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FULL ARTICLE

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Clinical application of a cocoa and unripe banana flour beverage for overweight women with abdominal obesity: Prospective, double-blinded and randomized clinical trial

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Abstract

Objective

To assess the effect of a cocoa and unripe banana flour beverage (UBF) on fecal short-chain fatty acids (SCFA), gastrointestinal symptoms, fecal characteristics and inflammation, in overweight women with abdominal adiposity.

Methods

This prospective, double-blinded, randomized clinical trial involved 60 female volunteers aged between 20 and 50 years. One group received a cocoa beverage ($n = 30$) and one group received a cocoa and UBF beverage ($n = 30$), for 6 weeks. Intestinal microbiota was indirectly assessed by consistency, shape, and color of feces, determination of fecal SCFA, and gastrointestinal symptoms.

Results

Both beverages increased the production of propionic acid ($p < .05$) and decreased gastrointestinal symptoms ($p < .05$). The cocoa beverage decreased indigestion ($p < .05$) and the pro-inflammatory cytokine, IL-17.

Conclusion

Cocoa and cocoa with UBF beverages decreased the symptoms of dyspepsia, improved gastrointestinal symptoms, and increased production of propionic acid, favoring healthy intestinal microbiota. Only the cocoa beverage showed an anti-inflammatory effect.

Practical applications

Unripe banana flour and cocoa have been widely used to decrease cardiovascular risk, by improving inflammatory parameters and gastrointestinal symptoms. However, the interaction between these two food ingredients and the implication of their interaction on human health remains unknown. Important health benefits may be achieved by assessing the synergism or antagonism of functional foods, particularly when they coexist in the same product. This study aims to attract the interest of the scientific community to conduct more studies on functional bioavailability. Both unripe banana flour and cocoa can have therapeutic potential but it may not be a good idea to associate them. This article provides relevant information to the scientific and broader community regarding the preparation of these foods, to maximize their health benefits on a daily basis, and, additionally, offers the food industry valuable knowledge that can be used to develop healthier food products.

KEYWORDS

flavonoids, gastrointestinal symptoms, interleukin, short-chain fatty acid, starch resistance

1 | INTRODUCTION

Currently there is growing interest in studies on intestinal microflora due to its action in the body, which occurs through a variety of mechanisms, such as activation of the immune response, the synthesis of bacteriocins, production of short-chain fatty acids (SCFA), nutritional and physical competition with pathogens as well as maintaining an acidic environment (Liu et al., 2014; Montalto, Gallo, & Gasbarrini, 2009).

Evaluation of the microbiota should be considered in the clinical evaluation to be expressed indirectly by the composition of SCFA, color, shape and consistency of stools (Sierra et al., 2015). Food is one of the main factors that cause alteration of intestinal microbiota, as well as in the production of SCFA and cytokines (Teixeira, Collado, Ferreira, Bressan, & Peluzio, 2012). Tzounis et al. (2011) suggest that consumption of foods rich in flavonoids and fibers can restore the intestinal balance with increased growth of beneficial bacteria and inhibition of pathogens.

Among the foods that have high flavonoid and fiber content, cocoa and unripe banana flour (UBF) are considered good sources of these compounds (Albertini et al., 2015;. Fernández-Millán et al., 2015; Lamport et al., 2015).

Cocoa is one of the main sources of polyphenols (Albertini et al., 2015; Fernández-Millán et al., 2015; Sarriá et al., 2015). Phenolic compounds present in cocoa act on the intestinal microbiota, anti-inflammatory action, antioxidant, and reduction of cardiovascular disease in women (Dower et al., 2015; Sansone et al., 2016; Yoon et al., 2016.). And the UBF is considered a good source of resistant starch. Ramos, Leonel, and Leonel (2009) found values of up to 40%. Resistant starch, when fermented by colonic microflora, has also shown benefits in women, both systemic and local (Behall, Scholfield, Hallfrisch, & Liljeberg-Elmståhl, 2006; Silva et al., 2014), and increases fecal bulk and production of SCFA (Cummings, Beatty, Kingman, Bingham & Englyst, 1996; Jekins et al., 1998; Santos, 2010).

Despite the fact that previous studies have shown the beneficial effects of cocoa and UBF, there are no studies that have examined this interaction. Thus, the use of combinations of natural products to enhance the effect of both, or uncover possible synergistic or antagonistic effects can be an interesting approach. The combination of active biomolecules can also be a tool to prevent chronic no communicable diseases (Giménez-Bastida, Zielinski, Piskula, Zielinska, & Szawara-Nowak, in press), such as obesity (Zhang et al., 2015).

The incidence of obesity is increasing in all countries, the prevalence of overweight and obesity is higher in regions of the Americas, with 61% overweight or obese in both sexes, and women are more likely to be obese than men (Balakumara, Maung-Ub, & Jagadeesh, 2016).

The objective of the study was to evaluate the effect of two beverages—the first cocoa and the second based on cocoa and UBF—indirectly on the intestinal microbiota and the levels of inflammatory cytokines in women with overweight and abdominal adiposity. The study also addresses relevant issues concerning the interaction of functional foods.

