Physicochemical profile of commercial margarines marketed in Brazil and the impact of *trans* fatty acid elimination

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Abstract

This study aimed to evaluate the physicochemical composition, the lipid profile, and the technological and nutritional impact of Brazilian margarines. Thirteen margarine samples were characterized for fatty acid composition, technological properties, and thermal stability. The margarines presented total fat from 20 to 82% and low *trans* fatty acids (TFA) levels, except for two samples (5-7% of elaidic acid), allowing labels to declare "zero *trans*-fat", according to the current Brazilian legislation (Resolution 54 of November 12, 2012). All margarines had similar fatty acids composition, with predominance of linoleic (23-46%), oleic (20-46%) and palmitic acids (7-14%), probably from soy and palm-based fats. The saturated fat content ranged from 23 to 31%. All lipid phases had a similar fat solid profile, with a melting point between 22 and 28 °C. The margarines with higher lipid content and saturated fatty acids (SFA) showed greater hardness and lower spreadability, and the presence of TFA provided greater plasticity. Changes in the lipid profile of this product are still necessary, as there is a global transition scenario aimed at healthy aspects with the elimination of TFA and reduction of SFA intake to reduce deaths from coronary heart disease.

Keywords: Margarines, Saturated fatty acids, *Trans* fatty acids, Melting point, Partially hydrogenated fat, Cardiovascular diseases.

1. Introduction

Margarines are considered water-in-oil emulsions that have been produced as a more economically viable alternative to butter [1]. The emulsion consists of at least 80 wt% fat and at most 16 wt% aqueous phase and can be classified based on the fat content into high-fat (70-82 wt%), medium-fat (48-60 wt%), low-fat (35-42 wt%) and spreads (<30 wt%) [2].

The solid characteristic of margarines is provided by fat crystal aggregates and water droplets trapped in the crystalline matrix [3]. This network of solid fat crystals provides structure and contributes to the stability of the product [4]. The sensory properties of margarines (spreadability, overall appearance, and organoleptic properties) are determined by the composition and microstructure of the product (i.e. shape, size, number of fat crystals and water droplets, and strength of the crystal network). The microstructure is determined by the interaction between the product composition and the production process [3]. High-quality margarine melts quickly in the mouth, with no oil loss or persistent wax [5]

For many years, margarines have been produced from partially hydrogenated fat (PHF). This process gives vegetable oils physical properties of saturated fats such as higher melting point, plasticity, thermal and oxidative stability. However, the partial hydrogenation leads to the formation of geometric and positional fatty acids isomers, that is, cis isomers are converted to *trans*, which are more thermodynamically stable [6–8]. Epidemiological studies have shown that TFA have adverse effects on the development of cardiovascular diseases, as they lead to an increase in total cholesterol and low-density lipoprotein (LDL) levels, and a decrease in high-density lipoprotein (HDL) [9, 10]. In Brazil, margarines are considered the food with a great contribution of TFA consumption, representing more than 30% of the total intake, followed by stuffed cookies [11].

According to the World Health Organization (WHO), several countries have already eliminated the use of PHF in processed foods. Denmark was the first country to impose this restriction, leading to a drastic decrease in the number of deaths from cardiovascular disease. Diets rich in TFA increase the risk of heart disease by 21% and deaths by 28%. In view of this, the WHO has called on governments to eliminate industrially-produced *trans* fat from the food supply by 2023 [12]. In Brazil, industries must adapt not to use PHF in food, Resolution 332 of 23 December 2019 [13]. The elimination of the global food supply containing PHF has been considered as one of the priority goals of the WHO strategic plan, a project of the 13th General Programme of Work (GPW13), which will guide WHO's plan from 2019 to 2023 [12].

Several industries have developed foods with interesterified fat as an alternative to partial hydrogenation, and margarines were the first lipid-based products to undergo this modification. However, studies have reported the development of isomers with SFA at the *sn-2* glycerol position,

rather than the typical regiospecific distribution of fatty acids in unmodified oils [7, 8]. Thus, whereas TFA causes negative health impacts, the high intake of SFA promotes an increase in the production of LDL, VLDL and platelet aggregation, with an impact on atherogenesis and cardiovascular events [9, 10].

The replacement of fats in food products is quite challenging, once they provide excellent organoleptic properties and are also responsible for the physical characteristics (hardness, spreadability, and melting) and stability of colloidal systems [14, 15], which is observed mainly in high-fat foods, like margarines. Several countries have shown concern with the lipid profile of commercialized margarines. In the American market, in 2013, only 15% of margarines contained PHF with a concentration of 0.1-21.7% TFA and 0.5-46% SFA [16]. In Latin America, conventional margarines have a TFA content of approximately 28.6% [17]. In the European market, around 3% of margarines have lower TFA levels, as reported by some authors in Portugal [18, 19]. Often, these data do not correspond to the levels reported in the nutrition labels of the product. Therefore, the present study evaluated Brazilian margarines with different lipid contents and their respective lipid phases, concerning the fatty acids composition and the physical properties of fats used, and their effect on the final product aimed to identify possible alternatives to make these products healthier and with appropriate technological properties.

