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Influence of salt content on the triglyceride composition of Idiazabal cheese during ripening

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Abstract — The influence of salt content on the triglyceride (TG) composition of Idiazabal cheese during ripening has been studied. Cheeses were brined for 12, 24 and 36 h, with a higher salt content ($g \cdot kg^{-1}$ of cheese) and lower moisture percentage recorded in the longer-brined cheeses. The lower saturated, unsaturated, short-, medium- and long-chain TG contents recorded for the low-salted cheeses (brined for 12 h) and their progressive decrease during ripening were attributable mainly to differences in the rate of moisture loss in the cheeses. The differences in the composition of the TG profile of the low-, medium- and high-salted cheeses that were observed could be due to differences in the levels of lipolytic activity in the cheeses, with the higher salt concentrations (~3–4 %) contributing to higher levels of lipolysis. © Inra/Elsevier, Paris.

salt content / triglyceride / lipolysis / ripening / Idiazabal cheese

Résumé — **Influence de la teneur en sel sur la composition des triglycérides du fromage Idiazabal pendant l'affinage.** On a étudié l'influence de la teneur en sel sur la composition des triglycérides (TGs) pendant l'affinage. Les fromages ont été placés en saumure pendant 12, 24 et 36 h. Les fromages saumurés le plus longtemps avaient une plus forte teneur en sel ($g \cdot kg^{-1}$) et une plus faible teneur en eau. Les teneurs plus faibles en TGs saturés, insaturés, à courte, moyenne et longue chaîne observées pour les fromages peu salés (saumurés 12 h) et leur diminution progressive pendant l'affinage seraient à attribuer principalement à des différences dans le taux de perte d'eau dans les fromages.

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Les différences observées dans la composition du profil des TGs des fromages à faible, moyenne et forte teneur en sel pourraient être dues aux différences d'activité lipolytique, les concentrations les plus élevées en sel (~3–4 %) donnant les taux de lipolyse les plus élevés. © Inra/Elsevier, Paris.

teneur en sel / triglycéride / lipolyse / affinage / fromage Idiazabal

1. INTRODUCTION

Salting exerts a major influence on cheese ripening and quality. However, a review of the literature has yielded very few studies describing the effect of salt on lipolysis in cheese during ripening. Some authors have shown that sodium chloride (NaCl) has an important effect on regulating lipase production and activity and on the subsequent metabolism of fatty acids by microorganisms [1, 4, 13, 25].

Lipolytic activity has been reported to be higher in unsalted than in salted cheeses; and among the latter, in those with the lowest salt levels [14, 16, 22, 23]. Nevertheless, it has also been reported that a minimum salt concentration in cheeses is required for optimum cheese quality, although certain NaCl concentrations should not be exceeded. Therefore, each type of cheese has its own minimum and maximum reference values for internal salt levels [3, 12, 21].

In the literature reviewed, no study has been made on the triglyceride (TG) composition of cheese. However, several investigators have found that the TGs in the milk of different animal species have partition numbers (PNs; PN = CN–2ND, where CN is the total carbon number and ND the total number of double bonds of the constituent fatty acids) ranging from 22 to 54, the principal of these having PN values of 34 and 36. Recent work in our laboratory on the TG composition of Idiazabal cheese has shown that the main individual molecular species are butyroyl-dipalmitin, butyroyl-myristoyl-palmitin and butyroyl-palmitoyl-olein [19].

The object of the present study was to examine the influence of salt content on changes in the TG profile of Idiazabal cheese during ripening. No guidelines on salting method and NaCl content are described in the procedures for cheese-making established by the Denomination of Origin of Idiazabal Cheese [18]. The present study will therefore contribute to a better definition of the quality of this Basque traditional cheese.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Cheeses were made at the pilot plant of Queserías Araia (Araia, Alava, Spain). Bulk ewes' milk was obtained from several local commercial flocks of *latxa* sheep, and was collected in March. All cheeses were manufactured from the same vat of milk, according to the traditional method for the industrial production of Idiazabal cheese approved by its Denomination of Origin [18], using lamb rennet. After moulding and pressing, the cheeses were brined in a saturated solution of NaCl 1.2 $g \cdot mL^{-1}$ at 13 °C. Three batches of cheeses were selected according to brining time: low-salted (brined for 12 h), medium-salted (24 h) and high-salted cheeses (36 h). The cheeses were ripened at 8–10 °C and at a relative humidity of ~85 % for 360 d. Cheeses (~13 cm in diameter and 10 cm in high) weighed between 1–1.2 kg.

