Evaluation of Volatile Compounds of Red and White Wine Treated Dil Cheese during Refrigerated Storage

Volatile compounds in wine treated cheeses

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***Abstract-* The purpose of this study was to analyze volatile compounds (VCs), basic chemical composition and overall acceptability of wine-treated-cheeses (WTCs) during 28 days refrigerated storage. Red and white wines were used for cheese immersion. VCswere determined using a Headspace Solid Phase Microextraction (HS-SPME) and GC-MS methods. Wine-treatment processesresulted in a significant (P < 0.05) decrease in total solid, ash and salt contents of Dilcheese compared with control cheese (CC). The major VCs in WTCs and wine sampleswere alcohols, mainly ethanol, and ethyl esters, whereas the most abundant VCs in CC were free fatty acids, ketones, phenolics as well as alcohol and esters.The ethanol, benzeneethanol and diethyl butanedionate were higher in WTCs than CC and wineused.Diethyl butanodionate, benzeneethanol and 16-oxosalutaridine compounds were identified for the first time in the cheeses. Except for ethanol, most VCsidentified in CC were not detected in WTCs.Although the ethanol concentrations were similar inboth types of WTCs,whitewinetreatedcheeses(WWTCs)contained higher levels of isoamyl alcohol, 2-phenyl methanol and benzenol, which was much more preferred by the sensory panel. Therefore, WWTCs may be a good alternative to cocktail foods for restaurants, hotels and other venues.**

Keywords-* *Dil cheese; Wine; Volatile Compounds; HS-SPME

I. INTRODUCTION

Dil cheese is a scalded and stretched hard cheese variety. Itis usually produced from raw cow milkwithout starter culture addition. Dil cheese is manufactured in two steps: curdmakingand cooking/stretching. The curd is scalded to 55°C and above, and is stretched on table. Stretching causes curds to become fibrous and malleable. The cheese is dry-salted and stored at 4-6°C. Dil cheese looks like Mozzarella. It is often consumed at breakfast as fresh cheese. In general, wine and cheese is seperately consumed at hotels, restaurants and other eateries.Flavour of wine is dependent mainly on the variety of grape,fermentation process and the aging stage. Volatile compounds (VCs) have an impact on the both wine and cheese quality [1-4]. Thus, accurate and precise analytical methods are neccessary to determine volatile compounds in food samples. Solid phase microextraction (SPME) technique is widely used in cheese and wine [2,4-5]. SPME is a simple, fast, solvent-free and sensitive technique [6-7]. As the heating of sample is minimal at SPME, the recovery of volatile compounds is closer to real value in a sample than those obtained by simultaneous steam-distilation extraction (SDE) and hydrodistilation (HD) [8]. SPME technique can be successfully applied for polar and non-polar compounds in gas, liquid and solid samples [9]. Novel fibers such as PDMS (polydimethylsiloxane), DVB (divinylbenzene) and CAR (carboxene) have been developed for non-polar and polar volatile compounds. SPME fibers coated two adsorbant phases such as CAR/PDMS and PDMS/DVB have been previously used for cheese volatiles [5, 10]. Fiber carbowax (CWAX)/divinylbenzene (DVB) was used for extraction of wine volatiles[2]. We have chosen a fiber consisting of DVB/CAR/PDMS for wine and cheese volatile compounds since this fiber is characteristic for odours compounds including non-polar and polar volatiles [11].On the otherhand, no literature has been available on the determination of volatile compounds (VCs) in wine-treated-cheese (WTC) compared to control cheese (CC), as well as wine samples (both red and white) used for immersionof cheeses by using a DVB/CAR/PDMS fibre SPME analytical method. To our knowledge, there is only one publication on WTC,in which has not been reported the fiber characteristics used for VC extractionand was tested a mixture of red and white wine for cheese treatment [12]. Recently, consumers have demanded new products having different flavour and functional properties. In order to ascertainthe feasibility of increased product diversity for the dairy food industry, wehave conducted ourstudies on determination of nutritional and flavourcharacteristics of white and red wine-treated-cheeses (WWTCs and RWTCs).

For this end, the basic chemical properties,VCs and overall acceptability of WTCs were analyzed in comparison withthose of control cheese (CC). Therefore, the aims of the present study were: (i) to determinethe changes in basic chemical composition and volatile compounds (VCs) of CC, and WTCs during 28 days of refrigerated storage, and (ii) to evaluate the acceptability of red and white wine treated cheeses in comparison with that of CC by sensory panelists.

