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Physical Properties and Bioactive Constituents of Powdered Mixtures and Drinks Prepared with Cocoa and Various Sweeteners

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In the present study the physical properties of powdered cocoa drink mixtures prepared from two cocoa powders with various fat contents and different sweeteners, as well as the bioactive content and sensory properties of cocoa drinks prepared from them, were investigated. Particle size and bulk density of the used sugars and sweeteners, as well as the formulated mixtures, were determined and their influence on cohesion index was evaluated. To compare the content of polyphenols in the formulated cocoa drink mixtures, UV−vis spectrophotometric methods were applied. Antioxidant capacity of cocoa drinks was evaluated by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and ferric reducing/antioxidant power (FRAP) assays. The analyzed cocoa drinks prepared from cocoa powder and different sugars or sweeteners delivered a substantial content of cocoa antioxidants, whereas the content and the type of sugar or sweetener did not affect the polyphenolic constituents of the prepared cocoa mixtures. Cocoa powder mixtures prepared with the cocoa powder containing higher fat content (16−18%) generally provided lower total polyphenol, total flavonoid, flavan-3-ol, and proanthocyanidin contents, compared to the mixtures prepared with cocoa containing lower fat content (10−12%). Total phenol content of cocoa drinks prepared from experimental mixtures ranged from 320.45 to 480.45 mg of GAE/L, whereas the ranking of the antioxidant capacities varied depending on the used assay, and the fat content of cocoa powder did not affect the antioxidant capacity of cocoa mixtures. As determined, the addition of sugar to cocoa powder increases the solubility and dispersibility of the mixtures; on the basis of their cohesion index all mixtures can be classified as very cohesive or hardened/extremly cohesive. Results of the sensory evaluation, using the 9-point hedonic scale, showed that there was a preference for the cocoa drinks made with sweeteners (aspartame/acesulfame K and stevia extract), and there was a significant difference in the sensory attributes between the experimental mixtures and the control. The displayed results indicate the significant potential of using alternative sweeteners for the preparation of cocoa drink mixtures, which may provide good physical and sensory properties and also enhance the already existing beneficial effects of cocoa.

KEYWORDS: Antioxidant capacity; cocoa; physical properties; polyphenols; sensory evaluation; sweeteners

INTRODUCTION

Cocoa is nowadays one of the most appreciated food commodities, available in a wide variety of products and used in numerous applications. Accompanied by the continuously increasing popularity, cocoa is appreciated by almost all ages, owing to the fact that its consumption is not limited to any particular part of the day (*1*). Also, growing evidence has emerged confirming the health benefits of cocoa antioxidants and other bioactive compounds of cocoa in the diet, especially polyphenols. Due to a great number of various cocoa products available on the market and growing consumer demands, there is an increasing interest in improvement of cocoa drinks, aimed to reducing the sugar content and enhancing the content of beneficial bioactive constituents.

Sweeteners, and especially sugars, are particularly important as constituents of cocoa-based beverages due to the bitter taste of cocoa powder. Because sucrose and glucose are relatively cheap and abundant, cocoa products are readily available to the consumer. The consumption of cocoa products and chocolate contributes to the intake of sugar in the diet, which is considered to be implicated in the development of dental caries, obesity, and metabolic diseases associated with obesity (*2*). Because other sugars and noncarbohydrate sweeteners are much more expensive than standard sugar ingredients and as they vary in sweetness compared to sugar, they often need to be supplemented with

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intense sweeteners, or filler ingredients, thus slightly further increasing the total cost (*3*). Recently, there has been increasing interest in the use of a natural sweetener obtained from the leaves of the plant called *Stevia rebaudiana*, which contain nine known leaf sweetening diterpenic glycosides, among which stevioside and rebaudiosides are up to 100−300 times sweeter than sucrose (*4*,*5*).

In the production of ready-to-drink beverages, manufacturers are confronted with some difficulties, especially in achieving an acceptable mouthfeel and a good suspension of ingredients in milk or other base liquids. Besides color and flavor, a range of other characteristics define cocoa powder and have an important functional impact on the end product in which the cocoa is used. Manufacturing parameters and other ingredients in the formula of a certain application may distinctively influence the overall performance of cocoa powder in the final product as well (*1*). Some properties, such as bulk density, compressibility (compaction), and flowability are essential to product handling, processing, and transportation; some others, such as particle size and instant properties, are related to quality and consumer acceptance (*6*). When a cocoa powder is chosen for a certain application, the desired characteristics of the end product, as well as the overall recipe and the processing parameters, need to be taken into consideration.

Although there are some patent applications available addressing the use of different sweeteners for the production of powdered cocoa drinks, there is a lack of scientific research studies addressing that issue. The present study concerns several formulations of water-soluble or water-dispersable solid substances that provide, when stirred with warm water or milk, a cocoa-based drink of desirable sensory properties. The purpose of this study was to provide a cocoa drink with good physical properties to enable good flow properties and, as well, to achieve a high content of polyphenolic compounds that exhibit positive health effects. Therefore, in this study the physical properties of 10 prepared cocoa drink mixtures were evaluated comprising several different sweeteners and 2 cocoa powders with differing fat contents. Also, the effect of different sweeteners on the content of total polyphenols and several subclasses of polyphenols as well as their antioxidant capacity and sensory properties in beverages prepared from the expermental cocoa drink mixtures was determined.

MATERIALS AND METHODS

Chemicals. Folin−Ciocalteu, formic acid, potassium peroxodisulfate, sodium carbonate, formaldehyde, and hydrochloric acid were of analytical grade and supplied by Kemika (Zagreb, Croatia). Methanol (HPLC grade) was supplied by J. T. Baker (Deventer, The Netherlands). Vanillin, *p*-dimethylaminocinnamaldehyde, Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH), as well as gallic acid and cyanidin chloride were obtained from Sigma-Aldrich (Steinheim, Germany). Cocoa powders were obtained from a local chocolate industry Zvečevo (Požega, Croatia), sucrose, glucose, maltodextrin, and erythritol were supplied by Cargill (Krefeld, Germany), fructose was supplied by Merck (Darmstadt, Germany), isomaltulose was obtained from Beneo-Palatinit (Manheim, Germany), inulin and oligofructose were obtained from Beneo-Orafti (Tienen, Belgium), aspartame/acesulfam K sweetener blend (50%/50%) was obtained from Brenntag (Wien, Austria), and stevia sweetener was purchased from a local pharmacy (Kal, USA).

Formulation of Experimental Cocoa Drink Mixtures. Because cocoa drink powders usually consist of about 70% sugar and 30% cocoa powder (*7*), this ratio served as a basis for the preparation of cocoa drink mixtures. The cocoa powder to sugar ratios selected for experimental blends were adjusted according to the relative sweetness of selected sugars and sweeteners (**Table 1**). The cocoa powder and sugar fractions were mixed in a T2F Turbula mixer (Willy A. Bachofen Maschienenfabrik, Muttenz, Switzerland) for 10 min to obtain homogeneous cocoa drink powder blends.

