ISSN: 2347-467X, Vol. 12, No. (3) 2024, Pg. 1106-1118

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Cocoa Shell as a Bulking Agent in Nutritionally Improved Cocoa Spreads with Sugar Substitutes

VERONIKA BARIŠIĆ[1], ĐURĐICA AČKAR[1]*, IVANA LONČAREVIĆ[2], JOVANA PETROVIĆ[2], MIRTA BENŠIĆ[3], DANIELA PAULIK[1], KRISTINA DOKO[4], ANTUN JOZINOVIĆ[1], JURISLAV BABIĆ[1], DRAGO ŠUBARIĆ[1], IVANA FLANJAK[1]

[1]Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Franje Kuhača, Osijek, Croatia.
[2]Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara, Novi Sad, Serbia.
[3]School of Applied Mathematics and Informatics, Josip Juraj Strossmayer University of Osijek, Trg Ljudevita Gaja, Osijek, Croatia.
[4]Federal Agro Mediterranean Institute, Biskupa Čule, Mostar, Bosnia and Herzegovina.

Abstract

Due to unhealthy lifestyle, Western countries are dealing with pandemic of overweight and obesity. To tackle the problem, industry has been looking for solutions to produce nutritionally improved foods. In the present research, sucrose was fully substituted with xylitol, or by combination of xylitol and stevia with cocoa shell as a bulking agent. Apart from reducing caloric value of the sweet spreads, protein, ash, methylxanthine and phenolic compound contents were increased, without major influence on fatty acid composition. The spreads with added cocoa shell may be declared as a source fibre. Although stability of sweet spreads during storage was not compromised, which is visible from low water activity (between 0.132 for control sample and 0.233 for sample with 52 % xylitol) and high colloidal stability (above 99), rheological properties and texture of the spreads deteriorated (increase of plastic viscosity from 3.708 Pas up to 18.280 Pas and decrease of spread ability from 1180.43 gs up to 334.670 gs), which could pose an issue from the technological and sensorial points of view. Overall, satisfactory products were obtained with potential for further upgrade for industrial production.

Article History
Received: 19 July 2024
Accepted: 26 September 2024

Keywords
Cocoa Shell;
Nutritional Value;
Physicochemical Properties;
Stevia;
Xylitol.

CONTACT Đurđica Ačkar dackar@ptfos.hr Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Franje Kuhača, Osijek, Croatia.

Doi: https://dx.doi.org/10.12944/CRNFSJ.12.3.10

Introduction

Overweight and obesity are burning issues in Europe. According to WHO European regional obesity report 2022,[1] obesity prevalence among age groups in European countries is as follows: 7.9% of children under 5 years, app. 33% of school-aged children, 25% of adolescents and app. 60% of adult population. Even more worrying is the fact that not only none of the Member states has reached the track of halting the obesity by 2025, but the obesity prevalence is on the rise.

Two compounding driving mechanisms for obesity are identified in the WHO Report:1 preconception and gestational exposure, and unhealthy life style (poor dietary habits and lack of exercise). The latter one is highly driven by obesogenic environment, including energy dense foods of poor nutritional value.

Sweet spreads are one of these foods, largely consumed by all age groups. They are characterized by large contents of sugar (app. 50%) and fats (min 25%), which determine high caloric value of the products. On the other hand, they have minor contribution in the intake of nutritionally valuable compounds, such as vitamins, minerals and non-essential bioactive compounds.

According to the Market Data Forecast,[2] chocolate based sweet spreads became the second most popular spread on the global market, with significant raise of sales in recent years. The increased purchase power of adult population, which buy the products for themselves, the consumption "on the go", and developing "ready-to-eat" products containing chocolate based sweet spreads are among main reasons of growth of the chocolate based sweet spreads market. Additionally, chocolate based sweet spreads have become an integral part of breakfast menus, slowly pushing out jams and marmalades.

Since the consumers' habits are hard to influence, there is obvious need for improvement of the nutritional value of sweet spreads, as well as other foods of poor nutritional value preferred by consumers. Recently, different approaches have been involved, from the substitution of fat with fibre,[3] aerogels,[4] or oleogels,[5] enrichment of sweet spreads6 to replacement of sucrose.[7]

In this research, cocoa sweet spreads in which sucrose was fully substituted were produced in laboratory conditions. Firstly, sucrose was replaced by xylitol, natural sugar replacer obtained from birches. It has sweetness similar to sucrose and replaces it in 1:1 ratio. It gives 240 kcal per 100 g, which is app. 60% of caloric value of sucrose. Additionally, it has been reported that xylitol has caries-preventive properties. It has advantage over other polyols as well, due to reducing proportions of mutans streptococci in plaque in addition to non-fermentability by dental plaque.[8]

In the following step, stevia and cocoa shell were used to replace 10, 20, 30, 40 or 50 % of xylitol, respectively. Cocoa shell is a by-product of the chocolate industry, as a bulking ingredient in cocoa based sweet spread. Proximate composition of cocoa shell is: proteins 116 – 181 g/kg, fat 20.2 – 181 g/kg, dietary fibre 504 – 606 g/kg (with one reference "not detected"), carbohydrates mostly not detected, with one reference reporting 178 g/kg, ash 73 – 114.2 g/kg.[9] These values show the potential of cocoa shell for improvement of nutritional values of cocoa spreads. Stevia was used to adjust the sweetness of the product, since it is 200 – 350 times sweeter than sucrose and may be used in small quantities.

Materials and Methods

Cocoa shell was obtained after deshelling roasted cocoa beans in industrial conditions. Sugar (Viro, Croatia), xylitol (Nutrigold, Finland), stevia (Nutrimedica, Croatia), hazelnut paste (DGF, France), cocoa powder (Kandit, Croatia), coconut oil (Zvijezda, Croatia), milk powder (26% milk fat) (Dukat, Croatia), lecithin (A.C.E.F., Italy) and salt (Solana Pag, Croatia) were purchased in local stores.

Sample Preparation

Two control samples without cocoa shell were produced: one with sugar (52%) and one with xylitol (52%). In subsequent samples, part of xylitol (10, 20, 30, 40 or 50%) was replaced by a combination of cocoa shell and stevia. Stevia was used to adjust the sweetness, since it has much larger sweetness than sucrose and xylitol. The aim was to completely replace sucrose used in the recipe and to increase the content of dietary fibre, while maintaining desired firmness and spreadability of the spreads.

