdef	2.27±0.02 ab	2.66±0.13 defg	0.72±0.03 bcdefg	2.6±0.03 bc	1118±1.57 efg	23.78
S-18	135.9±0.14	13.20±0.00 e	45.69±0.09 hi	69.90±0.37 fg	5.55±0.00 o	3.47±0.00 bcd	2.19±0.02 ab	2.38±0.01 fg	0.85±0.04 bcdef	2.6±0.03 bc	1121±0.49 efg	23.78
S-19	138.75±0.21	13.30±0.14 e	46.98±0.12 ghi	69.41±0.31 fg	6.08±0.00 i	3.47±0.01 bcde	2.11±0.12 ab	2.25±0.13 g	1.00±0.02 abc	2.9±0.01 abc	1171±3.66 defg	21.88
S-20	139.55±0.21	13.20±0.00 e	47.265±0.32 ghi	67.77±0.11 fg	6.08±0.00 i	3.46± 0.00 bcdef	2.02±0.29 b	2.08±0.36 g	1.06±0.10 abc	3±0.01 abc	1201±3.64 cdefg	21.71
S-21	141.10± 0.00	13.80±0.00 d	45.38±0.10 hi	72.92±0.30 ef	5.78±0.00 l	3.44±0.00 efgh	2.04±0.18 b	2.08±0.18 g	0.76±0.21 bcdefg	2.7±0.03 bc	1098±1.96 fg	23.88
S-22	168.20±0.00	15.80±0.00 b	60.86±1.21 f	82.54±1.05 bc	6.15±0.00 h	3.44±0.00 efgh	2.33±0.04 ab	2.51±0.09 efg	0.72±0.04 bcdefg	2.8±0.01 bc	1122±10.78 efg	25.69
S-23	169.80±0.00	15.80±0.00 b	62.97±0.71 ef	82.58±0.52 bc	6.3±0.00 g	3.42±0.01 hij	2.40±0.00 ab	2.49±0.10 efg	1.19±0.05 ab	3±0.01 abc	1242±2.39 bcdefg	25.08
S-24	172.25±0.21	16.10± 0.14 b	62.64±0.49 f	85.65±0.63 bc	6.30±0.00 g	3.44±0.00 efgh	2.37±0.03 ab	2.41±0.02 fg	1.12±0.17 abc	3±0.01 abc	1195±0.99 cdefg	25.56
S-25	176.50±0.14	17.00±0.00 a	70.34±4.38 bcd	91.74±5.41 a	6.49±0.00 ef	3.41±0.01 ij	2.32±0.08 ab	2.35±0.06 fg	0.21±0.05 g	2.9±0.02 abc	1251±8.68 abcdefg	26.19

found for beverages S-3 and S-4, which contained higher quantities of apple juice. The Brix/acid ratio (Table 3) is an important parameter usually used to control fruit quality. In this study a positive correlation (r = 0.54, $p \leq 0.01$.) resulted between it and the overall pleasantness in accordance with JAYASENA AND CAMERON (2007). These authors reported that the °Brix/acid ratio compared with the °Brix alone demonstrated a higher degree of association with the consumer acceptability and it appeared a very useful maturity indicator. The peach juice exhibited the highest potassium content. This characteristic determined an increase in the content of this important component in the beverages containing high percentages of peach, e.g., sample S-5. The lowest value was determined for sample S-12, which was obtained without peach juice. The total polyphenols content ranged between 265.5 mg L^{-1} for beverage S-5 and 407 mg L^{-1} for beverage S-24, with a mean value of 359.30 mg L^{-1}. According to the total polyphenol contents of fruit juices, higher values were exhibited by beverages with high percentages of pear juice. The same beverage also displayed some of the highest values for the flavonoid content (627.5 mg L^{-1} , the highest) and antioxidant capacity (585 mg L^{-1} , the second highest one). For this parameter, the result for S-24 was similar to that of beverage S-1 (593 mg L^{-1}). The lowest value for the antioxidant capacity (464.41 mg L^{-1}) was displayed by beverage S-5. These results highlighted that the most interesting findings were obtained with a high quantity of apple or pear juice in the beverage, while a high content of peach juice led to a reduction of this parameter. The ANOVA and Tukey's test performed for each parameter of the beverages displayed high variability among all samples and strictly corre-

Table 4 - Polyphenol composition (PHEN - total polyphenols; TAI - anthocyanins; TFI - flavonoids; VCEAC - antioxidant capacity) and CIELab values of samples obtained by mixing grape juice and fruit juices of pear, peach and apple and results of ANOVA analysis with Tukey's test. Data are expressed as mean ± SD. For sample code see Table 1. Values in each column having different letters are significantly different at $p<0.05$.

Sample code	PHEN (mg L^{-1})	TAI (mg L^{-1})	TFI (mg L^{-1})	VCEAC (mg L^{-1})	L*(D65)	a*(D65)	b*(D65)
S-1	384.5±4.95 cd	84±1.41 abc	540.5±44.55 cdefgh	593.83±10.40 a	68.14±2.86 ab	32.18±0.46	11.20±0.43
S-2	389.5±2.12 bcd	84.5±2.12 abc	478±4.24 ghijkl	578.38±1.04 ab	64.75±0 .01 b	33.9±0.10	11.675±0.15
S-3	366±1.41 efg	82±2.82 abcd	407.5±20.51 l	526.17±4.16 abcd	66.28±0.45 ab	33.56±0.19	11.36±0.26
S-4	362±0.7 gh	82.5±1.06 abc	497±4.95 fghijk	468.09±20.28 cd	66.63±0.19 ab	32.72±0.51	10.52±0.27
S-5	265.5±6.36 o	80±0.00 abcde	447±2.83 ijkl	464.41±10.40 d	66.65±1.98 ab	34.84±1.72	11.23±0.30
S-6	402.5±4.95 ab	88.5± 0.71 a	612.5±0.71 abc	534.26±48.87 abcd	64.89±0.25 b	32.94±0.40	11.31±0.28
S-7	405.5±3.54 a	88±0.00 ab	576±1.41 abcd	476.91±7.28 cd	64.13±0.93 b	32.75±0.07	10.89±0.01
S-8	385.5±2.12 cd	80.5±0.71 abcde	546.5±7.78 bcdefg	492.35±47.83 bcd	63.85±1.52 b	33.52±2.33	10.97±1.19
S-9	380.5±2.12 de	81.5±0.71 abcd	545±5.66 cdefg	530.59±29.12 abcd	65.63±1.25 ab	33.85±0.76	11.46±0.59
S-10	362.5±2.12 fgh	80.5±0.71 abcde	517±1.41 defghi	480.59±10.40 cd	66.21±3.21 ab	34.4±2.77	11.25±1.00
S-11	338.5±4.95 ijk	79.5±0.71 abcde	490.5±.71 fghijk	537.94±18.72 abcd	67.96±2.23 ab	33.8±1.33	10.9±0.72
S-12	377±1.41 def	82±0.00 abcd	581±1.41 abcd	577.65±0.00 ab	66.62±0.40 ab	33.49±1.94	9.45±0 .47
S-13	334±0.00 jkl	67.5±0.71 gh	515.5±4.95 defghi	551.18±0.00 abcd	67.5±2.21 ab	31.66±0.46	9.81±0.01
S-14	325±4.24 klm	63.5±2.12 h	493.5±4.95 fghijk	510±2.08 abcd	68.51±1.62 ab	32.66±1.77	10.04±0.04
S-15	314.5±0.71 mn	68.5±2.12 gh	430±15.56 kl	506.32±11.44 abcd	70.09±0.14 ab	30.79±1.81	9.77±0.95
S-16	309.5±0.71 n	65.5±0 .71 gh	436±9.90 jkl	510.74±15.60 abcd	74.03±4.72 a	31.21±1.16	9.26±1.27
S-17	350±4.24 hi	72±1.41 efgh	552.5±6.36 bcdef	583.53±12.48 a	66.6±0.62 ab	32.86±1.14	11.09±0.09
S-18	342±1.42 ij	69.5±2.12 fgh	514±2.83 defghi	551.17±33.28 abcd	68±2.18 ab	33.46±1.28	10.93±0.60
S-19	333±1.14 jkl	67±0.00 gh	503±0.00 efghij	540.88±6.24 abcd	69.48±1.60 ab	32.71±1.05	10.76±0.69
S-20	321±1.41 lmn	64.5±2.12 gh	469±8.49 hijkl	551.18±29.12 abcd	67.82±2.71 ab	33.63±2.10	9.95±0.09
S-21	365±4.24 fg	68±0.00 gh	571±1.41 abcde	546.76±12.48 abcd	67.49±0.49 ab	32.6±1.43	10.56±1.41
S-22	395.5±0.71 abc	80±7.07 abcde	618±49.5 ab	576.91±7.28 ab	63.95±1.63 b	34.42±0.86	11.88±0.30
S-23	376±2.83 defg	73±1.41 defg	556±0.00 abcdef	548.24±0.00 abcd	70.73±5.76 ab	32.09±1.22	10.05±1.86
S-24	407±7.07 a	78±1.41 cdef	627.5±4.95 a	585±37.43 a	65.57±1.23 ab	32.27±0.23	11.68±0.23
S-25	390.5±7.78 bcd	79±5.66 bcde	576±46.67 abcd	556.32±1.04 abcd	66.51±0.28 ab	32.82±0.91	10.89±0.59

lated with the composition of each single fruit juice and the different percentages used for beverage production. In fact, there were no differences between the beverages for the CIELab parameters a* and b* only, and this is due to the high grape must percentage used.

Sensory aspects

Concerning the overall pleasantness, the ANOVA highlighted significant differences among the 25 experimental beverages (Table 5).

Even if the content of grape juice was kept constant in all of the beverages at 70%, the different percentages of other fruit juices can influence the acceptability. The most appreciated samples (S-22, S-24 and S-23) had the highest pear juice percentages, while the least appreciated (S-14, S-12, S-1 and S-16) had the lowest pear juice concentrations. The least appreciated was beverage S-14, which was obtained with a mix of apple, pear and peach juices at the same percentage (10%). Penalty analysis was used because with this test it is possible to identify the sensory attributes that have the largest influence on consumer liking and provides directions for product reformulation (ARES *et al.*, 2014) and also allows one to determine if a specific product attribute is "just about right" (TAYLOR, 2013). Penalty analysis combines JAR variables and overall liking tests to find correlations between a decrease in consumer acceptance and attributes not at the JAR level. This analysis, based on multiple comparisons, is aimed to identify and determine if the rankings on the JAR scale are related to significantly different results in the liking scores for each sensory attribute studied on the JAR scale. This can be achieved by evaluating the mean decrease in overall liking versus percentage of not-JAR variables (i.e., the low percentage of not-JAR evaluation determines a low mean decrease in overall liking). When some not-JAR categories receive at least 20% (Pareto principle) responses for an attribute, this becomes a candidate for penalty analysis. Penalty analysis uses the 20% cut-off theory on the percentage of not-JAR consumers based on the Pareto principle (i.e., the Pareto principle recognises that "80% of effects occur from 20% of causes" or the 80-20 rule) and signifies several common occurrences in everyday phenomena. Therefore, the 20% cut-off is used as a general rule for penalty analysis (NARAYANAN *et al.*, 2014). In Fig. 1 are reported the JAR scores for each parameter used in the beverage evaluation.

The colour was judged "just right" by 50% of the consumers, odour by 37%, aroma by 38% and persistence in the mouth by 39%. In general, the "JAR" value was chosen by a higher number of assessors: the higher frequency was highlighted by the "a little too low" val-

Table 5 - Mean values of overall pleasantness and results of ANOVA and Tuckey's test. Data are expressed as the mean ±SD. Values with different letters are significantly different at p<0.05.

Sample code	Fruit juices			Overall pleasantness	Tuckey test (p< 0.05)
	pear (%)	peach (%)	apple (%)		
S-22	20	5	5	56.98	a
S-24	25	0	5	55.3	ab
S-23	20	10	0	55.14	ab
S-8	5	10	15	54.18	abc
S-9	5	15	10	54.08	abc
S-2	0	10	20	54.04	abc
S-25	25	5	0	53.07	abcd
S-5	0	25	5	53.36	abcde
S-11	5	25	0	52.36	abcdef
S-3	0	15	15	50.82	abcdefg
S-4	0	20	10	50.56	abcdefg
S-6	5	0	25	50.54	abcdefg
S-7	5	5	20	49.72	abcdefg
S-10	5	20	5	48.9	abcdefg
S-21	20	0	10	48.38	abcdefg
S-15	10	15	5	46.36	abcdefgh
S-20	15	15	0	45.7	bcdefgh
S-17	15	0	15	43.8	cdefgh
S-18	15	5	10	42.9	defgh
S-19	15	10	5	42.68	efgh
S-13	10	5	15	42.6	efgh
S-16	10	20	0	42.46	fgh
S-1	0	5	25	41.96	fgh
S-12	10	0	20	40.86	gh
S-14	10	10	10	36.36	h

ue for only the odour. Fig. 1 also demonstrates that the "much too much" and "much too low", although they may affect the overall pleasantness, do not weigh significantly on it because of their low frequency in the responses of consumers. The variables can then be grouped into two main groups with "a little too much" or "a little too low". The first group corresponds to "much too much", while the second corresponds to "not enough" for the parameters of

