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Lipid phase characterization and reformulation of chocolate spreads to reduce saturated fatty acids

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Abstract

Given the high content of unsaturated fatty acids that high oleic sunflower oil has and thinking in terms of healthiness and innovation, this study aimed to characterize organogels and to investigate the effect of the addition of organogels in chocolate spreads to reduce saturated fatty acids (SFA). The characterization of the organogels was carried out in terms of solids fat content (SFC), crystallization kinetics, thermal behavior, polymorphism, microstructure, hardness, and rheological properties. The organogels analysed showed 5.22% to 6.20% solids at 10 °C, melted at 45 °C, and dropped to < 2.34% at 37.5 °C. Induction time was 4 to 8 minutes, with max solids at 2.29% to 4.72%. The melting and crystallization curves could be subdivided into different regions, which reflected the different classes of triacylglycerol present in the organogels. All organogels showed β polymorphism. The average diameter of the organogels was less than 30 μm, thus minimizing the perception of grittiness in the mouth resulting from the lipid phase. Hardness values varied between 3.53 and 4.58 N. The elastic modulus (G') value was greater than the viscous modulus (G"), indicating the behavior of a solid material. The results of chocolate spreads were characterized by consistency, distribution particle size, rheological properties and dynamic turbidimetry. The range of consistency of spreads ranged from 13.83 to 631.60 gF. All chocolate spreads formulated with organogels showed particle size between 14.59 and 46.23 μm at index d 0.5 and d 0.9, respectively. As for the rheological parameters, the G' value was observed superior to G", indicating properties of solid materials. The chocolate spreads with organogels analyzed showed characteristic stability. There was no exudation of liquid oil in the stabilization period for 36 days, thus the chocolate spreads showed a predominant elastic behavior. This characterization proves to be fundamental for the reformulation of products in terms of greater healthiness.

Keywords: Organogels; Particle size; Dynamic turbidimetry; Structuring agents; Crystal morphologies; Food products; Consistency.

Highlights

- Chocolate spreads formulated with organogels exhibited stability without any liquid oil exudation
- Various properties of chocolate spreads contributed to their stability
- Chocolate spreads with β polymorphism contained imperceptible particles
- The low concentrations of structurants in organogels enable the development of healthier chocolate spreads
- Organogels can be effectively used in chocolate spreads for structuring

1 Introduction

Chocolate spreads are dispersions of cocoa powder and sugar particles in a continuous lipid phase, with a considerable proportion of solid fat. This chocolate-favored paste contains specific rheological properties, as the spreads behave like a solid that prevents sedimentation of dispersed particles and phase separation (liquid oil), but also exhibits high spreading capacity (Patel et al., 2014). These products are very popular due to their flavor and high nutritional and energy value. The popularity of these foods is mainly associated with their potential to arouse sensory pleasure and positive emotions (Macht & Dettmer, 2006).

Chocolate spreads are fat-based products in which its physical properties are dependent on its crystalline structure. Consequently, the oils and fats used in the formulation have a significant effect on the quality of the product (Mayfield et al., 2015).

There is a great risk of exudation and oxidation of the lipid phase in chocolate spreads since the fat used has a liquid phase; and the technological process for obtaining these products includes grinding the ingredients. Thus, all fat trapping systems are required to be efficient, so that the oil is effectively retained in the crystal lattice (Norberg, 2006). Therefore, to reduce the content of saturated fatty acids (SFA), the plastic properties of lipids with different crystallization properties should be explored (Manzocco et al., 2014).

Palm oil and its stearins, with high SFA contents, are partially solid at room temperature and are among the most widely used lipid raw materials for the formulation of chocolate spreads (El-Hadad et al., 2011; Shin et al., 2010). Solid fats (partially hydrogenated vegetable oils or natural fats with high SFA contents) are used for chocolate spread formulations. In addition, lipid stabilizers (high melting triacylglycerols (TAGs) based on fully hydrogenated oils) are often incorporated into the formulation to prevent oil exudation at storage, distribution, and marketing temperatures (Patel et al., 2014).

Saturated fat is the main dietary cause of elevated plasma cholesterol, and reducing its intake is globally endorsed to lower the risk of cardiovascular disease (Wassell et al., 2010). Given the harmful effects of SFA and trans fatty acids on health, actions were carried out that direct the industries to increase food healthiness, by changing the raw materials conventionally used (Santos et al., 2013).

The utilization of organogel technology presents a promising alternative when juxtaposed with traditional lipid modification processes. This innovative approach avoids inducing any chemical alterations within the fatty acids and TAGs, thereby preserving the nutritional attributes of the oil, including the retention of unsaturated fatty acid contents (UFA) and the inherent natural stereospecific distribution, which remains unaltered (Sundram et al., 2007).

In the scope of unconventional structuring of organic phases, lipid systems composed by UFA (such as liquid vegetable oils) can be structured as gels. Such structures form continuous networks of small molecules that gather in liquid crystals, micelles, or fibrillar networks and develop various structural arrangements described in the literature (Pang et al., 2022; Chaves et al., 2019; Okuro et al., 2018; Siraj et al., 2015). This particular type of structuring characterizes systems known as organogels. They are viscoelastic materials composed of structuring agents and a non-polar liquid phase which differs from other gels formed by water-soluble compounds, often denominated as hydrogels. They are semi-solid systems in which an oil phase is immobilized by a self-supporting three-dimensional network of the structuring agent (Dassanayake et al., 2009; Rogers et al., 2009, 2007).

An effective structuring agent requires minimal concentrations to achieve the desired outcome, as higher concentrations can lead to escalated production costs. Furthermore, a comprehensive understanding of the potential physiological effects induced by these additives at elevated concentrations is currently lacking (Co & Marangoni, 2012).

The potential of waxes as structuring agents has become an alternative technique for structuring oils. Different edible structuring oil systems have been intensively researched (Marangoni & Garti, 2011). Candelilla wax is approved as a food additive by the Food and Drug Administration (FDA), recognized as safe (GRAS), and as a food ingredient in the human diet (U.S. Food & Drug Administration, 2016).

Other components, such as emulsifiers, were recently described in the scientific literature with the potential to structure complex lipid matrices (Siraj et al., 2015). Monoacylglycerols (MAG) are lipid molecules comprising only one fatty acid esterified to the glycerol molecule, which can vary in terms of chain size and degree of unsaturation (Chen & Terentjev, 2010). The structuring of vegetable oils by MAG occurs through the self-assembly mechanism, by the formation of micelles or reverse lamellar phases during the cooling of the formed system (Lopez-Martínez et al., 2015; Valoppi et al., 2017; Wang et al., 2016).

