PAPER

FREE FATTY ACIDS IN "PROVOLA DEI NEBRODI", A HISTORICAL SICILIAN CHEESE

GLI ACIDI GRASSI LIBERI DELLA "PROVOLA DEI NEBRODI", FORMAGGIO STORICO SICILIANO

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ABSTRACT

The FFA content of Provola dei Nebrodi during ripening and in different cheesemaking seasons was investigated. Twenty-eight components from C4 to C20 were quantified and myristic, palmitic, stearic and oleic acids were the major FFA in all the samples analysed. The level of FFA after 90 days of ripening ranged from 651.52 to 840.45 mg 100 g-1 of cheese, depending on the cheesemaking season. There were significant differences (P ≤ 0.05) between the average content of even-car-

RIASSUNTO

Gli Autori riportano il contenuto di acidi grassi liberi (FFA) della Provola dei Nebrodi, formaggio tipico Siciliano a pasta filata, in campioni a diversa maturazione durante un intero anno di produzione. Sono stati identificati acidi grassi da C_4 a C_{20}; tra questi, gli acidi miristico, palmitico, stearico ed oleico erano i più rappresentati. Il livello di FFA dopo 90 giorni di maturazione era compreso tra 651.52 e 840.45 mg 100 g^{-1} di formaggio; l'analisi statistica dei dati ottenuti ha evidenziato differenze significa-

- Key words: cheese ripening, free fatty acids, "Pasta filata" Sicilian cheese, Production season, Provola dei Nebrodi -

bon number fatty acids during ripening and within the production seasons. The high content of FFA and the significant differences observed are discussed and correlated with the artisanal cheese production technique.

tive ($P \leq 0.05$) per gli acidi grassi a numero pari di atomi di carbonio relative alla maturazione ed al periodo di produzione. Il contenuto elevato di FFA e le differenze riscontrate vengono discusse e correlate con la tecnica artigianale di produzione.

INTRODUCTION

Free fatty acids (FFA) composition is an important marker for cheese characterisation. FFA strongly contribute to cheese flavour which is one of the most important quality criteria of fresh and aged cheese. Since consumer acceptability of cheese depends mainly on its sensory qualities, the flavour is determinant.

Short chain fatty acids (SCFA), butyric (rancid, sour, repellent), caproic and caprylic acids (cheesy, rancid, sweatlike) are the most significant examples of cheese flavour notes (AKIN, *et al.*, 2003; HA and LINDSAY, 1991; URBACH, 1997; NAJERA *et al.*, 1994). Fatty acids with more than 12 carbon atoms have less influence on flavour, even though they are present in a fair amount in cheese (SABLE and COTTENCEAU, 1999). Medium chain free fatty acids (MCFA), especially branched ones, are responsible for mutton fat flavour, while long chain (LCFA) are perceived as oily, waxy and soapy (ASHURST, 1999). FFA also act as precursors of many aromatic compounds such as alcohols, aldehydes, methylketones, lactones, esters and thioesters (McSWEENEY and SOUSA, 2000).

Free fatty acids are released mainly from milk lipids through the lipolytic activity of lipases. Lipolysis in cheese is well documented and it is known that lipases may originate from milk, indigenous or added micro-organisms (STEAD, 1986), rennet and, if utilised, exogenous lipases. Furthermore short chain fatty acids can also be produced by nonenzymatic oxidation of long and/or unsaturated fatty acids, especially of linoleic and linolenic acids (MACEDO and MALCATA, 1996). Protein and carbohydrate metabolism by bacteria are other possible sources for free fatty acids in ripened cheese: branched chain fatty acids derive from reductive and oxidative deamination of amino acids (CONTARINI *et al.*, 1989); acetic and propionic acids and butyric acids, to some extent, arise from microbial fermentation of lactate (McSWEENEY and SOUSA, 2000).

