UNIVERSIDADE DA CORUÑA

Grao en Bioloxía

Memoria del Trabajo de Fin de Grado

Study of the quality of dark chocolate (70% cocoa) present in the market

Estudio de la calidad del chocolate negro (70% cacao) presente en el mercado

Estudo da calidade do chocolate negro (70% cacao) presente no mercado

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Julio, 2021

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UNIVERSIDADE DA CORUÑA

D. JOSÉ DÍAZ VARELA, CATEDRÁTICO DE FISIOLOGÍA VEGETAL, DOCTOR EN BIOLOGÍA, Y D. JAVIER VELOSO FREIRE, CONTRATADO POSTDOCTORAL, DOCTOR EN BIOLOGÍA, DEL DEPARTAMENTO DE BIOLOXÍA DE LA UNIVERSIDADE DA CORUÑA

INFORMAN:

Que el presente Trabajo de Fin de Grado presentado por la alumna YACINE DIEYE ZOTES y titulado:

"Study of the quality of dark chocolate (70% cocoa) present in the market"
"Estudio de la calidad del chocolate negro (70% cacao) presente en el mercado"
"Estudo da calidade do chocolate negro (70% cacao) presente no mercado"

ha sido realizado bajo su dirección y autorizan su presentación para que pueda ser juzgado por el tribunal correspondiente.

Asimismo, manifestamos que ambos PDI disponemos de la correspondiente acreditación para impartir materias en inglés (como es el caso del Trabajo Fin de Grado) de acuerdo con la vigente "Normativa reguladora de la Docencia en inglés en las titulaciones de la Universidad de A Coruña".

Y para que así conste, expiden y firman el presente informe en A Coruña, a 23 de Julio de 2021.

Fdo. José Díaz Varela

Fdo. Javier Veloso Freire

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ABSTRACT

During the last few decades, dark chocolate has increased its demand at a global scale, mainly due to the popularity of the idea of its multiple benefits. From this concept, the variety of the supply of dark chocolate (70% cocoa) present in the local market of the metropolitan area of A Coruña (Spain) is analyzed in this study, with the aim of studying the offer range. In addition, the quality of the offered products is also analyzed, taking into account the reliability of the commercial data and the presence of substances that are favorable or harmful for human health. Lastly, a population tasting survey is performed, in order to conclude (by using the mentioned analysis too) if there is a good offer of dark chocolate 70% cocoa in the mentioned area (as an example of spanish town)

RESUMEN

En las últimas décadas, el chocolate negro ha aumentado su demanda a escala global, debido en gran medida a la popularidad de la idea de sus múltiples beneficios para la salud humana. A partir de este concepto, en este estudio se analiza la variedad de oferta de chocolate negro (70% de cacao) presente en el mercado local del área metropolitana de A Coruña (España), con la finalidad de estudiar el rango de oferta. Además, también se analiza la calidad de los productos ofertados, teniendo en cuenta la fiabilidad de los datos comerciales y la presencia de substancias favorables y nocivas para la salud humana. Por último, en el estudio también se realiza una encuesta poblacional a modo de cata, para, junto con los análisis mencionados, concluir si existe una buena oferta de chocolate negro 70% cacao en dicha zona (como ejemplo de ciudad española)

RESUMO

Nas últimas décadas, o chocolate negro aumentou a súa demanda a escala global, debido nunha gran medida á popularidade da idea dos seus múltiples beneficios para a saúde humana. Partindo deste concepto, neste estudo analízase a variedade de oferta de chocolate negro (70% cacao) presente no mercado local da área metropolitana de A Coruña (España), coa finalidade de estudar o rango de oferta. Ademais, tamén analízase a calidade dos produtos ofertados, tendo en conta a fiabilidade dos datos comerciais e mais a presenza de substancias favorables ou daniñas para a saúde humana. Por último, no estudo tamén se fai unha enquisa poboacional a modo de cata, para, xunto coas análises mencionadas, chegar á conclusión de se existe una boa oferta de chocolate negro 70% cacao en dita zona (coma exemplo de cidade española)

KEYWORDS

Dark chocolate, lipid content, phenolic compounds, tasting survey

PALABRAS CLAVE

Chocolate negro, contenido en lípidos, compuestos fenólicos, cata,

PALABRAS CHAVE

Chocolate negro, contido en lípidos, compostos fenólicos, cata

1. INTRODUCTION

1.1. History of chocolate

As it is well known, cocoa is the main component of chocolate. This ingredient comes from cocoa seeds, present inside the pod of the cocoa plant (*Theobroma cacao*). The relationship between cocoa and the human being had its origin in the Maya civilization, around 400 AD. Furthermore, although the current crop varieties are not the same as in the past due to breeding (De la Cruz et al., 1996), this ingredient was the main component of a stimulant and bitter drink named “xocolatl” in Maya language. In fact, it was as such a beverage how cocoa was introduced in Europe some years later by Hernán Cortés, after the colonization of South America (after 1492).
Thus, some specific european countries were responsible for the mixture between cocoa seeds and sweet flavours (such as vanilla, sugar, etc), around 1660, when the commercial product was called. After that, some important dates in the advance of chocolate production were the processing of chocolate butter (firstly done in 1815 by Conread Van Houten), and the production of the first chocolate bar in 1847.
As a result, the demand for chocolate increased with time and has caused the expansion of cocoa cultures at a global level. In this way, cocoa originally grown in Central America, is currently produced in various countries, mostly placed in the intertropical zone (such as Ecuador, Brazil, Ghana, etc.) and some Pacific Islands (Verna, 2013; Rusconi and Conti, 2010).