Sorbitan monostearate is a non-ionic hydrophobic emulsifying surfactant used to modify crystallization properties in lipid systems (Marangoni & Narine, 2002). This emulsifier demonstrates the ability to form viscous dispersions in organic solvents and edible oils, through the self-assembly mechanism (Co & Marangoni, 2012; Smith et al., 2011). Recent studies on the structuring effect of sorbitan monostearate suggest its use as a potential structuring element in vegetable oils, in addition to a positive interaction with TAGs (Cerqueira et al., 2017; Oliveira et al., 2015b; Sonwai et al., 2017).

A low cost and high potential option for structuring lipid phases is the fully hydrogenated vegetable oils, called hardfats. These components are considered model systems in terms of fatty acid and TAG composition, which represent important factors in determining the structuring and modifying effect of crystallization processes in continuous or emulsified lipid phases (Omonov et al., 2010). In addition to their function as primary agents of crystallization, hardfats act by modifying the physical properties of continuous fat systems, enabling various adjustments related to the development of organogels, a property that has justified a series of studies on the conventional structuring of TAGs (Wassell et al., 2010; Smith et al., 2011).

These structuring agents allow two distinct phases to combine in an almost homogeneous state, modifying even the thermal behavior. The specific structurants, used alone or together, as well as their interactions, determine the structure of the final product, and consequently, its consistency and plasticity properties (Cerdeira et al., 2006; Silva et al., 2018).

The utilization of organogels in food products presents an appealing alternative, as these materials can provide characteristics such as texture and consistency, while simultaneously mitigating the presence of trans fatty acids and substantially reducing the content of SFA in the final product. This represents a significant advancement in the development of food products, showcasing both strong nutritional and technological appeal (Rogers, 2009).

High Oleic Sunflower Oil (HOSO) is considered a premium raw material, generally used in food applications that require the use of liquid oil with exceptional oxidative stability. It has a neutral flavor and aroma, a characteristic associated with its high potential for application in food, cosmetics, and pharmaceuticals (Gunstone, 2005).

In the development of new fat-based products, the biggest challenge is to mimic the sensory properties of the original fat, which are dependent on the lipid crystalline microstructure (Rush et al., 2008). Structural factors such as solid fat content and microstructure determine the macroscopic rheological properties of the final lipid system (Rombaut et al., 2009). Information on the rheological behavior and thermal properties (crystallization and melting behavior) of the lipid phase present in food products can provide better quality control at each processing step (Gonçalves & Lannes, 2010).

This study aimed to characterize different organogels and their respective chocolate spreads, aimed at reformulating the lipid phase of this category of products to reduce the content of SFA.

2 Material and methods

2.1 Material

The HOSO used to formulate the lipid bases was supplied by Cargill Agrícola SA (Brazil); refined Palm Oil (PO) was supplied by Agropalma (Brazil); Candelilla Wax (C) (Light Special Candelilla REAL®) was supplied by the company Multiceras SA, García - NL, Mexico; fully hydrogenated vegetable oil (hardfat) was obtained from palm oil (HP) was supplied by Cargill Alimentos Ltda, Brazil; Monoglyceride (M) Grindsted Crystallizer 100 was supplied by DuPont do Brasil SA, São Paulo – SP, Brazil; and Sorbitan Monostearate (S) was supplied by Sigma Aldrich, USA. The following ingredients were used to prepare the chocolate spreads: União glaçúcar, alkaline cocoa powder Cargill cal 70 and Arcolor hazelnut essence, which were acquired from a commercial supplier, and standard soy lecithin, supplied by Bunge Alimentos SA.

3 Experimental design

3.1 Characterization of organogels

The organogels were prepared by mixing the immobilized phase (HOSO) and the structuring agent (candelilla wax, sorbitan monostearate and monoglyceride (CSM), candelilla wax, sorbitan monostearate, and palm oil hardfat (CSH) and sorbitan monostearate, palm oil hard fat and monoglyceride (SHM) at 6%) under stirring and heating above the melting point of the structuring agent. The heating was turned off and the stirring was sustained for an additional 3 minutes to ensure complete homogenization (Rocha et al., 2013; Stahl et al., 2018). The physicochemical characterization of the organogels was performed regarding solids content, crystallization kinetics, thermal behavior, polymorphism, microstructure, hardness, and rheological properties. All analyzes were performed in triplicates.

Solids fat content (SFC) was determined using a Nuclear Magnetic Resonance (NMR) Spectrometer (Bruker pc120 Minispec). The temperature was controlled with the aid of a high precision dry bath (Tcon 2000, 0-70 °C). Then, the analyzes were carried out according to the AOCS Cd 16b-93 direct method, following sequential sample readings and tempering for unstabilized fats (American Oil Chemists' Society, 2009).

Crystallization kinetics were determined by initially tempering the samples (100 ºC for 15 min, 1 hour at 70 ºC), and the SFC was monitored at 25 ºC ($\pm$ 0.5 ºC) using an NMR Spectrometer (Bruker Mq20 Analyzer) (Wassell & Young, 2007). Data was acquired automatically, with measurements taken every minute, for 1 hour. The characterization of crystallization kinetics was carried out in terms of the induction period (τSC), which is relative to the beginning of crystal formation, and the maximum solid fat content (SFCmax) (Campos, 2005; Stahl et al., 2018).

Thermal behavior was performed using a differential scanning calorimeter (DSC) (TA Q2000, coupled with the RCS90 Refrigerated Cooling System, TA Instruments, Waters LLC, New Castle). The data processing system employed was Universal V4.7A (TA Instruments, Waters LLC, New Castle). The analysis conditions included sample mass: ~ 10 mg; a modified AOCS Cj 1-94 method (American Oil Chemists' Society, 2009): temperature ranging from -60 ºC to 100 °C, with a ramp of 5 °C/min (for both crystallization and melting). The following parameters were used to assess the results: onset temperature of crystallization and melting (T_{oc} and T_{of}), peak temperatures of crystallization and melting (T_{pc} and T_{pf}), crystallization and melting enthalpies (ΔH_c and ΔH_f) and crystallization and melting completion temperature (T_{fc} and T_{ff}) (Barbosa et al., 2018; Campos, 2005).