Several authors have studied the FFA composition of different types of cheese and significant changes during ripening have been observed (WOO *et al.*, 1984a; FARKYE and FOX 1990; MACEDO and MALCATA, 1996; POVEDA *et al.*, 1999; PAVIA *et al.*, 2000; MALLATOU *et al.*, 2003; ADDIS *et al.*, 2005; MOATSOU *et al.*, 2004; GEORGOLA *et al.*, 2005; VIRTO *et al.*, 2003). Due to its increase during ripening, the FFA content has been suggested as a ripening index, even if it has been reported to be less useful than proteolytic and glycolytic indicators (FARKEY and FOX, 1990; WOO and LINDSAY, 1984b; WOO *et al.* 1984a). In contrast, there are fewer reports of changes during time of the year (MACEDO and MALCATA, 1996; CHAVARRI *et al.*, 1999; COLLOMB *et al.*, 2003).

"Provola dei Nebrodi" is classified as a historical and typical Sicilian dairy product; it is artisanally produced in several areas in the Nebrodi Mountains, near

Messina, Sicily, Italy. It is a "pasta filata" cheese derived from full-fat raw cow milk with the addition of farmhouse rennet paste. Salting takes place in saturated brine. Well known for its size (4-5 kg), it has a peculiar oblong, pear-like shape, with a short neck and a small round top; it can be eaten fresh (one month) or aged (3-4 months) since it is the only "Provola" subjected to ripening.

As part of a larger research project, which aims at the chemical characterisation of typical Sicilian cheeses (VERZERA *et al.*, 2004; ZIINO *et al.*, 2005), the FFA composition of "Provola dei Nebrodi", and its dependence on ripening and on the cheesemaking season were investigated. Samples produced in winter, spring, summer, and autumn were analysed immediately after cheesemaking and during ripening. Statistical analysis gave interesting information on the similarities and differences in the FFA composition during the ripening time and throughout different cheesemaking seasons.

MATERIALS AND METHODS

Cheesemaking

The cows grazed on wet pasture from April to June (spring) and from October to December (autumn), and on dry pasture from July to September (summer), and with hay and concentrated feed from January to March (winter).

A flowchart for the production of "Provola dei Nebrodi" is reported in Fig. 1. "Provola dei Nebrodi" was manufactured from evening and morning milk; the evening milk was refrigerated (4 °C) overnight and mixed with the morning milk of the following day. Milk was coagulated with kid or lamb rennet paste without addition of starter culture. The native microflora of the raw milk provided the acid-producing bacteria for cheesemaking. The rennet was prepared by the same cheesemaker in the following way:

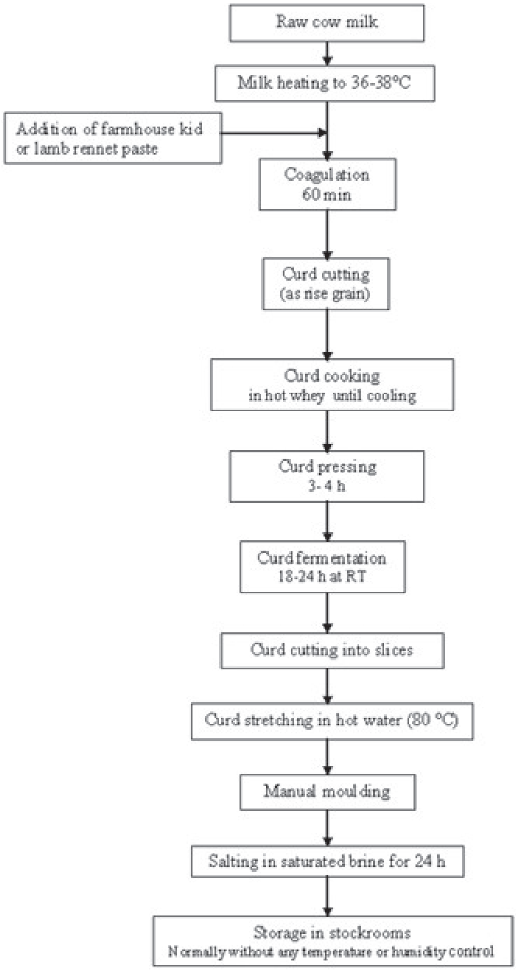

Fig. 1 - Flowchart of "Provola dei Nebrodi" production.

