Research Article

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Changes in bioactive compounds present in beef burgers formulated with walnut oil gelled emulsion as a fat substitute during *in vitro* gastrointestinal digestion

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Abstract

BACKGROUND: The partial or total substitution of animal fat by a gelled emulsion elaborated with cocoa bean shell and walnut oil in beef burgers was assessed in terms of the stability of the bioactive compounds (polyphenolic and methylxanthines compounds, and fatty acid profile), bioaccessibility, colon-available indices (CAIs), and lipid oxidation after *in vitro* gastrointestinal digestion (GID).

RESULTS: No free polyphenolic compounds were detected in the soluble fraction after the GID of reformulated beef burgers. Reductions were obtained in the bound fraction with respect to the undigested sample from 47.57 to 53.12% for protocatechuic acid, from 60.26 to 78.01% for catechin, and from 38.37 to 60.95% for epicatechin. The methylxanthine content decreased significantly after GID. The theobromine content fell by between 48.41 and 68.61% and the caffeine content was reduced by between 96.47 and 97.95%. The fatty acid profile of undigested samples was very similar to that of digested samples. In the control burger the predominant fatty acids were oleic acid (453.27 mg g^{-1}) and palmitic acid (242.20 mg g^{-1}), whereas in reformulated burgers a high content of linoleic acid (304.58 and 413.35 mg g^{-1}) and α-linolenic acid (52.44 and 82.35 mg g^{-1}) was found. As expected, both undigested and digested reformulated samples presented a higher degree of oxidation than the control sample.

CONCLUSIONS: The reformulated beef burgers with cocoa bean shells flour and walnut oil were a good source of bioactive compounds, which were stable after *in vitro* gastrointestinal digestion.

Keywords: bioaccessibility; fat replacer; co-product; gelled emulsions; vegetable oil

INTRODUCTION

Burgers are currently one of the most frequently sold meat products worldwide due to their practicality, pleasant flavor, low cost, and nutritional value. However, in recent years, the consumption of this type of meat product has been stigmatized by several health organizations owing to its high saturated fat, cholesterol, and sodium chloride content, which has been linked commonly with the development of chronic non-communicable diseases including overweight, obesity, cardiovascular diseases and several types of cancer.[1,2] These health claims have reached consumers, who have increased their demand for healthier meat products in general and burgers in particular. This has pushed the meat industry and the scientific community to look for and develop ingredients that are accepted by consumers and that could partially or totally replace saturated fat in these types of products without radically modifying their sensory attributes.[3]

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Several strategies have been used to accomplish this objective, apart from reducing the fat, such as the use of encapsulated vegetable oils with healthier lipid profiles, and the use of oleogels or gelled emulsions.[4-6] One of the most successful strategies is the use of gelled emulsions. Gelled emulsions are semi-solid systems consisting of a polymeric network of proteins and/or polysaccharides with embedded oil droplets that could be used to mimic the textural properties that saturated fat imparts to foods, improving the lipid profile by incorporating oils with polyunsaturated fatty acids.[7] A huge variety of vegetable oils with a healthier fatty acid profile such as chia oil, soybean, hemp oil, and linseed oil have been used in the development of gelled emulsions.[8-10]

In recent decades there has been much interest in the use of walnut oil in the food industry due to the recognition of some of its components such as phenolic acids, tocopherols, and mono- and polyunsaturated fatty acids like linolenic acid, linoleic acid and oleic acid.[11] In particular, walnut oil contains between 2.4–5.3% palmitic acid, between 1.4–4.1% stearic acid, between 17.66–20.7% oleic acid, between 48.50–53.20.4% linoleic acid, and between 13.7–15.90% linolenic acid.[11,12]

Among the substances that can be used as emulsifying agents to elaborate gelled emulsions the application of agro-industrial co-products is being promoted because they are proving to be a technologically viable strategy and contribute to the much-needed sustainability of the agro-food industry. These co-products, apart from their high polysaccharide and/or protein content, are rich in bioactive compounds that can exert a beneficial effect on health once released from the food matrix. A very interesting co-product is cocoa bean shells. In their composition is possible to find several bioactive compounds including dietary fiber, polyphenols, and methylxanthines, which may have beneficial health effects.[13]

However, in order for the food to exert a beneficial effect on health, the bioactive compounds present must be able to withstand food processing and be bioavailable. Not all of them, when consumed, can enter into the bloodstream and induce beneficial health effects.[14,15] For this reason, it is very important to know how these compounds are released from the matrix and are bioaccessible in the different phases of the digestive tract.[16] The *in vitro* gastrointestinal digestion system is a tool for investigating the behavior of bioactive compounds present in food products during human digestion.[17]