II. MATERIALS AND METHODS

A. Experimental Design

The study was conducted as 2 x 4 factorial experiment, where two types of cheeses (red and white wine treated) and four storage periods (1, 7, 14 and 28 days). All wine-treated-cheeses (RWTCs and WWTCs) were storaged at 4ºC for 28 days. Two batches of WTCs were produced, and two replicates of each type of cheeses were sampled at four storage periods for nutritional, flavor and sensory analyses.

B. Red and White Wine

Red and white wine used in this study were produced from *Vitisvinifera Emir* (grapes provided from Aegean and Central Anatolia Regions in Turkey) and *Vitislabrusca*Kalecikkarası and Boğazkere (grapes obtained from Denizli province in Aegean Region), respectively. Red and white wine samples have been for three and twoyearsold, respectively.

C. Preparation of Experimental DilCheese

Dil cheeses were provided by a producer, which were traditionally produced from cow milk in a small dairy plant in Hatay. A 250 L of milk was coagulated at 32ºC for 50 min with rennet (strenght, 1:17 000) (Yörük, Peyma& Chr. Hansen, İstanbul, Turkey) without the addition of starter culture. The native microflora of raw milk provided the acid–producing bacteria for cheesemaking. Following coagulation, the coagulum was cut into about 1cm^3 cubes, and whey was drained. The curds were left to drain and ripened for 24 h at room temperature. After ripening, the curds were cut into thick slices (about 1 cm), stretched in hot water (75-80°C), and then manually stretched on a table. Finally, it was dry-salted,and on the following day, cheeses were placed in glass jars (1000 g). Wine-untreated cheese was coded as control cheese (CC). The other glass jars placed cheese were filled up to red or white wine and closed.Red and white wine-treated-cheeses were coded as 'RWTC' and 'WWTC', respectively.

D. BasicChemical Analysis

The cheeses were analyzed for total solid [13], salt and titratable acidity[14], pH using a pH meter (Orion Thermo, 3 stars, Beverly, MA, USA) and fat content [13].Total nitrogen content was measured by micro-Kjeldahl method [15], using the Gerhardt KB 40S digestion and Vapotest distillation systems (C. Gerhardt, Bonn, Germany).Ash content was quantitated by dry ashing the samples in a muffle furnace at 550 ^{o}C for 24 h. Before samples were placed in a muffle furnace, they were pre-dried in an oven at 105^{o}C for 8 hours.

E. Volatile Compound Analysis

The extraction and characterization of the volatile compounds were carried out by the headspace (HS)-solid phase microextraction (SPME)-Gas Chromatography (GC)-Mass Spectrometry (MS) analysis. Volatile samples were prepared in triplicate from wine samples, and wine treated cheeses or non-treated control cheese samples. In each sampling time, each cheese sample was cut into small pieces and placed in a chilled mortar and ground with a pestle. After several preliminary tests to optimize solid phase microextraction (SPME) system, 10g of the homogenized cheese and wine sample was immediately transferred in 20 mL head space vial (Agilent, Palo Alto, CA, USA). For wine samples, the vials contained 3 g NaCl to inhibit enzyme reactions as described inCarrillo et al. [16]. The vials were sealed using crimp-top caps with TFE/silicone headspace septa (Agilent, Palo Alto, CA, USA) and immediately frozen at -20ºC until analysis. Prior to analysis, frozen samples were thawed at 4ºC overnight. At the time of solid phase microextraction analysis, the vials were placed in a water bath with temperature control and stirring. For cheeses, the sample vials were equilibrated for 30 min at 60ºC in the water bath, then a 50/30 μm DVB/CAR/PDMS fibre (Supelco, Bellefonte PA, USA) was exposed to the sample headspace for 40 min at 60ºC. For wine, vials were kept at 45ºC for 15 min, then DVB/CAR/PDMSfibre (Supelco, Bellefonte PA, USA) was exposed to the sample headspace for 15 min at 45ºC. The fiber was conditioned for maximum performance at 260 °C for 1 h before being placed in the subsequent sample. A similar extraction procedure was previously carried out by Pozo-Bayon,Pueyo, Martín-Alvarez and Polo[6] for wine and Ziino,Condurso, Romeo, Giuffridaand Verzera [3] for cheese. These sampling temperatures were chosen after preliminary trials at different temperatures. After sampling, desorption of the volatile compounds from the fibre coating was carried out in the injection port of GC at 250 ºC during 3 and 5 min in splitless mode for wine and cheese, respectively. The identification and quantification of volatile compounds were carried out on Agilent model 6890 GC and 5973 N mass spectrometry (MS) (Agilent, Palo Alto, CA, USA) equipped with a HP-INNOWAX capillary column (60 m x 0.25 mm id x 0.25μm film thickness). Helium was used as carrier gas at a flow rate of 1mL min^{-1}.