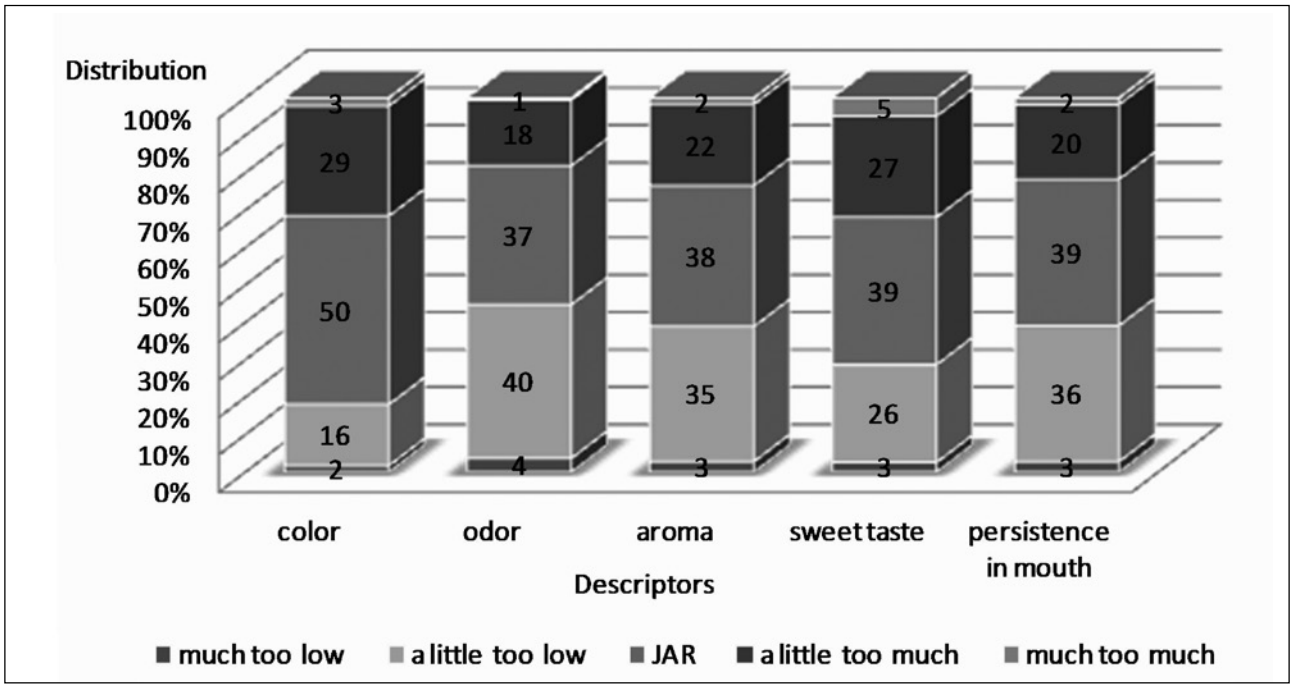

Fig. 1 - Distribution of JAR scores for each sensory attribute evaluated.

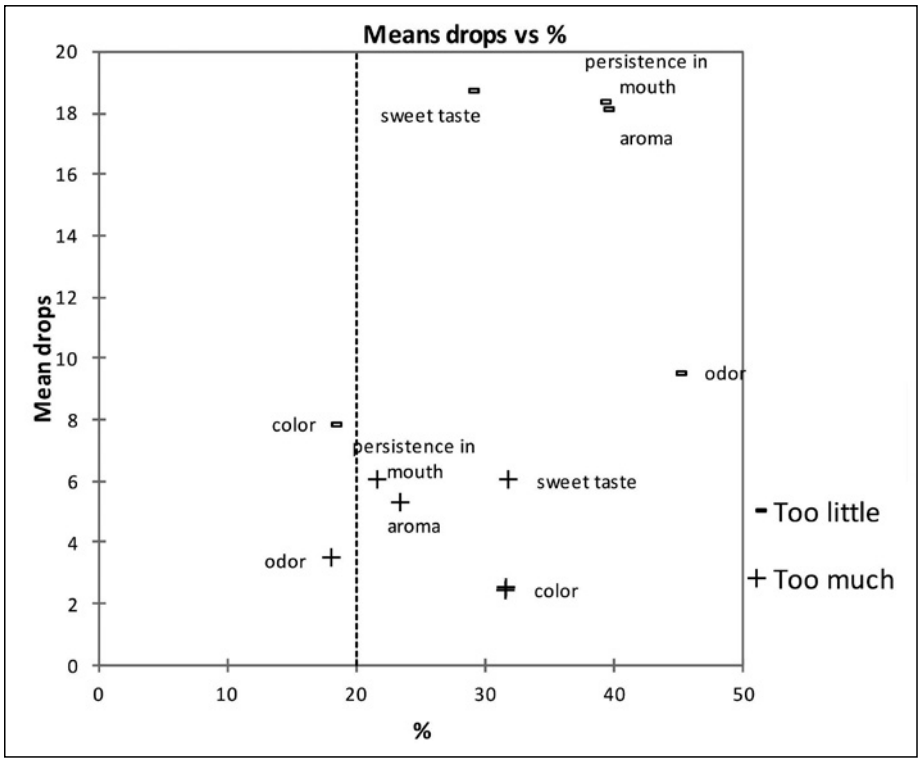

Fig. 2 - Penalty analysis from JAR data. Not-JAR data with a frequency <20% of total responses are not considered significant.

colour, aroma, sweet taste and persistence in the mouth. In Fig. 2 are displayed the distribution of frequency and then their effect on the mean drop in overall pleasantness.

Sweet taste, aroma and persistence in mouth exhibited a higher effect on the overall pleasantness if classified as "not enough". Also important for determining the overall pleasantness was the odour, if classified as "not enough". When the sensory parameters were classified as "too much", they had less impact on the overall pleasantness. A principal component analysis was also performed to highlight the correlation between chemical-physical parameters and overall pleasantness. The first two components explained 72.82% of the variance (Fig. 3).

The first component explained 50.74% of the variance and was mainly correlated with the to-

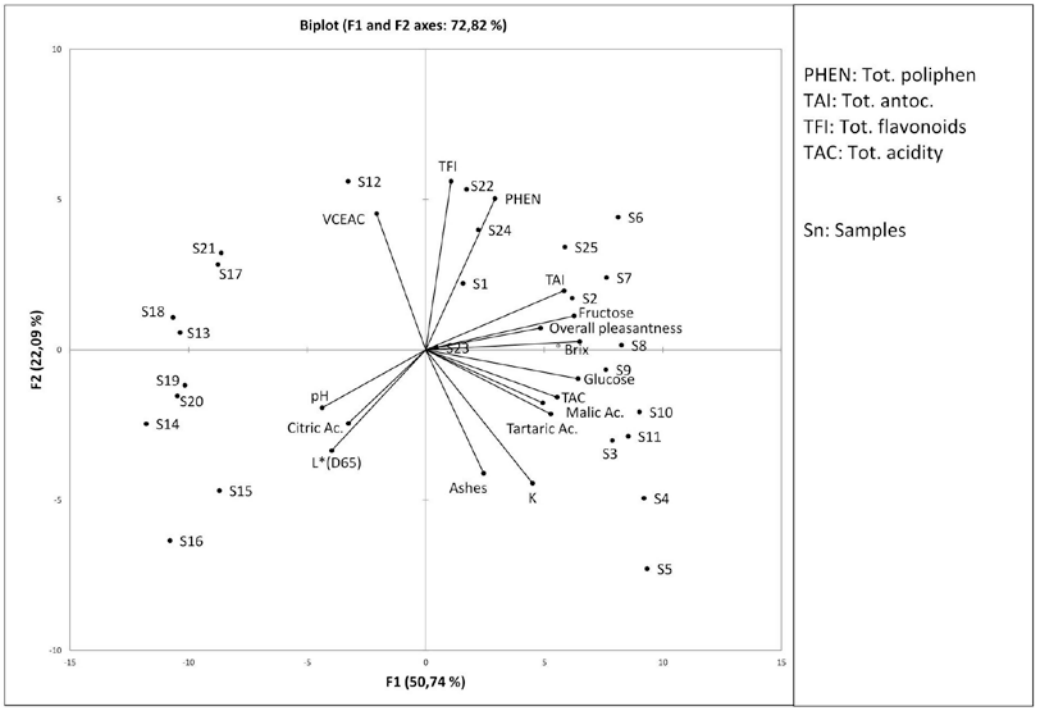

Fig. 3 - Distribution on plane defined by the first two components of chemical-physical parameters, overall pleasantness and beverage samples.

tal soluble solids, glucose and fructose contents, which corresponded to 12.1, 11.8 and 11.2%, respectively, of the total variance explained by this axis. The second component that explained 22.08% of the total variance is associated with the flavonoids, total polyphenols and antioxidant capacity, accounting for 20.7, 16.7 and 13.5%, respectively, of the total variance explained by this axis. The overall pleasantness was positively correlated with the contents of the total soluble solids and fructose and negatively correlated with the pH, citric acid content and L*. Because the overall pleasantness is located in the upper right graph quadrant, all of the beverages placed in the same quadrant are the most appreciated. In particular, the highest appreciation was found for the S-22, S-24 and S-23 samples, as also demonstrated by Table 5. The less appreciated samples are on the lower left side of the graph. They can be grouped into two groups: S-16, S-15, S-14, S-20 and S-19 in the lower left quadrant of the PCA graph and S-13, S-18, S-17, S-21 and S-12 in the upper left quadrant. The first group exhibited a more transparent colour, with a high value for L* and higher pH and citric acid contents. The second group demonstrated a low overall pleasantness but a high antioxidant capacity. In this group, it must be highlighted that beverage S-14, with the same percentages of fruit juice (10-10-10), had the lowest appreciation and the highest pH.

CONCLUSIONS

One of the first internationally accepted descriptions of functional food has been provided by DIPLOCK *et al.* (1999) according to which: "a food product can be considered functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism...". Taking this into account, and also of the scientific evidence regarding the benefits of the products based on grapes to human health, the results obtained in this study have shown that these experimental fruit juices have functional characteristics. Additionally, as reported by BHARDWAJ and PANDEY (2011), it may be concluded that the formulation of mixed beverages can satisfy consumer tastes and preferences. In particular, the overall pleasantness results indicate a tendency of consumers to prefer samples with the highest percentage of pear juice, followed by samples containing mixtures of peach and apple juices.

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PAPER

FREEZE DRYING OF KIWI (*ACTINIDIA DELICIOSA*) PUREE AND THE POWDER PROPERTIES

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ABSTRACT

In this study, it was intended to investigate the production of freeze dried kiwi (*Actinidia deliciosa*) puree in the form of powder that can be used as a natural alternative to synthetic additives used in food products such as pudding, instant tea, and sauces for improving their flavour. In order to obtain the powder product, kiwi puree as plain and with maltodextrin (Dextrose Equivalence of 10-12, as 10 % by weight) addition were freeze dried. Drying behaviour of plain kiwi puree and kiwi puree with MD were explained by Logarithmic model (R^2=0.994, RMSE=0.024, χ^2=0.0008) and Wang and Singh model (R^2=0.999, RMSE=0.012, χ^2=0.0002), respectively. The effective moisture diffusivity (D_{eff}) value was calculated as $7.3x10^{-10}$ m^2/s and it was observed that it was not affected by the addition of MD. The vitamin C content of fresh kiwi fruit was evaluated as 66.3 mg/100 g kiwi and there was a loss of 17.1% for plain and 19.8% for MD containing powders respectively after freeze drying. It was also observed that, the addition of maltodextrin decreased cohesiveness, on the other hand, increased bulk and tapped densities, average time values for wettability and solubility, and glass transition temperature of the powder products.

- Keywords: kiwi, kiwi puree powder, freeze drying, maltodextrin, vitamin C -

INTRODUCTION

Kiwi fruit contains high amounts of vitamins (vitamin C (100-400 mg vitamin C/100 g), A, B_2, and E), minerals (calcium, iron, copper, phosphorus, magnesium, and potassium), carotenoids (beta carotene, lutein, and xanthophyll), phenolic compounds (flavonoids and anthocyanins) and antioxidant compounds (CASSANO *et al.*, 2006). Kiwi fruit is being processed to obtain juice, frozen food, wine, jam, marmalade, and canned and dried slices. Drying might be a suitable technique to prolong the shelf life of kiwi, which is susceptible for microbial spoilage and softening due to its high moisture content. Fruit juices, purees and powders are being marketed due to an increased demand for ready-to-eat foods. In addition, powder products, with a long-term ambient shelf life and microbiological stability can reduce the transportation, and storage costs as well (JINAPONG *et al.*, 2008). Thus, alternatives to conventional processing technologies are being explored to produce better quality products. Due to high content of vitamin C, it is essential to protect vitamin C during drying of kiwi (KAYA *et al.*, 2010). Freeze drying is an important process for the protection of sensitive compounds such as vitamin C, phenolic compounds, biological activity, appearance, color, texture, aroma, and nutritional values of foods which compensates its high operating costs for drying of foods (ZEA *et al.*, 2013; WANG *et al.*, 2006). In addition, FERNANDES *et al.* (2011) reported that for producing whole fruit powder, drying fruits at low temperature and reduced pressure with low amounts of carrier is apparently the best alternate. Because, there exist some difficulties for drying of food extracts, juices, and purees because of the stickiness problems resulted by low glass transition temperatures of their components such as sugars and organic acids. In order to prevent problems in drying and obtaining powder products with acceptable properties, the drying aids that have high T_g is to be used. The use of drying agents such as gum arabic, maltodextrin, whey protein, sucrose etc. improves the drying process, and leads to an effective drying (NADEEM *et al.*, 2011).

Numerous studies were carried out with freeze drying of foods which contain sensitive compounds such as carrot (LIN *et al.*, 1998), pumpkin (QUE *et al.*, 2008), kiwi (ERGÜN, 2012) mango (SHOFIAN *et al.*, 2011) pineapple (Marques *et al.*, 2011), papaya (SHOFIAN *et al.*, 2011; MARQUES *et al.*, 2011) and guava (WANG *et al.*, 2006). Several researchers studied on drying of kiwi fruits such as convective, microwave, vacuum microwave, and freeze drying (KAYA *et al.*, 2010; ERGÜN, 2012; DOYMAZ *et al.*, 2009; KIRANOUDIS *et al.*, 1997) methods.