Recipes of the spreads are shown in Table 1.

Table 1: Recipes of cocoa based sweet spreads

	Cocoa powder (%)	Hazelnut paste (%)	Cocoa shell (%)	Sweetener (%)
N0	6.3	17	-	sugar: 52.0
N6	6.3	17	-	xylitol: 52.0
N5	6.3	17	5.1792	xylitol: 46.8 stevia: 0.0208
N4	6.3	17	10.3576	xylitol: 41.6 stevia: 0.0424
N3	6.3	17	15.5376	xylitol: 36.4 stevia: 0.0624
N2	6.3	17	20.7168	xylitol: 31.2 stevia: 0.0832
N1	6.3	17	25.8960	xylitol: 26.0 stevia: 0.104

(All samples contained 10% palm oil, 10% coconut oil, 4.1% milk powder, 0.4% lecithin (emulsifier), 0.1% vanillin and 0.1% salt.)

Cocoa spreads were produced in the laboratory ball mill equipped with circulating water bath for maintenance of the temperature at 50 °C. Shearing speed was set to 60 RPM, and 3 kg of stainless-steel balls suitable for chocolate production were used for production of 500 g of cocoa spread. After the addition of the balls to the mill, raw materials were added following the order: palm oil, coconut oil, cocoa shell (when used), hazelnut paste, milk powder, cocoa powder, salt, xylitol, stevia. An hour before the end of milling lecithin was added, and 30 min prior to the end vanillin. When cocoa shell was used, it was mixed for 30 min with fats prior to the addition of other ingredients, in order to reduce the particle size of the shell. Processing time after the addition of hazelnut paste, milk powder, cocoa powder, salt, xylitol, stevia was 3 h.

Chemical Analyses

Protein content was determined according to ISO 5983-2:2005[10] method (Kjeldahl method with factor of conversion 6.25), dietary fibre according to AOAC 991.43[11] method (soluble, insoluble and total dietary fibre using Megazyme® kit) and ash according to ISO 5984:2022[12] method (at 550 °C). Measurements were done in 2 replicates.

Total phenolic compounds (TPC) were determined by original[13] and modified[14] spectrophotometric Folin-Ciocalteu method. TPC is commonly determined using Folin-Ciocalteu reagent, with addition of Na_2CO_3. However, sugars interfere with the results of spectrophotometric measurement, and modified method, in acidic conditions, gives more reliable results, as we have previously shown on chocolate.[15] Phenolic components and methylxanthines were determined as described previously.[16] Briefly, samples were defatted with n-hexane, dried and phenolic components and methylxanthines were extracted with 70% methanol with ultrasonication. Analysis was performed on liquid chromatographic system with photodiode array detector (Shimadzu Corporation, Japan). All measurements were done in 3 replicates.

Fatty acid profile was determined after the extraction of lipids by Folch,[17] which is commonly used method for extraction of lipids from biological samples.[18] The extraction was performed with the mixture of HPLC grade solvents: chloroform : methanol (2:1), using the volume 20 times of the mass of the sample, with mixing for 20 min at 400 rpm (IKA KS 260 Basic, Germany) at ambient temperature. Liquid phase was separated by filtration through filter paper, washed with 0.9% NaCl solution and left until layers separated. The upper layer was removed using micropipette and the chloroform from the bottom layer was evaporated at rotavapor (Laborota 4010, Heidolph Instruments; Germany) at 60 °C. The residue was dried in the laboratory oven (Binder FED 53, USA) at 105 °C until constant mass. Methyl esters were prepared according to Annex X.B of Commission Regulation No 796/2002[19] and analysed at gas chromatograph (Shimadzu GC-2010 Plus) with flame-ionisation detector (FID). Carrier gas was nitrogen, injector temperature 240 °C, separation ratio 1:100, injection volume 2 µL. Column (FAMEWAXTM, 30 m × 0.32 mm × 0.25 µm) temperature was set to 120 °C for 5 min, increased to final temperature 220 °C (5 °C/min), which was held for 20 min. Temperature of the FID was 250 °C. Flow rate of hydrogen was 40 mL/min, air 400 mL/min, and make-up gas (nitrogen) 30 mL/min. Identification of FAME was done with reference standard (Supelco FAME Mix, C4-C24, St. Louis, USA), and results were expressed as % of identified fatty acid in total fatty acids. Measurements were done in 3 replicates. Values for SFA (short-chain

fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids) were calculated as the sums of mean values, and SFA:MUFA and SFA:PUFA, IA (index of atherogenicity) were calculated from the sums of mean values for detected fatty acids.

Physical Properties

Water activity was measured using HygroLab 3 (Rotronic) in 2 replicates, because it is directly linked to susceptibility of food to microbial spoilage. It is considered that growth of microorganisms is inhibited in foods with water activity of 0.85 and below.[20]

Rheological properties, texture and particle size of foods are directly related to its sensory properties.[21,22] Rheological properties (Casson yield stress and Casson plastic viscosity) were determined using rotational rheometer Rheo Stress 600 (Haake, Germany). Sweet spreads were melted at 40 °C, and the rheological properties were measured at the same temperature. During first three minutes shearing speed was increased from 0 s-1 to 60 s-1, kept for 1 min at 60 s-1, and reduced to 0 s-1 during the last three minutes of analysis. Via computer software, values for Casson yield stress (Pa) and Casson plastic viscosity (Pas) were obtained.

Texture properties (firmness and spreadability) were determined using TA.XT Plus texture analyzer (Stable Micro System) with TTC Spreadability Rig, and 5 kg load cell. Measuring speed was set to 3 mm/s, and height 25 mm. All measurements were done in 5 replicates. The values for firmness (maximum force, g) and spreadability (area below the curve, gs) were obtained from the plot generated by the analysis software.

Density (g/cm^3) was determined by weighing the known volume of the sweet spread. All measurements were done in 3 replicates.

Colloidal stability was determined as follows. 15 g of the sweet spread was heated in water bath at 80 °C for 30 min and cooled to ambient temperature during 15 min. Cooled samples were centrifuged (Centrifuge Centra MP4P, IEC, USA) at 2900 g and 20 °C for 20 min. The separated oil was decanted and the residue was weighed. Colloidal stability (CS) was calculated using the Eq. 1. All measurements were done in 2 replicates.

$$CS = (m2 - m1) \times 100 \quad ...(1)$$

Where: m2, mass of the sample without oil (g); m2, initial mass of the sample (15 g).