The oven temperature program was initially held at 50ºC for 1 min and then programmed from 50ºC by a ramp of 5ºC min^{-1} up to 100ºC and then at 10ºC min^{-1} to reach a final temperature of 230ºC, which held for 10 min. The mass selective (MS) detector was operating in the scan mode within a mass range 33 to 330 m z^{-1} at 1 scan s^{-1}, with electron energy of 70eV. The interface line to MS was set at 250ºC. The total analysis time was 30 min. The volatile compounds were preliminarily identified by a computer-matching of their mass spectral data supplemented with a Wiley7n.1 and Nist 02.L. GC-MS libraries, and then the identifications of the molecular mass were confirmed by GC retention time (RT) and MS ion spectra of authentic standards (Sigma-Aldrich, Milwaukee, WI, USA). The retention indices were also determined for all constituents by using homologous series of *n*-alkanes C_5–C_{25}. Results from the volatile compound analyses were expressed as the percentage of each compounds integrated area relative to the total integration of compounds identified.

F. Sensory Analysis

Sensory evaluation was performed by 16 experienced panelists highly familiar with both dil cheese and wine. The panel consisted of academic staff and students from Food Engineering Department of Mustafa Kemal University, Hatay, Turkey. Cheeses were removed from refrigerator (4 ºC), and kept at room temperature (22±2ºC) for 1 h prior to sensory evaluation. By using a 9-point hedonic sale (1 = dislike extremely, *5* = neither like nor dislike, 9 = like extremely,the sensory panel rated acceptability of the experimental cheeses in duplicates.

G. Statistical Analysis

Statistical analysis on chemical composition, volatilecompoundsand sensory scores of all experimental data were performed using SPSS statistic program (SPSS version 17.00 software, 2009). The effects of main factors, storage times (1, 7, 14, 21, 28 days), types of cheeses (wine treated: red and white) and CC, and their interaction effects between storage time x types of cheeseswereanalyzed by two-way analysis of variance (ANOVA). The mean differences were separated using Duncan's multiple-range test. The least significant differences(P<0.05) between treatment means were also tested.

III. RESULTS AND DISCUSSION

The basic chemical composition and the volatile compounds (VCs)as well as overall acceptability of wine-treated-cheese (WTC)during storage are shown in Table 1.The Dil cheese in our study contained 55.2% total solids, which is in agreement with that ofFox, Guinee, Cogan and McSweeney [17], where it is shown as a semi-hard cheese variety having 55% total solids content. The low acidity (0.35% L.A.) in the control cheeseis probably due to the microorganism inactivation during cooking process of the curds.On day 1, the pHdecreased and titratable acidityvalues increased inWTCscompared with those of CC. However, titratable acidity was decreased from day 1 to the end of storage, while pH was unchanged, which may be attributed to the formation of HCO_3 as a result of the reaction between H^+ and CO_2 and/or the neutralizing effect of alcohol in wine.

TABLE I. THE BASIC CHEMICAL PARAMETERS IN RED OR WHITE WINE TREATED CHEESES (WTCS) DURING 28 DAYS REFRIGERATED STORAGE

		Storage days of wine-treated-cheeses									
	Control cheese (CC)	**Day 1**		**Day 7**		**Day 14**		**Day 21**		**Day 28**	
		RWTC	**WWTC**	**RWTC**	**WWTC**	**RWTC**	**WWTC**	**RWTC**	**WWTC**	**RWTC**	**WWTC**
Dry matter (%)	55,20^c	51,04^b	51,06^b	50,42^a	49,99^a	49,87^a	50,03^a	50,10^a	49,53^a	50,10^a	49,53^a
Total fat (%)	24,00^b	24,00^b	24,00^b	23,00^{ba}	22,00^a	23,00^{ba}	23,00^{ba}	22,00^a	22,00^a	22,00^a	22,00^a
Total protein(%)*	19,60^b	18,38^a	18,12^a	18,95^a	18,67^a	18,24^a	18,62^a	18,88^a	18,50^a	18,88^a	18,50^a
Salt (%)	6,43^c	5,26^b	5,26^b	3,51^a	3,50^a	3,51^a	3,49^a	3,51^a	3,50^a	3,46^a	3,50^a
Ash (%)	9,22^b	4,78^a	5,05^a	4,76^a	4,81^a	4,97^a	4,64^a	4,69^a	4,76^a	4,69^a	4,76^a
Titratable acidity(%L.A.)	0,35^b	0,43^c	0,43^c	0,35^b	0,35^b	0,35^b	0,35^b	0,35^b	0,35^b	0,25^a	0,25^a
pH	5,42^b	5,31^a	5,33^a	5,37^{ab}	5,24^a	5,35^{ab}	5,33^{ab}	5,39^b	5,35^{ab}	5,39^b	5,35^{ab}

^{a,b,c} Means with different superscript within the same row were significantly different from each other for storage period.

