

## Study of free fatty acid profiles in Brie type cheese

### Estudio del perfil de ácidos grasos libres en queso tipo Brie

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#### Resumen

*En este estudio experimental, se empleó la Regresión Logística Binomial para el modelado de la aceptabilidad global del consumidor y el análisis de Varianza para el estudio de los Perfiles de Ácidos Grasos Libres (AGL) en quesos tipo Brie elaborados con distintos contenidos de grasa (3,1%; 4,0%, y 4,5%) y tiempos de maduración (8, 16 y 25 días). Estos quesos fueron sometidos a un análisis sensorial con 35 panelistas semientrenados para determinar la aceptabilidad global y un análisis del grado de lipólisis mediante la determinación del contenido de AGL por cromatografía de gases, utilizando una columna capilar de sílice, previa extracción con éter/heptano (1:1) y separación en columna CP SIL 8 de poco sangrado para masas. Los resultados mostraron que estos quesos se caracterizan por presentar, bajos contenidos de AGL de cadena cortas y medias de C4:0 a C12:0 (menor a 320 mg/Kg queso) destacando el alto contenidos de C4:0 (hasta 178,04 mg/Kg de queso), y alto contenido de AGL de cadenas largas desde C14:0 hasta C18:1 (mayor a 1900 mg/Kg queso), resaltando por su alto contenidos el C18:1 (superior a los 3000 mg/Kg queso). Los AGL de cadena larga mostraron una tendencia creciente hasta los 16 días y luego una disminución al final de la maduración. Cuando el contenido de estos ácidos grasos es muy bajo (menos de 750mg/Kg queso) producto de una lipólisis avanzada, los quesos resultaron desagradables, contrastando con los quesos de mayor aceptación que mostraron altos contenidos de AGL de cadenas largas. Esto se podría lograr a nivel de planta, elaborando quesos con bajos niveles de grasa en la leche y largos tiempos de maduración, en condiciones controladas, o altos contenidos de grasa, pero con poco tiempo de maduración.*

**Palabras clave:** Ácidos Grasos Libres, Brie, lipólisis, análisis sensorial, *Penicillium candidum*.

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#### Abstract

*In this research, the analysis of the degree of lipolysis and the modeling of the global acceptability of the consumer was carried out based on the fat content of the milk and the maturation time of the Brie type cheese. The research is quantitative, with an experimental design, in which Binomial Logistic Regression was used to model global consumer acceptability and Variance Analysis to study the Free Fatty Acid Profiles (FFA) in cheeses. Brie type made with different fat contents (3.1%, 4.0%, and 4.5%) and maturation times (8, 16 and 25 days). These cheeses were subjected to a sensory analysis with 35 semi-trained panelists to determine overall acceptability and an analysis of the degree of lipolysis by determining the FFA content by gas chromatography using a silica capillary column, after extraction with ether/heptane (1:1) and separation in CP SIL 8 column. The results of the research showed that these cheeses are characterized by presenting low contents of short and medium chain FFA from C4:0 to C12:0 (less than 320 mg/Kg cheese), highlighting the high contents of C4:0 (up to 178.04 mg/Kg of cheese), and a high content of long-chain FFA from C14:0 to C18:1 (greater than 1900 mg/Kg cheese), highlighting C18:1 for its high content (greater than 3000 mg/Kg cheese). Long-chain FFA showed an increasing trend until 16 days and then experienced a decrease at the end of ripening. When the content of these fatty acids is very low (less than 750 mg/kg cheese) as a result of advanced lipolysis, the cheeses were unpleasant, contrasting with the more widely accepted cheeses that showed high contents of long chain FFA. This could be achieved at the plant level, making cheeses with low levels of milk fat and long maturation times, under controlled conditions, or high fat contents but with a short maturation time.*

**Keywords:** free fatty acid, brie cheese, lipolysis, sensory analysis, *Penicillium candidum*.

## 1 Introduction

Brie is a cheese of French origin that is distinguished by its texture and soft consistency resulting from the effect of the growth of the *Penicillium candidum* mold in its rind. The preparation is similar to that of Camembert cheese, but larger molds are used.

