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Characterization of a traditional semi-hard Italian cheese produced by soaking in wine

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Abstract

A traditional type of semi-hard cheese which, after a ripening period in the warehouse, is soaked in wine or fermented must, was studied. In particular, the effect of the soaking phase on the characteristics of the cheese and on the profiles of the volatile compounds was investigated. The study proved that it is the alcohols and the short-chain ethyl esters which chiefly contribute to the distinctive aromatic fraction of the product. These compounds migrate from the wine into the cheese as a result of the different concentrations existing between the two systems.

Keywords: Cheese characterization; Flavour profile; HS-SPME

1. Introduction

Over recent years, various efforts have been made at European level to protect traditional food products (EC Reg., 2006). As part of this process, Italy has already issued Ministerial Decree n.130/2000 in which a list of traditional products produced in different regions of Italy has been published (MD, 2000). Among the products covered in this decree, is an unusual semi-hard cheese type, produced in north-eastern Italy which is soaked in wine, or fermented must, after a variable ripening period in the warehouse. The product is usually made from pasteurised cow's milk which is inoculated with selected starters and coagulated using calf rennet. The curd is cut into hazelnut-sized pieces and then cooked at 45–46 °C. After salting in brine (16–18% w/w of sodium chloride) the cheese is ripened for a period ranging between 2 and 8 months in the warehouse at a temperature of 13–15 °C and a relative humidity of 75–85%. At the end of this time the cheese is soaked in a mixture of wine or must and marc, which is kept in wooden vats at 13–15 °C. A variety of different grapes (*Vitis labrusca* and *Vitis vinifera* Cabernet, Merlot, Refosco, Sauvignon, Tocai Friulano and Verduzzo) can be used, in association with different wine or must/marc ratios. The immersion time varies according to the experience and judgement of the cheesemaker, but usually a soaking phase of about 7 days is required for the characteristic flavour to develop. A one-month period in the warehouse follows to equilibrate the cheese. The final aged product has a purple or intense yellow rind (depending on whether red or white grape varieties are employed) but the colour does not fully penetrate into the paste, which is straw-yellow. The flavour is distinctive and is characterized by different flowery and fruity notes, again according to the grape cultivars used (Corradini, Bonessi, & Innocente, 2002; Innocente, Biasutti, & Corradini, 2006).

The aim of this work was to study this traditional product in an attempt to define the effect of the specific post-processing soaking phase on its characteristics. Accordingly, the main composition parameters, the proteolysis index and the fatty acid content were determined. Particular attention was also paid to the analysis of the volatile fraction.

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2. Materials and methods

2.1. Cheese samples

Three cheesemaking trials were performed. In each trial, thirty cheeses produced from the same vat, using the traditional process described above, were ripened in the warehouse for 8 months. After this time, they were divided into two groups. One group was soaked for 7 days in the fermented must and then equilibrated in the warehouse for 20 days (*experimental cheeses*) while the other group continued ripening in the warehouse during the same period (*control cheeses*). For each trial, initial samples (at the start of immersion), control samples and experimental samples were taken. The following analyses were performed on each sample: pH, acidity and moisture (MD, 1986), NaCl by FIL-IDF Standard 17A (1972), fat by FIL-IDF Standard 5A (1969), total and water-soluble nitrogen by Kjeldahl method to evaluate the proteolysis index (AOAC, 1980; Innocente, 1997), free volatile fatty acids (Innocente, Moret, Corradini, & Conte, 2000) and volatile compounds using the headspace solid-phase microextraction method (HS-SPME) coupled with gas chromatography with mass spectrometric detection (GC–MS) (Biasutti & Innocente, 2006).

To obtain a representative sample of cheese, at each sampling time a whole segment was cut from the centre to the edge of the cheese, the rind (2 cm) was removed and the sample (about 500 g) was finely grated.