the abomasus of suckling lamb or kid was salted, dried until 13-15% of water content remained and then ground up with the addition of salt in the ratio 1:5 w/w salt/ rennet paste. The amount of rennet used for the milk coagulation was in the range of 20-40 g 100 L^{-1} of milk. After coagulation, the curd was broken to the size of a rice grain, and the whey drained. The curd was then covered with the hot whey (70-80°C) for about 3 h until it cooled to room temperature. It was then removed from the vat and left to drain and ripen (18-24 h depending on room temperature).

After maturation, the curd was cut into thick slices (ca. 1 cm), stretched in hot water (80°C) and manually moulded. Finally, it was salted in saturated brine (24 h, RT).

The cheese was ripened in farmhouse stockrooms, tied with a cord across a wooden rafter, usually without any temperature or humidity control.

Sampling

The cheese samples analysed were provided by three producers. Sampling was carried out in the months of January, April, July and October in the year 2003. For each producer, in each month four successive cheese productions were followed, within the same week. For each production one sample was analysed immediately, whereas three samples were stored away and analysed after 7, 30 and 90 days of ripening. The ripening took place in each producer's farmhouse stockrooms. Each cheese, prior to the analyses, was divided in two portions and each portion was analysed in duplicate.

Compositional analysis

Samples of cheese were analysed for moisture, protein and fat content according to the International Dairy Federation (IDF) methods. The moisture content was determined gravimetrically after oven drying at 102°C (IDF, 1982), protein content by the Kjeldahl method (IDF, 1993) and fat content gravimetrically (IDF, 2004). The pH was measured with an electrode for solids connected to an Inolab pH level 1 standard pH meter (WTW GmbH, Weilheim, Germany).

Free fatty acid analysis

Free fatty acids were extracted according to the method described by CONTARINI *et al.* (1989): 20 g of cheese were homogenised with anhydrous Na_2SO_4, suspended in ethanol acidified to a pH < 2 and extracted with three aliquots of diethyl ether. The organic extracts were combined and potassium hydroxide (0.1 N) was added to make the solution basic. The FFA were then extracted with distilled water; the aqueous solution was dehydrated at 105 °C, the FFA methylated with sulphuric acid (96%)/methyl alcohol 3:1 v/v and analysed by gas chromatography.

A Shimadzu 17A gas chromatograph with a flame ionisation detector (FID) (Shimadzu Italia s.r.l., Milano, Italy) was used to analyse the FFA. Injector temperature: 200 °C; injection mode: splitless; column: RTX-225 (Chrompack Italia s.r.l., Milano, Italy), 30 m, 0.32 mm i.d., 0.25 μm film thickness; oven temperature: 50 °C held for 2 min, then increased to 100 °C at a rate of 10 °C min^{-1} and to 200 °C at a rate of 3 °C min^{-1}. Carrier gas: helium at constant pressure of 10 psi; detector temperature: 210 °C; integrator: Hewlett Packard HP3394.

FFA were identified by comparing the retention time of each known standard with that of each unknown. The quantitative determination was carried out using internal standards, $C_{7:0}$ and $C_{13:0}$ (Sigma-Aldrich, Milan, Italy), which were absent in the sample analysed or present as traces.

Statistical analysis

The SPSS 11.5 (2002) software package (SPSS Inc., Chicago, IL, USA) was used for statistical treatment of the data. One way analysis of variance (ANOVA) was applied to the data to determine the presence of significant differences (Duncan test, significant level $P \leq 0.05$) in the FFA content during the ripening time and throughout the cheesemaking seasons.

