

**SENSORY QUALITY OF MILK FAT WITH LOW CHOLESTEROL CONTENT
FRACTIONED BY MOLECULAR DISTILLATION**

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ABSTRACT

BACKGROUND

Anhydrous Milk Fat (AMF) is a valuable ingredient in several industries, but its cholesterol content is a disadvantage because it is associated to several diseases. The objective of this study was to remove the largest amount possible of cholesterol from anhydrous milk fat (AMF), using molecular distillation, and to analyze the sensory properties of the obtained product. AMF was subjected to various molecular distillation arrangements.

RESULTS

The first set of experiments involved molecular distillations performed over a range of evaporation temperatures. Then, according to the outcome of the first set of experiments, a

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second set of experiments was carried out in order to study the effect of the feeding flow rate. Finally, the number of distillation stages was modified.

CONCLUSIONS

The best results were obtained in a three-stage arrangement, in which $\cong 60\%$ of cholesterol was removed with a 30.48% distillate yield. The sensory properties of this distillation cut were also the most similar to those of the untreated milk fat.

Keywords: *Molecular distillation; Anhydrous Milk Fat; cholesterol; sensory analysis.*

INTRODUCTION

Anhydrous milk fat (AMF) is a product obtained from cream or butter and consists of <997 g kg⁻¹ milk fat. The remaining (~ 3 g kg⁻¹) matter consists of water, and non-fat solids. AMF is an important source of lipids and it imparts an excellent flavor and mouthfeel to food. Due to its low water content, AMF remains safe over extended periods and requires no refrigeration during its shelf life. AMF occupies a smaller volume than common butter, which has 160 g kg⁻¹ water in its structure, and, consequently, its storage and transportation is less costly. Because of its unique physical properties, AMF is widely used for recombination of various dairy products and can be part of the continuous fat phase in several food preparations. It can also be injected in-line in a continuous operation, thus, it is widely used in the bakery and ice cream industry⁽¹⁾.

Despite its good nutritional image and excellent technical properties, the presence of cholesterol in AMF, has been a disadvantage for its consumption and marketability. Cholesterol intake is strongly connected to cardiovascular diseases, such as atherosclerosis⁽²⁾ and it has also been linked to neurological disorders, like Alzheimer's disease⁽³⁾. No-cholesterol substitutes for AMF, such as hydrogenated vegetable oils, have been developed but the particular flavor and mouthfeel of AMF are irreplaceable. Several scientific methods have been proposed to reduce cholesterol in fats. Boudreau & Arul⁽⁴⁾ reviewed the various physical, chemical and biological technologies that are aimed at reducing the cholesterol content of AMF. Campos⁽⁵⁾ studied the influence of evaporation temperature (T_{evap}) in short-path distillation, with the intention of obtaining fractions with distinct physical properties, such as melting behavior, kinetics of crystallization, and rheology.

Cholesterol separation from milk fat by molecular distillation, has been studied by Lanzani et al.⁽⁶⁾, who achieved almost complete removal of cholesterol from AMF at temperatures between 190 and 250 °C and residual pressures between 10^{-3} and 10^{-4} Torr. However, the sensory consequences of molecular distillation have not yet been extensively analyzed.

The current study focused on removing the largest possible amount of cholesterol from AMF, using molecular distillation, and analyzing the sensory properties of the final product.

MATERIALS AND METHODS

Materials

AMF preparation and storage conditions. Corlasa S.A. (Santa Fe, Argentina) donated the raw material used for these experiments. Before the molecular distillations, AMF was heated up to 42 °C, to confirm that it was fully melted, so that no solid fraction was fed into the apparatus. All samples were collected in glass amber bottles and stored at -10 °C for the duration of the study.

Methods

Description of the molecular distillation process

Molecular distillation apparatus. A KDL4 molecular distillator, manufactured by UIC (Hörstein, Germany), equipped with a 0,4 m² falling film evaporator and a 0,2 m² internal condenser, was used for the molecular distillation experiments, as illustrated in Figure 1. The unit consists of a falling film evaporator, connected to a vacuum system. It also has a hot oil heating system, as well as separate heating and chilling systems.

The percentage of distillate yield and cholesterol removal were calculated, using Equation 1 and Equation 2.

$$\% \text{ Percentage distillate yield} = D/F \cdot 100 \quad (\text{Equation 1})$$

$$\% \text{ Cholesterol removal} = (D \cdot x_D) / (F \cdot x_F) \cdot 100 \quad (\text{Equation 2})$$

Where: D = amount of distillate obtained by molecular distillation; x_D = cholesterol fraction in the distillate; F = amount of AMD fed into the apparatus, and x_F = cholesterol fraction in the AMF.