Polymorphism was determined by X-ray diffraction, according to procedures outlined by Sawalha et al. (2012). The analyzes were performed in a Philips diffractometer (PW 1710), employing the Bragg-Bretan geometry (θ:2θ) with Cu-ka radiation (l = 1.54056 Å, voltage 40 KV and current 30 mA). Measurements were taken with 0.02° increments in 2θ and a 2-second acquisition time, with scans conducted from 15º to 30° (2θ scale) at a temperature of 25 °C. The identification of crystalline forms was performed from the short spacings (SS) characteristic of the crystals (American Oil Chemists' Society, 2009).

The microstructure of the organogels was assessed by microscopy under polarized light (Model BX51, Olympus America Inc., United States) coupled to a digital video camera (Media Cybernetics). A droplet of the sample was placed onto a glass slide, with the aid of a capillary tube, covered with a coverslip, and maintained at a temperature of 5 ºC for 24 hours and then at 25 ºC for 24 hours. The organogels were analyzed at 25 °C with a 20x magnification. Images were captured using the Image Pro-Plus 7.0 software (Media Cybernetics, United States) in four different visual fields of each slide for every sample and the mean particle diameter result was expressed as the mean and standard deviation of these values (De Cindio & Cacace, 1995; Toro-Vazquez et al., 2013).

Hardness was determined using a microcomputer-controlled texturometer (TA-XTi2, Stable Microsystems, England). Thirty milliliters (30 mL) of organogels were placed in 50 mL beakers and conditioned in a BOD oven, at a temperature of 5 ºC, for 24 h throughout the analyzes. A compression/extrusion test was performed employing a cylindrical acrylic probe 25 mm in diameter and 35 mm in length, at a speed of 1.0 mm/s, and a fixed probe penetration distance of 15 mm. The maximum strength achieved was considered for the evaluation (Rocha et al., 2013).

The rheological analyzes were carried out according to the methodology proposed by Rocha et al. (2013) using a controlled tension rheometer (Physica MCR 301, Anton Paar, Germany). A parallel stainless steel plate with a rough surface (50 mm in diameter and 200 µm gap) was used. Temperature was controlled using a Peltier system. Temperature sweeps were performed at a rate of 5 °C/min, ranging from 5 °C to 100 °C, followed by cooling from 100 to 5 °C and reheating from 5 to 100 °C. A frequency (f) of 1 Hz and a deformation of 1% were applied, within the linear viscoelasticity range. The organogels were evaluated by tension and frequency scanning to assess their mechanical strength and behavior under different observation times, respectively. Shear stress tests were performed within the range of 0.1 to 10 Pa (f = 1 Hz). Frequency sweeps spanned from 0.01 and 10 Hz, staying within the linear viscoelasticity range, and matching the stress sweeps range. All analyzes were conducted at 25 °C. The following parameters were determined: elastic modulus (G'), viscous modulus (G''), complex modulus (G*), phase angle (δ), and complex viscosity (η*).

3.2 Characterization of chocolate spreads

Chocolate spread formulations were prepared in an amount of 3 kg, according to the three organogel formulations (Table 1). The characterization of the chocolate spreads included assessments of their consistency, particle size distribution, rheological properties and the dynamic turbidimetry technique.

Organogels were incorporated into the chocolate spreads. The formulation and processing of the standard chocolate spreads were based on Ambiel (2013) to ensure proper incorporation. The ingredients and their respective proportions for the standard formulation and the chocolate spreads with organogel were as follows: Refined Sugar (39.50%), Alkaline Cocoa Powder (25.00%), Palm Oil or Organogel (35.03%), Soy Lecithin (0.40%) and Chocolate Aroma (0.07%).

Table 1. Formulations of the lipid phases of chocolate spreads.

Ingredient (%)	F1	F2	F3	F4	F5	F6	F7	F8
PO	99.60	-	-	-	-	-	-	-
HOSO	-	96.60	93.60	96.60	93.60	96.60	93.60	99.60
C	-	1.00	2.00	1.00	2.00	-	-	-
HP	-	-	-	1.00	2.00	1.00	2.00	-
M	-	1.00	2.00	-	-	1.00	2.00	-
S	-	1.00	2.00	1.00	2.00	1.00	2.00	-
Soy lecithin	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

F1: PO standard; F2: 3% (C, M and S); F3: 6% (C, M and S); F4: 3% (C, HP and S); F5: 6% (C, HP and S); F6: 3% (HP, M and S); F7: 6% (HP, M and S); F8: HOSO standard; PO: palm oil; HOSO: high oleic sunflower oil; C: candelilla wax; HP: palm oil hardfat; M: monoglyceride grindsted crystallizer 100; S: sorbitan monostearate.

The processing to obtain chocolate spreads occurred by mixing dry ingredients (sugar and cocoa powder), which were previously mixed in a Caotech ball mill (Wormerveer, Holland) type CAO - B5. At this stage, the lipid phase considered in the different formulations was added to the dry mixture. The ingredients were homogenized and refined in a Caotech ball mill (Wormerveer, Holland) type CAO - B5, to reduce the particle size at a temperature of 60 ºC. The refined product was placed in plastic containers with a lid and subsequently cooled in BOD at a temperature of 5 ºC for 24 hours and at a temperature of 25 °C for 24 hours for carrying out the analyzes. The physicochemical characterization of chocolate spreads was performed regarding consistency, particle size distribution, rheological properties, and dynamic turbidimetry.

The consistencies of the chocolate spreads were determined by the texture analyzer TA-XT Plus (Stable Micro Systems, Surrey, UK). A cone probe with 45° non-truncated tip angle was used and the penetration depth applied was 10 mm with a probe speed of 2 mm/s. The compression force obtained is given in gram force (gF) (Campos, 2005). The samples were analyzed in quadruplicate and the results expressed as the means of the repetitions.