2. Material and Methods

2.1. Sampling

Thirteen representative brands of margarine available in different Brazilian supermarkets, widely consumed by the Brazilian population, were randomly selected.

The margarines were classified concerning the total fat levels as low-fat (lower than 50%, corresponding to the samples A20, B20, C35, D36, E38, and F50) and high-fat (higher than 50%, corresponding to the samples G51, H55, J65, K70, L80, M80, and N82). The different margarines were coded with capital letters accompanied by a number representing the total fat levels of the respective sample (Example: the sample coded as A20 referred to margarine with 20% total fat). All margarines were stored at 5 °C in the laboratory, and evaluated after 3 months from the date of manufacture, to prevent interference due to the product's shelf life.

2.2. Lipid phase extraction

The margarines were homogenized and placed in a separating funnel to extract the lipid phase, which was extracted by heating in an oven at 60-70 ºC for a period sufficient for phase separation. Usually, when the emulsion breaks, the aqueous phase remains at the bottom of the funnel and is eliminated. Then, the remaining portion, the lipid phase, was removed from the funnel

and filtered on filter paper containing anhydrous sodium sulfate to remove the remaining water. After extraction, the lipid phase was stored for analysis Silva et al. [20] with adaptations.

2.3. Fatty acid composition

The fatty acid composition was determined by gas chromatography, after esterification using the methodology described by [21]. The methyl esters were separated according to the AOCS method Cf 1-96 [22]. A gas chromatograph equipped with an Agilent DB23 column (50% cyanopropyl-methylpolysiloxane), dimensions 60 m x 0.25 mm x 0.25 µm was used. The following conditions were adopted: oven temperature = 110 °C / 5min, 110 °C-215 °C (5 °C / min), 215 °C / 24 min, detector temperature = 80 °C; injector temperature = 250 °C, carrier gas = helium, injected volume = 1.0 µL, split ratio 1:50. The qualitative composition was determined by comparing retention peak times with the respective fatty acid standards. The quantification was calculated, in triplicate, by the percentage of the corrected area.

2.4. Solid fat content (SFC) and melting point

The SFC of the margarines and the lipid phase was determined using Bruker pc120 Minispec Nuclear Magnetic Resonance (NMR) spectrometer (Bruker, Germany), and a Tcon 2000 dry bath with a temperature range from 0 to 70 °C, (Duratech, USA). The readings were performed at temperatures of 2, 5, 10, 15, 20, 25, 30, 35, 40 and 45 °C, according to the AOCS method Cd 16b-93 [22]. The melting point was calculated for the temperature corresponding to 4% solids content, obtained from the SFC curve given by NMR [23]. All determinations were performed in triplicate.

2.5. Crystallization kinetics

The lipid phase was evaluated for the crystallization kinetics. The fats were kept in a Tcon 2000 dry bath (Duratech, USA) at 70 °C for the complete destruction of the crystalline history. The solid fat content as a function of time was monitored by a Bruker pc120 Minispec NMR spectrometer (Bruker, Germany), with a reading compartment stabilized at 15 °C. Data acquisition was automatic, with measurements taken every minute, for 40 minutes. The determinations were carried out in triplicate. The crystallization kinetics was determined according to the induction period (t_{SFC}) and maximum solid fat content (SFCmax). The Avrami equation was used to calculate the *k* and *n* (Equation 1). The Half-time of crystallization reflects the magnitude *k* and *n*, as shown in Equation 2 [24].

$$SFC = SFC_{max}\left(1 - e^{-kt^n}\right) \qquad \text{(Equation 1)}$$

$$t_{1/2} = \left(\frac{0.693^{\frac{1}{n}}}{k}\right) \qquad \text{(Equation 2)}$$

where: *SFC* is the solid fat content (%) as a function of time; SFC_{max} corresponds to the maximum SFC achieved at a particular temperature (%); *k* is the Avrami constant (min^{-n}), which considers both nucleation and crystal growth; *n* is the Avrami exponent, which indicates the mechanism of crystal growth; and $t_{1/2}$ is the half-time of crystallization.

2.6. Thermal behavior

To determine the thermal behavior, a differential scanning calorimeter (DSC) (Q2000 -TA Instruments, New Castle, DE, USA) was used, using indium as a calibration standard. The lipid phases were evaluated for crystallization and melting behavior according to the AOCS method Cj 1-94. The lipid phase was heated to 80 °C/10min, and the temperature was gradually reduced to -60 °C/30 min (rate of 10 °C/min) and then heated again to 80 °C (rate of 5 °C/min) [22]. The melting profile (from margarine) was determined as reported by [25] with adaptations, for that, the margarines were equilibrated at 5 °C/10 min, and heated to 80 °C (10 °C/min). The parameters onset temperature, peak temperature, final temperature and enthalpy were determined.

2.7. Texture profile analysis

The texture profile of the margarines was determined using a TA-XT2 texture analyzer. The sample was placed in a conical probe (female), remaining for 24 hours at 5 ºC (for analysis at refrigeration temperature) or 25 °C (for analysis at room temperature) for stabilization. During the analysis, the male cone, at a 90º angle, penetrated the sample, which was forced to flow between the male and female cones. A pre-test speed of 10 mm/s, test speed of 3 $mm.s^{-1}$, and a post-test speed of 10 mm.s were used. The parameters hardness (maximum force applied to the sample), spreadability (negative area), stickiness (maximum negative force) and adhesiveness (negative area) were determined [26].

