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*Ingeniería de alimentos*

# VOLATILE COMPOUND CHARACTERIZATION OF CAPRINE MILK BY MULTIVARIATE OPTIMIZATION OF HEADSPACE SOLID PHASE MICRO-EXTRACTION (HS-SPME)

# CARACTERIZACIÓN DE COMPUESTOS VOLÁTILES DE LA LECHE DE CABRA POR OPTIMIZACIÓN MULTIVARIABLE DE LA FASE SÓLIDA MICROEXTRAIDA DEL ESPACIO DE CABEZA (FSME-EC)

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## Abstract

A Central Composite Design in conjunction with response surface methodology was used to optimize the extraction conditions of volatile compounds in caprine milk using *headspace* Solid Phase Micro-Extraction (HS-SPME). Through this statistical methodology, it was possible to identify the best conditions to maximize the response variables which were 15 minutes for equilibrium time, 30 minutes for extraction time and 45 °C for extraction temperature. Using these conditions, 19 different volatile compounds were identified, with a profile that comprises compounds belonging to the following classes: carboxylic acids, alcohols, aldehydes, ketones, hydrocarbons, terpenoids and furans. Amongst these compounds, octanoic acid (caprylic acid) and decanoic acid (capric acid) can be highlighted with having larger relative areas, and they are volatile fatty acids that relate to the characteristic flavor presented in caprine milk.

*Keywords*: flavor, gas chromatography, goat, response surface.

## Resumen

Un Diseño Composito Central (DCC) en combinación con la metodología de superficie de respuesta fue utilizado para optimizar las condiciones de extracción de compuestos volátiles en la leche caprina mediante el uso de Fase Sólida Microextraida (FSME) del espacio de cabeza. Mediante esta metodología estadística, fue posible identificar las mejores condiciones para maximizar la variable de respuesta y el número de compuestos volátiles, que corresponde a 15 minutos para el tiempo de equilibrio, a 30 minutos de tiempo de extracción y 45 °C para la temperatura de extracción. Utilizando estas condiciones, se identificaron 19 compuestos volátiles diferentes, con un perfil que comprende compuestos que pertenecen a las siguientes clases: ácidos carboxílicos, alcoholes, aldehídos, cetonas, hidrocarburos, terpenos y furanos. Entre estos compuestos, el ácido octanoico (caprílico) y ácido decanoico (cáprico) son destacados por tener áreas relativas más grandes, y son ácidos grasos volátiles que están relacionados con el sabor característico presentado en la leche de cabra.

*Palabras clave*: sabor, cromatografía de gases, cabra, superficie de respuesta.

## 1 Introduction

One of the most important criteria that determine the choice and acceptance of a food product by consumers is the flavor, which results from a complex balance between taste and aroma. Volatile compounds play a key role in this context, as they influence the aroma of the food, and their presence, content and composition have an influence on product quality (Milosavljevic *et al*., 2012; Delgado *et al*., 2010, 2011).

Milk flavor is formed by a variety of chemical compounds, including carboxylic acids, alcohols, aldehydes, ketones, free fatty acids, sulfur compounds. These compounds are possibly derived from plants consumed by the animal, being transferred from its fodder to the milk through the rumen, or are products of the digestion of precursors (Kurka & Rankin, 2006;

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Queiroga *et al*., 2005).

The results of the analysis of aromatic compounds depend on the detection and isolation techniques. Volatile compounds are commonly analyzed by gas chromatography (GC) coupled with mass spectrometry (MS), with a step of prior extraction and pre-concentration of the volatile fraction (Tunick *et al*., 2013; Delgado *et al*., 2010).

Solid phase micro extraction (SPME) technique is currently the predominant method for extraction of volatile compounds in some foods. The reason is that this technique requires only a small amount of a sample, allowing the isolation of volatile compounds from solid and liquid matrices in a fast and simple way (Jelèn *et al*., 2012; Delgado *et al*., 2010).