2 | METHODS

2.1 | Participants

The study population comprised 60 healthy female volunteers, aged between 20 and 50 years, with a body mass index (BMI) from 25 to 35 kg/m^2 and waist circumference greater than or equal to 80 cm (Xavier et al., 2013). Exclusion criteria were women who regularly take anti-inflammatory drugs, or in the last 6 months took dietary supplements, laxatives, and/or antibiotics. Other ineligibility criteria were participation in a weight loss program in the last 6 months, medications or herbal supplements used for weight loss in the last 6 months (diuretics, anti-obesity, antidepressive, glucocorticoids), or allergies to any of the beverage components.

The formula used to calculate the sample was according to Santo and Daniel (2015) and Hulley, Cummings, Browner, Grady and Newman (2008), the gastrointestinal symptom rating scale (GSRS) total score was the clinically important effect for the calculation, thus considering a standard deviation of 2.7 score and difference between the groups of 2.19 score. Thus, we determined 30 volunteers per group guaranteeing 80% power and alpha of 0.05 level to obtain meaningful result, and to allow for a possible 15% attrition from the study. Sample size was determined based on time, cost, and the ability to detect clinically important effect (Kalman et al., 2009).

The ethical committee of the Federal University of Alfenas, under process number 778,562, approved this research. All volunteers read and understood the objectives of the research, and were recruited only after reading and signing the consent form.

2.2 | Study design

This is a prospective, double-blinded randomized clinical trial, neither the volunteers nor those responsible for assistance and evaluation know which treatment each person was receiving. The participants were distributed into two groups, matched for age and BMI. The first group (cocoa group) received a functional cocoa beverage and the second group received a functional cocoa and UBF beverage (cocoa and UBF group). The experiment lasted 6 weeks and was designed according to Figure 1.

2.3 | Clinical trial products

Daily servings of powder were given to the volunteers to make at home, preparing the drink with cocoa (39.1 g) and cocoa and UBF (54.1 g). The drinks were homogenized in a blender with 150 mL of water. Each package of the product was delivered to volunteers with instructions for preparation and storage. The portions were based on phenolic content and resistant starch respectively present in cocoa (3 g/portion) and UBF (30 g/portion).

The drinks were made from whole milk powder, sucralose, xanthan gum, cocoa powder (Armazém São Vitto Comércio de Produtos Alimentícios, São Paulo, SP, Brazil) and UBF (Relva Verde Produtos Naturais, Londrina, PR, Brazil). Both drinks had the same ingredients, except UBF had excluded from the cocoa drink. The cocoa drink contained