Lipids are the most important components of the technological and nutritional quality of milk and its derivatives, being involved in the firmness, color, flavor and performance of cheeses (Chilliard et al., 2003). During ripening, lipolysis makes the greatest contribution to flavor development in strong cheeses such as those ripened by molds (Leclercq, 2011; Marshall, 1992). The short and medium chains of free fatty acids together with the volatile fractions seem to contribute to the development of the typical lipolytic flavor of these cheeses. However, excessive lipolysis can produce unpleasant odors and flavors (Pinho et al. 2003).

The most important chemical and physical changes for the development of the organoleptic quality of cheeses occur during their ripening. This can be followed through the sensory evaluation of smell, flavor or texture among other parameters, but the difficulty of having a panel of expert tasters, as well as the inability to analyze a wide range of samples without fatigue, make the scope very limited. So, the cheese industry is increasingly interested in the development of ripening and quality indices that complement sensory evaluation (Ibañez, Barcina, & Torres, 1994). For the evaluation of the degree of lipolysis, the most used indices are based on the measurement of the content of fatty acids released during ripening and for the classification of the sample based on organoleptic perception, the individual amounts of free fatty acids are estimated. (FFA) by instrumental techniques such as mass-coupled gas chromatography (GC/MS) (Fuentes Soriano, 2019; Panizzolo et al., 2011; Serhan et al., 2010; Sánchez, 2004)

In this article, the study of the effect of milk fat levels and cheese maturation time on the FFA profiles of Brie-type cheeses manufactured by the University Food Producer Lácteos Santa Rosa C.A, of the University of Los Andes with the purpose of improving the quality during the manufacture of these cheeses, allowing both the researcher and the producer to evaluate the response and sensitivity of the quality of the cheese to the different levels of the factors that were analyzed.

## 2 Theoretical Framework

### 2.1 Brie cheese

Mold-ripened cheeses, especially Camembert and Brie, are produced throughout the world and have gained great popularity in the last decade. In addition to the

unique taste, recent studies have revealed a beneficial effect of consuming Camembert cheese on human health, which suggests that fermented cheeses such as Camembert could be consumed daily without increased atherosclerosis and reduced nitrate activity that prevents conversion to toxic nitrite. (Adamska et al., 2017).

Brie cheese of French origin. It is obtained by inoculating cultures of the mold *Penicillium candidum* and the bacteria *Brevibacterium linens* at the time of acidification of the milk. These cheeses are distinguished by their texture and soft consistency. Curd is obtained by enzymatic coagulation with lactic acidification. Lactic culture is a combination of mesophilic and thermophilic ferments. *Penicillium candidum* has a high lipolytic activity which gives this type of cheese its characteristic flavor, it develops well at temperatures between 22 to 30°C, it needs high relative humidity (90%) and a pH of 5.3 to 7.8, tolerates salt concentrations of 0.5 to 1.5%, its activity decreasing from 1.5% and stopping at 4%. The maturation of these cheeses is generally carried out in chambers at 10/15 °C and relative humidity of 85 to 90%, where the fungi or white molds on the surface develop rapidly, growing from the outside to the inside of the mass (Battro, 2010 ; Leclercq, 2011; However, the optimal ripening conditions may vary in the Brie-type cheeses of each manufacturer, so the microflora developed in the different cheeses varies from one region to another.

### 2.2 Lipids

Lipids are the most important components of the technological and nutritional quality of milk and its derivatives. Specifically, the profile of fatty acids present in the lipid fraction modulates the physical properties of milk such as the melting point, crystallization and fractionation of fat, as well as the nutritional and organoleptic properties of the milk. milk and its derivatives (Chilliard et al., 2003).

The milk fat, which passes into the cheese with minimal losses, is essential for the adequate development of the organoleptic properties of the cheeses during their maturation, with the free fatty acid (FFA) profiles being responsible for their flavor. and aroma (Foda et al., 1974).

Cow's milk has a low concentration of short chain acids such as butyric (C4:0), caproic (C6:0), caprylic (C8:0) and capric (C10:0), however, it has high acid contents. medium chain such as lauric (C12:0), myristic (C14:0), palmitic (C16:0) and long chain such as linoleic (C18:2), stearic (C18:0) and oleic (C18:1). (De La Torre et al., 2018).