Table 1. Experimental Formulations for Cocoa Powder Drink Preparation (Percent)

	RS[a]	A	B	C	D	E	F	G	H	I	J
cocoa powder		30	30	30	30	30	30	30	30	30	30
sucrose	1	70	60	35	35	30					
glucose	0.7				35		29.5		20		
fructose	1.1						10				
trehalose	0.45		10				30		9		10
isomaltulose (palatinose)	0.45					20		10		20	19
eritrythol	0.7			35		20		10		9	
stevia	300						0.5		1	1	1
aspartame/ acesulfame K	130−250							0.7			
maltodextrin								30	20	20	
inulin								19.3	20	10	20
oligofructose										10	20

[a] RS, relative sweetness (*10*).

Moisture Content. Moisture content of the used sweeteners and experimental mixtures was determined according to the official AOAC method (*8*) by drying the samples at 102 °C to constant weight. The moisture content was determined by weight difference and expressed as a percentage of the initial sample weight.

Particle Size. The particle size of sugars and sweeteners was investigated because of its effect on cohesion, bulk density, dispersibility, and solubility of the formulated blends. The particle size of the used sugars was characterized using conventional sieving analysis. Sieves were manufactured by Fritsch, Germany. The powders were sieved for 15 min using an Analysette 3 PRO laboratory shaker manufactured by Fritsch with a 2.5 mm vibration amplitude and a 3 s interval time. The results of the sieving analysis were tabulated to show the particle size range of sugar samples.

Bulk Density. Bulk density of the samples was determined following a modified method of Murakami et al. (*9*). Sample was poured into a 100 mL measuring cylinder, and the masses of the empty and filled measuring cylinders and the volume occupied by the sample were recorded. Bulk density was determined by dividing the net weight of the sample with the volume occupied by the sample in the cylinder. Bulk density was calculated as an average value of 10 measurements.

Dispersibility, Solubility, and Wettability. Dispersibility was determined following a modified method of Shittu and Lawal (*10*). Five grams of each sample was dissolved in 50 mL of distilled water at 27 °C. The mixture was stirred manually for 1 min and allowed to rest for 24 h before the supernatant was decanted. The density of the supernatant was determined by filling 25 mL of the supernatant in a density bottle. The weight of the dispersed sample was calculated as twice the difference between the mass of the supernatant and an equal volume of distilled water.

Solubility was determined as described by Takashi and Seibi (*11*), with some modifications. An amount of 5 g of each sample was suspended in 50 mL of distilled water at 30 °C. The suspension was stirred occasionally for 30 min and centrifuged at 9500 rpm for 10 min. The supernatant was drained into an evaporating dish and dried at 105 °C to constant weight. The weight of the solids recovered after drying was used to calculate the water solubility (percent).

Wettability (or wetting time) was determined as described by Schubert (*12*), being considered as the time (in seconds) required for all of the powder to become wetted and penetrate the surface of the distilled water at 27 °C. Analyses were done in triplicate.

Cohesion Index (CI) of Cocoa Drink Mixtures. CI values of cocoa drink powders were determined using a TA.HD Plus Powder Flow Analyzer manufactured by Stable Micro Systems, Godalming, U.K. The Powder Flow Analyzer is constituted by a vertical glass container (120 mm height and 50 mm internal diameter) and a rotating specific blade (48 mm diameter and 10 mm height), which is able to go up and down, in right or left rotation (*13*). The flowability properties were evaluated during the displacement in a controlled manner of the rotating blade inside the container, filled with the powder sample (*14*). Before testing, samples were weighed and placed into a powder container in which the tests were

performed. A quick test is used to determine the cohesion coefficient. The CI was calculated by dividing the cohesion coefficient with sample weight, and the cocoa powder mixtures were categorized on the basis of the CI.

Determination of Quality Parameters of Cocoa Drinks. *Preparation of Cocoa Drinks*. To simulate household preparation conditions, cocoa drinks were prepared according to a procedure described by Lee et al. (*15*): 7.3 g of cocoa blend (equivalent to 2 tablespoons) was dissolved in 200 mL of boiling, distilled water and stirred with a glass rod. The samples were cooled and centrifuged at 3500 rpm for 5 min, and the resulting supernatants were used as the final samples.

Determination of Total Phenol (*TPC*) *and Flavonoid Contents* (*TFC*). TPC of cocoa drinks was determined spectrophotometrically according to a modified method of Belščak et al. (*16*). To determine the TFC, these compounds were precipitated using formaldehyde, which reacts with C-6 or C-8 on 5,7-dihydroxy flavonoids. The condensed products of these reactions were removed by filtration, and the remaining nonflavonoid phenols were determined according to the previously mentioned procedure for the determination of TPC. Flavonoid content was calculated as the difference between total phenol and nonflavonoid contents. Gallic acid was used as the standard, and the results were expressed as milligrams of gallic acid equivalents (GAE) per liter (*17*). All measurements were performed in triplicate.

Determination of Flavan-3-ol Content. (*a*) *Vanillin Assay*. Cocoa drinks were analyzed for their flavan-3-ol content using a method described by Belščak et al. (*16*). For the analysis, a working solution of 4% vanillin in methanol was prepared daily. Cocoa extract (500 μL) was added to 3 mL of the previously prepared vanillin reagent, and after 5 min, 1.5 mL of concentrated HCl was added. The mixture was allowed to react for 15 min, in a cold water bath, whereafter absorbance readings were taken at 500 nm. The blank was prepared by replacing the 4% vanillin solution with methanol. Absorbance of the blank was subtracted from the absorbance of the corresponding vanillin-containing sample (ΔE). The content of flavan-3-ols was calculated according to the formula (+)-catechin = 290.8ΔE, and the results were expressed as milligrams of (+)-catechin per liter.

(*b*) *Reaction with p-Dimethylaminocinnamaldehyde*. A procedure reported by Belščak et al. (*16*) was also used to estimate the flavan-3-ol content. Reagent was prepared by dissolving 100 mg of *p*-dimethylaminocinnamaldehyde in a mixture of concentrated HCl (25 mL) and methanol (70 mL), and the resulting solution was made up to 100 mL with methanol. For the analysis, 1 mL of cocoa extract was added to 5 mL of *p*-dimethylaminocinnamaldehyde reagent in a glass test tube and thoroughly shaken. After 10 min, an absorbance reading was taken at 640 nm, along with two blank samples prepared separately for each cocoa sample. The first blank consisted of 5 mL of *p*-dimethylaminocinnamaldehyde reagent and 1 mL of distilled water, and the second one consisted of 5 mL of distilled water and 1 mL of cocoa extract. The content of flavan-3-ols was calculated according to the formula (+)-catechin = 32.1ΔE, where ΔE is the difference of absorbance between the tested cocoa extract and appropriate blanks. The results were expressed as milligrams of (+)-catechin per liter.

Quantitative Determination of Proanthocyanidins. Proanthocyanidins (i.e., condensed tannins) were analyzed according to the procedure described by Porter et al. (*18*), with some modifications. Briefly, a butanol/HCl assay was carried out by mixing 2 mL of cocoa extract with 4 mL of a solution of *n*-BuOH/concentrated HCl (95:5, v/v) and 0.2 mL of a 2% solution of $NH_4Fe(SO_4)_2 \cdot 12H_2O$ in 2 M HCl. The solution was capped and thoroughly mixed and heated for 45 min at 95 °C in a water bath. The sample was cooled and the visible spectrum recorded at λ = 550 nm. The blank value of the BuOH/HCl/Fe^{III} solvent was subtracted. The quantity of condensed tannins was determined from a standard curve of cyanidin chloride treated with BuOH/HCl/Fe^{III} mixture and expressed as milligrams of cyanidin chloride equivalents (CyE) per liter.