1.2. Economic importance

When talking about the chocolate industry, it must be pointed out that its development is crucial for the global market. This is due to the fact that cocoa (the main chocolate component) is one of the main primary products exported by economically developing nations (located in Asia, South America and Africa) (Quintero and Morales, 2004). Furthermore, cocoa is grown in small farms runned by local families. As a consequence, cocoa production helps to create job positions in underdeveloped countries, and it is supposed to be a source of enrichment for them. According to Beg et al. (2017); up to six million farmers were involved in this labour in 2017, meaning that between 40 and 50 millions of people are currently financially surviving thanks to this labour. However, in the last few years some issues have arised, due to the fact that the positive evolution of the global economy has caused an important increase in cocoa demand. Thus, worldwide chocolate-related multinationals are dealing with the lack of resources of primary producers, and are considering the idea of improving the neglected and poor working conditions of their suppliers (Fold, 2001; Beg et al., 2017). Furthermore, it must be said that, although cocoa is often used in several industries (not only food industry, but also cosmetic or even pharmaceutic), chocolate is not considered a basic good, therefore only afforded by people with a good income. As a

consequence, the chocolate industry is considered to be one of the first that would be damaged if changes of the global food industry happened (Quintero and Díaz, 2004). Thus, the cocoa crop has been commonly affected by other external factors (Schmitz and Shapiro, 2012). For instance, it has been influenced by political conflicts; that might be the cause of the decreasing global cocoa production that took place in 2001, due to these types of disturbances in the main cocoa producer country: Côte d'Ivoire. On the second place, several biologic factors are able to alter the whole chain equilibrium, such as the yield decrease associated to the witches' broom disease in 2003 (Quintero and Díaz, 2004); or the recent outbreaks of plagues affecting the cocoa crop in Ghana or Ivory Coast (Goergen, 2020).
This high variability in yield and production dependent on several external factors (diseases, critical weather conditions or government problems, etc.), explains the continuous historical variation of cocoa manufacturing, and thus, the instability of the economy of the chocolate fabrication. For instance, according to Beg et al. (2017) the highly productive conditions given in the year 2000, caused a decrease on the prices of cocoa (up to $714 per tonne), whereas adversal conditions raised the tonne price until $3775 in 2011. In figure 1 the price increase in the mentioned period of time can be observed for one of the main global cocoa producer countries (Côte d'Ivoire).

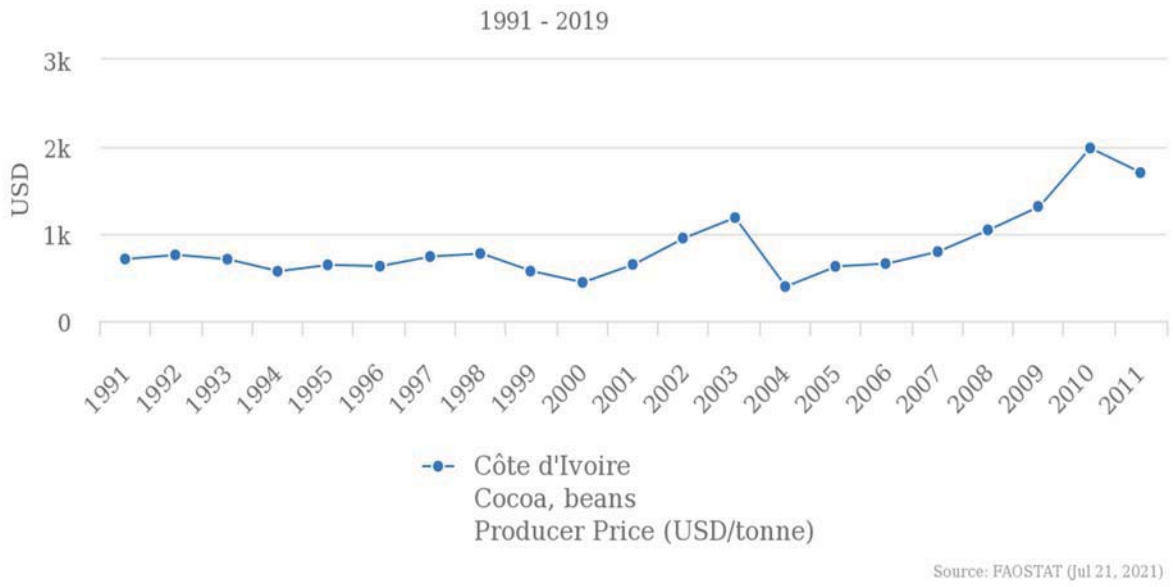

Figure 1.: **Evolution of the annual producer price of cocoa beans from Côte d'Ivoire between 1991 and 2019 (FAOSTAT, 2021).** X axis: Year (values from 1991 to 2011); Y axis: price of cocoa beans expressed in USD per tonne (range from 0$ to 3.000$)

However, in the last few years, it must be said that dark chocolate demand is clearly increasing (Zugravu and Otelea, 2019).

1.3. Main chocolate producers

According to Quintero and Díaz, 2004; Beg et al., 2017; Côte d'Ivoire has been the first country in the global chart of cocoa production during the last decades. In fact, Africa is the main continent producing cocoa, followed by Asia, Oceania, and South America. In table 1 and figure 2, the main countries that produce cocoa according to reference sources are shown.

Table 1. **Main cocoae producers and their contribution to global production.** Data collected from Beg et al. (2017)

Regions	Countries	Production Share
Africa	Ivory Coast, Ghana, Nigeria, Cameroon	68%
Asia and Oceania	Papua New Guinea, Malaysia, Indonesia	17%
America	Colombia, Brazil, Ecuador	15%

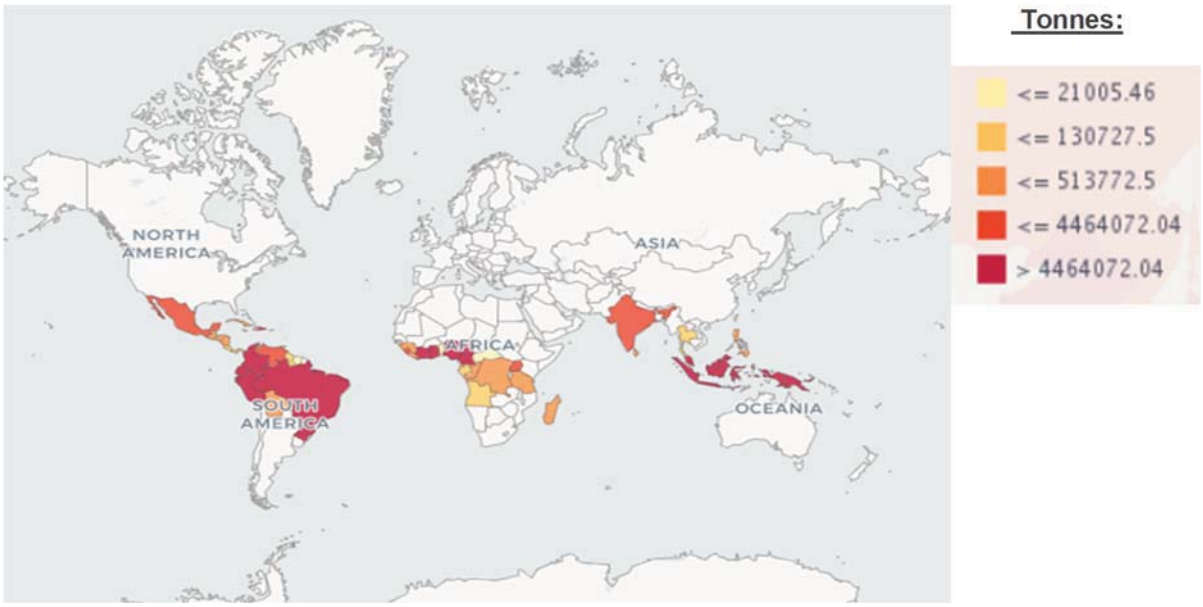

Figure 2: World map signaling the main cocoa producer countries between years 1990 and 2019 (FAOSTAT, 2021)