In cheese, it is considered that FFA with chains greater than 12 carbon atoms play a minor role in flavor, due to their high perception thresholds, while those with short and medium chains (C4:0-C12:0) have lower perception thresholds and each contributes a characteristic flavor note. Bu-

tanoic acid contributes to the "rancid" and "cheesy" flavor, hexanoic acid has "spicy" and "blue cheese" notes, octanoic acid has "wax", "soap", "goat", "goat" notes, "musty", "stale" and "fruity". Depending on their concentration and perception threshold, volatile fatty acids can contribute positively to cheese aroma or to a rancidity defect (Molimar and Spinnler, 1996).

The importance of butanoic acid in the flavor of Camembert cheese was indicated by the preparation of a Camembert-flavoured cheese base, which contained a mixture of butanoic acid, methyl ketones, alcohols (e.g. oct-1-en-3-ol) and other compounds (Spinnler et al., 1992).

### 2.3 The ripening

Cheese ripening takes place through a long series of primary enzymatic reactions through which the components of fresh, concentrated and preserved milk are transformed into cheese. In specific varieties ripened by molds such as Camembert and Brie, fat can be a decisive factor, which is degraded into fatty acids by the action of several lipases (esterases) that can subsequently be transformed by various enzymes into aromatic components. (Calzada et al., 2014; Leclercq, 2011; Battro, 2010).

Lipolysis makes the greatest contribution, direct and indirect, to the development of flavor in strong cheeses such as hard Italian cheeses, mold-ripened varieties and cheeses. The greatest flavor effect due to lipolytic activity is due to the short chains that form free fatty acids (FFA), especially butyric, caproic and caprylic acids, which give strong and characteristic flavors. However, long chain free fatty acids can be degraded into various aldehydes, alcohols and keto acids, which in turn give characteristic cheese flavors, such as those produced by the ketones 2-heptanone, 2-nonanone and 2-butanone, with aromas especially known in blue cheeses (Calzada et al., 2014; Leclercq, 2011; Battro, 2010).

### 2.4 Lipolysis indices

The evaluation of the maturation process is carried out using different indices, depending on the biochemical process of interest. The FFA resulting from lipolysis are concentrated in the fatty phase, although a certain proportion of the short-chain ones can pass into the aqueous phase along with other organic acids, especially lactic acid, which sometimes interfere with the determination of fat FFA. The measurement, not only of the overall content, but also of the FFA profile of dairy products, is of great help for understanding their properties. Global methods for determining FFA only allow estimating the degree of lipolysis. However, to establish the classification of the sample based on organoleptic perception, it is necessary to estimate the quantities of individual fatty acids by instrumental techniques such as high-performance liquid chromatography (HPLC), capillary electrophoresis. (CE), nuclear magnetic

resonance (NMR) and mass-coupled gas chromatography, which is the most used technique (Fuentes Soriano, 2019; Panizzolo et al., 2011; Serhan et al., 2010; Sánchez, 2004).

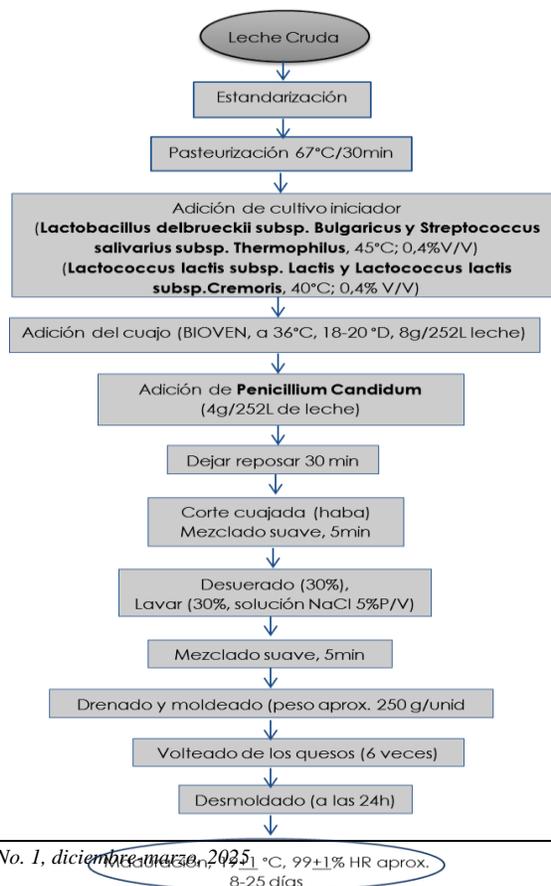
Free fatty acids must be present within an optimal concentration level, avoiding excessive amounts that could cause hydrolytic rancidity and unpleasant flavors. Among the factors that influence the release of FFA, the physical-chemical conditions of the cheese should be highlighted, especially pH, acidity, chlorides and proteins, microbiological conditions, prior treatment, and maintenance temperatures (Sánchez, 2004; De La Fuente and Juárez, 1993; Marshall, 1992).