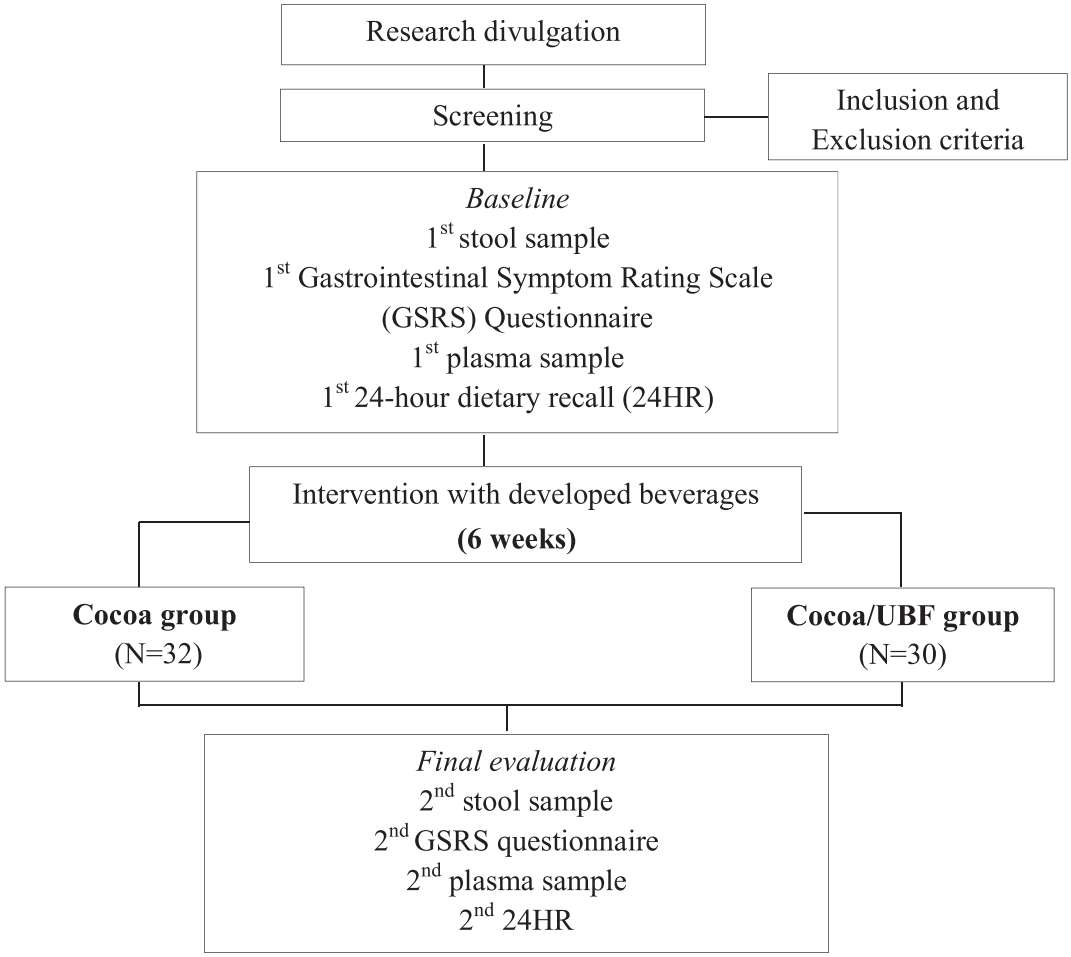

FIGURE 1 Experimental design

3.07 total g fiber/serving, 29.55 mg of gallic acid equivalents (GAE)/portion (GAE/portion) and 184.37 kcal/portion. Cocoa and UBF beverage contained 8.48% resistant starch, 4.37 g fiber/full portion, to 69.24 mg GAE/portion and 212.73 kcal/portion (Table 1).

The UBF and powder for preparation of drinks were evaluated for chemical composition according to the methodologies described by the Association of Official Analytical Chemistis (AOAC, 2000). The determination of the content of resistant starch product was conducted by direct method AOAC (2002), by means of a commercial Resistant Starch Assay Kit (Megazyme K-Rstar 08/11).

The beverages were produced according to the Brazilian food safety management system. The UBF and whole milk powder were weighed using an electronic table scale. Cocoa powder, sucralose, and xanthan gum were weighed on an semi-analytical scale at 0.001 g precision (Marte, model AL 500C).

The volunteers attended the weekly Nutritional Assessment Laboratory of the Federal University of Alfenas to collect the drinks that would be used the following week, repeating this procedure during the 6-week study. This moment was opportune to verify adherence to product use and to clarify any doubts and difficulties.

Each week the volunteers receved seven sachets with the individual portions of the beverages. The remaining leftovers were returned to the study site and counted at the end of each week. It was assumed that all non-returned portions had been taken. So the volunteers consumed a serving once a day for 6 weeks (42 servings). Compliance was recorded as missed doses of a total of 42 servings.

TABLE 1 Centesimal composition of the powder for the preparation of the beverages developed

Variables	Beverage with cocoa	Cocoa with UBF
Energetic value (kcal/100)	467.96 ± 0.96	393.23 ± 1.88
Total carbohydrates (g/100)	28.54 ± 0.95	57.71 ± 0.40
Proteins (g/100)	20.79 ± 0.82	10.15 ± 0.15
Total lipids (g/100)	30.07 ± 0.25	13.49 ± 0.04
Total fibers (g/100)	7.80 ± 0.69	8.08 ± 0.16
Insoluble fibers (g/100)	4.7 ± 0.91	5.64 ± 0.19
Soluble fibers (g/100)	3.18 ± 1.60	2.44 ± 0.34
Ashes (g/100)	5.49 ± 0.13	3.43 ± 0.03
Humidity (g/100)	7.21 ± 0.16	7.27 ± 0.20

2.4 | Stool characterization

2.4.1 | Stool sample

Participants were instructed to collect 10 g of fresh stool in a sterilized sealed container. Stool samples were collected on two occasions, including the baseline (week 0) and last day of the intervention (week 6). Samples were collected at the study site and immediately stored at −80°C.

2.4.2 | Stool consistency

Stool consistency was evaluated as described by Canani et al. (2007), using a grading scale of 1 (normal), 2 (soft), 3 (semi-liquid), and 4 (liquid). In addition, the Bristol Stool Scale for the consistency of feces, validated for use in Brazil (Martinez & Azevedo, 2012) was used as an alternative method of stool classification. Use of the Bristol Stool Scale can offer a visual and numeric reference, and provide researchers with a rough estimate of stool consistency. Low scale numbers (1, 2) mean a harder stool, whereas medium values (3, 4) support a normal stool consistency and higher values (5, 6, 7) liquid consistency. Stool consistency was measured on two occasions, including the baseline (week 0) and last day of the intervention (week 6).

2.4.3 | Stool color

The color, evaluated by study researchers, was determined by macroscopic observation and classified as dark brown, light brown, yellow, bleached, black, red, or greenish (Silveira Júnior, 1988).

2.4.4 | SCFA analysis

Stool samples were stored at −80°C until analysis. High-performance liquid chromatography (HPLC) analysis was performed using a Shimadzu HPLC equipped with a C_{18} (30 × 7.9 mm) column by ultraviolet detection at a fixed wavelength of 210 nm, and a flow rate of 0.6 mL/min at 32°C. Ultrapure water and ultrafiltered 1% phosphoric acid were used to prepare the mobile phase. Utilized patterns were acetic acid, propionic acid, and butyric acid (Sigma) (Smiricky-Tjardes, Grieshop, Flickinger, Bauer, & Fahey, 2003).

2.4.5 | GSRS questionnaire

A questionnaire based on a GSRS, with 15 questions about gastrointestinal symptoms, was used. The Likert scale, with a grading range from 0 (absence of symptoms) to 3 (high rate of symptoms) was also used. The scales could be assigned to the symptoms, such as abdominal pain, reflux, diarrhea, constipation, and indigestion (Svedlund, Sjodin, & Dotevall, 1988). The questionnaire was used at weeks 0 and 6.