2.8. Thermal stability by cyclization

The thermal stability of the margarines was evaluated for oil and/or water exudation as described by [27], which consisted of two cyclization steps. In the first cyclization, the sample (40 mL) remained at 5 °C for 48h for stabilization. After stabilization, the margarine was kept in a temperature-controlled incubation chamber at 35 °C for 24 h and visually evaluated using a photographic camera. Then, the margarine was stored again at 5 °C for 24 h and visually evaluated for oil and/or water exudation. In the second cyclization step, the margarines were kept at 35 °C for 48h and subjected to analysis. Finally, the samples were stored at 5 °C for 72 h and evaluated again.

2.9. Statistical analysis

Data were analyzed by analysis of variance (ANOVA) and Tukey's comparison test, at the significance level of 5% ($p < 0.05$). The statistical analysis was performed using the Sisvar software (version 5.6, Build 86- DEX-UFLA, Brazil).

3. Results and Discussion

3.1. Fatty acids composition

The great consumption of margarine is due to its low cost when compared to butter, in addition to its spreading properties at refrigeration temperature. However, from a nutritional point of view, there are many discussions about its composition. The fatty acid composition of margarines varies according to the oil or fat used in the manufacture and the lipid modification method. For a long time, margarines were formulated with PHF with high TFA levels. As established by the Brazilian law RDC 360, of December 23, 2003, the partially hydrogenated fat bases have been gradually replaced by interesterified fats, thus leading to important technological and nutritional changes of the margarines marketed in the country [28]. Table 1 shows the fatty acid composition of thirteen Brazilian margarines, with different total fat levels. The atherogenic index (AI) and thrombogenic index (TI) was calculated based on the fatty acid composition of the margarines, as described by [10].

All margarines showed a similar fatty acids composition, with a predominance of linoleic acid (C18:2ω-6), followed by oleic acid (C18:1). Therefore, probably the margarines may have been produced with soy-based fats since Brazil is a great soybean producer, thus soybean oil is less expensive and more available. However, before using in margarine formulations, soybean oil must undergo a lipid modification, with changes in the physical properties. The partial hydrogenation or interesterification of oils is usually adopted for application in margarines. It is important to highlight that the margarine H55 presented oleic acid as the major fatty acid (46.33%), indicating that unlike the others, it may have been produced with canola-based oil or high oleic sunflower oil.

Clinical and epidemiological data indicate that besides the low *trans* fats levels, foods should have lower saturated fats contents, with an adequate ratio of omega 3 and 6 polyunsaturated fatty acids [29]. Although the labels of the margarines M80 and L80 informed that the product was a source of the essential omega-3 fatty acids, the results showed that the C18:3 concentrations were similar to the other samples. Soybean oil contains 4.5 to 11% C18: 3ω-3 in its composition [30]. In margarines with a higher total fat content, the fatty acids of the lipid phase are in greater concentration in the final product, thus the presence of essential fatty acids is highlighted on the label. However, these margarines cannot be characterized as a source of omega-3, because its concentration is low since these fatty acids come only from soybean oil. The dietary intake of C18:3ω-3 can have positive effects on multiple risk factors, including blood pressure, blood vessel

function, cardiac function, and blood lipids, besides presenting antithrombotic, anti-inflammatory and antioxidant properties. In addition, foods rich in C18:3ω-3 can be an alternative for individuals who do not consume fish regularly. The low-fat margarines of this study had a higher content of monounsaturated fatty acids (MUFA), with the highest levels observed for the margarine H55 (47.43%).

Brazilian margarines contain around 20% oleic acid and 30 to 40% linoleic acid. Oleic acid is the main component of olive oil and is considered beneficial for the cardiovascular system. Its increased consumption may improve the ω-3: ω-6 ratio to the detriment of oils that contribute to the ω-6 intake. Emerging evidence from experiences and epidemiological studies have associated the increased intake of oleic acid with reduced risk of cardiovascular diseases, type 2 diabetes, and obesity [10, 31].

The Food and Drug Administration (FDA), in 2015, imposed a ban on the use of PHF (which contains high TFA levels formed during the partial hydrogenation process) in foods. In 2019, a public consultation on *trans* fats was performed in Brazil, determining a gradual implementation of a maximum limit of 2% TFA on the total fat content of foods. Then, Resolution 332 of 23 December 2019 was approved, which defines the requirements for the use of industrial *trans* fats in foods [13]. However, the presence of elaidic acid, a *trans*-isomer of oleic acid, which is produced during the partial hydrogenation was observed in two margarines of this study, A20 and B20, with values of 7.81% and 5.11%, respectively. The dietary intake of TFA is associated with several disorders, such as cardiovascular disease, breast cancer, prostate cancer, diabetes, obesity, and others. WHO and the Food and Agriculture Organization of the United Nations have recommended that the amount of TFA in human dietary should be reduced to less than 4% [32].