The principle of *headspace* SPME is based on partition equilibrium of analytes among fiber, sample and *headspace* (Mitelut *et al*., 2011). The analysis consists in capturing the analyte in a chemically modified fused silica fiber, coated with an appropriate film material, which is exposed to the environment where the volatile compounds are to be extracted. The extraction phase can be placed directly in the liquid phase or in contact with the vapor in equilibrium (*headspace*) above the liquid or solid sample. After a certain period of time required to establish equilibrium between the phases involved, the fiber is repositioned inside the needle (Dórea *et al*., 2008).

According to Liu and Yang (2002), the reproducibility and sensitivity of the analysis of volatile compounds by *headspace* SPME is greatly influenced by the vapor pressure of the flavor compounds in the bottle containing the sample. Thus, to optimize the extraction of volatile compounds by SPME technique, thermodynamic knowledge of this method is fundamental, allowing for the identification of the factors that influence its kinetic (Dórea *et al*., 2008). Time and temperature are the two most important factors affecting the vapor pressure and equilibrium of volatile compounds in the bottle (Liu & Yang, 2002; Pellati *et al*., 2005).

Statistical optimization of experiments is employed to improve system performance and increase the yield of the process without increasing the cost (Bay and Boyaci, 2007). Central composite design (CDD) in conjunction with response surface methodology (RSM) enables the evaluation of the effects of many independent variables and their interactions by using a small number of experiments, overcoming the disadvantages of other statistical techniques that evaluate only one parameter at a time.

The use of SPME for analysis of volatile compounds has been previously reported in bovine milk (Karatapanis *et al*., 2006) and ovine milk (Vasta *et al*., 2012; Povolo *et al*., 2007), but data for caprine milk are scarce. Queiroga *et al*. (2005) performed an optimization of extraction conditions of volatile compounds in caprine milk, however using the extraction and simultaneous concentration technique. Thus, this study aimed to carry out the optimization of extraction conditions of volatile compounds in caprine milk by using the SPME technique, in order to promote preliminary knowledge of some variables that affect this process, as well as to identify some compounds that contribute to caprine milk flavor.

## 2 Materials and methods

### 2.1 Samples

The milk was obtained from twelve Saanen goats, raised in an intensive system at the Department of goat and cattle breeding of the Centre for Humanities, Social and Agrarian Sciences at the Federal University of Paraíba (UFPB - Paraíba, Brazil). The animals were manually milked, and about 200 mL of milk from each animal was collected. The raw milk was then mixed and homogenized, packaged in polypropylene bottles and stored at 4 °C until the analysis. The analytical procedures were carried out at the Laboratory of Food Chemistry and at the Laboratory of Flavor of the Food Engineering Department, Technology Center of the Federal University of Paraíba (DEA/CT/UFPB).

### 2.2 Optimization of extraction of volatile

Central composite design (CCD) for three independent variables was used in this study, where the independent variables selected were the equilibrium time (min), the extraction time (min) and the extraction temperature (°C). The codified levels of the variables and their real values are shown in Table 1. The experimental design included 17 assays, eight factorial (the combinations of variables at -1 and +1), six axial (one variable set to $\pm\alpha$ and another set to 0) and three repetitions at the central point (three variables set to 0) (Table 2). The response variables were the number of chromatographic peaks and the sum of the areas of the chromatographic peaks. Due to systematic errors, all the experiments were carried out randomly to minimize the effect of unexplained variability on the observed responses.

Table 1. Factors and levels used for the CCD.

| Independent variables | Levels | | | | |
|---|---|---|---|---|---|
| | -1.68 | -1 | 0 | 1 | 1.68 |
| Equilibrium time (min) | 7 | 10 | 15 | 20 | 23 |
| Extraction time (min) | 14 | 20 | 30 | 40 | 46 |
| Extraction temperature (ºC) | 28 | 35 | 45 | 55 | 62 |

$\alpha = (2^n)^{1/4} = 1.68$; where $n$ = number of independent variables.