Experiments performed over a wide range of T_{evap} . As a result of a preliminary analysis of the molecular distillation of AMF, the conditions for the experiments were set at: $T_{\text{condenser}} = 45\text{ }^{\circ}\text{C}$, $T_{\text{preheater}} = 40\text{ }^{\circ}\text{C}$, wiper speed = 0.1 rad s^{-1} , and feeding flow rate = 1 mL/min . According to previous evidence⁽⁵⁾, the T_{evap} is the most relevant variable in molecular distillation. No separation was achieved below $120\text{ }^{\circ}\text{C}$. Hence, the lowest temperature was fixed at $120\text{ }^{\circ}\text{C}$. The molecular distillation unit used for this investigation restricted the highest possible temperature for the experiments, which was $200\text{ }^{\circ}\text{C}$.

Feeding flow variations. An independent set of experiments were done to study the impact of various feeding flow rates on cholesterol removal.

Successive distillation arrangements. Two arrangements of successive molecular distillations were proposed (see Figure 2).

- Arrangement number 1:
 - o Stage I: $T_{\text{evap}} = 120\text{ }^{\circ}\text{C}$
 - o Stage II: $T_{\text{evap}} = 150\text{ }^{\circ}\text{C}$ (residue re-distillation)
 - o Stage III: $T_{\text{evap}} = 180\text{ }^{\circ}\text{C}$ (residue re-distillation)
- Arrangement number 2:
 - o Stage I: $T_{\text{evap}} = 150\text{ }^{\circ}\text{C}$
 - o Stage II: $T_{\text{evap}} = 165\text{ }^{\circ}\text{C}$ (residue re-distillation)
 - o Stage III: $T_{\text{evap}} = 180\text{ }^{\circ}\text{C}$ (residue re-distillation)

Analytical methods

Cholesterol concentrations were determined in all samples by gas chromatography. A Hewlett Packard 5890 gas chromatograph (Wilmington, United States), with a flame ionization detector and a Hewlett Packard 3395 integrator (Santa Clara, United States) was used to perform the chromatographic analysis. Samples were prepared based on the method proposed by Fletouris, Botsoglou, Psomas, & Mantis⁽⁷⁾. The chromatographic conditions were as follows: oven temperature: $285\text{ }^{\circ}\text{C}$, injector temperature: $300\text{ }^{\circ}\text{C}$, detector temperature: $300\text{ }^{\circ}\text{C}$; gas flow: $\text{N}_2 = 1\text{ mL/min}$, $\text{H}_2 = 30\text{ mL/min}$, air = 400 mL/min ;

column: HP-5 (30 m × 0.25 mm internal diameter × 0.25 μm film thickness). The injection volume was 1 μL.

As a means to estimate free fatty acid content, Thin Layer Chromatography was carried out for the most important samples. The plate had 60G Silica and the solvents were hexane-ethyl ether-acetic acid (80:20:1,5). Samples were developed in resublimated iodine. The procedure was repeated in triplicate. Each TLC plate was scanned using a HP Deskjet F4100 scanner which was connected to a personal computer. Data were analyzed and integrated using Christin software⁽⁸⁾.

Sensory analysis

It is important to determine if the unique attributes of AMF are altered when it is passed through the molecular distillator. Descriptive sensory analysis was performed for the following three samples:

- 1) Sample 1: untreated AMF
- 2) Sample 2: residue obtained at 180 °C and 1 mL/min feeding flow, in a single operating stage
- 3) Sample 3: residue of arrangement number 2

Ten (seven female and three male) trained panelists, were used for the evaluation of sensory descriptive attributes. They were recruited, selected, trained and monitored by the rules stated in ISO 22935-1/IDF 99:1⁽⁹⁾. Panelists had to meet the following criteria: aged 18–64 years, no AMF allergies, natural dentition, non-smokers, available for all sessions, interested in participating, and able to verbally communicate their observations, regarding the product. Selected panelists showed a perfect score in a taste sensitivity test and the ability to identify five of seven commonly found food flavors⁽¹⁰⁾.

Training. All ten panelists were trained and their evaluations calibrated in 4 four training sessions, performed over 4 days. Each training session lasted 2 h for a total of 8 h. Descriptive analysis test procedures, as described by Gayol et al.⁽¹¹⁾, were used to train the panelists.

On the first day of training, panelists were given a review of sensory analysis concepts. Then, they were asked to taste standard solutions of sucrose, sodium chloride, citric acid, and caffeine, at varying concentrations and intensities that corresponded to points on a 150-mm unstructured line scale.