The consistency characteristics of margarines are provided by the SFA, which are used to confer structure and palatability, and to minimize the undesirable technological effects. The presence of TFA in the margarines A20 and B20, probably due to the addition of *trans* fat *to* an interesterified base, had a positive effect on the structural properties. The low-fat margarines showed 23.40 to 28% SFA, while those with fat levels presented from 21.94 to 31.84% SFA. The margarine H55 showed a lower SFA content (21.94%), due to its composition associated with canola oil. The predominant SFAs were palmitic acid (C16:0) and lauric acid (C12:0) [33]. The SFAs are also the subject of studies related to health implications. Studies have shown that diets with a high SFA content promote an increase in the serum cholesterol level, increasing the risk of cardiovascular disease and other chronic diseases [34–36]. The atherogenic and thrombogenic effects are represented by the AI and TI. The high SFA levels are related to the increase in AI and TI, and also related to high TFA, which was observed for the margarines A20 and B20 (high in

TFA) when compared with J65 and L80 (high in SFA). Therefore, the replacement of TFA by SFA is also undesirable for the consumers' health.

Palmitic acid was the fatty acid that most contributed to the increase in saturated fat. The main source of palmitic acid is palm oil, which represents one of the best alternatives to *trans* fats due to its technological properties. Although the margarine A20 did not contain palm fat, it presented the highest concentration of *trans*-fat. Palmitic acid can increase the HDL concentration and the total cholesterol/HDL ratio, in addition to increasing triglycerides [29], which was observed by the high TI (0.54) of the margarine B20 containing a higher palmitic acid level when compared to the other samples. Stearic acid was the second most abundant saturated fatty acid. Unlike palmitic acid, stearic acid has discrete effects on LDL-cholesterol, as it is metabolized to oleic acid by the human body [37].

3.2. SFC and melting point

The market demands have led to an increasing need to obtain industrial fats by other technological alternatives since the partial hydrogenation has been eliminated [38]. SFC is a physical property that measures the amount of solid fat in a lipid at a given temperature. In food, SFC can affect the physical properties such as hardness, spreadability, mouthfeel, and melting, and is used as a quality parameter to define the applicability of fats [39]. Therefore, the SFC is widely used to study the properties of food and its applications, as well as its behavior under different storage and consumption conditions [40]. Fig. 1a and 1b shows the SFC curves of the lipid phase and their respective margarines. In margarines, the solid fat content defines several characteristics including the appearance, ease of packaging, spreadability, and organoleptic properties [41].

Each temperature provides a different SFC, which is related to a specific physical property of the final product. The ideal fat for application in margarines has unique properties in relation to the SFC. At temperatures between 4 and 10 ºC, the SFC indicates the spreadability under refrigeration, and should not exceed 32% at 10 ºC. The fat of all margarines of this study showed SFC between 10 and 20% at 10 °C, indicating ideal spreadability at the storage temperature. The SFC of table margarines during storage has a strong effect on consistency and yield of margarines and fats [42].

SFC between 20 and 22 °C is related to the product's stability and its resistance to oil exudation, which should be greater than 10% [7, 41, 43]. At temperatures greater than 25 °C, the SFC determines the fat hardness. Fats containing *trans*-isomers showed higher solids content at this temperature. In the *trans* configuration, two hydrogen atoms attached to the carbon atoms that form the double bond are located on opposite sides of the carbon chain, creating a linear and more rigid molecule and consequently with greater hardness, melting point, and stability [44, 45].

The lipid phases showed very similar behavior, except for the margarines A20 and B20, which showed higher SFC from 20 to 30 °C, with a slight change in their SFC curves. These data corroborate the fatty acids composition, with values of 9.14% and 6.22% TFA for A20 and B20, respectively. Therefore, the use of PHF was again confirmed by the SFC curve, demonstrating its best technological properties (such as resistance to oil exudation, firmness, and spreadability) when compared to the interesterified fats, which are bases of the other margarines.

The use of those fats under study as the basic ingredient of margarines led to different SFC behavior. Margarines showed maximum SFC at 2 °C from 4 to 15%. High-fat margarines showed high SFC and low plasticity. In contrast, the low-fat margarines showed lower SFC at room temperature and maximum solids of around 8%. The high proportion of water in these margarines can reduce fat functionality, leading to rapid melting. All margarines were already melted at 35 ºC, with a solid fat content of less than 4%.

The great diversity of margarines in the market is due to the lipid modification methods such as hydrogenation, fractionation, interesterification or blends, which allows producing materials with SFC profiles necessary for processing with ideal melting behavior [46]. The melting point was calculated for the temperature corresponding to 4% SFC. Low-fat margarines had a lower melting point (for example the sample D36 exhibited a melting point of 8.52 ± 0.48 °C), which is not observed when analyzing its lipid phase (22.02 ± 0.11 °C). The margarines D36 and H55 (13.57 ± 0.44 ºC) showed a lower melting point due to the higher content of unsaturated fatty acids, corresponding to 74.47% and 77.15%, respectively. The lipid phase of A20 and B20 showed a higher melting point when compared to the others, thus melting at body temperature, 33.87 ºC and 37.27 ºC, respectively. The melting point determines the melting properties of margarines when consumed in bread and biscuits or food preparations as an ingredient. At 37 °C, margarines should have low SFC for adequate melting at body temperature, as they should melt when in contact with the mouth. Attention is drawn to the margarine D36, which had a melting point of 8 ºC. This margarine contains a high PUFA level with lower melting points.