Determination of Antioxidant Capacity. *DPPH Radical Scavenging Assay*. The antioxidant capacity of the experimental mixtures was determined using the DPPH$^{\bullet}$ radical scavenging assay described by Brand-Williams et al. (*19*). The free radical scavenging capacity using the DPPH$^{\bullet}$ radical reaction was evaluated by measuring the absorbance at 515 nm after 30 min of reaction at room temperature. The results were expressed as millimolar Trolox equivalents, using the calibration curve of Trolox (0−1 mM). All measurements were performed in triplicate.

ABTS Radical Scavenging Assay. The Trolox equivalent antioxidant capacity (TEAC) of cocoa drinks was estimated by using the ABTS radical cation decolorization assay (*20*). The results, obtained from triplicate analyses, were expressed as Trolox equivalents and derived from a calibration curve determined for Trolox (100−1000 μM).

Ferric Reducing/Antioxidant Power. The ferric reducing/antioxidant power (FRAP) assay was carried out according to a standard procedure by Benzie and Strain (*21*). All measurements were performed in triplicate. Aqueous solutions of $FeSO_4 \cdot 7H_2O$ (100−1000 μM) were used for the calibration curve, and the results are expressed as millimolar Fe^{II}.

Sensory Evaluation. The experimental cocoa drink mixtures were subjected to sensory evaluation using a laboratory type panel, comprising 15 people with previous experience in taste panel procedures. Cocoa drinks for sensory evaluation were prepared as for the experimental analyses, by mixing 7.3 g of cocoa powder mixtures with 200 mL of boiling water. Each panelist was given about 30 mL of warm (60 °C) reconstituted cocoa drink. Warm water (about 60 °C) was provided for rinsing between samples. Five attributes (cocoa odor, mouthfeel, bitter, sweet, balanced) were scored using the 9-point hedonic scale. The scale ranged from "like extremely" to "dislike extremely", corresponding to the highest and lowest scores of "9" and "1", respectively (*22*). The list of attributes was developed from attributes in the literature and discussions with the panelists.

Statistical Analysis. The results were analyzed statistically using the Statistica 7.0 software to determine the average value and standard deviation. Variance analysis, with a significance level of α = 0.05%, was performed to determine the influence of composition of powdered cocoa drink mixtures on their physical properties, polyphenolic content, and antioxidant potential, as well as to establish the differences in the content of polyphenols among the cocoa mixtures, regarding the fat content of cocoa powder. Correlation analysis was also run with the same statistical package.

RESULTS AND DISCUSSION

Sugars have many different functions in foods, such as sweetening, bulking, preserving, texturing, and flavoring. Because the application of sugars in food processing depends on their particle size, it is usual to use coarse or fine powder in baked goods and even finer powders in confectionery goods (*23*). The particle size range of sugar samples used in this study is shown in **Table 2**. As can be seen, the particle size of sugars used in the preparation of cocoa drink mixtures ranged from 56 μm for isomaltulose and aspartame/acesulfame K to 630 μm for eritrythol. Particle size correlated well with bulk densities (r = 0.686) of sugar samples.

Sugar samples are classified on the basis of the cohesion index as follows: Stevia and aspartame/acesulfame K are hardened/extremely cohesive with cohesion indices of 23.36 and 26.21, respectively (**Table 2**). Glucose (CI = 16.46), fructose (CI = 16.58), and maltodextrin (CI = 16.78) are very cohesive, followed by sucrose (CI = 15.37), which is cohesive, whereas trehalose, isomaltulose, erythritol, inulin, and oligofructose are easy- or free-flowing. Previous research (*24*) has shown that cohesion increases with the decrease of particle size, due to the increased surface area per unit mass of the powder and a higher probability of particle/particle interactions. However, statistical analysis of our experimental results showed no significant influence of particle size on cohesion index ($p > 0.05$). Besides particle size, many other factors can affect cohesion, such as moisture, temperature, electrostatic forces, and particle shape. Further study of the above-mentioned factors should show which of them have the biggest influence on cohesion of sugar samples.

An important quality indicator of cocoa powder is its moisture content, because in the presence of an excessive level of moisture, flavor may deteriorate, and the possibility of microbiological spoilage will arise. Generally, the moisture content of cocoa powder must be maintained at up to 5% (*7*). The moisture content of cocoa powders used in this study was 3.73% for $CP_{10-12\%\ fat}$ and 3.62% for $CP_{16-18\%\ fat}$. The moisture content of

Table 2. Physical Properties of Used Sugars and Sweeteners[a]

	moisture content (%)	particle size, range (μm)	"loose" bulk density (kg/m^3)	wettability (s)	solubility (%)	dispersibility (%)	cohesion index
sucrose	0.13 ± 0.01	500−355	914.40 ± 19.47	0.10 ± 0.00	9.30 ± 0.23	28.52 ± 0.78	−15.37 ± 0.07
glucose	8.27 ± 0.45	250−180	665.07 ± 18.94	1.34 ± 0.01	8.75 ± 0.17	54.58 ± 1.20	−16.46 ± 0.79
fructose	0.44 ± 0.05	450−315	829.91 ± 20.00	0.25 ± 0.02	9.31 ± 0.46	50.81 ± 1.45	−16.58 ± 1.02
trehalose	8.60 ± 0.52	180−100	852.38 ± 25.68	0.15 ± 0.02	9.24 ± 0.25	41.40 ± 1.71	−12.55 ± 0.88
isomaltulose	1.25 ± 0.11	100−56	787.75 ± 18.98	0.76 ± 0.05	9.17 ± 0.18	50.51 ± 1.67	−11.06 ± 0.52
erytrithol	0.06 ± 0.01	630−450	945.62 ± 16.34	0.14 ± 0.01	9.44 ± 0.27	43.44 ± 0.92	−9.74 ± 0.12
stevia	3.59 ± 0.23	140−80	515.56 ± 20.04	15.10 ± 0.87	8.79 ± 0.33	50.42 ± 1.33	−23.36 ± 1.22
aspartame/acesulfame K	1.78 ± 0.17	100−56	761.67 ± 19.76	4.09 ± 0.26	10.07 ± 0.24	65.97 ± 2.01	−26.21 ± 1.95
maltodextrin	3.86 ± 0.45	140−80	549.27 ± 5.34	1.19 ± 0.04	9.08 ± 0.37	53.87 ± 1.98	−16.78 ± 0.99
inulin	3.16 ± 0.21	250−180	678.33 ± 5.47	1.16 ± 0.07	5.53 ± 0.19	23.40 ± 0.64	−9.57 ± 0.09
oligofructose	3.49 ± 0.13	450−315	856.72 ± 45.23	0.64 ± 0.03	9.14 ± 0.36	47.44 ± 1.59	−11.51 ± 0.12

[a] Results are expressed as mean (n = 3) ± SD.