* Total nitrogene x6.38.; (P< 0.05), P: significantlevel

Significant decreases (P<0.05) in total solids, ash and salt contents of WTCs were observed up to days 7. due to the status of equilibrium of soluble compounds. These results may account for the salt or mineral diffusion from cheese to wine. After day 7, no significant differences in the basic chemical properties of WTCs were observed. This may be occurred between wine and cheese samples.

There were considerable differences in VCs compositionsamong wine,CC and WTC samples, as shown in Table 2. A total of 52 volatile compounds were identified in headspace of wine and cheese extracts by solid phase microextraction technique (SPME) using GC-MS. Thirty-seven (37) VCs were found in wine which were much highernumber thanthose in WTCs (24) orCC (17). All cheese samples contained ethanol, benzyl alcohol, 2-nonanone, 2-undecanone, hexanoic acid ethyl ester, octanoic acid ethyl ester, oximemetoxy phenyl and octanoic acid. Compounds such as 2-heptanol, 2-heptanone, ethyl acetate, butanoic acid, hexanoic acid, benzene-1-methyl-2-(1-methylethyl), methyl trisulfide and 16-oxosalutaridine identified in CC had not been found in WTCs (Table II). However, the results of VCs in our study could not be compared with other reports, since no other datahas been available for the comparison.

Table 3 demonstrates that the major VCswere ethanol (range of values of 83.7% - 88.0%), 3-methyl butanol (3.0% - 6.0%), benzene ethanol (1.8% - 3.6%) andbutanedioic acid diethyl ester (1.9% - 4.9%), which accounted for 96.5% of the total volatiles quantified in WTCs. However, the main VCs in wine were hexanoic acid ethyl ester (2.0% - 3.8%), octanoic acid ethyl ester (7.7% - 11.4%) and decanoic acid ethyl ester (2.2% - 3.3%) as well as ethanol (63.4% - 69.0) and 3-methyl butanol (10.8% - 11.7%). The components representing for 84 % of the total VCs quantified in CC were the free fatty acids, the ketones, the phenolics as well as the alcohol and the esters (Table 3).These results showed that, the number of major VCsin WTCs was decreased when compared with those in CC.

Other researchers have reported on VC compositions in wines alone [1, 5, 18], without cheese immersion studies.It was reported that they detected four-ethylphenol, 4-vinylguaiacol, ethylvanilate[5], 3-methyl-1-butanol and 3-methyl-1-butyl acetate [3], and isoamylalcohol and ethyl acetate [1] in both white and red wine as the majorVCs.

This may be due to analyticaltechinique, variety of grapes, fermentation conditions and aging process of wine. In the present study, the VCs were influenced by the type of wine. The red wine had significantly (P<0.01) higher ethyl acetate and lower ethyl hexanoate, ethyl octanoate and ethyl decanoatethan the white wine. This finding was similar to the data reported by Ortega et al. [1].We have observed thatVCs of cheeses were influenced by the wine treatment process on the first day of the storage.

Ethanol significantly (P<0.001) increased in WTCs compared with CC and wine (Table 3). A similar result was obtained by Innocente et al. [12]. Ethanolis probably formed from glucose fermentation or from acetaldehyde reduction by yeasts. Ethanolis the predominant alcohol in various hard type cheeses such as Appenzeller, Gabriel, Gruyére[20]. At same time, it is the precursor of ethyl esters relevant to cheese flavour [19]. A marked difference in ethanol concentrations of WTCswasnotobserved during refrigerated storage. Even though 3-methyl butanol (Isoamylalcohol) was not detected in CC, it was the second highest compoundin WTCs (Table 3).However, 3-methyl butanoldecreasedin WTCs compared with those in wine samples used. During storage, WWTCs had significantly (P<0.05) higher 3-methyl butanol than RWTCs.This may be due to the differences in yeast strains of red and white wine.