RESULTS AND CONCLUSION

The average values for moisture, protein and fat contents, and pH of all the samples analysed, grouped according to the ripening time and the production season, are shown in Table 1. Average values for fat and protein were between 26.35-27.95 and 31.66-33.02 g 100 g^{-1} of cheese at 90 days of ripening; fat content values were similar to average values of Provolone and, Caciocavallo Pugliese and Silano cheeses, but protein contents were higher than the average values of the same cheeses, as reported by BATTISTOTI and CORRADINI (1993) and GOBBETTI *et al.* (2002). Moisture content decreased during ripening and reached the final values of 32.73-36.37 g 100 g^{-1} of cheese after 90 days of ripening, as expected for a semi-hard cheese. pH values varied from 5.05 to 5.38 in all the samples analysed, and they are similar to those of other types of "pasta filata" cheeses (GOBBETTI *et al.*, 2002).

Table 2 shows the average concentrations (mg 100 g^{-1} cheese) of individual FFA for all the analysed samples grouped according to the ripening time and the production season. Saturated, monounsaturated and polyunsaturated fatty acids from C_4 to C_{20} were quantified and the long chain fatty acids constituted most of the fat fraction, followed by medium and short chain. Myristic, palmitic, stearic and oleic acids were the major FFA in all the samples analysed, which is typical of bovine milk fat (ALAIS, 1984).

Table 1 - Chemical composition (g 100 g^{-1} cheese) of "Provola dei Nebrodi" throughout ripening in different production seasons.

Production season	Ripening time/days	Moisture g 100 g^{-1} X^d	pH X	Fat g 100 g^{-1} X	Protein g 100 g^{-1} X
spring	0	42.92 a	5.21 A	21.97 cB	28.08 bB
	7	41.31 ab	5.38 A	23.90 cb	28.67 b
	30	38.76 b	5.09	24.17 bAB	29.12 b
	90	32.73 cB	5.15	26.35 a	33.02 a
summer	0	43.61 a	5.21 A	21.94 bB	28.64 bAB
	7	40.33 b	5.32 AB	22.76 b.	28.80 b
	30	39.64 b	5.35	21.04 bB	29.64 b
	90	33.24 cB	5.22	26.58 a	31.94 a
autumn	0	40.80 a	5.08 B	24.80 bA	29.94 abA
	7	40.67 a	5.05 C	23.99 b	28.99 b
	30	37.49 b	5.15	26.38 aA	28.87 b
	90	34.01 cAB	5.30	27.95 a	31.66 a
winter	0	42.43 a	5.14 B	22.36 bB	27.41 bB
	7	42.05 a	5.10 CB	23.26 b	30.01 b
	30	40.44 a	5.10	24.29 ab AB	28.94 b
	90	36.37 bA	5.24	27.27 a	32.03 a

[d] mean value: 12 cheeses (4 cheeses/ producer), for each cheese two samples, each sample in duplicate.
Means with different letters (a-c) in the same column are significantly different from each other for the ripening time ($P \leq 0.05$). Means with different capital letters (A-C) in the same column are significantly different from each other for the production season ($P \leq 0.05$) at the same ripening time. Letters are not reported where significant differences did not result ($P > 0.05$).

Table 2 - Free Fatty Acid concentration (mg 100 g^{-1} cheese) in "Provola dei Nebrodi" throughout ripening in different production seasons.

