

# IDENTIFICATION OF VOLATILE COMPOUNDS, FREE AMINO ACIDS THROUGH CHROMATOGRAPHY AND SENSORY PROPERTIES OF THE PASTA FILATA CHEESES KNOWN AS MOMPOSINO

## IDENTIFICACIÓN DE COMPUESTOS VOLÁTILES, AMINOÁCIDOS LIBRES POR CROMATOGRAFÍA Y LAS PROPIEDADES SENSORIALES DE LOS QUESOS DE PASTA HILADA CONOCIDO COMO MOMPOSINO

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### ABSTRACT

The cheese known as Momposino is a Colombian variety of pasta filata cheese. For this investigation, several cheese-production technologies were identified as follows: techniques 1 and 2, for cheeses elaborated from raw milk (such as autochthonous cheeses): technique 3, for cheeses made with pasteurized milk and with a culture; and technique 4, for cheeses made with raw milk. Volatile compounds and free amino acids were examined through chromatography and sensory evaluation tests performed by the members of laboratory. Cheeses made with techniques 1 and 2 presented more volatile compounds (with 60 and 73 respectively) than the cheeses made with technique 3 (for which 52 volatile compounds were found), and the ones made with technique 4 (which presented 40 volatile compounds). The fat content for cheeses from Technique 1 was 29.8%, 30% for cheeses from Technique 2, 27% for cheeses from Technique 3, and 25% for the ones made with Technique 4. These results lead to conclude that the higher the fat content is, the higher the volatile compound production will be. The volatile profiles that were found include acids, alcohols, hydrocarbons and esters. Through the Thin Layer Chromatography test, it was found that cheese proteins spread to the following amino acids: arginine, valine, tryptophan, histidine, threonine, lysine, tyrosine, alanine, and cysteine. In the cheeses from all treatments, the most outstanding flavor was the acid flavor, and in the most outstanding texture was the elastic texture. No significant differences were found in the sensory evaluation, for the free amino acids ( $p > 0.05$ ), but free fatty acids and fat did show significant differences ( $p < 0.05$ ).

**Keywords:** Momposino autochthonous cheese, momposino type cheeses, bacteria, amino acids, sensory evaluation.

### RESUMEN

El queso Momposino es una variedad de queso de pasta hilada que se encuentra comúnmente en Colombia. Para esta investigación fueron identificadas varias tecnologías de producción de queso de la siguiente manera: técnicas 1 y 2, para quesos elaborados de leche cruda (tales como quesos autóctonos), técnica 3, para quesos hechos con leche pasteurizada y con un cultivo; y técnica 4, para quesos hechos con leche cruda. Los compuestos volátiles y aminoácidos libres fueron examinados por cromatografía y la evaluación sensorial desempeñada por los miembros del laboratorio. Los quesos hechos con las técnicas

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1 y 2 presentaron más compuestos volátiles (con 60 y 73 respectivamente) que los quesos hechos con la técnica 3 (para el cual 52 compuestos volátiles fueron encontrados), y los hechos con la técnica 4 (el cual presentó 40 compuestos volátiles). El contenido de grasa para la técnica 1 fue 29,8%, 30% para quesos de la técnica 2, 27% para quesos de la técnica 3, y 25% para los hechos con la técnica 4. Estos resultados llevan a concluir que en el más alto contenido de grasa será la más alta producción de compuestos volátiles. Los perfiles volátiles que fueron encontrados incluyen ácidos, alcoholes, hidrocarburos y ésteres. Por la prueba de Cromatografía en Capa Fina, se encontró que las proteínas de los quesos se dividían en los siguientes aminoácidos: arginina, valina, triptófano, histidina, treonina, lisina, tirosina, alanina y cisteína. En todos los tratamientos de los quesos, el sabor más destacado fue el sabor ácido, y la textura más destacada fue la textura elástica. No fueron encontradas diferencias significativas en la evaluación sensorial, para los aminoácidos libres ( $p > 0,05$ ), pero ácidos grasos libres y grasa mostró diferencias significativas ( $p < 0,05$ ).