Describing dehydration kinetics is important in the design and optimisation of drying processes (SIMAL *et al.*, 2005). Thin layer drying models, generally means to dry as one layer of sample which provide uniform temperature assumption and suitable for lumped parameter models, are important in mathematical modelling of drying. Although, models depend on the process conditions, they are practical and provide sufficiently good results (ERBAY and ICIER, 2009). The properties of food powders such as bulk density, hygroscopicity, degree of caking, dispersibility, wettability, solubility, particle size, and size distribution are useful for design, and control of processing, handling, storage operations, and product quality control. Properties of powder products are usually studied in two groups such as particle properties (particle size, shape, distribution, density and morphological properties), and bulk properties (bulk density, wettability, solubility, porosity, cohesiveness, and flowability).

In this study it was intended to investigate the production of freeze dried kiwi (*Actinidia deliciosa*) puree in the form of powder that can be used as a natural alternative to synthetic additive used in food products such as pudding, instant tea, and sauces for improving their flavour. Also, an alternative product with the advantages of high nutritional value, long durability, easiness for usage in dry mixture formulations, being portable easily, and a healthier food additive for the consumers consumption will be obtained. In addition to the mentioned purposes: it was also aimed to determine the drying behaviour of kiwi puree (pure and with 10% MD) during freeze drying and the effect of maltodextrin addition and the properties of the powder product.

MATERIAL AND METHODS

The fresh kiwi fruits were obtained from a local supermarket in Izmir, Turkey. They were peeled and grounded into puree by using a home type blender (Tefal Smart, MB450141, Turkey). In order to obtain the puree with maltodextrin addition, maltodextrin (MD) with Dextrose Equivalence (DE) value of 10-12 (AS Chemical Industry and Commerce Limited Company, Turkey) was added directly to puree in suitable amounts (10% by weight).

Freeze drying

The freeze drying experiments were performed in a pilot scale freeze dryer (Armfield, FT 33 Vacuum Freeze Drier, England). Prior to drying kiwi puree was frozen in a layer of 3 mm in the petri dishes at - 40°C in an air blast freezer (Frigoscandia, Helsinborg, Sweden) for two hours, then freeze dried under vacuum (13.33 Pa absolute pressure), at - 48°C condenser temperature. The temperature of the heating plate was set to 30°C, which was constant during the drying process. The powder was obtained by grind-

ing the dried material, obtained as pellets of diameter of petri size, in a blender (Tefal Smart, MB450141, Turkey), and powder was stored in glass jars in the dark at 20±1°C until further tests were carried out.

Physical and chemical analyses

The moisture content of kiwi puree and freeze dried kiwi puree powders (KPP) were determined according to AOAC (2000). For this process, each experiment for increasing time periods was carried out with new samples of equal mass, and moisture loss was determined gravimetrically by using a digital balance with 0.01 precision (Ohaus AR2140, USA). Moisture ratio was calculated according to equation (1).

$$MR = \frac{M_t - M_e}{M_0 - M_e} \quad (1)$$

Where the M_t, M_0 and M_e are the moisture content at any time, initial, and equilibrium moisture content (kg water/ kg dry matter), respectively. Drying data was fitted to ten well-known thin layer drying models (Lewis, Page, Modified Page I, Henderson and Pabis, Logarithmic (Asymptotic), Midilli, Modified Midilli, Two-term, Two-term Exponential, and Wang and Singh) (ERBAY and ICIER, 2009). Nonlinear regression analysis was used to evaluate the parameters of the selected model by using statistical software SPSS 16.0 (SPSS Inc., USA). The goodness of fit was determined using the coefficient of determination (R^2), root mean square error (RMSE), and the reduced chi-square (χ^2) that can be described by the equations given by ERBAY and ICIER, 2009.

Where $MR_{exp,i}$ and $MR_{pre,i}$ is the experimental, and predicted moisture ratio at observation i; N is number of the experimental data points, and n is number of constants in model.

The effective moisture diffusivity (D_{eff}) of freeze dried kiwi slices were calculated by Fick's diffusion model (Eq. 2).

$$MR = \frac{M_t - M_e}{M_0 - M_e} = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} exp\left[-(2n-1)^2 \pi^2 \frac{D_{eff}}{4L^2} t\right] \quad (2)$$

Where t is the time (s), D_{eff} is the effective diffusivity (m^2/s) and L is the thickness of samples (m). For long drying times, a limiting case of Eq. (3) is obtained, and expressed in a logarithmic form;

$$lnMR = ln\left(\frac{8}{\pi^2}\right) - \left(\frac{\pi^2 D_{eff}}{4L^2}\right) t \quad (3)$$

The effective diffusivity was calculated by plotting experimental moisture ratio in logarithmic form versus drying time. From Eq.(3), a plot of ln MR versus drying time gives a straight line with a slope of:

$$Slope = \frac{\pi^2 D_{eff}}{4L^2} \quad (4)$$

Water activity was measured by using Testo-AG 400, Germany, water activity measurement device. The pH values of kiwi puree and the powders were measured using a pH meter (Inolab WTW pH 720, Germany) directly and after dissolving the powder in deionised water (1 g/1 g) respectively.

The color values (L*, a*, and b* values) of fresh kiwi fruits, and the powders were measured with Minolta CR-400 Colorimeter, Japan, calibrated with white standard plate three times and results as the average of three measurements were expressed in accordance with the CIE Lab. System. The L* value, is a measure of lightness which ranges between 0 and 100. Increases in a* value in positive, and negative scales correspond to increases in red or green color, respectively. The b* value represents color ranging from yellow (+) to blue (-).

The vitamin C content of fresh kiwi fruits was determined according to HIŞIL (2007). Freeze dried powders were rehydrated to the initial moisture content prior to the analysis. The indication principle of vitamin C value is based on extraction with 10% oxalic acid afterwards adding of 2,6-dichlorophenolindophenol solution. The absorbance was measured at 518 nm by a Varian Cary 50 UV/Vis spectrophotometer.

Glass transition temperature

Glass transition temperature of the powder samples was determined by a Differential Scanning Calorimeter (TA Instruments, Q10, USA) equipped with a thermal analysis station. An empty sealed aluminum pan was used as a reference in each test. Nitrogen gas at a flow rate of 50 ml/min was used as the purge gas to avoid water condensation around the samples. About ten milligrams of kiwi sample was sealed in aluminum pans and cooled from room temperature to -40°C at 10°C/min for formation of glassy state in kiwi sample and equilibrated for 10 min. The heating rate was 10°C/min and the temperature range varied between -40 and 120°C, depending on sample moisture content. DSC thermograms, presenting the heat flow (W/g) and temperature relationship were used to analyze the thermal transitions in samples during heating and cooling. TA Instruments Universal analysis software was used to analyze the onset, mid and end points of the glass transition. The glass transition temperature (Tg) was calculated as the average of the onset and end point values.

Thermo gravimetric analysis

Thermo Gravimetric Analysis (TGA) was carried out by Perkin Elmer Diamond TG/DTA (Canada) under nitrogen flow. The assay con-

ditions were as follows: isotherm at 30 °C and heating from 30.00°C to 1000.00°C at 10.00°C/min. Five milligrams of equilibrated samples was introduced into the apparatus and the measurements were plotted during the heating.

Scanning electron microscope (SEM)

The morphology of the powder samples, prepared by placing the powders on aluminium stubs using a double-sided adhesive tape and then coating with gold, were examined with a scanning electron microscope (SEM- Phillips XL-30S FEG, Eindhoven, Netherlands) operating at 5kV accelerating voltage.

Analysis of the powder properties

For the determination of bulk density, the method explained by JINAPONG *et al.* (2008) was used. The average wettability and solubility times of freeze dried kiwi puree powders were determined by using the method explained by GONG *et al.* (2008) and GOULA and ADAMOPOULOS (2008), respectively. Flowability and cohesiveness values of the powders were evaluated in terms of Carr index (CI) and Hausner ratio (HR), respectively. Both CI and HR were calculated from the bulk (ρ_{bulk}), and tapped (ρ_{tapped}) densities of the powder as shown below Eqs. (5) and (6), respectively.

$$CI = \frac{(\rho_{tapped} - \rho_{bulk})}{\rho_{tapped}} x\ 100 \quad (5)$$

$$HR = \frac{\rho_{tapped}}{\rho_{bulk}} \quad (6)$$

Statistical analysis

Data were analyzed by using statistical software SPSS 16.0 (SPSS Inc., USA). The data were subjected to analysis of variance (ANOVA), and Duncan's multiple range test (α=0.05) to determine the difference between means. The drying experiments were replicated twice and all the analyses were triplicated.

RESULTS AND DISCUSSION

Results of physical and chemical analyses

Kiwi is harvested through a long season. However, due to its high moisture content, storage period and its direct use in food compositions are limited and this makes necessary the drying to obtain pure, minimally processed, decreased in volume and easy to use form of the kiwi. The results of the experimental study showed that, it was possible to dry the fresh kiwi puree under the freeze drying condition. In order to improve the drying process, to see the effect of maltodextrin addition and to obtain a more stable powder, maltodextrin was used as a drying aid. The amount of MD to be used to prevent quality losses during drying and to obtain powder which has almost the same properties with fresh kiwi was determined by the preliminary tests. For this purpose, MD with amounts of 5, 10, 15, and 20% of the puree weight were added to the fresh kiwi puree. The addition of MD as 5% of the puree weight was not suitable since there was no decrease in the drying time of kiwi puree. For the MD amounts being more than 10 %, the powders lost their quality characteristics such as specific color, vitamin C content etc. Similar results were observed by QUEK *et al.* (2007). It was reported that after addition of the 10% MD watermelon powders lost their red-orange color. Therefore, as a result of the preliminary tests, the concentration of MD in the puree necessary for successful drying and powder production was determined as %10 of the puree weight. ZEA *et al.* (2013) reported that powder obtained by freeze drying of guava and pitaya pulp was found to be very hygroscopic and difficult to compact. In order to minimize this problem the researchers added 10% maltodextrin to guava and pitaya mash.

The drying behaviour of the freeze drying process was determined from the mass loss in samples of known initial moisture content. For the drying process, the total drying time was determined to be nine and ten hours respectively for the samples of kiwi puree, and kiwi puree with maltodextrin until getting constant weight of the samples. Similar results were obtained by MARQUES and FREIRE (2005) in their freeze drying study on pulps of tropical fruits as ten to thirteen hours.

The average values of the experimental results of the analysis applied on fresh kiwi puree and freeze dried powders are given in Table 1. The initial moisture content of kiwi puree was found to be as 81.19 % (wet basis, wb), and this result was consistent with KAYA *et al.* (2010) (81% wb). The final moisture content of kiwi powder is 9.55 % (wb) after removal of 88.24% of water. For the sample with MD, 94.31% of water was removed where the initial dry matter content of the sample was higher than the plain sample due to maltodextrin addition and the amount of water to be removed at the same drying time decreased. The residual moisture in the powder decreased, and the moisture content of the sample with MD was found to be 56% lower than the plain sample, and this differences between samples was found to be statistically significant ($P<0.05$).

The moisture ratio were calculated by using the determined moisture content values and the data were fitted to ten thin layer drying models (Lewis, Page, Modified Page I, Henderson and Pabis, Logarithmic, Midilli, Modified Midilli, Two-term, Two-term Exponential, and Wang and Singh). The coefficient of correlation

Table 1 - The physical and chemical properties of kiwi puree and freeze dried kiwi puree powders.

Properties	Fresh kiwi puree	Freeze dried kiwi puree powder	Kiwi puree with MD	The freeze dried kiwi puree powder with MD
Moisture content (% wb)	81.19 ±0.02^{b}	9.55±0.64^{r}	73.82±0.04^{a}	4.20±0.05^{p}
Water Activity	0.98 ±0.01^{b}	0.28±0.03^{r}	0.96±0.01^{a}	0.22±0.01^{p}
pH	3.16±0.01^{a}	3.37±0.01^{p}	3.38±0.01^{a}	3.60±0.02^{r}
Color				
L*	47.37±0.35^{a}	77.93±0.53^{p}	48.84±0.34^{a}	78.12±0.44^{p}
a*	-0.67±0.24^{b}	1.16±0.09^{r}	-0.74±0.08^{a}	-6.53±0.12^{p}
b*	17.5±0.29^{a}	21.77±0.17^{p}	17.85±0.18^{a}	22.08±0.11^{p}
Vitamin C (mg/100g, wb)	66.3±0.28^{b}	54.97±0.13^{r}	51.07±0.09^{a}	40.95±0.51^{p}

$^{a-b}$ Different letters in the same row indicate significant difference between averages of puree and puree with MD at P<0.05.
$^{p-r}$ Different letters in the same row indicate significant difference between averages of powder and powder with MD at P<0.05.

(R^2) was accepted one of the primary criterion for selecting the best model to define the freeze drying curves of kiwi puree powders. For freeze drying process of kiwi puree the highest R^2 value (0.994), and the lowest RMSE (0.02459), and $\chi 2$ (0.00083) values were obtained from logarithmic model (Fig. 1). However, for freeze drying of kiwi puree with MD the best fit was obtained from Wang and Singh model (R^2=0.999, RMSE=0.012, χ^2=0.0002) (Fig. 2). In the literature, the convective drying characteristics of kiwi slices were explained with two term exponential (KAYA *et al.*, 2010), Page (CEYLAN *et al.*, 2007; SIMAL *et al.*, 2005), and Henderson and Pabis (DOYMAZ, 2009) models.