The generally intertropical placing of cocoa crops is due to the climatic conditions needed for its growth. Cocoa trees grow under conditions of equatorial to tropical wet climates. Thus, the optimal conditions for its growth (temperature ranging from 21ºC to 32ºC annual rainfall among 1.150 and 2.500 mm) are only given in the area between latitudes 20ºN and 20ºS (Quintero and Díaz, 2004). In addition, these trees must be grown in low altitude zones, because besides the conditions mentioned above, they

need to be protected from extreme climate factors such as wind, that could cause a huge physical injury on the plant, and cause damage to it.

1.4. Production process

The first step in the chocolate production process is the manual harvesting of cocoa pods and the extraction of cocoa beans. Depending on the area, this process can be done regularly or twice a year. Then, beans are incubated to allow anaerobic fermentation and then dried by different traditional processes (depending on the country). After being exported, beans are roasted in order to obtain the nibs. This objective is reached after making mechanical impact over them (kibbling), and separating the obtained nibs from the shell (winnowing). Then, cocoa liquor is obtained from the high temperatures reached at the grinding process. At this part, the first variety of chocolate of the process is obtained, and this liquid substance is often sold in order to make baking chocolate. It must be pointed out that during chocolate production, the dutching process (reduction of the acidity) takes place, and the location of this activity determines specific qualities of the product obtained. Finally, the fat-rich fraction (cocoa butter) is separated by the usage of an hydrostatic press, releasing cocoa liquor too. In this way, the fat proportion of each chocolate type is directly related with the characteristics of this last step (Beg et al., 2017).

1.5. Benefits and inconvenients on dark chocolate ingestion

Although chocolate may first be considered as an unhealthy food, several studies point to specific benefits of its consumption for human well-being, especially when talking about dark chocolate instead of the milky one (Serafini et al., 2003). According to Laličić-Petronijević et al. (2016); this aliment provides a remarkable proportion of antioxidants to consumers (up to 20% of the total adult ingestion of antioxidants in The Netherlands, for instance). Such antioxidants are phenolic compounds (anthocyanins, proanthocyanidins and flavan-3-ols), that reduce the presence of free oxidative radicals by reacting with them. Thus, such substances may favour human health mainly improving cardiovascular activity. These components play a key role in processes such as cholesterol metabolism, fat concentration in blood vessels and blood pressure regulation (interfering in the system renin-angiotensin, and improving the endothelium activity and thus its release of nitric oxide). However, on the other hand, some studies question the truthfulness of these effects when phenolic compounds are ingested via chocolate bars, instead of natural pure cocoa (Rusconi and Conti, 2010). Besides the benefits of these substances, chocolate also presents other chemical compounds which have been demonstrated to produce positive effects in human health, such as methylxanthines. In this way, caffeine supply also improves the cardiovascular system’s health, apart from being a stimulator of the central nervous system. On the second place, phenylethylamine may work as an activator for the sensation of well-being. Lastly, theobromine might also present some benefits for

the human being, like reducing the risks of malignant glioma formation, or increasing the proportion of HDL (Gammone et al., 2018; Langer et al., 2011).

However, it must be admitted that this nourishment presents also an important proportion of hurtful components. Firstly, chocolate bars show a high percentage of saturated fats that are not healthy. On average, a general piece of dark chocolate might have 40% of these lipids (Gammone et al., 2018), posing a potential risk when it is ingested in large amounts, since these acids highly contribute to the appearance of cardiovascular diseases (Kris-Etherton and Krauss, 2020). Furthermore, for instance it has been demonstrated that a high content of palmitic acid (more than 20%) is a relevant indicator of the presence of palm oil within chocolate components, and that this percentage was found in 100% of the dark chocolate samples of previous studies (Suzuki et al., 2011). Secondly, this food also shows a relevant sugar proportion. Since it has been proven that high sugar ingestion may substantially alter the glucose regulation processes, low sugar chocolates are recommended. In addition, it has also been proven that some of the previously mentioned methylxanthines may have an adverse effect on cardiovascular health of some people presenting related previous issues. (when it is consumed in big amounts) (Gammone et al., 2018).
Thus, in this way, it is deduced that the higher the cocoa proportion, the more benefits (polyphenols) may the chocolate have in comparison with the disadvantages (fats and sugar). Hence, dark chocolate is considered to be healthier than milky chocolate; since the first one presents almost twice the weight of polyphenols than the second one (Laličić-Petronijević et al., 2016). In addition, the antioxidant activity of phenolic compounds can be inhibited by the presence of milk proteins, due to the establishment of secondary bonds (Serafini et al., 2003).

Currently, although the mentioned variables have been analyzed on multiple occasions, there is a lack of literature regarding the analysis of different dark chocolate bars commonly present in Spanish local markets. Furthermore, it is known that, for instance, in 2016 the average annual chocolate consumption in spanish adults was 2kg per year, and 43’3% of spanish chocolate consumers preferred dark chocolate instead of the milky one (Revista ARAL, 2016).

Thus, taking into account all of the previously mentioned data, this variety of chocolate has been chosen for the study due to several reasons:

- Potential in the global food market. Since chocolate demand has increased in the last decades (Quintero and Díaz, 2004), and production mechanisms are starting to be improved, in the direction to a more sustainable and respectful way for small-farmers (Beg et al., 2017)
- Healthy benefits. In comparison with other chocolate varieties that have a lower cocoa content.

- Availability in the market. The selection of a standard percentage (70%) allows a wider sample variety. Furthermore, it increases the interest of the study, since the obtained conclusions may be highly useful and applicable to our daily life.

2. OBJECTIVES

- To know the varierty of the offer of dark chocolate (70% cocoa) present in the average market of a spanish village (metropolitan area of A Coruña, Spain), sampling different dark chocolate (70% cocoa) brands.
- Checking the veracity of the weight stated in the package tags of the samples.
- Determine the quality of the samples by performing a tasting survey in a group of non-professional tasters.
- To analyze two variables indicating both unhealthy components (fats) and quality components (phenolic compounds) of the sample.

