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Original article

Influence of milk pasteurization and scalding temperature on the volatile compounds of Malatya, a farmhouse Halloumi-type cheese

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Abstract – Malatya cheese, a farmhouse Halloumi-type cheese, was made from raw or pasteurized milk and the curds were scalded in hot whey at 60, 70, 80 or 90 °C. After ripening for 30 or 90 d, the cheeses were analyzed for characterization of volatile composition. One hundred and two volatile compounds including 11 acids, 13 esters, 15 ketones, 6 aldehydes, 26 alcohols, 2 lactones, 5 sulfur compounds, 5 terpenes and 19 miscellaneous compounds were identified using solid phase micro extraction and gas chromatography-mass spectrometry. The use of raw milk in the manufacture enriched the volatile profile of the cheese and the majority of volatiles were more abundant in raw milk cheeses than in pasteurized milk cheeses. The cheeses made with raw milk contained higher levels of acids, esters and lactones and lower levels of aldehydes and sulfur compounds than did the cheeses made from pasteurized milk. Principal component analysis (PCA) was applied to simplify interpretation of the GC-MS data and distinguished the raw and pasteurized milk cheeses on the plot. The samples were also classified based on scalding temperature by PCA, but no regular distribution was observed. The results suggest that the pasteurization of cheese milk had a greater effect on volatile composition of cheese than scalding temperature of the curd.

pasteurization / raw milk / Malatya cheese / volatile compounds / scalding / aroma

摘要 – 原料奶的巴氏杀菌和热烫温度对一种农家 Halloumi 干酪——Malatya 干酪中挥发性化合物的影响。 Malatya 干酪属于农家 Halloumi 干酪中的一种。由未杀菌奶和巴氏杀菌奶生产的凝块分别在 60、 70、 80 和 90 °C 的乳清中热烫 3 min。干酪成熟 30 d 和 90 d 后分析了其中挥发性化合物的特性。经固相微萃取技术和 GC/MS 分析，共检测出 102 种挥发性化合物成分，其中包括 11 种有机酸、 13 种酯类化合物、 15 种酮类化合物、 6 种醛类化合物、 26 种醇类化合物、 2 种内酯、 5 种硫化物、 5 种萜烯和 19 种其他类化合物。未杀菌奶生产干酪中挥发性化合物成分丰富，与巴氏杀菌奶生产的干酪相比，前者干酪中主要挥发性化合物含量高，其中有机酸、酯、内酯的含量明显高于后者，但是醛类和含硫化合物含量则低于后者。采用主成分分析法 (PCA) 对 GC-MS 测定的数据进行了简化分析，分析了未杀菌奶和巴氏杀菌奶生产的两种干酪的特性。对不同热烫温度下测定的数据采用 PCA 分析法将样品进行了分类，证明他们之间没有规律性的分布。试验结果表明原料奶的巴氏杀菌对干酪中挥发性化合物的影响要显著地高于凝块的热烫温度。

巴氏杀菌 / 原料奶 / Malatya 干酪 / 挥发性成分 / 热烫 / 芳香气味

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Résumé – Influence de la pasteurisation du lait et de la température de cuisson sur les composés volatils du Malatya, fromage fermier de type Halloumi. Du Malatya, un fromage fermier de type Halloumi, a été fabriqué à partir de lait cru et de lait pasteurisé, et les caillés obtenus ont été cuits dans du lactosérum chauffé à 60, 70, 80 ou 90 °C. Après 30 ou 90 jours d'affinage, la composition en volatils des fromages a été caractérisée. Cent deux composés volatils incluant 11 acides, 13 esters, 6 aldéhydes, 26 alcools, 2 lactones, 5 composés soufrés, 5 terpènes et 19 composés divers ont été identifiés par micro-extraction en phase solide et chromatographie gazeuse couplée à la spectrométrie de masse (CG-MS). L'utilisation du lait cru pour la fabrication a abouti à un profil en volatils plus riche et la majorité des volatils étaient présents en quantité plus importante dans les fromages au lait cru par rapport aux fromages au lait pasteurisé. Les fromages au lait cru contenaient des teneurs plus élevées en acides, esters et cétones, et des teneurs moins élevées en aldéhydes et composés soufrés. Une analyse en composante principale (ACP) a permis de simplifier l'interprétation des données de CG-MS et de différencier les fromages au lait cru des fromages au lait pasteurisé. Les échantillons ont également été classifiés sur la base de la température de cuisson par ACP, mais aucune distribution régulière n'a pu être observée. Ces résultats suggèrent que la pasteurisation du lait de fabrication a un effet sur la composition en volatils plus marqué que celui de la température de cuisson du caillé.

pasteurisation / lait cru / Malatya / composé volatil / cuisson du caillé / arôme

1. INTRODUCTION

Cheese ripening is a complex and dynamic biochemical process that includes protein breakdown, fat hydrolysis and lactose metabolism [25]. Hydrolysis of casein by proteolytic enzymes produces peptides and free amino acids. These play a critical role in the development of cheese flavour. Many volatile compounds which contribute to the characteristic aroma and flavour occur during the ripening of cheeses. Some factors affect the composition of volatile fractions in cheese such as animal feeding, the types of milk, coagulant, starter or secondary starters, heat treatment of milk, ripening time and temperature [7, 12, 14, 31, 39]. Heat treatment of milk alters the flavour profile of cheese due to a decrease in the number of non-starter lactic acid bacteria (NSLAB) and possibly inactivation of indigenous milk enzymes [15, 40]. Heat treatment causes delaying in the flavour development in cheese. Lau et al. [22] reported that a cheese made from pasteurized milk took twice as long as that made from raw milk to develop the same flavour intensity and lower levels of soluble nitrogen and free amino acid were obtained in cheese made from pasteurized milk. The native microorganisms and indigenous milk enzymes are main contributors for the formation of the characteristic aroma components in cheese. Heat treatment of milk prior to cheese making had an effect on microbial flora, proteolysis, free amino acids, free fatty acids, volatile fractions and sensory characteristics [6, 12, 17, 26, 31, 39]. However, heat treatment of milk prior to cheese making is an essential process in many cheeses due to the presence of undesirable microorganisms which causes some defects in texture and flavour. In addition, it can ensure the hygienic and standard quality of cheese, the ripening at higher temperature and the reduction of some risks including blowing caused by butyric fermentation, control over lactic acid production and off-flavours [30].

Malatya, a farmhouse Halloumi-type cheese, is traditionally made from raw ewe's or cow's milks or their appropriate mixtures and no starter culture is employed. The traditional method is still used in farms in small scale and villages. Recently, some cheese makers used the pasteurization process in its manufacture and added a starter culture to standardize the production and to eliminate undesirable microorganisms. The characteristics of this type of cheeses are the scalding process which produces an elastic and compact texture after the pressing of curd at 80–90 °C. So, a second heat treatment is applied in the manufacture of Malatya cheese and the method of manufacture influences the physical, chemical and sensory characteristics of the cheese.

A number of volatile components belonging to the chemical groups acids, esters, aldehydes, ketones, alcohols, sulfur compounds and other aromatic hydrocarbons have been identified in different cheeses [27–29, 36]. To the authors' knowledge, no previous studies were carried out to identify the volatile compounds contributing to the characteristic volatile profiles of Malatya cheese; consequently, a study would be useful for the characterization of this cheese which is currently manufactured both from raw and pasteurized milk. The objective of the study was to investigate the influence of pasteurization prior to cheese making and scalding temperatures on volatile composition of Malatya cheese during ripening.

