Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.1590/fst.10519

Health benefits of chocolate consumption with high concentration of cocoa incorporated from triterpenic acids, isolated from *Mansoa Hirsuta* DC.

Maria Patrícia MILAGRES[1], Daniel Melo SILVA[1], Ivan de Oliveira PEREIRA[2], Ludimila Mascarenhas SENHORINHO[2], Antônio Euzébio GOULART SANT'ANA[3], Talita Batista MATOS[1]*

Abstract

The objective of the work was to develop a 70% cocoa chocolate containing ursolic and oleanolic acids isolated from *Mansoa hirsuta* DC and to evaluate the effects of its consumption on the health of individuals. Physical-chemical, microbiological and sensorial analyzes were made in the chocolates. In order to evaluate the health of individuals, a clinical trial was conducted with the developed chocolate, in which 45 volunteers, during 04 weeks, were divided into three groups: a test group, a placebo group and a control group. The individuals were submitted to laboratory and anthropometric tests on two occasions, before and after the clinical trial weeks. The analyzes revealed that the developed chocolate presented physical-chemical and microbiological characteristics within the standards accepted for bitter chocolate and presented good sensorial acceptance. The results of the clinical trial showed that the volunteers presented reduction in the anthropometric measurements and changes in the laboratory tests.

Keywords: food choices; healthy eating; innovation; chocolate.

Practical Application: Functional chocolate contributes to increasing the quality of health and the well-being of consumers.

1 Introduction

Chronic non-communicable diseases (CNCD) are now the leading causes of morbidity and mortality worldwide (Zhou et al., 2018). Estimates indicate that this number is expected to increase significantly over the next few years, reaching 52 million deaths by 2030 (Arokiasamy et al., 2017).

Thus, it is important to prevent the risk factors associated with these diseases, which includes changes in eating habits (Libman et al., 2015; Ding et al., 2016). However, several studies (Gough & Conner, 2006; Cheval et al., 2017) have mentioned the lack of time and sensory pleasure as motivators for unhealthy eating. The sensory properties of food (intrinsic attributes), which encompasses taste, texture, appearance and odor, are among the most important predictors of food choice by humans (Asioli et al., 2017).

Thus, chocolate can be a tasty and practical alternative for this issue, besides minimizing the lack of pleasure in healthy eating. The benefits of using bitter chocolate for health are widely known; it is a good source of flavonoids and its consumption is associated with decreased risk of death from cardiovascular disease, decreased blood pressure, inhibition of platelet aggregation and antioxidant activity (Carvalho et al., 2018). In addition to flavonoids, it has some phytochemicals that have well-being-stimulant effects in the brain, methylxanthines (Quelal-Vásconez et al., 2019), and there is evidence that bitter chocolate consumption produces anti-inflammatory effects (Colombo et al., 2015).

In addition to cocoa, other Brazilian plants have beneficial effects, among which the *Mansoa hirsuta* DC stands out, a plant in the semi-arid region of Brazil rich in triterpene acids. The use of natural foods containing oleanolic acid or ursolic acid, which are triterpenes widely distributed in the plant kingdom, in folk medicine is diverse and very old. These are known to possess anticholesterolemic, hypoglycemic, anti-hepatotoxic, antioxidant, anti-inflammatory, antifungal and antibiotic activity. Also, they are said to inhibit the growth of tumors and pathogens (Pereira et al., 2017; Zhang et al., 2019).

Although it is present in numerous plants, obtaining these two acids is usually done through its isolation from apple peels, an expensive process (Yin et al., 2018). However, recently, these same compounds were extracted and isolated from *Mansoa hirsuta* DC, a Brazilian semi-arid plant, in a process of extraction and isolation carried out under patent deposit nº BR 1020150081804, which will be used in this work.

Thus, the objective of this work was to develop a 70% cocoa chocolate incorporated with ursolic and oleanolic acids and to evaluate the effects of its consumption on the health of individuals.

2 Materials and methods

The experiments were carried out in the laboratories of the Instituto Federal Baiano Campus de Uruçuca and in the city of Itajuípe, Bahia. The study population consisted of a convenience

Received 18 Apr., 2019
Accepted 28 Sept., 2019
[1] *Universidade Estadual do Sudoeste da Bahia – UESB, Jequié, BA, Brasil*
[2] *Instituto Federal Baiano, Uruçuca, BA, Brasil*
[3] *Universidade Federal de Alagoas – UFAL, Maceió, AL, Brasil*
*Corresponding author: tali.matos@hotmail.com

and non-probabilistic sample, composed of men and women aged over 18 years, totaling 145 (one hundred and fortyfive) volunteers. Of these, 100 volunteers were from the city of Uruçuca and participated in the sensory analysis (acceptance test) and 45 volunteers were from the city of Itajuípe and participated in the clinical trial.

Inclusion criteria were people older than 18 years who wanted to participate in the whole study process, had availability of time, liked to eat chocolate and correctly signed the Informed Consent Form (ICF). Volunteers who presented intolerance/allergy to any components of the product were excluded from the study, as well as those using medications that interfered with the perception of odors and flavors and those who had any pathology that could be aggravated by research and/or that impaired sensory perception.