3. MATERIALS AND METHODS

3.1 Sampling

Chocolate bars of 13 different brands were analysed. They were purchased in duplicate (matching bars had to have the same lot number) at different markets of one single zone (metropolitan area of A Coruña, Spain). Thus, they highly reflect part of the variety offered in the local market. The main criteria for their selection was a 70% of cocoa content stated in the package tag. In addition, the bars showed a relevant diversity because they belong not only to different brands, but also to different cocoa origins. Furthermore, in order to maintain confidentiality, each chocolate bar variety was assigned a sample code (number).

3.2. Veracity of the tag data check

In order to verify the weight stated in the package tag of each chocolate bar, they were weighed after removing the package. Then, the mean between two matching chocolate bars and the percentage of change among declared and real values were calculated.

3.3. Tasting survey

The process of data collection for this part of the experiment was performed following mainly the study of Sukha (2016). Thus, we recruited 20 volunteers ranging from 18 to 25 years old. The volunteers were not professional tasters.

3.3.1. *Sample preparation*

For each bar, 20 samples of approximately 2 grams each one were separated. Then, they were individually packed in aluminium foil, and the corresponding code was written on it with a black permanent marker. Finally, these samples were distributed into hermetic plastic bags, and one bag of each sample was delivered to each volunteer. The same process was done with the 13 bars. However, 3 bar samples were included twice in the process as a checkpoint of the blind experiment, in order to check the reliability of the survey and the possible influence of other external variables affecting the volunteer. Thus, in the case of the repeated samples, the samples separated from each bar were 40 instead of 20.

In order to do the process and to offer the maximum protection and reduce risky conditions (especially related to the possibility of transference of COVID-19), the sampling process was performed by the author of this dissertation, in a small ventilated room, and the sampler was equipped with lab coat, latex gloves, and face mask. Furthermore, the materials were constantly disinfected with ethanol 70%, between samples and bar changes.

The samples were kept in their corresponding bags, under dry and temperate to cold conditions (environmental temperature) until the moment of the test.

3.3.2. Survey design

The tasting survey consists of two parts: one related with perceiving the aroma, and the second one regarding the flavour of the samples.

For the selection of the participants, important data were needed:

- Age: Between 18 and 25 years old. It has been confirmed that the perception of the different flavours (sweet, sour, etc.) changes with age, and normally decreases as we get older. Therefore, in order to prevent the interference of other variables like this one, it was crucial to assess the samples by tasters within short range of ages. It has also been demonstrated that taste is influenced by gender (Barragán et al., 2018). Therefore, 11 females and 9 males were selected as tasters.
- Allergies: For the sake not only the study results, but also the volunteers, it was indispensable to select non-allergic tasters that could not experience any adverse reaction to components of the chocolate bars such as cocoa, milk, dry fruits, etc.

3.3.3. Procedure

Due to the risks of the contemporary world wide pandemic caused by COVID-19, the performance of the survey was done by videoconference using Microsoft Teams. Thus, each corresponding bag was delivered to the volunteers, and after that, the next procedure was followed:

-First of all, personal data was asked in order to check the validity of the candidates.

-Then, the tasting process took place, following these steps:

A. *Aromal:*
    1. *Open the sample A.*
    2. *Maintaining it on the piece of aluminum foil, approach it to the nose, with a distance of approximately 5 cm between the sample and the tip of the nose.*
    3. *With the eyes closed, smell it until being sure of the aroma of the sample.*
    4. *Rate the aroma according to:*
        a. *Intensity of the aroma (from 0 to 10)*
        b. *Mention aromas other than chocolate if perceived (free answer).*

B. *Taste:*
    1. *Enter the sample into the mouth without chewing it.*
    2. *With the eyes closed, when indicated, start chewing (30 seconds, measured by the pollster)*
    3. *Remove the sample:*
        a. *Swallowing it*
        b. *Splitting it*
    4. *Rate the flavour according to:*
        a. *Sweet (from 0 to 10)*
        b. *Bitter (from 0 to 10)*
        c. *Salty (from 0 to 10)*
        d. *Sour (from 0 to 10)*
        e. *Cocoa (from 0 to 10)*
        f. *Mention flavours other than the above stated, if detected (free answer)*
    5. *Remove sample remainings from the mouth rinsing with water*
        a. *Swallowed*
        b. *Splitted*

Once the previous steps are performed, continue with sample B and so on (in alphabetic order of the sample code).

Notice that no extra information was given to the volunteers, in order to avoid bias in the survey results. Thus, no other questions or remarkable physical gestures were performed by the pollster.

3.3.4. Data analysis

First of all, in order to check the reliability of the results of each tester, the variation of the given punctuation between paired samples was estimated. Then, the average of these values was calculated for each tester, and the values obtained from volunteers that showed a variation larger than 10% were not considered valid for the performance of the rest of the analysis.

In the second place, a contingency table was done for each one of the variables evaluated (Fragrance intensity, flavour sweetness, flavour bitterness, flavour saltiness, flavour sourness and cocoa flavour), taking into account the tester code and the frequency of each punctuation given per sample.
In order to analyze the correlation of the results (by performing a chi-square test), punctuations were grouped in 3 ranges of values (from 0 to 3, from 4 to 6 and from 7 to 10). In the case of existence of significant differences between results of different testers, a more detailed study was done. Thus, chi-square tests were performed by pairs of data, for all the possible combinations between testers.

3.4. Quality and unhealthy components evaluation

Related to one of the aims of the experiment (study of fats and phenols of dark chocolate), the main procedure of this analysis was carried out according to Laličić-Petronijević et al. (2016), with some modifications.

3.4.1. Lipids

The initial step was the separation of fats, and the remaining defatted samples were used in order to determine the phenolic compound content. In brief, the separation of fats was based on these steps: sample preparation, separation, drying and final weighing.

- Sample preparation:

Previously to the experiment, a sample of 4 g of each chocolate bar was separated and frozen at a temperature -80 ºC until the analysis, order to preserve its fat and phenolic content.
Then, a sample of 1g of each variety was weighed, placed into a 12mL tube with a polypropylene cap.

- Extraction:

Chemicals: n-Hexane

- 8mL of *n*-Hexane were added to each tube and once closed, tubes were heated in the thermoblock at 45ºC during 5 minutes. Then, tubes were shaken and placed again in the thermoblock for 5

additional minutes, and were shaken when half of the time had passed and also at the end of incubation.