Table 3. Physical Properties of Cocoa Powder (CP) Drink Mixtures[a]

	moisture content (%)	"loose" bulk density (kg/m^3)	wettability (s)	solubility (%)	dispersibility (%)	cohesion index
Cocoa Powder with 10−12% Fat						
CP	3.73 ± 0.23	383.31 ± 9.42	157 ± 5	2.12 ± 0.11	6.65 ± 0.37	−19.64 ± 0.68
A	1.22 ± 0.09	818.96 ± 30.56	57 ± 9	7.13 ± 0.24	10.43 ± 0.68	−29.10 ± 0.61
B	2.03 ± 0.12	773.25 ± 42.92	138 ± 13	7.30 ± 0.28	32.19 ± 1.03	−30.39 ± 1.41
C	2.48 ± 0.21	747.15 ± 19.29	328 ± 11	7.06 ± 0.18	29.79 ± 0.19	−31.52 ± 1.03
D	3.97 ± 0.31	678.25 ± 11.71	190 ± 8	6.55 ± 0.21	23.20 ± 0.48	−30.92 ± 1.59
E	3.04 ± 0.21	862.20 ± 84.53	158 ± 12	7.55 ± 0.34	39.78 ± 0.67	−23.23 ± 0.64
F	6.45 ± 0.36	712.39 ± 12.98	253 ± 14	6.56 ± 0.20	28.17 ± 0.89	−25.45 ± 1.12
G	4.30 ± 0.29	585.65 ± 13.99	905 ± 23	6.81 ± 0.32	20.18 ± 0.23	−21.73 ± 0.61
H	5.99 ± 0.38	637.07 ± 11.08	1327 ± 56	6.83 ± 0.27	22.12 ± 0.48	−24.72 ± 0.59
I	5.13 ± 0.42	608.23 ± 13.03	383 ± 12	6.89 ± 0.11	14.00 ± 0.59	−22.99 ± 0.59
J	5.77 ± 0.55	638.97 ± 11.38	1521 ± 64	6.90 ± 0.19	31.62 ± 1.10	−24.20 ± 1.56
Cocoa Powder with 16−18% Fat						
CP	3.62 ± 0.28	441.76 ± 37.40	234 ± 21	2.02 ± 0.12	4.37 ± 0.29	−19.36 ± 1.82
A	1.09 ± 0.05	828.34 ± 14.08	435 ± 24	7.07 ± 0.36	25.99 ± 0.76	−27.31 ± 0.84
B	1.88 ± 0.15	800.82 ± 18.96	759 ± 35	7.37 ± 0.24	26.14 ± 0.38	−20.42 ± 0.85
C	2.57 ± 0.29	763.97 ± 22.16	50 ± 5	6.86 ± 0.13	31.80 ± 0.43	−26.58 ± 1.85
D	3.95 ± 0.24	688.56 ± 29.93	108 ± 9	6.70 ± 0.32	18.27 ± 0.18	−21.96 ± 1.98
E	3.11 ± 0.19	739.20 ± 21.14	1207 ± 45	6.89 ± 0.48	12.73 ± 0.21	−20.47 ± 0.82
F	6.07 ± 0.34	657.68 ± 40.20	447 ± 37	6.61 ± 0.17	38.77 ± 1.14	−20.26 ± 0.87
G	5.02 ± 0.41	589.15 ± 10.58	227 ± 29	6.88 ± 0.11	22.75 ± 0.64	−19.70 ± 0.03
H	5.81 ± 0.39	584.30 ± 19.45	177 ± 12	6.78 ± 0.25	12.12 ± 0.35	−26.02 ± 0.31
I	5.01 ± 0.42	628.49 ± 16.04	4277 ± 125	6.97 ± 0.31	21.51 ± 0.27	−20.93 ± 0.31
J	5.53 ± 0.45	619.42 ± 5.49	166 ± 8	6.92 ± 0.38	22.19 ± 0.32	−18.15 ± 0.64

[a] Results are expressed as mean (n = 3) ± SD.

cocoa drink mixtures differed depending on the moisture content of the sweeteners used for the preparation of experimental mixtures. Mixture A exhibited the lowest moisture content (1.22% for $A_{10-12\%\ fat}$ and 1.09% for $A_{16-18\%\ fat}$), which corresponds to the low moisture content of sucrose (0.13%) used for the formulation of mixture A. Accordingly, mixture F formulated from glucose and trehalose, which were characterized with the highest moisture contents (8.27 and 8.60%, respectively), exhibited the highest moisture content (6.45% for $F_{10-12\%\ fat}$ and 6.07% for $F_{16-18\%\ fat}$). It was also observed that fat content affects the moisture content of cocoa, because the cocoa drink mixtures prepared from cocoa containing higher fat content exhibited lower moisture percentage. Because cocoa powder is hygroscopic, good packaging and storage conditions are essential to preventing the uptake of moisture.

A high content of cocoa powder in chocolate drinks contributes to a desirable chocolate taste, but it may affect the physical properties such as increasing the sedimentation of cocoa particles and decreasing their wettability and dispersibility, due to its cocoa butter content. Cocoa drink powders face some physical problems, that is, poor wettability, low dispersibility, and sedimentation of cocoa particles, after reconstitution (*25*). These are more pronounced when cow's milk is used as the solvent. Because cocoa powder is an insoluble, hydrophobic powder, which is hard to disperse in milk or in water, we also present some physical characteristics of the cocoa powder mixtures. **Table 3** presents the powder properties of experimental cocoa drink mixtures.

It is well-known that particle size is one of the most important characteristics of powders. The measurement of particle size, besides their distribution, is a method to characterize powders in the industry (*26*). These analyses are useful in the quality control of the final product, and they can be related to the other physical properties already mentioned (*27*). Cocoa powder particle size influences flavor, mouthfeel, color, texture, viscosity, and solubility (*28*). The actual particle size of cocoa powder is achieved during the cocoa liquor grinding process (*7*). The cocoa cake pulverizer merely reduces the size of the compacted aggregates formed during the hydraulic pressing to the true size of the cocoa

powder. Despite the difference in particle sizes and bulk densities of cocoa powders (cocoa powder with 10−12% fat, average particle size = 19 μm; cocoa powder with 16−18% fat, average particle size = 30 μm) and added sugars, the mixtures were homogeneous and no segregation occurred during storage.

Cocoa powders showed significantly ($p < 0.05$) lower bulk densities than cocoa drink mixtures (**Table 3**), which is understandable, considering that sugars, which exhibit higher bulk density values, were mixed with cocoa powders. Bulk densities of our analyzed cocoa drink mixtures differed significantly ($p < 0.05$), depending on various particle shapes that can interlock to form a rigid structure and, hence, different densities (*23*). The highest bulk density was determined in mixture $E_{10-12\%\ fat}$ (862.20 kg/m^3) containing sucrose, palatinose, and erythritol, which exhibited the highest particle size range. Generally, mixtures A, B, C, and E, consisting of a higher content of sucrose and erythritol, exhibited the highest bulk densities. These observations were confirmed by the statistical analysis, which indicated a significant influence of the addition of sucrose on bulk densities of mixtures with 16−18% cocoa powder. As opposed to previous findings (*29*), which showed no relationship between the bulk density and particle size of chocolate drink powders, our results showed a high correlation between the particle size and bulk density of cocoa drink mixtures.