This research was carried out in accordance with the guidelines and standards of Resolution 466 of December 12, 2012 (Brasil, 2012), sent to the Research Ethics Committee of the Universidade Estadual do Sudoeste da Bahia and approved under No. 46718615.0.0000.0055.

2.1 Obtaining ursolic and oleanolic acids

The ursolic acid and its isomer, the oleanolic acid, were isolated and extracted from the leaves of the *Mansoa hirsuta* DC plant, whose patent was registered at the National Institute of Industrial Property (INPI) under the number BR102015008180 (Silva et al., 2015). The acids were provided by Laboratory of Pharmacognosy of the Pharmacy course of the Universidade Estadual do Sudoeste da Bahia after authorization from the patent's author.

2.2 Chocolate processing

Two chocolate formulations were developed; one containing the triterpenic acids to be used in the clinical trial of the test group, and another not containing triterpenic acids, to be used in the clinical trial of the placebo group.

The chocolate was developed from a formulation containing cocoa liquor (64%), cocoa butter (6%), refined sugar (29.54%), liquid soy lecithin (0.4%) and ursolic and oleanolic acids (0.06%), corresponding to 150 mg of acids/25 g bar of 70%chocolate for the test group of the clinical trial. While the chocolate formulation for the placebo group was cocoa liquor (64%), cocoa butter (6%), refined sugar (29.60%) and liquid soya lecithin (0.4%). The test and placebo chocolates were produced by following the same methodology.

After weighing the ingredients, they were transferred to a Melanger-type roller mill, in which the steps of refining, mixing of ingredients and conching took place. The ingredients in the roller mill were homogenized and kept under stirring at a temperature of about 54 °C for a 24-hour period until the mass turned into chocolate.

Then the chocolate was transferred to a marble table for the tempering step for cooling to 29 °C. After the tempering step, the chocolate was molded manually into specific 25g molds (bars) which were then cooled at approximately 5 °C for two hours. The chocolate was then removed from the molds, packed and stored at 25 °C.

2.3 Physical-chemical characterization

The analyzes for physical-chemical characterization of chocolates (test and placebo) were done three times for each repetition, according to the methodology described in the Adolfo Lutz Institute's Manual of Analytical Standards (Instituto Adolfo Lutz, 2004). It was also performed analyzes of water content (moisture); waste by incineration (ash); pH; total titratable acidity and lipids by Soxhlet with modifications.

Protein quantification (nitrogen content) was performed by using a modified Micro-Kjeldahlm (Association of Official Analytical Chemists, 1990) methodology, and the determination of the total carbohydrates was done by means of the difference in the sum values of moisture, ashes, proteins and lipids. The mean particle size was determined using a digital micrometer with a 0-25 μm scale, according to Sampaio (2011).

A qualitative analysis was carried out to verify the presence of the acids in the chocolate samples by using the methodology of thin layer chromatography, using the ursolic and oleanolic acidspatterns for comparative purposes, under the following conditions: silica gel chromatographic plates, mobile phase chloroform solution: methanol (5% v/v), iodine vapors and sulfuric vanillin as developing agents.

2.4 Microbiological analyzes

Microbiological analyzes were performed to ensure food safety for the sensory tests in the two chocolates (test and placebo), following Annex III of Normative Instruction 62 (2003) and in Petrifilm3M™ plates, according to the recommendations of the manufacturer (3M 1997).

For the preparation of the sample, 25 g of chocolate were used in 225 mL of 0.1% (w/v) sterile peptone saline solution to obtain a 10^{-1}dilution. This solution was used to carry out all analyzes performed under a flat surface within the previously prepared flow chamber and forthree times.

The analysis of mesophilic aerobes was performed in PCA medium (Plate Cont Agar). 1mL of the 10^{-1}dilution was inoculated into sterile Petri dish. 20 mL of the hot (water bath) and molten medium was poured into the plate on the diluted sample (10^{-1}). The plates were inverted and incubated in B.O.D.-type incubators at 25 °C for 48 hours.

For the determination of molds and yeasts from the chocolate samples, it was performed on Petrifilm YM 3M™ (Ref.6407). Then, 1mL of the diluted sample was transferred to Petrifilm. After homogenization, the sample was incubated in B.O.D. incubators at 25 °C for 5 days.

For the determination of coliforms with differentiation for *E. coli* in the samples of chocolate, it was carried out inPetrifilm EC 3M™ (Ref.6404). 1mL of the diluted sample was transferred to Petrifilm. After homogenization, the sample was incubated in B.O.D. incubators at 35 °C for 48 hours.

In all analyzes the colonies were counted in colony counts and the results were expressed by the number of Colony-Forming Units per gram of the sample (CFU/g).

2.5 Sensory analysis

In order to verify the opinion of the people regarding the sensorial characteristics of the chocolate developed for the test group (containing acids), an evaluation was carried out, using a sensorial acceptance analysis of the product. For this purpose, a 9-pointhedonic scale of was used, according to Amaral et al. (2018) apud Belsito et al. (2017), (9 = extremely liked; 5 = neither liked nor disliked, and 1 = disgusted extremely).