		Free Fatty Acids[d]										
Production Season	Ripening time/days	C4:0	C5:0	C6:0	C8:0	C9:0	C10:0	Total SCFA	C11:0	C12:0	iC14:0	C14:0
Spring	0	7.94 cA	0.10	12.52 bB	8.73 bB	0.15	21.29 dB	50.74	0.27	19.85 bB	0.58 b	44.58 bC
	7	12.18 bAB	0.11	15.04 bB	11.20 bA	0.21	26.42 cA	65.16	0.27	18.07 bB	0.55 b	43.44 bC
	30	14.95 aB	0.09	17.03 aB	14.68 aB	0.20	30.67 bAB	77.62	0.25	23.74 bC	0.62 bB	58.62 bC
	90	16.23 aB	0.11	17.14 aB	15.83 aB	0.32	40.85 aB	90.48	0.45	41.48 aAB	0.82 aB	74.10 aB
Summer	0	8.41 cA	0.10	15.17 bA	11.74 bA	0.14	26.87 dA	62.43	0.30	22.33 bAB	0.73 b	54.57 bB
	7	14.05 bA	0.14	17.36 bA	11.65 bA	0.19	31.80 cA	75.19	0.27	20.41 bB	0.66 b	54.37 bB
	30	20.23 aA	0.08	20.85 aA	16.13 aA	0.31	39.94 bA	97.54	0.47	39.58 abA	0.78 bB	78.20 abAB
	90	24.14 aA	0.15	23.15 aA	18.53 aA	0.31	45.17 aA	111.45	0.50	39.35 aAB	1.07 aB	87.14 aB
Autumn	0	5.66 bB	0.09	9.52 bB	7.80 bB	0.12	21.63 cB	44.80	0.25	24.73 bA	0.86 b	67.43 bA
	7	9.92 bB	0.05	12.76 bB	8.61 bB	0.17	22.02 cB	53.54	0.26	25.54 bA	0.71 b	52.22 bB
	30	18.95 aAB	0.11	14.82 aB	12.53 aB	0.18	27.31 bB	73.89	0.33	27.63 abB	1.05 bA	85.40 abA
	90	21.89 aB	0.14	16.60 aB	12.39 aB	0.19	30.10 aC	81.32	0.37	37.33 aB	1.28 aA	107.76 aA
Winter	0	9.21 bA	0.14	11.96 bB	9.11 bB	0.21	21.74 cB	52.37	0.37	22.13 bAB	0.51 b	58.21 bB
	7	11.78 bAB	0.12	14.05 bB	9.77 bAB	0.29	22.12 cB	58.12	0.37	24.42 bAB	0.49 b	62.82 bA
	30	13.65 aB	0.17	16.03 aB	12.08 aB	0.28	27.63 bB	69.85	0.52	27.91 bB	0.58 bB	65.44 bBC
	90	15.66 aB	0.15	16.11 aB	12.42 aB	0.46	36.36 aB	81.16	0.77	44.22 aA	0.62 aB	86.02 aB

Table 2 Continued.

		Free Fatty Acids[d]											
Production Season	Ripening time/days	iC15:0	aiC15:0	C15:0	C14:1	iC16:0	C16:0	C16:1w7 MCFA	Total	iC17:0	aiC17:0	C17:0	C17:1
Spring	0	1.22 B	2.45	4.96 B	0.51 b	1.19 b	115.02 bC	1.70 cB	192.31	0.40	0.79 B	1.69 B	0.37
	7	1.11	2.27 B	4.57 B	0.51 bB	1.00 bB	102.40 bC	1.50 cB	175.69	0.33 B	1.30 B	1.94	0.28
	30	1.42 B	2.79 B	5.67 B	0.52 bB	1.58 bB	125.60 bC	1.80 bB	222.59	0.58 B	1.49 B	2.42 B	0.77
	90	1.90 B	3.48 B	7.43 B	3.54 aA	1.83 aB	186.92 aC	3.97 aB	325.92	0.69 B	1.81 B	2.84 C	1.26
Summer	0	1.48 AB	2.77	6.28 A	0.38 b	1.54 b	153.60 bAB	2.06 bAB	246.04	0.35	1.46 AB	2.42 AB	0.37
	7	1.33	2.54 B	5.46 AB	0.62 bB	1.38 bB	142.57 bB	2.72 bAB	232.33	0.59 B	1.50 B	2.12	0.35
	30	1.72 AB	3.14 B	6.12 B	0.92 bA	1.76 bB	161.35 bB	3.34 bAB	297.37	0.58 B	1.83 B	2.51 B	0.76
	90	2.31 AB	4.39 AB	9.25 AB	1.68 aB	2.52 aAB	261.25 aB	4.65 aAB	446.10	1.59 B	2.47 B	3.66 B	0.94
Autumn	0	1.73 A	2.64	4.97 B	0.52 b	1.50 b	154.15 cAB	1.69 bB	260.46	0.27	2.40 A	2.98 A	0.28
	7	1.41	3.44 A	6.30 A	0.83 bA	1.96 bA	180.22 cA	1.99 bB	274.89	1.21 A	2.18 A	2.26	0.49
	30	2.31 A	4.58 A	8.48 A	0.60 bB	2.61 bA	238.36 abA	2.91 bAB	374.27	1.46 A	2.67 A	3.97 A	0.73
	90	3.11 A	6.17 A	11.21 A	1.64 aB	3.35 aA	295.35 aA	4.24 aAB	471.81	1.61 A	3.87 A	5.13 A	0.81
Winter	0	1.20 B	2.78	5.44 AB	0.71 b	1.21 b	182.99 bA	2.50 bA	278.06	0.69	1.12 B	2.81 A	0.56
	7	1.09	2.51 B	5.16 B	0.98 bA	1.56 bAB	160.75 bAB	3.23 bA	263.37	0.43 B	1.79 B	2.63	0.64
	30	1.41 B	3.47 B	7.96 A	0.90 bA	1.54 bB	180.08 bB	3.19 bA	292.99	0.77 B	2.57 A	3.78 A	0.69
	90	1.49 B	3.26 B	6.90 B	2.11 aAB	1.97 aB	234.55 aB	5.63 aA	387.53	0.54 B	2.06 B	3.19 B	0.93