On the second day of training, panelists reviewed descriptors, definitions, and reference standards, to describe AMF samples. Panelists tasted each reference and provided a rating. The panel was calibrated by obtaining an average panel rating in a 10-point scale with a standard deviation of 1 point. Panelists not rating within ± 1 point of the mean rating, were asked to re-evaluate the sample and adjust their rating, until a consensus was reached.

On the third day of training, panelists finalized the definitions, descriptors, and reference standard intensities to describe the products. Then, the list of definitions, and warm-up and reference intensity ratings, was finalized. Panelists described the following attributes in AMF: brightness, yellowness, cooked odor, cheese odor, artificial butter flavor, vegetal oil odor, milk fat odor, rancid odor, granular texture, hardness, crumbliness, spreadability, melting rate, sweetness and residual mouthfeel⁽¹²⁾. Panelists also identified references to be used to describe each attribute. Each panelist gave an intensity rating of each reference between 0 and 150 for each attribute. The definitions of attributes and standard references used in the descriptive analysis of AMF, are shown in Table 1. Standard references were employed as a reference point of intensity rating, for a determined sensory attribute used during training and evaluation sessions.

Afterward, panelists evaluated three AMF samples that possessed various degrees of oxidized flavors, using paper ballots, in order to calibrate themselves.

On the last day of training, panelists continued evaluating AMF samples that presented various degrees of oxidized flavors, to practice and to calibrate themselves within ± 10 points of the mean ratings for each attribute of the samples.

Sample evaluation. All samples were evaluated in partitioned booths under fluorescent light, at room temperature. The samples were presented to the judges in $2.15 \times 3.25 \times 2.4$ cm³ cubes. The sample and the tasting room temperatures were established in accordance with ISO 22935-1 (9) (i.e. sample temperature: 14 ± 2 °C; tasting room temperature: 20 °C).

Each sample was coded with a three-digit random number. Samples were tested using a complete randomized block design. Data were registered on paper ballots.

Statistical analyses

Analytical determination results, were the average of triplicate measurements from three independent samples. Data were analyzed using Infostat software, version 2012.p (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Córdoba, Argentina). Statistical differences were estimated by analysis of variance (ANOVA) at the 95% level of significance ($p < 0.05$). Whenever ANOVA indicated a significant difference, the least significant difference (LSD) test was used, to detect pair-wise differences among the means. Principal component analysis (PCA) and biplot graphics were performed.

RESULTS AND DISCUSSION

Variation of operating conditions of molecular distillation

Effect of temperature in cholesterol separation and distillate yield

Varying the T_{evap} , allowed obtaining distinct fractions of AMF, presenting different volatilities and physical properties. The amount of distillate increased exponentially in the working range of the T_{evap} , as illustrated in Figure 3. Cholesterol is one of the most volatile compounds in AMF. Thus, it is evaporated and, then, collected with the distillate fraction. Therefore, the residue is the fraction of interest (depleted in cholesterol)⁽¹³⁾.

The cholesterol compositions of the residue fractions are presented in Table 2.

At temperatures above 170 °C, for which the percentage of cholesterol removal was higher, the amount of residue decreased considerably (reaching up to 50%), suggesting that in order to remove higher quantities of cholesterol, up to a half of the AMF has to be lost in the distillation procedure. Based on the results of the statistical analysis, it is possible to confirm, at a significance level of 0.01% that the T_{evap} has a statistically significant influence on cholesterol removal. Considering the confirmed influence of temperature, it is possible to identify the statistical significance between groups of means by using the Scott-Knott test⁽¹⁴⁾, available in Infostat. The influence of T_{evap} on cholesterol removal obtained

by the Scott-Knott test is shown in Table 2. This test was chosen because it did not present overlapping in the results. The residue obtained at 180°C, was sensorially analyzed.

Effect of feeding flow rate on cholesterol separation and distillate yield

It is possible to differentiate three levels of classification (A, B and C) for the T_{evap} that produce statistically significant differences in cholesterol removal, which include A: $T_{\text{evap}} = 120$ °C; B: $T_{\text{evap}} = [130, 140, 150, 160, \text{ and } 170]$ °C, and C: $T_{\text{evap}} = 180, 190 \text{ and } 200$ °C. Given that molecular distillation aims to prevent thermal damage⁽⁶⁾, the lowest temperature of each group was selected to repeat the experiments but using another treatment for the feeding flow. The cholesterol concentrations (g kg^{-1}) in the distillate obtained from the feeding flow variations, are presented in Figure 4.