**Palabras clave:** quesos autóctonos momposinos, quesos tipo momposinos, bacteria, aminoácidos, evaluación sensorial.

## INTRODUCTION

Momposino cheese is an artisanal cheese original from Mompos, Colombia. It is a pasta filata cheese elaborated with raw milk, which becomes acidified due to the lipolysis and proteolysis of the native acid-lactic bacteria present in the milk. A wide variety of cheeses around the world are made from raw milk, and present a better final flavour than the cheeses that are made from pasteurized milk due to the high level of lipolysis and proteolysis (1).

Lactic acid bacteria show different properties in the elaboration of cheeses, such as milk acidity and the yield of flavor, which is caused by the variety in the enzymatic activities and by the production of exopolisaccharide compounds (2) with *Streptococcus Termophilus* cultures for hard cheese varieties such as the Swiss cheese (3). An important contribution of starter cultures to the ripeness of cheese is their proper autolysis and releasing of enzymes that are usually peptidases or lipases, which are enzymes responsible for flavor development. During the ripening of hard cheeses, the proteinases of lactobacillus (*Lactobacillus delbrueckii ssp*) are very important for the breakage of casein. *Lactis* are frequently used in the elaboration of hard cheeses due to their balanced activity, compared with the traditionally used *Lactobacillus helveticus* (3).

Exopolysaccharides (EPS) that produce lactic acid bacteria (LAB) have been used in several dairy products. Most of the studies that involve this kind of bacteria have been performed in yogurt, obtaining several advantages such as an increase in the viscosity and a diminution of the product syneresis. Most of the research works that involved

cheeses made with EPS that produced cultures have been carried out with low fat mozzarella cheese (4).

The presence of volatile compounds and their relative concentrations determine the quality and typical flavor of each cheese variety. The main agents involved in cheese flavor formation are indigenous milk enzymes, rennet and microbial enzymes from wild microflora and/or from the commercial starter or adjunct cultures used (5).

The volatile fraction of the cheese and, consequently, its sensory characteristics are affected by climatic conditions and the quality of raw milk, which depends on the animal species, breeding, feeding and farming (6).

Several different studies have shown that the enzymatic system of the indigenous microflora in raw milk is considerably more complex than the one of the starter bacteria that is added to the milk during the cheese-making process. Therefore, this indigenous microflora has a strong influence in cheese proteolysis (7).

Milk pasteurization ensures a higher uniformity of the product and improves the sanitary conditions of cheesemaking, but it also eliminates some of the indigenous microbiota of milk, which is partly responsible for the development of typical cheese flavor (8).

Lipolysis is usually understood as the accumulation of FFA during the ripening process, with most of the free fatty acids (FFA) being released by triglycerides. Total FFA concentration and short/long-chain FFA ratio have been related to the type and the amount of lipase used during cheese ripening and to the sensory characteristics of cheese.

The characteristic flavor of some Mediterranean cheeses (such as Provolone, Romano, Parmesan, Idiazabal and white pickled cheese) is developed by adding pregastric lipase that contains lamb rennet paste. In the Roquefort cheese, the fungal lipase produced by *penicillium roqueforti* is necessary for the development of the characteristic flavor of this cheese variety. Lipolysis and  $\beta$ -oxidation, which are biochemical routes especially important in blue cheeses due to the activity of the thiol ester hydrolase for  $\beta$ -keto-acyl-CoA, are sources of compounds such as FFA and ketones (2-heptanone and other methyl ketones) (9). FFA are released upon lipolysis and they contribute directly to the development of cheese flavor, especially short and intermediate chain FFA. The proportions of free C6:0 to C18:3 in the Cheddar cheese appear to be similar to the ones in milk fat. However, free butanoic acid (C4:0) is present at a greater relative concentration in cheese than it is in milk fat, which suggests its selective release by the lipases that are present in cheese or its synthesis by the cheese microflora tri- or diacylglycerides (i.e., in the positions sn-1 and sn-3). Lipases in cheese originate from six possible sources: milk, rennet preparation (rennet paste), starter, adjunct starter, non-starter bacteria and possibly, their addition as exogenous lipases (10).