2. MATERIALS AND METHODS

2.1. Cheese-making

Malatya cheese was made in duplicate using both raw and pasteurized cow's milk in a local dairy plant (Karlidag Dairy Products, Malatya, Turkey). A volume of 200 L raw cow's milk was used in the manufacture of Malatya cheese with 100 L of the milk used to make raw milk cheese (C cheeses) and the rest pasteurised at 72 °C for 30 s and used for the manufacture of pasteurized milk cheeses (P cheeses). After pasteurization, the milk was cooled to 32 °C and the commercial culture consisting of selected strains of *Lc. lactis* ssp. *lactis* and *Streptococcus thermophilus* (Sacco, srl, Cadorago, Italy) was added at the manufacturer's recommended dose and held for 30–45 min. Both C and P cheeses were coagulated using commercial calf rennet (>85% chymosin, REN–NA®, Mayasan, Istanbul, Turkey) at the level of 15 g·100 L^{-1}. The coagulation took place at 32 °C for 45 min. Following coagulation, the curd was cut and stirred for about 30 min, and transferred into cloth bags and then left for 30 min to drain its whey with no pressing. The bags which contain approx. 250 g of curd were tied up and moulded as a ball, and then they were pressed between two wooden blocks for 2 h. The cheeses were scalded at 60, 70, 80 or 90 °C for 3 min by means of dipping their wheys and then the cheese blocks were re-pressed between the same wooden blocks for 3 min and then immediately cooled to room temperature. Then, the cooled blocks were immersed in brine (10% NaCl) and ripened for 90 d at 6–8 °C.

2.2. Chemical analysis

Cheeses were analyzed in duplicate for moisture, fat, total protein, salt, pH and titratable acidity as described in Hayaloglu et al. [16]. Statistical evaluation of the chemical data was analyzed by ANOVA with significant differences for $P < 0.05$ using SPSS package program version 9.0 for Windows (SPSS Inc., Chicago, IL).

2.3. Analysis of volatile components in cheese using Solid Phase Microextraction (SPME)

Cheese samples were sliced, frozen in liquid nitrogen, pulverized into small granules and stored in glass bottles in a freezer at –20 °C. Samples were analyzed within max. 14 d. A 3-g portion of sample was then placed in a 15-mL vial and allowed to equilibrate at 40 °C for 30 min. Essentially, extraction is achieved by injecting a 75-µm Carboxen–Polydimethylsiloxane fiber (Sigma-Aldrich, Poole, England) into the vial and exposing to the headspace for 30 min at 40 °C. The fiber was positioned at 3.0 scale units in each run. Desorption of the extracted volatiles was carried out on an Agilent 5890 Gas Chromatography-5972 Mass Spectrometry System (Agilent Technologies, UK Ltd, Cheshire, UK) run in splitless mode. During desorption the fiber remained in the injector for 2 min at a temperature of 250 °C, with helium as the carrier gas at a flow rate of 1.0 mL·min^{-1}. The components were separated on an Agilent FFAP column, 50 m × 0.2 mm × 0.33 µm (Crawford Scientific, Lanarkshire, Scotland). The oven was held at 40 °C for 2 min (desorption period), then ramped at 5 °C per min to 70 °C, which was held for 1 min. The temperature was then raised at 10 °C per min to 240 °C to give a run time of 30 min. The mass spectrometer was set to

Table I. Chemical composition and pH of Malatya cheese made from raw (C) and pasteurized (P) milk after 1 d of ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C).

Cheeses	Titratable acidity[1]	pH	Total solid (%)	Salt (%)	FDM[2] (%)	Total protein (%)
C60	0.21 ± 0.08^{ab}	6.02 ± 0.5^{a}	39.85 ± 0.55^{a}	2.70 ± 0.0^{a}	42.03 ± 0.05^{a}	14.03 ± 0.32^{ab}
C70	0.14 ± 0.0^{a}	6.03 ± 0.42^{a}	40.20 ± 0.30^{a}	2.81 ± 0.0^{a}	41.64 ± 2.79^{a}	15.04 ± 014^{bcd}
C80	0.22 ± 0.09^{ab}	6.01 ± 049^{a}	38.79 ± 0.70^{a}	2.70 ± 0.0^{a}	45.74 ± 1.10^{a}	16.20 ± 0.28^{d}
C90	0.23 ± 0.07^{ab}	5.96 ± 0.39^{a}	38.90 ± 1.55^{a}	2.70 ± 0.0^{a}	49.48 ± 0.04^{a}	15.68 ± 0.05^{d}
P60	0.37 ± 0.03^{b}	5.62 ± 0.16^{b}	39.22 ± 0.17^{a}	1.93 ± 0.0^{a}	44.60 ± 2.35^{a}	13.74 ± 0.50^{a}
P70	0.39 ± 0.01^{b}	5.57 ± 0.03^{b}	39.20 ± 0.75^{a}	1.76 ± 0.0^{a}	44.68 ± 2.13^{a}	14.94 ± 0.69^{bcd}
P80	0.30 ± 0.02^{ab}	5.81 ± 0.06^{ab}	40.75 ± 2.55^{a}	2.70 ± 0.0^{a}	41.72 ± 0.15^{a}	14.25 ± 0.0^{abc}
P90	0.29 ± 0.04^{ab}	5.81 ± 0.03^{ab}	40.22 ± 0.52^{a}	2.46 ± 0.0^{a}	42.33 ± 5.52^{a}	15.36 ± 0.01^{cd}

Mean ± SD of duplicate determination in two cheesemaking trials. Means in the same column followed by different letters differ ($P < 0.05$).
[1] Titratable acidity expressed as g lactic acid/100 g cheese.
[2] FDM: fat-in-dry matter.

record 33–450 atomic mass units, threshold 1000, at a sampling rate of 1.11 scans per s.

2.4. Data analysis of SPME

Components were identified using the data obtained from the mass spectrometer in full scan mode. A database was then set up to quantitate relative amounts of each. The database was constructed using selected ion monitoring with specific ions selected in order to allow quantitation of co-eluting peaks. Response factors were not used in the quantitation of the samples as these would require the determination of rate of release from the cheese into the headspace, and the efficiency of extraction of each component. Thus actual concentrations of the components in the cheese are not known but for the purpose of this project comparative values were used to show differences between the varying treatments. Instead the data was in the form (Area of peak/10^5) and was normalized to a weight of 1 g sample. This comparative data was then analyzed using ANOVA whereby the mean response of the data, transformed to give the square root (SQRT), was calculated. ANOVA of the SQRT transformed data was carried out using Minitab Statistical Software version 13 (Minitab Ltd., Coventry, England) on the basis of cheese type and age. To simplify interpretation of the results, principal component analysis (PCA) was performed using the varimax rotation between the aroma characters of the cheeses on the ANOVA results. PCA was carried out using The Unscrambler v9.6 free-trial version (CAMO, Software AS, Oslo, Norway).

3. RESULTS AND DISCUSSION

3.1. Chemical composition

The chemical composition and pH of Malatya cheese at 1st d of ripening are shown in Table I. No significant differences were found between cheeses in total solids, salt and fat-in-dry matter. However, a higher level of titratable acidity and lower pH values were found in the cheeses made from pasteurized milk with a starter culture ($P < 0.05$). The addition of starter culture to the pasteurized milk resulted in significantly lower pH in pasteurized milk cheeses (P) in comparison to raw milk cheeses (C). The gross composition of the cheese samples at the beginning of the ripening period are in the normal ranges for a salted cheese. These results are in accordance with those of Ozer et al. [34] for Urfa cheese and Kahyaoglu and Kaya [21] for Gaziantep

Table II. Concentrations of fatty acids in Malatya cheese made from raw (C) and pasteurized (P) milk during ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C). The results were expressed as SQRT [Area/10^5] from triplicate analysis of each cheese.