Table 2 Continued.

		Free Fatty Acids[d]								
Production Season	Ripening time/days	C18:0	C19:0	C18:1w9	C18:1w7	C20:1	C18:2w6	C18:3w3	Total LCFA	Total FFA
Spring	0	49.63 bB	0.31	70.73 cC	4.27 bB	0.37	4.83 bB	0.66 b	134.06	377.11
	7	40.06 bC	0.25	80.59 bB	4.30 bB	0.29 B	3.30 bB	0.98 b	133.62	374.47
	30	53.10 bB	0.31	98.27 abB	4.87 bB	0.25 B	13.81 aA	2.18 aA	178.05	478.26
	90	75.09 aB	0.53	128.60 aB	5.37 aC	0.84 B	12.12 aAB	2.98 aB	231.13	647.52
Summer	0	49.19 bB	0.18	82.71 cB	5.91 bB	0.69	5.35 bB	0.77 b	149.40	457.87
	7	47.86 bB	0.28	84.95 bB	6.04 bAB	0.59 B	6.55 bAB	0.80 b	151.63	459.15
	30	50.40 bB	0.42	108.98 abB	6.27 bAB	0.55 B	7.91 aB	1.30 aB	181.51	576.42
	90	75.44 aB	0.52	146.66 aAB	11.89 aAB	0.82 B	11.78 aAB	1.47 aC	257.25	814.80
Autumn	0	53.73 cA	0.24	80.12 bB	5.33 bB	0.26	4.54 bB	0.46 b	150.61	455.87
	7	63.31 cA	0.18	93.96 bA	4.65 bB	0.74 A	5.75 bAB	0.58 b	175.32	503.74
	30	75.58 abA	0.47	141.09 aA	5.25 bB	1.12 A	6.28 bB	0.80 bC	239.41	687.57
	90	96.97 aA	0.48	149.05 aAB	9.53 aB	1.05 A	10.42 aB	1.05 aC	279.96	833.10
Winter	0	51.15 bB	0.15	111.04 bA	8.01 bA	0.45	8.25 bA	0.50 b	184.73	515.16
	7	58.59 bA	0.28	100.02 bA	8.84 bA	1.02 A	9.42 bA	0.63 b	184.29	505.78
	30	59.49 bB	0.32	121.03 abAB	9.78 bA	1.06 A	9.84 abA	0.71 abC	210.03	572.87
	90	62.61 aB	0.36	164.90 aA	14.88 aA	1.15 A	14.30 aA	3.27 aA	248.19	716.89

[d] mean value: 12 cheeses (4 cheeses/producer), for each cheese two samples, each sample in duplicate.
Means with different letters (a-c) in the same column are significantly different from each other for ripening time ($P \leq 0.05$). Means with different capital letters (A-C) in the same column are significantly different from each other for the production season ($P \leq 0.05$) at the same ripening time. Letters are not reported where there were no significant differences ($P > 0.05$).

