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EVALUATION OF BIOCHEMICAL CHANGES OCCURING IN "NĂSAL" CHEESE DURING THE RIPENING STAGES

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ABSTRACT. "Năsal" cheese is the only sort of Romanian cheese with mould, being unique also by its processing technology and sensorial characteristics. This study is the first complex investigation which focuses on the biochemical changes occurring during the ripening stages of this cheese pointing out its nutritional value. This type of cheese is characterized by a high total solids (45.92%), fat (40.3%) and protein (19.3%) contents at the end of the ripening process. All these biochemical compounds increased during the ripening process, starting from day 20 until day 60, the values being statistically different ($p<0.05$). The increase in values was noticed also in the fatty acids profiles, the most abundant saturated fatty acids being C14:0, C16:0 and C18:0. The most statistically significant increase ($p<0.001$) in quantity during the three stages of ripening analysed, was noticed in case of C4:0, C8:0, C14:0. The ripening applied influences significantly the concentrations of unsaturated fatty acids, favouring the increase in C16:1, C18:1, C18:2, C18:3n3. We concluded that "Năsal" cheese holds a great nutritional value, the ripening period having a strong influence on the amounts of biochemical compounds. Compared to other type of not-ripened cheese, this type stands out by its higher amount of unsaturated fatty acids, essential in human diet.

Keywords: *fatty acids, ripen, unsaturated, protein, total solids.*

INTRODUCTION

Along the years, cheese processing has evolved from traditional art to science. A lot of cheese varieties have been developed and tested for different environmental conditions in order to meet the highly pretentious

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requests of the consumers. It is estimated that currently there are over 2000 cheese varieties [1], and this list is in continuous growing. Each of these sorts of cheese has a specific particularity, either due to the milk used, processing technology or ingredients.

The uniqueness of cheese sorts develop especially during the ripening stages, being influenced by the starter cultures used [2; 3]. One of the most complex biochemical events that take place during ripening is proteolysis. Researches concerning the pathways for the catabolism of free amino acids during ripening have been published in previous years [4; 5; 6; 7] but still it has not been fully elucidated [8]. Lipolysis is another process that occurs during the ripening process. The free fatty acids released during lipolysis along with the volatile compounds and the proteolysis products contribute directly to cheese flavour [4, 9]. Cheese flavour is one of the most important criteria determining consumer choice and acceptance [10].

In Romania, the ripened dairy products originate from various areas of production, each having their characteristics and nutritional qualities. "Telemea" cheese represents 60% of all kinds of cheeses produced in Romania, having particular nutritional characteristic influenced by the milk, processing technology and area of production. For that matter there are a number of researches that have studied the dynamics of biochemical compounds during the ripening stages.

"Năsal" cheese is part of this category of fermented cheeses, soft content, made from cow milk. On its surface there is a bacteria substrate due to the development of *Brevibacterium linens*, which gives the particularity of this product. This bacterium transforms the cheese components offering its specific taste and consistency, not being necessary the artificial adding of mould spores which often are needed in specific French cheeses. This cheese holds an important value to the traditional production of cheese in Romania, given the fact that it is the only one with this particular technology of processing. The ripening is made in a special environment (Ţaga cave) that favours the development of *Brevibacterium linens.* In this cave, the temperature and air humidity are constant during the entire year, ensuring the production of characteristic cheese, appreciated of having exquisite sensorial features [11].

Given the particularities of "Năsal" cheese, the aim of our study was to evaluate the changes in the biochemical composition during the ripening stages and to assess its nutritional value compared to another type of cheese.

RESULTS AND DISCUSSION

The biochemical analysis of the „Năsal" cheese has revealed that the fat percentage shows a consistent upper trend during the ripening stages, from 28.4 ± 0.76 g% within day 20 of ripening to 40.3 ±1.37 in the last stage (day 60). The differences noticed were statistically different ($p<0.05$) when comparing the three stages of „Năsal" cheese ripening.