Statistical Analysis

For each examined parameter, the average value and standard deviation are calculated, and the results are presented in tables and graphics. Statistical data processing was performed using the computer programs STATISTICA (StatSoft) and Microsoft Excel 2016, with $p < 0.05$. Similarity of the nutritionally improved sweet spread samples to control sample (N0) was determined using R software. Based on mean values of the results of physicochemical, biological and rheological parameters; Cosine, Euclid, Manhattan and Maximum distances were calculated as the measures of similarity/dissimilarity. The higher value of distance presents more similarity to control sample.

Results and Discussion

All samples contained app. 30% of total lipids (results not shown). The amount of protein ranged from 5.944% in the control sample with sucrose (N0) to 10.243% in the sample with 50% replacement of xylitol (N1) (Table 2). The amount of protein in control sample with sucrose (N0) and the control sample with xylitol (N6) was very similar, as expected, since the only difference between these samples is in the sweetener, and the sources of protein in these samples are cocoa powder, hazelnut paste and milk powder. Other authors reported between 6.1 and 6.4 g/100 g of protein in commercial and laboratory-made chocolate spreads,[23] and 6.54% protein in functional chocolate spread.[24] In the present research, these values are exceeded already with the addition of app. 5 % cocoa shell, and with the maximum amount of app. 25% of cocoa shell, the amount of protein raised to 10.243%, which is the increase by 72%. However, this is still below the limit for declaring food as a source of protein. Protein contents of cocoa bean shell is similar to the proteins of cotyledons (part of the bean used in the production of cocoa products), and it varies significantly, depending on the cocoa bean variety and origin. For example, 178.0 g/ kg,[25] and 10.30–27.40 g/ 100 g[26] have been reported. Fermented cocoa nibs contain 287 mg of essential amino acids/g crude protein, with predicted protein efficiency ratio of 2.55 and essential amino acid scores between 0.43 (for Met + Cys) and 0.96 (for Lys) compared to whole hen's egg,[27] showing a satisfactory quality of protein, compared to the standard value of 2.7.[28]

Table 2: Water activity, protein, fibre and ash contents of cocoa sweet spreads

Sample	Water activity	Protein (%)	Insoluble fibre (%)	Soluble fibre (%)	Ash (%)
NO	0.132±0.006	5.944±0.190	2.657±0.037	0.722±0.074	1.099±0.011
N6	0.233±0.016	6.165±0.118	2.433±0.083	0.833±0.125	1.070±0.003
N5	0.212±0.003	6.990±0.280	4.990±0.279	1.831±0.141	1.559±0.006
N4	0.163±0.008	7.983±0.150	7.567±0.187	2.449±0.067	2.040±0.005
N3	0.168±0.007	8.745±0.153	10.526±0.428	3.073±0.062	2.442±0.016
N2	0.153±0.001	8.921±0.180	13.107±0.977	3.606±0.412	2.877±0.050
N1	0.149±0.005	10.243±0.644	14.149±0.611	4.225±0.383	3.365±0.003

N0, sample without cocoa shell with 52% sugar; N6, sample without cocoa shell with 52% xylitol; N5 sample with 10% xylitol replaced by cocoa shell and stevia, N4 sample with 20% xylitol replaced by cocoa shell and stevia, N3 sample with 30% xylitol replaced by cocoa shell and stevia, N2 sample with 40% xylitol replaced by cocoa shell and stevia, N1 sample with 50% xylitol replaced by cocoa shell and stevia

Table 3: Contents of methylxanthines and phenolic compounds in cocoa sweet spreads

Sample	TEO	CAF	GA	CA	p-CA	CAT-(+)	EPI-(-)	EPG-(-)	TPC	TPC modified
	mg/g								mg GAE/g	
N0	3.022±0.022	0.253±0.003	0.015±0.001	0.004±0.000	0.017±0.000	0.217±0.002	0.205±0.003	0.305±0.008	2.31±0.10	0.90±0.03
N6	3.073±0.045	0.257±0.002	0.016±0.000	0.005±0.000	0.018±0.000	0.227±0.003	0.219±0.002	0.197±0.004	2.82±0.23	1.09±0.04
N5	3.498±0.014	0.323±0.003	0.018±0.001	0.007±0.001	0.015±0.002	0.234±0.003	0.223±0.001	0.248±0.001	2.98±0.33	1.10±0.02
N4	3.767±0.036	0.390±0.008	0.022±0.000	0.009±0.001	0.014±0.001	0.243±0.005	0.230±0.006	0.390±0.097	3.58±0.10	1.26±0.06
N3	3.932±0.015	0.438±0.008	0.024±0.001	0.012±0.001	0.013±0.000	0.245±0.007	0.234±0.004	0.589±0.022	3.47±0.09	1.22±0.04
N2	4.052±0.018	0.476±0.005	0.021±0.000	0.012±0.001	0.012±0.000	0.241±0.008	0.230±0.006	0.608±0.026	3.58±0.029	1.25±0.08
N1	4.129±0.063	0.503±0.020	0.025±0.002	0.010±0.001	0.013±0.000	0.229±0.012	0.224±0.008	0.596±0.019	3.65±0.035	1.26±0.10

N0, sample without cocoa shell with 52% sugar; N6, sample without cocoa shell with 52% xylitol; N5 sample with 10% xylitol replaced by cocoa shell and stevia, N4 sample with 20% xylitol replaced by cocoa shell and stevia, N3 sample with 30% xylitol replaced by cocoa shell and stevia, N2 sample with 40% xylitol replaced by cocoa shell and stevia, N1 sample with 50% xylitol replaced by cocoa shell and stevia; TEO, theobromine; CAF, caffeine; GA, gallic acid; CA, caffeic acid; p-CA, p-coumaric acid; CAT-(+), catechin; EPI-(-), epicatechin; EPG-(-), epicatechin gallate; TPC, total phenolic compounds by original Folin-Ciocalteu method; TPC modified, total phenolic compounds obtained by Folin-Ciocalteu modified method