Analysis of variance of the data obtained showed statistically significant differences ($P \leq 0.05$) during the ripening time and within the cheesemaking seasons. During ripening, since the P-value was less than 0.05, there were statistically significant differences between the mean of all fatty acids from 0 to 90 days (95% confidence level), except for the odd-carbon-number fatty acids and eicosenoic acid ($P > 0.05$). Using homogeneous groups (Duncan's test), for each free fatty acid ($P < 0.05$), the multiple-range test determined statistically significant differences as a result of ripening and production season, which are summarised as follows: 1) between 0 and 7 days no statistically significant differences resulted for all free fatty acids considered, except for butanoic, decanoic acids and for oleic acid in summer and spring; 2) there were significant differences between 7 and 30 days for even-carbon number fatty acids from C_4 to C_{10} and between 30 and 90 days from C_{10} to $C_{18:3}$; 3) in ripened samples even-carbon number fatty acids from C_4 to C_{10} had a significantly higher content in summer, all saturated even-carbon number fatty acids from C_{14} to C_{18} in autumn, while those unsaturated in winter; 4) the amount of myristic, palmitic and stearic acids were significantly lower in spring.

The total content of free fatty acids was higher than that reported by WOO *et al.* (1984 b) for other Italian cheese varieties. The significant increase of all the even carbon number fatty acids observed during the ripening period has also been reported for a great majority of cheeses (NAJERA *et al.*, 1994; CONTARINI and TOPPINO, 1995; MACEDO and MALCATA, 1996; SOUSA and MALCATA, 1997; FREITAS and MALCATA, 1998; MALLATOU *et al.* 2003; AKIN *et al.* 2003; GEORGALA *et al.*, 2005).

The increase of the FFA content during ripening is a consequence of the progressive lipolytic process that involves the hydrolysis of glycerides. The lipolytic system, responsible for lipolysis in Provola dei Nebrodi, at first hydrolyses the external position sn-3 of triglycerides, producing butyric and caproic acids, then during ripening hydrolyses the other external position sn-1, producing oleic, stearic and palmitic acids.

Although Provola dei Nebrodi manufacture involved the utilisation of rennet paste contained the lipase, pregastric esterase (PGE), apparently the PGE action is not evident. In fact PGE is highly specific for SCFA at the sn-3 position of mono, di, and triglycerides of milk fat.

The lipolytic action in Provola dei Nebrodi seems to be due to other less selective lipolytic enzymes, such as microbial enzymes. These non specific enzymes can release indiscriminately short chain fatty acids from the sn-3 position, long chain fatty acids from the sn-1 position and medium chain fatty acids from the sn-2 position by conversion in sn-1 or sn-3 isomers (SOMERHARJU *et al.*, 1978; NILSSON-EHLE *et al.*, 1973).

The main differences in the SCFA composition were between 7 and 30 days in all the production seasons considered as previously reported by VERZERA *et al.* (2004), and the highest amount of each SCFA were observed in summer. Probably the higher temperature of the summer season forces the hydrolysis of triglycerides, moreover, in these samples lactic acid bacteria, enterococci and propionic acid bacteria reached their maximum value (8.68-8.96, 5.41-5.75, 4.18-4.70 log CFU g^{-1} cheese, respectively) in the second week of ripening (unpublished data). Lactic acid bacteria are weakly lipolytic (STADHOUDYERS and VERINGA, 1973), but enterococci, which are part of the dominant flora in raw milk cheese (CENTENO *et al.*, 1996), have been used on numerous occasions to accelerate cheese maturation due to their proteolytic and lipolytic activity (CENTENO *et al.*, 1999) and propionic acid bacteria are well known for their high lipolytic activity, 10-100 times more than lactic acid bacteria (CHAMBA and PERREARD, 2002). In this light, the microbial population could contribute to the lipolysis of "Provola dei Nebrodi". Regarding the concentration of butanoic, decanoic and oleic acids it was noted that they continuously increased throughout the ripening period. For these fatty acids, in analysing white pickled cheese from raw cow milk, AKIN *et al.* (2003) observed a similar behaviour in cheese samples made with the addition of pregastric lamb lipase.