The number of esters increased in WTCs compared with CC,whiletheir concentrations decreased. This may be attributed to the suppression in recovery of volatile compounds of high ethanol since an increase in ethanol concentration resulted in a decrease in absolute areas of volatile compounds in food and also lower extraction efficiency [7, 19]. For example,butanodioic acid diethyl ester (diethyl succinate) having high moleculer weight was determined in wine at trace level, but it was the most abundant ester in WTCs. Control cheese had the three ester compounds. Ethyl hexanoate was the most abundant ester, followed ethyl butanoate and ethyl octanoate. The similar results were observed by Ziino et al. [3] for Pasta filate cheese, which is similar to Dil cheese. Esters can occur as a result of reaction between short- to medium-chain fatty acids and primary orsecondery alcohols derived from lactose fermantation or from amino acid catabolism [20]. Esters can contribute to the flavour of cheese minimising the sharpness and the bitterness imparted by fatty acids and amines, respectively [7]. Mainly esters identified in CC play an important role in the flavour profiles of various hard-type cheeses such as natural Gorgonzola, Cheddar, Grana Padano and Flor de Guia [21]. To our knowladge, the major ester diethyl butanodionate (diethyl succinate) identified in WTCs has not been previously found in various cheeses.This compound could occur in wine as a byproduct of sugar fermantation. However, its considerable increase in WTCs may be due to the catalytic effect of salt in cheese.Free fatty acids including butanoic, hexanoic and octanoic acid were the second most abundant compounds in CC with a value of 23.05% (Table 3). Hexanoic acid was the predominant free fatty acid in CC, followed bybutanoic and octanoic acids. The similar results were obtained by Bellesia et al. [22] for Parmigiano cheese. Free fatty acids contribute tothe flavour development of many cheese types, which are not only flavour compounds by themselves, but also serve as precursors of methyl ketones, alcohols, lactones and esters. Within free fatty acids identified in CC, octanoic acid alone was found in WTCs at trace levels. As mentioned previously, the high level of ethanol may suppress the volatility of compounds with low moleculer weight.

TABLE II. THE VOLATILE COMPOUNDS IDENTIFIED IN WHITE AND RED WINE, CONTROL CHEESE AND WINE TREATED CHEESES

			Wine		**Cheese**	**Wine Treated Cheese**	
Volatile Compounds	**RT**	**RI**	**Red**	**White**	**CC**	**RWTC**	**WWTC**
Alcohols							
Ethanol[A]	5.78	<900	+	+	+	+	+
1-Propanol 2-methyl	9.24	1112	+	+	-	-	+
3-Methyl butanol (isoamylalcohol) [A]	11.82	1209	+	+	-	+	+
1-Hexanol	15.09	1358	+	+	-	-	-
3-Hexen-1-ol *(Z)*	15.33	1370	+	+	-	-	-
1-Heptanol	16.96	1461	+	-	-	-	-
2-Heptanol	14.37	1322	-	-	+	-	-
2,3-Butanediol	18.41	1552	+	-	-	+	-
1-Octanol [A]	18.57	1562	-	+	-	-	-
1-Nonanol[A]	19.99	1665	+	-	-	-	-
Esters							
Acetic acid ethyl ester[A]	5.65	1000	+	+	-	-	-
Butanoic acid ethyl ester[A]	7.71	1032	-	-	+	-	-
3-Methyl butanol acetate	9.8	1132	+	+	-	-	-
Benzoic acid, 2-[trimethylsilyl)oxy] -trimethylsilyl ester	10.26	1149	-	-	-	-	+
Hexanoic acid ethyl ester[A]	12.48	1237	+	+	+	+	+
2-Hydroxi-propanoic acid ethyl ester	15.04	1356	+	+	-	+	+
Octanoic acid methyl ester [A]	15.9	1399	+	+	-	-	-
Octanoic acid ethyl ester[A]	16.7	1445	+	+	+	+	+
3-Methylbutyl hexanoate	17.1	1469	-	+	-	-	-
Furancarbonic acid-(2)-Ethylester	19.72	1644	-	+	-	-	-
Octanoic acid, 3-methylbutyl ester	20.05	1669	+	+	-	-	-
4-Hydroximandelic acid (Hydroxy phenyl acetic acid), ethyl ester, di-TMS	17.68	1503	-	-	-	+	-
Decanoic acid ethyl ester[A]	19.8	1650	+	+	-	+	+

Butanedioic acid diethyl ester[A]	20.34	1691	+	+	-	+	+
Ethyl 9-decenoate	20.48	1702	+	+	-	+	+
Benzeneacetic acid ethyl ester	21.83	1813	+	+	-	-	-
Benzoic acid, 2-hydroxy-, methyl ester	21.91	1820	+	+	-	-	-
Dodecanoic acid ethyl ester [A]	22.31	1856	+	+	-	+	+
Acids							
Butanoicacid[A]	19.73	1645	-	-	+	-	-
Hexanoicacid[A]	22.38	1892	-	-	+	-	-
Octanoicacid[A]	24.68	>1900	-	+	+	+	+

