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Fatty acid and sodium contents of commercial milk chocolate – analytical aspects and nutritional information

Teor de ácidos graxos e sódio em chocolate ao leite comercial – aspectos analíticos e informação nutricional

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Summary

Chocolate consumption is usually associated with enjoyment, milk chocolate desserts being a very popular choice. Besides, the literature provides data suggesting health benefits for chocolate products as compared to non-chocolate candies. However, the lipid composition of cocoa and its commercial products has yet to be completely elucidated and understood, although much research has been carried out with this objective. Contributions to this objective frequently face difficulties in the field of Analytical Chemistry due to the complexity of the composition of such a food. On the other hand, the sodium content of foods is currently a major concern. Thus, this work aims to provide information concerning the composition of commercial milk chocolate in terms of its fatty acid profile and sodium content. To achieve this purpose, analytical adjustments and improvements to the methodology were made and described in this paper. Sodium (FAAS) and a total of 50 fatty acids (GC-FID) were determined in eight samples of milk chocolate bars from different manufacturers. The samples were purchased from retailers in Porto Alegre – Brazil. In the determination of the fatty acids, possible losses during methylation deserved special attention and were studied. Nevertheless, large differences were not found in comparison with the nutritional facts declared on the label. However, the results obtained for sodium demonstrated the importance of food inspection, considering the discrepancies found.

Key words: *Milk chocolate; Lipid composition; Lipid profile; Fatty acid contents; Sodium contents; Nutritional information; GC-FID; FAAS.*

Resumo

O consumo de chocolate é normalmente associado a prazer, sendo sobremesas à base de chocolate ao leite uma escolha muito popular. Além disso, a literatura mostra dados que sugerem benefícios à saúde, provenientes de produtos feitos com chocolate quando comparados a doces sem chocolate. Contudo, a composição do cacau e seus derivados comerciais está ainda por ser completamente demonstrada e entendida, embora muitos esforços de pesquisa tenham sido feitos. Contribuições para esse objetivo frequentemente enfrentam dificuldades no campo da Química Analítica, por causa da complexidade da composição química desse tipo de alimento. Por outro lado, o conteúdo de sódio dos alimentos em geral é atualmente motivo de interesse e preocupação. O presente trabalho objetiva adicionar informações sobre a composição de chocolate ao leite comercial, em termos de perfil de ácidos graxos e teor de sódio. Com essa finalidade, ajustes e melhoramentos analíticos foram feitos e são descritos. Um total de 50 ácidos graxos (GC-FID) e sódio (FAAS) foram determinados em oito amostras de barras de chocolate ao leite de diferentes fabricantes. As amostras foram compradas em estabelecimentos comerciais, em Porto Alegre/RS – Brasil. Na determinação dos ácidos graxos, ficou evidente que perdas por volatilização na etapa de metilação são importantes e não devem ser negligenciadas. Porém, não foram encontradas discrepâncias relevantes ou generalizadas em relação às informações nutricionais contidas nos rótulos. Já os resultados obtidos para sódio mostram como são necessárias as ações de vigilância sanitária, considerando as disparidades encontradas.

Palavras-chave: *Chocolate ao leite; Composição lipídica; Perfil lipídico; Teor de ácidos graxos; Teor de sódio; Informação nutricional; GC-FID; FAAS.*

1 Introduction

Reviewing recent data regarding the association of chocolate consumption and health, it is possible to conclude that experimental and clinical studies continue to suggest cardiovascular benefits and antioxidant importance. Although dark chocolate may be more protective than milk chocolate, the latter is still preferable to non-chocolate candies (DJOUSSE et al., 2011; LETTIERI-BARBATO et al., 2012; VILLARREAL-CALDERON et al., 2012). Research has also been done on the neurobiological impact of cocoa, as well as its preventive effects on cancer (MARTIN et al., 2013; SOKOLOV et al., 2013). The challenge for the future is to establish a correlation between all these properties and the chemical composition of chocolate.

In Brazil, milk chocolate desserts are a very popular choice, justifying research to obtain compositional data on commercial products. Being a fatty food, the lipid composition is of importance. On the other hand, the sodium content of foods is currently a major concern due to its recognized effect on the blood pressure, with consequences for the heart, kidneys, brain and other organs. Government agencies do not establish maximum or recommended limits for sodium and fatty acids, but health services indicate moderate consumption.