2.5 | Interleukins analysis

Blood samples, collected using heparin vacuum tubes, were taken from each volunteer and a 1 mL aliquot of the plasma was stored at −80°C until analysis. The plasma was obtained by centrifugation (Sigma 3K15 refrigerated centrifuge, Germany) at 3000 rpm, 4°C, for 15 min. The samples were performed in triplicate.

The plasma cytokines (IL-2, IL-4, IL-6, IL-10, and IL-17) and tumoral necrosis factor alfa (TNF-α) were quantified by cytometric beads array (BD Biosciences, CA, USA). All tubes were homogenized and incubated at room temperature for 3 hr, in the dark and analyzed in the FL3 channel of an FACScalibur flow cytometer (BD Biosciences) using CellQuest software (BD Biosciences).

2.6 | Anthropometric evaluation

Anthropometric measurements were taken in accordance with Duarte (2007). Body weight was assessed by scale portable electronic digital calibrated by Inmetro, Kratos (maximum capacity of 150 kg, minimum of 1.25 kg and precision of 50 g). Height was measured using a portable stadiometer Alturexata (maximum range of 213 cm and precision of 1 mm). The body waist circumference was obtained using an inelastic and inextensible tape measuring 1 mm for comparison of absolute values.

The cutoff points recommended by WHO were used (2000) for classification of nutritional status according to BMI.

2.7 | Food consumption analysis

A food consumption survey was used for 24-hr dietary recall (24-HR) (Gibson, 1990). Volunteers filled in the 24-HR form 3 days before the intervention began and 3 days during the last week of the intervention. The food consumption analysis was designed to verify whether the participants had changed their diet after the intervention.

The analysis of the nutritional composition of the diet of each participant was performed using Software Avanutri® Revolution, version 4.0. The diet of the volunteers was the standard Brazilian diet, consisting of breads, cereals, legumes, fruits, vegetables, coffee, and milk. None of the participants drank tea.

2.8 | Statistical analysis

The data were analyzed by SPSS 19.0 software. Stool consistency and color was analyzed by Fisher's exact test. The Shapiro–Wilk normality test was used for the SCFA, GSRS variables, cytokines data, profile of participants, anthropometric data and analysis of food consumption. The paired *t* test was used to analyze the SCFAs and anthropometric data. The Student's t-test was used to compare the effect of the treatments. Analysis of the GSRS questionnaire, cytokines and analysis of food consumption used the Wilcoxon test for nonparametrical variables and the Mann−Whitney test to compare the effect of the treatments. A significance level of $p < .05$ was used.

3 | RESULTS

Among the 60 volunteers that started the experiment, 54 answered the GSRS questionnaire. During the stool characterization and SCFA determination phase, samples were obtained from 22 volunteers, while the cytokines analysis phase had 25 volunteers (Figure 2). Table 2 provides the profiles of the volunteers that participated in the entire study, the characteristics of the groups in the steps that occurred withdrawal remained identical to that.