Table 4: Fatty acid profile of cocoa sweet spreads

Fatty acid	N0 (% of total fatty acids)	N6	N5	N4	N3	N2	N1
C6:0	0.22±0.00	0.17±0.01	0.19±0.01	0.18±0.01	0.18±0.02	0.18±0.01	0.16±0.00
C8:0	2.11±0.03	1.65±0.06	1.80±0.08	1.71±0.03	1.66±0.10	1.74±0.02	1.46±0.02
C10:0	1.64±±0.02	1.26±0.06	1.50±0.21	1.31±0.03	1.28±0.06	1.33±0.01	1.12±0.01
C12:0	13.41±0.17	10.62±0.30	11.22±0.38	10.73±0.16	10.52±0.27	10.78±0.07	9.47±0.03
C14:0	5.54±0.01	4.68±0.09	4.76±0.10	4.66±0.05	4.64±0.04	4.64±0.04	4.37±0.02
C16:0	20.26±0.80	19.80±0.13	19.63±0.03	19.73±0.14	20.01±0.33	20.05±0.13	19.88±0.04
C16:1	0.15±0.01	0.14±0.01	0.15±0.00	0.15±0.00	0.16±0.01	0.17±0.01	0.17±0.00
C18:0	4.07±0.09	4.50±0.04	4.45±0.01	4.56±0.05	4.61±0.03	4.71±0.03	5.02±0.01
C18:1n9c+t	44.83±0.70	48.89±0.58	48.13±0.51	48.69±0.23	48.65±0.09	47.84±0.04	49.42±0.07
C18:2n6c	7.28±0.03	7.75±0.06	7.64±0.04	7.71±0.05	7.75±0.03	8.03±0.08	8.36±0.05
C18:3n3	0.12±0.02	0.14±0.01	0.14±0.01	0.14±0.02	0.15±0.03	0.11±0.02	0.13±0.01
C20:0	0.18±0.00	0.21±0.00	0.18±0.05	0.21±0.01	0.21±0.01	0.22±0.01	0.24±0.00
C20:1	0.10±0.01	0.10±0.00	0.11±0.01	0.10±0.00	0.10±0.00	0.11±0.01	0.11±0.01
C22:0	0.09±0.01	0.10±0.01	0.11±0.01	0.11±0.02	0.09±0.01	0.09±0.01	0.10±0.01
SFA	47.52	42.98	43.83	43.20	43.19	43.73	41.81
MUFA	45.07	49.13	48.38	48.94	48.90	48.12	49.69
PUFA	7.4	7.88	7.78	7.85	7.89	8.14	8.49
SFA:MUFA	0.94	1.14	1.10	1.13	1.13	1.10	1.18
SFA:PUFA	0.15	0.18	0.17	0.18	0.18	0.18	0.20
IA	0.74	0.62	0.64	0.63	0.62	0.63	0.59

N0, sample without cocoa shell with 52% sugar; N6, sample without cocoa shell with 52% xylitol; N5 sample w ith 10% xylitol replaced by cocoa shell and stevia, N4 sample with 20% xylitol replaced by cocoa shell and stevia, N3 sample with 30% xylitol replaced by cocoa shell and stevia, N2 sample with 40% xylitol replaced by cocoa shell and stevia, N1 sample with 50% xylitol replaced by cocoa shell and stevia; values for SFA (short-chain fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), SFA:MUFA and SFA:PUFA, IA (index of atherogenicity) were calculated from the sums of mean values for detected fatty acids.

Table 5: Physical properties of cocoa sweet spreads

Sample	Casson yield stress (Pa)	Casson plastic visco -sity (Pas)	Firmness (g)	Spreada- bility (gs)	Density (g/cm3)	Colloidal stability
N0	6.385	3.708	1762.220±35.990	1180.430±24.020	1.266±0.007	99.430±0.030
N6	26.01	2.792	465.650±17.050	334.670±18.580	1.217±0.002	99.300±0.030
N5	22.21	3.002	711.240±24.050	486.780±17.410	1.213±0.004	99.330±0.000
N4	20.58	3.847	525.090±11.860	343.280±8.820	1.220±0.001	99.100±0.030
N3	18.28	18.28	891.400±25.520	654.010±47.020	1.206±0.006	99.400±0.130
N2	24.01	4.447	921.610±24.180	672.700±53.000	1.217±0.008	99.300±0.170
N1	26.68	7.380	1100.230±18.000	837.310±49.640	1.181±0.013	99.170±0.100

N0, sample without cocoa shell with 52% sugar; N6, sample without cocoa shell with 52% xylitol; N5 sample with 10% xylitol replaced by cocoa shell and stevia, N4 sample with 20% xylitol replaced by cocoa shell and stevia, N3 sample with 30% xylitol replaced by cocoa shell and stevia, N2 sample with 40% xylitol replaced by cocoa shell and stevia, N1 sample with 50% xylitol replaced by cocoa shell and stevia