Lipolysis plays an important role in cheese ripening, especially in blue cheese varieties, and a large number of studies that deal with the acceleration of lipolysis via the addition of free lipolytic enzymes to either cheesemilk or curd have been published (11).

Proteolysis contribute to the flavor of cheeses by releasing peptides and amino acids, providing a substratum for the transamination, deshydrogenation, decarboxilation and reduction, and producing a wide variety of compounds of flavors, such as: acid fenilacetic, dimethyl disulfur, 3 metyl butirate, 3 metyl butanal, 3 metyl butanol, 2 metyl butirate y 2 metyl butanol, which are the main compounds of cheese flavor that have been identified (12).

The comparison of the effect of different coagulants on primary proteolysis could be developed through the urea-polyacrylamide gel electrophoresis (urea-PAGE) or the capillary electrophoresis (CE) of the pH 4.6-insoluble fraction (or cheese), followed by the electroblotting, sequencing and identification of the products of the primary proteolysis. The peptide profiles of the

pH 4.6-soluble fraction (or ethanol-insoluble and soluble fractions there from) should be determined through a reversed-phase high-performance liquid chromatography (RP-HPLC) (13).

Some researchers have reported that the freezing process did not affect the cheese proteolysis. The Mozzarella cheeses that underwent the freezing process before ripening showed no significant differences in the primary proteolysis due to the coagulant (14).

Cheese proteolysis, primarily of casein components, is considered to result from several proteinase and peptidase activities. The principal proteolytic agents in cheese are proteinases of rennet, indigenous milk proteinases (especially plasmin), and proteinases and peptidases from the starter. Non-starter and secondary starter bacteria in cheese are affected by the pH of the curd, salt-in-moisture content of the cheese, and ripening time. Primary proteolysis in cheese may be defined as the changes in  $\alpha$ 1- and  $\beta$ -caseins, and peptides, which can be detected through a PAGE technique. Lactic acid bacteria are weakly proteolytic, but they possess a considerably comprehensive proteinase / peptidase system, which is capable of hydrolysing the casein derived peptides into small peptides and amino acids (15).

The proteolysis in cheese during ripening is an important process, as it plays a direct role in the formation of cheese flavor and texture development for most cheese varieties. Proteinases and peptidases from different origins catalyze this process: residual coagulant, milk, starter and non-starter lactic acid bacteria, and adjunct cultures. The influence of probiotic lactobacilli on the Pategra cheese proteolysis was assessed. The impact of the probiotic culture changed from one probiotic strain to another and, in one case, it significantly modified the proteolytic pattern of the standard cheese. On the contrary, the methodology of culture addition did not affect the chemical composition of the cheese. The two strains of probiotic bacteria studied in this research work distinctly influencing the proteolysis pattern of semi-hard cheeses, probably as a consequence of their different proteolytic systems and their activity through the alimentary matrix. The observed effects were an increase in the production of short peptides and free amino acids (FAAs), as well as the modification of peptide profiles (16).

Free amino acid composition has been evaluated to serve as a typicality and quality index of several cheese varieties. The concentrations of the different amino acids in a cheese are related to the manufacturing technology (type of curd, addition of proteinases, starters, ripening conditions), the duration of ripening, and the extent and type of proteolysis (17).

The acceptance of cheeses depends on their appearance and sensorial properties (flavor, texture and color); but flavor is the most important attribute for the customer (18).

Among the principal cheese flavor compound formation pathways, amino acid catabolism by cheese-related microorganisms is a major process. For Swiss-type cheeses, such as Gr uyere and Emmental, the role of *Propionibacterium* ssp. and thermophilic lactic acid bacteria in the production of flavor compounds is beginning to be elucidated. The contribution of other microorganisms that are present in these cheeses and that are involved in the formation of aroma compounds, such as mesophilic lactobacilli and enterococci, appears to have been overlooked (19).

This investigation looks for alternative technologies for the reproduction and improvement of the study of this cheese, as well as all the technical and scientific information. The chromatographic research is very important because it allows determining volatile compounds from the Momposino cheese that have not been discovered yet, moreover, it compares the relationship between the fat and the volatile compounds that exist in the cheese.