The particle size distribution of spreads of chocolate was determined using the Horiba LA-960 Laser Particle Size Analyzer – High Performance Laser Diffraction Analyzer. The spreads of chocolate pre-dispersed (0.4 g in 10 mL of sunflower oil) at room temperature (25 ºC) were added directly to the sampling unit until obtaining an obscuration value of 15%. The samples were dispersed with the aid of an ultrasonic bath for 2 minutes to ensure that the particles were individually dispersed and suspended. Then kept under agitation. The particle size distribution was expressed by the values of volume mean diameter D[4.3], median D(0.5) and mode. Parameters D(0.5) and D(0.9) were also evaluated, which represent the diameter values below which are located at 50% and 90% of the accumulated distribution (in volume), respectively (Afoakwa et al., 2009). This analysis was performed in triplicate. As an indication of the amplitude of the particle size distribution, the Span index was used, calculated by Equation 1.

$$Span = \frac{D(0.9) - D(0.1)}{D(0.5)} \tag{1}$$

The rheological analyzes were performed according to the methodology proposed by Rocha et al. (2013) using a controlled tension rheometer (Physica MCR 301, Anton Paar, Germany). A parallel stainless steel plate with a rough surface (50 mm in diameter and 200 µm gap) was used. Temperature was controlled using a Peltier System. Frequency (f) of 1 Hz and deformation of 1% were used, within the range of linear viscoelasticity. The spreads of chocolate were analyzed by voltage and frequency scanning, to assess their mechanical strength and behavior under different observation times, respectively. Shear stress tests were performed from 0.1 to 10 Pa (f = 1 Hz). Frequency sweeps were obtained between 0.01 and 10 Hz, within the linear viscoelasticity range, and in the same range as the voltage sweeps. Both analyzes were conducted at 25 °C. The following parameters were determined: elastic modulus (G'), viscous modulus (G"), complex modulus (G*), phase angle (δ) and complex viscosity (η*). This analyzes was performed in triplicate.

3.3 Dynamic turbidimetry technique

The kinetic stability of the chocolate spread was monitored using infrared light backscatter (NIR) near 880 nm (Turbiscan LAB, Formulaction, Ramonville St. Agne, France). For this, 15 mL of chocolate spread were transferred immediately after processing to borosilicate glass tubes (12 mm in internal diameter and 30 mm in height). The light source digitized the samples at intervals of 0, 8, 15, 22, 29 and 36 days and measured the percentage of backscattered light at a temperature of 25 °C. The backscatter variation (ΔBS) was determined from the difference in backscatter between control samples (spread of chocolate produced with OP and HOSO without structuring) and spreads of chocolate with organogel. Data was analyzed using Turbisoft 2.0 software. This analysis was performed in triplicate (Zhao et al., 2014).

3.4 Statistical analyzes

The objective results were evaluated using the statistical program Statistica 8.0 - Statsoft, USA (StatSoft, 2007) to calculate the regression coefficient, probabilities (p-value) and analyzes of variance (ANOVA) with a significance level of 5%. The same program was used to compare means to compare chocolate spreads using the Tukey's test.

4 Results and discussion

4.1 Characterization of organogels

4.1.1 Solids fat content (SFC)

Figure 1 presents the solid profiles determined for the organogels between 10 °C and 50 °C. The organogels showed SFC ranging from 5.22% to 6.20% at 10 °C. All showed complete fusion at 45 ºC and SFC less than 2.34% at 37.5 °C. In this range, the SFC must be less than 5%, to minimize a possible waxy sensation (Oliveira et al., 2014; Masuchi et al., 2014). The organogel SHM showed an SFC of 6.20% at a temperature of 10 ºC, higher than the total content of structurants (6%) added in the formulation, followed by CSM (5.70% solids). These formulations contain 2% of M and probably due to the different compositions in triacylglycerol classes of M, this structurant may have induced fraction crystallization triacylglycerol saturated from HOSO. The CSH organogel showed lower SFC at a temperature of 10 °C (5.22% solids), a percentage lower than the 6% of structurants added to the lipid phase. This formulation contains 2% S, which in previous studies showed lower SFC in isolated or combined form with C or M.

The SFC at different temperatures describes the melting profiles of organogels and, therefore, qualifies the organogels for application in chocolate spreads. This analyzes determined the solid fraction of each organogel during fusion. At low temperatures (4 to 10 °C), SFC values typify the spreadability of refrigerated organogels. At room temperature (20 to 22 °C), a minimum of 10% solid fat is essential to guarantee the resistance to oil exudation and stability of the products when referring to conventional fat. The SFC of organogels ranged from 4.69 (CSM) to 5.43% (SHM) at 20 ºC, since they are non-conventional lipid bases. At temperatures between 30 °C and 35 °C, general purpose fats, such as palm oil, are distinguished by melting, with concomitant release of flavor; and SFC provides an estimate of sensory attributes in the tasting (Wassell & Young, 2007). Body temperature (37.5 ºC) is critical for the sensory quality of lipid-based products. During lipid phase crystallization, materials with similar chemical composition and molecular structure are more prone to co-crystallization (Marangoni, 2005).
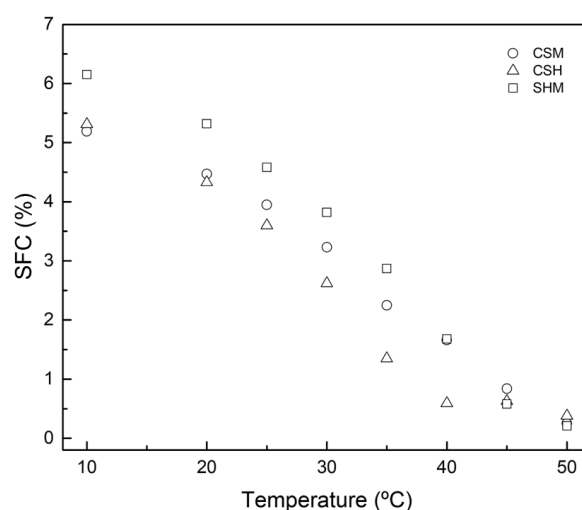

Figure 1. Solid profile of organogels. SFC: solids fat content; CSM: candelilla wax, sorbitan monostearate, monoglyceride; CSH: candelilla wax, sorbitan monostearate, palm oil hardfat; SHM: sorbitan monostearate, palm oil hardfat, monoglyceride.

4.1.2 Crystallization kinetics

Table 2 shows the induction time (IT) and the SFCmax at 25 ºC of the organogels. IT ranged from 4 to 8 minutes and SFCmax from 2.29 to 4.72%. The CSH organogel showed slower crystallization, with IT of 8 minutes, followed by CSM (6 minutes) and SHM (4 minutes) organogels. The SFCmax at 25 ºC of the SHM organogel was the highest, probably due to the different compositions in the triacylglycerol classes of M, in agreement with the results of SFC.