Table 2. Central Composite Design for the three independent variables tested

| Assay | Variable | | | Response |
|---|---|---|---|---|
| | $T_{eq}$ (min) | $T_{ext}$ (min) | T (ºC) | $Y_1$ |
| 1 | -1 (10) | -1 (20) | -1 (35) | 11 |
| 2 | 1 (20) | -1 (20) | -1 (35) | 6 |
| 3 | -1 (10) | 1 (40) | -1 (35) | 13 |
| 4 | 1 (20) | 1 (40) | -1 (35) | 9 |
| 5 | -1 (10) | -1 (20) | 1 (55) | 9 |
| 6 | 1 (20) | -1 (20) | 1 (55) | 6 |
| 7 | -1 (10) | 1 (40) | 1(55) | 19 |
| 8 | 1 (20) | 1 (40) | 1 (55) | 11 |
| 9 | $-\alpha$ (7) | 0 (30) | 0 (45) | 12 |
| 10 | $+\alpha$ (23) | 0 (30) | 0 (45) | 11 |
| 11 | 0 (15) | $-\alpha$ (14) | 0 (45) | 5 |
| 12 | 0 (15) | $+\alpha$ (46) | 0 (45) | 8 |
| 13 | 0 (15) | 0 (30) | $-\alpha$ (28) | 3 |
| 14 | 0 (15) | 0 (30) | $+\alpha$ (62) | 15 |
| 15 | 0 (15) | 0 (30) | 0 (45) | 17 |
| 16 | 0 (15) | 0 (30) | 0 (45) | 18 |
| 17 | 0 (15) | 0 (30) | 0 (45) | 16 |

$Y_1$: number of chromatographic peaks

The estimate of the optimal conditions for the extraction of volatile compounds was also performed based on the proposed statistical models and with the help of simultaneous optimization technique called "desirability". The software *Statistica* 7.0 was used for the calculation and the results were analyzed by Response Surface Methodology (RSM).

## 2.3 HS-SPME-GC/MS Analysis

Raw caprine milk (20 mL) was placed in a 100 mL flask and heated, following the experimental conditions presented in Table 2, before exposure of the fiber. After the heating period, the gray fiber (Divinylbenzene/Carboxen/Polydimethylsiloxane - DVB/CAR/PDMS-50/30 mM-Supelco, Bellefonte, PA) was exposed to the *headspace*, with the gas phase above the sample remaining exposed for a period of 14 to 46 minutes (according to Table 2). This entire procedure was performed in a water bath

with magnetic stirring at 700 rpm. The fiber was then retracted into the needle and transferred to the injector gas chromatograph (Varian Saturn 3800 2000R), VF-5MS column (60m x 0.25mm x 0.25 mM) coupled with a mass detector (Varian Saturn 2000R 2000) where the analytes were desorbed for 10 minutes. The oven temperature programming chromatograph started at 40 °C for 10 minutes, then increased 5 °C per minute until reaching 240 °C, and maintaining this temperature for 11 minutes with total run time equal to 61 minutes. The injector temperature was maintained at 250 °C, and Helium gas was used as the carrier gas at a constant flow rate of 1.0 mL per minute. The mass spectrometer was operated by impact electrons with a source temperature of 200 °C, and ionization energy of 70 V, scan range of m/z 29 to m/z 400 to 3.33 scans/s. For the identification of the volatile compounds, a standard solution of n-alkane counterparts (C8-C25 - Sigma Aldrich Chemie GMbH. Steinheim, Germany) was examined before sample injection

in order to obtain the subsequent calculation of the Linear Retention Index (LRI) of volatile compounds. For the identification of compounds, the spectra bank's own library of GC/MS, NIST/EPA/NIH Mass Spectral Database (Version 1.7) was used, and the spectra and values of LRI volatile were also compared with the literature data. After identification of compounds, normalization of the volatile areas was performed.

## 2.4 Statistical analysis

The regression coefficients for linear, quadratic, and interaction terms were determined by multiple linear regression (MLR). The significance of each regression coefficient was statistically assessed by computing the t-value from pure error obtained from the replicates at the central point of this experiment. Analysis of variance (ANOVA), with a confidence level of 95% ($p < 0.05$) was used to validate the model. The regression coefficients were then used to generate response surfaces. All calculations and graphics were performed using the software Statistica®, version 7.0 (Statsoft, USA).