Some information about the chemical composition of commercial chocolate products is available, but is normally present in general approaches. For example, the Brazilian Table of Food Composition (TACO), based on national sampling and analysis (UNICAMP, 2011), shows data for milk chocolate, including total fat and 13 fatty acids. Other components are also informed including sodium.

From the analytical point of view, the determination of sodium in food may pose some difficulties since it is a ubiquitous element. Usually, the sample is digested prior to instrumental determination, and in this case, care must be taken to prevent contamination of the digests. Thus, a closed digestion system is preferred, instead of an open apparatus and the use of high purity water and reagents is necessary.

On the other hand, after digestion of the sample, sodium can be quantified following a simple instrumental procedure. One of the most used techniques for determining sodium is the classical flame atomic absorption spectrometry (FAAS) method.

The determination of the fatty acid composition of a food is normally a complex analysis (BRONDZ, 2002; ROSENFELD, 2002). The fat and fatty acids are usually extracted from the food by hydrolytic methods, followed by derivatization to improve chromatographic response.

Currently methylation to the fatty acid methyl esters (FAMEs) and quantitation by gas chromatography using a flame ionization detector (GC-FID) is one of the most used procedures. Due to the high separation capability of this technique, along with great instrumental sensitivity, it is possible to identify and quantify dozens of such esters simultaneously.

Some reference methods for the determination of the fatty acid composition in foods are available, but are not specific. For instance, the Official Methods of Analysis includes method 996.06 for the determination of fat in foods, which is intended for use in the quantitation of a large number of fatty acids (AOAC, 2012). However, the analysis of commercial milk chocolate samples is especially cumbersome.

This work proposes an analytical procedure for milk chocolate samples, which can be carried out using well-known instrumentation, FAAS for sodium after microwave digestion, and GC-FID for the fatty acid composition after methylation. The GC-FID procedure allows for the quantitation of 48 fatty acids simultaneously, plus two other fatty acids which can be determined separately. The method was applied to eight samples of commercial milk chocolate from different manufacturers (named here alphabetically as “A” to “H”), bought from Brazilian retailers at Easter, 2013.

2 Material and methods

2.1 Instrumentation

Sodium was determined using a SpectrAA 55 (Varian, Australia) atomic absorption spectrometer, with a Photron hollow cathode lamp, at a wavelength of 589.6 nm with a slit of 0.2 nm. An acetylene-air flame was used and the burner height and flow rates were optimized.

The fatty acids were determined using a Shimadzu GC-2010 gas chromatograph (Shimadzu, Japan) equipped with a split/splitless injector inlet and a flame ionization detector. The output was recorded using the LabSolutions/GCsolution version 2.30.00 SU7 software to integrate the chromatographic data. A Restek SP2560 capillary column (100 m × 0.25 mm of internal diameter × 0.25 µm of film thickness) was used for the analysis. Nitrogen was employed as the carrier gas and as the detector make up gas. The purge of the septum was at 1.0 mL min^{-1} and a split of 1:100 was used.

2.2 Reagents

All solutions were prepared using ultrapure water (Milli-Q water purification system, Millipore, Bedford, USA).

The nitric acid used in the sodium determination was of analytical grade (Suprapur, Merck), and the calibration standards were prepared by diluting 1000 mg L^{-1} stock solution in Titrisol (NaCl, Merck).

The fatty acid composition was determined using the following analytical grade reagents and solvents: 95% ethanol (Merck); 50% ammonium hydroxide (Dinamica); hydrochloric acid (Merck); pyrogallic acid (Sigma-Aldrich); sodium chloride (Vetec); and 7% boron trifluoride in methanol prepared using a 14% methanolic boron trifluoride solution (Aldrich) and sodium sulphate (Merck). The following chromatographic grade solvents were also used: petroleum ether (Vetec), diethyl ether (Vetec), toluene (Sigma-Aldrich), chloroform (Merck), n-hexane (Merck) and methanol (Merck).