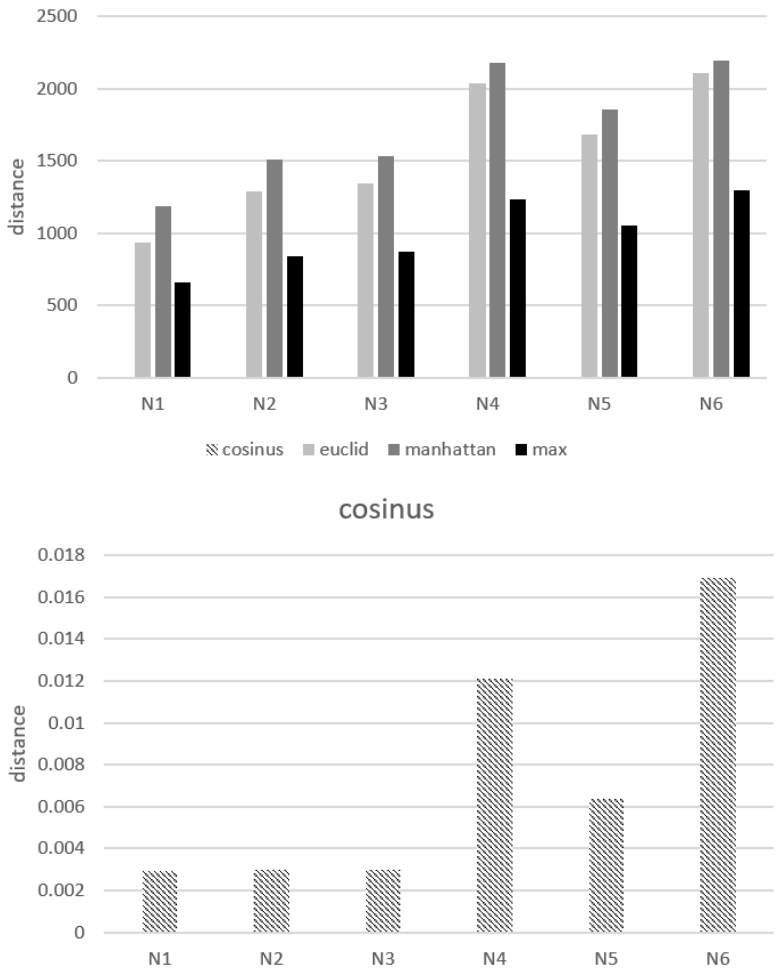

Fig. 1: Statistical analysis of similarity of the samples N6 (without cocoa shell with 52% xylitol), N5 (with 10% xylitol replaced by cocoa shell and stevia), N4 (with 20% xylitol replaced by cocoa shell and stevia), N3 (with 30% xylitol replaced by cocoa shell and stevia), N2 (with 40% xylitol replaced by cocoa shell and stevia), N1 (with 50% xylitol replaced by cocoa shell and stevia) to the control sample (N0), expressed through cosinus (enlarged in the 2nd graph), Euclid, manhattan and maximum distance from the control sample

Total fibre content of the control samples (N0 and N6) was app. 3.3% (Table 2). The amount doubled already with 10% replacement of xylitol with cocoa shell and stevia (N5), and at the maximum replacement (N1) it was 18.375(±0.229)%, which presents the increase by 443%. 9.8 g fibre/100 g of laboratory made chocolate spread has been reported, with no fibre reported in commercial sample.[23] All spreads with cocoa shell may be declared as products rich in fibre, since they exceed the limit of 6 g fibre/100 g of the product. The majority of fibre in all samples, but esp. when cocoa shell was used, came from insoluble fibre, which increase faecal bulk and faecal nitrogen excretion due to increased excretion of cell bound protein.[29] Even at the smallest proportion of added cocoa shell, the content of insoluble fibre nearly doubled, and at the maximum level it increased 5 times. Soluble fibre was even more affected: the contents increased 2.5 times by the addition at the lowest, and 5.8 times at the highest level of addition. Soluble fibres are fermented by colonic bacteria, they absorb water and thereby influence the intestinal motility and microbiota in the colon.[29] McBurney[30] distinguishes nutritional benefits of fibre-rich foods in small and large intestine. In the small intestine, fibre delay

gastric emptying and digestion and absorption of nutrients into the bloodstream. The amount of digesta entering the large bowel is increased, thus stimulating bacterial fermentation, production of short-chain fatty acids and increasing faecal mass. Consequently, the postprandial satiety is increased, glycemic control improved, insulin resistance and obesity prevented.

Ash contents also increased by the substitution with cocoa shell and stevia (Table 2). In our previous study,[31] we determined significant amounts of Zn (32803.[33] µg/kg), Cu (50180 µg/kg), Mn (53133.33 µg/kg), Ca (1139.99 mg/kg), Fe (1708.57 mg/kg), Mg (3995.37 mg/kg) in cocoa shell. All these elements have significant role in maintaining health, from metabolic to immune effect and cell growth. For example, they are important in brain function: Zn is glutamatergic neuron receptor modulator, Fe contributes in myelin synthesis, Ca is important for neurotransmitter release, Cu, Zn and Mn are cofactors for antioxidant enzymes, and Cu is involved in dopamine, norepinephrine synthesis, activation and release of neuropeptide hormones. In addition, Ca is, along with P, important in the crystalline matrix of bones, it stabilises membranes, activates muscle contractions etc. Mg is the fourth most abundant mineral in the body, deposited in bones and muscles. Along with the control of muscle function, it is important for control of hypertension, diabetes and other nutritionally related disorders. The majority of Fe in human body is located in blood cells and app. 80% of daily Fe intake is used for haemoglobin synthesis.[32] Taking into consideration recommended daily intake of these elements, the sweet spreads produced in this research could significantly contribute to their daily intake, even though this is a type of product that should be consumed in smaller amounts.

Another nutritional benefit of sweet spreads produced in the present research is the increase of phenolic compound contents (Table 3). The difference in total phenolic compounds determined by classical Folin Ciocalteu method is not so pronounced (2.31 mg GAE/g in the classic recipe compared to 3.65 mg GAE/g in the sample with the highest proportion of cocoa shell and stevia). However, when the method disannulling influence of reducing sugars on the result is applied (TPC modified), the difference is more visible. The amounts of caffeic acid and epicatechin gallate nearly doubled by the maximum substitution with cocoa shell and stevia. Contents of gallic acid, catechine and epicatehine also increased, whereas the content of p-coumaric acid decreased (Table 3). Compared to TPC content in control chocolate spread of 19850 mg/kg reported by other authors,[33] sweet spreads in the present research are significantly better sources of polyphenols. Although not essential elements, cocoa polyphenols have important nutritional role and, according to EFSA[34] they contribute to maintenance of cardiovascular health and healthy blood pressure. Although results of studies of effect of cocoa polyphenols on human adults are inconsistent, cocoa polyphenols have ameliorated non-alcoholic fatty liver disease in obese mice,[35] have had protective effect on auditory senescent cells and prevention of hearing loss *in vitro*,[36] and it has been reported that "bioactive cocoa metabolites can enhance gut health, displaying anti-inflammatory activities, positively affecting immunity, and reducing the risk of various diseases".[37]

Methylxanthines are another group of biologically active compounds, with two components represented in cocoa: theobromine and caffeine. The contents of theobromine in the present research varied from 3.022 mg/g in the control sample to 4.129 mg/g in the sample with the highest proportion of cocoa shell (Table 3), whereas caffeine contents varied from 0.253 mg/g (control) to 0.503 mg/g (sample with the highest proportion of cocoa shell). It has been established that methylxanthines enhance concentration and arousal levels, due to action on adenosine receptors in the central nervous system. In addition, it has been reported that theobromine may be used as a cough medicine,[38] caffeine has been reported for having decreasing effect on hypoxic depression of breathing and that combination of theobromine and caffeine act as a bronchodilatator, easing the symptoms of apnea of prematurity and asthma. The proportions of these methylxanthines in cocoa are such that they have psycho-stimulant effect, but the cocoa consumption is not linked to sleeping disturbances. There are evidences that theobromine from cocoa increases plasma HDL and decreases plasma LDL cholesterol levels.[38]

All of the above changes in chemical composition (protein, fibre, ash, polyphenolic compounds, methylxantines) are the result of cocoa shell addition.