Table 1
Chemical parameters measured in the fermented must over the operating period

	0 days	7 days	14 days	21 days
	M[A]	*M*	*M*	*M*
Relative density	0.9955c[B]	1.0024b	1.0103a	1.0152a
Alcoholic degree (% v/v)	10.28a	9.97a	9.38b	7.10c
Total dry extract (g/L)	24.1c	39.1b	62.9a	65.5a
Volatile acidity (g/L)	2.22c	3.90b	3.88b	4.53a
Total acidity (g/L)	7.05a,b	7.40a	7.20a,b	6.00b
pH	3.35d	3.95c	4.09b	4.62a
Reducing sugars (g/L)	4.4a	4.8a	4.2a	4.6a
Total nitrogen (g/L)	4.34d	8.88c	18.85b	24.31a

[A] *M*, mean value of three measurements.
[B] Values within the same row followed by the same letters do not differ significantly at $P \leqslant 0.05$ according to Tukey's HSD test.

2.2. Fermented must samples

A mixture consisting of marc and fermented must made with *Vitis labrusca* and *Vitis vinifera* (Merlot) grapes was employed. The mix was in use for a total of 21 days and during this period 3 groups of cheese were soaked (7 days for each soaking trial).

Samples of fermented must were taken at the start of each soaking phase and were analysed for pH, relative density and total dry extract, alcoholic degree, total and volatile acidity (EC Reg., 1990), total nitrogen content by Kjeldahl method (AOAC, 1980), reducing sugars by Fehling method (Sudario, 1982) and volatile compounds (Biasutti & Innocente, 2006).

To obtain a uniform sample, the must was always thoroughly stirred before 500 mL was removed. After sampling, the fermentation processes were stopped by adding 1 mL of 5% (v/v) ethyl bromoacetate solution in ethanol.

2.3. Statistical analysis

Three different cheesemaking batches were studied and each time all the analyses were carried out three times on the cheeses and on the fermented must samples. The data shown are therefore the averages of the nine values obtained. One-way analysis of variance and Tukey's HSD test for multiple comparison were used and the differences between the means were considered statistically significant for P values $\leqslant 0.05$. All statistical analyses were conducted using the software Statistical Discovery JMP 3.0 for Windows.

Table 2
Chemical and compositional parameters measured in the cheese samples

	Initial cheese			Experimental cheese			Control cheese		
	M[A]	SD[B]	RSD[C]	*M*	SD	RSD	*M*	SD	RSD
pH	5.2a,b[D]	0.07	1.26	5.15b	0.04	0.80	5.24a	0.04	0.70
Acidity (Lactic acid g/100 g)	1.35b	0.04	2.63	1.45a	0.02	1.66	1.42a	0.01	0.88
Dry matter (g/100 g)	68.94b	0.13	0.19	67.70c	0.19	0.29	69.53a	0.19	0.28
Moisture (g/100 g)	31.06b	0.13	0.42	32.30a	0.19	0.60	30.47c	0.19	0.63
NaCl (g/100 g dry matter)	3.52a,b	0.02	0.64	3.45b	0.05	1.56	3.65a	0.07	1.92
Fat (g/100 g dry matter)	50.32a	0.34	0.67	50.47a	0.57	1.13	50.55a	0.71	1.41
Proteins (g/100 g dry matter)	37.62a	0.20	0.54	37.56a	0.95	2.52	38.48a	1.66	4.30
Proteolysis Index (WSN/TN* 100)[E]	25.55b			27.28a			26.08a		

[A] *M*, mean value of nine measurements.
[B] SD, standard deviation.
[C] RSD, relative standard deviation.
[D] Values within the same row followed by the same letters do not differ significantly at $P \leqslant 0.05$ according to Tukey's HSD test.
[E] WSN/TN= water-soluble nitrogen/total nitrogen.

Table 3
Free fatty acid content measured in the cheese samples

	Initial cheese			Experimental cheese			Control cheese		
	M[A]	SD[B]	RSD[C]	*M*	SD	RSD	*M*	SD	RSD
Acetic acid (μg/g dry matter)	1585b[D]	51	0.32	1868a	55.2	3	1927a	70.6	3.70
Propionic acid (μg/g dry matter)	25a,b	0.4	1.69	27a	0	0	19b	0.40	2.10
Butyric acid (μg/g dry matter)	125a	1.1	0.89	114b	1.0	0.89	112b	1.30	1.20
Caproic acid (μg/g dry matter)	21a	0.4	1.90	23a	0.20	0.90	19a	0.50	2.63

[A] *M*, mean value of nine measurements.
[B] SD, standard deviation.
[C] RSD, relative standard deviation.
[D] Values within the same row followed by the same letters do not differ significantly at $P \leqslant 0.05$ according to Tukey's HSD test.