The standards employed were C4 to C24 from Supelco (CRM47889); the cis/trans mix C18:3 from Sigma-Aldrich (L6031); the cis/trans mix C18:2 from Supelco (47791); (C18:1t; 6-octadecenoic acid (E)) from Supelco (47199); (C18:1c) from Supelco (46904); and (C18:1t; 11-octadecenoic acid, (E)) from Supelco (46905-U).

The standards were purchased as methyl esters for the qualitative tests. For quantitative purposes, the internal standard (I.S.) undecanoic acid from Sigma-Aldrich (171476) was also purchased and employed in the form of its fatty acid.

2.3 Sample preparation

Prior to the sodium determination, representative grated chocolate samples were digested in an Ethos Touch Control microwave labstation with temperature control (Milestone, Italy). Aliquots of 0.15 g (three replicates) were reacted with 10 mL of nitric acid and 5 mL of water. The labstation allows for 12 reaction flasks to be installed for each digestion program, three always being blanks. For the microwave procedure, the following four-step program was developed, always at 1000 W: times of 15, 10, 5 and 10 min; and corresponding temperatures of 80, 150, 200 and 200 °C. The digests were filtered and diluted to 50 mL with water in volumetric flasks. A spiked sample was also analysed with three replicates.

For the determination of the fatty acid profiles, the sample preparation was based on Method 996.06 – (c) Cheese - of the AOAC Official Methods of Analysis (AOAC, 2012), with some modifications as explained below. The preparation consisted of hydrolysing the sample and extracting the lipids, followed by methylation to the FAMEs.

For the hydrolysis step, 0.3 g of the grated and homogenized samples were weighed into Mojonnier flasks containing boiling granules, and 100 mg pyrogallic acid and 2.00 mL I.S. undecanoic acid internal standard solution added to each. A volume of 2.0 mL ethanol was then added and mixed until the entire test portion was in solution. Additions of 4.0 mL H_2O (well mixed) and 2.0 mL NH_4OH (also well mixed) followed. The flasks were placed in a water bath at 80 °C for 20 min, with manual homogenization of the contents at intervals. In sequence, 10.0 mL 12 M HCl were added and the flasks placed in a boiling steam bath for 20 min, keeping the contents well mixed. The flasks were then removed from the steam bath, cooled to room temperature (20–25 °C) and enough ethanol added to the flasks to fill the bottom reservoir. The contents were then gently mixed.

For the extraction, 25 mL of diethyl ether were added to the Mojonnier flasks, the flasks stoppered with silicon corks, and manually shaken for 8 min. An aliquot of 25 mL of petroleum ether was then added, the flasks stoppered again and shaken for a further 8 min. After allowing the contents to settle, the clear upper layer (ether layer) was decanted into a 100 mL beaker. The flasks were rinsed with 20 mL of a diethyl ether-petroleum ether mixture (shaken for 2 min) and the upper layer transferred to the beaker. The cork was also rinsed into the beaker using the same mixture. The ether was then slowly evaporated (40 °C steam bath) using a nitrogen stream to aid evaporation, and the residue, which contained the extracted fat, submitted to methylation as described below.

The extracted fat was dissolved in 2-3 mL chloroform in the beaker, and the contents transferred to a 3 dram glass vial. In order to avoid losses, this step was repeated. The solvent was then evaporated to dryness (40 °C steam bath) using a nitrogen stream, and 2.0 mL of 7% methanolic BF_3 reagent plus 1.0 mL toluene added. Each vial was sealed with a Sun-Sri 200835 lid and heated in an oven for 45 min at 100 °C, with homogenization at 10 min intervals. After cooling to room temperature (20–25 °C), 5.0 mL H_2O, 1.0 mL hexane and 1.0 g ± 0.1 Na_2SO_4 were added and the vials sealed and shaken for 1 minute. Time was allowed for separation of the layers, and the top layer then transferred to another vial containing 1.0 g ± 0.1 Na_2SO_4. This top layer contained the FAMEs, and was injected onto the GC column. A control was always prepared and analysed with the objective of guaranteeing that the glassware, solvents and chromatographic system were free from interferences. Moreover, a test portion without the addition of the internal standard was always analysed. The chocolate samples were analysed for their lipid profiles with three replicates.