The fats extracted from the margarines showed a higher melting point when compared to their respective margarines. This result shows that fats with a melting point greater than 27 °C are required during the manufacturing process of margarines, so that they can melt at temperatures close to room temperature, thus preventing excessive melting or other undesirable properties, such as the release of liquid oil. Margarines with a fat content greater than 65% showed similar melting points for lipid phase and margarine, with values of 27 ºC and 29 ºC, respectively. This result suggests that high-fat margarines require fat bases with a lower melting point. On the other hand, margarines with lower fat content require harder fats since the high content of aqueous phase reduces the melting point of the lipid phase during the emulsification process.

3.3. Crystallization kinetics

From an industrial point of view, crystallization kinetics plays a fundamental role in fat-based products. Fig. 2 shows the crystallization isotherms of the lipid phases extracted from margarines, at 15 °C. The crystallization isotherm allows understanding the differentiation of fats for various applications, according to parameters induction period, crystal growth rate, and SFC during stabilization (Table 2).

Crystallization is characterized by the structural transformation of the solid fat at a specific temperature. The margarines A20, N82, and K70 showed lower t_{SFC}, with values of 36.67, 36, and 38 min, respectively, with SFC_{max} between 9 and 11%. Fats with high TFA levels showed higher SFC_{max} and rapid crystallization profile. However, the margarine B20 presented a different profile when compared to A20, with high t_{SFC} (55.67 min), probably due to the presence of palmitic acid and the lower TFA levels.

The parameter $t_{1/2}$ allows assessing more effectively the time required for 50% crystallization to complete, which is very important for the product application, as it represents the overall performance of fats. The margarines A20 and B20 showed the lowest $t_{1/2}$, with values of 15.76 min and 12.32 min, respectively, due to their high TFA levels. The other fats showed $t_{1/2}$ of about 20 min. Therefore, in addition to the lower t_{SFC}, PHF also presented a lower $t_{1/2}$, which can lead to cost reduction for the industry and formation of more uniform crystal networks.

The crystal morphology is another important parameter, represented by the factor n. All samples showed values between 1 and 2, except for N82 (n value of 3), indicating that the nucleation may be sporadic or instantaneous with a disc-like to needle-like crystal growth [24]. The Avrami exponent, n, sometimes referred to as a crystallization index, indicates the crystal growth mechanism. Nucleation is instantaneous, with the nuclei appearing at the onset of the process, or sporadic with the number of nuclei increasing linearly over time [47].

3.4. Thermal behavior

The crystallization and melting profiles obtained by DSC are shown in Fig. 3. All lipid phases showed similar crystallization behavior, with the onset of crystallization occurring from 17 °C to 22 °C, peak temperature around 15 °C, and final crystallization temperature from 4 to 12 °C. The margarines A20 and B20, with PHF, showed different thermal behavior, with the onset of crystallization between 24.84 °C and 25.37 °C and final crystallization temperature between 4.06 °C and 15.39 °C, respectively. The presence of TFA also resulted in greater crystallization enthalpy (data not shown), with values of 4.92 and 5.60 J/g for the margarines A20 and B20, respectively. The other samples exhibited peaks with crystallization enthalpy ranging from 2.12 to 3.93 J/g. It

is believed that the sharp peak observed in B20 with a height of 0.39 W/g may be due to the presence of TFA.

Concerning the melting of the lipid phase (Fig. 3b), although a second peak was observed, all samples exhibited a wide melting temperature range, which can be associated with specific triacylglycerols, mainly monosaturated. The melting behavior of fats was compatible with the crystallization peaks, with average melting ranges at -21 °C and 25 °C. Again, the lipid phases of the margarines A20 and B20 differed from the others, with the maximum melting peak displaced from the others, between -4.04 and 6.00 °C, respectively, with final temperatures between 5.26 °C and 11.18 °C.

The commercial margarines exhibited different melting profiles when compared to their respective lipid phases, with the presence of a single and wide melting peak of all triacylglycerols classes. The melting peaks were evident in high-fat margarines, while the margarines with PHF showed a small peak, despite presenting displacement of the curve similar to that observed for fats.

3.5. Texture properties

The properties of the lipid phase directly affect the texture of the final product. Margarines should present satisfactory texture properties both at the temperature of storage (under refrigeration) and room temperature, giving ideal hardness and spreadability for use as a spread or in culinary preparations. To assess the behavior of the samples at different consumption temperatures, the margarines of this study were evaluated at 5 °C and 25 °C for hardness, spreadability, stickiness, and adhesiveness (Fig. 4).

A significant decrease in the texture parameters of the margarines was observed at room temperature, probably due to the low solid fat content at 25 ºC. The decrease in SFC is due to the melting of low melting triacylglycerols, which affects hardness, spreadability, stickiness, and adhesiveness of the final product. However, for the margarines studied, the decrease in texture was greater for margarines with a higher fat content, which was more evident in the margarine H55. The high oleic acid content from canola oil or high oleic sunflower oil in the margarines contributed to a higher level of unsaturated fatty acids (77.15%), which have a lower melting point, leading to a lower hardness (3.56 N), greater spreadability (4.01 N.s), lower stickiness (2.81 N), and greater adhesiveness (1.07 N.s).