Fatty acid	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
Formic acid	30	1.27	1.58	1.92	2.10	3.14	2.58	2.40	1.98	NS	**
	90	1.37	1.51	1.38	1.15	1.70	1.25	1.13	1.32		
Ethanoic acid	30	16.93	18.86	22.62	23.38	23.16	21.65	22.04	17.67	NS	**
	90	23.96	31.37	28.39	26.36	25.00	20.63	18.75	23.11		
Propanoic acid	30	1.25	1.20	1.37	1.64	1.58	0.99	0.95	0.80	***	***
	90	3.88	4.08	4.39	3.27	1.37	1.30	1.31	1.32		
2-Methyl propanoic acid	30	1.19	1.57	1.84	1.89	1.32	1.31	1.16	1.14	**	***
	90	4.79	3.20	3.53	2.43	2.09	2.01	2.06	1.93		
Butanoic acid	30	12.99	13.61	15.15	14.77	7.16	7.25	6.47	5.22	***	***
	90	21.76	25.03	23.91	22.30	9.50	7.90	7.66	7.71		
2-Methyl butanoic acid	30	0.71	0.61	0.71	0.80	0.63	0.58	0.50	0.48	**	***
	90	2.41	1.79	2.01	1.36	1.02	0.93	0.96	0.89		
3-Methyl butanoic acid	30	2.67	2.96	3.69	3.90	3.06	3.36	3.04	2.87	*	***
	90	6.96	6.36	6.34	4.97	4.30	4.24	4.54	3.91		
Pentanoic acid	30	1.73	1.85	1.83	1.81	1.20	1.20	1.08	0.87	**	**
	90	2.27	2.67	2.43	2.35	1.28	1.20	1.19	1.10		
Hexanoic acid	30	12.45	11.53	11.40	11.98	7.05	6.08	5.30	4.56	**	**
	90	16.08	18.44	16.59	17.49	6.86	6.03	5.76	4.52		
Octanoic acid	30	5.55	5.52	4.74	5.42	4.02	3.34	2.71	2.61	**	NS
	90	5.85	6.54	6.89	7.22	2.75	2.85	2.84	1.96		
Decanoic acid	30	1.96	2.03	2.12	2.36	1.84	1.52	1.34	1.22	**	NS
	90	2.02	2.20	2.39	2.40	1.21	1.27	1.22	0.89		
Total	30	58.70	61.33	67.37	70.05	54.14	49.86	46.98	38.21		
	90	91.33	103.18	98.25	91.29	57.07	49.60	47.42	48.66		

* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$; NS: non significant.

cheese; these cheeses are scalded and ripened in brine like Malatya cheese.

3.2. Volatile composition

One hundred and two compounds were identified in the volatile fractions of Malatya cheese including 11 fatty acids, 13 esters, 15 ketones, 6 aldehydes, 26 alcohols, 2 lactones, 5 sulfur compounds, 5 terpenes and 19 miscellaneous compounds. The compounds identified from the cheeses were listed by chemical group in Tables II–X. Identification of volatile fraction has not previously been conducted in Malatya cheese or other cheeses where scalding is carried out on the curd in hot whey. In the present study, the use of pasteurised milk in the manufacture and ripening time has significantly influenced the volatile fraction in Malatya cheese. Some of the volatile compounds (forty) were significantly influenced by using pasteurized milk in the manufacture or by scalding the curd in hot whey and sixty-three compounds were significantly influenced by cheese age ($P < 0.01$).

3.2.1. Fatty acids

Fatty acids are one of the main chemical groups in the volatile fraction of Malatya cheese (Tab. II). Ethanoic and propanoic are produced by the fermentation of lactose or lactic acid; in contrast, 2-methyl propanoic, 2- and 3-methyl butanoic acids are produced by the metabolisms of Val, Leu and Ile amino acids, respectively [29, 40]. Use of pasteurized milk in the production of Malatya cheese and scalding the curd in hot whey have significantly influenced all fatty acids present in the cheese except formic and ethanoic acids. Concentration of the fatty acids with the exception of octanoic and decanoic acids changed with age. Formation of the fatty acids in raw or pasteurised milk cheeses exhibited a different trend during ripening. On 90 d of ripening, raw milk cheeses had the highest concentrations of ethanoic, propanoic, 2-methyl propanoic, butanoic, 2-methyl butanoic, 3-methyl butanoic, pentanoic, hexanoic, octanoic and decanoic acids probably due to numerous other microflora could be involved in cheese lipolysis. The results obtained are in agreement with studies by Buchin et al. [6] and Shakeel-Ur-Rehman et al. [38] who found that the concentration of acids was higher in raw milk cheese than those of pasteurised milk cheese during ripening. Pasteurization of cheese milk had a greater effect than scalding the curd in hot whey on the formation of acids in Malatya cheese. In general, the concentration of the fatty acids decreased as scalding temperature increased; however, a strong correlation or relationship between scalding temperature and concentration of the fatty acids cannot be found. The concentration of formic and ethanoic acids were not affected by heating of the curd in hot whey. These compounds are produced by several metabolic pathways such as lactose or butyric acid fermentation, catabolism of some amino acids or hydrolysis of glycerides [29]. Butanoic (butyric) acid has a strong effect on cheese flavour, since it is found only in milk fat and is described as having a cheesy and sweaty odour.

3.2.2. Esters

Esters contribute to cheese flavour by minimizing sharpness of fatty acids and bitterness of amines [35]. Table III shows the mean concentrations of esters identified in Malatya cheeses during ripening. Ethyl esters (seven esters) are the principal esters in the cheese samples and the other esters were methyl, propyl and butyl (Tab. III). The highest level of ethyl esters in the cheeses can be correlated with higher concentrations of primary alcohols and fatty acids [11]. Concentration of ethyl acetate, the most abundant of esters, was higher in raw milk cheese on 30 d of ripening and its concentration was the same in both raw and pasteurized milk cheeses. Methyl lactate, propyl hexanoate and 3-methylbutyl butanoate were not detected in pasteurized milk cheeses, while ethyl propionate was not identified in raw milk cheeses. Ethyl butanoate, ethyl hexanoate, ethyl octanoate and ethyl lactate were identified at the highest concentrations in raw milk cheeses and their concentrations increased significantly during ripening.

3.2.3. Ketones

A total of 15 ketones were identified in Malatya cheeses made from raw or pasteurized milk. Methyl ketones are principal compounds in blue cheeses and are formed by enzymatic oxidation of free fatty acids to β-ketoacids and their consequent decarboxylation to methyl ketones [11, 25], contributing to the pungent aroma [31]. The majority of ketones identified in Malatya cheeses were not different between cheeses made from raw or pasteurized milk except 2-propanone, diacetyl and 2,3-pentanedione. Major ketones were 2-propanone, 2-butanone, 2-pentanone, 2-heptanone, 3-hydroxy 2-butanone (acetoin) and diacetyl (Tab. IV) in the cheeses. Diacetyl (2,3-butanedione) is one of the most important ketones and it denotes a buttery and nutty flavours in cheese [29]. Diacetyl is produced by metabolisms of lactose and citrate by cit^{+} *Lactococcus lactis* ssp. *lactis* and *Leuconostoc* species [11]. Changes in ketone concentration showed different trends during ripening. That is, some fluctuations were observed during ripening. The concentration of the majority of methyl

Table III. Concentrations of esters in Malatya cheese made from raw (C) and pasteurized (P) milk during ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C). The results were expressed as SQRT [Area/10^5] from triplicate analysis of each cheese.