The research group enrolled in a food analysis proficiency test, (PEP-SENAI/SC: Programa de Ensaios de Proficiência - Serviço Nacional da Indústria/State of Santa Catarina-Brazil) was used in 2012 and 2013. In 2012, PEP-SENAI/SC distributed a farinaceous matrix for analysis, with the objective of determining the total trans-fat. In 2013, the sample analysed was a freeze-dried meat matrix. The group achieved good results on both occasions. The z-scores obtained in 2012 and 2013 were equal to ***0.00*** and ***1.53***, respectively (satisfactory results must have a z-score ≤ |2|). It is important to note that this proficiency test was the only one available for fat at the

time the study was carried out. No chocolate proficiency test could be found.

2.4 Chromatographic conditions

Two chromatographic programs were established. The main one was capable of determining 48 fatty acids simultaneously. For this procedure, the temperature of the injector and detector were kept at 250 °C and 260 °C, respectively. The carrier gas flow rate was 0.89 mL min^{-1} and the linear velocity 18 cm s^{-1}. The temperature gradient was as follows: 140 °C (isotherm 5 min) to 170 °C at 4 °C min^{-1} (isotherm 37 min), then at 10 °C min^{-1} to 185 °C (isotherm 20 min), and finally at 25 °C min^{-1} to 240 °C (isotherm 9 min).

This main procedure was also capable of detecting the presence of two other fatty acids, $C_{22:1n9}$ and $C_{20:3n3}$, but could not separate and quantify them. Thus, a complementary program was run for this purpose.

For the complementary program, the temperature of the injector and detector were also kept at 250 °C and 260 °C, respectively. The carrier gas flow rate was 0.97 mL min^{-1} and the linear velocity 19 cm s^{-1}. The temperature gradient was as follows: 140 °C (isotherm 5 min) to 190 °C at 4 °C min^{-1} (isotherm 27 min), then at 10 °C min^{-1} to 200 °C (isotherm 20 min), and finally at 25 °C min^{-1} to 240 °C (isotherm 9 min).

Thus considering both programs, the method developed was capable of identifying and determining 50 fatty acids.

3 Results

3.1 Sodium results

The calibration curve for sodium can be represented by the equation ***y= 0.30 x + 0.001*** with a quadratic correlation coefficient equal to ***0.9999***. The blank absorbance was typically ***0.03***. The spiked chocolate samples gave about ***99%*** recovery, and the RSD varied from ***1 to 2%***.

Figure 1 shows the results obtained for sodium as compared to the values declared on the labels. The values obtained were very close for the majority of the samples, within a variation of ± 20%, in agreement with the Brazilian regulation (BRASIL, 2003). However one sample had an unexpectedly high sodium content, about twice the value declared on the label, and in one other case, the sodium concentration was considerably lower than the reported value.

The Brazilian Table of Food Composition gives a value of 77 mg 100 g^{-1} of sodium for milk chocolate, which is a number very similar to the majority of the results obtained. In contrast, sample "G" showed a sodium concentration more than three times this value. There is

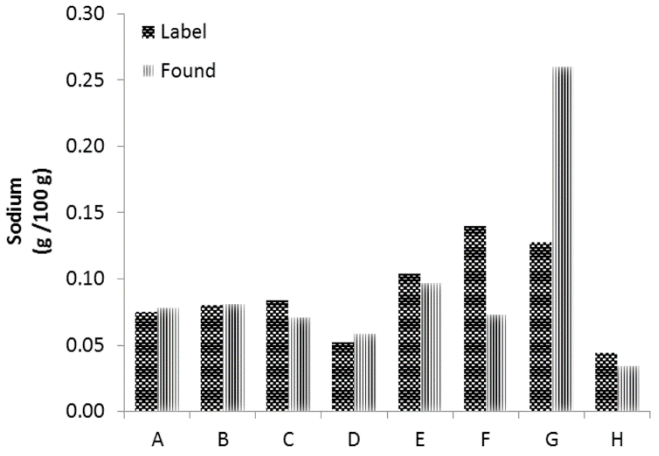

Figure 1. Sodium contents in milk chocolate: concentration found value stated on the package.

reason to suspect that some kind of problem occurred in the industrial process of this manufacturer.