- After 5 minutes at room temperature for tempering, tubes were centrifuged at 6.700 xg during 5 min.
- The supernatant was discarded.

These 3 steps were repeated once more, adding 8 mL of fresh *n*-hexane to the pellet fraction.

- Drying:

The tubes with the pellet were left opened at room temperature overnight.

- Final weighing:

Finally, once dried, the pellets were were weighed.

Notice that all processes involving *n*-hexane exposition were performed in the fume hood for security reasons.

In order to perform the data analysis, the average percentage of lipids was calculated for each sample, by subtracting the weight of the dry pellet from the starting weight. Two independent analysis were carried out for each sample. In order to compare samples, a Kruskal Wallis analysis and a post-hoc Dunn test was performed. In addition, final results were compared with the commercial declared lipid content in the tag of the sample.

3.4.2. Phenolic compounds

This process was done using the dried samples of the pellet tubes mentioned above.

- Phenolics extraction:
  Chemicals: Methanol 80%
  - 50mg of each sample were weighed and placed into Eppendorf tubes of 2mL. 1.5mL of Methanol 80% were added to each tube.
  - Tubes were shaken and placed in the thermoblock at a temperature of 70ºC for 15 minutes, shaking them every 5 minutes.
  - Tubes were centrifuged at 12000 xg during 1 minute.
  - The supernatant was extracted and placed into Eppendorf tubes of 2 mL.
  - 0.5 mL of Methanol 80% were added to the pellet tubes, tubes were shaken and centrifuged again under the same conditions as described.
  - The obtained supernatant was extracted and added to the first one.
  - The supernatant Eppendorf tubes of 2mL were flushed with the Methanol 80% up to 2mL.
- Phenolics determination:
  Chemicals: Distilled water, Folin-Ciocalteu reagent, NaCO□ 20% solution
  - The reaction mixtures were made by adding to an Eppendorf tube 5µL of the supernatant sample, 795 µL of distilled water and 50µL of the

Folin-Ciocalteu reagent. They were made in triplicate for each sample. In addition, two blank solutions were obtained.

- Tubes were shaken. After 3 minutes, 150µL of NaCO□ solution were added to each tube. Then, the tubes were shaken again.
- After 2 hours, the absorbance was measured in the spectrophotometer at 760 nm wavelength.

- Calibration curve:
  Chemicals: Gallic acid, distilled water,
  - By making solutions of different known concentrations of gallic acid and measuring their absorbance, a calibration curve was obtained.

As a checkpoint of drying efficiency in the initial pellet tubes, the whole obtained samples from each tube were weighted before and after being submitted to a dry process in the oven at 90ºC for at least 30 minutes.

Two independent analysis were carried out for each sample. With the results from the previous steps, the phenolic compound content was calculated for the defatted chocolate and the original chocolate sample. Data were analysed using a Kruskal Wallis test.

4. RESULTS

4.1. Sampling of different dark chocolate varieties and comparison with declared values

The differences between declared bar weights and real ones are in all cases lower than 5% (Table 2). Furthermore, in the cases where the differences were higher than 1%, the actual weight was higher than the announced.

Table 2. Chocolate sampling and weight comparison. Actual weight data correspond to average values ± standard error (n=2). Positive values of Variation (%) correspond to an increment of actual weight compared with the declared tag values whereas negative values correspond to a decrease. SE, standard error.

Sample code	Declared weight (g)	Actual weight (±SE) (g)	Variation (%)
1	90	91.535 (±0.193)	1.706
2	90	91.218 (±0.267)	1.353
3	90	90.6765 (±0.529)	0.752
4	100	99.1495 (±1.708)	-0.851
5	70	72.4735 (±0.027)	3.534
6	70	71.855 (±0.643)	2.650
7	100	101.094 (±0.157)	1.094
8	100	100.685 (±0.064)	0.685
9	120	119.977 (±0.029)	-0.019
10	125	126.537 (±0.610)	1.299
11	100	101.378 (±0.286)	1.378
12	100	99.2725 (±1.109)	-0.727
13	200	209.706 (±0.207)	4.853

4.2. Tasting survey to non-professional tasters

According to the results of chi-square analysis, there were significant differences among the punctuations given to the aroma intensity (Figure 3) of the samples (p-value<0.05). Chi square tests done between pairs of samples for aroma intensity determined that the samples that had more aroma showing the higher number of significant differences with other samples, were those with these codes: 5, 9 and 13 (Figure 3). The three ranges of punctuation (0 to 3, 4 to 6 and 7 to 10) were present in the evaluation of all the samples.

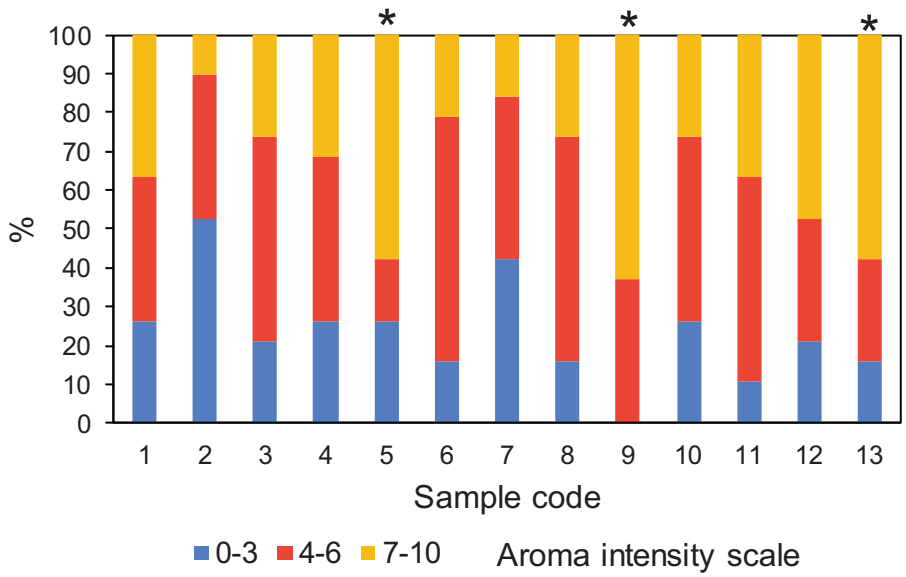

Figure 3. Frequency of aroma intensity values per sample type. 0-3, punctuations between values of 0 and 3 (both included); 4-6, punctuations between values of 4 and 6 (both included); 4-6, punctuations between values of 7 and 10 (both included).