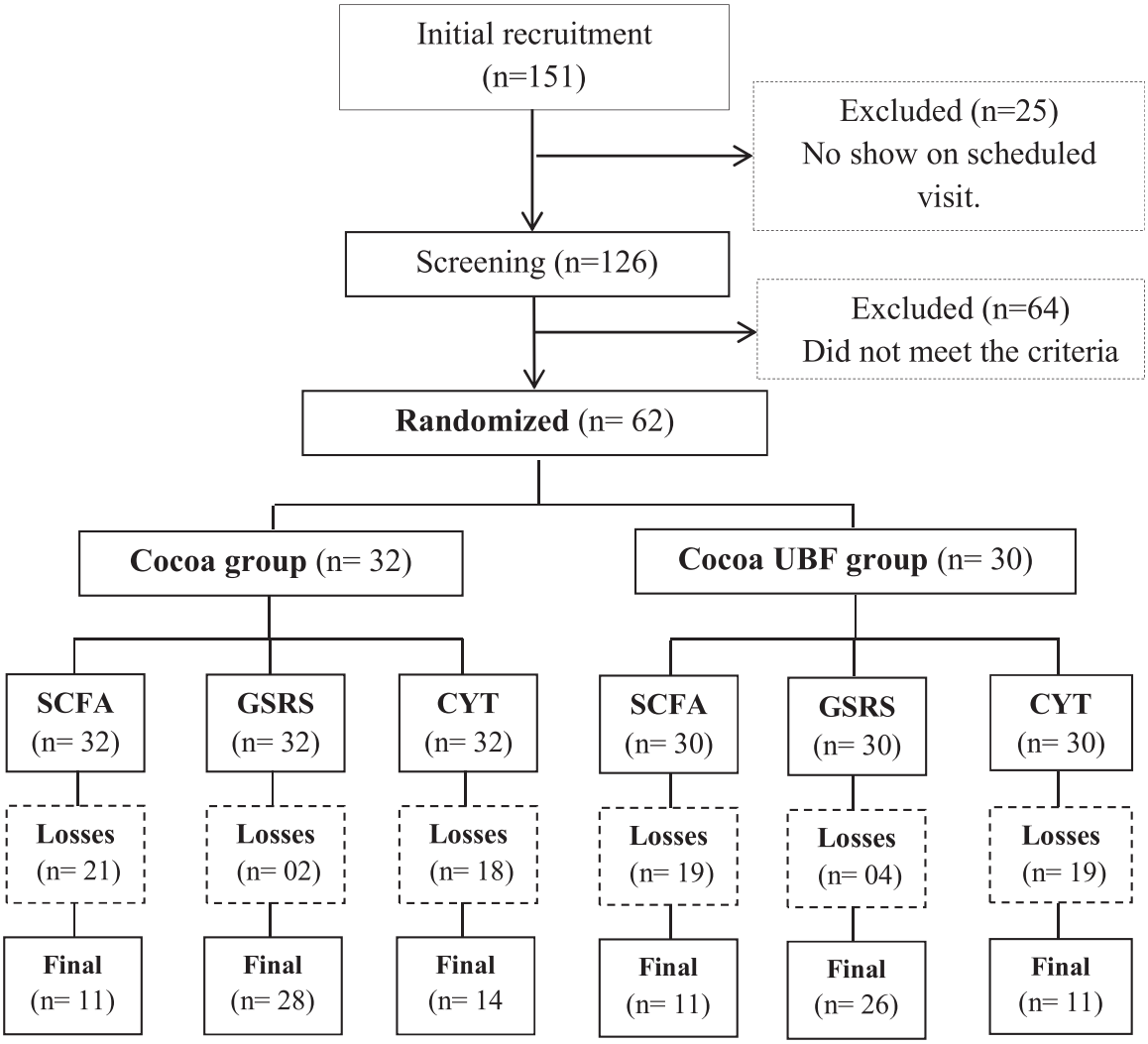

FIGURE 2 Study consort flow diagram (*n*: volunteers number; SCFA: short chain fatty acids analysis; GSRS: Applied gastrointestinal Symptom Rating Scale questionnaire; CYT: cytokines analysis)

Stool consistency, shape, and color were not significantly different ($p > .05$) between the two groups (Table 3).

Regarding fecal SCFAs, there was an increase in propionic acid ($p < .05$) in both groups (Table 4).

Both groups (cocoa and cocoa and UBF) had a significant improvement in dyspeptic syndrome, increase in stool frequency, decrease in gastrointestinal total score, and increase in IL-2 ($p < .05$) (Tables 5 and 6).

The cocoa group also had a significant improvement in indigestion and IL-17 reduction ($p = .004$).

The weight and BMI of the volunteers showed no statistical difference in either group. Before intervention the weight of the cocoa group was 76.31 ± 11:44 kg and after intervention it was 76.25 ± 11.1 kg ($p = .628$). The weight of the cocoa/UBF group before the intervention was 77.32 ± 13:42 kg and after intervention it was 77.15 ± 12.5 ($p = .584$). There was no statistical difference between treatments ($p = .584$).

Before and after the intervention there was no statistical difference in the levels of marconutrientes, fiber and micronutrients in both groups (Table 7).

4 | DISCUSSION

This study focused on the potential benefit of cocoa and UBF in overweight and abdominal adiposity. Beverages with cocoa and cocoa with UBF promoted increased propionic acid, reduced the symptoms of dyspeptic syndrome, gastrointestinal symptoms improved, favoring healthy intestinal microflora. The effect was also observed in the increase in IL-2 levels.

However, only the drink with cocoa caused a significant reduction in indigestion and intestinal dysfunction and also reduced IL-17, factors that were not observed in the beverage with UBF. When observing the effect of the treatments, it was observed that only the cocoa group showed a significant reduction in indigestion. Thus, there was no potentiation of the effects with the addition of the UBF.

It is known that many factors can interfere with the effect of the bioactive compounds of foods, such as complex food matrix, the chemical form of each substance, the structure and amount of other compounds present in the diet, the rate of gastric emptying and individual metabolism (Cifuentes-Gomez, Rodriguez-Mateos, Gonzalez-Salvador,

TABLE 2 Baseline of the volunteers who drank the drinks until the sixth week

	Cocoa group ($n = 28$)	Cocoa/UBF group ($n = 26$)	p^a
Age (years)	32.7 ± 8.4	37.7 ± 8.57	**.018**
Weight (kg)	76.31 ± 11.44	77.32 ± 13.42	.678
BMI (kg/m^2)	29.22 ± 3.73	29.78 ± 5.04	.897
Waist circumference (cm)	92.88 ± 8.92	91.12 ± 10.0	.572
Bowel habits (%)			
1–3x/day	71.42	76.92	
3–4x/week	0	11.53	.456
1–2x/week	28.57	11.53	
Consistency of the feces (%)			
Consistency normal	71.42	73.07	
Consistency pasty	0	11.50	.152
Dry consistency	28.57	15.38	
Water intake (%)			
>2 L/day	7.14	15.38	
2–1.5 L/day	25.00	15.38	.401
1.5–1 L/day	42.85	34.61	
< 1 L/day	24.99	34.61	
Physical activity (%)			
Daily rate	3.57	3.84	
1–3x/week	21.42	15.38	1.000
4x or more/week	7.14	7.69	
None	67.85	73.07	

Note. [a]Results obtained using the Wilcoxon test or Fisher's exact test ($p < .05$).