3.2 Fatty acid profile

During sample preparation prior to the chromatographic determination of the lipid profile, one aspect is considered to be of great importance. Possible losses during methylation deserve a special discussion, as explained below, and an experiment involving the lids was carried out.

3.2.1 Lid testing

During methylation, evaporation of liquid from the vials must be avoided, and it is very important to keep the losses of esters formed from the fatty acids as low as possible, by employing a secure lid. The total losses can be quantified, and the resulting data used to improve the procedure.

Three different types of seal were tested, searching for the best choice to reduce evaporation from the vials. The lids 1.Fischerbrand Fish-03-338D, 2.National Scientific B7807-15 and 3.Sun-Sri 200835 were submitted to the same conditions described in sample preparation (2.3) for the methylation step, with the same amounts of the same reagents, but without the contents originating from the sample. Three lids of each type were tested. The vials with lids were weighed before and after addition of the solvents, and at the end of the methylation process. The test was carried out avoiding the application of too much pressure to the lids, or to leave them loose.

Lid types numbers 1 and 2 were found to allow losses of more than 10% of the vial contents, and also showed changes in shape. On the other hand, the Sun-Sri 200835 lids, made of PTFE, showed good performance, and less than 3% was lost. This lid is capable of being submitted to high temperatures with no changes in shape,

and one can repeat the procedure at least six times without considerable losses, as shown in Table 1.

3.2.2 Fatty acid profile results

Table 2 shows the fatty acid profiles obtained for the milk chocolate samples, with individual results considering each lipid whose value was above the limit of quantitation (0.01 g 100 g^{-1}). As stated before, the method could determine 50 fatty acids, but only 20 of the total of 50 compounds had concentrations above this limit. For concentrations above 1 g 100 g^{-1}, the RSD varied from ***1 to 7%***. For concentrations from 0.1 to 1 g 100 g^{-1}, the RSD varied from ***2 to 16%***. The other values (<0.1 g 100 g^{-1}) are near the limit of quantitation and are therefore considered to be semi-quantitative numbers.

It is apparent that the principal fatty acids are: C18:0, C18:1n9c and C16:0, which are also present in high concentrations in milk, thus their presence must be due to the milk used in the manufacture of the milk chocolate. It is interesting to note that some countries have strict regulations about the presence of fat in chocolate products, and count on a comprehensive database to enforce the regulations (BUCHGRABER et al., 2007). Such work is still to be carried out in Brazil, and thus it is not possible to provide an accurate description of the origin of each fatty acid.

When compared with the Brazilian Table of Food Composition, the results were very similar for almost all the compounds quantified. However there were a few exceptions, for instance, the concentration of C18:3ccc was found to be twice that informed in TACO, whereas C18:1t was found to be half the amount cited in TACO. Such discrepancies may be due to the use of different methods. Anyway, a complete comparison is not possible, since TACO presents a smaller number of quantified fatty acids.

Due to their origin, chocolates are not expected to contain poly-unsaturated fatty acids in significant concentrations, including those regarded as quite desirable, for instance, omega 3 (C18:3ccc), omega 6 (C18:2n6cc) and omega 9 (C18:1n9c) acids. The results found for the milk chocolate samples were in agreement with this expectation, since the values obtained were under 0.1, 1.0 and 0.1 g 100 g^{-1}, respectively. In the case of C20:2, the GC-FID peak should be confirmed using more advanced analytical techniques, since it is an unusual unsaturated fatty acid.

Table 1. Solvent losses from each vial+lid ensemble, using Lid 3.Sun-Sri 200835.

Experiment Number	Solvent loss (g 100g^{-1})		
	replicate 1	replicate 2	replicate 3
1	2.6	0.23	*
2	*	*	2.8
3	1.4	2.0	*
4	*	*	*
5	1.8	*	1.3
6	*	1.3	1.7

*no apparent loss.

Table 2. Fatty acid contents in milk chocolate samples from the different brands (concentrations found above the quantitation limits).