The investigation objectives consisted in identifying the volatile compounds and the free amino acids, and to sensorially evaluate the cheeses by means of a panel of judges expert in flavor and texture.

## MATERIALS AND METHODS

### Milk, culture, rennet, whey

Four techniques were used in this research. 50 kg of milk were used in all the techniques and all the samples were stored in a refrigerator at 4 C until the moment of performing the treatments. The first technique was performed in Mompos, using raw milk from zebu cattle at an environment temperature of 36 C (treatment 1). The second technique was also performed in

Mompos, using raw milk from zebu cattle at an environment temperature of 39 C (treatment 2). The third technique was performed in the dairy plant of Universidad Nacional, sede Medellin, using pasteurized milk with culture from Holstein cattle and at an environment temperature of 24 C (treatment 3). The fourth technique was performed in the dairy plant of Universidad Nacional, sede Medellin, using raw milk from Holstein cattle and at a temperature of 24 C (treatment 4).



**Figure 1.** Transverse cut of the Momposino cheese shaped as a cabbage.

Figure 1 shows the autochthonous pasta filata cheese known as Momposino cheese. The image shows the cross section of the cheese, which has the shape of a cabbage in order to make it desirable; also, this was the same type of cheese that was given to the panelists. This shape is achieved by wrapping layer by layer, thus achieving a 500-gram ball of cheese.

### Methods

**Statistical analysis:** An experimental design lead to evaluate the influence of the four treatments on the characteristics of volatile compounds and cheese amino acids. Also, the sensorial evaluation was performed in duplicate and triplicate. The analysis of variance (ANOVA) was performed by means of the STATHGRAPHIC<sup>®</sup> CENTURION (VERSION XV) software.

**Analysis of free fat acids:** Fat acids were determined through a GC-MS with a ZB-35 column of 30 m of length at a temperature of 340 C. Each sample was finely grated, homogenized and sampled in triplicate. Also, each sample (6 g) was suspended in 12 mL of water in a 40 mL vial.





Table 1 shows the yield of compounds of acids, alcohols, esters, acetones and aromatic for each technique. Cheeses made with techniques 1 and 2 presented more volatile compounds (with 60 and 73 respectively) than the cheeses made with technique 3 (for which 52 volatile compounds were found), and the ones made with technique 4 (which presented 40 volatile compounds). The fat content for cheeses from Technique 1 was 29.8%, 30% for cheeses from Technique 2, 27% for cheeses from Technique 3, and 25% for the ones made with Technique 4. These results lead to conclude that the higher the fat content is, the higher the volatile compound production will be.

Hexadecanoic acid was produced in all treatments, and volatile acids (such as hexadecane, octadecene, and heptadecene) were found in Momposino autochthonous cheeses, as well as in cheeses elaborated with raw milk as it was reported by Barron *et al.*, 2007 (24).

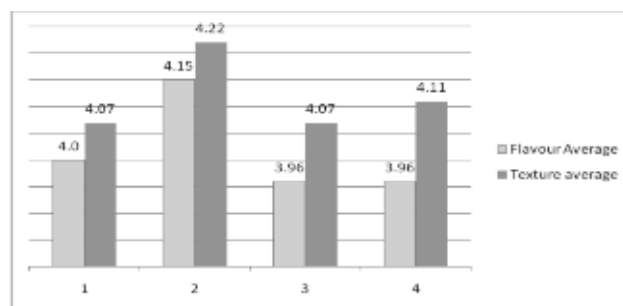
In the fontina cheese study, volatile compounds were analyzed and alcohols, esters, cetones, aldehydos, hidrocarburs, and organic acids were found. This study can be compared with the investigation of the Momposino cheeses, in which similar volatile compounds were found (25).