The chocolate samples were evaluated as for overall impression by 100 untrained evaluators. Thus, containers encoded with three random digits containing 10g of chocolate at room temperature were served to the evaluators in individual booths using white light and individually.

Together with chocolate, the evaluator received a hedonic-scale sensory acceptance form, where he/she was asked to mark his/her overall impression on chocolate according to the proposed scale (Belsito et al., 2017).

The results of the acceptance test were submitted to descriptive statistical analysis by using the Statistical Packpage for the Social Sciences® software (SPSS, version 21.0).

2.6 Clinical trial to evaluate chocolate consumption in the health of individuals

A prospective, randomized, blinded, placebo-controlled clinical trial was conducted to evaluate the effect of 70% cocoa consumption added with ursolic and oleanolic acids on individuals' health. The population was composed of volunteers who were registered in a basic health unit of the city of Itajuípe-Bahia, and the study was conducted from July to August 2016.

Of these volunteers, only the ones who were found at their homes during the study period and who met the inclusion criteria were included in the clinical trial, which made up a total of 45 people. All the volunteers were visited by the researcher in their homes to explain all phases of the research. The volunteers were divided into three groups (a test group, a placebo group and a control group) through a drawing, aiming at the randomization of the group.

The test group consisted of 15 people who received in daily visits at their homes a 25 g bar of 70% cocoa chocolate added with ursolic and oleanolic acid, individually packaged, for four weeks. On the other hand, the placebo group consisted of 15 people who received in daily visits at their homes a 25 g bar of 70% cocoa chocolate individually packaged, for four weeks. The control group consisted of 15 people who were instructed not to consume any form of chocolate during four weeks of the study.

All participants were instructed to maintain their daily activities and continue their usual diet, not inserting new medications or new physical activities into their routine. The 24-hour Recall (Buzzard, 1998) was applied in all visits to establish the daily consumption of each individual, as well as to evaluate if the usual diet was not modified.

After all the instructions, an anthropometric evaluation was performed by measuring weight (kg), height (m) and waist circumference (cm). Based on these measurements, the Body Mass Index (BMI=kg/m^2) was obtained. Weight measurements were carried out in scales having a maximum capacity of 150 kg and a minimum of 2 kg, and a 200-cm tape was used to obtain height and waist circumference.

In addition to the nutritional and anthropometric evaluation, clinical tests were also performed to evaluate the lipid profile - total cholesterol and its fractions (high density lipoprotein - HDL and low density lipoprotein - LDL) and triglycerides and fasting glycemia. For that, participants' venous blood was collected after a 12-hour fasting period, in an outpatient clinical laboratory (LL Laboratório - Itajuípe, Bahia). For the analysis of the laboratory tests the methods according to Lima et al. (2001).

The participants were submitted to laboratory and anthropometric tests on two occasions: before the first week of the experiment and immediately after the fourth week of the experiment, in order to evaluate whether there were changes in the indicators mentioned above.

In order to compare whether there were differences in the variables (weight, BMI, waist circumference, glucose, HDL, LDL, triglycerides, total cholesterol) before and after the chocolate consumption, a Kolmogorov-Smirnov test was initially performed to test the normality of data, and after that definition, we used the average test for comparison, using Wilcoxon test in the variables that did not have a normal distribution, and the t test for paired samples in the variables that followed the normal distribution.

The Kolmogorov-Smirnov test was used to verify the normality of the dataand to verify whether there were differences in the variables (weight, BMI, waist circumference, glucose, HDL, LDL, triglycerides, total cholesterol), followed by analysis of variance at 5% probability.

All average tests were performed at 5% probability using Statistical Packaged for Social Sciences (SPSS, version 21.0).

3 Results and discussion

3.1 Physico-chemical characterization of chocolates

Table 1 shows the physico-chemical analysis of 70% cocoa chocolate with addition of the mixture of ursolic acids and oleanolic acid.

Table 1. Water content, ashes, pH, acidity, lipids, proteins and average particle size of the chocolate.

	Value ± Standard deviation
Water content (%)	1.72 ± 0.16
Ashes (%)	2.30 ± 0.05
pH	5.58 ± 0.04
Titratable acidity (%)	8.20 ± 0.40
Lipids (%)	29.43 ± 0.65
Proteins (%)	8.79 ± 0.48
Total Carbohydrate (%)	57.76 ± 0.00
Averageparticlesize (μm)	19.00± 0.01

Source: Directsurvey.

The ursolic and oleanolic acids were inserted into the chocolate together. This is due to the difficulty of separating them because they are isomers So, the similarity of physical and chemical properties between these pairs makes their differentiation and separation very difficult. Howeverthey share many properties (Ding et al., 2018), which add value to developed chocolate.