## 3 Experimental procedure

### 3.1 Cheese making

Cheeses were made by hand from the milk of milking cows at the Santa Rosa Dairy Plant, according to the process indicated in Figure 1.

5 batches of cheese were made with the same raw milk, which was standardized for 5 fat contents (3.1%; 3.5%; 4.0%, 4.3% and 4.5%), by means of addition of heavy cream extracted from the same milk of the day. The cheeses from each batch were left to mature for 8, 12, 16, 22 and 25 days in the ripening cellars at 19+1 °C and 99+1% relative humidity (see figure 1). Once the respective period had expired, samples of the cheeses were taken for physicochemical and sensory analyses, as indicated in the experimental design.



binomial in the 3 experiments that were conducted. The second group contains the remaining scores.

**Table 1** Composition and pH of experimental and imported Brie cheeses

Parameters	3.1% Fat			4% Fat			4.5% Fat			Brie imported
	8 d	16 d	25 d	8 d	16 d	25 d	8d	16 d	25 d	
<b>Solids %</b>	47.8 <sup>a</sup>	48.1 <sup>a</sup>	50.9 <sup>a</sup>	45.3 <sup>a</sup>	50.9 <sup>a</sup>	45.8 <sup>a</sup>	54.2 <sup>a</sup>	47.8 <sup>a</sup>	52.2 <sup>a</sup>	47.1
<b>NaCl %</b>	2.1 <sup>ab</sup>	2.3 <sup>ac</sup>	2.0 <sup>ad</sup>	2.3 <sup>ba</sup>	2.1 <sup>bc</sup>	3.5 <sup>bd</sup>	3.0 <sup>ca</sup>	2.6 <sup>cb</sup>	2.0 <sup>cd</sup>	1.5
<b>Fat %</b>	46.0 <sup>ab</sup>	51.0 <sup>ac</sup>	49.0 <sup>ad</sup>	51.0 <sup>ba</sup>	50.0 <sup>bc</sup>	59.0 <sup>bd</sup>	46.0 <sup>ca</sup>	56.0 <sup>cb</sup>	59.0 <sup>cd</sup>	47.0
<b>Protein %</b>	47.6 <sup>a</sup>	45.9 <sup>a</sup>	42.3 <sup>a</sup>	42.5 <sup>a,b</sup>	38.9 <sup>a,b</sup>	38.4 <sup>a,b</sup>	37.5 <sup>b</sup>	41.6 <sup>b</sup>	38.6 <sup>b</sup>	43.3
<b>pH</b>	5.4 <sup>ab</sup>	5.8 <sup>ac</sup>	5.2 <sup>ad</sup>	5.4 <sup>ba</sup>	5.8 <sup>bc</sup>	5.6 <sup>bd</sup>	5.2 <sup>ca</sup>	6.0 <sup>cb</sup>	5.8 <sup>cd</sup>	5.4

Protein, fat and NaCl content are reported on a dry basis.

**a,b,c:** Different letters in the same row indicate significant differences at a 1% significance level, for the fat factor (only in the percentage of protein) and the fat-time interaction term (for the content of solids, NaCl, fat and pH).

**Fig. 1** Artisanal production process of experimental cheeses.

### 3.2 Chemical Analysis

The samples were analyzed for their fat content (Babcock), protein (Kjeldahl), total solids (drying in an oven at 100°C), sodium chloride (Titration with AgNO<sub>3</sub>) and pH, following the methodology described by Marshall (1992). The acid degree value (VDA) was determined in samples prepared by homogenizing 7g of cheese in 15mL of a 2% sodium citrate solution and then following the procedure described by Marshall R. (1992) for milk. All data were subjected to an analysis of variance to determine if there are significant differences between the different levels of the study factors using the SPSS version 25 program.