Under typical food conditions, cocoa powder is about 30% soluble in water. The remaining part does not disperse well in water, owing to the presence of hydrophobic fat crystals on the outer side of the cocoa particles. The wettability is also poor. The wettability and the dispersibility can be improved by mixing cocoa with lecithin, mixing with sugar, adding an agglomeration step, or a combination of these steps (*1*). The most important factor that is expected to affect solubility is the sugar content, because it is the major soluble component of the mixtures (*10*). From the results shown in **Table 2**, a strong correlation was observed betwen solubility and dispersibility of sugars ($r = 0.736$), indicating that the solubility of sugars increases with higher dispersibility values. Pure cocoa powder with 10−12% fat has a solubility value of 2.12% and a dispersibility of 6.65%, whereas the mixtures exhibit solubility above 20%, with the exception of mixtures A and I. The solubility of cocoa powder with 16−18% fat is 2.02% and the dispersibility is 4.37%, whereas the solubility increases significantly with the addition of sugars and sweeteners to all mixtures (**Table 3**).

Nonagglomerated cocoa powders exhibit very poor reconstitutional properties, including wettability. According to the results obtained by this study (**Table 3**), cocoa powder with 10−12% fat and powder mixtures with cocoa containing 10−12% fat exhibited better wettability than cocoa powder and the mixtures made with cocoa powder containing 16−18% fat. In the case of most mixtures, the addition of sugars increased the wettability time. The addition of maltodextrin, inulin, and oligofructose had a significant negative influence on the wettability of mixtures G−J. Wettability times for all the mixtures are extremly high, which was expected for nonagglomerated cocoa powder mixtures. Therefore, we suggest improvement of wettability for all of the mixtures by means of agglomeration.

Powders with higher fat content (particularly free fat) are usually more cohesive than powders with lower fat content (*30*). In our study, we used cocoa powders with two different fat contents, 10−12% fat and 16−18% fat. Both powders exhibited similar cohesion indices. However, we must emphasize the difference in particle sizes of these powders (cocoa powder with 10−12% fat, average particle size = 19 μm; cocoa powder with 16−18% fat, average particle size = 30 μm) and, therefore, the inability to determine the effect of fat content on cohesiveness. On the basis of the cohesion index (**Table 3**), all cocoa drink mixtures, as well as both cocoa powders, are classified as hardened/extremely cohesive. A significant influence ($p < 0.05$) on cohesion index was detected in the mixtures of cocoa powder with 10−12% fat and the addition of sucrose, palatinose, maltodextrin, and inulin (mixture G). Maltodextrin and inulin also exhibited a significant influence on the bulk densities of mixtures prepared with cocoa powder containing 10−12% fat, implying that these two filler ingredients affect the physical properties of a powdered mixture, which can be interpreted by the previously stated influence of the particle size and particle shape of a compound. In general, powdered cocoa drink mixtures did not exhibit satisfying physical properties, which implies that an agglomeration process should be applied after the mixing step to achieve the required flowability and reconstitutional properties.

According to previous studies on various cocoa products, the content of polyphenols depends on cocoa solids content of a cocoa-based product (*16*). Because cocoa powder usually comprises 82−90% nonfat cocoa solids (NFCS), the consumption of cocoa drinks and beverages contributes to the daily intake of antioxidants in a diet, especially with regard to polyphenolic compounds. Total phenol content (TPC) of cocoa powders prepared with aqueous extraction (equal to preparation of cocoa drinks) amounted to 977.27 mg of GAE/L for cocoa powder with 10−12% fat and 970.45 mg of GAE/L for cocoa powder with 16−18% fat. As can be seen, cocoa powder with higher fat content exhibits slightly lower TPC. Because the main source of polyphenols in cocoa products is nonfat cocoa solids, whereas cocoa butter represents the hydrophobic, fat phase of cocoa products and does not provide any polyphenolic compounds, the lower content of TPC in the cocoa powder with higher fat content is not surprising. Compared to the results of previously conducted studies on cocoa products, the TPC of cocoa powders used in this study, expressed as milligrams of GAE per gram of cocoa powder, was in accordance with the results of other authors. In a study by Lee et al. (*15*) cocoa drink prepared according to the same procedure as in our study contained 611 mg of GAE of total phenolics per serving, which is considerably lower than the results obtained in this research. Waterhouse et al. (*31*) determined 20 mg of GAE/g of cocoa powder using 95% aqueous methanol as the extraction solvent, whereas Serra Bonvehi and Ventura Coll (*32*) estimated TPC of 58 mg of GAE/g of cocoa powder using 70% methanol for the extraction. In our study, the TPC of cocoa powders amounted to 26.77 mg of GAE/g of cocoa powder with 10−12% fat and 26.59 mg of GAE/g of cocoa powder with 16−18% fat.

The content of total phenols in cocoa drink mixtures (**Figure 1**) ranged from 320.45 mg of GAE/L in sample $E_{10-12\%\ fat}$ to 480.45 mg of GAE/L in sample $I_{10-12\%\ fat}$, which is about one-third of TPC of cocoa powder, as could be expected from the experimental formulations of cocoa drink mixtures. A significant difference ($p > 0.05$) of TPC between the prepared cocoa drink mixtures was observed with regard to the fat content of cocoa powder. According to the results, the mixtures prepared with cocoa powder containing lower fat content (10−12%) exhibit higher TPC than the mixtures prepared with cocoa powder containing higher fat content (16−18%). Higher fat content accounts for higher hydrophobicity of cocoa powder and lower solubility and dispersibility (**Table 2**), which consequently results in lower extraction efficiency of phenolic compounds.

The highest TPC, as well as total flavonoids content (TFC), was determined again in sample $I_{10-12\%\ fat}$, which is a complex system consisting of seven compounds: cocoa powder, several sweeteners (isomaltulose, erythritol, stevia), and fillers (maltodextrin, inulin, oligofructose). Again, a statistically significant difference ($p < 0.05$) between the TFC of mixtures prepared with different fat contents

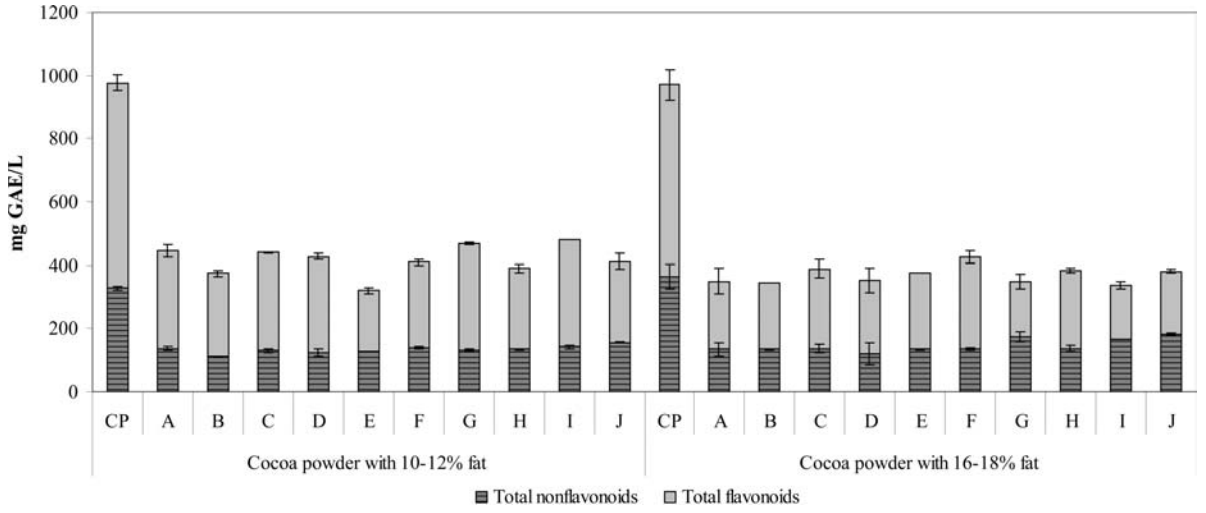

Figure 1. Total phenol (TPC) and flavonoid contents (TFC) of cocoa powders (CP) and cocoa drink mixtures. Results are expressed as mg of GAE/L ± SD.