It is evident that substitution of sucrose with cocoa shall and stevia results in larger nutritional benefit compared to substitution of sucrose with xylitol, which contributes only to energy reduction, without significant influence on the proximate chemical composition, reported also by Rad and Pirouzian.[39]

Considering the fact that sweet spreads are high-fat products, and that the composition of fatty acids highly influence the effect of fats on health, we analysed fatty acid profile of the spreads (Table 4). The addition of cocoa shell resulted in decrease of contents of medium- and long-chain saturated fatty acids, with the exception of docosanoic acid, which was not influenced by the substitution, just like palmitoleic and eicosenoic acid among unsaturated fatty acids. Linoleic acid content was also similar in all analysed samples, while palmitic acid content slightly decreased by the addition of cocoa shell, although not significantly. However, resulting ratios od saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) in cocoa sweet spreads was kept at the same level in all samples, app. 1:1.1:0.18. This is not in line with recommended balance of 1:1.3:1 (SFA:MUFA:PUFA). However, Chen and Liu[40] claim that short- and medium chain fatty acids (C4:0, C6:0, C8:0, and C10:0) do not influence LDL levels due to rapid oxidation to acetyl-CoA in the liver, while C12:0, C14:0, and C16:0 can increase the cholesterol concentration in serum. Therefore, one of the indicators they propose is index of atherogenicity, calculated from the formula: IA = [C12:0 + (4 × C14:0) + C16:0]/ΣUFA. The lower IA, the lower atherogenicity of food. In our samples, IA values ranged from 0.59 to 0.74, which is lower than lamb meat (1.1 – 1.34) or cow milk (2.2 – 4.6), and in narrower range than chicken meat (0.28 – 1.69), for example.[40] However, IA also has some imperfections and should not be used as a sole indicator of nutritional quality of fat.

When developing new product, other than nutritional quality, sensory properties are very important, in order to make the product appealing to consumers. Among them, physical properties that determine the ease of consumption (spreadability, chewiness) are highly influential. Therefore, we determined rheological (Casson yield stress, Casson plastic viscosity) and textural properties (firmness, spreadability), and density of the products. From the results presented in Table 5, it is visible that substitution of sucrose resulted in much larger stress needed for the spreads to start the flow, with the most pronounced effect of xylitol addition (Casson yield stress of 26.01 Pa compared to 6.385 for the control sample). Combining cocoa shell and stevia did not show any trend, but in majority of cases, Casson yield stress of the samples with these components was lower than in the sample with sole xylitol. Homayouni Rad and Pirouzian[39] also reported increase of Casson yield stress viscosity by substitution of sucrose with maltitol, xylitol and galactooligosaccharide (GOS) in chocolate, but they observed the most prounced effect when GOS was used. Casson plastic viscosity of the samples with cocoa shell was higher than both of the control samples – sucrose (N0) and xylitol (N6) and this is the property that directly influences mouthfeel of the spread. Namely, it shows how easy material flows once it has started to flow and will be reflected through sensation of stickiness in the mouth. Homayouni Rad and Pirouzian39 reported the increase of Casson viscosity by substitution of sucrose with maltitol, xylitol and galactooligosaccharide (GOS) in chocolate, with the most pronounced effect of GOS. Apparently, flow properties are influenced by particle size – smaller particles have larger surface area, making them less coverable by cocoa butter. Additionally, hydroxyl groups may absorb water and/or form macromolecules through inter- and intra-molecular interactions, and thus elevate viscosity. Although samples with cocoa shell in our research had larger particle size (results not shown), fibre in the shell have influenced viscosity as macromolecules. Water activity of samples with sucrose substitutes was slightly higher than the one of the control sample, which also contributed to viscosity increase, as reported by other authors,[41] who attributed higher viscosity values to higher moisture contents in chocolates with polyols. Viscosity increase caused by xylitol has been associated to its hygroscopicity, crystallinity and specific surface area, along with the higher solid volume.[42] The same authors also stated that increase of temperature during conching activated hydrophilic active sites and caused agglomeration of the hydrophilic particles, since fat phase is hydrophobic.

The textural properties improved by the substitution of sucrose, although both firmness and spreadability

values generally increased by the increase of proportion of cocoa shell. This was to be expected since the high amount of fibre had been introduced into the product. For example, the increase of sweet spread stiffness was observed after the addition of fiber-rich currant pomace,[43] and increase of hardness and reduced spreadability of sweet spreads with apple pomace used as a substitute for sucrose.[7] The addition of fibre increases the amount of total solids, and the affinity of the product for moisture, which additionally contributes to the increase in hardness. The influence of substitution on density and colloidal stability (Table 5), as well as water activity (Table 2) was not pronounced, which is a positive characteristic, from technological point of view. Low water activity shows high stability towards microbial spoilage, while high colloidal stability shows low tendency of fat to leek to the surface, which in turn keeps fat in limited contact with oxygen and slows down the oxidation.

All recipe changes influenced the properties of the cocoa spreads (Figure 1). The Euclidian, Manhattan and maximum distances are influenced by the large values of some analysed properties, which is avoided by the cosinus distance, however, all used methods show similar trend: N1<N2<N3<N5<N4>N6, with cosinus distance showing approximately same values for N1, N2 and N3, revealing of the potential of further development of these products.

Conclusion

In the present research, sucrose in cocoa sweet spreads was fully substituted with natural sweeteners (xylitol and/or stevia), with cocoa shell as a bulking agent. The sucrose substitution resulted in decrease of caloric value of the products, and the cocoa shell introduced fibre, increased contents of protein, phenolic and mineral components, and methylxanthines, which, in the end, improved the nutritional quality of the spreads. However, although the sweet spreads with the addition of cocoa shell may be declared as sources of fibre, the exact nutritional benefit regarding this nutrient is questionable, taking into account the recommended portions and frequency of intake of this type of food. In addition, rheological and textural properties of the product were significantly changed, posing potential technological issues. Namely, products with increased viscosity require more energy for pumping and flowing, which influences production costs. In addition, they are directly linked to consumer preferences. Although consumers may be willing to compromise sensory properties of foods with nutritional benefits, the quality of the products should be as high as possible. Therefore, further research regarding the adjustment of these properties by the selection of different fats and/or milk components, as well as emulsifier should be conducted. It is well established that fats with lower melting points and milk fat increase spreadability, while emulsifiers, such as PGPR, may additionally improve rheological properties of the spreads. However, there is potential of different interactions between ingredients, and finding their optimal combinations will yield optimum results.