Alañon, & Spencer, 2015; Oliveira & Bastos, 2011). The cocoa drink in which UBF was added showed a higher fiber content, which may have reduced the gastric emptying rate, the slower access of cocoa flavonoids in the intestine and reduced absorption of epicatechin free. UBF Fiber may also have caused an increase in the formation of conjugated metabolites, which may have contributed to reducing the potential of flavonoids (Vitaglione et al., 2013). Thus, there was potentiation of the anti-inflammatory effects and the intestinal microbiota when the cocoa bioactive compounds were mixed with the UBF in beverage form, indicating a better effect when the product only had cocoa.

The effects on gastrointestinal symptoms showed the drinks had a positive and significant action. The impact of functional foods on health and well-being is an important clinical finding, but often this dimension is ignored. It is noteworthy that gut health and well-being in general are connected through the gut–brain axis (Clarke et al., 2016). This paper presents drinks that could help control these gastrointestinal symptoms. However, it should be noted that the improvement of

TABLE 3 Consistency, color, and shape of the feces before and after intervention

	Cocoa group ($n = 11$)			Cocoa/UBF group ($n = 11$)		
	Before the intervention (%)	After intervention (%)	p^a	Before the intervention (%)	After intervention (%)	p^a
Consistency						
Normal	45.45	72.72		90.9	45.45	
Soft	45.45	27.27	1.000	0	45.45	.091
Semi-liquid	9.09	0		9.09	9.09	
Forms—Types						
1 and 2	0	9.09		0	0	
3 and 4	45.45	81.81	.714	90.90	72.72	.455
5 and 6	54.54	9.09		9.09	27.27	
Color						
Dark brown	81.81	81.81	.655	81.81	63.63	.109
Light brown	18.18	18.18		18.18	18.18	
Yellowish	0	0		0	18.18	

Note. [a]Results obtained using the Fisher's exact test ($p < .05$).
Legend: 1 and 2: harder stool; 3 and 4: normal stool consistency; 5 and 6: liquid consistency.

TABLE 4 Mean and standard deviation of the content of short-chain fatty acids before and after the intervention in overweight women

SCFA	Cocoa group ($n = 11$)			Cocoa/UBF group ($n = 11$)			
	Before the intervention (umol/g feces)	After intervention (umol/g feces)	p^a	Before the intervention (umol/g feces)	After intervention (umol/g feces)	p^a	p^b
Acetic acid	7.34 ± 1.61	6.66 ± 1.37	.179	6.42 ± 1.45	6.76 ± 1.74	.523	.879
propionic acid	5.58 ± 1.40	6.45 ± 1.75	**.017**	5.41 ± 0.97	7.15 ± 2.33	**.012**	.434
Butyric acid	4.44 ± 0.92	4.53 ± 1.10	.739	4.68 ± 0.82	4.88 ± 1.10	.572	.459

[a]Comparison at baseline (before ingestion of the beverage) and after 6 weeks of ingestion of the products in each intervention, results obtained by the paired Student's *t* test ($p < .05$).
[b]Comparison of effect between treatments—using the Student *t* test.

gastrointestinal symptoms observed in both groups may also be associated with a possible placebo effect. Studies have observed the interference of this effect on outcomes (Kalil et al., 2010; Weckx, Hirata, Abreu, Fillizolla, & Silva, 2009). Geers, Helfer, Weiland, and Kosbab (2006) report that there is a correlation between the expectations created by individuals and the placebo effect. Thus, there may have been an expectation bias in the study participants.

The analysis of intestinal microflora was performed indirectly through the GSRS questionnaire, a validated and widely used method for diagnosis of gastrointestinal disorders (Laurikka et al., 2016; Nakamura et al., in press; Takenaka et al., 2016), which is low cost compared to quantifying the population of bacteria.

The consistency of the feces did not change between groups receiving the cocoa drinks and cocoa drinks and green banana flour. However, the volunteers reported that they liked consuming the drinks because they were feeling less constipated and their stools appeared to be less dry, which caused them a sense of well-being. Furthermore, there was an increase in the frequency of soft feces as described by Canani et al. (2007) and the Bristol Scale (Martinez & Azevedo, 2012). The frequency of soft feces may have occurred due to the presence of the fibers in both beverages, because these can change the characteristics of feces through the following mechanism: Selective fermentation and growth of the species Lactobacillus and bifidobacterium and subsequent production of SCFAs can increase the amount of water in fecal mass, resulting in softer faeces, and alter the color of the same (Scholtens, Goossens, & Staiano, 2014).

Regarding color, there was a visible decrease in the frequency of brown color and an increase in yellowing in the feces of the volunteers in the UBF group. Tateyama et al. (2005) conducted a study with constipated pregnant women who used prebiotics and observed an increase in the frequency of yellowish color and softness of the stool.

The increase in IL-2 may be attributed to the presence of functional compounds in beverages. In vitro studies have demonstrated that cocoa polyphenols exert negative regulation on IL-2 activation effect (Ramiro et al., 2005; Sanbongi, Suzuki, & Sakane, 1997). However, there is still inconsistency and contradictory data in in vivo studies (Mathur, Devaraj, Grundy, & Jialal, 2002).