In the case of sweetness, bitterness and saltiness, significant differences were observed according to chi-square analysis including all the samples (p-value<0.05).
Chi square tests done between pairs of samples for sweetness also determined that the samples that were sweeter, showing the higher number of significant differences with other samples, were those with these codes: 2, 7 and 10 (Figure 4A).
In the case of bitterness and sourness, the results of these pairs comparison showed the some trend in both cases: samples with codes 5, 11 and 12 were the more bitter and sour (Figure 4B,C).

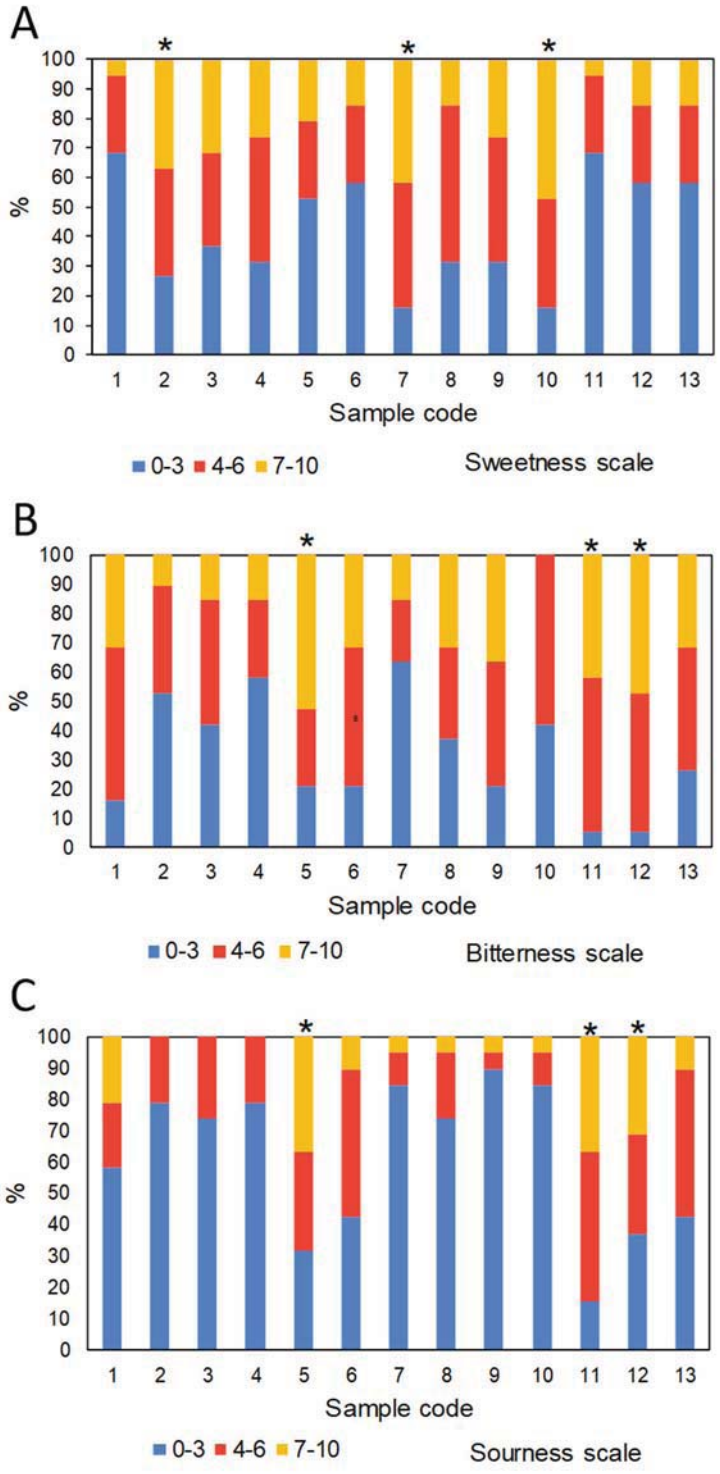

Figure 4. Frequency of sweetness values (A), bitterness values (B) and sourness values (C) per sample type. Codes marked with * indicate the 3 samples that present a higher number of significant differences when compared with the rest. 0-3, punctuations between values of 0 and 3 (both included); 4-6, punctuations between values of 4 and 6 (both included); 4-6, punctuations between values of 7 and 10 (both included).

According to the results of chi-square analysis, there were no significant differences among the punctuations given to the saltiness of the samples (p-value>0.05). However, sample 1 shows a lack of scores between 7 and 10 points, whereas sample 7 was only punctuated with values from 0 to 3 points (Figure 5).

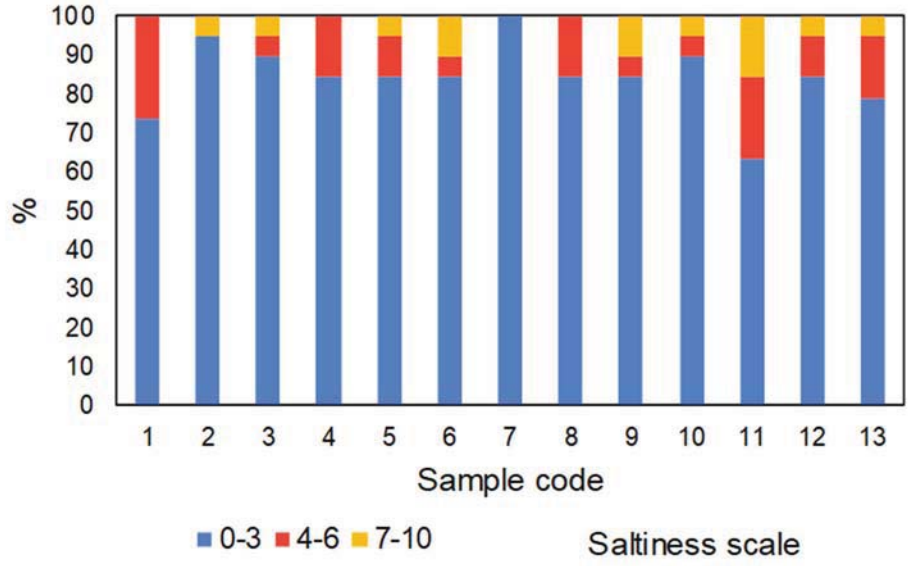

Figure 5. Frequency of saltiness values per sample type. 0-3, punctuations between values of 0 and 3 (both included); 4-6, punctuations between values of 4 and 6 (both included); 4-6, punctuations between values of 7 and 10 (both included).