The cheeses were analysed after 1, 180 and 360 d of ripening. Additional cheeses were also taken on d 15, 30, 60, 120 and 270 for NaCl, moisture and total fat content analyses. Samples were taken from two different cheeses on each sampling date and physicochemical analyses were performed in duplicate.

The samples were prepared by cutting the cheese into triangular portions weighing ~100 g each. The rind (~0.5 cm) was removed from the cheese portions, which were then comminuted to a uniform grain size at room temperature in a model A10 grinder (Janke & Kunkel, Staufen, Germany).

2.2. Physicochemical analyses

International Dairy Federation Standards No. 4, 88 and B-3 5A [9-11] were used to analyse cheeses for moisture, NaCl and total fat content, respectively.

Triglycerides were extracted from the cheese in accordance with Standard No. 32 of the International Dairy Federation [8] using *n*-pentane (Panreac, Barcelona, Spain) as solvent. The TGs were analysed and quantified by high-performance liquid chromatography (HPLC) according to the procedure of Nájera et al. [19] on two Nucleosil 120 C-18 columns (20 cm × 4.6 mm each, 3 μm particle size; Symta, Madrid, Spain), connected in series. The chromatograph comprised two Kontron pumps (models 422M and 422S, Milan, Italy), a Rheodyne injector (model 7161, Cotati, CA, USA) and a Sedere light-scattering detector (model Sedex 45, Alfortville, France).

The results were assessed for TG groupings with the same PN value. The TGs were also grouped as saturated, unsaturated, short-, medium- and long-chain TGs according to the criteria used by Nájera et al. [19] for cheese TGs.

2.3. Statistical analysis

Analysis of variance (ANOVA) and multiple comparison tests were applied to establish the existence of significant differences ($P \leq 0.05$) in the NaCl and TG content of the cheeses with different brining times on each sampling date (1, 180 and 360 d) during the ripening period. The 7D programme from BMDP Statistical Software, Inc. (Los Angeles, CA, USA) was used in the statistical analysis.

3. RESULTS AND DISCUSSION

Significant differences in the NaCl content, moisture and total fat content of the cheeses brined for different times at each sampling date were observed. *Figures 1* and *2* respectively show the NaCl (g·kg^{-1} of cheese) and moisture (%) content of the low-, medium- and high-salted cheeses during ripening. In all cases,the salt content increased with ripening time due to moisture loss from the cheeses. As expected, the highest salt concentrations were recorded in the longest brined cheeses (*figure 1*), which also showed the lowest moisture content during ripening (*figure 2*). Total fat content (g·kg^{-1} of cheese) of the low-, medium- and high-salted cheeses increased during ripening (*figure 3*). The low-salted cheeses had, except on d 120, the lowest total fat content during ripening as this batch had the highest moisture content (*figure 2*).

Significant differences for all the TG groupings studied at each sampling date were found mainly between the low-salted batch and the other two batches, except on

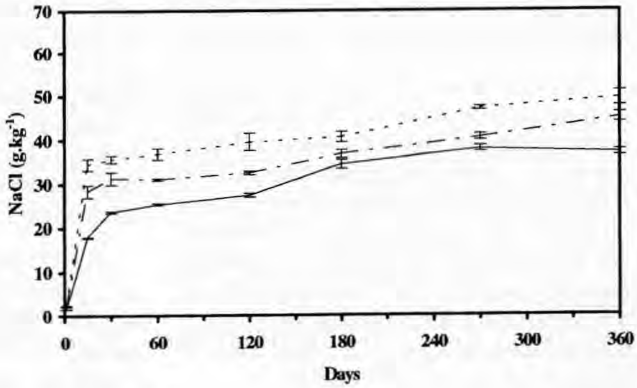

Figure 1. Changes in the NaCl content of the 12-h (—— low-salted), 24-h (— - — medium-salted) and 36-h brined (- - - high-salted) cheeses during ripening.