### 3.3 Sensory evaluation

The degree of consumer acceptability was evaluated at 8, 16 and 22 days of ripening by 35 semi-trained panelists, following a factorial design. To measure this variable, an instrument with an ordinal scale was used that included the following response categories for the degree of acceptability:

I really dislike it = 1; I dislike it a little = 2; I neither like it nor dislike it =3; I like it a little = 4; I like it a lot = 5.

A Logistic Regression analysis for the binomial case was performed on the data obtained from the tasting of the experimental cheeses using the statistical package SAS version 9. The design included 16 sample points of combinations of fat levels and ripening time. The 5 scores were grouped into 2 groups, such that the first was formed by the first three score values (1, 2, and 3), which was used as the event of interest and was modeled by Logistic Regression.

### 3.4. Determination of Free Fatty Acids (FFA).

Free fatty acid profiles from C4 to C18:1 were determined by gas chromatography coupled with mass spectrometer (GC/MS). The extractions and separations of the FFA were carried out according to the method described by Sánchez María (2004) and the chromatographic profiles obtained were subjected to a two-factor multiple variance analysis to determine the variation of the contents of the FFA with the fat content of the milk and the maturation time of the cheese. For this study, the SPSS version 25 program was used.

#### 3.4.1 Extraction of lipids from cheese.

2 g of grated cheese was mixed with 6 g of hydrated sodium sulfate to absorb moisture and 0.6 mL of 2.5 mol/L sulfuric acid. The lipids were extracted with 6 mL of ether/heptane (1:1 v/v) in a 75 mL centrifuge tube by centrifugation at 2,500 rpm/min for 2 min. This extraction was repeated twice by adding the same amount of ether/heptane (1:1 v/v) to the residue. The extracts from the three extractions were mixed.

#### 3.4.2 Separation of the FFA.

Pelargonic acid (C9) was used as an internal standard (1mL of C9 with a concentration of 0.5mg/mL), which was added to the lipid extract before proceeding to isolate the free fatty acids. For this purpose, 3 mL aminopropyl columns (Supelclean LC-NH<sub>2</sub> from Supelco, Inc) conditioned with 2 mL of heptane were used. The total volume of the extract was applied to the column with positive pressure. Neutral lipids were eluted with 3 mL of 2:1 v/v chloroform/2-propanol. Next, the free fatty acids ad-

sorbed on the column were eluted with 3 mL of diethyl ether with 2% formic acid. The first mL was discarded because it did not contain free fatty acids. Of the next 2 mL eluted, with all the FFA, 1  $\mu$ L was injected into the chromatograph for determination. Four extractions were made from each cheese sample and each extraction was injected in duplicate into the chromatograph.

### 3.4.3 Quantification of the FFA.

For the quantification of FFA, a Varian 3400 gas chromatograph coupled to a Saturn 2000 mass detector and a CP SIL 8 low-bleed column was used for masses of 30 m in length, 0.25 mm in internal diameter and 0.25  $\mu$ m film thickness, calibrated with standard solutions of the fatty acids to be analyzed (Sigma). Direct injection was adapted due to the low concentrations of the fatty acids. Quantification was carried out by relating the areas of the peaks to the areas of the internal standard (C9) using an integrator. The operating conditions in the chromatograph were: It started at 100 °C for 4 min, then with a ramp of 10 °C/min it was taken to 150 °C, left for 10 min and then with a ramp of 15 °C/min was brought to 240 °C for 15 min, using helium as carrier gas with a flow of 1.0 ml/min.