High concentrations of acetoin has been identified in cheeses elaborated with pasteurized milk in comparison with the ones elaborated with raw milk (26). Metyl cetones are considered to be derived from free fat acids, which are enzymatically oxidized into B-ceto- acids. Consequently, those products are descarboxils to alcans- 2 ones with the loss of an atom of carbon (27). Diacetyl is metabolized by the activity of the adventitious bacteria. The secondary or adventitious lactic flora, named NSLAB (abbreviation of non-starter lactic acid bacteria) spontaneously develops in all cheeses, with the purpose of industrially obtaining an environment. Such flora is mainly composed by *Lactobacillus casei*, *L. paracasei*, *L.rhamnosus*, and *L. plantaru*. (26, 27). It has also been stipulated that this flora can play an important role in the generation of flavor (28).

High levels of octane have been reported in the cheeses elaborated with raw milk (29), as well as in autochthonous Momposino cheeses. The following are the hydrocarbons found in the investigation: pentadecane, heptadecane, octadecane, ciclohexadecane, among others.

**Amino acid results:** The thin layer chromatography (TLC) is a technique used for separating mixtures (30), in this case amino acids in cheeses. This technique showed that proteins are divided into amino acids so that they can use the solvent that drags the amino acid by solubility. In the aminogram, RF measures the distance traveled by the amino acid according to the distance traveled by the solvent ( $RF = Dm / Df$ ). For this investigation, the solvent was propanol and ammonia in a 7:3 ratio. In Treatments 1 and 2, the amino acid tyrosine travelled the longest distance (0.96), which indicates that this amino acid is more soluble in the solvent. Arginine was the one that travelled the shortest distance (0.28), probably because it is less soluble in the solvent. In Treatments 3 and 4, cysteine travelled the longest distance (0.93), and lysine travelled the shortest distance (0.13). In conclusion, the samples showed all the amino patterns, which indicates that the protein is divided into these amino acids through proteolysis. Samples are rising in the aminogram as an electrophoresis and some have more vivid colors than others. In Treatments 1 and 2, the colors are all pink; and in Treatments 3 and 4, the colors are all yellowish pink, due to the reaction of amino acids and ninhydrin.

### Qualification average of flavor and texture



**Figure 3.** Qualification average of flavor and texture by 9 panelists.

Figure 3 shows that the highest value in flavor and texture are found in Treatment 2 with 4.16 for flavor, and 4.22 for texture in the autochthonous cheese. These values correspond to a good flavor and texture, ranging between 4 and 5. According to the results, it could be observed that, in all treatments, the most outstanding flavor is the acid one. Moreover, there was no significant statistical differences among the 4 treatments with  $p > 0.05$ .

## Evaluation of texture

**Table 2.** Texture evaluation averages.

Treatment	Breakable	Gritty	Elastic	Sticky
1	1.9	1.37	2.2	2
2	2.07	1.5	2.4	2.1
3	1.53	1.3	2.3	1.7
4	1.4	1.37	2.3	1.8

Table 2 shows that the elastic texture is the most outstanding characteristic for all treatments. There was no significant differences in texture with  $p > 0.05$  among all treatments.

## CONCLUSIONS

Momposino cheeses are pasta filata cheeses shaped as cabbages. Two different techniques were implemented for making Momposino cheeses at Universidad Nacional of Colombia, sede Medellin, which allowed obtaining cheeses that are very similar to the autochthonous ones. One of the cheese types was elaborated with culture and pasteurized milk, which has a larger quantity of volatile compounds than the one that did not have added culture. The cheeses that were elaborated with culture showed that the technique used for making them was better because it avoids contamination and keeps an adequate quality of the pasta filata cheeses. Autochthonous Momposino cheeses have a larger quantity of fat contents, therefore, they present more volatile compounds than the other Momposino cheese types. The volatile compounds found were acids, alcohols and hydrocarbons. Oleic acid is found in all cheeses, showing a higher protection effect against flavor-changing bacteria. In the thin layer chromatography (TLC), it was observed that cheese proteins spread into the following amino acids: arginine, valine, tryptophan, histidine, threonine, lysine, tyrosine, alanine, and cysteine. And finally, it was found that the most outstanding flavor is the acid flavor, and the most outstanding texture is the elastic texture for the cheeses from all treatments.

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