Fatty acid*	A	B	C	D	E	F	G	H
C6:0	0.08	0.07	0.05	0.06	0.08	0.08	0.07	0.06
C8:0	0.04	0.03	0.03	0.03	0.04	0.05	0.04	0.03
C10:0	0.09	0.10	0.07	0.07	0.11	0.11	0.07	0.07
C12:0	0.12	0.12	0.07	0.09	0.14	0.12	0.10	0.10
C14:0	0.41	0.38	0.28	0.32	0.50	0.43	0.35	0.33
C14:1	0.04	0.03	0.03	0.03	0.04	0.03	0.03	0.03
C15:0	0.04	0.03	0.03	0.03	0.04	0.05	0.04	0.03
C16:0	8.2	6.9	6.9	7.8	9.9	6.5	7.6	7.7
C16:1	0.12	0.10	0.10	0.10	0.15	0.09	0.10	0.10
C17:0	0.08	0.07	0.07	0.07	0.08	0.06	0.07	0.06
C18:0	9.4	8.3	8.2	9.5	11.7	6.7	7.9	9.4
C18:1t	0.09	0.03	0.04	0.06	0.08	0.06	ND	0.07
C18:1n9c	9.95	7.76	8.84	10.45	12.42	7.44	8.58	9.87
C18:1c	0.04	0.06	0.03	0.07	0.04	0.03	0.04	0.03
C18:2n6cc	0.98	0.69	0.89	1.15	1.12	0.79	0.84	0.94
C18:3ttt	0.22	0.19	0.19	0.22	0.27	0.15	0.18	0.23
C18:3ccc	0.08	0.07	0.07	0.07	0.08	0.06	0.06	0.07
C20:2	0.04	ND	0.03	0.03	0.02	ND	0.04	0.03
C22:0	0.04	0.03	0.03	0.05	0.07	0.03	0.04	0.04
C24:0	0.06	0.05	0.05	0.07	0.08	0.04	0.05	0.06

ND = not detected. *(g 100 g^{-1}).

Table 3. Fat (total, saturated and trans) expressed as the fatty acid contents in the milk chocolate samples.

Brand	Total fat*		Saturated fat*		Trans fat*	
	VP	VF	VP	VF	VP	VF
A	41.0	30.1	25.0	18.5	0.0	0.3
B	31.5	25.1	20.3	16.2	1.0	0.2
C	30.0	26.0	18.0	15.8	0.0	0.2
D	31.6	30.3	18.0	18.1	0.0	0.3
E	38.4	37.0	23.2	22.8	0.0	0.4
F	28.4	22.8	17.2	14.2	0.0	0.2
G	34.4	26.2	20.0	16.4	0.0	0.2
H	30.8	29.4	17.6	18.0	0.0	0.3

VP - Value stated on the package, calculated in percentage, instead of being expressed in the portion (20 - 30 g); VF - Value Found. *(g 100 g^{-1}).

It was possible to detect and quantify trans fat in the samples. This was not generally declared on the label, even when quantifiable due to the size of the portion considered, normally 20 to 30 g.

Table 3 shows that the total fat found was generally close to the value declared on the label. In terms of saturated fat, which is one of the principal health concerns, there was a certain difference (–26%) between the value on the label and the value found for sample "A", the concentration found being a better value for the consumers than the value declared on the label.

4 Conclusions

This work provided nutritional information about the milk chocolate bars available for consumers on the Brazilian market. A total of 50 fatty acids (GC-FID) and the sodium contents (FAAS) were determined in eight samples of milk chocolate bars from different manufacturers in 2013.

Whilst the results obtained for sodium were, in general, close to the values informed on the labels, and also similar to those found in the Brazilian Table of Food Composition, one important exception was found, where the sodium contents were more than twice the value stated on the label. This may suggest that some problem occurred during the industrial process, and demonstrates the importance of food inspection.

A GC-FID procedure was described to determine the liid profiles, which allowed for the quantitation of 48 fatty acids simultaneously, and a further two fatty acids were determined separately. In the sample preparation step, possible losses during methylation deserved a special discussion, considering that the evaporation of liquid from the vials must be avoided. An experiment for the comparison of different types of lid was carried out, which confirmed the importance of adequate sealing.

This work aimed to add data to the Brazilian Table of Food Composition, specifically in terms of the presence of more fatty acids than those originally determined in milk chocolate. In addition conclusions were made about the accuracy of the information on the labels, that is, to what extent the consumer is receiving the correct information. Finally, since it is highly descriptive, the work may help other analysts in future projects.

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