The effective moisture diffusivity (D_{eff}) of freeze dried kiwi puree and pure with MD were evaluated as $7.3x10^{-10}$ m^2/s. The difference between calculated values was $0.002x10^{-10}$ m^2/s and this was not considered to be effective. KAYA *et al.* (2010) reported that the effective moisture diffusivity values of kiwi slices which were dried under different drying conditions (air velocity, temperature, and relative humidity) varied between 0.589 and 6.574 $x10^{-10}$ m^2/s. SIMAL *et al.* (2005) reported that the effective moisture diffusivity of hot air dried kiwi slices (30-90°C) ranged between 3.00 and 17.21 $x10^{-10}$ m^2/s. The D_{eff} value of kiwi powder was found to be similar to the D_{eff} value ($7.13x\ 10^{-10}$ m^2/s) of kiwi slices which were dried at 50 °C hot air temperature (SIMAL *et al.*, 2005). The effective moisture diffusivity values in foods are in the range of 10^{-12} to 10^{-6} m^2/s.

Water activity is considered as one of the most important quality factors especially for long term storage and also it is related to moisture content, and responsible for biochemical reactions. The values of water activity under 0.6 is generally considered as microbiological-

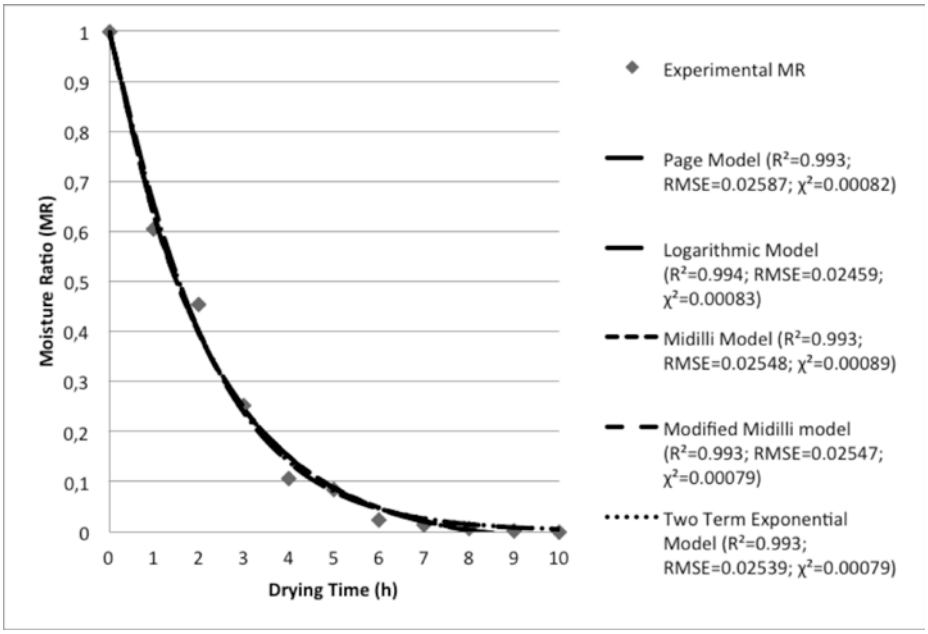

Fig. 1 - Experimental and computed moisture ratio values obtained by selected models for pure kiwi puree powder (R2≥0.993).

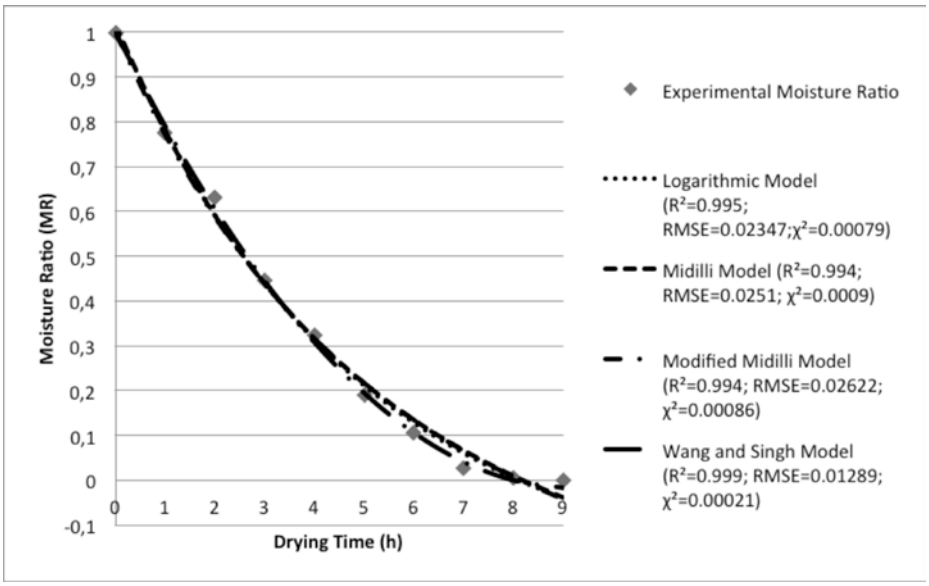

Fig. 2 - Experimental and computed moisture ratio values obtained by selected models for kiwi puree powder with MD (R2≥0.994).

ly stable (QUEK, 2007) and between 0.20, and 0.40 ensure the stability of the product against browning, and hydrolitical reactions, lipid oxidation, auto-oxidation, and enzymatic activity (AMRQUES *et al.*, 2007). The water activity values of freeze dried kiwi puree powders (plain powder and powder with MD) were found to be as 0.287, and 0.225, respectively. In literature water activity values around 0.28 was also expressed for freeze dried guava and pitaya powders with 10% MD (ZEA et al., 2013). Drying process and addition of MD showed the significant effects on the water activity of freeze dried kiwi puree powders (P<0.05).

The pH value of kiwi puree was measured as 3.16. SOUFLEROSA *et al.* (2001) reported that the pH value of kiwi ranges between 3 and 4, due to the content of including the acids such as gluconic, galacturonic, oxalic, succinic, fumaric, oxcaloacetic, and p-coumaric acids. HARDER *et al.* (2009) and ARROQUI *et al.* (2004) measured the pH value of kiwi nectar and puree as 3.50 and 3.41, respectively. The pH values of powders (kiwi puree powder and powder with MD) were found to be as 3.37 and 3.60, respectively. Results showed that the drying process and addition of MD caused a significant increase in the pH value of powders (P<0.05). The increase in the pH values was found as 6.65% and 6.51% for plain and MD containing powders, respectively. This increase was comparable with the increase in 3.64% in freeze drying of guava concentrate MAHENDRAN (2010) and the reason for the increase can be explained with the loss of some acidic compounds during drying.

Color of the dried products is an important quality factor, which reflects the sensory attractiveness, and the quality of the powders (QUEK *et al.*, 2007). Thus, the color of the processed products should ideally remain unchanged after production. The color values (L*, a* and b*) of kiwi puree were measured as 47.37, -0.67, and 17.5 respectively. These values are quite different than the measurements of ANCOS (1999) reporting the color values (L*, a*, and b*) of kiwi puree 36.01, -12.35 and 23.03, respectively and this shows the differences between the cultivars and the storage time after harvest. The variation of color values for plain and MD containing samples depending on the drying time were shown in Figs. 3 and 4, respectively. As shown in Fig. 3, the L*, b* and a* values of freeze dried kiwi puree powder increased throughout the drying period and reached the final values as 77.93, 1.16, and 21.77, respectively. CHOPDA and BARRETT (2001) reported that the increase in L* (brightness), a* (redness) and b* (yellowness) values following production of guava puree powder was most likely a result of non-enzymatic browning during freeze drying which produced a darker product. The addition of MD in freeze drying, increased the L* (78.12), and b* (22.08) values, but decreased a* value (-6.53) (Table 1). Results showed that, drying process increased the brightness values of samples (P<0.05); addition of MD caused superior bright color but it was not found to be statistically significant (P>0.05). The same effect was also observed for yellow-blue (b*) value. Nevertheless, both drying process, and addition of MD showed a significant effect on the green-red (a*) value of the samples (P<0.05).

For the determination of vitamin C, freeze dried powders were rehydrated to the initial moisture content prior to the analysis to obtain comparable results. The vitamin C content of kiwi was found

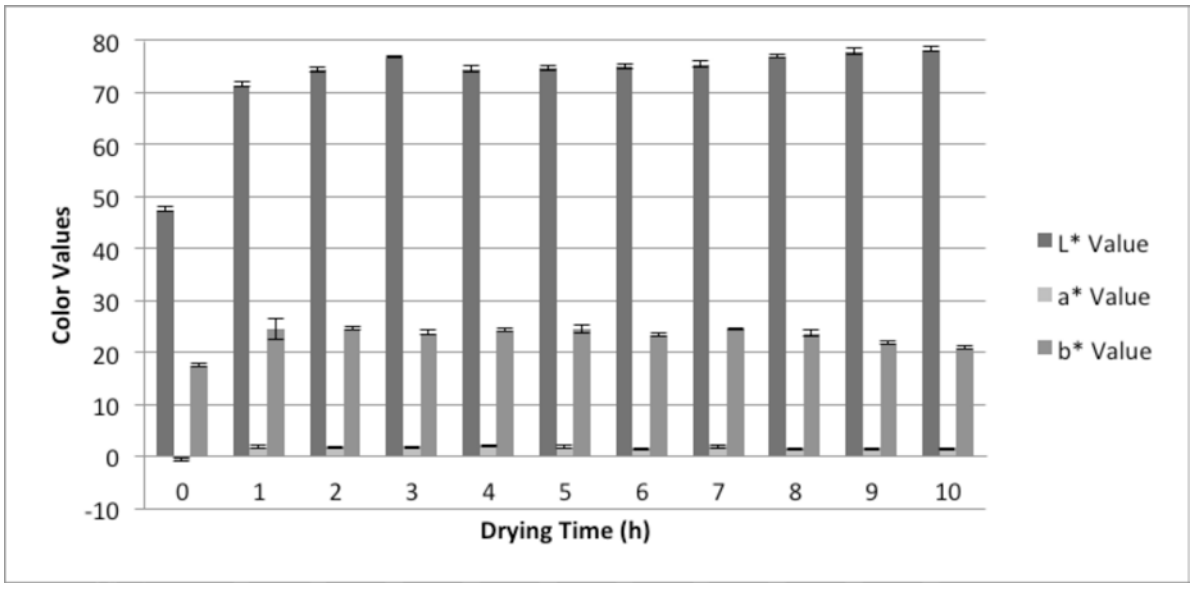

Fig. 3 - The variation of color values of plain samples depending on the drying time.

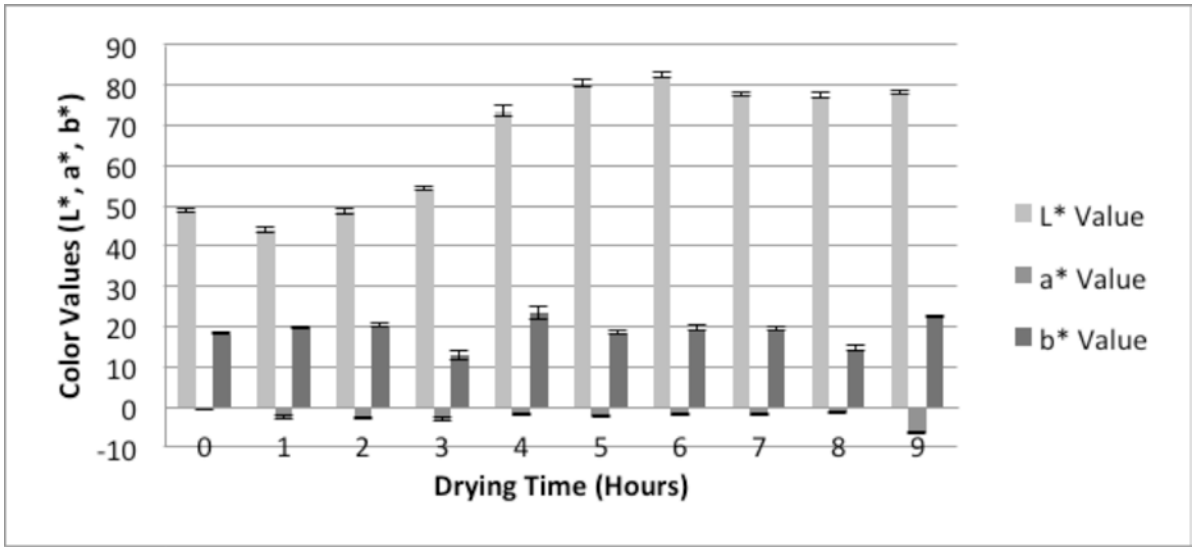

Fig. 4 - The variation of the color values of samples with MD depending on the drying time.

to be as 66.3±0.28 mg/ 100 g (wb) kiwi. The freeze drying process caused a significant (17.1%) decrease on the vitamin C content of kiwi powder ($P<0.05$). The vitamin C loss during drying is similar to losses of 18.8% (MAHENDRAN, 2010) and 16% (MARQUES *et al.* 2006) during freeze drying of some other fruit concentrate and pulps. Also, the addition of MD caused an insignificant loss in the vitamin C content (19.82%) ($P>0.05$). This decrease may occur due to the dilution effect. Exposure to heat, light, oxygen and metals may also lead to vitamin C losses. LIN *et al.* (1998) did not observed significant loss of Vitamin C in freeze-dried carrots. The vitamin C losses can be due to not only the freeze drying, but also by the operations before drying such as cutting, slicing and freezing. Therefore, grinding process, preparation of maltodextrin and kiwi puree blend may cause more vitamin C losses for the kiwi puree. MARQUES *et al.* (2011) reported that the vitamin C losses for freeze dried fruits are considerably smaller when compared the vitamin C losses caused to others drying methods due to the low temperatures, and to the use of vacuum in the process.