The developed chocolate has low water content (1.72%) and a 5.58 pH due to these characteristics; it is not necessary to use additives or preservatives, besides being more durable, which is a beneficial factor for health (Pandey et al., 2014). The pH value is close to the pH found in other bitter chocolates (Leite et al., 2013), as a larger amount of cocoa liquor decreases the pH in relation to milk chocolates. A lower pH was also expected because two acids were added to the formulation.

The developed chocolate has an 8.8% protein content. Foods rich in good quality proteins are important because they provide essential amino acids to promote growth, especially for the maintenance and development of lean body mass (Devries & Phillips, 2015). The lipid concentration is the 29.43% and for example, regarding total carbohydrates, the developed chocolate has 57.76% of carbohydrates.

Samples of the analyzed chocolates presented value of fixed mineral residue (2.30 ± 0.05) allowed by the legislation for chocolate, which is a maximum of 2.5% w/w of fixed mineral residue. An amount of ash greater than that allowed by the legislation may suggest adulteration or contamination by some mineral residue in chocolate (RESOLUTION-CNNPA No.12, 1978).

The size of the particles of chocolate may interfere with its palatability, directly influencing taste and texture (Saputro et al., 2019). Ideally, this size should be between 20-25 μm. The analyzed chocolates obtained a 20 μm particle size.

The presence of ursolic and oleanolic acids in the chocolate after processing was confirmed by the thin layer chromatography method using iodine and vanillin vapors as developing agents and the acid standards for comparison. There was presence of acids in allfractions. This technique was chosen because it is easy to apply, simple, low cost and efficient.

3.2 Microbiological analyzes

Microbiological analyzes were performed with the purpose of providing safety for sensory analysis and for the clinical trial. The dilution used was at 10^1. The other smaller dilutions were not able to indicate the presence of any microorganism studied. The values of colony forming unitsof mesophilic bacteria per gram of chocolate ($7x10^1$) and molds and yeasts ($2.5x10^1$) are within the limits allowed by the legislation,which are 10^4 and 10^3 UFC/g, respectively (Resolution CNNPA No.12, 1978).

The analysis for total coliforms and thermotolerant coliforms resulted in the absence of microorganisms at a 10^1dilution, being within the quality standard required by the legislation (CNNPA Resolution No. 12, 1978). Thus, the result of these analyzes revealed that the chocolate bars were safe for human consumption, either for sensory analysis or for the clinical trial.

3.3 Sensory analysis

According to the evaluators' answers, the chocolate sample obtained a mean score of 7.29 ± 1.43, ranging from the hedonic terms "I liked it a little" and "I liked it a lot", corresponding to the acceptance region of the hedonic scale. The chocolate was well-accepted, presenting 83% of the answers between the scales 07 and 09.

In addition, difficulty in maintaining a balanced diet is often linked to deprivation of tasty foods (Justo & Ferreira, 2019). That is why the food industry needs innovation to meet this demand of tasty, practical and healthy foods. The 70% chocolate added with ursolic and oleanolic acids can return the pleasure of eating and increase adherence rates to healthy diets because of its practicality and good sensory acceptance.

3.4 Clinical trial

The study population consisted of 45 volunteers, 24 male (53.3%) and 21 female (46.7%). The mean age of the population was 52 ± 15.01 years. There was no significant difference between the groups in the demographic variables. The intervention took place during four weeks, from July to August 2016. During this period, volunteers maintained their usual activities throughout the research process, including feeding and practicing/not practicing physical activity.

The trial was characterized by the daily consumption of 25 g of 70% cocoa chocolate plus 150 mg of the mixture of ursolic and oleanolic acids for the test group; 25 g of 70% cocoa chocolate without addition of acids for the placebo group and no consumption of chocolate for participants in the control group.

The recommended daily dose of ursolic and oleanolic acids ranges from 150-300 mg. We chose to analyze the effect of the lowest recommended dose (150 mg/day), although the acids are non-toxic (National Center for Biotechnology Information, 2016).

After the four weeks of experiment it was possible to observe changes in the health of the volunteers according to Table 2.

In the placebo group, it was noted that this food inserted daily in the diet caused significant changes in the variables weight ($\rho = 0.014$), BMI ($\rho = 0.015$) and waist circumference ($\rho = 0.02$), by the t-test for paired samples, a 5% probability, when comparing before and after the intervention. These modifications occurred in a negative way for the health of the individuals, that is, the consumption of 70% cocoa chocolate in the amount used and in the intervention period increased the weight, BMI, and waist circumference of the people.

This negative modification in the placebo group may have been caused by the increased amount of sugar in the formulation, since the sugar was used to replace the concentrations of the triterpenic acids of the test chocolate, so the acid chocolate had 29.54 g/100 g sugar while the placebo chocolate had 29.60 g of sugar.

When analyzing the same variables in the test group (weight, BMI and WC) before and after the consumption of 70% cocoa chocolate incorporated with ursolic and oleanolic acids through t-test for paired samples at 5% probability, significant changes

Table 2. Parameters of volunteers before and after the clinical trial.