The attributes hardness and spreadability are the main parameters studied in margarines, as they represent the necessary force to break its structure and maintain the spreadability, resulting in intermediate properties between mayonnaise and butter. The high-fat margarines showed greater resistance to spreading. High SFA levels (31.34%) and the presence of TFA (2.94%) affected hardness and spreadability, mainly for the margarine J65, which showed higher values for these

parameters at 5 ºC when compared to the other samples ($p < 0.05$). This result demonstrates that the replacement of *trans*-fat and/or saturated fats in margarines is a major technological challenge, once they directly contribute to the texture of the final product.

High-fat margarines showed higher hardness and stickiness values when compared to low-fat margarines, with a reduction of up to 42% at 25 °C, which was not observed for the low-fat margarines. Emulsifiers play an important role in the texture profile of margarines made with higher incorporation of water. The emulsification system must have sufficient strength to prevent the destabilization of the margarine, keeping the crystalline network also stable [48]. These properties were confirmed in the thermal stability analyses of this study.

All margarines showed similar adhesiveness, with low values for all samples, and little changes with the increase in temperature. The adhesiveness has little effect on the typical characteristics of margarines, once the force necessary for the product to present an adequate adhesiveness on the food is relatively low.

3.6. Thermal stability by cyclization

Temperature cycling analysis is very important to the food industry, once it determines the ability of margarines to withstand temperature cycling without oil exudation or water release. The margarines containing high TFA levels (A20 and B20), high unsaturated fatty acids, mainly PUFA (D36 and G51) and those produced with fat high in C18:1 (H55) proved to be more stable when compared with the others. In the first cyclization, the stability of the system was studied (image not shown), which was confirmed in the second cyclization.

The other margarines showed no thermal stability, as can be seen in Fig. 5. It is suggested that fat provided structure for the high-fat margarines, not requiring emulsifiers with great emulsifying properties, which were responsible for the stability of the low-fat margarines. The margarine F50 showed greater oil exudation, due to the lower efficiency of the emulsifying system with the increase in temperature of systems containing 50% oil and 50% water.

4. Conclusion

Brazilian margarines have been subjected to various technological changes in recent years to adapt to the TFA and SFA reduction required by the legislation. This study gives a brief outline of the profile of the margarines marketed in Brazil. The margarines presented different total fat levels (20 to 82%), and those with 20% fat still required the use of PHF to maintain their technological properties. The probable future scenario will be a gradual reduction of PHF in margarine formulations until the elimination from the Brazilian market. Concerning the characteristics of the lipid phase, although presenting different fat levels, the lipid phase consisted of the same fatty acids, including oleic, linoleic, and palmitic acids, and SFA content of 23 to 31%.

From the fatty acid profile, the results suggested that the fats used in the manufacturing process of Brazilian margarines were from an interesterified base consisting mainly of soybean oil and palm oil, while canola oil or high oleic sunflower can be used when the increase in oleic acid level is required. All fats had a melting point of approximately 25 °C. Finally, concerning the effect of the oil content on the overall properties of margarines, the higher this content, the greater the hardness, spreadability, and stickiness values. In addition, the oil content can also affect stability, and low-fat margarines were the most stable when compared to the others. The margarines made with the same oil and water ratio (50:50), had lower stability. Although the majority of Brazilian margarines have low TFA levels, the high SFA levels can be reduced, considering that the substituted lipid phase provides the same characteristics as the inter-esterified fats currently used.

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Figure Captions

Fig. 1 (a) Solid fat content of the lipid phases, and **(b)** their respective margarines obtained, by Nuclear Magnetic Resonance.

Fig. 2 Crystallization kinetics at 15 °C of the lipid phases extracted from margarines.

Fig. 3 (a) Crystallization profiles and **(b)** melting of the lipid phases, and **(c)** melting profiles of the margarines, obtained by Differential Scanning Calorimetry.

Fig. 4 Textural properties (hardness, spreading, stickiness and adhesiveness) of margarines stored at 5 °C and 25 °C for 24 hours. Superscript letters represent statistically significant ($p < 0.05$) differences between the values of each column (different letters within each column denote significant differences). Capital letters represent the comparison between margarines at 25 °C, and lower case letters represent the comparison between margarines at 5 °C.

Fig. 5 Thermal stability of margarines after second cyclization at 35 °C for 48 hours.