Glass transition temperature

In order to have safety storage, and stability of powders, the powders should be kept below glass transition temperature (T_g). So the T_g value of kiwi powders was determined. Kiwi powder exhibited well defined T_g (average -18°C) represented by an endothermic change in the base

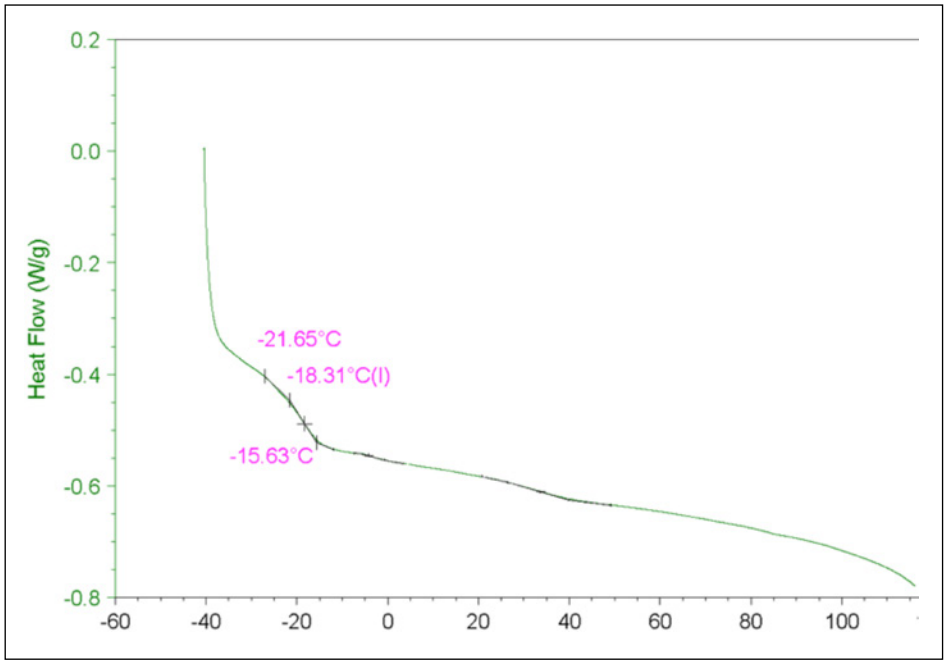

Fig. 5 - DSC thermogram for freeze dried kiwi puree powders.

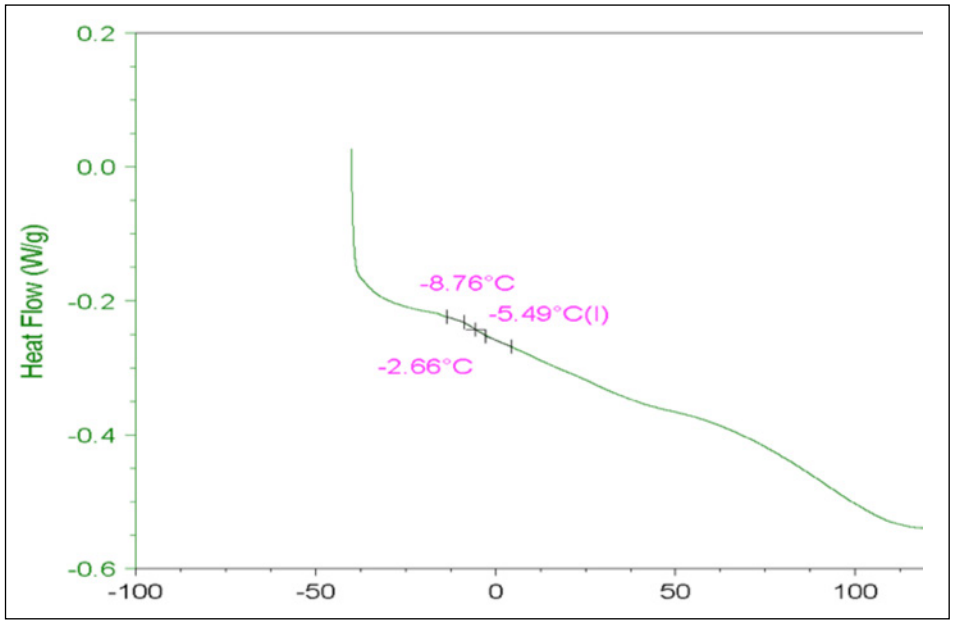

Fig. 6 - DSC thermogram for freeze dried kiwi puree powders with MD.

line (Fig. 5). Moisture content and water activity are the main factors affecting T_g of materials. However, in the consideration of food materials with similar moisture content and water activity values, the high acid and sugar content may decrease the T_g value. The increases in T_g values of kiwi puree powders with carriers possibly due to the addition of carriers, and the lower moisture content of carrier-incorporated powders. T_g of kiwi puree powders with MD (T_g average -5°C) was found to be higher (Fig. 6). SILVA *et al.* (2006) reported that, addition of 30% MD (w/w, DE20) increased Tg of freeze dried camu-camu pulp from -58.8°C to -40.1°C for the moisture content values between 0.2 to 0.5 (g dry solid/ g sample). After this value, T_g increased rapidly with decreasing moisture content. In their study, MOSQUERA *et al.* (2010) observed an increase in Tg with the addition of MD and this increase was slightly more where MD with low DE was used.

Thermo gravimetric analysis

The results of the analysis of the samples of kiwi puree powders by TGA are shown in

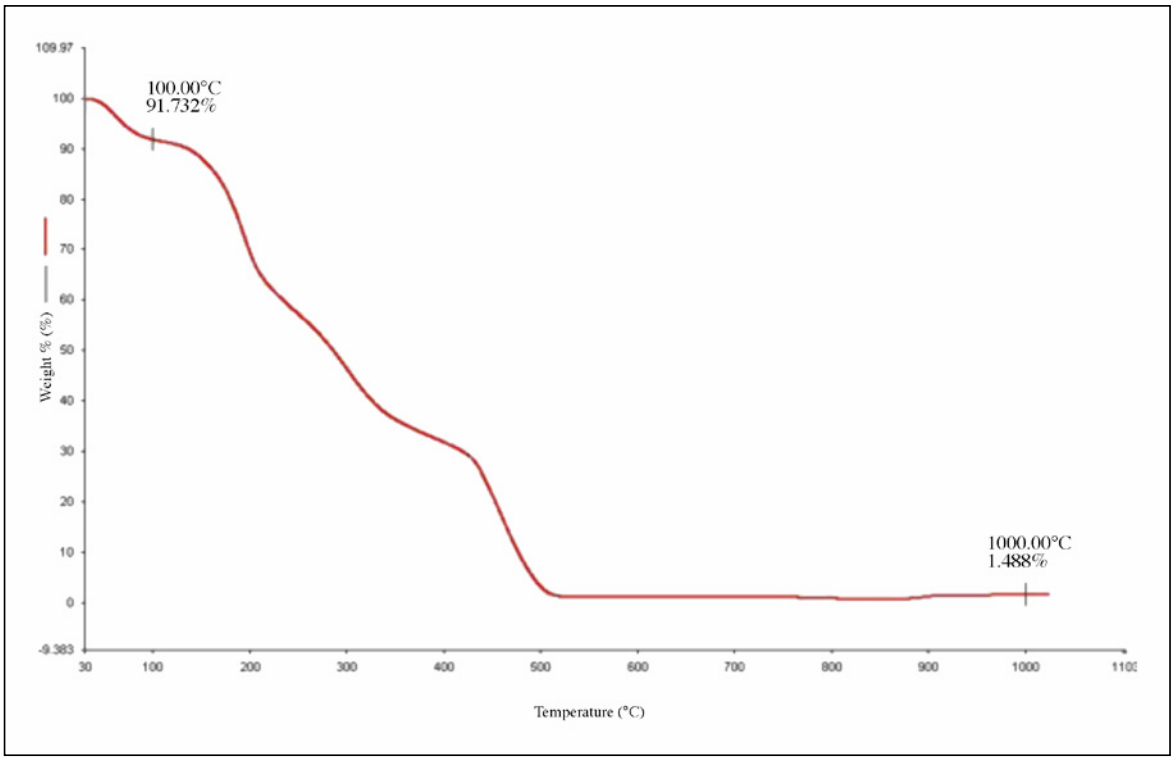

Fig. 7 - The variation of the weight of freeze dried kiwi puree powder with respect to time.

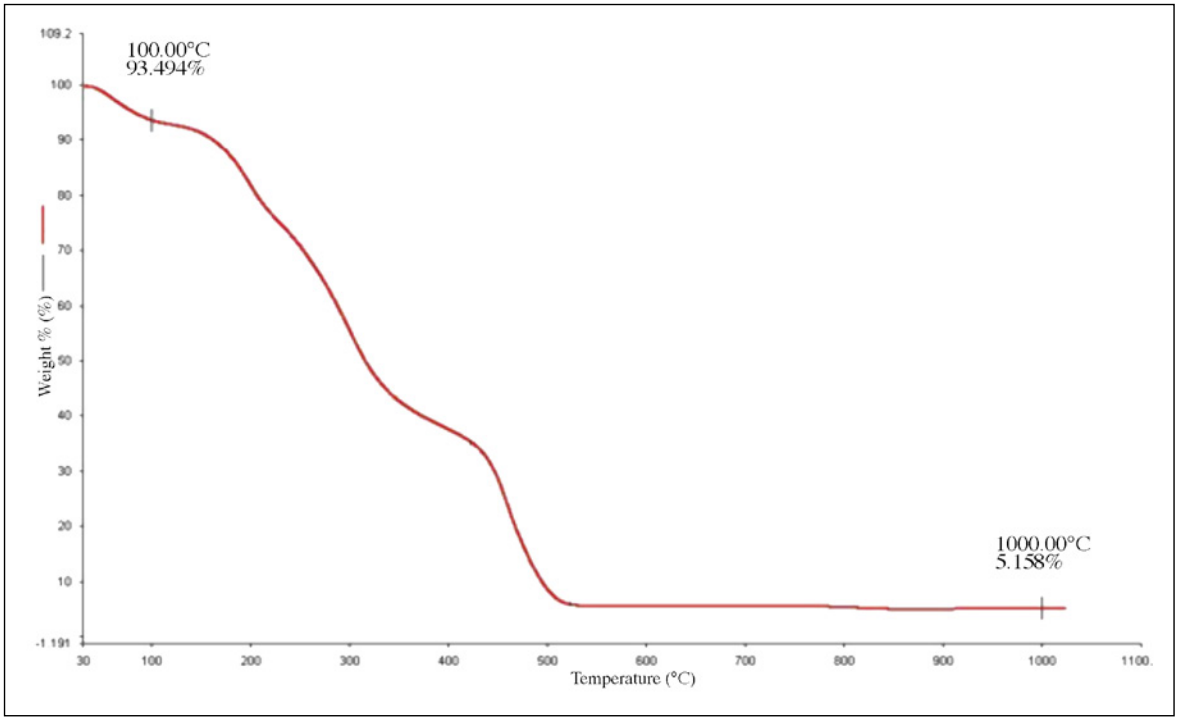

Fig. 8 - The variation of the weight of freeze dried kiwi puree powder with MD with respect to time.

the Figs. 7 and 8. These spectra determine the changes of weight in relation to change of temperature that the samples experiment when exposed to heating from room temperature to 1000°C. TGA spectra showed that the loss of matter began around 50°C for both samples but the kinetics of thermal decomposition is different for them. At 100°C, the sample with 10% MD lost around 6.5% of its own weight, but the sample that was dried without MD lost around 8.5% of its own weight. Their components were considerably stable until 150°C because the loss of matter is not significant. However, between 100 and 220°C, reactions such as Maillard's

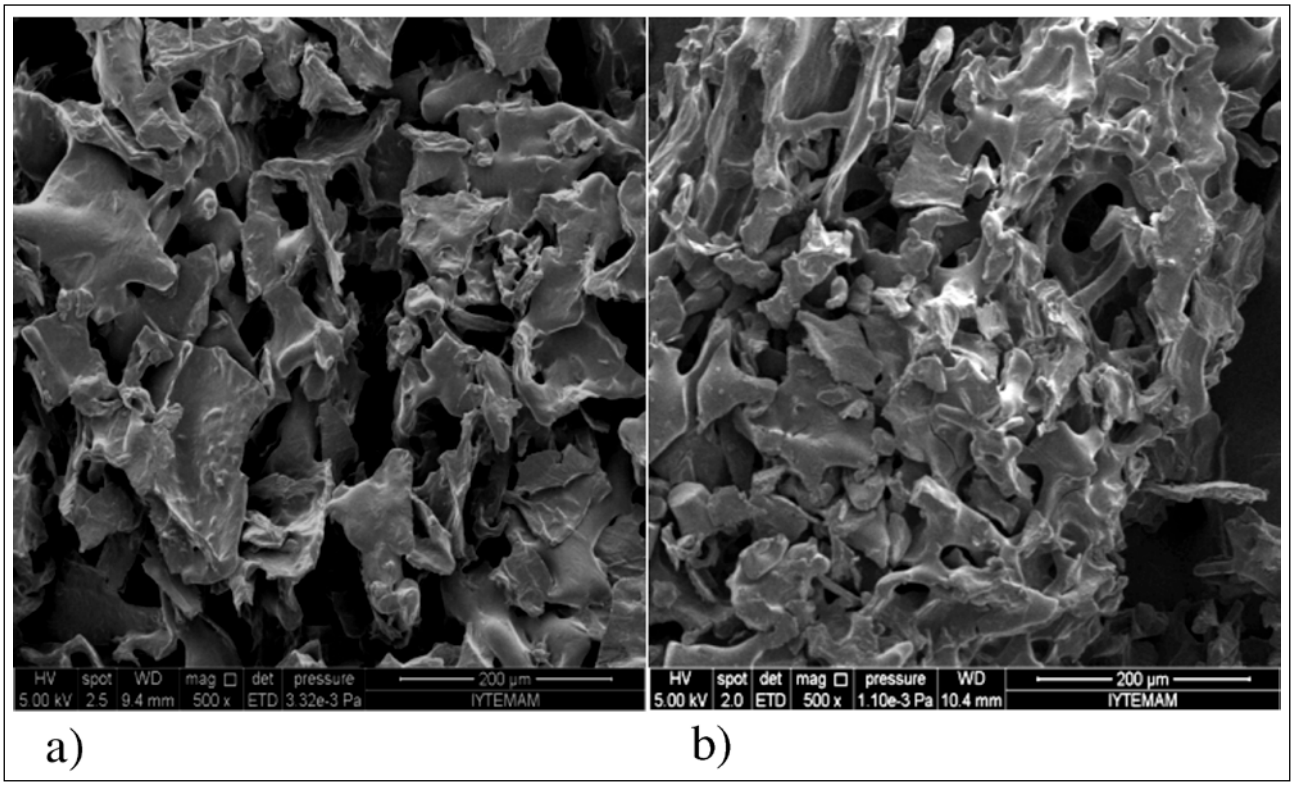

Fig. 9 - Scanning electron micrographs of freeze dried plain (a) and MD containing (b) kiwi puree powder at 500x magnification.

reaction or the condensation between phenolic acids and proteins may occur. As of 150°C, the loss of matter is significant, and the phenomena are exothermic for all samples.