# 3 Results and discussion

## 3.1 Optimization by RSM

HS-SPME is an equilibrium technique that requires a preceding step of sampling optimization conditions in order to obtain high recoveries of volatile compounds and satisfactory precision of the method. The responses for the 17 assays performed are shown in Table 2. The three independent variables studied influenced ($p < 0.05$) the number of chromatographic peaks, and it was possible to obtain adjusted quadratic model that describe the effects of these independent variables on the evaluated response. However, interaction effects were not observed ($p > 0.05$).

To represent the variable $Y_1$ (number of chromatographic peaks) as a function of the codified values of independent variables that were significant at 95% confidence, one adjusted quadratic model were generated (Eq. 1). That model presented determination coefficient ($R^2$) of 0.80, indicating that 80.0 % of the experimental data variability is explained by that model. In the ANOVA results for $Y_1$, where it is observed that the F value calculated for the regression was greater than the tabulated F value, indicating that

the models offered significant statistics (Rodrigues and Iemma, 2014). Response surfaces were generated from these obtained quadratic model (Fig. 1), in order to identify the optimal conditions. The response surfaces were generated considering two factors at a time, setting the third factor at the central point.

$$Y_1 = 16.87 - 1.59Teq - 1.50Teq^2 + 1.83Tex$$
$$- 3.27Tex^2 + 1.92T - 2.38T^2 + 1.25TexT \quad (1)$$

The solid phase micro-extraction is based on kinetic mass transfer between phases and on the thermodynamics that describes the partition equilibrium of analytes between the sample and the extraction phase (Valente & Augusto, 2000). The thermodynamic knowledge of the SPME technique and the identification of factors that affect its kinetics are useful to achieve the objective of the analysis in the shortest time possible (Dórea *et al.*, 2008). In this study, the SPME was carried out in *headspace* mode, which comprises three phases: the liquid sample, the vapor phase over the liquid sample (*headspace*) and the extraction phase (fiber). According to Pellati *et al.* (2005), the equilibrium time (Teq, min), the extraction time (Tex, min) and the extraction temperature (T, °C) are the three most important variables that affect the vapor pressure and the balance of volatile compounds in *headspace*.

By analyzing the response surfaces generated by the model, it was possible to visualize optimum ranges, since the quadratic effect of independent variables were significant, showing negative values, it was possible to obtain concave-up surfaces exhibiting maximum points, hence allowing process optimization. Temperature is a very important parameter that can affect the extraction of volatile compounds in SPME, as the solute is needed from the sample during extraction to vaporize into the gas phase by heating the sample (Yilmazcan *et al.*, 2013). In the work of Flores-Martínez *et al.* (2016), temperature was one of the process parameters with the greatest effect on the yield of antioxidants from Mexican oregano (*Lippia graveolens* HBK) where the optimum conditions were also identified by using response surface methodology (RSM).