Tables

560 Table 1 - Fatty acid composition, atherogenic and thrombogenic index of commercial margarines marketed in Brazil.

Fatty acids (%)	A(20)	B(20)	C(35)	D(36)	E(38)	F(50)	G(51)	H(55)	J(65)	K(70)	L(80)	M(80)	N(82)
C6:0 (Caproic)	0.00±0.00	0.17±0.02	0.09±0.02	0.00±0.00	0.00±0.00	0.16±0.02	0.00±0.00	0.19±0.05	0.18±0.01	0.00±0.00	0.00±0.00	0.17±0.05	0.00±0.00
C8:0 (Caprylic)	0.00±0.00	0.13±0.00	0.26±0.01	0.22±0.01	0.32±0.01	0.31±0.00	0.22±0.00	0.24±0.01	0.42±0.01	0.33±0.02	0.45±0.01	0.32±0.01	0.31±0.01
C10:0 (Capric)	0.02±0.01	0.12±0.00	0.25±0.01	0.21±0.00	0.31±0.01	0.30±0.00	0.21±0.00	0.24±0.01	0.40±0.01	0.31±0.01	0.44±0.01	0.32±0.01	0.30±0.01
C12:0 (Lauric)	0.07±0.02	1.58±0.03	3.27±0.04	2.57±0.03	4.17±0.07	4.02±0.03	2.79±0.01	3.19±0.04	5.49±0.08	4.10±0.08	5.80±0.08	4.44±0.07	4.01±0.07
C14:0 (Myristic)	0.15±0.01	0.75±0.01	1.20±0.01	0.96±0.01	1.53±0.04	1.53±0.03	1.05±0.00	1.20±0.01	2.00±0.01	1.52±0.02	2.10±0.02	1.67±0.02	1.49±0.02
C15:0 (Pentadecanoic)	0.02±0.00	0.04±0.00	0.02±0.01	0.02±0.00	0.03±0.01	0.02±0.00	0.03±0.00	0.02±0.00	0.03±0.00	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.00
C16:0 (Palmitic)	11.66±0.05	14.54±0.12	11.26±0.08	10.88±0.02	11.78±0.15	11.94±0.01	10.98±0.01	7.91±0.07	12.07±0.03	12.42±0.01	12.01±0.02	12.27±0.08	11.68±0.06
C16:1 (Palmitoleic)	0.07±0.00	0.09±0.02	0.10±0.02	0.09±0.01	0.07±0.00	0.08±0.01	0.09±0.01	0.23±0.01	0.08±0.00	0.08±0.01	0.08±0.00	0.09±0.01	0.19±0.01
C17:0 (Margaric)	0.12±0.00	0.12±0.00	0.11±0.00	0.11±0.01	0.09±0.00	0.11±0.00	0.11±0.00	0.10±0.01	0.11±0.00	0.11±0.01	0.12±0.00	0.10±0.02	0.09±0.00
C17:1 (Margaroleic)	0.05±0.00	0.05±0.00	0.05±0.00	0.04±0.00	0.04±0.00	0.05±0.00	0.05±0.01	0.11±0.00	0.05±0.00	0.10±0.01	0.09±0.00	0.05±0.01	0.05±0.00
C18:0 (Stearic)	9.94±0.03	8.87±0.08	8.52±0.04	8.29±0.04	8.58±0.10	8.57±0.02	9.89±0.03	7.54±0.28	9.67±0.02	9.47±0.04	9.89±0.05	9.25±0.8	9.12±0.04
C18:1t (t-Elaidic)	7.84±0.10	5.11±0.16	0.04±0.06	0.00±0.00	0.26±0.00	0.27±0.01	0.00±0.00	0.00±0.00	0.19±0.00	0.00±0.00	0.26±0.01	0.22±0.20	0.27±0.00
C18:1c (Oleic)	28.80±0.27	29.06±0.32	22.26±1.39	21.48±0.03	23.09±0.36	23.17±0.05	20.88±0.01	46.33±0.44	20.82±0.06	22.07±0.09	20.44±0.06	21.18±0.39	22.98±0.23
C18:2t (t-Linoleic)	0.34±0.00	0.38±0.00	0.26±0.00	0.30±0.00	0.35±0.01	0.27±0.00	0.27±0.00	0.15±0.00	0.86±0.00	0.44±0.00	0.36±0.00	0.39±0.03	0.35±0.01
C18:2c (Linoleic)	34.89±0.10	33.69±0.24	45.08±1.27	47.16±0.03	41.95±0.21	42.37±0.09	46.03±0.04	23.92±0.36	41.19±0.03	42.02±0.07	40.68±0.02	42.77±0.42	41.86±0.11
C18:3t (t-Linolenic)	0.96±0.00	0.73±0.01	0.79±0.03	0.90±0.00	1.01±0.05	0.82±0.00	0.85±0.01	0.76±0.15	1.89±0.02	1.15±0.01	1.01±0.00	0.98±0.05	0.82±0.01
C18:3 (Linolenic)	3.45±0.01	3.25±0.03	5.20±0.17	5.51±0.01	5.13±1.02	4.76±0.02	5.35±0.00	5.80±0.12	3.40±0.01	4.58±0.01	5.07±0.01	4.51±0.04	5.17±0.04
C20:0 (Arachidic)	0.50±0.00	0.43±0.00	0.42±0.00	0.43±0.01	0.43±0.01	0.41±0.00	0.43±0.00	0.68±0.01	0.38±0.00	0.43±0.00	0.41±0.00	0.43±0.01	0.43±0.01
C20:1 (Gadoleic)	0.19±0.01	0.20±0.00	0.18±0.01	0.19±0.01	0.21±0.01	0.21±0.01	0.17±0.00	0.76±0.02	0.18±0.00	0.20±0.00	0.16±0.00	0.18±0.01	0.19±0.00
C22:0 (Behenic)	0.70±0.01	0.49±0.01	0.46±0.00	0.47±0.01	0.46±0.01	0.45±0.00	0.46±0.00	0.42±0.01	0.42±0.00	0.49±0.01	0.43±0.00	0.46±0.00	0.47±0.01
C24:0 (Lignoceric)	0.21±0.00	0.19±0.00	0.17±0.03	0.17±0.00	0.18±0.00	0.18±0.00	0.15±0.02	0.21±0.00	0.17±0.00	0.19±0.00	0.17±0.00	0.17±0.01	0.19±0.00
∑TFA	9.14±0.10	6.22±0.15	1.10±0.04	1.20±0.01	1.62±0.06	1.36±0.01	1.12±0.01	0.91±0.15	2.94±0.02	1.59±0.12	1.63±0.08	1.59±0.21	1.44±0.02
∑SFA	23.40±0.10	27.44±0.21	26.04±0.03	24.34±0.01	27.88±0.38	28.00±0.08	26.31±0.05	21.94±0.37	31.34±0.08	29.39±0.13	31.84±0.08	29.63±0.24	28.12±0.10
∑UFA	67.46±0.17	66.34±0.09	72.86±0.07	74.47±0.01	70.50±0.44	70.64±0.08	72.57±0.05	77.15±0.22	65.71±0.10	69.02±0.01	66.53±0.01	68.78±0.24	70.44±0.08
∑MUFA	29.11±0.07	29.4±0.09	22.59±0.36	21.8±0.01	23.41±0.09	23.51±0.02	21.19±0.01	47.43±0.12	21.13±0.02	22.45±0.03	20.77±0.02	21.5±0.11	23.41±0.06
∑PUFA	38.34±0.06	36.94±0.14	50.28±0.72	52.67±0.02	47.08±0.62	47.13±0.06	51.38±0.02	29.72±0.24	44.59±0.02	46.6±0.04	45.75±0.02	47.28±0.23	47.03±0.08
AI	0.32±0.00	0.38±0.00	0.30±0.00	0.25±0.00	0.34±0.01	0.33±0.00	0.26±0.00	0.22±0.00	0.43±0.00	0.35±0.00	0.42±0.00	0.36±0.01	0.33±0.00
TI	0.34±0.01	0.54±0.00	0.37±0.00	034±0.00	0.39±0.03	0.39±0.00	0.38±0.00	0.23±0.00	0.51±0.00	0.43±0.00	0.45±0.00	0.43±0.01	0.39±0.00