Statistical analysis performed confirms that both T_{evap} and feeding flow rate have a statistically significant influence on cholesterol removal but there is no interaction between them ($p = 0.1813$). The lowest feeding flow rate offered better results for cholesterol removal at all temperatures in the studied range. There was still a considerable loss of AMF (~30%) and the amount of cholesterol removed in the residue fraction is lower. These samples were not subjected to sensory analysis because cholesterol removal was significantly lower than those obtained at a lower feeding flow.

Variation of the number of stages used for molecular distillation

As a means to improve the removal of cholesterol with lower yields of distillate (i.e. less raw material loss), two arrangements of successive distillations of the residue were proposed. Previous authors have used successive distillations of the residue fractions, with various objectives, such as concentrating antioxidants⁽¹⁵⁾ and ω -3 fatty acids⁽¹⁶⁾. In the past, successive distillations of the residue have allowed obtaining four fractions of AMF, in, with a 43.6% global yield⁽¹⁷⁾.

Results for the proposed schemes are presented in Table 3. Both arrangements led to a significant reduction in the cholesterol concentration of the residue at the third stage, which is the fraction of interest. Arrangement number 1 led to 22.75% cholesterol removal,

with 14.38% distillate yield, while arrangement number 2, led to 57.92% cholesterol removal, with 30.48% distillate yield.

Descriptive sensory analysis

The results of the sensory profiles are presented in Table 4. The scores were statistically analyzed. Brightness, hardness, crumbliness and cooked odor, were the most different variables. Cheese odor, granular texture and residual mouthfeel were not significantly different for all three samples (see Table 4). As illustrated in the principal components analysis (Figure 5), the right upper quadrant was characterized by attributes found in heated AMF, such as residual mouthfeel and cooked odor. The lower right quadrant was characterized by favorable attributes typically associated with AMF, such as sweetness and milk fat odor. The upper left quadrant was characterized by softness and texture associated attributes. The lower left quadrant included brightness and crumbliness.

As demonstrated, the AMF obtained in the second three-stage arrangement, not only has a lower cholesterol concentration than the one obtained in a single 180 °C stage but its sensory behavior is more similar to non-treated AMF. The sensory analysis results, are comparable to the ones that Jinjarak, Olabi, Jiménez-Flores, Sodini, & Walker⁽¹⁸⁾ obtained in their work, for untreated butters. As Rohm⁽¹⁹⁾ proposed on his work, butter colour affects sensory perception of spreadability. During the present work, samples with higher scores for “Yellowness” were found more “Spreadable” than the other and also got a lower score at “Hardness”. According to previous work by Krause⁽²⁰⁾ the key discriminating sensory characteristics of butters include color intensity, cooked, grassy, and distinctive milk fat flavor, which are all present in the three samples analyzed in the present work. Krause found that negative aspects of butter include cholesterol, which in this case is not a disadvantage but a good aspect of the studied product since it is a novel low-cholesterol product. As Drake points out in her work⁽²¹⁾, principal component analysis (PCA) is applied to assess how several products were differentiated by several sensory descriptors. In this case, PCA (Figure 5) was useful to demonstrate how descriptive analysis can be applied to understand flavor variability within a product category, and to visualize in a clear way how attributes are related among each other. For example “Milk fat flavor”, “Artificial butter

flavor”, “Sweetness” and “Cheese odor” are almost overlapped, which means that they cause similar sensory effects.

Fatty acids

Results are presented in Table 5. ANOVA Test was performed and it concluded that Untreated Anhydrous Milk Fat was significantly different from the other samples, which were not statistically different from each other.

CONCLUSIONS

The best way to accomplish the objective of removing cholesterol with a good distillation yield was achieved by the three-stage distillation arrangement, in which the residue is re-distilled two times, in a temperature range between 150 and 180 °C (arrangement 2). Using this approach, ~60% of the original cholesterol is removed, whilst preserving ~70% of the prime matter. These results can be contrasted with the ones obtained in a single stage distillation, where, in order to remove the same amount of cholesterol ($T_{\text{evap}} = 190$ °C), more than 40% of the raw material is wasted. The descriptive sensory analysis results, show that the three-stage arrangement has less impact on the sensory properties of milk fat than a single stage.

Molecular distillation constitutes an alternative procedure to produce a milk butter product with low cholesterol content.