Ester	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
Methyl acetate	30	0.35	0.35	0.34	0.41	0.35	0.39	0.48	0.54	NS	NS
	90	0.37	0.34	0.39	0.31	0.50	0.53	0.55	0.63		
Methyl lactate	30	ND	ND	ND	ND	ND	ND	ND	ND	***	***
	90	1.59	1.53	1.84	1.83	ND	ND	ND	ND		
Ethyl acetate	30	11.34	10.93	10.99	11.24	7.22	7.24	9.78	8.84	NS	NS
	90	8.17	11.26	10.66	11.28	10.62	10.42	8.81	8.92		
Ethyl propanoate	30	ND	ND	ND	ND	ND	ND	ND	ND	***	***
	90	ND	ND	ND	ND	1.20	1.09	1.07	1.04		
Ethyl butanoate	30	4.66	4.19	4.32	4.11	1.95	2.25	2.73	2.57	*	*
	90	3.78	4.92	4.72	5.32	3.66	3.36	2.92	3.09		
Ethyl hexanoate	30	3.18	2.77	2.36	2.41	1.39	1.16	1.11	0.93	**	**
	90	3.78	4.62	4.24	4.93	1.27	1.52	1.13	1.06		
Ethyl octanoate	30	1.37	1.45	0.98	1.32	1.00	0.74	0.43	0.52	**	**
	90	1.85	2.13	2.34	2.54	0.81	0.79	0.72	0.49		
Ethyl decanoate	30	0.55	0.59	0.52	0.58	0.48	0.69	1.00	0.78	NS	NS
	90	0.66	0.76	0.84	0.87	0.39	0.42	0.41	0.33		
Ethyl lactate	30	1.20	1.41	1.74	1.89	1.80	1.74	1.64	1.32	**	***
	90	2.90	6.44	4.24	4.15	2.14	1.50	1.31	1.81		
Propyl acetate	30	1.25	1.47	1.19	1.16	1.14	1.03	1.38	1.33	NS	NS
	90	1.33	1.94	1.76	1.84	1.03	1.04	1.13	0.95		
Propyl hexanoate	30	0.91	0.59	0.00	ND	ND	ND	ND	ND	*	NS
	90	0.09	0.34	0.38	ND	ND	ND	ND	ND		
3-Methylbutyl acetate	30	2.07	1.40	1.31	1.77	1.26	1.22	1.37	1.23	NS	*
	90	2.37	2.83	3.46	2.84	1.84	1.64	1.58	1.83		
3-Methylbutyl butanoate	30	0.68	0.49	ND	ND	ND	ND	ND	ND	*	**
	90	ND	ND	ND	ND	ND	ND	ND	ND		
Total	30	27.56	25.64	23.75	24.88	16.57	16.46	19.91	18.05		
	90	26.89	37.11	34.85	35.89	23.46	22.32	19.63	20.13		

* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$; NS: non significant; ND: not detected.

ketones decreased during ripening, while the concentration of 2-propanone and 2-undecanone increased with ripening time in all cheeses. Interestingly, 1-hydroxy 2-propanone was not identified in pasteurized milk cheeses either at d 30 or 90; however, 1-hydroxy 2-propanone and 2,3-pentanedione were not determined in 90-d old raw milk cheeses. Concentrations of 3-hydroxy 2-butanone and diacetyl were higher in raw milk cheeses at 30 d but showed a marked decrease at 90 d as compared to the trend for pasteurized cheeses milk. This correlated well with studies by Bintis and Robinson [3] who found higher levels of 3-hydroxy 2-butanone and diacetyl in fresh Feta cheese than those of 60-d old Feta cheeses, probably due to the action of starters, while they declined during ripening. Raw milk cheeses contained the highest levels of 2,3-pentanedione at 30 d of ripening; however, it was not identified at 90 d of ripening. The concentration of 2,3-pentanedione in pasteurized milk cheese was half the amount of raw milk

Table IV. Concentrations of ketones in Malatya cheese made from raw (C) and pasteurized (P) milk during ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C). The results were expressed as SQRT [Area/10^5] from triplicate analysis of each cheese.

Ketone	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
2-Propanone	30	7.02	6.71	6.40	7.63	9.38	11.21	10.64	11.57	**	**
	90	9.60	7.59	8.39	7.75	11.92	12.99	14.72	13.25		
2-Butanone	30	10.23	10.01	9.07	11.59	15.31	9.90	10.52	10.33	NS	**
	90	9.41	8.47	8.82	8.84	9.57	9.71	9.44	9.89		
2-Pentanone	30	8.00	6.71	6.30	5.78	5.63	9.15	8.85	7.13	NS	*
	90	5.09	4.75	5.41	6.83	6.72	7.15	6.31	6.62		
2-Hexanone	30	1.01	0.97	0.81	0.93	0.92	1.43	1.19	1.12	NS	NS
	90	0.75	0.78	0.82	1.04	1.01	1.08	0.97	1.03		
2-Heptanone	30	7.97	7.66	6.14	8.07	8.44	15.25	10.96	8.82	NS	*
	90	6.17	5.72	6.16	9.70	6.51	8.00	5.72	6.44		
2-Octanone	30	0.75	0.60	0.39	0.51	0.58	1.25	0.83	0.76	NS	NS
	90	0.50	0.45	0.63	0.71	0.45	0.60	0.70	0.41		
2-Nonanone	30	2.94	2.92	2.08	2.77	3.25	5.32	3.62	3.18	NS	*
	90	2.85	2.32	2.45	3.50	2.04	2.36	2.46	1.93		
2-Decanone	30	0.35	0.35	0.12	0.29	0.28	0.21	ND	ND	NS	NS
	90	0.21	0.10	0.20	0.26	ND	ND	ND	ND		
2-Undecanone	30	0.45	0.49	0.42	0.49	0.46	0.51	0.41	0.41	NS	*
	90	0.55	0.56	0.57	0.62	0.45	0.48	0.52	0.44		
1-Hydroxy	30	1.49	1.57	1.68	1.78	ND	ND	ND	ND	***	***
2-propanone	90	ND	ND	ND	ND	ND	ND	ND	ND		
3-Hydroxy	30	8.18	10.57	11.12	10.66	7.65	7.61	7.65	7.97	NS	***
2-butanone	90	2.76	2.71	2.29	2.98	6.14	4.66	5.19	5.37		
3-Hydroxy	30	3.09	3.14	3.70	2.26	2.28	2.73	2.62	2.66	NS	***
2-pentanone	90	0.50	0.38	ND	0.33	1.44	0.97	1.09	1.19		
2-Hydroxy	30	2.92	2.92	3.42	2.17	2.08	3.04	2.52	2.60	NS	***
3-pentanone	90	0.52	0.32	ND	ND	1.34	0.96	1.12	1.12		
Diacetyl	30	13.80	13.72	15.62	12.29	7.74	9.19	9.50	9.30	*	***
	90	4.57	5.72	5.82	7.58	6.80	6.71	7.12	6.53		
2,3-Pentane-	30	6.77	6.18	7.25	4.37	2.32	2.84	3.03	3.09	**	***
dione	90	ND	ND	ND	ND	1.48	1.19	1.72	1.45		
Total	30	74.96	74.51	74.49	71.57	66.31	79.62	72.33	68.94		
	90	43.45	39.88	41.55	50.14	55.87	56.85	57.07	55.67		

* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$; NS: non significant; ND: not detected.

cheeses at 30 d of ripening, but their concentration markedly declined at 90 d. Imhof et al. [19] detected high levels of 2,3-pentanedione in cheeses inoculated with thermophilic cultures and suggested that it might be produced by metabolism of Ile.

Table V. Concentrations of aldehydes in Malatya cheese made from raw (C) and pasteurized (P) milk during ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C). The results were expressed as SQRT [Area/10^5] from triplicate analysis of each cheese.