Figure 1. Modifications de la teneur en NaCl des fromages salés 12 h (—— faible teneur en sel), 24 h (— - — moyenne teneur en sel) et 36 h (- - - forte teneur en sel) pendant l'affinage.

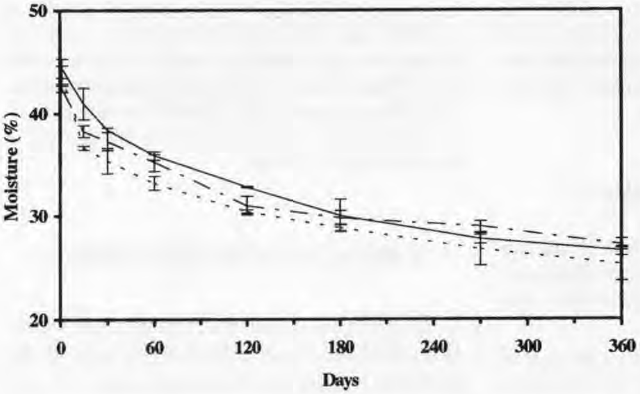

Figure 2. Changes in the moisture content of the 12-h (—— low-salted), 24-h (— - — medium-salted) and 36-h brined (- - - high-salted) cheeses during ripening.

Figure 2. Modifications de l'humidité des fromages salés 12 h (—— faible teneur en sel), 24 h (— - — moyenne teneur en sel) et 36 h (- - - forte teneur en sel) pendant l'affinage.

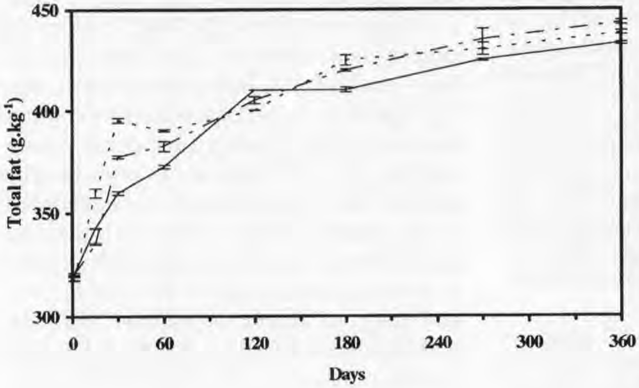

Figure 3. Changes in the total fat content of the 12-h (—— low-salted), 24-h (— - — medium-salted) and 36-h brined (- - - high-salted) cheeses during ripening.

Figure 3. Modifications de la teneur en matière grasse totale des fromages salés 12 h (—— faible teneur en sel), 24 h (— - — moyenne teneur en sel) et 36 h (- - - forte teneur en sel) pendant l'affinage.

d 1 (*table I*). In this case, significant differences were mainly found between the high-salted batch and the other two batches.

In view of the results obtained, the TG composition of the low-salted cheeses was compared to that of the medium- and high-salted cheeses considered as one batch, from thereon termed medium-high-salted cheeses.

Figure 4 shows the mean percentage increase (%I) or decrease (%D) in the saturated, unsaturated, short-, medium- and long-chain TGs in the low-salted cheeses with respect to the medium-high-salted cheeses on each of the sampling dates (1, 180 and 360 d). All the TG groupings except those regarding medium- and long-chain TGs on d 1 decreased in the low-salted cheeses, and that decrease became appreciably larger as ripening advanced. Accordingly, the percentages of decrease were found to be ~13, 25 and 49 % on d 1, 180 and 360, respectively.

The lower TG content recorded for the low-salted cheeses and the progressive decrease during ripening could be ascribable to the differing behaviour of the cheeses in response to water loss. Various investigators have reported that diffusion of salt inwards towards the centre of the cheese is greater in cheeses with a low salt content which is associated with a lower rate of moisture loss, especially in the later stages of ripening [2, 15, 24, 26].