The induction period is the time taken by the system to crystallize. The lower the induction time is, the faster the formation of lipid crystals will be. Another factor associated with crystallization is the incorporation of low concentrations of structuring agents into organic solvents, which grants good gelling properties to the organogel (Hwang et al., 2014; Rocha et al., 2013; Wang et al., 2016). The formation of stable triacylglycerol crystals is also linked to crystallization, thus being an important indicator of thermal stability, as well as the induction of solid material formation in unsaturated systems (Godoi et al., 2019).

Table 2. Induction time, maximum solids and SFC at 25°C preferential polymorphism and hardness of organogels.

Organogel	IT (minutes)	SFCmax at 25 °C (%)	Short spacings (Å)			SS	Hardness (N)
			4.6	4.2	3.8		
CSM	6 ± 0.71	3.50 ± 0.05	4.48	4.14	3.72	β	4.35 ± 0.47
CSH	8 ± 1.41	2.29 ± 0.03	4.51	4.16	3.71	β	4.58 ± 0.43
SHM	4 ± 0.00	4.72 ± 0.16	4.52	n.d	n.d	β	3.53 ± 0.15

IT: induction time; SFCmax at 25 °C: maximum solid fat content at 25 °C; SS: short spacings; CSM: candelilla wax, sorbitan monostearate, monoglyceride; CSH: candelilla wax, sorbitan monostearate, palm oil hardfat; SHM: sorbitan monostearate, palm oil hardfat, monoglyceride; n.d: not detected.

4.1.3 Thermal behavior

Table 3. Thermal behavior of organogels.

	Crystallization							
Samples	**Peak 1**				**Peak 2**			
	T_{oc} (°C)	T_{pc} (°C)	T_{fc} (°C)	H_c (J/g)	T_{oc} (°C)	T_{pc} (°C)	T_{fc} (°C)	ΔH_c (J/g)
CSM	46.22	26.23	7.80	2.85	-32.33	-37.93	-49.92	40.36
CSH	36.79	15.14	3.05	3.47	-31.70	-38.30	-50.60	42.81
SHM	25.53	19.75	7.07	1.90	-30.38	-33.95	-50.04	53.31
	Fusion							
Samples	**Peak 1**				**Peak 2**			
	T_{of} (°C)	T_{pf} (°C)	T_{ff} (°C)	H_f (J/g)	T_{of} (°C)	T_{pf} (°C)	T_{ff} (°C)	ΔH_f (J/g)
CSM	-14.24	-4.24	7.23	52.25	18.18	26.37	59.73	2.49
CSH	-15.51	-4.95	11.45	53.69	26.31	33.71	46.56	1.49
SHM	-15.00	-4.40	8.16	55.85	28.28	38.49	51.81	2.93

CSM: candelilla wax, sorbitan monostearate, monoglyceride; CSH: candelilla wax, sorbitan monostearate, palm oil hardfat; SHM: sorbitan monostearate, palm oil hardfat, monoglyceride.

The thermal behavior of the organogels is shown in Table 3. The melting and crystallization curves can be subdivided into different regions, reflecting the different triacylglycerol classes present in the organogels.

As for the thermal behavior in crystallization, the CSM, CSH, and SHM organogels showed lower onset temperature of crystallization (T_{oc}) (46.22, 36.79 and 25.53 ºC, respectively), which is consistent with the data presented in Table 2 related to crystallization times of 6, 8, and 4 minutes for the organogels, respectively. Probably due to the high concentration of TAGs with the highest melting point of H, which is

the structurants present in the highest concentration. Hardfats play an interesting role in the structuring of TAGs, due to their insolubility, or limited solubility, in polyunsaturated vegetable oils and the ability to form a solid network of crystals. The different types of hardfats have similar melting characteristics. High melting point, high melting enthalpy compared to conventional fats and the ability to form a matrix that will crystallize at high temperatures forming a fine dispersion of stable crystals (Alander & Lidefelt, 2007; Norberg, 2006). The presence of hardfats as additives modifies the crystalline habit and alters the crystallization behavior, thus reducing the crystallization induction period and acting as crystallization germs (Oliveira, 2011). This behavior is related to the physical characteristics and fatty acid composition of the oil from which the hardfat was obtained (Alander & Lidefelt, 2007).

The crystallization curves showed two broad peaks (representing fractions of low and high melting point components) and overlapping. They are associated with the presence of these different triacylglycerol classes (Manzocco et al., 2014; Saberi et al., 2011). All structurants co-crystallized with each other and with HOSO. The thermal behavior parameters of the organogels indicated a first melting peak. Related to the more unsaturated TAGs, probably from the HOSO, and a second melting peak related to the structurant(s) used and also to the saturated fraction of the HOSO.

4.1.4 Polymorphism

The typical fat content of chocolate spreads is approximately 30%, characterizing the product as a continuous fat system, where sugar and other particles are dispersed, making the properties of the fat have a great influence on the sensory behavior, providing creaminess and softness. Typically, β-polymorphic-prone fats form large crystals and provide desirable snap in chocolate products, while β'-type fats promote small crystals and a smooth feeling during tasting (Norberg, 2006).

Crystalline forms are characterized by specific short spacings (SS) (Table 2). The characteristic SS corresponds to 4.15 Å for the polymorphic forms α, 3.8 and 4.2 Å for β' and 4.6 Å for β, respectively; and are used to determine the relative proportion and type of polymorphs present in lipid bases (Stahl et al., 2018).

For all organogels, SS equal to 4.6 Å was verified, characterizing the β polymorphic habit. Although structured M has a high concentration of behenic acid (C22:0) (Silva et al., 2018), which would favor the β polymorphic habit, its molecular structure is heterogeneous due to the regiospecific distribution typical of MAG. Tripalmitin and behenic acid MAG act as accelerators in the palm oil crystallization process, very important in the chocolate industry, where β-type crystals are desired (Basso et al., 2010). However, in the lipid phase of spreads and margarines, the crystals should preferably be stabilized in the β' form to favor the spread of the product (Wassell & Young, 2007), however, as organogels contain a high amount of HOSO in the formulation, they already have greater spreadability due to the high content of AGI, regardless of the polymorphic form.