Aldehyde	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
Acetaldehyde	30	1.32	1.37	1.25	1.33	2.34	2.29	2.57	2.47	*	***
	90	3.06	2.33	3.10	2.03	3.26	4.14	3.30	3.47		
2-Propenal	30	0.58	0.61	0.42	0.62	0.92	ND	ND	ND	**	*
	90	1.20	1.35	1.21	1.00	ND	ND	ND	ND		
2-Methyl propanal	30	0.52	0.65	0.79	0.78	0.65	0.81	1.00	0.96	NS	*
	90	0.52	0.51	0.62	0.55	0.57	0.72	0.58	0.58		
2-Methyl butanal	30	0.32	0.45	0.44	0.48	0.31	0.49	0.57	0.48	NS	NS
	90	0.39	0.37	0.52	0.41	0.43	0.53	0.54	0.34		
3-Methyl butanal	30	2.71	3.30	4.05	4.25	3.11	4.40	6.24	4.49	NS	***
	90	1.06	0.95	1.35	1.01	1.20	1.12	1.05	1.36		
Benzaldehyde	30	1.53	1.50	0.87	1.11	1.38	1.55	1.27	1.28	NS	NS
	90	1.26	1.26	1.49	1.64	1.06	1.21	1.52	1.09		
Total	30	6.97	7.87	7.82	8.57	8.71	9.52	11.65	9.68		
	90	7.48	6.76	8.27	6.64	6.51	7.72	6.99	6.83		

* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$; NS: non significant; ND: not detected.

3.2.4. Aldehydes

Six aldehydes were identified in Malatya cheeses (Tab. V) and are produced by the catabolism of fatty acids or amino acids via decarboxylation or deamination. Aldehydes are transitory compounds and do not accumulate in cheese because they are transformed rapidly to alcohols or to corresponding acids [10, 23]. No significant differences in concentrations of 2-methylpropanal, 2-methylbutanal and 3-methylbutanal branched-chain aldehydes were found among the cheeses made from raw or pasteurized milk. These aldehydes are produced from Val, Ile and Leu, respectively, by Strecker degradation or transamination and are responsible for unclean and harsh flavours in Cheddar cheese [10]. The most abundant aldehyde was 3-methylbutanal in 30-d old cheeses; however, its concentration decreased at 90 d and acetaldehyde was the highest in 90-d old cheeses. The concentration of acetaldehyde in the cheeses increased during ripening as observed in other types of cheese such as Roncal [32], Emmental [5]. Benzaldehyde is formed in cheese by α-oxidation of phenyl acetaldehyde which is derived from Phe via the Strecker reaction or β-oxidation of cinnamic acid [25]. The concentration of benzaldehyde was not influenced by pasteurization and ripening processes.

3.2.5. Alcohols

The cheeses made from raw milk contained higher levels than those of the cheeses made from pasteurized milk for most of the alcohols, especially after 90 d of ripening (Tab. VI). The concentrations of 1-propanol, 2-propen-1-ol, 3-methyl 1-butanol, 3-methyl 2-buten-1-ol, 2-propanol, 2-butanol, 2-pentanol, 2-methoxy ethanol and phenethyl alcohol were significantly higher in raw milk cheeses, suggesting the pasteurization process negatively influenced the production of alcohols in cheese during ripening. Primary alcohols such as 1-propanol, 1-butanol, 1-pentanol, 1-hexanol, 1-heptanol, 1-octanol are produced mainly by the reduction of aldehydes and

Table VI. Concentrations of alcohols in Malatya cheese made from raw (C) and pasteurized (P) milk during ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C). The results were expressed as SQRT [Area/10^5] from triplicate analysis of each cheese.

Alcohol	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
Ethanol	30	37.89	36.73	40.02	40.24	38.03	36.76	40.02	38.99	NS	**
	90	29.07	34.89	32.20	31.07	39.92	35.81	33.58	42.59		
1-Propanol	30	3.95	4.04	4.48	6.14	6.43	3.47	4.11	4.20	**	***
	90	9.18	10.35	8.78	8.64	3.12	3.32	3.34	3.62		
2-Propen-1-ol	30	0.57	0.85	0.70	1.35	1.31	0.57	0.55	ND	***	***
	90	2.31	3.04	2.89	3.24	ND	ND	ND	ND		
2-Methyl 1-propanol	30	1.67	3.11	3.23	3.83	1.52	1.54	1.69	1.66	NS	***
	90	4.55	4.39	5.62	3.97	4.49	6.35	6.60	4.56		
1-Butanol	30	4.50	4.74	3.83	3.70	3.61	3.78	4.45	3.88	NS	***
	90	3.35	3.57	3.54	3.31	2.58	2.79	3.00	3.15		
2-Methyl 1-butanol	30	2.99	3.81	3.90	4.34	3.30	3.44	3.64	3.44	NS	***
	90	6.55	6.56	7.44	6.04	5.51	6.13	6.20	5.36		
3-Methyl 1-butanol	30	7.84	10.17	10.63	11.86	8.96	9.35	9.86	9.44	*	***
	90	15.85	16.10	17.21	14.81	12.33	12.94	13.24	12.14		
3-Methyl 3-buten-1-ol	30	1.20	1.33	1.38	1.39	1.35	1.35	1.32	1.22	NS	**
	90	2.27	3.03	2.27	2.05	1.91	1.59	1.56	1.94		
3-Methyl 2-buten-1-ol	30	0.90	0.88	0.87	0.98	1.22	1.01	0.94	0.80	**	***
	90	1.92	2.64	2.39	2.38	1.19	1.01	1.00	1.10		
1-Pentanol	30	1.59	1.67	1.55	1.56	1.45	1.63	1.79	1.61	NS	NS
	90	1.40	1.25	1.48	1.35	1.86	1.69	1.76	1.82		
1-Hexanol	30	2.29	2.53	2.10	2.22	2.12	2.66	2.57	2.30	NS	NS
	90	2.27	2.34	2.11	2.31	2.05	1.96	2.06	2.06		
2-Ethyl 1-hexanol	30	1.78	2.06	1.68	1.97	2.23	2.47	1.76	1.44	NS	*
	90	2.03	2.20	2.06	2.58	2.20	2.35	2.52	2.31		
1-Heptanol	30	0.57	0.57	0.53	0.58	0.54	0.54	0.49	0.47	NS	*
	90	0.64	0.65	0.62	0.72	0.59	0.59	0.61	0.49		
1-Octanol	30	0.81	0.95	1.02	1.22	0.88	0.65	0.65	0.54	NS	**
	90	0.56	0.55	0.51	0.60	0.45	0.50	0.55	0.41		
2-Propanol	30	4.83	4.76	4.62	4.20	3.04	2.73	2.65	2.70	**	**
	90	5.46	5.73	5.76	5.14	2.59	4.24	4.28	3.64		
2-Butanol	30	4.37	4.52	4.47	7.13	5.50	3.47	3.86	3.61	***	***
	90	20.18	22.76	20.87	19.11	4.48	4.96	5.14	5.52		
2-Pentanol	30	3.17	2.82	2.72	2.70	2.39	2.35	2.42	2.28	*	***
	90	4.68	5.66	5.12	4.86	3.06	2.75	2.96	3.56		
2-Hexanol	30	0.89	0.66	0.62	0.61	0.51	0.56	0.56	0.53	NS	*
	90	0.88	1.03	0.94	0.93	0.61	0.62	0.63	0.65		

Table VI. (continued).