Variable	Test group (mean ± deviation)		Placebo group (mean ± deviation)		Control group (mean ± deviation)	
	Before	After	Before	After	Before	After
Weight (kg)	70.07±15.99*	68.87±15.04*	73.73±17.70*	75.40±18.23*	68.19±16.02	68.10±15.48
BMI (kg/m²)	25.42 ± 4.62*	24.99±4.28*	26.12±4.87*	26.68±4.89*	24.59±4.15	24.57±4.01
WaistCircumference– WC (cm)	92.13±14.43*	88.73±13.26*	93.80±13.45*	97.13±13.30*	86.60±12.36*	87.07±11.61*

*Significant change in columns p<0.05.

(ρ<0.05) were noted. However, these occurred in a beneficial way for the group, since the consumption of that chocolate reduced the anthropometric measurements.

In the control group, the analysis of the variables: weight, BMI and WC before and after the experimental period through the t-test for paired samples at 5% probability showed no significant changes (ρ>0.05).

After consumption of 70% cocoa chocolate incorporated with ursolic and oleanolic acid, 53.3% (n = 8) of the subjects in the test group decreased weight measurements. Of these, 50% (n = 4) lost approximately 2 kg. On the other hand, in the placebo group, 86.6% (n = 13) of the individuals gained weight during the intervention, of which 23.7% (n = 3) acquired 3 kg. In the control group, 60% (n = 9) remained at the same weight.

The association between weight gain, abdominal increase, physical inactivity and the onset of chronic non-communicable diseases is evident, and eating practices are considered elements that can be modified in the prevention of comorbidities linked to overweight and obesity (Hernandez et al., 2017). Thus, the weight loss observed in the test group was a very relevant result.

As a consequence of weight reduction, the decrease in Body Mass Index (BMI) was observed in the same individuals who hadweight reduction in the test group. Thus, 53.3% (n = 8) of the individuals had a BMI loss, of which four had BMI reduction between 0.5 and 1.0 kg/m^2. The subjects in the placebo group gained weight (n = 13) and increase between 0.5 and 1.0 kg/m^2 was observed in 61.54% (n = 8) of the individuals. In the control group the majority (60%) of the subjects remained with no changes in the BMI.

This reduction in BMI seen in this study is considered fundamental for health maintenance, since this is a good indicator of obesity and overweight. Evidence suggests that high values (> 30 kg/m2) of this variable are associated with the risk of chronic non-communicable diseases. This result may be related to the incorporation of ursolic acids to chocolate, since the use of these is related to the decrease of body mass index in study for evaluate the effect of ursolic acid on metabolic syndrome (Ramírez-Rodríguez et al., 2017).

The analysis of the waist circumference of the individuals allowed verifying that 73.3% (n = 11) of the individuals in the test group had reduction in measurements. Of these, 27.7% (n = 3) had more than 8cm of reduction in waist circumference. In the placebo group the result was different, 46.6% (n = 7) of subjects had an increase of 1 to 3cm in waist circumference, while only one individual presented no changeinhis measurements. The control group also showed an increase in their WC measurement as 46.6% (n = 7) of the subjects presented increase of 1 to 3 cm in the circumference.

Considering that waist circumference measurements reflect the visceral fat content and are also strongly associated with total body fat and this fact correlates with the risk of CNCD (Hernandez et al., 2017), this reduction was very important for the health of individuals in the test group.

The increasing incidence of chronic noncommunicable diseases associated with poor diet is increasing and worrying, especially the increase in overweight, obesity and their associated complications (Arokiasamy et al., 2017). Thus, a balanced diet and food additions that may aid in weight, BMI and waist circumference loss are essential for the maintenance of a body mass within the parameters of normality. And it was observed in this study that the developed chocolate helped in the reduction of these anthropometric variables.

In the statistical evaluation of the clinical trials, no significant difference was observed in the variables glucose (ρ = 0.414) and LDL (ρ = 147), comparing the values collected before and after the consumption of 70% cocoa chocolate with ursolic and oleanolic acids in the test group, according to the Wilcoxon test at 5% probability. No difference was observed in this group between the values of total cholesterol (ρ = 0.580), triglycerides (ρ = 0.124) and HDL (ρ = 0.113) before and after the consumption of 70% cocoa chocolate with ursolic and oleanolic acids by the t-test for paired samples at 5% probability.

The results of the clinical trials of the placebo group also did not indicate statistical differences (ρ> 0.05) in the variables glucose, LDL, HDL, total cholesterol before and after consumption of 70% cocoa chocolate with placebo for ursolic and oleanolic acids by Wilcoxon's test at 5% probability, and in the variable triglycerides (ρ = 0.803) by the t-test for paired samples at 5% probability.

In the control group, no significant difference (ρ>0.05) was observed in the variables glucose, LDL, HDL, total cholesterol, and triglycerides before and after the intervention period without chocolate consumption by the t-test for paired samples at 5% probability, as shown in Table 3:

When comparing the test, control and placebo groups, the statistical difference (ρ<0.05) after intervention of the values of glucose, LDL, HDL, totalcholesterol and triglycerides (ρ<0.05) were observed by analysis of variance at 5% probability. In the test group, greater reductions were observed in the clinical variables after the consumption of 70% cocoa chocolate with

Table 3. Clinical parameters of the volunteers before and after the clinical trial.