The TG groupings by PN value (expressed as a percentage of the total TG content) in the low-salted cheeses were compared to those in the medium-high-salted cheeses to obtain information on the effect of salt con-

Table I. Significance levels for the differences in the TG content in the low- (L), medium- (M) and high-salted (H) Idiazabal cheeses after 1, 180, and 360 d of ripening

Tableau I. Niveaux de signification pour les différences concernant la quantité de TGs des fromages Idiazabal à faible (L), moyenne (M) ou forte (H) teneur en sel à 1, 180 et 360 j d'affinage.

Triglycerides	1 day			180 days			360 days		
	LM	LH	MH	LM	LH	MH	LM	LH	MH
PN 28	NS	**	**	***	***	**	***	***	***
PN 30	NS	NS	NS	***	***	***	***	**	NS
PN 32	*	***	NS	***	**	*	**	**	NS
PN 34	NS	*	*	**	*	NS	***	*	*
PN 36	NS	*	**	***	**	NS	***	*	NS
PN 38	NS	**	***	***	NS	***	***	*	**
PN 40	**	***	***	***	*	NS	**	*	NS
PN 42	***	***	***	*	NS	**	***	*	NS
PN 44	***	***	***	NS	*	NS	***	**	NS
PN 46	**	**	***	***	***	NS	***	***	NS
PN 48	*	***	**	***	***	NS	***	***	**
PN 50	NS	**	**	***	***	NS	***	***	***
Saturated TGs	NS	***	***	***	***	***	***	*	NS
Unsaturated TGs	**	*	**	***	***	**	***	**	NS
Short-chain TGs	NS	***	***	***	***	**	***	*	NS
Medium-chain TGs	***	***	***	***	NS	**	***	*	NS
Long-chain TGs	***	***	***	**	***	NS	***	***	*

*** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$; NS: not significant; PN: partition number.
NS : non significatif ; PN : nombre de partition.

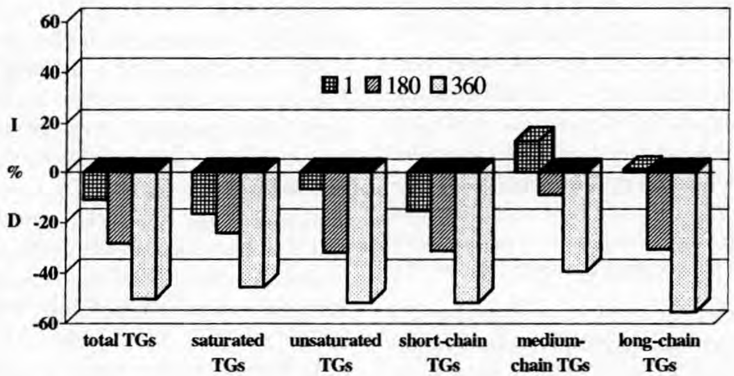

Figure 4. Mean percentage increase (%I) or decrease (%D) in the saturated, unsaturated, short-, medium- and long-chain TGs (g·kg^{-1} of cheese) in the low-salted compared to the medium-high-salted cheeses after 1, 180 and 360 d of ripening.

Figure 4. Pourcentages moyens d'augmentation (%I) ou diminution (%D) des TGs saturés et insaturés, à courte, à moyenne et à longue chaîne (g·kg^{-1} de fromage) des fromages à faible teneur en sel par rapport aux fromages à forte et moyenne teneur en sel à 1, 180 et 360 j d'affinage.