3. Results

In order to study the effect of the soaking phase on the characteristics of the cheese, the chemical composition and the volatile compounds of the cheese and of the fermented must itself were analyzed.

Table 1 shows the data regarding the chemical parameters measured in the fermented must over the operating time. The progressive decrease in alcoholic degree and the increase in volatile acidity and pH values prove that the system was subject to an acetification process, probably due to the presence of anomalous and uncontrolled fermentations or oxidative processes by acetic acid bacteria (Farkaš, 1988). The rise in the total nitrogen value is probably due to a migration process of water-soluble protein fractions from the cheese into the must–marc mixture. Regarding the other chemical parameters, no significant differences were observed.

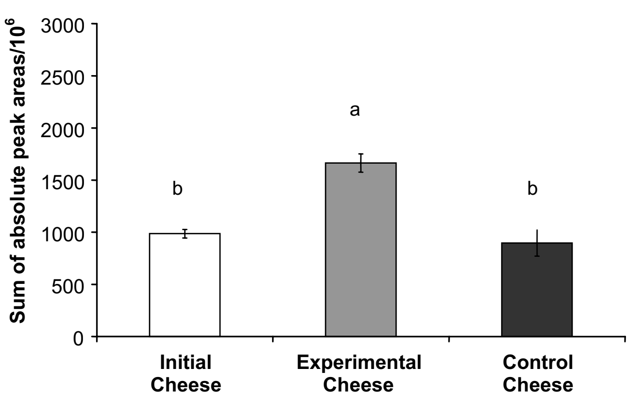

Fig. 1. Total volatile profiles, expressed as sum of the averages of the absolute area/10^6, measured in the cheese samples. Mean values superimposed with different letters differ significantly at $P \leqslant 0.05$ according to Tukey's HSD test.

Tables 2 and 3 compare the main compositional parameters and the free volatile fatty acids of the cheese soaked in the fermented must (experimental cheese) with those of the cheese at the start of the immersion and of the cheese which continued the ripening in the warehouse (control cheese). The experimental cheese does not display any significant changes in the values of the proteolysis index, pH, acidity and free volatile fatty acids compared with the control sample. So, it is clear that the soaking phase does not influence the regular behaviour of the proteolysis and of the fermentation processes of the cheese. No significant differences were detected in salt and fat content either. As expected, an increase in the moisture content of the experimental sample was measured as a result of water migration into the cheese over the period of immersion.

The total volatile fraction profiles, expressed as averages of the sum of the absolute areas of the peaks measured in the headspace of the cheese samples, are shown in Fig. 1. The total area value of the soaked cheese is significantly higher than the control sample ripened in the warehouse, which, by contrast, does not differ from the initial cheese ($P > 0.05$). It is clear, therefore, that the immersion phase in the fermented must has a direct effect on the volatile fraction of the cheese.

In the headspace of the cheese and the fermented must samples, about 50 compounds were identified, consisting

Table 4
Mean values of the absolute peak areas and relative percentages of the volatile compounds, grouped according to chemical classes, measured in the cheese samples

	Initial cheese		Experimental cheese		Control cheese	
	Absolute area	Relative percentage	Absolute area	Relative percentage	Absolute area	Relative percentage
Alcohols	565	57.30	953	57.27	508	56.70
Esters	136	13.79	288	17.31	151	16.85
Fatty acids	100	10.14	160	9.62	95	10.60
Terpenes	113	11.46	115	6.91	58	6.47
Ketones	72	7.30	148	8.89	84	9.38
Total sum	896		1664		896	