Acknwoledgement

Authors are grateful to the Croatian Science Foundation for granting the project, as well as Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek for the material and personnel support.

Funding Sources

This research was funded in part by Croatian Science Foundation under the project “Application of coca husk in production of chocolate and chocolate-like products” (UIP 2017-05-8709).

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study. Original data available upon request from corresponding author.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Cinical Trial Registration

This study did not involve clinical trial, and therefore, it is not registered in any Registry of Clinical Trials.

Author Contributions

- **Veronika Barišić:** Conceptualization, Experimental Research, Writing Original Draft.
- **Đurđica Ačkar:** Conceptualization, Funding Acquisition, Writing Original Draft.
- **Ivana Lončarević:** Experimental Research, Writing Review and Editing.
- **Jovana Petrović:** Experimental Research, Writing Review and Editing.
- **Mirta Benšić:** Experimental Design, Statistical Analysis, Writing Review and Editing.
- **Daniela Paulik:** Experimental Research, Writing Review and Editing.
- **Kristina Doko:** Experimental Research, Writing Review and Editing.
- **Antun Jozinović:** Experimental Research, Writing Review and Editing.
- **Jurislav Babić:** Experimental Research, Writing Review and Editing.
- **Drago Šubarić:** Supervision, Conceptualization, Writing Review and Editing.
- **Ivana Flanjak:** Conceptualization, Experimental Research, Writing Review and Editing, Project Administration.

References

1. WHO European Regional Obesity Report 2022. Copenhagen WHO Regional Office for Europe.
2. Market Data Forecast. Chocolate Based Spreads Market Size, Share | 2024 to 2029. Accessed July 18, 2024. https://www.marketdataforecast.com/market-reports/chocolate-based-spreads-market
3. Azab D. E. S. H., Mahmoud M. H. Ameliorate the quality of chocolate spread by using orange pectin as a fat substitute. *Egypt J Chem*. 2024;67(9):587-597. http://doi.org/10.21608/EJCHEM.2024.290205.9723
4. Plazzota S., Calligaris S., Manzocco L. Feasibility of protein aerogel particles as food ingredient: The case of cocoa spreads. *J Food Eng.* 2023;351:111522. https://doi.org/10.1016/j.jfoodeng.2023.111522
5. Marra F., Lavorgna A., Incarnato L., Malvano F., Albanese D. Optimization of hazelnut spread based on total or partial substitution of palm oil. *Foods* 2023;12:3122. https://doi.org/10.3390/foods12163122
6. Mostafa H. S. Formulation and evaluation of iron-rich chocolate spread from sugarcane syrup and sunflower seeds. *J Food Qual.* 2023;2023:9531768. https://doi.org/10.1155/2023/9531768
7. Büker M., Angin P., Nurman N., Pirouzian H. R., Akdeniz E., Toker O. S., Sagdic O., Tamtürk F. Effects of apple pomace as a sucrose substitute on the quality characteristics of compound chocolate and spread. *J Food Process Preserv.* 2021;45:e15773. https://doi.org/10.1111/jfpp.15773
8. Bond M., Dunning N. Xylitol. In Sweeteners and Sugar Alternatives in Food Technology 2006; (pp. 295-324). Blackwell Publishing.
9. Okiyama D. C. G., Navarro S. L. B., Rodrigues C. E. C. Cocoa shell and its compounds: Applications in the food industry. *Trends Food Sci Technol.* 2017;63:103-112. http://doi.org/10.1016/j.tifs.2017.03.007
10. ISO 5983-2:2005 Determination of nitrogen content and calculation of crude protein content.
11. AOAC 991.43 Total, soluble, and insoluble dietary fiber in foods: Enzymatic-gravimetric method, *MES-TRIS buffer.* In: AOAC 991; 1995:7-9.
12. ISO 5984:2022 Determination of crude ash. Edition 3, 2022-04. *International Standard Organisation*, 2022.
13. Singleton V. L., Orthofer R., Lamuela-Raventós R. M. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Oxidants and Antioxidants Part A. *Methods in enzymology* 1999; (pp. 152-178). Elsevier. http://doi.org/10.1016/s0076-6879(99)99017-1
14. Vinson J. A., Proch J., Bose P. Determination of quantity and quality of polyphenol antioxidants in foods and beverages. *In Methods in Enzymology* 2001; (pp 103-114). *Elsevier.* http://doi.org/10.1016/s0076-6879(01)35235-7
15. Barišić V., Stokanović Cvijetić M., Flanjak

I., Doko K., Jozinović A., Babić J., Šubarić D., Miličević B., Cindrić I., Ačkar Đ. Cocoa Shell as a Step Forward to Functional Chocolates—Bioactive Components in Chocolates with Different Composition. *Molecules* 2020;25(22):5470. https://doi.org/10.3390/molecules25225470