of cocoa powder was obtained. According to these results, the extraction efficiency of polyphenols in a system consisting of cocoa powder mixture dispersed in water is dependent not only on their physical properties but also on the fat content of the dispersed phase as well as on the interactions between both.

On the basis of the obtained results, the presence of NFCS can be used as a marker to predict the TPC, which was also observed by previous studies (*33*, *34*). Thus, the higher amount of NFCS indicates the higher phenolic content of a cocoa product.

The predominant polyphenolic compounds of cocoa and cocoa-derived products are flavonoids, with three classes being characteristic, especially flavan-3-ols (37%), anthocyanins (4%), and proanthocyanidins (58%), all of which possess high antioxidant potential and exhibit positive health effects, both in vitro and in vivo. The flavan-3-ols are present in monomeric form, more specifically (−)-epicatechin, and in oligomeric and polymeric forms, named procyanidins. Proanthocyanidins are polymers of flavan-3-ols linked through an interflavan carbon bond that is not susceptible to hydrolysis (*35*). To provide a better insight in the content of these beneficial constituents of cocoa drink mixtures, three simple and rapid assays were applied for the determination of the content of flavan-3-ols and proanthocyanidins. The results displayed in **Table 4** show the content of flavan-3-ols determined by vanillin and *p*-dimethylaminocinnamaldehyde assays. The vanillin assay is specific for flavan-3-ols, dihydrochalcones, and proanthocyanidins, which have a single bond at the 2,3-position and possess free metahydroxy groups on the B-ring (*36*). Flavan-3-ol content determined by vanillin assay ranged from 98.87 mg of CE/L in mixture $I_{16-18\%\ fat}$ to 149.76 in mixture $G_{10-12\%\ fat}$, whereas the content of flavan-3-ols determined by *p*-dimethylaminocinnamaldehyde assay varied between 39.96 mg of CE/L in mixture $D_{16-18\%\ fat}$ to 73.67 mg of CE/L in mixture $G_{10-12\%\ fat}$. As can be seen, the results obtained by the vanillin assay are higher in relation to the results obtained by the *p*-dimethylaminocinnamaldehyde assay, due to the structural diversity of flavan-3-ols in the sample (**Table 4**). The vanillin assay is based on a reaction between vanillin and hydroxyl side groups at the C-6 and C-8 positions in the flavan-3-ol molecules, whereas *p*-dimethylaminocinnamaldehyde reagent is able to react only with the hydroxyl group at the C-6 position in the benzene ring. According to the results, mixture G consisting of cocoa powder, three sweeteners (isomaltulose, erythritol, and aspartame/acesulfame K sweetener blend), and fillers (maltodextrin and inulin) exhibited the highest flavan-3-ol content.

Table 4. Content of Flavan-3-ols and Procyanidins in Experimental Cocoa Drink Mixtures[a]

	flavan-3-ol content (mg of CE/L)		
	vanillin index	*p*-dimethylaminocinnamaldehyde assay	procyanidin content (mg of CyE/L)
Cocoa Powder (CP) with 10−12% Fat			
CP	231.33 ± 4.32	120.86 ± 0.68	157.32 ± 10.16
A	111.96 ± 6.17	50.56 ± 0.23	60.99 ± 4.98
B	114.72 ± 22.41	51.84 ± 0.68	59.15 ± 0.40
C	135.22 ± 2.06	51.68 ± 0.91	59.44 ± 6.77
D	116.47 ± 0.62	42.53 ± 0.23	56.76 ± 4.18
E	147.87 ± 0.21	44.78 ± 0.23	54.23 ± 1.39
F	131.88 ± 0.21	56.01 ± 1.13	58.03 ± 8.37
G	149.76 ± 2.06	73.67 ± 2.04	71.83 ± 3.59
H	105.42 ± 19.95	61.47 ± 0.23	62.25 ± 4.38
I	104.69 ± 4.11	61.63 ± 2.27	73.66 ± 4.98
J	128.82 ± 2.88	57.62 ± 1.13	74.51 ± 2.19
Cocoa Powder with 16−18% Fat			
CP	284.98 ± 6.17	113.31 ± 2.95	93.24 ± 11.15
A	135.22 ± 8.23	44.06 ± 1.25	37.18 ± 2.79
B	128.82 ± 2.88	51.68 ± 1.36	23.94 ± 2.39
C	113.41 ± 4.11	45.26 ± 0.23	36.06 ± 5.58
D	102. 51 ± 1.03	39.96 ± 0.45	22.68 ± 0.60
E	117.77 ± 6.17	49.11 ± 1.82	55.21 ± 1.99
F	123.59 ± 6.17	52.64 ± 0.45	52.96 ± 4.78
G	137.55 ± 2.47	69.82 ± 3.40	53.38 ± 3.39
H	106.58 ± 9.66	66.61 ± 1.59	64.93 ± 4.58
I	98.87 ± 4.11	52.64 ± 7.72	51.97 ± 0.20
J	104. 69 ± 12.34	50.24 ± 3.40	57.89 ± 7.37

[a] Results are expressed as mean (n = 3) ± SD.

The content of proanthocyanidins (PAC) in cocoa powders and cocoa drink mixtures was determined by using the quantitative method of Porter et al. (*18*), modified from the method of Bate-Smith (*37*). The butanol−HCl reaction is specific to the proanthocyanidins and the rarely occurring flavan 3,4-diols and has therefore been proposed as a method for estimating the amount of matrix-bound PAC. The proanthocyanidin assay uses an acid-catalyzed oxidative depolymerization of condensed tannins (proanthocyanidins) to yield red anthocyanidins (*18*). The highest PAC was determined in mixture $J_{10-12\%\ fat}$ (74.51 mg of CyE/L), followed by mixture $H_{16-18\%}$ (64.93 mg of CyE/L), whereas the lowest PAC was determined in mixture $B_{16-18\%\ fat}$ (23.94 mg of CyE/L).