Alcohol	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
2-Heptanol	30	7.25	4.41	2.84	3.02	2.64	3.35	2.68	2.44	NS	NS
	90	4.76	5.46	5.04	5.17	2.57	2.69	2.86	2.60		
2-Octanol	30	0.47	0.68	0.47	0.57	0.55	0.62	0.62	0.64	NS	NS
	90	0.45	0.44	0.50	0.57	0.49	0.48	0.46	0.56		
2-Nonanol	30	1.40	1.07	0.66	0.79	0.80	1.33	0.79	0.70	NS	NS
	90	1.28	1.32	1.41	1.53	0.63	0.66	0.68	0.54		
2-Methoxy ethanol	30	2.54	2.55	2.43	2.30	2.23	2.23	2.14	1.99	*	***
	90	2.06	2.11	2.01	2.04	1.70	1.50	1.52	1.48		
1-Methoxy 2-propanol	30	3.14	3.85	1.43	1.86	1.94	2.22	2.20	1.98	NS	NS
	90	2.05	2.61	2.64	2.43	1.89	2.08	2.16	2.16		
2-Butoxy ethanol	30	1.27	1.11	0.86	0.94	0.87	0.83	0.79	0.71	NS	*
	90	0.84	0.84	0.82	0.82	0.65	0.68	0.76	0.77		
Benzyl alcohol	30	0.95	0.98	0.85	0.91	1.04	1.11	0.82	0.53	NS	NS
	90	1.05	1.06	1.10	0.93	0.51	0.67	0.87	0.60		
Phenethyl alcohol	30	1.12	0.89	0.58	0.67	0.65	0.60	0.55	0.52	*	***
	90	1.89	1.58	2.03	1.81	0.87	1.01	1.04	0.76		
Total	30	99.91	101.71	98.45	107.04	95.11	90.58	93.92	88.60		
	90	127.51	142.14	137.34	128.40	98.23	99.36	99.36	104.38		

* $P < 0.01$, ** $P < 0.001$, *** $P < 0.0001$; NS: non significant; ND: not detected.

methyl ketones [1] and they impart an alcoholic, winey, sweet, fruity and harsh notes in cheese [2]. The concentration of primary alcohols were higher in the cheeses than those of secondary alcohols, probably due to the higher concentrations of ethanol and 3-methyl 1-butanol. The highest amounts of ethanol were found at 30 d of ripening in the cheeses and its concentration decreased in 90-d old cheeses. Ethanol is also found as the principal alcohol in other types of cheeses such as Feta [3, 18], Roncal [31], Minas [8], Cheddar [1] and Hispanico [33]. The presence of the branched-chain primary alcohols including 2-methyl-1-butanol, 2-methyl-1-propanol and 3-methyl-1-butanol in the cheeses indicates conversion of aldehydes produced from Ile, Val and Leu, respectively [11]. Secondary alcohols such as 2-propanol, 2-butanol, 2-pentanol are formed by enzymatic reduction of methyl ketones [29] and their amounts changed with pasteurization of milk or ageing. The concentration of 2-butanol was highest within secondary alcohols in raw milk cheeses at 90 d of ripening. Of the secondary alcohols, the concentrations of 2-heptanol, 2-octanol, 2-nonanol which may be produced by reduction of methyl ketones were not influenced by pasteurization or ripening processes, suggesting no role of NSLAB or released enzymes of starter cultures used in the production. 2-Methoxy ethanol was present in all cheeses at the highest levels at 30 d of ripening, but its concentration was influenced by pasteurization and ageing processes. The formation of 1-methoxy 2-propanol, 2-butoxy ethanol and benzyl alcohol were not significantly affected by pasteurization or ageing, except 2-butoxy ethanol was affected with age. Raw milk cheeses contained a higher concentration of phenethyl alcohol than pasteurized milk cheeses at each stages of ripening. Phenethyl alcohol which may be produced by catabolism of Phe via transamination,

Table VII. Concentrations of lactones in Malatya cheese made from raw (C) and pasteurized (P) milk during ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C). The results were expressed as SQRT [Area/10^5] from triplicate analysis of each cheese.

Lactone	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
Butyrolactone	30	1.08	1.08	0.91	0.94	0.89	0.90	0.85	0.81	NS	*
	90	0.81	0.87	0.80	0.72	0.79	0.74	0.72	0.71		
γ-Hexanolactone	30	0.43	0.35	0.29	0.31	0.24	ND	ND	ND	*	NS
	90	0.26	0.34	0.36	0.37	ND	ND	ND	ND		
Total	30	1.51	1.43	1.20	1.24	1.13	0.90	0.85	0.81		
	90	1.07	1.21	1.15	1.10	0.79	0.74	0.72	0.71		

* $P < 0.01$; NS: non significant; ND: not detected.

decarboxylation or reduction reactions [25] provides a rose like note to cheese and it is one of the main volatiles in Camembert cheese [29]. Correa Lelles Nogueira et al. [8] reported that the floral character of Minas cheese correlated with the availability of phenethyl alcohol in the cheese.

3.2.6. Lactones

Only two lactones, butyrolactone and γ-hexanolactone were identified in Malatya cheeses (Tab. VII) and no significant differences were seen between cheeses for butyrolactone; its concentration was at a higher level at 30 d of ripening and then declined. γ-Hexanolactone was present in only P60 cheeses within pasteurized milk cheeses at 30 d of ripening and its concentration in raw milk cheeses did not change with age. Lactones contribute to cheese flavour giving fruity notes (peach, apricot, and coconut) [29] and may be formed by intramolecular esterification of hydroxy fatty acids by action of microorganisms; that is, they form spontaneously once the fatty acid is released by lipolysis [25]. This suggests that the lactones contribute to the creamy coconut note of the flavour of Malatya cheese.

3.2.7. Sulfur compounds

The concentrations of sulfur compounds in Malatya cheese declined with age (Tab. VIII). The most abundant compound was carbon disulfide at 30 d of ripening in the cheeses. Carbon disulfide was also the most abundant sulfur compound in Manchego cheese made from raw or pasteurized milk and no significant differences were found between cheeses [12]. The concentrations of sulfur compounds were not influenced by milk pasteurization except dimethyl disulfide. Dimethyl trisulfide was not identified in raw milk cheeses at any ripening period. Methanethiol was not present in any of the cheeses probably due to the extraction method of the volatiles; this compound is considered a key compound for Cheddar flavour [40, 41]. The lower levels of dimethyl trisulfide in cheeses (absent in raw milk cheeses) can be linked to the absence of methanethiol which is a precursor of dimethyl disulfide and dimethyl trisulfide [11].

3.2.8. Terpenes

Five terpenes were identified in Malatya cheese and α-pinene is the principal terpene in the cheese (Tab. IX). α-Pinene was also identified in Cheddar [9], Minas [8] and Manchego [12] cheeses. These compounds originate from plants which represent the feed mixture of pasture and are then transferred to milk and milk product via grazing animals [8]. Camphene was not identified in raw milk cheeses and it was found in pasteurized milk cheeses only at 90 d of ripening. β-Pinene was not identified at d 30 for all cheeses, while its concentration was higher in pasteurized milk cheeses than raw milk cheese at 90 d of ripening. Limonene and *p*-cymene were present in all cheeses

Table VIII. Concentrations of sulfur compounds in Malatya cheese made from raw (C) and pasteurized (P) milk during ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C). The results were expressed as SQRT [Area/10^5] from triplicate analysis of each cheese.

Sulfur compound	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
Carbon disulphide	30	2.19	1.66	2.11	1.77	1.78	2.77	1.81	3.08	NS	***
	90	0.84	0.89	0.93	1.19	0.91	0.88	0.69	0.80		
Dimethyl sulphide	30	0.83	0.70	0.80	0.70	0.73	0.90	0.65	0.82	NS	*
	90	0.77	0.77	0.84	1.27	0.93	0.99	0.61	0.96		
Dimethyl disulphide	30	0.57	0.55	0.40	0.43	0.59	0.85	0.82	0.92	*	NS
	90	0.38	0.54	0.46	0.56	0.83	0.76	0.69	0.75		
Dimethyl trisulphide	30	ND	ND	ND	ND	ND	0.42	0.39	0.46	NS	NS
	90	ND	ND	ND	ND	0.28	0.32	ND	0.21		
Dimethyl sulphone	30	0.45	0.51	0.62	0.62	0.68	0.63	0.61	0.49	NS	*
	90	0.37	0.41	0.44	0.39	0.39	0.31	0.35	0.32		
Total	30	4.04	3.42	3.92	3.52	3.79	5.55	4.27	5.77		
	90	2.35	2.61	2.68	3.41	3.34	3.25	2.34	3.03		

* $P < 0.01$; *** $P < 0.0001$; NS: non significant; ND: not detected.