RI= retention index based on identified compound retention times (RTs), calculated from linear equation between each pair straight alkanes (C5-C25) [A]Compounds verified with authentic standards. All compunds were also considered to be tentative (based on the MS library Wiley7n.1/ Nist02.L). CC: control cheese; RWTC: Red wine treated cheese; WWTC: white wine treated cheese. + and -: compounds are identified and unidentified in experimental samples, respectively.

TABLE II.(CONTINUED) THE VOLATILE COMPOUNDS IDENTIFIED IN WHITE AND RED WINE, CONTROL CHEESE AND WINE TREATED CHEESES

			Wine		**Cheese**	**Wine Treated Cheese**	
Volatile Compounds	**RT**	**RI**	**Red**	**White**	**CC**	**RWTC**	**WWTC**
Ketones							
3-Hydroxy-2-butanone [A]	14	1304	+	-	-	-	-
4'-(Trifluoromethyl)acetophenone	18.74	1574	+	-	-	-	-
p-cyanobenzophenone	13.36	1275	-	-	-	-	+
2-Heptanone[A]	11.37	1191	-	-	+	-	-
2-Nonanone[A]	15.94	1401	-	-	+	+	+
2-Undecanone[A]	19.32	1614	-	-	+	+	+
Phenolics							
Oxime- methoxy-phenyl-	20.95	1740	+	+	+	+	+
Benzyl alcohol[A]	22.89	1908	-	+	+	+	+
Benzenethanol (2-phenyl ethanol)	23.31	1949	+	+	-	+	+
Phenol (Benzenol) [A]	24.24	>1900	-	-	+	+	+
Phenol, 2-ethyl-	26.06	>1900	+	-	-	-	-
Alkaloids							
16-Oxosalutaridine	14.3	1319	-	-	+	-	-
Aldehydes							
Acetaldehyde[A]	4.26	<900	+	+	-	-	-
Benzaldehyde, 2,5-bis[(trimethylsilyl)oxy]	10.27	1150	-	+	-	-	-
2-Furancarboxaldehyde, 5-methyl-	19.15	1602	-	+	-	-	+
Hydrocarbons							
Benzene, 1-methyl-2-(1-methylethyl)	13.54	1283	-	-	+	-	-
Furfural	17.42	1487	+	+	-	+	+
Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	21.5	1785	+	+	-	-	-
[2.2]Paracyclophane	22.21	1847	-	+	-	-	-
Sulphurous							
Trisulfide, dimethyl [A]	16.04	1407	-	-	+	-	-
Alkanes							
2-Methoxycarbonyl-2-(cis-2'pentenyl)-3-methoxy carbonylcethylcyclopentane	17.58	1497	-	-	-	+	-

RI= retention index based on identified compound retention times (RTs), calculated from linear equation between each pair straight alkanes (C5-C25) [A]Compounds verified with authentic standards. All compunds were also considered to be tentative (based on the MS library Wiley7n.1/ Nist02.L). CC: control cheese; RWTC: Red wine treated cheese; WWTC: white wine treated cheese. + and -: compounds are identified and unidentified in experimental samples, respectively.

Like free fatty acids, ketones are common constituents of most dairy products, which contribute to cheese flavour due to their typical odour and their low perception thresholds [21]. Ketones were the third major class of VCs identified in CC, accounted for 14.04 % of the total volatiles. They can be a natural volatile compound or formed by β-oxidation of free fatty acids followed by decarboxylation as a consequance of heat treatment[5]. Within ketones identified in CC, 2-nonanone and 2-undecanone were found in WTCs at trace levels. Ketones 3-hydroxy-2-butanone and 4'-(trifluoromethyl) acetophenone were detected in red wine only, which may be natural compounds of red wine. However, *p*-cyanobenzophenone, is phytochemical, identified in WWTC only. Itmay be formed as a consequance of interaction between wine and cheese compounds.