Variable	Test group (mean ± deviation)		Placebo group (mean ± deviation)		Control group (mean ± deviation)	
	Before	After	Before	After	Before	After
Glucose	85.00±6.28	83.43±12.68	83.25±8.23	84.03±13.09	85.13±5.23	84.50±2.54
Total cholesterol	195.54±24.30	198.43±34.76	196.98±23.33	197.97±29.67	195.97±22.22	196.07±32.11
HDL	56.29±7.56	52.00±7.02	55.97±5.67	54.30±2.07	55.31±35.67	55.98±23.78
LDL	100.00±14.31	115.00±35.22	101.10±9.021	109.00±23.56	103.19±13.45	104.56±11.89
Triglycerides	197.14±24.08	155.79±58.72	195.13±24.08	182.89±32.15	186.23±34.56	183.83±36.79

Source: Directsurvey.

ursolic and oleanolic acid, when compared with the reductions in the clinical variables obtained with consumption of 70% cocoa chocolate with placebo and also when compared to the group without consumption of chocolate.

Although the use of 70% cocoa chocolate added with the acids did not cause significant differences in the clinical variables during the four weeks of observation, the clinical results of the test group showed improvement in relation to the group that consumed 70% cocoa without the acids during the same period. However, several authors (Crichton et al., 2017; Souza et al., 2017) have reported on the ability of 70% cocoa chocolate to improve the results of clinical testing of individuals.

In view of this, the findings of this work show that the incorporation of triterpenic acids can further enhance the benefits of 70% cocoa chocolate in the health of individuals. The study period limited the obtainment of significant differences in the clinical results of the individuals, which make further research with a longer intervention time necessary.

The results of this study show that it was possible to develop and characterize 70% cocoa chocolate with addition of ursolic acid and oleanolic acid, so that the formulation showed good sensory acceptance, which indicates its possible inclusion in the healthy foods market. It also showed that regular consumption of a 25 g bar of 70% cocoa chocolate containing 150 mg of ursolic acid and oleanolic acid may be useful to significantly reduce the anthropometric and clinical variables.

4 Conclusion

The findings of this study supported a significant association between the consumption of 70% cocoa chocolate added withursolic acid and oleanolic acid with the reduction of anthropometric measures of the individuals (weight, waist circumference and BMI). The results also suggest improvements in the clinical parameters, since the developed chocolate promoted benefits in the clinical parameters in relation to the other experimental groups. Further studies may be based on variations of cacao and of ursolic and oleanolic acids concentration, sample size and longer intervention time for a differentiated evaluation of the effects of the developed chocolate on individuals' health.

References

Amaral, G. V., Silva, E. K., Costa, A. L. R., Alvarenga, V. O., Cavalcanti, R. N., Esmerino, E. A., Guimarães, J. T., Freitas, M. Q., Sant'Ana, A. S., Cunha, R. L., Moraes, J., Silva, M. C., Meireles, M. A. A., & Cruz, A. G. (2018). Whey-grape juice drink processed by supercritical carbon dioxide technology: Physical properties and sensory acceptance. *LWT*, 92, 80-86. http://dx.doi.org/10.1016/j.lwt.2018.02.005.

Arokiasamy, P., Uttamacharya, Kowal, P., Capistrant, B. D., Gildner, T. E., Thiele, E., Biritwum, R. B., Yawson, A. E., Mensah, G., Maximova, T., Wu, F., Guo, Y., Zheng, Y., Kalula, S. Z., Salinas Rodríguez, A., Manrique Espinoza, B., Liebert, M. A., Eick, G., Sterner, K. N., Barrett, T. M., Duedu, K., Gonzales, E., Ng, N., Negin, J., Jiang, Y., Byles, J., Madurai, S. L., Minicuci, N., Snodgrass, J. J., Naidoo, N., &Chatterji, S. (2017). Chronic noncommunicable diseases in 6 low-and middle-income countries: findings from wave 1 of the World Health Organization's study on global ageing and adult health (SAGE). *American Journal of Epidemiology*, 185(6), 414-428. http://dx.doi.org/10.1093/aje/kww125. PMid:28399566.

Asioli, D., Varela, P., Hersleth, M., Almli, V. L., Olsen, N. V., & Naes, T. (2017). A discussion of recent methodologies for combining sensory and extrinsic product properties in consumer studies. *Food Quality and Preference*, 56, 266-273. http://dx.doi.org/10.1016/j.foodqual.2016.03.015.

Association of Official Analytical Chemists – AOAC. (1990). *Official Methods of Analysis of the Association of Official Analytical Chemists*. Arlington: AOAC.