Table IX. Concentrations of terpenes in Malatya cheese made from raw (C) and pasteurized (P) milk during ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C). The results were expressed as SQRT [Area/10^5] from triplicate analysis of each cheese.

Terpene	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
α-Pinene	30	0.66	0.72	0.61	0.56	0.48	0.57	0.57	0.60	**	***
	90	1.96	2.17	2.20	2.27	4.80	4.87	4.25	4.65		
Camphene	30	ND	ND	ND	ND	ND	ND	ND	ND	***	***
	90	ND	ND	ND	ND	0.55	0.54	0.50	0.50		
β-Pinene	30	ND	ND	ND	ND	ND	ND	ND	ND	NS	***
	90	0.34	0.21	0.33	0.37	0.70	0.73	0.62	0.72		
Limonene	30	1.26	1.25	0.77	0.75	0.72	0.70	0.80	0.83	NS	NS
	90	0.64	0.70	0.66	0.86	0.59	0.77	0.70	0.66		
p-Cymene	30	1.54	1.46	0.66	0.95	0.90	0.73	0.62	0.70	NS	NS
	90	0.83	0.68	0.72	0.84	1.06	1.29	1.25	0.99		
Total	30	3.46	3.43	2.03	2.26	2.09	2.01	1.99	2.13		
	90	3.77	3.75	3.90	4.34	7.70	8.20	7.31	7.52		

** $P < 0.001$; *** $P < 0.0001$; NS: non significant; ND: not detected.

and there were no significant differences among cheeses, suggesting no role of pasteurization and ripening process to the formation of limonene and *p*-cymene.

3.2.9. Miscellaneous compounds

A number of miscellaneous compounds were identified in Malatya cheese (Tab. X).

2,5-Dimethyl pyrazine was detected in 30-d old cheeses at very low levels, but not determined in C80 and P60 cheeses. The compound is produced in cheese by condensation of amino ketones, which are formed by Maillard and Strecker degradation reactions [13] and imparts roasted and hazelnut notes [27]. Phenolic compounds which are mainly present in sheep's milk as conjugates of phosphate and sulphate contribute to cheese aroma at about threshold concentration [40]. Phenol is an important compound for surface ripened cheese and its contribution to cheese aroma is perceived as sharp and medicinal notes [29]. The concentrations of phenol and acetophenone were not different among the cheeses made from raw or pasteurized milk. Their concentration increased significantly with age. Small differences, but not significant, were seen between acetophenone levels of raw or pasteurized milk cheeses at 90 d of ripening. Acetophenone is produced in cheese via β-oxidation of phenylpropionic acid followed by decarboxylation of the β-ketoacid [37]. 4-Methyl phenol (*p*-cresol) is formed via catabolism of aromatic amino acids and contributes to cheese flavour as unclean or off-flavours [25]. In Malatya cheese, *p*-cresol was not found in pasteurized milk cheeses and found only in 90-d old raw milk cheeses. Methyl salicylate and *p*-methyl anisole were present in all cheeses at similar abundances and their concentrations declined with age. Hydrocarbons are secondary products of lipid autoxidation and are precursors for the formation of other aromatic compounds [5]. Benzene, toluene and ethyl benzene were found in the volatile fraction of Malatya cheese and their concentrations were not influenced by pasteurization or ripening processes. Of these hydrocarbons, toluene was the most abundant compound and it was also identified at high levels in Feta-type [3] and a Spanish ewe's milk semi-hard [24] cheeses. In addition, benzyl nitrile which is a benzyl compound was identified only in 90-d old raw milk cheeses. The concentrations of carbon dioxide was similar in all cheeses, this compound is produced by catabolism of lactate, citrate and fatty acids [25]. Its concentration in raw milk cheeses did not change during ripening, but decreased in pasteurized milk cheeses. Styrene which has a strong plastic odour was found in Malatya cheeses made from raw or pasteurized milk and differences between cheeses were not significant. Styrene was also detected at trace levels in Provola dei Nebrodi cheese [42], Parmigiano [4], Roncal [20] and Camembert [29] cheeses. Diethyl ether, chloroform and bromoform which are likely external origin compounds were also identified in Malatya cheeses. Diethyl ether and bromoform were found in the cheeses at similar concentrations; however, chloroform had higher levels in pasteurized milk cheeses. Chloroform was also identified in Roncal [32], Feta-type [3] and Provola dei Nebrodi [42] cheeses.

3.2.10. Principal component analysis

Principal component analysis (PCA) was applied to those variables which presented significant ($P < 0.01$) differences among the cheeses to clarify separation of the cheese samples and interpretation of the results. Significant differences among the samples on the first two principal components on the PCA were determined by ANOVA. The results of PCA indicated that the cheeses were distinguished according to their GC-MS profiles. Two bi-plots of the sample scores and variable loadings for PC1 and PC2 is shown in Figures 1a and 1b for 30 or 90 d of ripening, respectively. PC1 and PC2 explained 88 and 6% of the variation between the volatiles of the cheeses, respectively, at 30 d. For 30 d, PC1 separated the cheeses on the basis of milk type used (raw or pasteurized) in the production of Malatya cheese. Samples were distributed into two main groups: raw milk cheeses located on the positive side on PC1 and pasteurized milk cheeses located on the negative side of PC1. The cheeses made with raw milk contained higher levels of acids, esters and lactones and lower levels of aldehydes and sulfur compounds than did the cheeses made from pasteurized milk. At 30 d, the cheeses made from raw milk were described as higher abundance of diacetyl, octane, pentanoic acid (5,0), 2-propenal, 2-propanol, 2-propen-1-ol and 1-hydroxy

Table X. Concentrations of miscellaneous compounds in Malatya cheese made from raw (C) and pasteurized (P) milk during ripening. Codes 60, 70, 80 or 90 refer to scalding temperature of the curd (°C). The results were expressed as SQRT [Area/10^5] from triplicate analysis of each cheese.