Scanning Electron Microscope (SEM)

Selected images from the SEM microstructure analysis of the freeze dried kiwi puree powders were shown in Fig. 9 (a and b). The microstructures of freeze-dried kiwi powder had a skeletal-like structure with void spaces previously occupied by ice prior to freeze drying. This is because the absence of liquid phase in the material during freeze drying process suppressed the transfer of liquid water to the surface and the ice was converted to vapor without passing the liquid state (KROKIDA and MAROULIS, 1997). Micrographs revealed that powder particles of all powders were irregular in shape. Irregular shape of powder particles may due to the fibrous and porous nature of the kiwi fruit powders since powder was prepared from whole fruits (ZEA *et al.*, 2013).

Powder properties

The powder properties of freeze dried kiwi puree powders are given in Table 2. The tapped and bulk densities of freeze dried kiwi puree powder were found to be as 0.257 and 0.161 g/ml, and the addition of MD significantly increased the tapped and bulk densities of powder (0.416 and 0.316 g/ml) ($P<0.05$). MARQUES *et al.* (2006) reported that, apparent density of the studied pulps has presented a linear relationship with moisture content where the apparent densities of fruit pulps decreased linearly with moisture

Table 2 - The powder properties of freeze dried kiwi puree powders.

Powder Properties	Freeze dried kiwi puree powder	Freeze dried kiwi puree powder with MD
Tapped Density (g/mL)	0.26±0.01[a]	0.42±0.02[b]
Bulk Density (g/mL)	0.16±0.01[a]	0.32±0.01[b]
Solubility (s)	26±3[a]	290 ±48[b]
Wettability (s)	78.5±2[a]	186 ±0.71[b]
Flowability (CI)	38±3[b] (Bad)	24.04±2.87[a] (Fair)
Cohesiveness (HR)	1.60±0.08[b] (High)	1.32± 0.05[a] (Intermediate)

[a-b]Different letters in the same row indicate significant difference between averages at $P<0.05$.

content (dry basis) during freeze drying and the real density increased. The researchers reported that the remaining solids after moisture removal have higher densities than water and the overall solid density tends to increase as moisture is removed. MAHENDRAN (2010) dried the guava concentrate with different drying methods (freeze drying, tunnel drying and spray drying with the 30, 40, 50 and 60% concentrations of MD) and the bulk density of guava powders were measured as 0.63 g/mL; 0.69 g/mL and 0.61, 0.60, 0.57 and 0.54 g/mL, respectively. In this study, on the contrary of the results given by MAHENDRAN (2010) the bulk density increased with the addition of the MD. Lower density of the dried product is recommended to increase its attractiveness for consumers (DURANCE and WANG, 2002).

The average solubility time of the freeze dried kiwi puree powder was found to be as 26 seconds. The reason for the addition of MD was to improve the drying process and at the same time maltodextrin is highly soluble in the water to be used as a carrier. However, addition of maltodextrin caused a significant increase in the average solubility time of the powder (290s) ($P<0.05$). In a study by MAHENDRAN (2010) guava concentrate was dried with spray, tunnel, and freeze driers and the freeze dried guava powder was found highly soluble (96%) compared with the other drying methods. The solubility of the powder is related with moisture content, particle size, and chemical conversions in the material (GOULA and ADAMOPOULOS, 2008). Wettability is the ability of the powder particles to overcome the surface tension between themselves, and water. Wettability depends on particle size, density, porosity, surface tension, surface area, and surface activity of particle. Besides the effects of physical properties, the chemical composition of the powders also influences wettability depending on the content of fats, proteins, and carbohydrates on their surface (FANG *et al.*, 2008). Also, GOULA and ADAMAPOULOS (2008) reported that the residual moisture content of the powder affects the bulk density, wettability, flowability, and cohesiveness. The residual moisture content of powders is significantly affected the operational conditions, and carrier concentrations. The average wettability time of freeze dried kiwi powder was found to be as 78.5 seconds. Addition of MD caused a significant increase in the average wettability time as 186s ($P<0.05$).

Flow difficulties and caking are common problems in industries producing food powders. The flowability and cohesiveness properties of kiwi powders in terms of Carr Index and Hausner ratio were evaluated. The classification of powder flowability based on Carr index (CI) is very good (<15), good (15-20), fair (20-35), bad (35-45), and very bad (>45). The powder cohesiveness based on Hausner ratio (HR) is classified as low (<1.2), intermediate (1.2-1.4), and high (>1.4) (JINAPONG *et al.*, 2008). Kiwi powder with higher moisture content showed bad flowability (37.15±3.15) and high (1.59±0.08) cohesiveness. However, addition of MD caused a significant decrease in cohesiveness (1.29), and significant increase in flowability (22.36) behaviours of powder ($P<0.05$). The kiwi powder containing MD with low moisture content showed superior flow properties compared to kiwi powder.

CONCLUSIONS

The present work describes the possibility of producing kiwi puree powder by freeze drying, and the changes in some physicochemical and powder properties of powders which were affected by drying process and addition of MD. The results showed that freeze drying can satisfactorily be applied for drying of kiwi puree to obtain powders that can be used as an ingredient which have high vitamin C content for flavoring and improving nutritional value purposes. The possible uses of this dried product as a food supplement with valuable constituents of kiwi fruits and storage test might be studied in future projects.

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PAPER

MINERAL CONTENTS AND PHYSICAL, CHEMICAL, SENSORY PROPERTIES OF ICE CREAM ENRICHED WITH DATE FIBRE

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ABSTRACT

Date samples of Amber cultivar straining from Medina region (Saudi Arabia) were analysed for their chemical compositions and physicochemical properties of date fibre in the present study. Fibre rich date pieces were found to contain 80.2 g/100 g total dietary fibre, 16.32 g water/g sample water-holding capacity while 9.50 g oil/g sample oil-holding capacity. It can be stated from these results that fibre content of date is a valuable dietary fibre source and used in food production as an ingredient. Effects of the addition of date fibres at different concentrations (1, 2, 3 and 4%) were investigated on the physical, chemical, sensory properties and mineral content of ice cream in the present study. It was found that elemental composition of ice cream samples was affected significantly by the addition of date fibre concentrations ($p<0.05$) and the rates of K, Mg and Zn especially increased in the samples depending on the content of date fibre while the content of Ca and P decreased. It was determined from the sensory results that ice cream sample containing date fibre in the rate of 1 and 2% received the highest score from panellists.

- Keywords: date fibre, ice cream, elemental composition, nutraceutical ingredient -

INTRODUCTION

Dietary fibre as a class of compounds includes a mixture of plant carbohydrate polymers, both oligosaccharides and polysaccharides (cellulose, hemicelluloses, pectic substances, gums, resistant starch, inulin) that may be associated with lignin and other non-carbohydrate components (polyphenols, waxes, saponins, cutin, phytates, resistant protein; ELLEUCH *et al.*, 2011). Over the last decades, knowledge on dietary fibre has increased considerably, both in the physiological and analytical areas. Health benefits of dietary fibre are associated with bowel function, reduced risk of coronary heart diseases, type 2 diabetes and improved weight maintenance (AGOSTONI *et al.*, 2010; HAUNER *et al.*, 2012; WESTENBRINK *et al.*, 2012). In addition, dietary fibre can provide a multitude of functional properties when they are incorporated in food systems. Several advantages of using fruit fibres in ice cream production are improvement in body due to the fibrous framework and melting properties, reduction of cold impression, reduction of recrystallization causing prolonged shelf-life, and enhancing mixed viscosities allowing freezing at higher overrun, causing no negative effect on the ice crystal sizes, and leading to a more homogenous air-bubble formation (ANONYMOUS, 2000; DERVISOGLU and YAZICI, 2006). Thus, fibre addition contributes to the modification and improvement of the texture, sensory characteristics and shelf-life of food due to their water binding capacity, gel-forming ability, fat mimetic, anti-sticking, anti-clumping, texturising and thickening effects (DELLO STAFFOLO *et al.*, 2004; GELROTH and RANHOTRA, 2001; THEBAUDIN *et al.*, 1997, chap. 23; SOUKOULIS *et al.*, 2009). There is little data dealing with the study of the functionality of dietary fibre in ice creams (SOUKOULIS *et al.*, 2009). Date (*Phoenix dactylifera* L.) provides a good source of dietary fibre content, is also considered to be a commercially important agricultural commodity as well as vital element of the daily diet and a nutritious food in the Arabian world (KHAN *et al.*, 2008; AL-FARSI and LEE, 2008) generally being consumed fresh or processed into various products (SINGH *et al.*, 2013). Annual production rate of date all across the world in 2010 was about 7.91 million tons, which increased in the rate of 6.6% in when compared to 2009 (FAO, 2011; AHMED *et al.*, 2013). Different varieties of date vary in their chemical composition especially in sugars and dietary fibre (MUSTAFA *et al.*, 1986; AHMED *et al.*, 1995; RAHMAN and AL-FARSI, 2005; SINGH *et al.*, 2013). The importance of date in human nutrition comes from its rich composition of carbohydrates (70-80%), salts and minerals, dietary fibres, vitamins, fatty acids, antioxidants, amino acids and protein (EL-BELTAGY *et al.*, 2009; AL-SHAHIB and MARSHALL, 2003; EL-NAGGA and ABD EL–TAWAB, 2012; AL-FARSI *et al.*, 2005, 2007; BIGLARI *et al.*, 2008; HONG *et al.*, 2006; MANSOURI *et al.*, 2005; VAYALIL, 2002; KCHAOU *et al.*, 2013). In fact, date fruit has been used in traditional medicine as immune system stimulator (PURI *et al.*, 2000), and as treatment for various infectious diseases (DUKE, 1992; MARTÍN-SÁNCHEZ *et al.*, 2013).

However, such a valuable nutrient is generally discarded or used in animal feeding. A serious economic loss can be experienced unless such a useful fruit and its products are used in human diet and food since it is rich in bioactive compounds, which can be extracted and used as value added materials. Development of new food products using date flesh is the topic of very limited number of studies. The objective of the present study is to characterize and evaluate the functional properties of the us date fibre (DF) taking into account the quality and nutritional content of ice cream.

MATERIALS AND METHODS

Materials

Cows' milk and cream were obtained by the Research and Application Farm of Atatürk University. Amber dates were purchased from the palm garden in Medina city of Saudi Arabia. Sugar, salep and emulsifier (mono- and diglycerides) were obtained from local markets. Skim milk powder was supplied by Pınar Dairy Products Co. (Turkey).

Preparation of date flour

Date fibre concentrates were extracted from the Medina cultivar 'Amber' as described previously (ELLEUCH *et al.*, 2008). DF from whole fruits were extracted in boiling water for 15 min, using a magnetic stirrer. After solubilisation of the sugars (sucrose, glucose and fructose), date fibres and pits were recovered through filtration using a 0.2 mm sieve. The pits were then removed. The fibres were concentrated by successive rinsing (water at 40°C) and filtration until the residue was free of sugar as described. The residues obtained were pressed dried, in oven at 65°C for 24 h and milled in a Mill Laboratory at 2890 rpm, then at 5000 rpm until they could pass through a 0.2 mm sieve to recover the date fibre concentrate, and stored at -18°C for subsequent physicochemical analyses and incorporation studies.

Chemical composition

Moisture content was determined according to the Association of Official Analytical Chemists (AOAC, 1997) method. Ash was analysed by combusting the sample in a muffle furnace at 550°C for 4 h. The residue was dissolved in HNO_3 and

the mineral constituents (Ca, K, Na, Mg, P and Fe) were determined using an inductively couple plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT, USA). The Bligh and Dyer method (HANSON and OLLEY, 1963) was used to determine the lipid content. Protein content was determined by micro kjeldahl method (AOAC, 1990) and expressed as: % N_2x6,23. Total sugars were extracted through ethanol (80%) (NINIO *et al.*, 2003). After centrifugation, the supernatant was collected and the total sugar content was analysed using phenol/sulphuric acid reagent (DUBOIS *et al.*, 1956). The total phenolic content was analysed according to the Folin-Ciocalteu method developed by AL-FARSI *et al.* (2005). The extract (200 µL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (previously diluted 10-fold with distilled water) for 5 min at room temperature. 1.5 mL of aqueous sodium bicarbonate (60 g/L) was added and the mixture was vortexed and allowed to stand at room temperature. After 90 min, the absorbance was measured at 725 nm. The total phenol concentration was expressed as the mean ± SD as mg of gallic acid equivalent (mg GAE) per 100 g of fresh weight of date for two replicates. AOAC enzymatic-gravimetric official method (991.43; AOAC, 1995) was used to determine dietary fibres while dry matter content, fat, ash, acidity (°SH) and pH of ice cream samples were determined as in DEMIRCI and GUNDUZ (1994). Mineral contents (Ca, K, Na, P, S, Mg, Fe, Mn, Zn, Ni) of ice cream samples were determined using an Inductively couple plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT, USA) and following the method described by GULER (2007). Samples were decomposed in a microwave oven (Berghof speed wave MWS-2, Eningen, Germany). For this purpose, about 0.5 g ice cream sample was weighed into the digestion vessels, added concentrated nitric acid (10 mL) and digestion process was realized over each sample at 210°C and under 176 psi pressure for 10 min. After cooling, the carousels were removed from the oven, 30% hydrogen peroxide (2 mL) was added to samples and then second digestion was applied at 195°C and under 95 psi pressure for 5 min. The vessels were immediately closed after the addition of oxidants. At the end of the digestion process, the samples were diluted in with distilled water to an appropriate concentration and filtered through Whatman No. 42 filter paper. All diluted digests were eventually analysed using an Inductively couple plasma spectrophotometer (ICP-OES).