## 4 Discussion and Results

### 4.1 Composition and pH of cheeses

Table 1 shows the analysis of variance for the compositions and pH of the experimental and imported Brie-type cheeses, indicating that the fat content of the milk has a significant influence with a significance level of 0.01 in the protein content of cheese, which decreases as the percentage of fat in milk increases. Likewise, we can observe that the fat-time interaction term interacts significantly with a significance level of 0.01 in the chloride, fat and pH contents of the experimental cheeses, which indicates that these factors do not act individually, but in an associated manner on the analyzed parameters, which show for all the fat contents of the milk a tendency to increase up to 16 days and then decrease towards the end of maturation. In the

same table it is observed that the content of total solids, chlorides, fat, protein and pH of the imported cheese acquired in the local market is within the ranges reported for the experimental cheeses, indicating that the formulations of these cheeses are like the imported Brie cheese used as a reference.

**Table 2** Acid degree value (VDA) and pH of the Brie type cheeses that obtained the highest and lowest scores in the tasting panel

Factors Parameter	Highest score		Lowest score
	3.1% fat	4.5% fat	4% fat
	16 d	8 d	25d
pH	5.8	5.2	5.6
VDA (meq KOH /100 g fat)	0.8	2.5	2.9

### 4.2 Sensory evaluation

Table 2 shows the VDA and pH values for the cheeses that achieved the highest scores through the tasting experiment modeled by Binomial Logistic Regression (3.1% fat and 16 days, and 4.5% of fat with 8 days of maturation), with these values the optimal region was established where the VDA levels belonging to the cheeses chosen by the tasters are found. The preference range was in the region of medium to low VDA values (between 0.8-2.5 meq of KOH/100 g of fat and pH between 5.2 and 5.8), cheeses with Very low or high values were considered unpleasant or even unpleasant by the evaluation panel, resulting in the lowest scores for the cheese with 4.0% fat and 25 days of maturation with a VDA of 2.9 meq of KOH /100 g of fat and pH of 5.6 and imported Brie cheese used as reference.

When comparing the preference range with that reported by Marshall R. (1992), it can be considered that these cheeses present a normal to extreme hydrolysis, which added to their high pH values (between 5.2-5.8) allow advanced hydrolysis to be tolerated without detecting unpleasant flavors, which coincides with what was reported by De La Fuente and Juárez (1993) who attribute a limit value between 1.3-1.5 meq of KOH/100 g of fat, above which rancidity defects are detected in cheeses, however,

**Table 3** Fatty Acid Profile (mg/Kg of cheese)

G (%)	3.1	3.1	3.1	4.0	4.0	4.0	4.5	4.5	4.5	
M (days)	8	16	25	8	16	25	8	16	25	Imported
C4:0	84.46 <sup>a</sup>	148.57 <sup>ab</sup>	99.11 <sup>b</sup>	121.18 <sup>c</sup>	178.04 <sup>cd</sup>	17.82 <sup>d</sup>	122.08 <sup>e</sup>	105.09 <sup>ef</sup>	115.86 <sup>f</sup>	5.47
C6:0	48.54	67.85	63.49	62.16	67.61	45.50	60.23	59.38	55.94	43.78
C8:0*	0.76 <sup>a</sup>	2.36 <sup>ab</sup>	2.08 <sup>b</sup>	1.17 <sup>c</sup>	3.00 <sup>cd</sup>	1.27 <sup>d</sup>	1.90 <sup>e</sup>	4.09 <sup>ef</sup>	3.06 <sup>f</sup>	0.77
C10:0	64.07 <sup>a</sup>	82.79 <sup>ab</sup>	80.68 <sup>b</sup>	70.33 <sup>c</sup>	96.37 <sup>cd</sup>	59.67 <sup>d</sup>	71.95 <sup>e</sup>	108.83 <sup>ef</sup>	93.88 <sup>f</sup>	60.41
C12:0	45.46 <sup>a</sup>	-	68.62 <sup>b</sup>	57.68 <sup>c</sup>	-	36.15 <sup>d</sup>	-	60.47 <sup>ef</sup>	95.82 <sup>f</sup>	39.35
C14:0	149.03 <sup>a</sup>	346.08 <sup>ab</sup>	268.15 <sup>b</sup>	222.50 <sup>c</sup>	517.63 <sup>cd</sup>	41.71 <sup>d</sup>	169.74 <sup>e</sup>	570.23 <sup>ef</sup>	445.84 <sup>f</sup>	77.86
C16:0*	192.30 <sup>a</sup>	334.35 <sup>ab</sup>	279.20 <sup>b</sup>	237.83 <sup>c</sup>	500.46 <sup>cd</sup>	109.67 <sup>d</sup>	272.12 <sup>e</sup>	652.29 <sup>ef</sup>	543.79 <sup>f</sup>	171.64
C18:0	1017.60 <sup>a</sup>	593.85 <sup>ab</sup>	641.24 <sup>b</sup>	556.72 <sup>c</sup>	1376.25 <sup>cd</sup>	38.55 <sup>d</sup>	206.53 <sup>e</sup>	781.10 <sup>ef</sup>	1574.56 <sup>f</sup>	235.13
C18:1*	3978.91 <sup>a</sup>	3228.62 <sup>ab</sup>	3278.79 <sup>b</sup>	1738.70 <sup>c</sup>	4739.77 <sup>cd</sup>	763.46 <sup>d</sup>	1962.69 <sup>e</sup>	4420.61 <sup>ef</sup>	2887.19 <sup>f</sup>	494.44