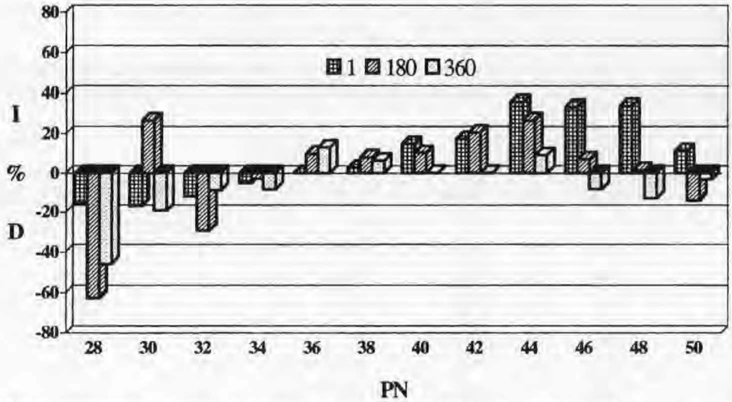

Figure 5. Mean percentage increase (%I) or decrease (%D) in the TG profile according to the partition number (PN) (percentages of the total TG content) in the low-salted cheeses compared to the medium-high-salted cheeses after 1, 180 and 360 d of ripening.

Figure 5. Pourcentages moyens d'augmentation (%I) ou diminution (%D) dans le profil TG selon le nombre de partition (PN) (pourcentages de la quantité totale des TGs) des fromages à faible teneur en sel par rapport aux fromages à forte et moyenne teneur en sel à 1, 180 et 360 j d'affinage.

tent on the TG profile in Idiazabal cheese. *Figure 5* shows that the behaviour of the TGs differed according to the PN and sampling date. Except for the grouping with a PN value of 30 at 180 d of ripening, the groupings with PN values of 28, 30, 32 and 34 showed percentage decreases in the low-salted cheeses, although the behaviour of the groupings varied as the ripening period advanced. The groupings with PN values of 36, 38, 40, 42 and 44 showed percentage increases in the low-salted cheeses. The groupings with PN values of 46, 48 and 50 showed a behaviour pattern that was similar to that of the latter groupings, with percentage increases in the low-salted cheeses early in ripening giving way to percentages of decrease as ripening advanced (*figure 5*).

The differences observed in the TG groupings with different PN values might reflect varying levels of lipolytic activity in the cheeses with a different salt content. As reported previously by certain workers, the salt content of cheese exerts an influence on the lipolytic activity during ripening, and a minimum salt content appears to increase lipolysis; in turn, lipolysis decreases when the salt content exceeds certain limits, though the minimum and maximum values concerned appear to be specific to each type of cheese [1, 5–7, 17, 20, 22, 25]. In the present study, salt levels attained 3.4 % by weight in the low-salted cheeses and 4.1 % in the high-salted cheeses after 6 months of ripening (*figure 1*). In previous work carried out at our laboratory [20], the free fatty acid (FFA) content was higher in medium-high-salted cheeses up to d 180 of ripening. The mean percentage increase in the medium-high-salted cheeses with respect to the low-salted cheeses was ~15 % for short-chain FFAs (C_4, C_6, C_8 and C_{10}), ~15 % for medium-chain FFAs (C_{12} and C_{14}), and ~23 % for long-chain FFAs (C_{16} and C_{18}). After 6 months of ripening, mean percentage decreases were recorded for FFAs, except for butyric ($C_{4:0}$) and stearic ($C_{18:0}$) acid [20]. These results suggested that higher NaCl contents led to an increase in FFA content, although certain lipolytic activity appeared to be limited by the NaCl content of the medium-high-salted cheeses after 180 d of ripening. According to this previous work, higher levels of lipolytic activity were to be expected in the medium- and high-salted cheeses, particularly during the first 6 months of ripening. Therefore, the lower levels of the TG groupings with PN values between 36 and 44 in the medium- and high-salted cheeses (*figure 5*) could be a result of higher levels of lipolytic activity in those cheeses.

4. CONCLUSION

In conclusion, the results of this study have shown that salt content had a significant effect on the TG composition of cheese. The differences in the TG content ($g \cdot kg^{-1}$ of cheese) between the low-, medium- and high-salted cheeses recorded during ripening were attributable mainly to differing rates of moisture loss from the different cheese batches. On the other hand, the differences in the relative percentages of the TG groupings by PN value in the cheeses with different salt content that were observed could be the result of differences in the levels of lipolytic activity in the cheeses, with the higher salt concentrations (~3–4 %) contributing to higher levels of lipolysis.

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