561 ±standard deviation. Values in parentheses represent total fat percentage in margarine. ∑TFA: total *trans* fatty acid. ∑SFA: total saturated fatty acid. ∑UFA: total unsaturated fatty acid. ∑MUFA: total monounsaturated fatty acid. ∑PUFA:
562 total polyunsaturated fatty acid. AI: atherogenic index. TI: thrombogenic index

563 Table 2 - Induction period (t_{SFC}), maximum solid fat content (SFCmáx), Avrami constant (k),
564 Avrami exponent (n), half-time of crystallization ($t_{1/2}$), and respective coefficients of determination
565 (R^2) for lipid phases of margarines, obtained at 15 °C.

Lipid phase	t_{SFC} (min)	SFC_{max} (%)	*k* (min^{-1})	*n*	$t_{1/2}$ (min)	R^2
A20	36.67±0.44	11.00±0.06	1.06E-03±0.00	2.36±0.05	15.76±0.13	1.00±0.00
B20	55.67±3.78	12.26±0.07	7.21E-03±0.00	1.83±0.07	12.32±0.61	0.98±0.00
C35	55.00±0.00	11.62±0.19	4.23E-04±0.00	2.43±0.07	21.14±0.00	0.98±0.00
D36	51.33±2.89	5.05±0.05	2.58E-03±0.00	1.81±0.10	22.72±0.13	0.96±0.00
E38	59.67±0.44	8.93±0.10	6.06E-04±0.00	2.18±0.06	25.62±0.13	0.98±0.00
F50	48.67±5.78	7.28±0.45	1.16E-04±0.00	2.73±0.17	25.48±0.51	0.99±0.00
G51	57.33±1.78	8.39±0.03	9.46E-03±0.00	1.52±0.04	16.91±0.20	0.98±0.00
H55	53.33±1.56	7.11±0.07	1.43E-03±0.00	2.14±0.10	18.51±0.27	0.97±0.00
J65	44.00±4.00	10.06±0.33	1.11E-04±0.00	2.82±0.06	22.26±0.28	0.99±0.00
K70	38.00±0.67	9.19±0.12	4.40E-04±0.00	2.52±0.05	18.71±0.36	0.99±0.00
L80	52.00±6.00	9.63±1.16	1.05E-04±0.00	2.89±0.05	21.46±1.30	0.99±0.00
M80	58.00±2.67	9.72±0.28	6.75E-04±0.00	2.26±0.10	22.13±0.45	0.98±0.00
N82	36.00±4.00	9.49±0.63	5.89E-05±0.00	3.14±0.03	20.05±2.01	0.99±0.00

566 ±standard deviation