Belsito, P. C., Ferreira, M. V. S., Cappato, L. P., Cavalcanti, R. N., Vidal, V. A. S., Pimentel, T. C., Esmerino, E. A., Balthazar, C. F., Neto, R. P. C., Tavares, M. I. B., Zacarchenco, P. B., Freitas, M. Q., Silva, M. C., Raices, R. S. L., Pastore, G. M., Pollonio, M. A. R., &Cruz, A. G. (2017). Manufacture of Requeijão cremoso processed cheese with galactooligosaccharide. *Carbohydrate Polymers*, 174, 869-875. http://dx.doi.org/10.1016/j.carbpol.2017.07.021. PMid:28821142.

Brasil, Conselho Nacional de Saúde. (2012, Dezembro 12). Diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos (Resolução CNS nº 466, de 12 de dezembro de 2012). *Diário Oficial [da] República Federativa do Brasil*.

Buzzard, M. (1998). 24-hours dietary recall and food record methods. In W. C. Willett. *Nutritional epidemiology* (2. ed., Vol. 1, pp. 50-73). Oxford: Oxford University Press.

Carvalho, J. C. S., Romoff, P., & Lannes, S. C. D. S. (2018). Improvement of nutritional and physicochemical proprieties of milk chocolates enriched with kale (Brassica olereacea var. acephala) and grape (Vitisvinífera). *Food Science and Technology*, 38(3), 551-560. http://dx.doi.org/10.1590/fst.15018.

Cheval, B., Audrin, C., Sarrazin, P., & Pelletier, L. (2017). When hunger does (or doesn't) increase unhealthy and healthy food consumption through food wanting: The distinctive role of impulsive approach tendencies toward healthy food. *Appetite*, 116, 99-107. http://dx.doi.org/10.1016/j.appet.2017.04.028. PMid:28455261.

Colombo, A. M. J., Valente, J. M. Fo, & Moreira, D. M. (2015). Efeitos do chocolate na função endotelial de pacientes com síndrome

coronariana aguda. *Internacional Journal of Cardiovascular Sciences*, 28(2), 89-94. http://dx.doi.org/10.5935/2359-4802.20150022.

Crichton, G. E., Elias, M. F., Dearborn, P., & Robbins, M. (2017). Habitual chocolate intake and type 2 diabetes mellitus in the Maine-Syracuse Longitudinal Study: (1975-2010): Prospective observations. *Appetite*, 108, 263-269. http://dx.doi.org/10.1016/j.appet.2016.10.008. PMid:27725277.

Devries, M. C., & Phillips, S. M. (2015). Supplemental protein in support of muscle mass and health: advantage whey. *Journal of Food Science*, 80(Suppl. 1), A8-A15. http://dx.doi.org/10.1111/1750-3841.12802. PMid:25757896.

Ding, D., Lawson, K. D., Kolbe-Alexander, T. L., Finkelstein, E. A., Katzmarzyk, P. T., van Mechelen, W., & Pratt, M. (2016). The economic burden of physical inactivity: A global analysis of major non-communicable diseases. *Lancet*, 388(10051), 1311-1324. http://dx.doi.org/10.1016/S0140-6736(16)30383-X. PMid:27475266.

Ding, H., Hu, X., Xu, X., Zhang, G., &Gong, D. (2018). Inhibitory mechanism of two allosteric inhibitors, oleanolic acid and ursolic acid on α-glucosidase. *International Journal of Biological Macromolecules*, 107(Pt B), 1844-1855. http://dx.doi.org/10.1016/j.ijbiomac.2017.10.040. PMid:29030193.

Gough, B., & Conner, M. T. (2006). Barriers to healthy eating amongst men: a qualitative analysis. *Social Science & Medicine*, 62(2), 387-395. http://dx.doi.org/10.1016/j.socscimed.2005.05.032. PMid:16011867.

Hernandez, D. C., Reesor, L. M., & Murillo, R. (2017). Food insecurity and adult overweight/obesity: Gender and race/ethnic disparities. *Appetite,* 117, 373-378. http://dx.doi.org/10.1016/j.appet.2017.07.010

Instituto Adolfo Lutz. (2004). *Métodos físico-químicos para análise de alimentos* (4. ed.). São Paulo: Instituto Adolfo Lutz.

Justo, G. F., & Ferreira, J. T. (2019). Tortura da dieta versus prazer de comer. *Equatorial: Revista do Programa de Pós-Graduação em Antropologia Social*, 6(11), 1-16. http://dx.doi.org/10.21680/2446-5674.2019v6n11ID16184.

Leite, P. B., Silva Lannes, S. C., Rodrigues, A. M., Soares, F. A. S. D. M., Soares, S. E., & Silva Bispo, E. (2013). Estudo reológico de chocolates elaborados com diferentes cultivares de cacau (Theobroma cacao L.)/Rheological study of chocolates madewith diferente cocoa (*Theobroma cacao* L.) varieties. *Brazilian Journal of Food Technology*, 16(3), 192. http://dx.doi.org/10.1590/S1981-67232013005000024.