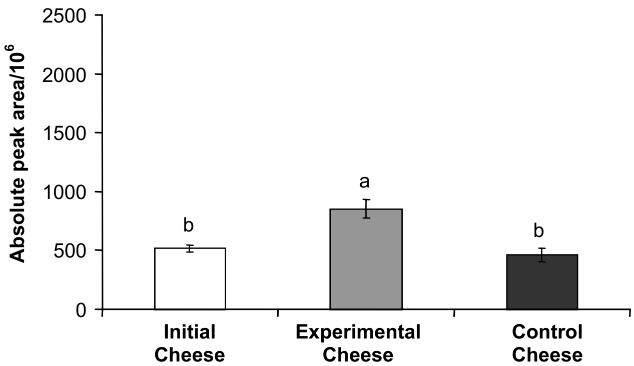

Fig. 2. Ethanol profiles, expressed as mean value of the absolute peak area/10^6. Mean values superimposed with different letters differ significantly at $P \leqslant 0.05$ according to Tukey's HSD test.

of 24 esters, 11 alcohols, 7 fatty acids, 4 ketones, 1 aldehyde and 4 terpenes. Table 4 reports the averages of the sum of the absolute areas of the peaks and the relative percentages of the different chemical classes detected in the cheese samples. We can see that alcohols and esters were the most significant contributors to the volatile profile of cheese. This is very important if we consider that these compounds impart flowery, fruity and wine-like aromatic notes (Dubois, 1994; Thierry & Maillard, 2002; Marilley & Casey, 2004).

More specifically, with regard to the alcohol class, ethanol was the most abundant compound in all the samples. Fig. 2 shows the ethanol profiles in the cheese samples. It increased in the experimental cheese, while it did not significantly change in the control sample compared with the initial cheese. We suggest that the ethanol migrated from the fermented must, which is rich in ethanol, into the soaked cheese as a result of the concentration differential existing between the two systems.

Fig. 3 shows the profiles of the most significant higher alcohols (2-methyl-1-propanol, 3-methyl-1-butanol, 1-hexanol and 2,3-butanediol) identified in the headspace of the cheese samples (A) and of the fermented must (B). These compounds are commonly present in wine after the fermentation phase and this could explain the high peak area measured in the fermented must samples (Dubois, 1994). It is clear that, among these higher alcohols, 3-methyl-1-butanol was the most abundant in the must, even if it progressively decreased over the time. On the contrary, the same compound significantly increased in the experimental cheese in comparison with the initial and the control samples. Therefore, we may conclude that this alcohol, too, migrated into the cheese during the soaking phase. The same is probably also true for the other alcohols, although they are present in lower amounts.

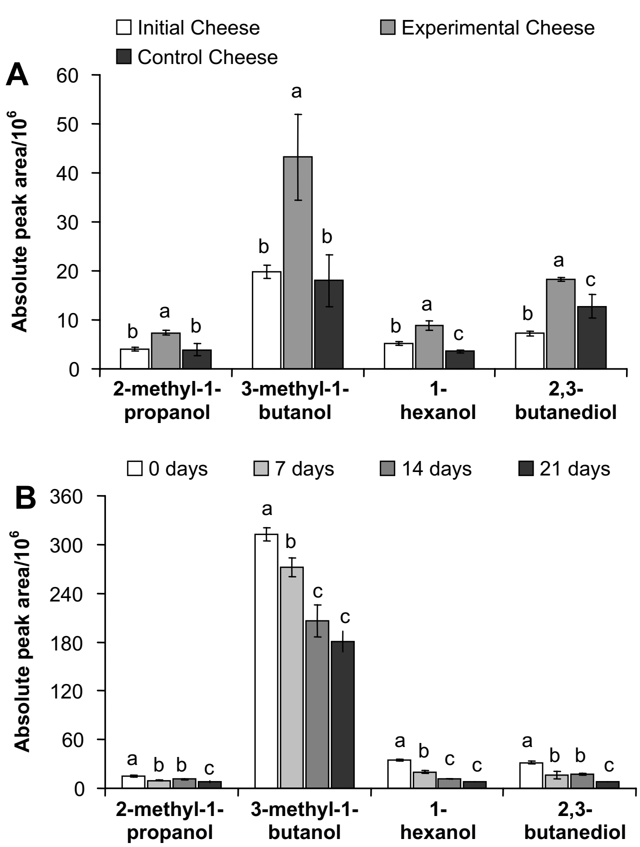

Fig. 3. Profiles of the higher alcohols, expressed as mean value of the absolute peak area/10^6. (A) cheese samples and (B) fermented must samples. In each graph, mean values superimposed with different letters differ significantly at $P \leqslant 0.05$ according to Tukey's HSD test.