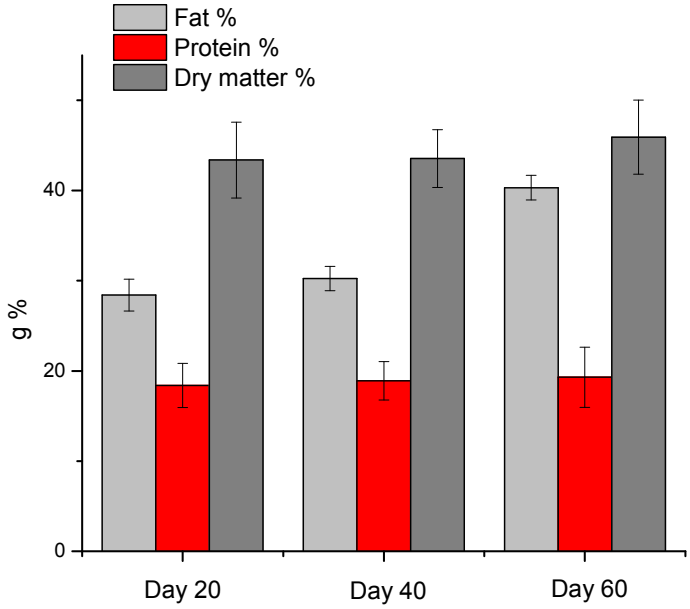

Figure 1. The compositional parameters percentage values according to the ripening stages

The protein values showed also a slight increase from day 20 of ripening (18,39 ± 0.45) compared to day 60 (19,3 ± 0.34), possibly due to the specific environmental conditions of Ţaga cave which has a specific humidity. Our results are in conformity with the ones found by Pappa et al. (2007) [12], which noticed that in the first day of „Telemea" cheese ripening, the average value of proteins was 15.03±0.52% and in two months of ripening increased to 16.74±0.13%. Not all studies obtained the same results, some of them showing a decrease in values during the ripening stages of cheese. Hui and Evranuz (2012) [13] showed a decrease in the content of protein during the ripening stages, explaining that this process is a consequence of various humidity values and the loss of protein components in the brine. We noticed that due to the environmental rippening condition particularities of „Năsal" cheese (higher humidity) the protein content is affected, the caseins being prevented from fragmenting.

The dry matter increased during the ripening stages from 43.38±1.40 g% to 45.92±1.68 g%. During the ripening process, it was noticed that in the first stage of ripening, 5.49% from the total amount of samples, showed lower values compared to the limits imposed by the legislation ((minimum 42%) [11]. In the second stage (day 40), only 2.19% showed lower values than the standards, and at the end of the ripening stages, all samples were in conformity.

From the statistical evaluation, the fat/dry matter fraction presented a uniform trend during the entire rippening stage, from 47.13±0.80 in the first stage of rippening to 47.03±2.79 in the last stage (day 60). From the total amount of examined samples, only 1.1% were in between the minimum admissible limits for this type of cheese (fat/dry matter > 50). This is a concerning fact given that almost all samples examined were not in conformity with this parameter. Pappa et al. (2007) [12] showed higher values regarding this parameter (54%). The same upper trend was noticed by Pappa (2006) [14], the value increasing from 43.2% in the first day of ripening to 55.4% after two months of ripening.

The processing technology of this particular type of cheese allows the mainting of a high proportion of fatty acids. However, as seen in table 1, there are a series of significant changes statistically interpreted ($p<0.05$) between the values obtained before ripening (day 0) and after ripening (day 60).