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Figure 1. Molecular distillation apparatus

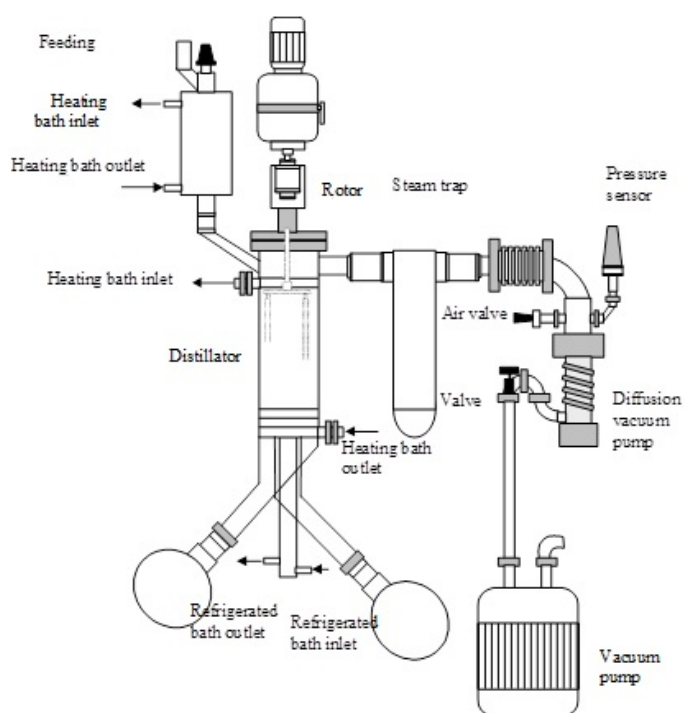


Figure 2. Successive distillation arrangements, a) Arrangement Number 1 and b) Arrangement Number 2

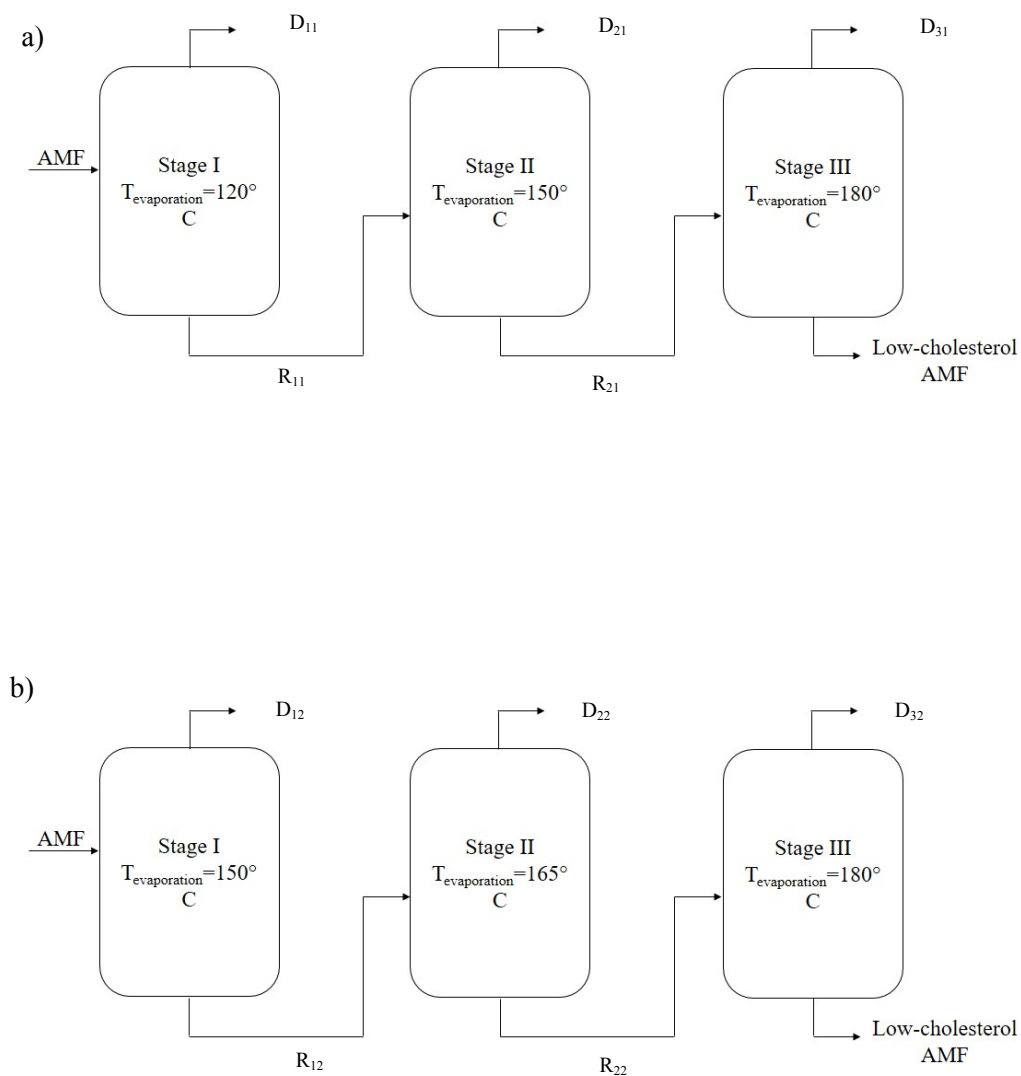


Figure 3. Percentage yield of distillate as a function of the evaporation temperature