Water and oil holding capacities, and pH of fibre

Water and oil holding capacities (WHC and OHC) of the fibres were determined according to the methods of MAC-CONNELL *et al.* (1997) and CAPREZ *et al.* (1986), respectively. WHC and OHC values represented the amount of water and oil absorbed per gram of sample, respectively. pH of DF was measured using a pH meter (WTW 340-1) and following the method described by SUNTHARALINGAM and RAVINDRAN (1993).

Ice cream manufacture

The ice cream samples were prepared in the Pilot Plant of Food Engineering Department, Agriculture Faculty, Atatürk University. Initially, the fat ratio of cows' milk was adjusted to 6% by adding cream. Then, the milk was divided into five equal parts of 2 kg. For each mix, skim milk powder (125 g), sugar (405 g), salep (stabilizer) (16.2 g), emulsifier (mono- and di-glycerides) (6.75 g) were added to each mix. Then prepared date fibres were added at four different concentrations: 1, 2, 3 and 4% to mixture weight. The mixes were pasteurized at 85°C for 25 min and stored at 4°C for 24 h. Then, they were iced in ice cream machinery (-5°C; Ugur Cooling Machineries Co., Nazilli, Turkey) and hardened at -22°C for 24 h and stored at -18°C and used for physical, chemical, mineral and sensory analyses. Ice cream samples were produced in duplicate.

Ice cream analysis

Physical measurements. Overrun was determined using a standard 100 mL cup, according to the equation [(volume of ice cream)-(volume of mix)/volume of mix×100] given by JIMENEZ-FLOREZ *et al.* (1993). First dripping and complete melting times were measured according to GUVEN and KARACA (2002) 25 g of tempered samples were left to melt (at room temperature, 20°C) on a 0.2 cm wire mesh screen above a beaker. First dripping and complete melting times of the samples were accepted to be seconds. The viscosities of the ice cream mixes were determined at 4°C using a digital Brookfield Viscometer, Model DV-II (Brookfield Engineering Laboratories, Stoughton, MA, USA). Before measuring the viscosity, the samples were stirred gently to remove the air from the mixes (AKIN *et al.*, 2007). The color analyses (L*, a* and b* values) of the ice cream mix were carried out using in Minolta colorimeter (Chroma Meter, CR-200, Osaka, Japan; ANONYMOUS, 1979). The colorimeter was calibrated using a white reference plate before measurements. Light source for the colorimeter was standard daylight (C) and the standard observer was 2°.

Sensory evaluation

Eight professional panellists from the Food Engineering Department of Atatürk University, Erzurum, Turkey, participated in the study to determine some properties using a score test for flavour, body and texture, color and appearance,

resistance to melting and general acceptability. Hardened ice cream samples were tested at a serving temperature of -10°C and scored their sensory characteristics in a scale ranging from 1 (poor) to 9 (excellent). Warm water and bread were also provided to the panellists to cleanse their palates between samples. All panellists were non-smokers, had prior testing experience with a variety of dairy products including milk, cheese and ice cream and had previously used flavour profile procedures adapted from ROLAND *et al.* (1999).

Statistical analysis

All statistical analysis was performed using SAS for windows (SAS, 1998). Analysis of variance was performed using the routine Proc ANOVA. Significant treatment was separated using Duncan's Multiple Range Test (DUZGUNES *et al.*, 1987).

RESULTS AND DISCUSSION

Physical and chemical characteristics of date fibre

Dry matter, fat, acidity (°SH) and pH values of milk, skim milk powder and cream used in the production of the ice cream are given in Table 1. Date and date fibre were analyzed for moisture, ash, fat, total sugars, color, total phenolic content, WHC and OHC (Table 2). Date fibres are rich in protein (9.01 g/100 g). Earlier, EL-LEUCH *et al.* (2008) reported 9 g/100 g protein contents for Tunisian dates and similar to the present work. Presence of high protein content in fruit fibres (11.6-14.4 g/100 g) is reported in the literature (BRAVO and SAURA-CALIXTO, 1998; SAURA-CALIXTO, 1998). In the present study, calcium, sodium, potassium and magnesium contents of date fibre were measured to be 1925, 56.5, 981 and 1807 mg/kg, respectively. AHMED *et al.* (2013) reported that the sodium content was significantly lower than other minerals (55-86 mg/kg); however, the fibres were rich in potassium. The Barhee cultivar possessed exceptionally higher amount of potassium (2600 mg/kg), and the maximum sodium was found in Owadi cultivar. These results are significantly different from the reported values for date flesh (EL-LEUCH *et al.*, 2008). The variation could originate from the cultivar, and agro-climatic as well as environmental conditions.

Date contains high proportions of total dietary fibre (80.2 g/100g) similarly to those reported in Deglet-Nour and Allig (two varieties of date) between 88 and 92%, respectively (EL-LEUCH *et al.*, 2008). In addition, the contents of dietary fibre in dried apricots, prunes, figs, and raisins were 7.7, 8.0, 12.2, and 5.1 g/100 g, respectively (CAMIRE and OUGHERTY, 2003; MARLETT *et al.*, 1994; VINSON, 1999). Thus, dates and their by-products serve as good sources of fibre compared with syrups and other fresh and most dried fruits. In addition, these DF contents are close to levels measured for DF preparations from apple (Liberty cultivars) (89.8%), but notably higher than those of other fruit DF concentrates reported for grapefruit, lemon, orange, apple and mango (28-78.2%) (FIGUEROLA *et al.*, 2005; VERGARA-VALENCIA *et al.*, 2007),

Table 1 - The gross chemical, physical properties and mineral contents of raw milk, skim milk, cream.

Analysis	Milk	Skim milk powder	Cream
Dry matter (%)	11.37	95.17	63.76
Fat (%)	3.5	1.00	65.00
Ash (%)	0.67	-	-
Acidity (°SH)	5.81	-	13.98
pH	6.40	-	4.95
Minerals (mg kg^{-1})			
Ca	1224.00		
K	1397.00		
Mg	91.67		
P	869.54		
Na	327.90		
Fe	13.56		

- Not determined.

Table 2 - Chemical and physical properties of date fibre and date.

Chemical analysis	Date	Date fibre
Moisture (g/100 g)	13.61±0.11	3.87±0.13
Ash (g/100 g)	1.79±0.07	2.06±0.04
pH	6.00±0.21	5.71±0.02
Protein (g/100 g)	1.23±0.16	9.01±0.75
Fat (g/100 g)	3.41±0.03	0.98±1.21
Total sugars	78.20	0
Total phenolic content[c]	186±2.30	0.73±0.01
Total dietary fibre (g/100 g)	8.75±0.96	80.2±1.06
Minerals (mg kg^{-1})		
Ca	23.40±0.51	1925±1.84
K	428±0.14	981±2.04
Mg	84.51±0.22	1807±0.82
P	90.19±1.36	1325±0.51
Na	17.65±0.12	56.5±0.05
Fe	2.03±0.07	24.82±1.36
Physical Analysis		
L*	23.8±0.04	61.08±0.05
a*	11.0±0.03	6.35±0.01
b*	8.9±0.07	14.72±0.01
WHC[a]	-	16.32±0.47
OHC[b]	-	9.50±0.23

[a]Water holding capacity (g water/g, sample);
[b]Oil holding capacity (g oil/g, sample);
[c]g/100 g of DF concentrates;
L* = lightness; a* = redness (+) and blueness (–); b* = yellowness.

grape skins (54.1–64.6%) (BRAVO and SAURA-CALIXTO, 1998; SAURA-CALIXTO, 1998), citrus peel (57%; CHAU and HUANG, 2003), or fibre from lime peels (66.7% and 70.4%; UBANDO *et al.*, 2005) and mango peel (71%; LARRAURI *et al.*, 1996).

WHC was found to be 16.32 (g water/g, sample) in date fibre in the present study. WHC in of date fibres was reported to be significantly higher than those of fruit and vegetable fibres (FEMENIA *et al.*, 1997; GAN and LATIFF, 2011; LOPEZ *et al.*, 1996; VERGARA-VALENCIA *et al.*, 2007; AHMED *et al.*, 2013), but similar to those found in date (15.5 g/g, dry matter) by ELLEUCH *et al.* (2008). OHC is another functional property of some ingredients used in formulated food. In general, date fibre showed significantly higher OHC (9.5 g oil/g, sample) when compared to other fruit and vegetable derived fibres (GAN and LATIFF, 2011; VERGARA-VALENCIA *et al.*, 2007). The highest OHC was observed for Allig cultivar (9.9 g oil/g sample) followed by ELLEUCH *et al.* (2008). Higher OHC of date fibre indicated that it could be used as an ingredient to stabilize foods with a high percentage of fat (ELLEUCH *et al.*, 2008; AHMED *et al.*, 2013). The mean L*, a* and b* values were found to be 61.08, 6.35 and 14.72, respectively. This could be due, on the one hand, to the wash operations during the extraction and concentration of DF and, on the other hand, to the solubility of pigments responsible for the dark units of color. ELLEUCH *et al.* (2008) reported that L*, a* and b* values were 61.92, 7.11 and 14.85, respectively for Allig, which are convenient with the present study. GOÑI *et al.* (2009) informed that PP associated with polysaccharides and proteins in cell walls are significant constituents of date fibre. Table 2 shows date fibre polyphenol (PP) contents (0.73 g/100 g).

Physical and chemical characteristics of ice cream samples

The results of some physical, chemical analyses and mineral contents of ice cream samples are given in Tables 3 and 4. The dry-matter content of control sample was lower than other samples at statistically significant levels ($p<0.05$). The dry matter rates of ice cream increased with the addition of DF concentration. The highest fat and acidity ratios were found to be in control sample (4.63%). pH values of ice

Table 3 - Some chemical and physical properties of ice cream samples with date fibre.

Analysis	C	DF1	DF2	DF3	DF4
Moisture (%)	33.15±0.02a	33.32±0.29b	33.49±0.10a	33.63±0.36b	34.05±0.01c
Ash (%)	0.89±0.01a	0.92±0.01ab	0.95±0.01b	1.06 ±0.02c	1.10±0.01c
Fat (%)	4.63±1.41d	4.17±0.14c	4.15±0.03c	3.91±0.01b	3.86±0.02a
Acidity(°SH)	8.99±0.00e	6.23±0.02a	6.38±0.01b	6.54±0.01c	6.73±0.02d
pH	6.20±0.02e	5.62±0.02d	5.56±0.01c	5.23±0.03b	5.09±0.01a
L*	83.33±0.01d	82.26±0.04d	80.27±0.03c	77.64±0.91b	75.45±0.07a
a*	1.62±0.05a	2.54±0.04b	2.73±0.01c	3.21±0.04d	3.90±0.01e
b*	9.15±0.02a	9.20±0.01a	11.60±0.14b	12.40±0.01c	12.50±0.02c
Overrun (%)	40.51±0.00e	39.32±0.21d	37.20±0.01c	32.24±0.19b	29.87±0.06a
Complete melting time (s)	0.43±0.02b	0.50±0.00c	0.46±0.02b	0.35±0.01a	0.38±0.00a

Mean values followed by different letters in the same row are significantly different ($p<0.05$).
C: Control (without date fibre); DF1: ice cream with made date fibre 1% (w/w); DF2: ice cream with date fibre 2% (w/w); DF3: ice cream with date fibre 3% (w/w); DF4: ice cream with date fibre 4% (w/w).

Table 4 - Elemental composition (mg kg^{-1}) of the ash in ice cream with date fibre.

Concentrations of minerals	C	DF1	DF2	DF3	DF4
Ca	1844.36±12.72e	1623.25±2.82d	1547±2.12c	1481.40±2.12a	1514.06±7.77b
K	1669.56±21.20a	1913.06±4.15b	1939.18±1.33bc	2043.46±80.63cd	2135.46±49.95d
Na	537.68±6.37b	572±0.04c	690±0.70d	528.5±0.70a	573±0.01c
P	1100.86±0.01c	1257.05±4.24d	1019±2.12b	1100±0.71c	1006±2.82a
S	875.24±1.41a	938.50±17.67b	980±0.70c	1015±7.07d	1103±2.12e
Mg	159.31±1.39a	161.32±1.15a	164.78±0.72b	171.06±0.12c	183.33±1.59d
Fe	10.82±0.24a	11.17±0.05b	14.73±0.02c	21.02±0.01d	29.65±0.22e
Mn	0.32±0.01b	0.35±0.07c	0.26±0.02a	0.30±0.01b	0.40±0.01d
Zn	57.84±0.86a	70.82±0.95b	84.03±0.89c	91.13±1.36d	94.56±3.93d
Ni	0.97±0.06a	1.20±0.14b	1.14±0.01ab	1.70±0.01c	1.61±0.01c

Mean values followed by different letters in the same row are significantly different ($p<0.05$).
C: Control (without date fibre); DF1: ice cream with made date fibre 1% (w/w); DF2: ice cream with date fibre 2% (w/w); DF3: ice cream with date fibre 3% (w/w); DF4: ice.