Table 1. Values of fatty acids in Năsal cheese during the ripening period

Free fatty acid	*Symbol*	*Ripening stage (%)*			
		Day 0	Day 20	Day 40	Day 60
Butyric	C4:0	0.69	0.75	0.82	1.12
Caproic	C6:0	1.74	1.68	1.79	1.97
Caprylic	C8:0	1.68	1.57	1.67	1.82
Capric	C10:0	5.20	5.45	5.55	5.45
Lauric	C12:0	1.64	1.72	1.75	1.55
Miristic	C14:0	11.73	11.64	11.94	12.15
Miristoleic	C14:1	0.32	0.22	0.24	0.34
Palmitic	C16:0	31.60	32.10	32.50	32.60
Palmitoleic	C16:1	1.13	1.23	1.43	1.53
Stearic	C18:0	12.15	12.22	12.62	12.64
Oleic	C18:1	13.78	13.82	13.84	13.86
Linoleic	C18:2	2.10	2.23	2.13	2.16
Linolenic	C18:3n3	1.03	1.13	1.23	1.53

The fatty acid profile in "Năsal" cheese samples taken before ripening (day 0) revealed essential concentrations of stearic, palmitic and oleic acids and lower values of linoleic, capric, butyric and linolenic acids. After only 20 days of ripening significant changes ($p<0.05$) were noticed. The C18:1, C16:0, C18:0 and C4:0 increased in values while the concentrations of C14:0, C8:0 and C6:0 decreased; these changes were similar during the entire period studied, no matter the season of sampling. After 40 days of ripening, statistically significant changes were revealed in case of C6:0, C10:0, C16:0, C16:1, C18:1 and C18:3n3 ($p<0.05$) which increased in values compared to day 0 of ripening. The period of ripening changes significantly the concentrations of fatty acids, favouring the increase in C16:1, C18:1, C18:2, C18:3n3 as seen from the results obtained after 60 days of ripening.

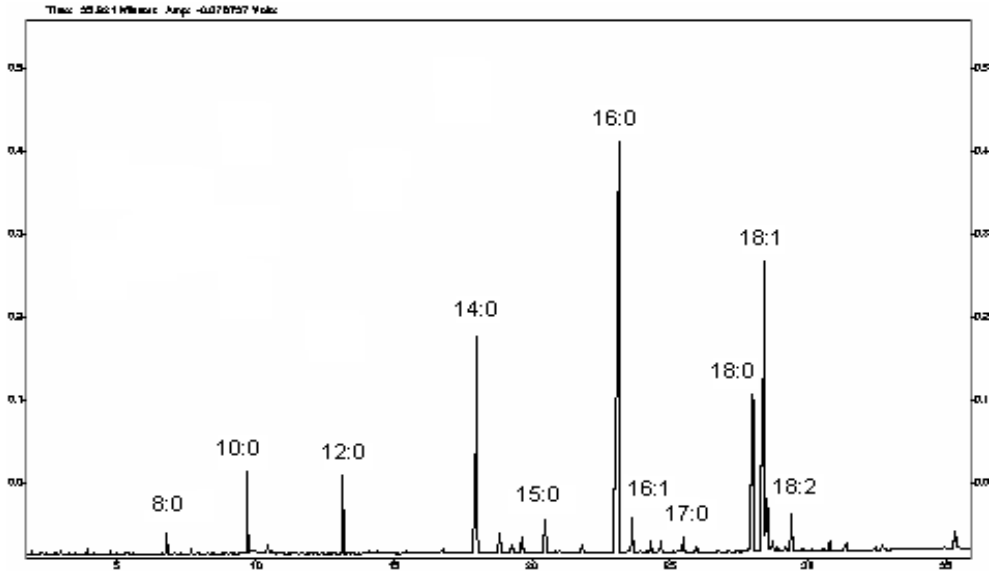

Figure 2. Chromatogram of fatty acids methyl esters in "Năsal" samples after 20 days of ripening

The most statistically significant increase ($p<0.001$) in quantity was noticed in case of C4:0, C8:0, C14:0. This increase can be explained by the fact that the lipases (originated mainly in milk and microorganisms) involved in cheese ripening, hydrolase preferentially the short and medium chain fatty acids [15,16].