Within phenolic compounds, oxime-methoxy-phenyl and benzenethanolwere the most abundant phenolic compoundsin CC and WTCs, respectively.They are probably a natural volatile in cheese, milk and wine [22] or may be originated from tyrosine since some microorganisms are capable of cleaving the side chain of tyrosine for releasing the phenol [23]. Benzeneethanol (2-phenyl ethanol) was not identified in CC, whereas it was higher in WTCs, in particular WWTC, than in wine samples alone. This aromatic compound is formed from phenylalanine by various yeast straines [24].This finding confirmed that the production of phenyl ethanol was related to the phenylalanine content and the yeast activity since substrat (phenylalanin) level for the activity of yeast could be increased in WTCs. In addition, the numbers of yeast were probably increased during refrigerated storage.

TABLE III THE PERCENT COMPOSITIONS OF THE MAIN VOLATILE COMPOUNDS IDENTIFIED IN WINE, CONTROL CHEESE, AND WINE TREATED CHEESES DURING 28 DAYS OF REFRIGERATED STORAGE.

				Storage Days of Wine Treated Cheeses (WTCs)									
	Wine	Cheese	Day1	Day 7	Day 14	Day 21	Day 28						
Volatile Compounds	**Red**	**White**	**CC**	**RWTC**	**WWTC**	**RWTC**	**WWTC**	**RWTC**	**WWTC**	**RWTC**	**WWTC**	**RWTC**	**WWTC**
Alcohols													
Ethanol^{A}	69,03	63,44	25,92^{a}	87,95^{c}	87,5^{c}	87,26^{c}	87,41^{c}	83,71^{b}	84,42^{b}	85,41^{bc}	86,37^{bc}	84,86^{bc}	84,81^{bc}
3-Methyl butanol (isoamylalcohol)^{A}	10,8	11,66	nd	2,99^{a}	4,74^{b}	4,09^{b}	4,74^{b}	5,15^{b}	6,01^{c}	4,38^{b}	5,07^{b}	4,25^{b}	5,63^{c}
2-Heptanol	nd	nd	2,65	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Esters													
Acetic acid ethyl ester^{A}	3,1	1,39	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Butanoic acid ethyl ester^{A}	nd	nd	1,62	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Hexanoic acid ethyl ester^{A}	1,99	3,28	6,84^{b}	0,23^{a}	0,17^{a}	0,26^{a}	0,22^{a}	0,4^{a}	0,51^{a}	0,33^{a}	0,36^{a}	0,33^{a}	0,59^{a}
Octanoic acid ethyl ester^{A}	7,66	11,43	1,27^{e}	0,56^{ab}	0,53^{ab}	0,41^{a}	0,53^{a}	0,7^{c}	0,84^{c}	0,58^{bc}	0,71^{bc}	0,95^{d}	0,98^{d}
Decanoic acid ethyl ester^{A}	2,24	3,25	nd	0,16	0,18	0,2	0,18	0,41	0,34	0,34	0,33	0,63	0,52
Butanedioic acid diethyl ester^{A}	0,06	0,04	nd	3,13^{b}	1,71^{a}	3,25^{c}	1,68^{a}	4,86d	2,32^{b}	4,02^{c}	1,91^{b}	4,33^{c}	2,1^{b}
Acids													
Butanoicacid^{A}	nd	nd	7,15	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Hexanoicacid^{A}	nd	nd	9,79	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Octanoicacid^{A}	nd	0,27	6,11^{b}	0,18^{a}	0,18^{a}	0,1^{a}	0,1^{a}	0,16^{a}	0,16^{a}	0,12^{a}	0,12^{a}	0,2^{a}	0,2^{a}
Ketones													
2-Heptanone^{A}	nd	nd	6,47	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2-Nonanone^{A}	nd	nd	6,28^{b}	0,05^{a}	0,06^{a}	0,04^{a}	0,05^{a}	0,06^{a}	0,08^{a}	0,05^{a}	0,06^{a}	0,08^{a}	0,06^{a}
2-UndecanoneA	nd	nd	1,29^{b}	0,04^{a}	0,04^{a}	0,03^{a}	0,03^{a}	0,04^{a}	0,04^{a}	0,04^{a}	0,03^{a}	0,05^{a}	0,04^{a}
Phenolics													
Benzenethanol (2-phenyl ethanol)	1,08	1,56	nd	1,79^{a}	2,97^{b}	1,85^{a}	3,0^{b}	2,6^{b}	3,59^{c}	2,23^{b}	3,13^{b}	2,2^{a}	3,26^{c}
Oxime- methoxy-phenyl-	0,04	0,19	8,82^{c}	1,69^{b}	0,91^{b}	1,16^{ab}	1,05^{ab}	0,52^{a}	0,6^{a}	0,94^{ab}	0,9^{ab}	0,83^{ab}	0,7^{ab}
Phenol (Benzenol)^{A}	nd	nd	2,08^{b}	0,03^{a}	2,18^{b}	0,04^{a}	0,1^{a}	0,05^{a}	0,16^{a}	0,06^{a}	0,12^{a}	0,05^{a}	0,2^{a}
Alkaloids													
16-oxosalutaridine	nd	nd	4,29	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Hydrocarbons													
Benzene, 1-methyl-2-(1-methylethyl)	nd	nd	1,6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sulphurous													
Trisulfide, dimethyl^{A}	nd	nd	2,24	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Values are mean of six analyses.^{a,b,c} Means with different superscript within the same row were significantly different from each other for storage period. CC: Control cheese; RWTC: Red wine treated cheese; WWTC: White wine treated cheese; nd: not detected.