Different letters in the same row indicate significant differences at a 5% significance level, for the time factor and the fat-time interaction term.

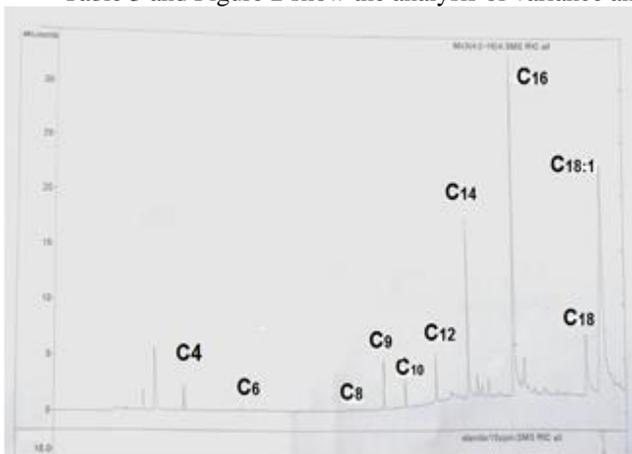
\*There are only significant differences at a 5% significance level, for the time factor.

the same author indicates that pH values play an important role in the organoleptic perception of rancidity, since As the pH increases, the detection threshold is located at higher concentrations of FFA. For experimental cheeses, this limit value would be located at 2.5 meq of KOH/100 g of fat with pH values between 5.2 and 5.8.

**Fig. 2.** Chromatogram of a replica of the experimental Brie type cheese with 4.5% fat and 16 days of maturation

#### 4.3 Quantification of the FFA.

Table 3 and Figure 2 show the analysis of variance and



a chromatogram of the FFA of the experimental Brie-type cheeses, where the presence of saturated FFA from C4 to C18 can be verified, as well as the mono-unsaturated C18:1, observing the predominance of high molecular weight FFA from C14 to C18:1. The analysis shows that for the time factor and the fat-time interaction term there are significant differences at a 5% significance level for all chains except for caprylic (C8), palmitic (C16) acids. ) and oleic acid (C18:1) which are only significant for the time factor and caproic acid (C6) where the factors of interest are not significant at a 5% significance level. This indicates that the maturation time produces an effect on most of the FFA chains and that the fat content of the milk has an effect that acts associated with the maturation time of the cheese and that cannot be analyzed separately.

In the same table we can see that comparing the short and medium chain FFA contents (from C4 to C12) for the experimental cheeses and an imported Brie type cheese obtained in the local market, the high content of butanoic acid (C4) stands out for most of the cheeses, except for the cheese with 4.0% fat and 25 days of maturation and the imported cheese which were considered unpleasant by the evaluation panel. This observation confirms the importance of butanoic acid in the flavor of Brie cheese, which coincides with what was reported by Woo and Lindsay (1984) for Camembert cheese.