Miscellaneous	Age (d)	Cheeses								ANOVA	
		C60	C70	C80	C90	P60	P70	P80	P90	*P*-type	*P*-age
2,5-Dimethyl pyrazine	30	0.26	0.28	ND	0.21	ND	0.25	0.25	0.10	NS	NS
	90	ND	ND	ND	0.26	ND	ND	ND	ND		
Phenol	30	0.70	0.68	0.55	0.64	0.65	0.60	0.59	0.55	NS	**
	90	0.86	0.90	0.89	0.89	0.79	0.89	0.87	0.85		
2-Methyl phenol	30	ND	ND	ND	ND	ND	ND	ND	ND	**	***
	90	0.47	0.47	0.52	0.55	ND	ND	ND	ND		
4-Methyl phenol	30	0.06	ND	ND	ND	ND	ND	ND	ND	**	***
	90	0.53	0.51	0.61	0.61	ND	ND	ND	ND		
Acetophenone	30	0.91	1.20	0.76	0.91	0.98	1.21	0.86	0.89	NS	*
	90	1.23	1.18	1.40	1.47	0.99	1.08	1.32	0.96		
p-Methyl anisole	30	0.52	0.72	0.47	0.73	0.72	0.92	0.80	0.79	NS	**
	90	0.69	0.55	0.54	0.71	0.37	0.52	0.51	0.35		
Methyl salicylate	30	0.98	1.02	0.65	0.78	0.77	0.69	0.55	0.57	NS	**
	90	0.61	0.53	0.54	0.56	0.32	0.39	0.38	0.23		
Benzene	30	1.01	1.21	0.99	0.99	0.75	0.87	1.14	1.08	NS	NS
	90	0.79	0.86	0.84	0.88	1.13	1.18	1.25	1.05		
Toluene	30	5.43	5.94	4.33	4.25	3.49	4.25	4.66	4.45	NS	NS
	90	3.18	3.52	3.69	3.91	4.71	4.89	4.88	4.75		
Ethyl benzene	30	3.67	3.80	2.88	2.68	2.52	2.93	2.82	3.07	NS	NS
	90	2.50	2.70	2.68	3.08	2.91	3.03	2.93	2.85		
Carbon dioxide	30	9.32	9.65	10.60	11.05	12.37	11.08	10.96	9.51	NS	*
	90	9.79	10.60	11.28	9.50	9.29	8.27	7.78	8.28		
Styrene	30	3.66	4.58	2.93	3.17	3.01	3.37	3.08	3.29	NS	NS
	90	3.04	2.69	3.07	3.43	3.00	3.51	3.79	3.21		
Benzyl nitrile	30	ND	ND	ND	ND	ND	ND	ND	ND	***	***
	90	0.37	0.46	0.50	0.48	ND	ND	ND	ND		
Pentane	30	5.11	6.02	4.34	4.25	4.13	3.55	4.55	4.45	NS	NS
	90	3.49	3.89	4.10	3.68	4.97	4.79	5.25	4.11		
Hexane	30	3.54	3.56	2.33	3.06	1.77	1.77	3.90	1.74	NS	**
	90	1.61	1.91	2.12	1.40	1.73	1.54	1.83	1.47		
Octane	30	0.59	0.38	0.63	ND	ND	ND	ND	ND	*	*
	90	0.60	0.53	0.60	0.72	ND	0.56	ND	0.49		
Diethyl ether	30	6.36	5.97	5.63	5.63	4.31	4.97	7.33	7.41	NS	NS
	90	4.24	4.94	5.59	4.15	6.74	7.07	6.02	6.51		
Chloroform	30	5.17	5.57	5.00	4.46	3.66	4.13	6.19	7.34	*	**
	90	3.41	3.59	3.72	3.07	4.37	5.07	5.56	4.78		
Bromoform	30	0.69	0.75	0.58	0.67	0.45	0.66	0.81	0.57	NS	**
	90	0.33	0.40	0.33	0.40	0.37	0.41	0.40	0.43		
Total	30	47.97	51.33	42.66	43.46	39.60	41.25	48.50	45.80		
	90	37.74	40.23	43.03	39.75	41.68	43.20	42.75	40.31		

* $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$; NS: non significant; ND: not detected.

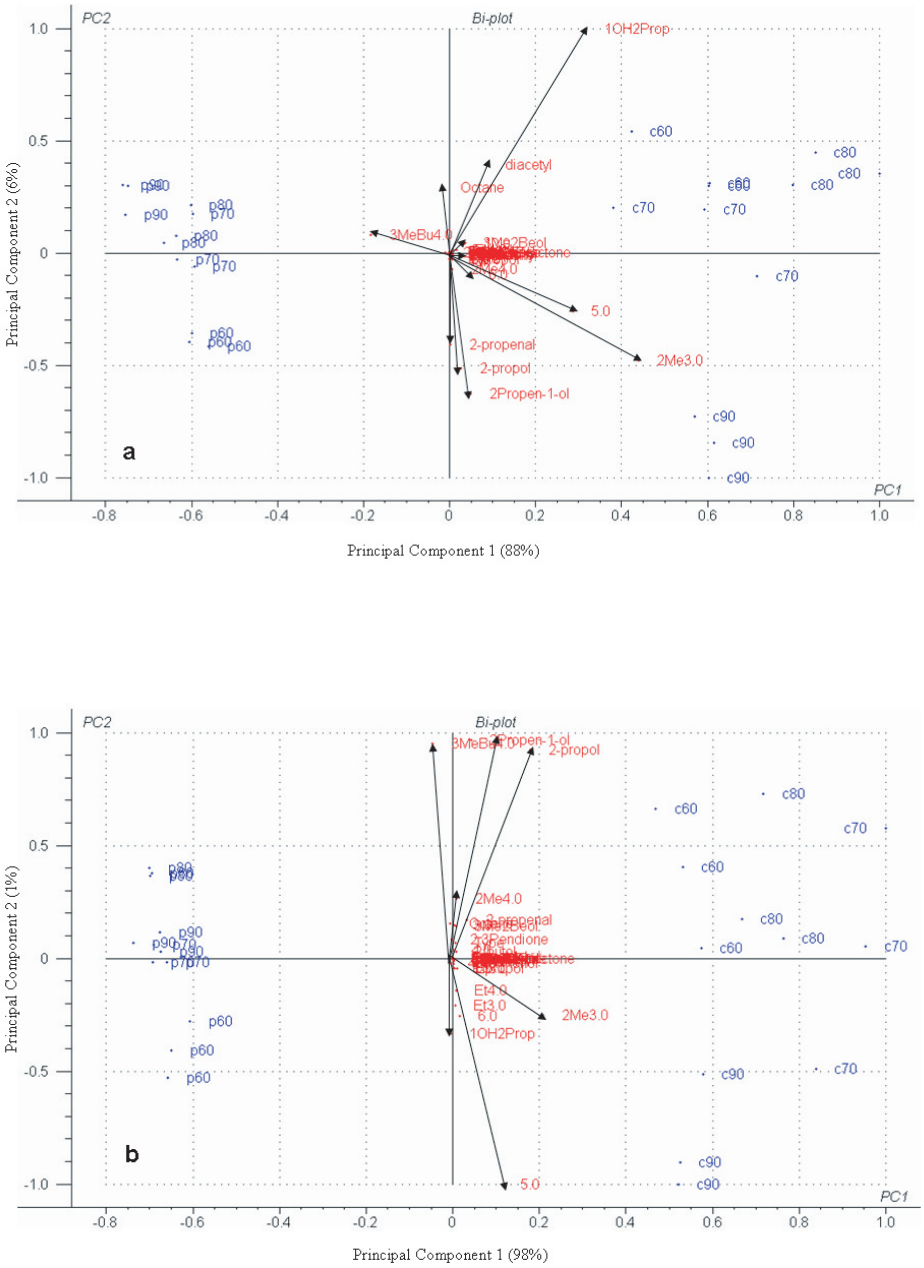

Figure 1. Bi-plots of principal components 1 and 2 showing the cheese sample scores and variable loadings from principal component analysis of the volatile data for Malatya cheeses made from raw (C) and pasteurized (P) milk and scalded at 60, 70, 80, or 90 °C after 30 (a) and 90 (b) d of ripening.

2-propanone than those of pasteurized milk cheeses. PCA was also applied to the GC-MS data obtained from the 90-d old cheeses. Samples were distributed into two groups (raw and pasteurized milk cheeses) as in 30-d old cheeses. PC1 and PC2 accounted 98% and 1% of the variance, respectively. PC1 separated the cheeses on the basis of use of raw or pasteurized milk in the cheese manufacture, while no regular distribution was observed by PC2 based on scalding temperature of the curd. Raw milk cheeses were described as containing higher levels of pentanoic acid, 2-methyl propanoic acid, 2-propan-1-ol, 2-propanal, 3-methylbutyl butanoate and 1-hydroxy 2-propanone than those of pasteurized milk cheeses. The similarity of the plots obtained for each ripening period showed that the pasteurization of milk had a greater effect on the volatile composition of the cheese than scalding temperature of the curd.

4. CONCLUSION

The results of this study showed that the use of raw milk in the manufacture enhanced the volatiles in cheese, especially acids, alcohols and esters. Also, it can be concluded that the pasteurization of the milk has a decreasing and/or delaying effects on the development of the volatile compounds of cheese. Scalding the curd in hot whey at different temperatures has a slight effect on the volatile compounds in Malatya cheese and pasteurization of cheese milk had a greater effect on the volatile composition of cheese than the scalding temperature of the curd. Many volatile compounds are responsible for the characteristic flavour of Malatya cheese.

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