The results indicated that temperatures ranging from 45 to 55 °C lead to a greater amount of volatile compounds, confirmed by the higher number of chromatographic peaks and by the higher total area. However, at lower or higher temperature (outside that range), the extraction efficiency starts to decrease.
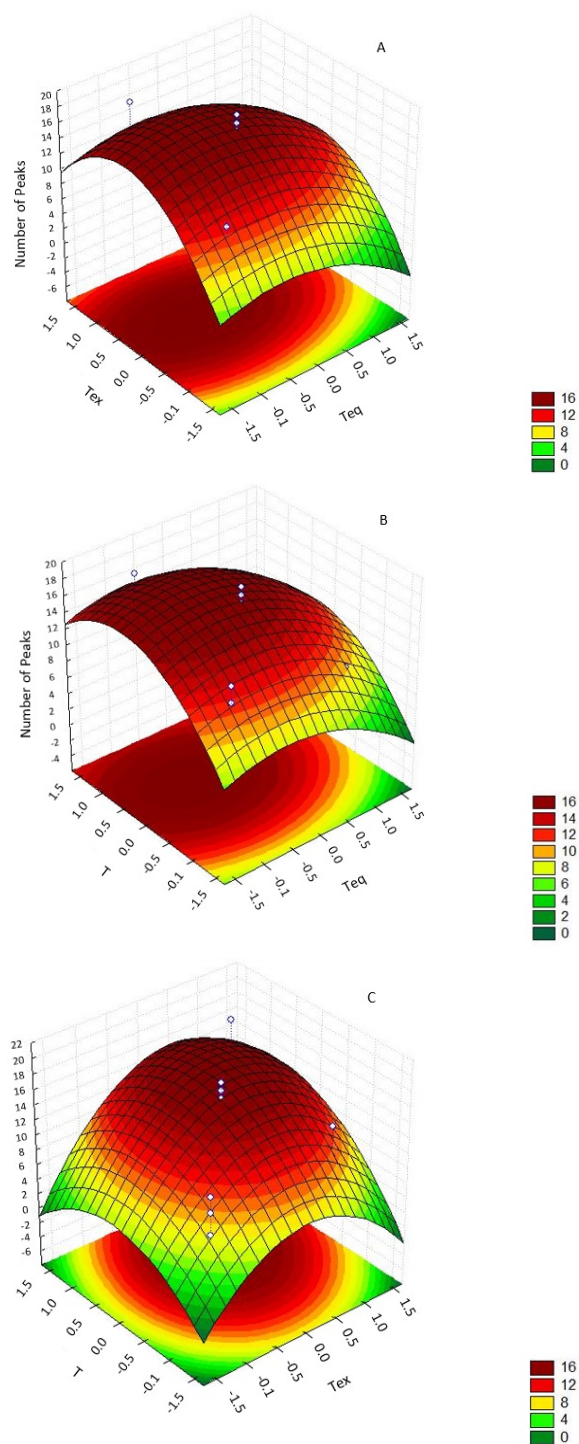
Fig. 1. Response surfaces for the number of peaks (Y1) obtained from goat milk as a function of A: Tex (min) and Teq (min); B: Teq (min) and T (ºC) and C: Tex (min) and T (ºC).

According to Feng *et al*. (2008), lower temperatures prevent the volatilization of volatile

compounds while increasing temperature is important to accelerate the transfer of the volatile compounds from the milk to the fiber. As temperature increases, more analytes are released from the matrix (sample) to the upper space, a process that increases the extraction (Zhang *et al*., 1994). This is because increasing temperature also increases the partitioning constant of the analytes in favor of the gas phase; thus favoring the analyte concentration between the sample and the *headspace*, thereby reaching the equilibrium in a shorter time (Heidari *et al*., 2008). However, at high temperatures, the partition coefficient of the *headspace* to the coating (fiber) decreases and the optimum extraction temperature is determined by interactions amongst the sample, the fiber and the analytes (Zhang *et al*., 1994).

The equilibration time can be defined as the time required to form a *headspace* (gas phase above the milk sample) and an equilibrium between the liquid phase and the gas phase. From the response surfaces, it can be observed that the number of chromatographic peaks and the total area of the peaks are higher when the equilibrium time ranges from 10 to 15 minutes. Riener *et al*. (2009) adopted a time of 15 minutes to equilibrate a pasteurized bovine milk sample prior to exposure of the fiber. The equilibration time decreases with the excitement of the array, as this makes the contact of the analyte with the fiber easier (Valente and Augusto, 2000).

The extraction time is defined as the time that the extraction phase (fiber) was exposed to the *headspace*. After equilibration in the *headspace*, the SPME sampler is inserted into the vial containing the sample and the fiber is exposed to adsorb the compounds present in vapor phase (Garruti *et al*., 2011; Valente & Augusto, 2000). According to Feng *et al*. (2008) the increase in adsorption (extraction) of volatiles is explained by the fact that the mass transfer of the analytes is accelerated and the balance amongst the three phases (liquid - gas - fiber) is established more quickly when the sample is shaken vigorously with a magnetic stirring bar, as observed in our study. The interpretation of the response surfaces indicated that the highest number of chromatographic peaks and a higher total area are observed when the extraction time used is in the range of 30 to 35 minutes. In the study of Povolo *et al*. (2007), an extraction time of 30 minutes was adopted to extract volatile compounds from the *headspace* in sheep milk.