16. Barišić V., Flanjak I., Križić I., Jozinović A., Šubarić D., Babić J., Miličević B., Ačkar Đ. Impact of high-voltage electric discharge treatment on cocoa shell phenolic components and methylxanthines. *J Food Process Eng.* 2020;43(1):e13057. http://doi.org/10.1111/jfpe.13057
17. Folch J., Lees M., Sloane Stanley G. H. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* 1957;226(1):497-509. http://doi.org/10.1016/s0021-9258(18)64849-5
18. Eggers, L. F., Schwudke, D. Liquid Extraction: Folch. In: Wenk, M. (eds) Encyclopedia of Lipidomics 2016; (pp. 1-6). Springer. https://doi.org/10.1007/978-94-007-7864-1_89-1
19. Amending Regulation (EEC) No 2568/91 on the Characteristics of Olive Oil and Olive-Pomace Oil and on the Relevant Methods of Analysis and the Additional Notes in the Annex to Council Regulation (EEC) No 2658/87 on the Tariff and Statistical Nomenclature and on the Common Customs Tariff; 2002.
20. Department of Health, Education, and Welfare Public Health Service Food and Drug Administration. *Water activity (aw) in foods.* https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/water-activity-aw-foods (accessed: 04/09/2024)
21. Sharma M., Pondicherry K. S., Duize L. Understanding relations between rheology, tribology, and sensory perception of modified texture foods. *J Texture Stud.* 2021;53(3):327-344. http://doi.org/10.1111/jtxs.12656
22. Ordonez E., Calderon-Pino J., Reategui, D. Effect of particle size on sensory and bioactive properties of chocolates with Physalis peruviana L and Vaccinium spp. *Rev Fac Nac Agron Medellin* 2023;76(2):10369-10379. https://doi.org/10.15446/rfnam.v76n2.103168
23. Tolve R., Tchuenbou-Magaia F. L., Verderese D., Simonato B., Puggia D., Galgano F., Zamboni A., Favati F. Physico-chemical and sensory acceptability of no added sugar chocolate spreads fortified with multiple micronutrients. *Food Chem.* 2021;364(130386):130386. http://doi.org/10.1016/j.foodchem.2021.130386
24. Kumar P. Process optimization for the preparation of chocolate spread incorporating whey protein concentrate, cocoa powder, olive oil and butterfat using response surface methodology. *J Food Process Preserv.* 2015;39(6):745-757. http://doi.org/10.1111/jfpp.12284
25. Cantele C., Rojo-Poveda O., Bertolino M., Ghirardello D., Cardenia V., Barbosa-Pereira L., Zeppa G. In Vitro bioaccessibility and functional properties of phenolic compounds from enriched beverages based on cocoa bean shell. *Foods* 2020;9(6):715. http://doi.org/10.3390/foods9060715
26. Rojo-Poveda O., Barbosa-Pereira L., Zeppa G., Stévigny C. Cocoa bean shell-A by-product with nutritional properties and biofunctional potential. *Nutrients* 2020;12(4):1123. http://doi.org/10.3390/nu12041123
27. Adeyeye E. I., Akinyeye R. O., Ogunlade I., Olaofe O., Boluwade J. O. Effect of farm and industrial processing on the amino acid profile of cocoa beans. *Food Chem.* 2010;118(2):357-363. http://doi.org/10.1016/j.foodchem.2009.04.127
28. Hoffman J. R., Falvo M. J. Protein - which is best? *J Sport Sci Med*. 2004;3(3):118-130. https://www.ncbi.nlm.nih.gov/pubmed/24482589
29. Schweizer T. F., Edwards C. A. Dietary fibre – a component of food nutritional function in health and disease 1992. Springer-Verlag.
30. Mcburney M. I. Dietary Fibre: Insights and Opportunities In Dietary fibre: new frontiers for food and health, 2010; (pp. 151-166). Wageningen Academic. https://doi.org/10.3920/978-90-8686-692-2_012
31. Barišić V., Kerovec D., Flanjak I., Jozinović A., Babić J., Lončarić Z., Šubarić D., Miličević B., Ačkar Đ. Effect of high-voltage electrical discharge treatment on multi-element content in cocoa shell and chocolates with cocoa shell. *LWT – Food Sci Technol.*2022;155:112944. http://doi.org/10.1016/j.lwt.2021.112944

32. Harris E. D. Minerals in Food Nutrition, Metabolism, Bioactivity 2014. DEStech Publications.
33. Acan B. G., Kilicli M., Bursa K., Toker O. S., Palabiyik I., Gulcu M., Yaman M., Gunes R., Konar N. Effect of grape pomace usage in chocolate spread formulation on textural, rheological and digestibility properties. *LWT – Food Sci Technol*. 2021;138:110451. http://doi.org/10.1016/j.lwt.2020.110451
34. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). Scientific Opinion on the modification of the authorisation of a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation pursuant to Article 13(5) of Regulation (EC) No 1924/2006 following a request in accordance with Article 19 of Regulation (EC) No 1924/2006. *EFSA Journal* 2014;12(5). http://doi.org/10.2903/j.efsa.2014.3654
35. Sun M., Gu Y., Glisan S. L., Lambert J. D. Dietary cocoa ameliorates non-alcoholic fatty liver disease and increases markers of antioxidant response and mitochondrial biogenesis in high fat-fed mice. *J Nutr Biochem*. 2021;92:108618. https://doi.org/10.1016/j.jnutbio.2021.108618
36. del Mar Rivas-Chacón L., Yanes-Díaz J., de Lucas B., Riestra-Ayora J. I., Madrid-García R., Sanz-Fernández R., Sánchez-Rodríguez C. Preventive effect of cocoa flavonoids via suppression of oxidative stress-induced apoptosis in auditory senescent cells. *Antioxidants* (Basel) 2022;11(8):1450. http://doi.org/10.3390/antiox11081450
37. Sorrenti V., Ali S., Mancin L., Davinelli S., Paoli A., Scapagnini G. Cocoa polyphenols and gut Microbiota interplay: Bioavailability, prebiotic effect, and impact on human health. *Nutrients* 2020;12(7):1908. http://doi.org/10.3390/nu12071908
38. Franco R., Oñatibia-Astibia A., Martínez-Pinilla E. Health benefits of methylxanthines in cacao and chocolate. *Nutrients* 2013;5(10):4159-4173. http://doi.org/10.3390/nu5104159
39. Homayouni Rad A., Rasouli Pirouzian H. Optimization of prebiotic sucrose-free milk chocolate formulation by mixture design. *J Food Sci Technol.* 2021;58:244–254. https://doi.org/10.1007/s13197-020-04536-w
40. Chen J., Liu H. Nutritional indices for assessing fatty acids: A mini-review. *Int J Mol Sci.* 2020;21(16):5695. http://doi.org/10.3390/ijms21165695
41. Homayouni Rad A., Pirouzian H. R., Toker O. S., Konar N. Effect of Various Bulk Sweeteners on the Survivability of Lactobacillus casei 431 in Milk Chocolate: Rheological and Sensory Properties Analysis. *Curr Pharm Biotech.* 2020;21:1224-1231. http://doi.org/10.2174/1389201021666200423105856
42. Rasouli-Pirouzian H., Peighambardoust S. H., Azadmard-Damirchi S. Rheological properties of sugarfree milk chocolate: Comparative study and optimisation. *Czech J Food Sci.* 2017;35(5):440-448. http://doi.org/10.17221/231/2016-CJFS.
43. Reißner A. M., Rohm H., Struck S. Sustainability on bread: How fiber-rich currant pomace affects rheological and sensory properties of sweet fat-based spreads. *Foods* 2023;12(6). http://doi.org/10.339