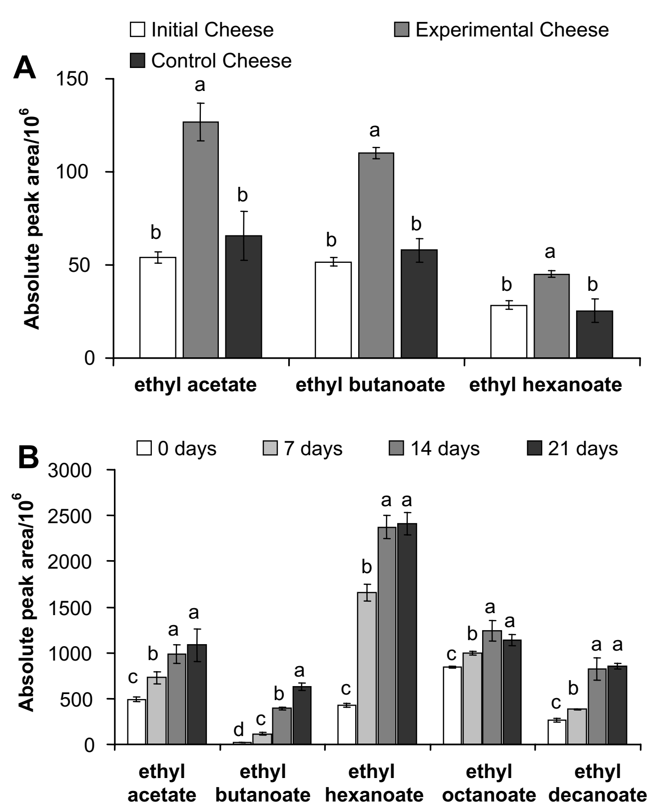

Fig. 4. Profiles of the ethyl esters, expressed as mean value of the absolute peak area/10^6. (A) cheese samples and (B) fermented must samples. In each graph, mean values superimposed with different letters differ significantly at $P \leqslant 0.05$ according to Tukey's HSD test.

Finally, with regard to the short-chain ethyl esters (Fig. 4A) significant amounts were measured in all the cheese samples. In fact, these compounds are known to derive from reactions occurring between the fatty acids, which are present in cheese as a result of amino acid catabolism, lactose fermentation or lipolysis, and the alcohols produced from lactose metabolism (Molimard & Spinnler, 1996). However, more elevated peak areas were measured in the experimental cheese, probably as the result of the migration process of these compounds from the fermented must, in which large amounts are present (Fig. 4B). This is true only for the short-chain ethyl esters, while longer-chain ethyl esters (such as C8 and C10) were found in the fermented must but not in the cheese, probably because they are unable to migrate due to their higher molecular weight. The increase in the number of ester compounds in the fermented must over time can be explained by the occurrence of metabolic processes effectuated by micro-organisms. Instead, we can suggest that in the studied environmental conditions wild yeasts play the major role in the development of these compounds (Bamforth, 2005).

4. Conclusions

The results proved that the post-processing phase of soaking cheese in the fermented must does not influence the regular behaviour of the proteolysis or of the fermentation processes occurring during cheese ripening. On the other hand, the aromatic fraction is strongly influenced by this unusual treatment. In particular, alcohols and short-chain ethyl esters were the most significant contributors to the volatile profile of the cheese soaked in fermented must. Since the fermented must also presents highly elevated profiles of those alcohols and esters, we can suggest that a migration process occurs from the immersion system into the cheese. These compounds are known to impart flowery, fruity and vinous aromatic notes. So, it is reasonable to suggest that the distinctive sensory profile that characterizes this product is closely related to the presence and the amount of these compounds in the cheese.

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