Table 2. The averages fatty acids values in "Năsal" cheese ripened 60 days compared to not ripened fresh cheese

Trait	*Symbol*	*"Năsal" cheese (g/100g)*				*Fresh cheese (g/100g)*				P value
		Min.	Max.	Mean	SD	Min.	Max.	Mean	SD	
Butyric	C4:0	0.5	1.33	1.12	0.14	0.2	0.96	0.82	0.11	*
Caproic	C6:0	2.30	3.11	1.97	0.31	1.47	2.75	1.74	0.40	*
Caprylic	C8:0	1.72	1.96	1.82	0.25	1.87	2.43	1.38	0.43	*
Capric	C10:0	4.71	6.43	5.45	1.31	3.18	6.55	5.40	0.41	NS
Lauryc	C12:0	1.3	1.78	1.55	0.31	0.87	1.94	1.44	0.05	NS
Miristic	C14:0	9.56	14.07	12.15	0.12	9.51	13.26	11.53	1.21	*
Miristoleic	C14:1	0.09	0.53	0.34	0.01	0.16	0.45	0.52	0.52	NS
Palmitic	C16:0	22.3	36.3	32.60	0.61	26.75	36.75	31.40	0.10	*
Palmitoleic	C16:1	0.23	1.87	1.53	0.30	0.93	1.27	1.13	0.21	**
Stearic	C18:0	11.23	13.62	12.64	1.28	11.46	13.31	12.55	0.34	NS
Oleic	C18:1	11.83	15.84	13.86	0.34	10.74	15.16	13.48	0.20	*
Linoleic	C18:2	1.38	3.81	2.16	0.37	1.33	3.45	2.20	0.05	NS
Linolenic	C18:3n3	0.70	1.95	1.53	0.21	0.37	1.51	0.53	0.51	***
SFA		60.25	72.34	69.3	0.23	60.11	62.95	66.26	1.90	**
MUFA		31.13	32.45	15.73	0.43	30.36	31.96	14.61	0.59	*
PUFA		3.14	4.11	3.69	0.52	6.21	8.67	2.73	0.18	***

SD = standard deviation; NS = not significant P > 0.05,
* Significant : P < 0.05; ** P < 0.01; *** P<0.001

Our results are similar with the ones found by Olmedo and Coll-Hellin (1976) [17] at the traditional ripened sheep cheese and those revealed by Gattuso and Fazio (1980) [18] in the Italian ripened cheese.

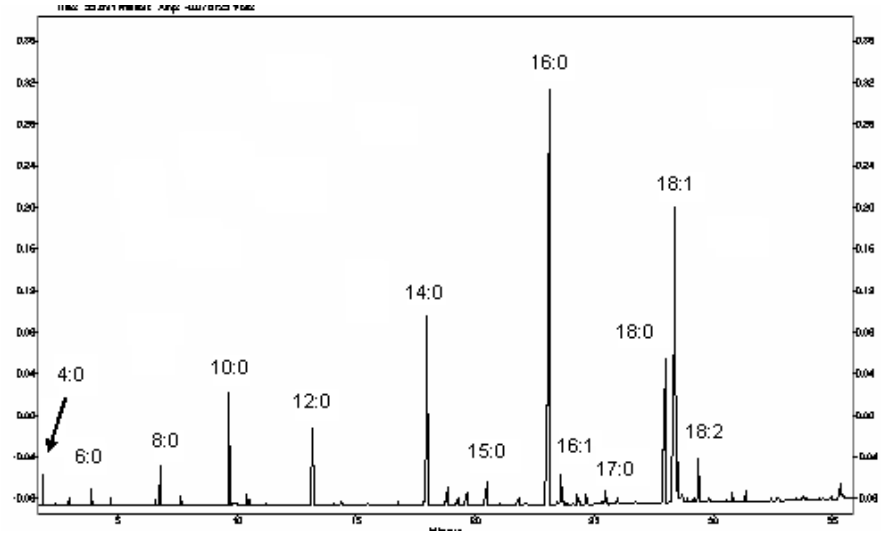

Figure 3. Chromatogram of fatty acids in "Năsal" cheese sampled after 60 days of ripening

After applying the Fisher test on the fatty acids values obtained we noticed that the concentrations of butyric, caprilic, lauric and miristic acids remain aproximatly constant within the 20 days of ripening but increase significantly until day 60; the concentration of caproic, capric, linoleic and linolein did not increase in values significantly only starting from the second half of the ripening process (day 40).