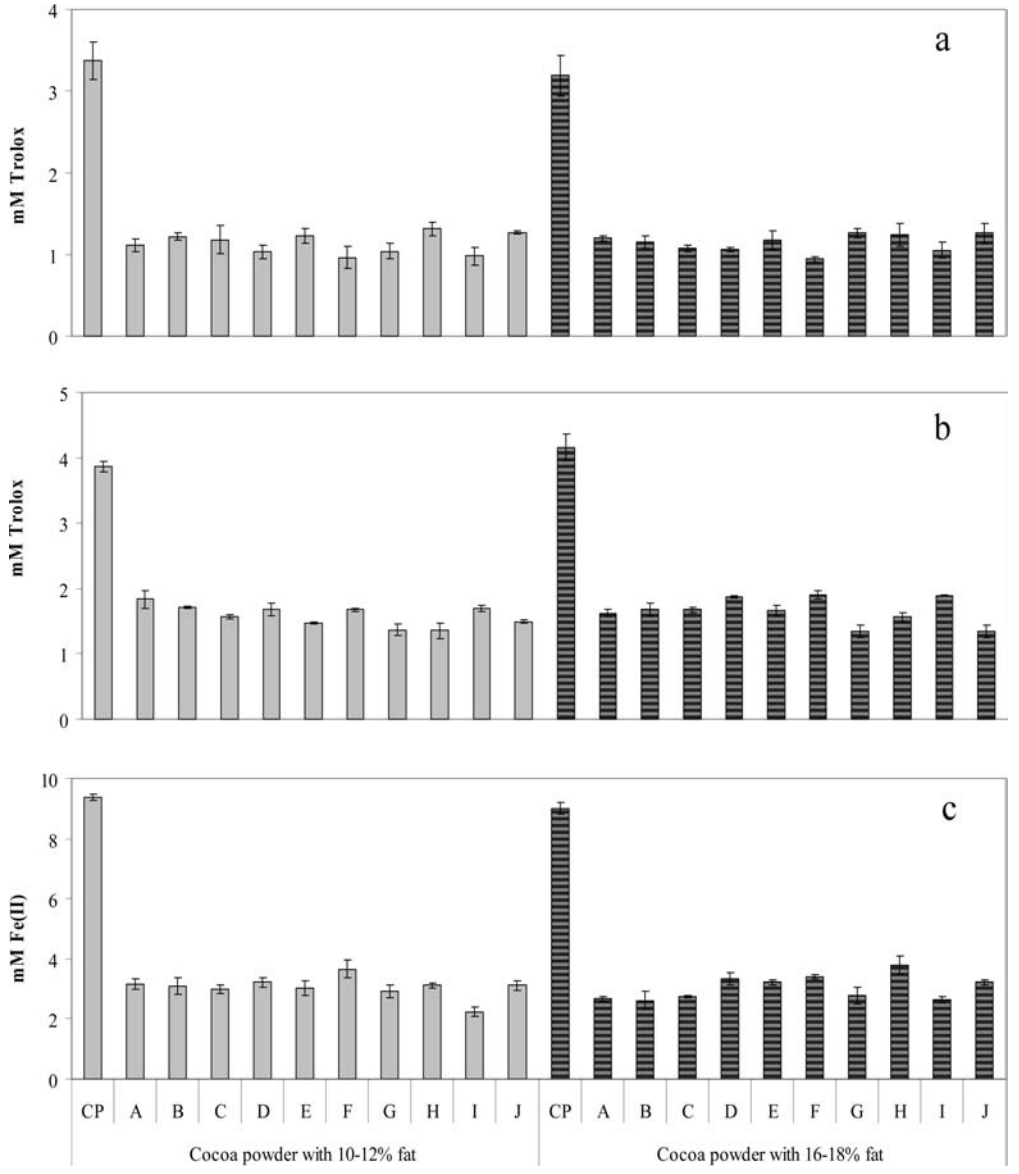

Figure 2. Antioxidant capacity of cocoa powders (CP) and cocoa drink mixtures determined by the DPPH (**a**), ABTS (**b**), and FRAP assays (**c**). Results are expressed as means ($n = 3$) in mM Trolox ± SD for the ABTS and DPPH assays and mM Fe(II) ± SD for the FRAP asay.

On the basis of the results of these assays it can be noted that cocoa powder mixtures deliver a high content of all evaluated polyphenolic compounds, and the type of sweetener does not influence the content of evaluated polyphenolic compounds in the prepared mixtures. Although it was observed that mixtures G, I, and J generally contain the highest content of polyphenolic compounds, which is determined in both mixtures with lower and higher fat content of cocoa powder; the statistical analysis found no significant difference ($p > 0.05$) between the TPC of the tested cocoa drink mixtures. The higher contents of phenolic compounds in some mixtures might be explained by the influence of some physical properties of cocoa drink mixtures on the extraction efficiency of polyphenols or by the fact that some sugars and sweeteners may interfere in some assays, such as the interaction of sugars, ascorbic acid, carotenoids, or amino acids with the Folin−Ciocalteu reagent (*38*, *39*).

The ranking of cocoa drink mixtures based on the strength of their antioxidative properties was determined using the DPPH (**Figure 2a**), ABTS (**Figure 2b**), and FRAP assays (**Figure 2c**). As can be seen in **Figure 2**, the arrangement of the mixtures based on their decreasing antioxidant capacities varies depending on the used assay. The DPPH radical scavenging capacities of pure cocoa powders were 3.37 mM Trolox for cocoa powder with 10−12% fat and 3.19 mM Trolox for cocoa powder with 16−18% fat, whereas the antioxidant capacities of cocoa drink mixtures ranged from 0.94 mM Trolox in mixture $F_{16-18\%\ fat}$ to 1.31 mM Trolox in mixture $H_{10-12\%\ fat}$.

In the case of the ABTS radical scavenging properties, mixture $I_{16-18\%\ fat}$ (1.89 mM Trolox) was the most efficient ABTS radical scavenger, followed by mixture $D_{16-18\%\ fat}$ (1.87 mM Trolox), whereas mixtures $G_{16-18\%\ fat}$ and $J_{16-18\%\ fat}$ exhibited the poorest ABTS radical scavenging capacities (1.34 mM Trolox). As can be seen, the results of ABTS assays indicate that cocoa drink mixtures prepared with cocoa powder containing higher fat content (16−18%) exhibit slightly higher antioxidant capacity than mixtures prepared with cocoa powder containing lower fat content. This might be explained by the fact that the $ABTS^{\bullet+}$ radical reacts with both hydrophilic and lipophilic antioxidants, whereas the DPPH radical reacts only with hydrophilic antioxidant compounds (*40*). This would also explain the higher Trolox values obtained in the ABTS assay with regard to the DPPH assay.

The reducing capacity of pure cocoa powders determined with the FRAP assay was 9.38 mM Fe(II) for cocoa powder with 10−12% fat and 9.01 mM for cocoa powder with 16−18% fat, whe reas the FRAP values of experimental mixtures ranged from 2.23 mM Fe(II) in mixture $I_{10-12\%\ fat}$ to 3.79 mM Fe(II) in mixture $H_{16-18\%\ fat}$. Although ranking of the antioxidant capacities of experimental mixtures differed in all three assays, high correlations were observed between the results ($r_{ABTS/DPPH}$ = 0.926, $r_{ABTS/FRAP}$ = 0.951, and $r_{DPPH/FRAP}$ = 0.969). According to the determined results, the fat content of cocoa powder does not significantly influence ($p > 0.05$) the antioxidant capacity of cocoa drink mixtures, which was determined with all three assays, although according to previous findings (*34*), a positive and significant relationship between NFCS and antioxidant properties of cocoa products was determined. Therefore, the antioxidant capacity of sugars was also determined to check if the applied sugars contribute to the antioxidant capacity of cocoa drink mixtures. The results displayed no or very low antioxidant capacity of sugar solutions (1 g/L), which confirmed the previous assumption.