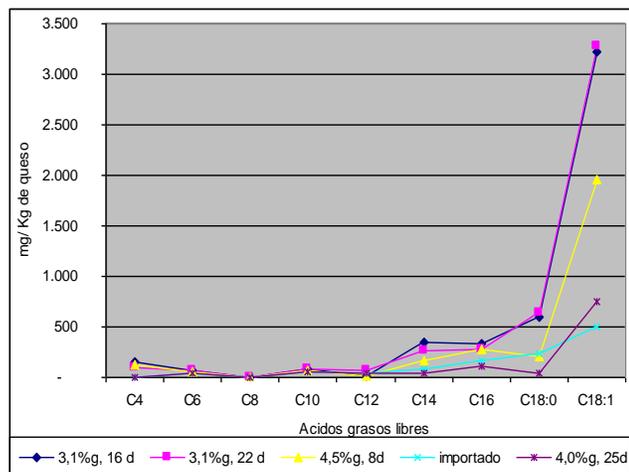
Table 3 also shows that the trend of FFA content with

maturation time is to grow up to 16 days and then decrease at the end of maturation. This is since FFA molecules also act as precursors for a series of catabolic reactions that lead to the production of flavor and aroma components such as methyl ketones, which are an important product of the catabolism of FFA by the action of lipases from the mold *Penicillium candidum* following the so-called  $\beta$ -oxidation pathway (Kinsella and Hwang, 1976).

Figure 3 shows the FFA profiles of the cheeses with the highest and lowest preference next to the imported Brie type cheese. If we compare these profiles with those reported by De La Torre et al. (2018) for bovine milk, it is observed that there is a higher proportion of C14 to C18:1 and that it is like that of the FFA that form the triglycerides of milk. This coincides with what was reported by Acuña et al. (2001), according to which Camembert and Brie cheeses have a high content of acids from C14 to C18, coming from a net lipolysis of milk fat. This behavior is also observed in some types of short-ripened cheese (Dambo, Gruyere, Munster), in which the FFA content comes almost exclusively from the hydrolysis of milk fat.

**Fig. 3.** Comparison of the FFA profiles of the most and least preferred cheeses, along with imported cheese.

From figure 3 we can also observe that for the long chains starting with 14 carbon atoms the curves begin to separate, highlighting the profile of the imported cheese



that is located below all of them, also being the most correct. ta, with a very low level of C18:1 (between 500-750 mg/Kg cheese), which shows a very advanced lipolysis with high levels of secondary metabolites product of the metabolism of chain FFA long. This profile is very similar to that of the cheese with 4.0% milk fat and 25 days of maturation that obtained a very low global acceptability score, similar to the imported one with VDA values above the limit value being considered. unpleasant by the tasting panel. This is because when lipolysis is very advanced and FFA metabolism is high, the levels of medium and long chain acids (from C14:0 to C18:1) decrease until they reach

a level below which the cheese is unpleasant. This observation agrees with what Mulder (1952) stated, according to which cheese is the product of a balance of flavors formed by different compounds that must be present at certain levels and in the correct balance, to produce the typical flavor of a variety. cheese.

From the FFA profiles of the cheeses that were most preferred in the sensory evaluation. It is clearly observed in Figure 3 that these cheeses present a very similar profile, with low FFA contents from C4:0 to C12:0 (less than 320 mg/Kg cheese), and a high level of long chains from C14: 0 to C18:1 (greater than 1900 mg/Kg cheese), highlighting the high contents of C18:1 (greater than 3000 mg/Kg cheese), which shows an advanced maturation but with low presence of catabolic reactions of long-chain FFA. This could be achieved, based on the behavior observed for the individual contents of the FFA, making cheeses with low levels of fat in the milk and long maturation times, under controlled conditions, or high fat content but with a short maturation time. The latter is of special interest to Brie-type cheese producers.

## 5 Conclusions

The studies carried out for the Brie type cheese manufactured by the University Food Producer Lácteos Santa Rosa A.C of the University of Los Andes, showed that the limit level above which these cheeses are considered unpleasant would be located at 2, 5 meq of KOH /100 g of fat with pH values between 5.2 and 5.8. Likewise, it was proven that these cheeses have a high content of butanoic acid (C4) and long-chain FFA from C14 to C18:1, the latter coming from a net lipolysis of milk fat, which grow up to 16 days and then experience a decrease at the end of ripening due to the catabolic reactions that use these FFA in the production of flavor and aroma components. When the decrease in these fatty acids is very great as a result of advanced lipolysis, cheeses become unpleasant. On the contrary, those that showed greater acceptance show high levels of high molecular weight FFA.

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