To our knowledge, compound 16-oxosalutaridine was identified for the first time in cheese. In a previous study [25], this compound was detected in Turkish pine honey samples. Salutaridine is a major alkaloid of *Papaver species*, in particular the opium poppy (*Papaversomniferum*) [26]. Therefore, Salutaridinemay be released from the forage by the metabolism of cows and probably transferred to milk and/or cheese.However, salutaridine was not detected in WTCs.

With regard to sensory properties, none of the cheeses received the maximum overall acceptability score of 9 (excellent). Control cheese received 6.2 score for overall acceptability. As shown in Figure 1, WWTCs received maximum score (8 out of 9) on day 14, after that the score decreased towards to the end of storage. This may be due to the non-identified compounds in cheese since no significant changes were observed for VCs and the other chemical parameters of WWTC on day 14. The overall acceptability scores of RWTCs unchanged up to 21 days refrigerated storage. Alcohol taste was intensively perceived for this cheese by panelists.

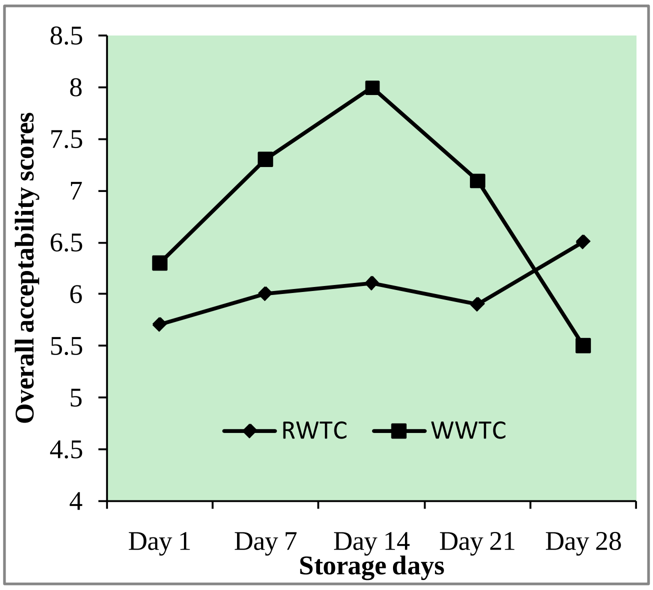

Fig. 1 The overall acceptability scores (out of 9 score) of wine-treated-cheeses (WTCs) during refrigerated storage of 28 days

The results of the sensory panel scores revealed that WWTC can eat at the breakfast up to days 7 or 14, whereas RWTC may be more suitable for cocktail.

IV. CONCLUSION

Meticulous analyses of all data relating to the CC and WTCs confirmed that wine-treatment affected considerably the basic chemical compositions, volatile compounds, and overall acceptability of the Dil cheeses. The WTCs contained less number of volatile compounds than the wine samples. Ethanol was the predominant VC in WTCs, followed by isoamylalcohol, diethyl butanodioate and benzene ethanol. The other VCs were detected at trace amounts. Most of VCs identified in CC were not detected in WTCs.

The concentrations of isoamylalcohol and benzenethanol were higher in WWTCs than RWTCs. Diethyl butanodioate (diethyl succinate) was the predominant ester in WTCs. WWTCs were more preferred by the panelists. In order to increase food diversity and convenience, food sector may provide WTCs to restaurants, hotel and other public eateries.

Increase in ethanol level and decrease in salt content of WTCs caused a decrease in the recovery of volatile compounds. It appeared to interfere with SPME analysis for the recovery or volatility of compounds in particular with low moleculer weight. Therefore, DVB/CAR/PDMS fibre used for SPME may be more suitable for ethanol containing products at low levels.

Further studies may be needed to investigate the application of SPME analysis using different fibers on unsalted Dil cheese as well as to determine the correlations between individual volatile compound and flavour attributes in WTCs.

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