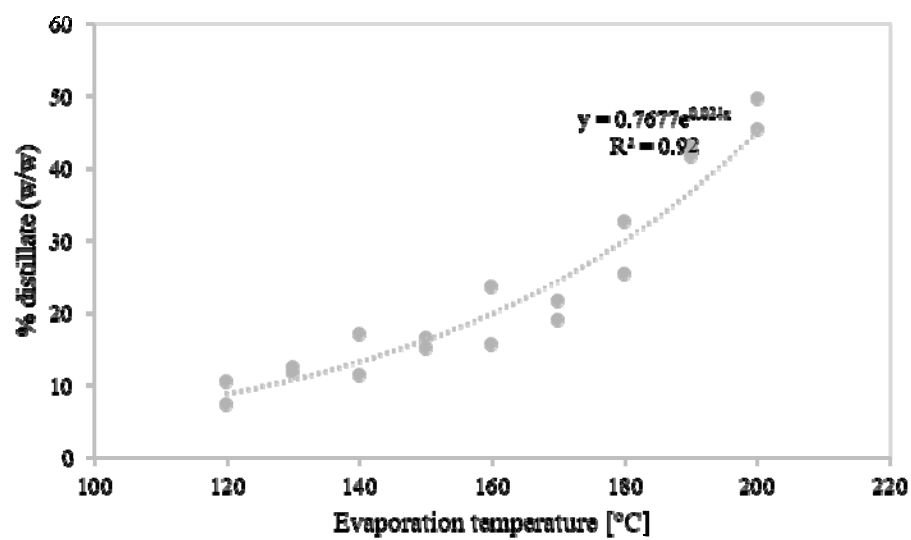


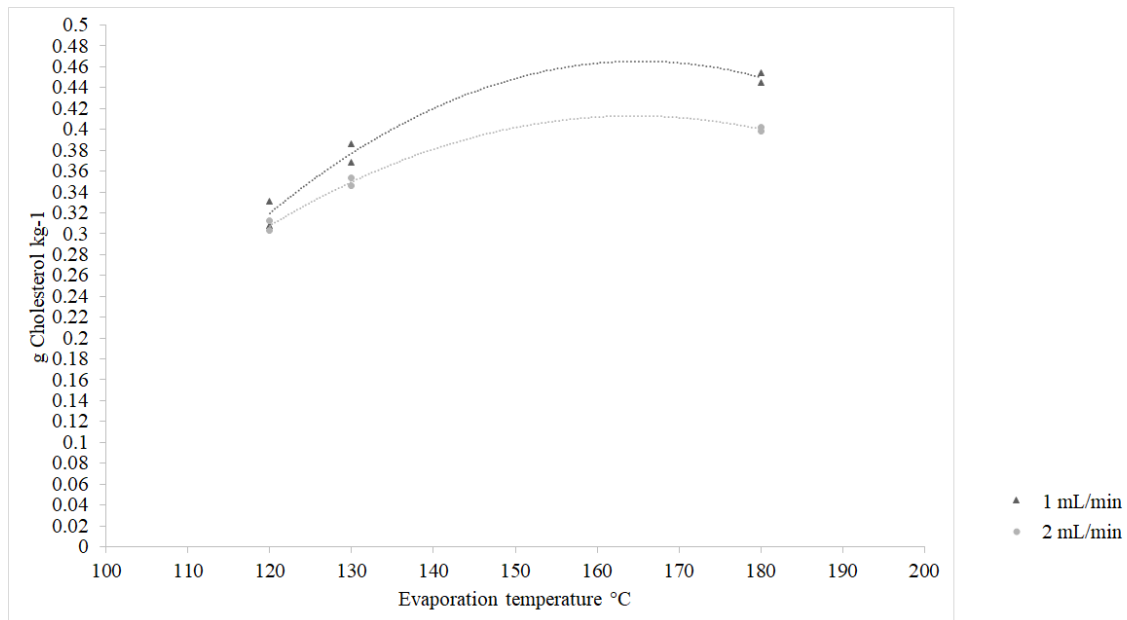
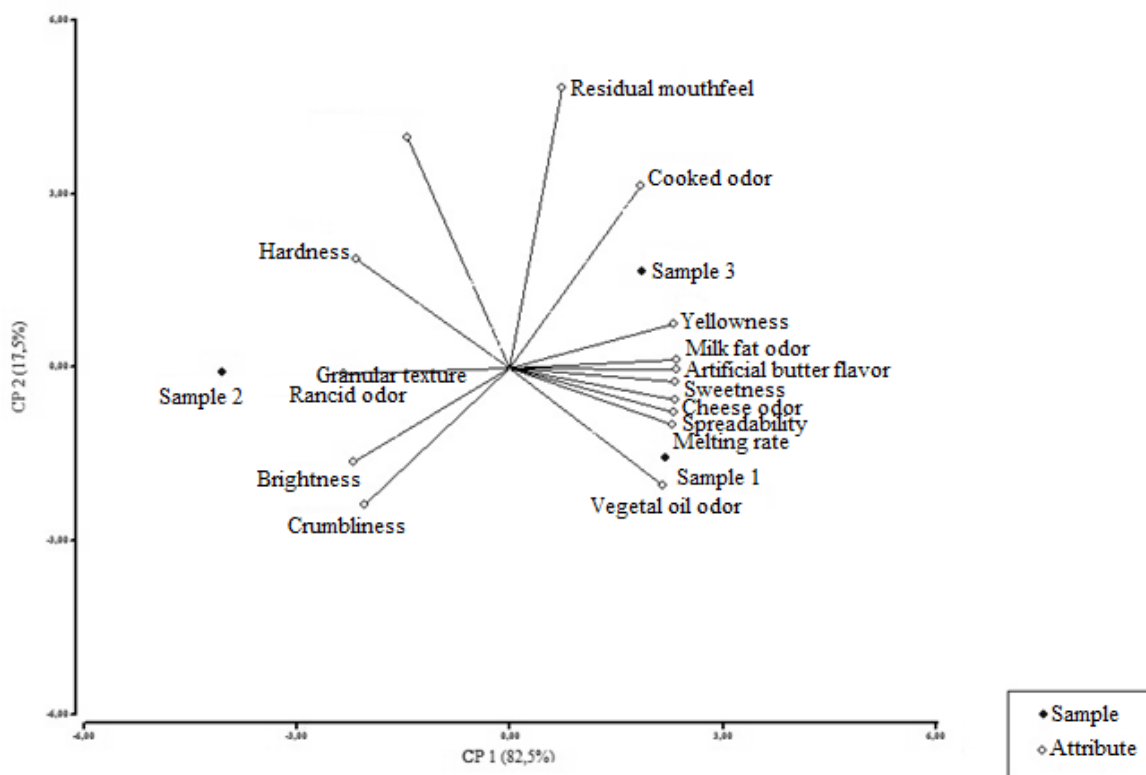
Figure 4. Cholesterol concentration in the distillate for feeding flow variations

Figure 5. Biplot of first and second principal components from different low cholesterol butters* obtained by molecular distillation in association with the sensory attributes



*Sample 1: untreated AMF, Sample 2: residue obtained at 180 °C and 1 mL/min feeding flow, in single operating stage, Sample 3: residue of arrangement number 2

Table 1. Definitions of attributes and standard references used in descriptive analysis of AMF

Attribute	Definition	Reference
Brightness	The amount of reflected light when observing a surface from a 45° angle	Craft paper Transparent vinyl
Yellowness	Yellow scale	Quinoline Yellow (E104) solutions in whole milk
Cooked odor	Odor related to cooked milk and canned corn	Whole milk, heated in a microwave for 3 minutes
Cheese odor	Odor of yellow, mature cheese	Parmesan cheese Butter
Artificial butter flavor	Unique odor of artificial butter.	Butter flavor cooking spray Butter flavor microwave popcorn
Vegetal oil odor	Aromatics related to vegetal oil (associated to possible adulterations)	Sunflower oil Extra virgin olive oil
Milk fat odor	Milk fat flavors and odors. Similar to lactones and coconut.	Cream at a room temperature
Rancid odor	Unique odor of rancid animal fats	Fresh butter Rancid butter (84 days at 70°C)
Granular texture	Size and definition of the granules /sold fat crystals) noticeable when the sample is pressed between the tongue and the palate.	Grated parmesan cheese Common “Type A” Sugar Powdered artificial sweetener (simple size: ½ teaspoon)