The polymorphism results reported in this study surpassed those of Fayaz et al. (2017), which replaced 50% of the palm oil with oleogels (5%) of monoglyceride (MAG), beeswax (BEW), and propolis (PPW) in the chocolate spreads. Findings showed that in PO oleogel systems, the chemical properties of MAG, BEW, and PPW contributed to the formation of diverse crystalline networks. Nonetheless, all samples did exhibit a β'-polymorphic form.

4.1.5 Microstructure

Lipid composition and crystallization conditions influence the crystal shape, and different polymorphic forms and crystal morphologies are possible. Crystals are aggregated into larger structures forming a network, which characterizes the microstructural level of fat. The concept of microstructure includes information about the state, quantity, shape, size, spatial relationship, and interaction between all components of the crystal lattice, and has an enormous influence on the macroscopic properties of fats (Marangoni & Hartel, 1998; Oliveira et al., 2015a; Ribeiro et al., 2009; Shi et al., 2005).

TAG usually crystallizes as spherulites, which correspond to the aggregation of crystalline lamellae, which grow radially from the same central nuclei and can develop branches during ripening (Rousset, 2002). Eventually, depending on the cooling conditions or even the characteristic melting profile of each fat, the TAGs can also crystallize in other morphologies, such as needles and discs (Oliveira et al., 2015a).

Organogels can be structured forming a 3D fibrous network, where the solvent is trapped in the structuring matrix, preventing solvent flow. The network is stabilized by weak interactions between the chains, such as hydrogen bonding, van der Waals forces, and π staking (Huang et al., 2014; Lupi et al., 2016; Pirner et al., 2016; Simsolo et al., 2018). Although it is already known that organogels are formed through weak intermolecular interactions between structurant molecules, which generate three-dimensional networks (Steed, 2011), there is still a lack of fundamental understanding regarding the type of interactions that are needed (Nikiforidis et al., 2015). The association between self-assembly and structuring of crystalline particles in vegetable oils constitutes hybrid systems with a high potential for the formation of organogels.

The CSM and CSH organogels have a higher number of crystalline elements (43845 and 47367, respectively) and a lower mean density (13.95 and 11.76 µm, respectively), which is directly related to higher IT and hardness and lower SFCmax values at 25 ºC (Table 2). The average diameter of the organogels was less than 30 µm, ranging from 1.30 (CSM) to 1.93 (CSH) µm, minimizing the perception of sandiness in the mouth resulting from the lipid phase (Beckett, 2008).

The organogels containing C showed the lowest net density in contrast to the SHM organogels, which had higher mean density and higher mean D crystals (18.94 µm). The CSM and SHM organogels showed smaller average crystal diameters (1.30 and 1.36 µm, respectively), evidencing that the presence of M as a structuring agent induces the formation of smaller crystals. Generally, crystal size is related to the firmness resulting from lipid bases, with smaller crystals being more resistant than larger crystals (Hwang et al., 2012), however, as the crystals of the organogels have a size smaller than 1.93 µm and little variation between them, it was possible to correlate the hardness with the average density of the crystals, and the greater the hardness of the organogels is, the greater the average density will be.

Hardfats Specific, derived from a certain oil source, presents a unique and differentiated triacylglycerol profile, which characterizes these materials as inducers of particular polymorphic habits. After the cooling of a lipid mixture added with hardfats, its trisaturated TAGs, with a high melting point (65 °C to 75 °C), promote the formation of crystallization nuclei for the ordering of a highly structured crystal lattice from the liquid system (Pernetti et al., 2007), as it was possible to observe for the organogel with CSH, as it presented the highest percentage of agglomerated crystals (23.64%) and the highest hardness (4.58 N) (Table 2).

4.1.6 Hardness

Hardness is the resistance of a material against permanent deformation, and its magnitude measures the mechanical strength of a structure as a result of the interaction forces between the components of the material (Walstra, 2003). The hardness values of organogels are presented in Table 2. Values ranged between 3.53 and 4.58 N, where the minimum value corresponded to organogel with SHM and the maximum to organogel with CSH. This characteristic can be favorable to lipid applications, where hardness and texture are important and can be beneficial in replacement of fats that require increased fluidity for products such as spreads (Manzocco et al., 2014).

The hardness results were inversely proportional to the SFC at 25 ºC. Higher percentages of agglomerated crystals can induce the formation of organogels with greater hardness. Organogels with these properties also exhibited a greater number of crystalline elements, as typical characteristics of more cohesive and harder crystal lattices (Campos, 2005). In general, the hardness parameters of organogels can be associated with the formation of small fat crystals dispersed in a high proportion of liquid oil, promoting the formation of less cohesive crystalline networks.

Organogels formulated with C (CSM and CSH) resulted in gels with greater hardness (4.35 and 4.58 N, respectively), being indicated for use in hybrid systems, this information is compatible with what is observed in the literature (Doan et al., 2015; Rocha et al., 2013; Toro-Vazquez et al., 2009).

4.1.7 Rheological properties

The frequency scan analysis to investigate the deformation behavior of organogels within the linear viscoelastic region is shown in Figure 2. All organogels showed similar behavior and frequency independent properties, with the G' value being higher than the G", indicating the behavior of solid material (Steffe, 1996). Frequency scan analyzes showed that CSH organogel had higher G' and G" values than SHM and CSM organogels, results consistent with hardness (Table 2) and number of crystalline elements.

The temperature scanning analyzes of the organogels can indicate a similar behavior, through the apparent viscosity, with an increase in the G' and G" within the same temperature range, with a value observed from G' higher than G", as observed for frequency sweep analyzes (Figure 2).
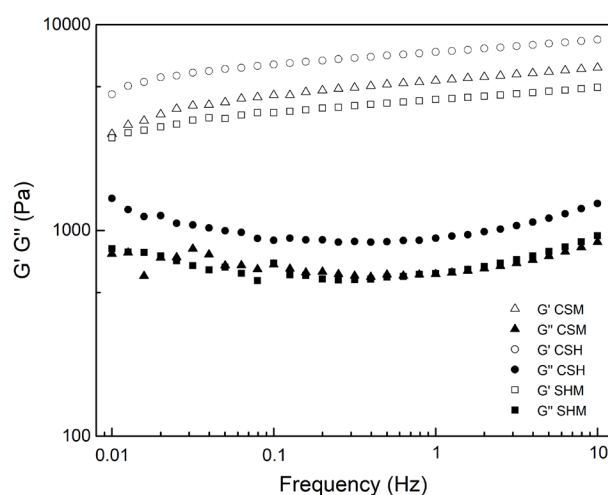

Figure 2. G' and G" of the organogel frequency scan. G': elastic modulus; G": viscous modulus; CSM: candelilla wax, sorbitan monostearate, monoglyceride; CSH: candelilla wax, sorbitan monostearate, palm oil hardfat; SHM: sorbitan monostearate, palm oil hardfat, monoglyceride.