Data on the optimization of parameters such as extraction temperature, equilibrium time and extraction time on the extraction of volatile

compounds in caprine milk by using SPME technique was not found in the available literature. However, other studies have been conducted with the optimization of these parameters in other foods. Pellati *et al.* (2005) also observed an improvement in the extraction of volatile organic compounds by increasing extraction temperature; however, they did not observe any changes in the qualitative and semi-quantitative composition of the volatile compounds from fruits of *Evodia* species at temperatures above 80 °C. The fiber exposure time (extraction time) showed a high effect on the extraction of volatile compounds from the *headspace* of a sugarcane alcoholic beverage (Garruti *et al.*, 2011). These authors also observed that increasing temperature from 25 °C to 50 °C caused a significant reduction in the number of chromatographic peaks and in the total area of the chromatogram, whereas the equilibration time had no significant influence on those responses.

## 3.2 Desirability

The diagram of the desirability function obtained via statistical function called "profiles values predicted and Desirability" describes the optimized conditions of extraction of volatile compounds within the ranges of values established in the experiments. In Fig. 2 it is observed that at the intersection of dashed lines is the optimal values which indicate the optimal parameters for this study are the values corresponding to the center point region for the three independent variables. The optimization desirability value was 0.84886, which is considered an excellent and acceptable value (Akhanazarova and Kafarov, 1982).

Thus, by analyzing the experimental results of response surface and desirability function, it was decided that the best conditions to maximize the response variables at the levels studied correspond to 15 minutes for equilibration time (level 0), 30 minutes for extraction time (level 0) and 45 °C for extraction temperature (level 0). These values were used for the extraction and identification of volatile compounds in caprine milk.
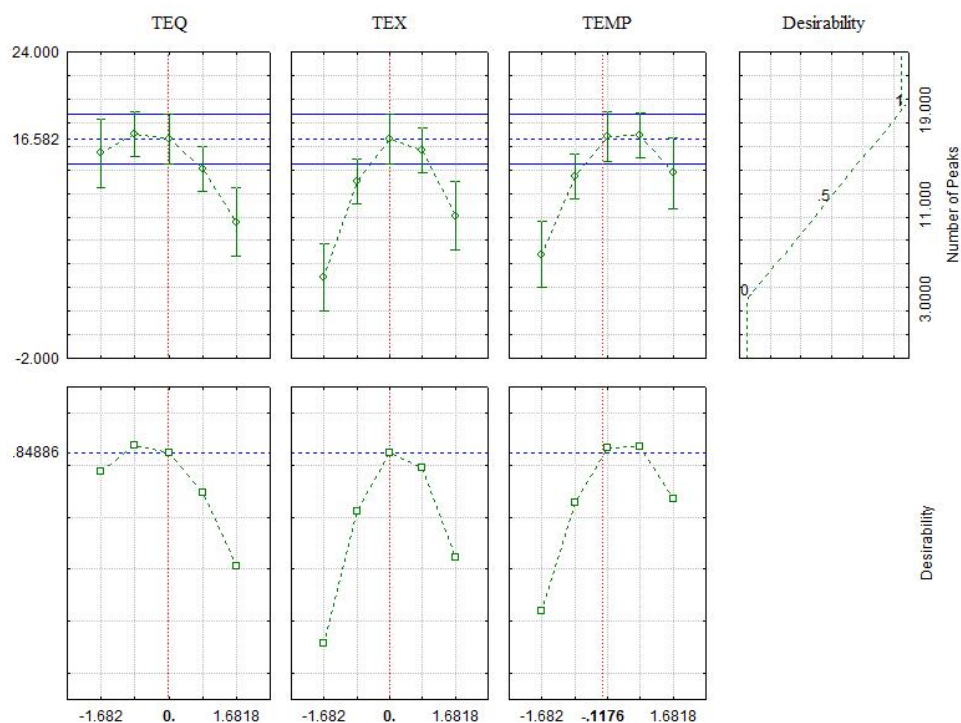