The arrangement of the antioxidant capacities of the experimental mixtures was somewhat different from that observed for total phenols and individual classes of polyphenols. The differences may be attributed to the presence of other compounds in the cocoa drink mixtures, which interacted in the assay, such as proteins, minerals, and even methylxanthines (*41*−*45*). It is well-known that polyphenols have a strong inclination to form complexes with proteins, polysaccharides, and alkaloids, in both unoxidized and oxidized forms (*46*). However, a high correlation was determined between the antioxidant properties and TPC of cocoa drink mixtures ($r_{TPC/DPPH}$ = 0.937, $r_{TPC/ABTS}$ = 0.941, and $r_{TPC/FRAP}$ = 0.952).

According to the experimental formulations, the phenolic content and antioxidant capacities of cocoa drink mixtures should exhibit approximately one-third of the antioxidant capacity of pure cocoa, because they contain 30% of cocoa powder. However, the results of all three antioxidant capacity assays vary from the expected values. Namely, it can be observed that the antioxidant capacity of mixtures determined with the ABTS assay are significantly higher than the expected one-third, which might be attributed to the previously mentioned fact that the $ABTS^{\bullet+}$ radical reacts with both hydrophilic and lipophilic antioxidants (*40*). Also, the variations observed in the FRAP assay might be due to the fact that in the presence of other metal chelators in food extract, Fe^{3+} could bind and form complexes that also react with antioxidants (*47*).

Table 5 reveals the sensory evaluation of the experimental cocoa drinks. Because commercial powdered cocoa drink mixtures are usually prepared with cocoa powder with reduced weight (10−12% fat) and sucrose and/or glucose syrup as the sweeteners, mixture $A_{10-12\%}$ prepared in our study served as the control sample. In general, the cocoa mixtures exhibited panelist acceptances closer to the points 3 "dislike moderately" and 6 "like slightly", for all of the evaluated sensory attributes, whereas mixture $B_{10-12\%\ fat}$ exhibited a high score of 7 "like moderately" for cocoa odor on a hedonic scale of 9 points. There were no significant differences ($p > 0.05$) in the evaluated sensory attributes between the cocoa drink mixtures prepared with cocoa powder containing 10−12% fat and cocoa drink mixtures prepared with cocoa powder containing 16−18% fat, which implies that the cocoa powder fat content did not influence the sensory perception of the consumers or that the differences observed by the descriptive analysis panel were insufficient to affect the preferences of this consumer panel. As one can see from **Table 5**, for all evaluated sensory attributes significant differences ($p < 0.05$) were observed between individual cocoa drink mixtures prepared under laboratory conditions. The sensory acceptance of the control sample varied significantly ($p < 0.05$) from the experimental cocoa drink mixtures, and most of the experimental drinks were preferred over the control sample in terms of all evaluated sensory attributes. With regard to mouthfeel and sweeteness as well as balanced attribute, the mixtures prepared with sweeteners (aspartame/acesulfame K and stevia extract) were preferred compared to control sample and the mixtures with sugars (sucrose, glucose, trehalose). According to the results, mixture G containing aspartame/acesulfame K sweetener was scored highest (average = 6.04 points) in terms of sweetness, whereas mixture C exhibited the lowest sweetness with an average of only 2.74 points achieved in the sensory evaluation. Also, the bitterness of mixture D differed (disadvantageously) from the others. The results are not surprising considering the difference in the relative sweeteness of the used compounds. However, mixtures containing stevia extract and fillers, especially mixture J, exhibited the most balanced attributes with an average 6.14 score determined by the panel. On the basis of these results, it can be seen that panelists preferred the drinks made with stevia sweetener, because it tasted well, was moderately sweet, and provided a well-balanced flavor and taste, which indicates a great potential of using this sweetener.

Table 5. Sensory Attributes of Cocoa Drink Mixtures[a]

	cocoa odor	mouthfeel	bitter	sweet	balanced
			Cocoa Powder with 10−12% Fat		
A	5.80 ± 0.64	4.20 ± 0.28	3.20 ± 0.64	4.07 ± 0.87	4.60 ± 0.14
B	7.13 ± 0.41	3.93 ± 0.18	3.00 ± 0.22	3.00 ± 0.58	3.73 ± 0.48
C	5.27 ± 0.31	3.73 ± 0.14	3.13 ± 0.20	2.87 ± 0.84	3.27 ± 0.14
D	5.13 ± 0.10	3.80 ± 0.45	2.80 ± 0.32	3.20 ± 0.30	4.00 ± 1.00
E	5.33 ± 0.55	3.87 ± 0.30	2.93 ± 0.17	3.13 ± 0.45	3.53 ± 0.14
F	5.20 ± 0.48	5.20 ± 0.52	3.60 ± 0.51	4.47 ± 0.52	5.40 ± 0.19
G	4.27 ± 0.29	5.13 ± 0.05	4.53 ± 0.79	6.07 ± 0.63	4.87 ± 0.64
H	5.67 ± 0.52	5.40 ± 0.38	4.00 ± 0.21	5.53 ± 0.82	5.07 ± 0.12
I	6.07 ± 0.22	6.07 ± 0.48	4.73 ± 0.30	5.47 ± 0.07	5.47 ± 0.34
J	5.27 ± 0.52	5.33 ± 0.07	4.53 ± 0.19	5.60 ± 0.95	6.00 ± 0.12
			Cocoa Powder with 16−18% Fat		
A	4.93 ± 0.22	4.40 ± 0.30	3.67 ± 0.70	4.07 ± 0.35	4.67 ± 0.35
B	5.87 ± 0.10	4.73 ± 0.48	3.13 ± 0.95	3.27 ± 0.30	4.60 ± 0.52
C	5.13 ± 0.84	3.13 ± 0.45	3.20 ± 0.30	2.60 ± 0.55	3.87 ± 0.48
D	4.80 ± 0.10	4.67 ± 0.89	2.53 ± 0.95	4.27 ± 0.10	4.73 ± 0.64
E	5.33 ± 0.55	3.93 ± 0.58	2.60 ± 0.52	3.33 ± 0.52	4.20 ± 0.79
F	5.40 ± 0.14	5.07 ± 0.58	4.87 ± 0.17	4.67 ± 0.67	4.47 ± 0.82
G	4.73 ± 0.30	5.27 ± 0.92	4.67 ± 0.30	6.00 ± 0.92	5.07 ± 0.23
H	5.73 ± 0.48	5.40 ± 0.89	4.13 ± 0.95	5.53 ± 0.82	5.13 ± 0.79
I	4.93 ± 0.41	6.07 ± 0.58	4.73 ± 0.79	5.13 ± 0.49	5.47 ± 0.30
J	4.67 ± 0.14	5.53 ± 0.67	4.60 ± 0.05	5.27 ± 0.64	6.27 ± 0.05

[a] Results are mean of scores of 15 panelists ± SD.

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