The results obtained in the temperature scan analyzes of the organogels showed that the behavior was the same as observed for the thermal behavior with similar start and end values of crystallization and melting (Table 3). The results indicate that the first peak observed for thermal analysis is related to the disruption of the organogel network. Results are consistent with other work that evaluated the rheological behavior of organogels (Rocha et al., 2013).

The crystallization temperatures were also higher than the temperatures found for melting, and the crossing of G'-G" can be observed, which can be used as a simple criterion for the gel point (Rocha et al., 2013). The gelation temperature refers to the phenomena of crystallization and the aggregation of crystals into clusters (Lupi et al., 2012). When the molten system is cooled, α-shaped fat crystals are obtained, and their size and number increase with decreasing temperature. Furthermore, during the cooling process, potential transitions ($\alpha \rightarrow \beta'$ polymorphic transformation) and aggregations occur forming a three-dimensional crystal lattice (Campos, 2005; Marangoni, 2005)

During heating, the values of G' and G" decreased with increasing temperature, but during the cooling of the organogels the values increased, probably due to the recrystallization and reorganization of the three-dimensional network of the organogels. The temperature scan shows that organogels can be used as fat substitutes in industrial processes using shear, even if the process temperature is higher than the organogel formation temperature, as they are thermo-reversible, as reported by other researchers (Alvarez-Mitre et al., 2012; Dassanayake et al., 2011; Pernetti et al., 2007; Smith et al., 2011).

4.1.8 Characterization of chocolate spreads

4.1.8.1 Particle size distribution

Table 4 presents the solid particle size distribution parameters, including Span (µm), d 0.5 (µm) and d 0.9 (µm), which corresponds to the maximum diameter for 50% and 90%, respectively, of the distribution of the chocolate spread particles.

Table 4. Particle size distribution and consistency of chocolate spreads.

Samples	Span (µm)	d 0.5 (µm)	d 0.9 (µm)	Consistency (gF)
F1	11.61 ± 1.34 [a]	18.05 ± 1.68 [a]	212.09 ± 8.57 [a]	631.60 ± 207.45 [a]
F2	2.95 ± 0.13 [ab]	14.59 ± 0.36 [ab]	46.23 ± 1.32 [b]	15.13 ± 1.65 [b]
F3	2.48 ± 0.05 [ab]	9.43 ± 0.39 [b]	26.57 ± 0.65 [e]	16.90 ± 1.04 [b]
F4	2.78 ± 0.19 [ab]	11.66 ± 0.45 [ab]	35.60 ± 3.26 [d]	13.83 ± 1.07 [b]
F5	3.09 ± 0.47 [ab]	13.50 ± 0.58 [ab]	45.03 ± 0.80 [bc]	14.07 ± 1.86 [b]
F6	3.02 ± 0.28 [ab]	11.21 ± 0.33 [b]	36.98 ± 2.87 [cd]	15.27 ± 1.55 [b]
F7	2.29 ± 0.05 [b]	9.34 ± 0.19 [b]	24.47 ± 0.80 [e]	15.20 ± 3.38 [b]
F8	2.72 ± 0.11 [ab]	11.10 ± 0.26 [b]	33.27 ± 0.77 [de]	N.d

F1: PO standard; F2: 3% (C, M and S); F3: 6% (C, M and S); F4: 3% (C, HP and S); F5: 6% (C, HP and S); F6: 3% (HP, M and S); F7: 6% (HP, M and S); F8: HOSO standard; N.d: not detected.

Spreads of chocolate are refined to a particle size of less than 30 µm (Beckett, 2008) and the final particle size critically influences rheological and sensory properties (Afoakwa et al., 2007). Lipid particles above 30 µm cause a sandy texture in the mouth and particles smaller than 20 µm are substantially smooth and creamy. Particle size distribution plays a clear role in product fluidity but is generally restricted to empirical knowledge based on experience (Beckett, 2008). The values of the Span index showed a significant difference, at the level of 5%; and ranged between 2.29 (F7) and 11.61 (F1) µm. The remaining formulations did not show a significant difference at the 5% level.

All organogel chocolate spreads showed particle sizes below 14.59 and 46.23 µm at d 0.5 and d 0.9, respectively; therefore, the evaluated chocolate spreads with organogel probably have a low sensory perception for sandiness, since the particle size distribution has a direct influence on this characteristic and on the rheological properties in chocolates (Afoakwa et al., 2007). However, F1 exhibited 18.05 and 212.09 µm for d 0.5 and d 0.9, respectively, which may have occurred due to the presence of SFA in the composition of palm oil.

Tan & Kerr (2018) evaluated the size and distribution of chocolate particles and showed d 0.5 of 11.63, 7.64, 5.93 and 5.16 µm for the refining times 15, 30 minutes, 4 and 8 hours, respectively and d 0.9 of 46.41, 30.67, 15.50 and 11.75 µm for the 15, 30 minutes, 4 and 8 hours of refining, respectively.

4.1.9 Consistency

The consistency values of chocolate spreads are shown in Table 4. Values ranged between 13.83 and 631.60 gF, with the maximum value corresponding to the spread of chocolate with standard lipid phase containing palm oil (F1), the standard spread with HOSO presented instability and, therefore, it was not

possible to detect a minimum reading value in the equipment. F1 showed a significant difference, at the 5% level, compared to the other formulations. Chocolate spreads with organogel showed consistency ranging between 13.83 and 16.90 gF, this characteristic is favorable to the fluidity that the product requires. These results match the values of Span, d 0.5 and d 0.9 of chocolate spreads (Table 4).

However, in the alternative chocolate spreads designed as chocolate palm oil replacers, the concentration of the structuring agent is as low as 3 and 6%. Despite the considerably lower consistency values of spreads containing organogels in their formulations compared to F1, this formulation had a higher concentration of SFA due to the presence of palm oil. The chocolate spreads exhibited stability over time, which can be confirmed through frequency scan analysis and dynamic turbidimetry techniques.