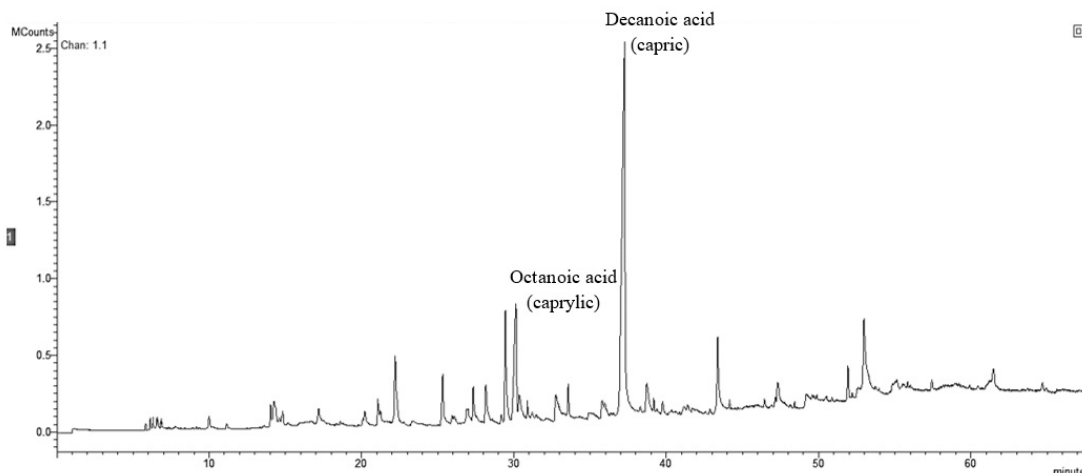Fig. 2. Profiles of predicted values and desirability to answer $Y_1$.

Fig. 3. Chromatogram of total ions in caprine milk.

## 3.3 Volatile compounds of caprine milk

Once the analytical conditions for the extraction of volatile compounds was established, the analysis of five caprine milk samples was carried out. Nineteen compounds of the following classes were identified: carboxylic acids (3), alcohols (3), aldehydes (3), ketones (2), hydrocarbons (5), terpenoids (1) and furans (2) (Table 3). Fig. 3 shows the chromatogram of total ion in caprine milk.

The same amount of volatile compounds was identified by Povolo *et al*. (2007) in ovine milk and by Mounchili *et al*. (2005) in bovine milk. The number of compounds detected in the milk samples was likely smaller than the number found in raw milk prior to analysis. This is because some micro-organisms and native milk enzymes may have been inactivated during the heating of the sample in the analysis, resulting in a decreased formation of volatile compounds (Tunick *et al*., 2013).

Amongst the identified compounds, octanoic acid (caprylic) and decanoic acid (capric) stand out with larger relative areas (%). These volatile fatty acids are reported to be responsible for the characteristic flavor in caprine milk (Ribeiro and Ribeiro, 2001). According by Mele *et al*. (2008), caprylic acid (8:0) and capric acid (10:0) are among the most characteristic fatty acids of goat's milk and are named after the species name. They are characterized by a sharp and persistent odor, typical of goats. Carboxylic acids are not only aroma compounds, but they are precursors of other compounds such as methyl ketones, alcohols, aldehydes, lactones, esters (Collins *et al*., 2003). In relationship to the ketones,

2-heptanone is produced from the β-oxidation and decarboxylation of free fatty acids (Marilley and Casey, 2004) and 2-nonanone was described as "hot milk" odor (Carpino *et al*., 2004), and possibly originated from the heating milk for analysis.

Some of the chemical compounds that contribute to the milk flavor are directly linked to the diet of the animal. Povolo *et al*. (2007) identified the volatile compound limonene in the grazing by sheep and also in their milk, suggesting that this monoterpene may be derived from animal feed. The aldehyde nonanal was described as "soapy" odor, and also was identified in some plants consumed by sheep and cheese made with the corresponding milk (Carpino *et al*., 2004). Nonanal and heptanal are aldehyde that are formed from the oxidation of polyunsaturated fatty acids, which are present in large quantities in plants consumed by animals (Morand-Fehr and Tran, 2001; Elmore *et al*., 2005).