In vitro studies employ pure standards in concentrations that are much higher than those observed in vivo; and the exposure period of the "biological target" to bioactive compounds occurs in the short-term. In in vivo studies there is a possible interaction between the various chemical compounds in the diet. It is noteworthy that these bioactive compounds are highly metabolized before and after absorption (Bastos, Rogero, & Arêas, 2009).

TABLE 5 Gastrointestinal symptoms evaluated before and after the intervention in overweight women

Symptoms	Cocoa group ($n = 28$)						Cocoa/UBF group ($n = 26$)						
	Before the intervention		After intervention				Before the intervention		After intervention				
	Mean ± *SD*	Median (P25; P75)	Mean ± *SD*	Median (P25; P75)	DM	p^a	Mean ± *SD*	Median (P25; P75)	Mean ± *SD*	Median (P25; P75)	DM	p^a	p^b
Dyspeptic syndrome	1.32 ± 1.7	1 (0; 2)	0.32 ± 0.7	0 (0; 0)	− 1.0	**.004**	1.65 ± 1.5	1.5 (0; 2)	0.27 ± 0.6	0 (0; 0)	−1.38	**.000**	.810
Indigestion	0.85 ± 1.1	0 (0; 1)	0.25 ± 0.5	0 (0; 0)	− 0.6	**.027**	1.19 ± 1.6	1 (0; 2)	0.69 ± 0.8	0 (0; 2)	−0.50	.183	**.040**
Intestinal dysfunction	1.35 ± 1.6	1 (0; 2)	0.75 ± 0.9	0 (0; 2)	− 0.6	**.021**	1.19 ± 1.7	1 (0; 1)	0.88 ± 1.1	1 (0; 1)	−0.31	.804	.651
Constipation	0.96 ± 1.3	0 (0; 2)	0.28 ± 0.6	0 (0; 0)	− 0.6	**.002**	0.5 ± 1.5	0 (0; 0)	0.34 ± 0.7	0 (0; 0)	−0.15	.672	.684
Looser stools	0.07 ± 0.2	0 (0; 0)	0.35 ± 0.6	0 (0; 0,5)	0.31	**.038**	0.03 ± 0.1	0 (0; 0)	0.38 ± 0.7	0 (0; 1)	0.35	**.024**	.910
Total Score	3.53 ± 3.1	2,5 (1; 6)	1.28 ± 1.6	0 (0; 2,5)	− 2.2	**.001**	4.03 ± 3.6	4 (0; 5)	1.84 ± 1.8	1,5 (0; 3)	−2.19	**.003**	.195

[a]Comparison at baseline (before ingestion of the beverage) and after 6 weeks of ingestion of the products in each intervention, results obtained by the Wilcoxon signed ranks test ($p < .05$).
[b]Comparison of effect between treatments—using the Mann–Whitney test.

TABLE 6 Levels of cytokines in the blood before and after the intervention

Cytokines (pg/mL)	Cocoa group ($n = 14$)		p^a	Cocoa/UBF group ($n = 11$)		p^a	p^b
	Before the intervention	After intervention		Before the intervention	After intervention		
IL-17	49.71 ± 34.2	22.15 ± 15.0	**.004**	41.81 ± 32.8	27.16 ± 17.85	.110	.826
INF-γ	2.17 ± 3.24	1.26 ± 2.55	.249	2.17 ± 4.35	1.29 ± 3.02	.499	.552
TNF-α	0.57 ± 1.80	1.21 ± 2.40	.176	3.07 ± 5.90	2.61 ± 4.14	.484	.092
IL-10	0.57 ± 2.02	0.61 ± 1.36	.463	0.82 ± 2.00	0.52 ± 1.28	.500	.660
IL-6	2.00 ± 1.67	3.08 ± 1.35	.101	2.13 ± 1.82	2.83 ± 1.35	.286	.709
IL-4	0.42 ± 1.57	0.78 ± 1.45	.144	0.59 ± 2.23	0.74 ± 2.09	.465	.392
IL-2	0.75 ± 1.69	2.29 ± 1.95	**.000**	1.25 ± 3.80	3.16 ± 3.21	**.022**	.079

[a]Comparison at baseline (before ingestion of the beverage) and after 6 weeks of ingestion of the products each intervention, results obtained using the Wilcoxon test ($p < .05$).
[b]Comparison of the effect between treatments—using the Mann–Whitney test.

Ramiro-Puig et al. (2007) also observed, in mice that received cocoa containing 32 mg of flavonoids/g for 3 weeks, an increase of IL-2. Thus the authors noted that there are other immunologically active compounds contained in cocoa, such as methylxanthines and fatty acids, which can contribute to the effects of in vivo cocoa if they differ in vitro effects.

The reduction of IL-17 in overweight and abdominal adiposity indicates the potential of cocoa beverage to reduce inflammation. Cocoa is a source of polyphenols, such as epicatechin, epigallocatechin, catechin, among others, that may have acted on the anti-inflammatory response, as Cai et al. (2015) observed that epigallocatechin-3-gallate, present in green tea, reduced inflammation in rats, and also affect IL-17.