4.1.10 Frequency scan

The analyzes of frequency scans to investigate the deformation behavior of chocolate spreads within the linear viscoelasticity region are shown in Figure 3. The chocolate spreads showed a similar behavior, with a higher value of G' than G", indicating a behavior of solid material. F1 showed higher consistency between samples, the impossibility of frequency scan analysis for this palm oil standard chocolate spread. Thus, the spreads of chocolate with organogel showed a predominant elastic behavior, as in the results observed by Doan et al. (2016), Fayaz et al. (2017), Patel et al. (2014) and Pușcaș et al. (2022).
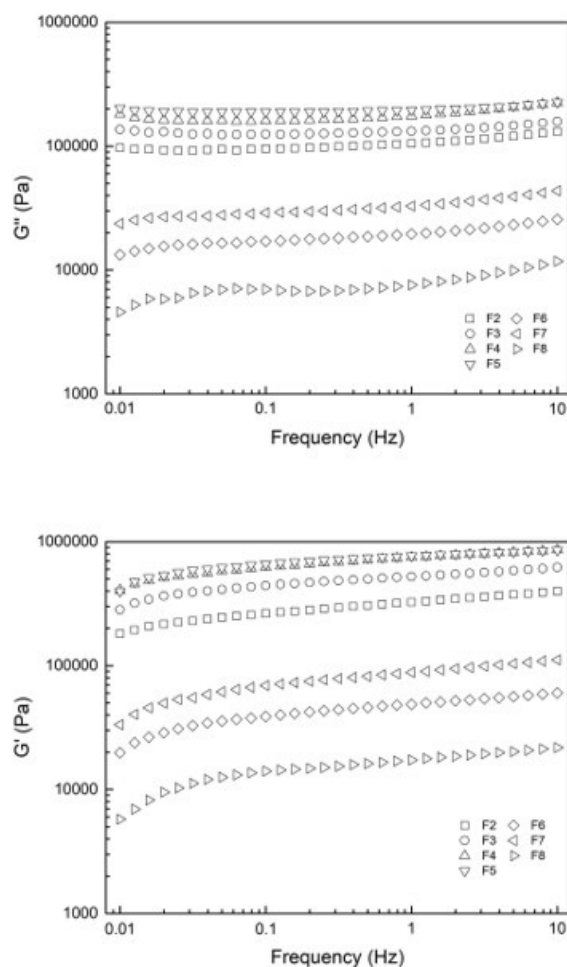

Figure 3. G' and G" of chocolate spread frequency scan. G': elastic modulus; G": viscous modulus; F2: 3% (C, M and S); F3: 6% (C, M and S); F4: 3% (C, HP and S); F5: 6% (C, HP and S); F6: 3% (HP, M and S); F7: 6% (HP, M and S); F8: standard HOSO.

Chocolate spreads with organogel had similar G' and G" values and F8 showed the lowest value for G' and G", results according to the consistency of the chocolate spreads (Table 4). It was observed that the storage modulus of the spreads dominated over the loss modulus during the frequency sweep test. The G' slightly increased over the frequency range, but with low dependence to the frequency. Thus, all the samples showed a semi-solid behavior with good tolerance to deformation.

4.1.11 Dynamic turbidimetry technique

The stability of the lipid phase of chocolate spreads can be monitored by scanning in the near infrared (880 nm) wavelength. Backscatter values were determined from the bottom (5 mm) to the top (35 mm) of the tubes. During the time analysis of the stability of chocolate spreads, the maximum difference of *backscatter variation* - ΔBS was 1%, indicating a low scatter of readings throughout the tube. Thus, the mean BS values obtained in scans from 5 to 35 mm were used, and these values were plotted throughout the experiment to obtain the curves.

The instability of the F8 observed by the ΔBS was probably due to the absence of structurants, results consistent with consistency (Table 4) and frequency scanning (Figure 3) of the chocolate spreads. The other spreads showed low variation in ΔBS, indicating that the use of organogels as a substitute for conventional lipid bases in chocolate spreads was efficient, as they presented similar behavior to the standard (F1) that contained palm oil, during the analyzes time (0, 8, 15, 22, 29 and 36 days), in addition, the concentration of 3 or 6% of structurants was indifferent concerning stability and these results are consistent with the consistency and frequency scanning of chocolate spreads, thus it is possible to produce a quality product using a low concentration of builder.

Spreads of standard chocolate and spreads prepared by total and partial replacement of palm oil (27%) with shellac organogels were evaluated for viscosity, with parameters similar to commercial spreads. Standard spreads and formulated with organogel did not show exudation when stored at 30 °C for more than 4 weeks (Patel et al., 2014).

5 Conclusion

The combination of structuring agents for the formation of CSM, CSH, and SHM organogels at 6% led to the creation of organogels. These products exhibited solid fat content profiles suitable for use in chocolate spreads and the desirable β polymorphic habit for chocolate spreads. Microstructural analysis of the organogels revealed the formation of a three-dimensional network that plays a fundamental role in their consistency.

The rheological properties of the organogels were in line with their thermal behavior, indicating their potential as substitutes for fats in industrial processes. Characterization of chocolate pastes formulated with organogels showed particle size distributions and consistencies conducive to smooth and creamy products. Frequency scans and dynamic turbidimetry techniques confirmed the stability of these chocolate pastes, underscoring the efficiency of organogels as substitutes for conventional lipid bases.

The chocolate spreads with organogels analyzed in this study showed characteristic stability since there was no exudation of liquid oil in the stabilization period at 25 °C for 36 days, a result that can be associated with the other properties of chocolate spreads, such as SFCmax at 25 °C, consistency, particle size and distribution, β polymorphism, the average density of crystals and behavior of solid material since they presented G' greater than G". Also, the β polymorphism, along with particle size less than 14.59 μm in d 0.5, present in the spreads of chocolate with organogel in this study, provides particles that are not sensory perceptible and it was possible to develop a product that meets the demand of consumers looking for healthier foods, using organogels with a low concentration of structurants. All organogels (CSM, CSH and SHM) can be used for application in chocolate spreads in concentrations of 3 and 6% of structuring.

In conclusion, the study supports the feasibility of using organogels to reformulate chocolate pastes, reducing saturated fat content while maintaining product quality and stability. These findings have significant implications for the development of healthier options for chocolate spreads that align with consumer preferences in terms of both texture and nutritional characteristics.

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