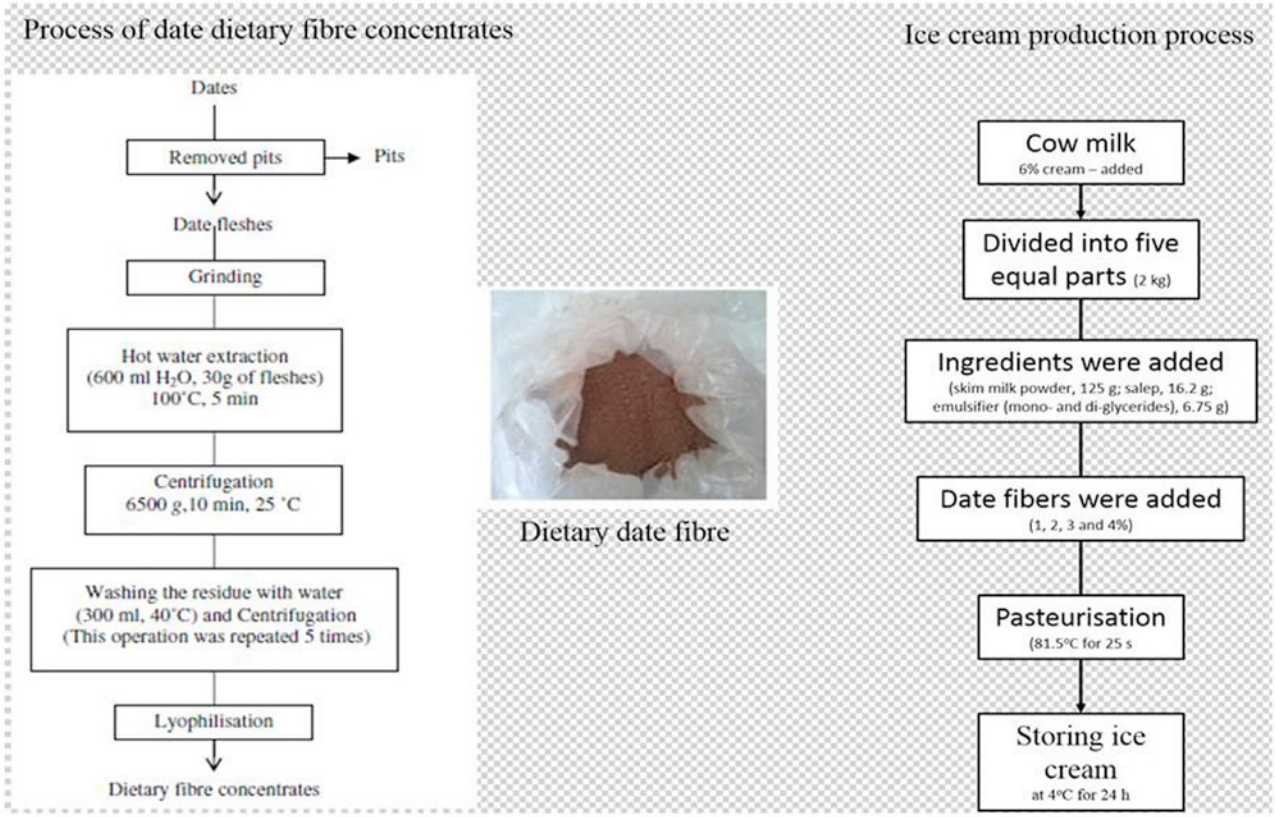

Fig. 1 - The obtain of date fibre and production of ice cream.

cream samples were not statistically different maybe due to pH of date (6.00).

Viscosity is one of the most important properties of an ice-cream mixture since it can result in a desirable body and texture in ice creams. Therefore, the measurement of viscosity is important to know the effect of DF on the characteristics of ice-cream mixtures. It could be seen in the present study that the addition of DF significantly ($p<0.05$) affected the viscosity behaviour of ice cream samples (Fig. 1). Viscosity of ice-cream samples increased significantly by adding DF (3 and 4%). As shown in Fig. 1, the lowest and highest viscosity rates value were obtained in DF1 sample the sample with 4% DF. The control sample had an average of 5175 viscosity. Similar results were reported in grape wine lees added in ice cream by HWANG *et al.* (2009), in frozen yogurt by GUVEN and KARACA (2002), in Cape gooseberry (*Physalis peruviana* L.) added in ice cream by ERKAYA *et al.* (2012) and the citrus fibre added in ice cream mixes by DERVISOGLU and YAZICI (2006).

Ice cream color was affected by the addition of DF. The date fibre had a brownish color. Ice cream fortified with date fibre had significantly higher a* and b* values and lower L* values compared to the control sample. Lightness (L*) values of ice cream samples were closer to each of dietary fibre but with DF1 and DF2 samples, it was found to be significantly higher than the other samples (Table 3). All samples had negative a* values and DF3 and DF4 samples had close but significantly higher values than other samples. Increase in the concentration of date fibre contributed to the color values of the samples ($p<0.05$). The addition of date fibre increased the b* values of all samples. The lowest b* value was obtained in DF1 samples while the highest b* was obtained in the DF4 samples. DERVISOGLU and YAZICI (2006) reported that the addition of citrus fibre increased the color properties similarly to the results of present study.

Overrun and melting time are associated with the amount of air incorporated during the manufacturing process. These features can define the structure of the end final product since the presence of air gives the ice cream an agreeable light texture and influences the physical properties of melting and hardness of the end product (SOFJAN and HARTEL, 2004; CRUZ *et al.*, 2009; DAGDEMIR, 2011). All ice cream samples had normally lower overrun values (29.87-40.51%) than those reported in literature (80-120%). Although the rate of DF lowered the overrun values of the ice-cream samples, control samples had higher overrun values than the DF added samples (Table 3). Since the viscosity of ice cream increased in DF added samples, it was possible that less air was incorporated in the ice cream mix with DF during batch freezing, which resulted in lower overrun than for control (with-

out DF). The decrease of overrun values for ice creams with DF was in agreement with the results indicated in literature (DERVISOGLU *et al.*, 2005; TEMIZ and YESILSU, 2010). EL-SAMAHY *et al.* (2009) reported that the decrement of overrun in ice cream containing concentrated cactus pear pulp might be attributed to increment of mix's viscosity that extremely affects whipping rate of mixes. HWANG *et al.* (2009) reported that the overrun values of ice-cream samples decreased significantly when grape wine lees was added. It was found by SUN-WATERHOUSE *et al.* (2013) that overrun rate of ice – cream containing green kiwi fruit was 90.5% and higher than that found in the present study. However, similar results with the present study were found with Cape gooseberry (*Physalis peruviana* L.) added in ice cream by ERKAYA *et al.* (2012).

As can be seen in Table 3, the complete melting times of the ice cream samples were significantly longer for DF4 samples (0.50 g min^{-1}) and the period got longer as the fibre content increased. This is due possibly to some compounds existent in DF4, which have the ability of water absorption. AKIN *et al.* (2007) reported that the decrease in melting rate of ice cream with inulin might originate from its ability to reduce the free movement of water molecules. DF (3 and 4%) concentration affected the first dripping times positively (Fig. 2). Results of the present study indicated that the first dripping times were prolonged as the fibre contents increased in the ice cream samples ($p<0.05$). It was found by DERVISOGLU and YAZICI (2006) that citrus fibre samples extended dripping times. These findings were similar to those in the present study. Statistically significant differences ($p<0.05$) were found in terms of major element contents such as Ca, K, Mg and S between the samples except for Mn concentration in all ice cream samples. Dairy products are known to be excellent sources of Ca, P and Mg and supply dietary fibre a significant amount of calcimine, a bioavailable form (MCKINLEY, 2005). Addition of date fibre lowered Ca content of the samples in the present study (Table 4). The highest Ca was found to be 1844.36 mg/kg in control samples. Mg and S values of ice cream samples increased with the addition of date fibre ($p<0.05$). Increasing K in human diet may provide protection from hypertension in people who are sensitive to high levels of Na. The highest rate of S and Na was fibre detected in the samples with 4% and 2% DF to be 1103 and 690 mg/kg, respectively, while the lowest rates were 875.24 in control and 528.5 mg/kg with 3% DF, respectively. Elements like Fe, Zn and Mn are classified as micro-nutrients. The addition of DF significantly increased Fe, Zn and Mn contents of the ice-cream samples ($p<0.05$). Similar results were reported by ERKAYA *et al.* (2012) in Cape gooseberry (*Physalis peruviana* L.) added ice cream samples. It can be suggested by considering such a result that date fibre may be a good source to enhance dairy products such as ice-cream, which is poor in minor elements like Fe and Zn. WU *et al.* (2005) reported that Zn acts as a non-enzymatic antioxidant, so that its consumption helps to prevent oxidative damage of the cell. The ice cream sample with 4% DF had the highest Zn content (94.56 mg/kg).

Sensory evaluations

Results of the sensory evaluation of the ice cream samples on a scale from 1 (poor) to 9 (excellent) are shown in a radar plot in Fig. 3. Fortifying ice cream with DF had a significant effect on all sensory properties except sweetness. All the fibre-enriched samples received lower scores for total evaluation in terms of sensory characteristics ($p<0.05$). Ice cream enriched with up to

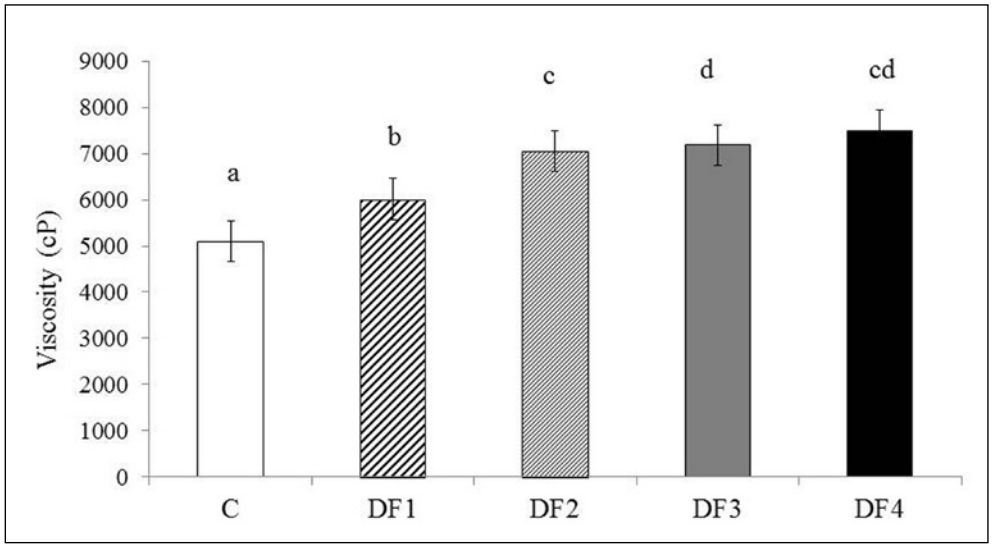

Fig. 2 - Viscosity values of ice cream containing date fibre and control.

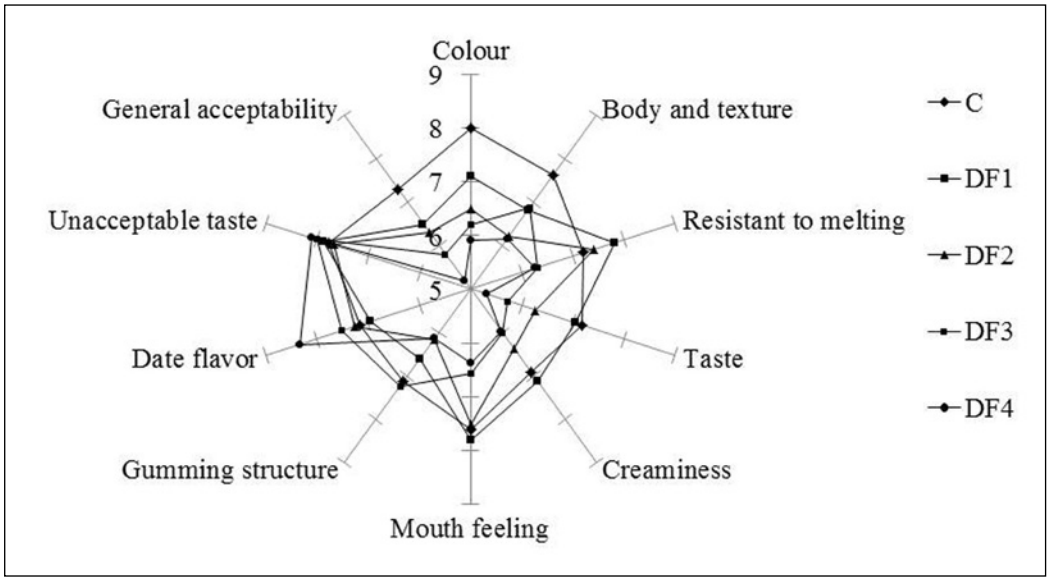

Fig. 3 - Effect of the addition of DF on the sensory profile of ice cream.
C: control; DF1: 1% (w/w) date fibre added; DF2: 2% (w/w) date fibre added; DF3: 3%(w/w) date fibre added; DF4: 4%(w/w) date fibre added.

1 and 2% DF had similar mouth feeling, showed resistant to melting and gave general acceptability ratings as control sample. Panellists preferred the ice cream samples more to the others.

CONCLUSIONS

It can be shown as the results of the present study that fibre of date (especially at 1 and 2%) may be successfully used as a good natural source of nutritive ingredients in ice cream production. The addition of date fibre improved the viscosity, first dripping times, complete melting times and mineral compositions, but had no significant effect on overrun of ice creams. The enrichment of food with date fibres is an effective way to enhance nutritional and physiological aspects and to promote functionality by influencing rheological and thermal properties of the final product.

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