Two main hypotheses are that they are originated from the plants consumed by the animal or they are produced through the digestion of precursors (Kurka and Rankin, 2006). Most of these volatile organic compounds (such as several low molecular weight alcohols and ketones) are considered to be of metabolic origin (Coppa *et al*., 2011). During digestion and metabolism, changes that occur in feed components result in the most part of the volatile compounds present in milk, due to microbial and enzymatic processes up to the mammary synthesis (Addis *et al*., 2006).

Table 3. Volatile compounds identified in caprine milk with their respective relative areas.

| Class and compound name | LRI | Identification method | Area (%) |
|---|---|---|---|
| | | | Mean ± Standard deviation |
| **Carboxylic acids** | | | |
| Etanoic acid (acetic) | Nc | MS + L | 8.09 ± 2.05 |
| Octanoic acid (caprylic) | 1196 | MS + LRI | 11.03 ± 4.88 |
| Decanoic acid (capric) | 1386 | MS + LRI | 16.11 ± 1.80 |
| **Alcohols** | | | |
| 1-Butanol | Nc | MS + L | 2.26 ± 0.43 |
| Maltol | 1146 | MS + LRI + L | 5.23 ± 1.54 |
| 1-Nonanol | 1177 | MS + LRI + L | 2.94 ± 0.36 |
| **Aldehydes** | | | |
| Heptanal | 914 | MS + LRI + L | 1.34 ± 0.71 |
| E-2-heptenal | 953 | MS + LRI + L | 0.93 ± 0.13 |
| Nonanal | 1117 | MS + LRI + L | 3.41 ± 0.32 |
| **Ketones** | | | |
| 2-Heptanone | 901 | MS + LRI + L | 0.46 ± 0.16 |
| 2-Nonanone | 1106 | MS + LRI + L | 2.77 ± 1.59 |
| **Hidrocarbons** | | | |
| Undecane | 1100 | MS + LRI + L | 2.22 ± 0.01 |
| 1-Decene | 1032 | MS + LRI + L | 7.55 ± 4.61 |
| Dodecane | 1199 | MS + LRI + L | 2.47 ± 0.03 |
| Tetradecane | 1405 | MS + LRI + L | 3.81 ± 0.03 |
| Pentadecane | 1498 | MS + LRI + L | 5.66 ± 0.19 |
| **Terpenoids** | | | |
| Limonene | 1044 | MS + LRI + L | 0.46 ± 0.11 |
| **Furans** | | | |
| 2-Furanmethanol | 886 | MS + LRI + L | 4.20 ± 3.00 |
| 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one | 1002 | MS + LRI + L | 3.26 ± 2.24 |

MS = Mass spectrometry; LRI = Linear retention index; L = Literature (www.odour.org.uk; Povolo *et al.*, 2007; Vasta et al. 2012; Madruga *et al.*, 2013; Cais-Sokolinska *et al.*, 2011); Nc = Not calculated.

# Conclusions

The CCD in conjunction with response surface methodology was shown to be an adequate statistical tool for the optimization of extraction conditions of volatile compounds in caprine milk by using SPME *headspace*. Through this statistical methodology, it was possible to identify the best conditions to maximize the the number of volatile compounds extracted, corresponding them to 15 minutes for equilibrium time, 30 minutes for extraction time and 45 °C for extraction temperature. Using these conditions, 19 different volatile compounds were identified, with a profile that comprises compounds belonging to the following classes: carboxylic acids, alcohols, aldehydes, ketones, hydrocarbons, terpenoids and furans. Amongst these compounds, octanoic acid (caprylic acid) and decanoic acid (capric acid) can be highlighted with having larger relative areas, and they are volatile fatty acids that relate to the characteristic flavor presented in caprine milk.

*Nomenclature*

| | |
|---|---|
| CCD | central composite design |
| SPME | solid phase micro extraction |
| GC | gas chromatography |
| MS | mass spectrometry |
| $Teq$ | equilibrium time in minutes |
| $Tex$ | extraction time in minutes |
| $T$ | extraction temperature in ºC |
| RSM | response Surface methodology |
| MLR | multiple linear regression |
| ANOVA | analysis of variance |
| $\alpha$ | $(2^n)^{1/4} = 1.68$; where n = number of independent variables |
| $Y_1$ | number of chromatographic peaks |
| LRI | linear retention index |
| L | literature |

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