Regarding the weight and food intake of participants, we found no statistically significant differences for any of the variables evaluated, therefore both groups started the research under the same conditions, showing an adequate randomization between them. After the intervention the weight and diet did not change.

Another issue to be raised concerns the age of the participants, since the UBF group averaged older compared to the cocoa group. Lei et al. (2006) recruited 793 women and 1091 men aged 20–40 years and concluded that age influences the body fat mass and other

TABLE 7 Comparison of macro and micronutrients in the diet of the volunteers before and after the intervention study

	Cocoa group ($n = 28$)		p^a	Cocoa/UBF group ($n = 26$)		p^a	p^b
	Before the intervention	After intervention		Before the intervention	After intervention		
Calories (kcal)	1672.7 ± 386.0	1522.1 ± 365.9	.102	1560.2 ± 233.2	1587.0 ± 289.0	.574	.478
Proteins (g)	72.9 ± 20.60	68.18 ± 21.4	.297	65.8 ± 17.2	64.61 ± 2.69	.671	.578
Carbohydrates (g)	217.66 ± 61.42	195.69 ± 65.5	.07	216.7 ± 45.7	205.79 ± 37.88	.329	.430
Lipids (g)	57.18 ± 17.98	51.04 ± 12.65	.175	55.9 ± 13.99	56.15 ± 17.98	.965	.651
Fibers (g)	10.04 ± 4.13	9.68 ± 3.90	.122	11.15 ± 4.63	12.85 ± 3.53	.144	.470
Vitamin A (RE)	733.23 ± 844.0	494.92 ± 189.2	.210	437.06 ± 194.2	443.25 ± 177.2	.886	.321
Vitamin C (mg)	37.24 ± 37.55	26.37 ± 20.89	.180	50.74 ± 54.19	34.39 ± 28.04	.189	.278
Vitamin E (mg)	11.63 ± 4.91	9.11 ± 4.91	.140	12.16 ± 6.89	10.19 ± 5.10	.142	.549
Vitamin B12 (mcg)	4.43 ± 8.12	1.55 ± 0.88	.123	1.87 ± 1.58	3.68 ± 8.43	.344	.267
Vitamin B1 (mg)	1.21 ± 0.47	1.15 ± 0.69	.656	1.07 ± 0.49	1.06 ± 0.37	.937	.511
Vitamin B2 (mg)	1.04 ± 0.50	1.09 ± 0.30	.664	0.79 ± 0.39	1.08 ± 0.39	**.006**	.535
Zinc (mg)	6.43 ± 2.90	6.29 ± 1.95	.842	5.70 ± 2.36	6.16 ± 2.15	.480	.760
Iron (mg)	9.06 ± 2.19	8.11 ± 2.22	.150	9.15 ± 2.71	9.80 ± 4.43	.582	.222
Magnesium (mg)	154.07 ± 61.39	148.58 ± 61.39	.641	121.23 ± 41.24	161.12 ± 24.55	**.000**	.589
Manganese (mg)	1.32 ± 0.55	1.08 ± 0.59	.144	1.45 ± 0.80	1.13 ± 0.38	.139	.870

[a]Comparison at baseline (before ingestion of the beverage) and after 6 weeks of ingestion of the products each intervention, results obtained using the Wilcoxon test ($p < .05$).
[b]Comparison of the effect between treatments—using the Mann–Whitney test.

anthropometric indices. Furthermore, aging influences the whole organism, such as gastric emptying, intestinal absorption and metabolism (Krishna, Stefanovic-Racic, Dedousis, Sipula, & O'Doherty, 2016; Santos, 2011), so age may have influenced the findings.

Some limitations of the study should be considered. The first limitation of the intervention was the impossibility of a placebo group. In addition, a fourth group with only UBF, not including cocoa, would also be useful to decipher its benefits and the issues around removing the possible inhibitory effects of the cocoa mixture.

It should be noted that the sample loss happened because some refused to participate in the collection of blood and many volunteers were unable to make the collection of feces on the last day of the intervention and therefore had to be excluded from the analyses of the characteristics of feces and fecal SCFA. But even after the losses, the groups remained balanced, and the profile of participants, such as BMI, waist circumference, bowel habits, showed no statistical difference between them. The same occurred in other studies without infringing on their results (Patel, Dibley, Mamtani, Badhoniya, & Kulkarni, 2009; Ralph et al., 2013). However, may be significant differences between the treatment groups that were not detected because of the sample size. Thus, the results of the GSRS questionnaire are more reliable, since the sample size provides a 80% chance to obtain a significant result.

5 | CONCLUSION

Both beverages have shown an effect in increasing propionic acid, improvement in gastrointestinal symptoms and increased IL-2. Only the cocoa drink caused an improvement in indigestion and reduction of IL-17. Thus, the UBF did not potentiate the effects of SCFA, gastrointestinal symptoms and cytokine levels in overweight women. Cocoa alone exercised more effects than with the UBF. More studies should be performed to elucidate the interactions between these and other functional compounds. This study is very important in demonstrating that two associated functional foods may not be as effective when used alone.

CONFLICT OF INTEREST

The authors report no conflict of interest.

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