Hardness	Force required for introducing a spoon into the butter sample with a 90° angle until reaching the center of the sample.	Melted Fontina cheese Butter Margarine
Crumbiness	Amount of crumbs that fall when the sample is cut with a saw knife in a 90° angle	Margarine Butter Mantecol
Spreadability	Ease of spreading a 0.3 cmx 1 cm layer of butter on toast	Margarine Butter Melted Fontina cheese
Melting rate	Rate at which the butter changes from a solid to a liquid while the sample is melting when it is pressed between the tongue and the palate.	Butter sample Milk chocolate (simple size: 1x1x0.3 cm ³)
Sweetness	Lactose flavor	Lactose solutions (0,05; 0.10 y 0.15 gr/mL)
Residual mouthfeel	Degree of residual mouth coating after expectoration of the sample	Sunflower oil Extra virgin olive oil

Table 2. Cholesterol concentration g kg^{-1} in the residue for evaporation temperature variations

Evaporation temperature [$^{\circ}\text{C}$]	g kg^{-1}
120	$0.2925 \pm 0.0000^{\text{a}}$
130	$0.2670 \pm 0.0001^{\text{b}}$
140	$0.4445 \pm 0.0000^{\text{b}}$
150	$0.2320 \pm 0.0000^{\text{b}}$
160	$0.2317 \pm 0.0000^{\text{b}}$
170	$0.1923 \pm 0.0001^{\text{b}}$
180	$0.2203 \pm 0.0002^{\text{c}}$
190	$0.1922 \pm 0.0002^{\text{c}}$
200	$0.1909 \pm 0.0005^{\text{c}}$

* Scott-Knott test: values followed by the same letter are not significant different ($p < 0.05$)

Table 3. Cholesterol concentration (g kg^{-1}) for successive distillations

		x_D	x_R	% Yield of distillate
Arrangement 1	Stage I	0.4714	0.2981	14.38
	Stage II	0.5607	0.2703	
	Stage III	0.7721	0.2548	
Arrangement 2	Stage I	0.4714	0.2981	30.48
	Stage II	0.6770	0.2236	
	Stage III	0.7922	0.1709	

x_D = Cholesterol concentration in the distillate
 x_R = Cholesterol concentration in the residue

Table 4. Descriptive sensory analysis results

	Sample 1	Sample 2	Sample 3
Brightness	3.16±0.75 ^b	2.16±0.75 ^b	5.66±2.13 ^a
Hardness	7.58±0.66 ^c	8.41±0.49 ^b	9.58±0.80 ^a
Crumbliness	4.25±0.41 ^c	1.50±0.54 ^b	7.91±0.58 ^a
Spreadability	7.00±0.83 ^a	6.33±0.60 ^a	3.83±1.47 ^b
Yellowness	12.00±0.89 ^a	12.66±1.36 ^a	6.75±1.25 ^b
Cheese odor	0.50±0.54 ^a	0.41±0.49 ^a	0.00±0.00 ^a
Artificial butter flavor	7.66±1.21 ^a	7.33±0.81 ^a	2.16±0.75 ^b
Vegetal oil odor	1.50±0.89 ^a	0.83±0.58 ^a	0.00±0.00 ^b
Milk fat odor	3.25±0.98 ^a	3.16±0.75 ^a	0.00±0.00 ^b
Rancid odor	0.87±0.77 ^b	1.12±0.86 ^b	9.83±1.72 ^a
Granular texture	1.95±1.43 ^a	2.95±1.72 ^a	3.08±0.66 ^a
Melting rate	12.75±1.89 ^a	11.08±2.45 ^a	6.08±2.01 ^b
Cooked odor	1.58±0.49 ^b	3.45±1.41 ^a	0.25±0.41 ^c
Sweetness	1.25±0.93 ^a	1.16±0.25 ^{a,b}	0.45±0.33 ^b
Residual mouthfeel	9.16±1.47 ^a	10.66±2.25 ^a	9.41±3.04 ^a

*Values in the same row followed by the same letter are not significantly different ($p < 0.05$, LSD Fischer test).

Sample 1: untreated AMF

Sample 2: residue obtained at 180 °C and 1 mL/min feeding flow, in single operating stage

Sample 3: residue of arrangement number 2

Table 5. Fatty acids estimation

Sample	fatty acids (g kg⁻¹)
Untreated Anhydrous Milk Fat	4.160±0.424
Distillate from experience at 180°C	5.124±0.278
Distillate from arrangement Number I	5.025±0.510
Distillate from arrangement Number II	4.532±0.441