According to the results of chi-square analysis, there were no significant differences among the punctuations given to the cocoa flavour of the samples (p-value>0.05). The three ranges of punctuation (0 to 3, 4 to 6 and 7 to 10) were present in the evaluation of all the samples (Figure 6).

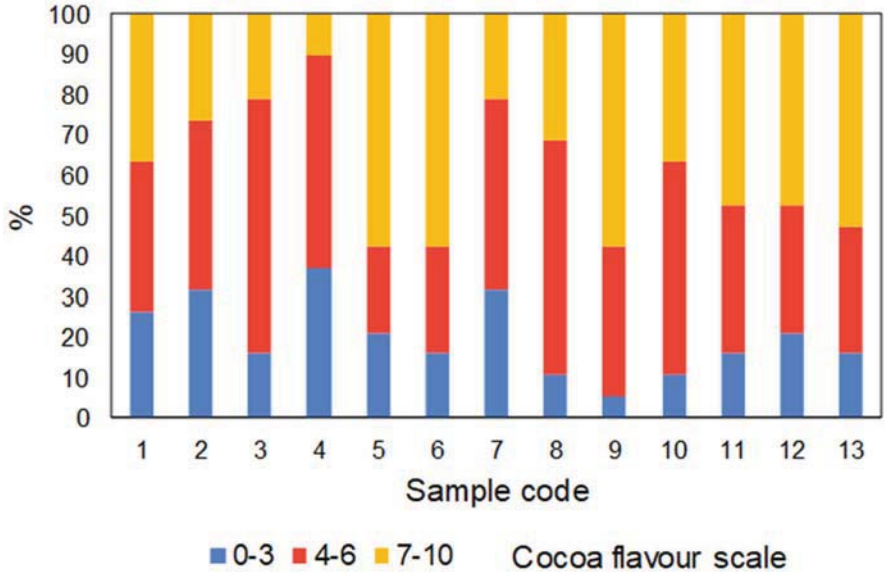

Figure 6. Frequency of cocoa flavour values per sample type. 0-3, punctuations between values of 0 and 3 (both included); 4-6, punctuations between values of 4 and 6 (both included); 4-6, punctuations between values of 7 and 10 (both included).

4.3. Fat and phenolic compounds analysis

4.3.1. Lipids (fat)

Most of the samples showed a higher fat content in our analysis than the lipid content stated in the package tag with the information for the consumer. The exception were samples 5 and 6, which did not follow this trend (Figure 6). However, the actual values are overall similar to those stated in the tag (Figure 7).

On the other hand, actual values were analyzed by a Kruskal-Wallis test, that revealed significant differences among samples (p-value<0.05). The post-hoc Dunn test estimated that there were significant differences between the groups of samples 4, 7 and 8 (with high fat content) and samples 5, 6 and 13 (with low fat content), which is in accordance with the differences among these samples that we could obtain from the data in the package tags.

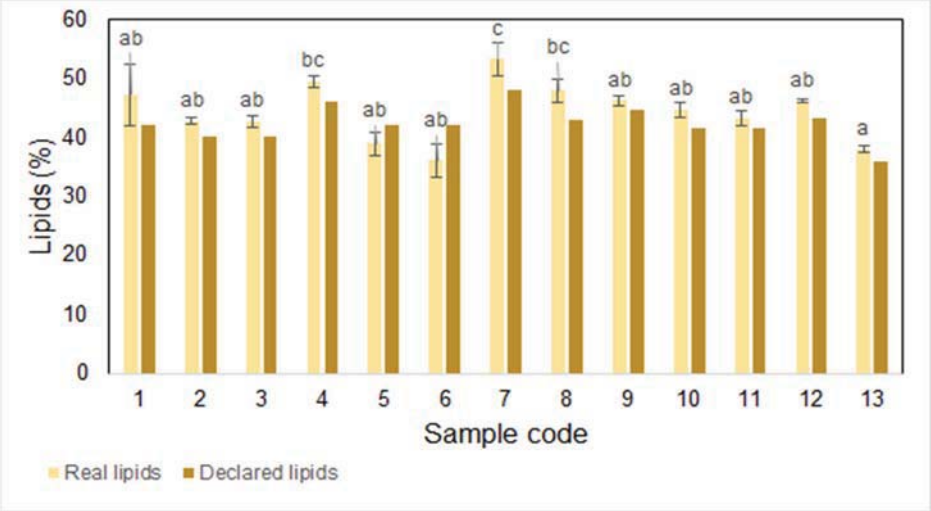

Figure 7. Lipid (fat) content. Actual lipid content data are shown as average values ± standard error (n=2). Different letters indicate significant differences between the different samples (α=0.05).

4.3.2.Phenolic compounds

Regarding the content of phenolic compounds, all values are included within the range of 40 to 80 mg per gram of commercial chocolate (Figure 8). The variation of this value among samples was not supported by Kruskal-Wallis test (p-value>0.05). Therefore, there were no significant differences when comparing the values of phenolic compounds among samples.

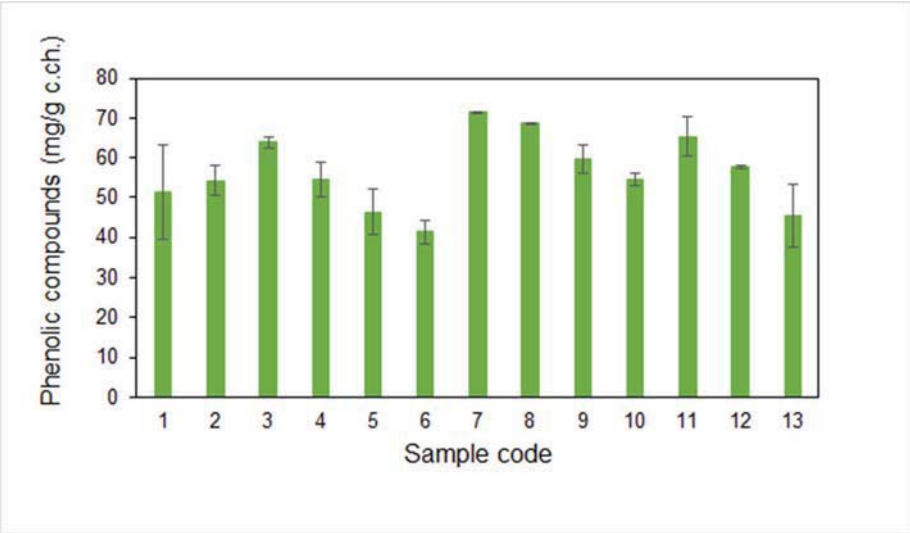

Figure 8. Phenolic compounds. Phenolic data are shown as average values ± standard error (n=2). c. ch., commercial chocolate.