The concentrations of C12:0 increased significantly within the first period of ripening but by the end the values lowered; although not in a very significant way, the concentrations of C18:1 increased slowly and constantly within the entire ripening period. As seen in table 2, there were significant differences between the fatty acids profile from "Năsal" cheese compared to the not ripened type of cheese (fresh cow cheese).

Although the lactic microflora has a relatively low lipolysis activity, it can hydrolyse milk fat in a significant way during the ripening period, especially if the bacterial level is high [19; 20]. The significant load of *Brevibacterium linens* in „Năsal" cheese gives the particular taste and consistency of this product; also, it might explain the high levels of fatty acids at the end of ripening stage.

The release of C16:0, C18:0, C18:1 was considerable starting from day 20 of ripening probably due to the indigenous lipases from ruminant's milk (not sufficiently characterized until now). Normally, the research concerning the lipolysis in cheese are focused on actual measured fatty acids during a certain period of ripening (which represents an indicator of lipolysis extend); however, supplementary information on the activity of lipases can be extracted from the effective rate of fatty acids release (estimated as being the rapport between the quantity of fatty acids in a certain period of time and the effective period).

CONCLUSIONS

We concluded that "Năsal" cheese has a high nutritional value mainly due to its unique processing technology. The ripening period applied influences the majority of the compounds in a positive way, increasing in quantities. This fact lead us to the idea that the ripening process determines a higher lipolytic and proteolytic activity and that not only the actual process holds importance but also the extent of it.

EXPERIMENTAL SECTION

Sample collection

The study focused on 20 samples of "Năsal" cheese taken from various stages of the ripening process as follows: day 0 meaning before the curd is prepared for ripening; day 20 (stage I) of ripening; day 40 (stage II) of ripening; day 60 (stage III) of ripening. The sample gathering started in the month of February and ended in May. They were kept at refrigeration temperatures (0…+4°C) until their further analysis. For comparison 10 samples of fresh cow cheese (not processed through ripening) were analysed for compositional analysis through the same methods as "Năsal" cheese samples.

The analysis of compositional parameters

All the compositional parameters analysed, such as fat %, protein %, dry matter %, were measured by Infrared Spectrometry using the FoodScan™ (Foss, Germany) apparatus. The instructions given by the producer were followed accordingly.

Dairy product fat extraction

Dairy fat was extracted by using the following protocol: About 0.5g of dairy product were mixed in a separator funnel with 50 ml chloroform and 25 ml of methanol and then agitated for 2-3 min. After this process the lower layer was discarded. Following this step the mixture was passed through a cellulose filter with Na_2SO_4 and then brought to dryness.

Transesterification

Fatty acids were converted to methyl esters by reaction with boron trifluoride/methanol at 80ºC for two hours in a closed Pyrex glass tube. The content was transferred into a separator funnel.

The methyl ester extraction

The extraction was made using 10 ml hexane. The hexanic fractions collected were dried using anhydrous sodium sulfate, filtered, concentrated under a nitrogen stream and finally re-eluted in 1 mL hexane. Fatty acids were analysed by gas chromatography (GC) with flame ionization detection (FID). A 1µL sample was injected into the Shimadzu GC-17A series gas-chromatograph, equipped with a 30m polyethylene glycol coated column (Alltech AT-WAX, 0.25mm I.D., 0.25µm film thickness). Helium was used as the carrier gas at a pressure of 147 kPa. The injector and detector temperatures were set at 260ºC. For the oven temperature the following program was used: 70ºC for 2 min. then raised to 150ºC at 10ºC/ min. rate and held at 150ºC for 3min., then further raised up to 235ºC at a 4ºC/min.

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