The period of lactation, animal feeding and the local weather are the major factors responsible for the differences observed among the seasonal cheeses. The major content of FFA in ripened cheese samples was observed in autumn and the lowest in spring (Fig. 2) which was also the case for the main free fatty acids: myristic, palmitic and stearic. In autumn, the end of lactation determined a significantly higher fat content of cheese samples produced in this season (Table 1) as previously reported by MACEDO and MALCATA (1996). This leads to a higher amount of FFA since lipolysis is expected to occur faster if the substrate concentration is increased (IRVINE *et al.*, 1948). In spring, cows which have been stalled throughout the winter are moved to the pasture and this sudden change causes a significantly lower fat content in the cheese samples (ALAIS, 1984). In winter the significantly higher amount of unsaturated fatty acids mainly oleic, linoleic and linolenic was probably due to the concentrated feed containing oil seeds.

In conclusion, "Provola dei Nebrodi" cheese is characterised by a high amount of FFA (up to 840.45 mg 100 g^{-1} cheese) which was higher than the values reported for other types of cheese (MALLATOU *et al.* 2003; POVEDA *et al.* 1999; PARTIDARIO, 1999; FREITAS and MALCATA, 1998) except for blue and smear cheeses and long ripened "pasta filata" cheeses (DE LA FUENTE *et al.*, 1999; WOO *et al.*, 1984a; BATTISTOTI and CORRADINI,

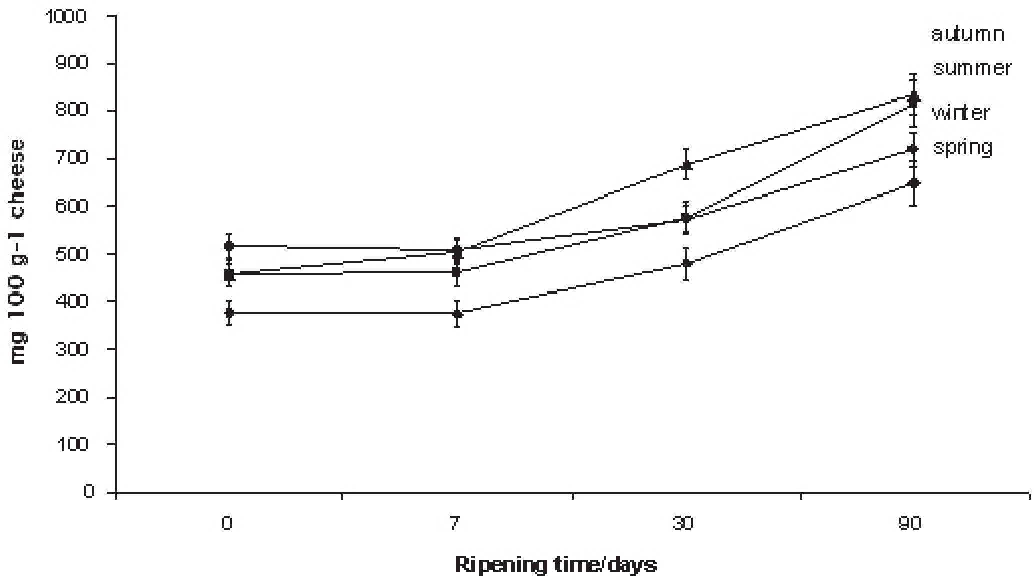

Fig. 2 - Total free fatty acid content (mg 100 $g^{-1}$of cheese) throughout ripening in the different production seasons.

1993). The statistically significant differences observed for samples from different seasons with equal ripening is coherent with the artisanal nature of Provola dei Nebrodi production, since the cheese makers do not standardise the milk. From the data obtained, the FFA composition could become a powerful tool to establish a protocol for the characterization of this product.

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