Libman, K., Freudenberg, N., Sanders, D., Puoane, T., & Tsolekile, L. (2015). The role of urban food policy in preventing diet-related non-communicable diseases in Cape Town and New York. *Public Health*, 129(4), 327-335. http://dx.doi.org/10.1016/j.puhe.2014.12.007

Lima, A. O., Soares, J. B., Greco, J. B., Galizzi, J., & Cançado, J. R. (2001). *Métodos de laboratório aplicados à clínica – técnica e interpretação* (8. ed.). Rio de Janeiro: Guanabara Koogan.

National Center for Biotechnology Information – NCBI. (2016). *PubChem Database: Ursolic Acid - CID=64945*. Bethesda: NCBI. Retrieved from https://pubchem.ncbi.nlm.nih.gov/compound/64945

Pandey, H., Kumar, V., & Roy, B. K. (2014). Assessment of genotoxicity of some common food preservatives using Allium cepa L. as a test plant. *Toxicology Reports*, 1, 300-308. http://dx.doi.org/10.1016/j.toxrep.2014.06.002. PMid:28962247.

Pereira, J. R., Queiroz, R. F., Siqueira, E. A., Brasileiro-Vidal, A. C., Sant'Ana, A. E., Silva, D. M., & Affonso, P. R. D. M. (2017). Evaluation of cytogenotoxicity, antioxidant and hypoglycemiant activities of isolate compounds from *Mansoa hirsuta* DC (Bignoniaceae). *Anais da Academia Brasileira de Ciências*, 89(1), 317-331. http://dx.doi.org/10.1590/0001-3765201720160585. PMid:28423086.

Quelal-Vásconez, M. A., Lerma-García, M. J., Pérez-Esteve, É., Arnau-Bonachera, A., Barat, J. M., & Talens, P. (2019). Changes in methylxanthines and flavanols during cocoa powder processing and their quantification by near-infrared spectroscopy.*LWT*, 108598. http://dx.doi.org/10.1016/j.lwt.2019.108598.

Ramírez-Rodríguez, A. M., González-Ortiz, M., Martínez-Abundis, E., & Acuña Ortega, N. (2017). Effect of ursolic acid on metabolic syndrome, insulin sensitivity, and inflammation. *Journal of Medicinal Food*, 20(9), 882-886. http://dx.doi.org/10.1089/jmf.2017.0003. PMid:28598231.

Sampaio, S. C. S. A. (2011). *Chocolate meio amargo produzido de amêndoas de cacau fermentadas com polpa de cajá, cupuaçu ou graviola: características fisico-químicas, reológicas e sensoriais* (Dissertação de mestrado). Universidade Federal de Viçosa, Viçosa.

Saputro, A. D., Van de Walle, D., Caiquo, B. A., Hinneh, M., Kluczykoff, M., & Dewettinck, K. (2019). Rheological behaviour and microstructural properties of dark chocolate produced by combination of a ball mill and a liquefier device as small scale chocolate production system. *LWT*, 100, 10-19. http://dx.doi.org/10.1016/j.lwt.2018.10.039.

Silva, D. M., Sant'Ana, A. E. G., Castro, M. M. S., Queiroz, L. P., Soares, M. B., & Costa, J. F. O. (2015).*Isolamento de TriterpenosPentacíclicos: ácido ursólico e oleanólico, e fitoesteróides: estigmasterol e β-sitosterol extraídos das folhas de Mansoa hirsuta DC Bignoniaceae, para aplicação em formulações de suplementos, alimentos funcionais e fitoterápicos. BR Patente 102015008180*. 01 abr. 19 p.

Souza, S. J., Petrilli, A. A., Teixeira, A. M., Pontilho, P. M., Carioca, A. A., Luzia, L. A., Souza, J. M., Damasceno, N. R., Segurado, A. A., & Rondó, P. H. (2017). Effect of chocolate and mate tea on the lipid profile of individuals with HIV/AIDS on antiretroviral therapy: A clinical trial. *Nutrition*, 43, 61-68. http://dx.doi.org/10.1016/j.nut.2017.06.017. PMid:28935146.

Yin, R., Li, T., Tian, J. X., Xi, P., & Liu, R. H. (2018). Ursolic acid, a potential anticancer compound for breast cancer therapy. *Critical Reviews in Food Science and Nutrition*, 58(4), 568-574. http://dx.doi.org/10.1080/10408398.2016.1203755. PMid:27469428.

Zhang, L., Cai, Q. Y., Liu, J., Peng, J., Chen, Y. Q., Sferra, T. J., & Lin, J. M. (2019). Ursolic acid suppresses the invasive potential of colorectal cancer cells by regulating the TGF-β1/ZEB1/miR-200c signaling pathway. *Oncology Letters*, 18(3), 3274-3282. http://dx.doi.org/10.3892/ol.2019.10604. PMid:31452805.

Zhou, T., Guan, H., Yao, J., Xiong, X., & Ma, A. (2018). The quality of life in Chinese population with chronic non-communicable diseases according to EQ-5D-3L: A systematic review. *Quality of Life Research: An International Journal of Quality of Life Aspects of Treatment, Care and Rehabilitation*, 27(11), 2799-2814. http://dx.doi.org/10.1007/s11136-018-1928-y. PMid:29980994.