5. DISCUSSION

In the first place, it has been proven that, if they have budget enough, the average population of a representative Spanish area has access to a huge variety of dark chocolate, 70% cocoa. In the study 13 samples of dark chocolate from different origins were found in a metropolitan area of 38.3 km^2 (Ayuntamiento de A Coruña, 2016) during a short period of time (2-3 days). This is important since the demand for dark chocolate is increasing due to the popularity of its benefits (Zugravu and Otelea, 2019), especially when compared to the milky one (Serafini et al., 2003). As a result, the chocolate global market is currently becoming more powerful, and production is being centralized, resulting in the assumption of new future perspectives of the main companies, in order to ameliorate production conditions (Fold, 2001).

However, with the aim of checking the reliability of this continuously emerging trade, correspondence between important product describing data were checked in this investigation. Results of the comparison between declared data of weight and actual values showed a favorable conclusion for chocolate producers. In all of the cases, the variability was lower than 5%, and in situations of a higher value, it was offered a higher quantity of chocolate than declared. Nevertheless, although this last point might seem a benefit for the consumer, it is recommended to be constantly analyzed by the production business, especially when talking about chocolate. It is well known that dark chocolate presents important quantities of sugars and saturated fats (Suzuki et al., 2011) certain specific situations that are given sometimes can be harmful for the health of the consumer (Kris-Etherton and Krauss, 2020), or even phenolic compounds or methylxanthines (Zugravu and Otelea, 2019; Gammone et al., 2018). Following this idea, a slightly detrimental point for the consumer was also detected taking into account this concept since, in the case of lipids, most of the samples

showed higher values than declared (although we could not apply a statistical test to check it).

On the other hand, a tasting survey was performed in order to check how average consumers perceived the differences among different chocolate bars. In the case of aroma perception, no significant differences were detected. However, if we assumed that aroma intensity depends mainly on the qualities of the cocoa (Nouri et al., 2019), results would not show logical conclusions (since cocoa evaluation showed significant differences among samples). Nevertheless, if this last idea is taken into account, it suggests the consideration of other variables that influence aroma perception such as gender (Barragán et al., 2018), cigarette consumption (Mechtild et al., 2018), having passed COVID-19 disease (Aziz et al., 2021), etc., or even surrounding environmental conditions when the aroma perception is performed. In all cases, the huge variety of possibilities for the explanation of this result suggests that the performance of further studies will be done with some modifications (for instance, increasing the number of samples or the sample size, increasing the number of testers, etc.).

Results related with sweetness flavor showed a visible correlation with the values for bitterness perception. Thus, these two variables seem to be inversely proportional in the performed evaluation, and in the same way, bitterness and sourness might be directly related. The fact that the samples showing more significant differences are the same in the case of bitterness and sourness, but completely different in the case of sweetness support this idea. Furthermore, it has been observed that non-professional tasters (that in this case represent the average population) find it easier to perceive the changes in sweetness and also in bitterness or sourness.

In the case of saltiness, the absence of significantly differences could be due to two main reasons. On the one hand, it might be possible that average people have more difficulties to differentiate salt presence. However, in this case it is highly probable that there were no important differences in salt concentration in the samples, since chocolate main ingredients are more related with the other previously mentioned variables.

Referring to cocoa flavor, no significant differences among samples were detected. In this case, the main explanation might be that all bars theoretically contain the same cocoa percentage (70%). Therefore, reliability of the commercial information of dark chocolate bars was checked.

According to the results, all the dark chocolate bars have between 40% and 60% of lipids proportion. Taking into account that lipids pose a potential risk for human health (Zugravu and Otelea, 2019), this variable needs to be improved during the evolution of the trade market of this food. Unfortunately, our analysis did not distinguish between saturated and non-saturated fat.

However, important values of phenolic compounds were also present in the samples, meaning that dark chocolate may have benefits too, especially related with antioxidant capacity (Pimentel et al., 2010)

Taking into account these two variables it must be noticed that in several cases, a higher lipids proportion involved a higher amount of phenolic compounds too in the chocolate bar. This correlation can be due to the fact that cocoa beans present both

phenolic and lipidic compounds. Thus, in order to obtain a healthier product, further studies are suggested.

6. CONCLUSIONS

- Nowadays, there is a wide range of dark chocolate, 70% cocoa, brands available in the Spanish markets.
- In general, the different declared weight values of commercial chocolate bars correspond to the real ones.
- Model testers representing the average young population find difficulties at perceiving sample differences in fragrance intensity, saltiness and cocoa flavour in samples of dark chocolate 70% cocoa. However, variability among samples referring to aroma intensity, sweetness, bitterness and sourness were detected, suggesting relationship with the product ingredients.
- Dark chocolate 70% cocoa shows relevant proportions of both fats and phenolic compounds, and fat content is different among brands.

CONCLUSIONES

- En la actualidad, existe una amplia gama de marcas de chocolate negro, 70% cacao, disponibles en los mercados españoles.
- En general, los diferentes valores de peso declarados de las barras de chocolate comerciales se corresponden con los reales.
- Los catadores que representan la población joven promedio encuentran dificultades para percibir las diferencias de las muestras en la intensidad del aroma, el sabor salado y el cacao en muestras de chocolate negro, 70% de cacao. Sin embargo, se detectó variabilidad entre muestras con respecto a la intensidad del aroma, dulzor, amargor y acidez, lo que sugiere una relación con los ingredientes del producto.
- El chocolate negro con 70% de cacao muestra proporciones relevantes tanto de lípidos como de compuestos fenólicos, y el contenido de lípidos es diferente entre las marcas.
-

CONCLUSIÓNS

- Na actualidade, existe unha amplia gama de marcas de chocolate negro, 70% cacao, dispoñibles nos mercados españois.
- En xeral, os diferentes valores de peso declarados das barras de chocolate comerciais se corresponden cos reais.
- Os catadores que representan a poboación xoven promedio atopan dificultades para percibir as diferencias das mostras na intensidade do aroma, o sabor salgado y el cacao en mostras de chocolate negro, 70% de cacao. Sen embargo, detectouse variabilidade entre mostras con respecto á intensidade

do aroma, dozura, amargor e acidez, o que suxire unha relación cos ingredientes do produto.

- O chocolate negro con 70% de cacao amosa proporcións relevantes tanto de lípidos como de compostos fenólicos, e o contido de lípidos é diferente entre as marcas.

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