There are not many studies in the scientific literature determining the effect of *in vitro* digestion on meat products where the fat content is partially or totally replaced by a gelled emulsion. Thus, the aims of this work were: (i) to determine the stability of bioactive compounds, including free and bound polyphenolic compounds, free and bound methylxanthines, and the fatty acid profile; (ii) to assess the bioaccessibility and colon-available indices (CAI); (iii) to evaluate the lipid oxidation values during the *in vitro* gastrointestinal digestion of beef burgers where the fat content was partially or totally substituted by a gelled emulsion made with cocoa bean shell flour and walnut oil.

MATERIALS AND METHODS

Materials

The following ingredients were used to elaborate the gelled emulsions: walnut oil (α-linolenic acid 612.1 mg g^{-1}, oleic acid 145.2 mg g^{-1}, and linolenic acid 124.3 mg g^{-1}) obtained from Cooks & Co. (Isleworth, UK); cocoa bean shell flour (protein content 171.30 mg g^{-1}, dietary fiber 611.80 mg g^{-1}) provided by Chocolates Valor (Villajoyosa, Spain); gellan gum (a polysaccharide excreted by microorganism *Pseudomonas elodea*) and gelatin of animal origin (180 °Bloom) were obtained from Sosa Ingredients SL (Barcelona, Spain). To produce the beef burgers the following meat ingredients were utilized: beef (semimembranosus) with 725.1 g kg^{-1} moisture, 243.7 g kg^{-1} protein, 21.2 g kg^{-1} lipids, and 10.0 g kg^{-1} ash, and pork backfat (743.5 g kg^{-1} lipids, 150.5 g kg^{-1} proteins, 102.0 g kg^{-1} moisture, and 4.0 g kg^{-1} ash), acquired from a local butchery (Orihuela, Spain).

Preparation of oil in water gelled emulsions and burgers

To prepare the gelled emulsion, 1.5 g of the gelling agent was mixed with water (47 mL) for 2 min at 37 °C at high speed (approximately 5600 rpm), using a Thermomix 31 homogenizer (Vorwerk-España M.S.L., Madrid, Spain). Then 10 g of cocoa bean shell flour was added and mixed for 1 min at medium speed (approximately 3000 rpm). After that, 1.5 g of gellan gum was added and mixed for 3 min at medium speed. Finally, walnut oil (40 mL) was added until it was perfectly integrated, then the mixture was mixed for 5 min, at 37 °C.

To make the burgers (ten for each formulation) the original formula was used as a control sample (BC), and two more formulations, where 50 or 100% of pork backfat was substituted by gelled emulsion elaborated with walnut oil and cocoa bean shell flours (BWC), were obtained as presented in Table 1. Beef burgers were manufactured following the recommendations of Lucas-González *et al.*[18] In control burgers, beef meat and pork backfat were ground through an 8 mm plate in a mincer attached to a mixer, and then the water, salt, and pepper were added into the bowl and mixed with the spiral dough hook at medium speed (80 rpm) for 4 min. For BWC50 and BWC100, the corresponding amounts of pork backfat (50% or 100%) were substituted by emulsion gel elaborated with walnut oil and cocoa bean shell and then mixed again for 4 min. After obtaining the meat batter, 90 g portions were weighed and shaped (9 cm diameter, and 1 cm thick) using a commercial burger maker, packed into bags, and stored at 4 °C until analysis.

In vitro gastrointestinal digestion

In vitro gastrointestinal digestion (GID) was carried out following the standardized methodology described by Minekus *et al.*[19] and following the recommendations of Lucas-González *et al.*[20] using pancreatin CREON (25 000 U) instead of individual pancreatic enzymes. Before simulated gastrointestinal digestion, beef

Table 1. Formulation of beef burgers (control and reformulated)

	Treatments		
	BC	BWC50	BWC100
Beef	65.70	65.70	65.70
Pork backfat	28.15	14.07	0
Water	4.70	4.70	4.70
Salt	1.40	1.40	1.40
White pepper	0.05	0.05	0.05
BWC	0	14.07	28.15

Note: Values expressed in g 100 g^{-1}.
Abbreviations: BWC: gelled emulsion with walnut oil and cocoa bean shell flour; BC, burger control; BWC50, beef burger where the 50% of fat content was replaced with BWC; BWC100, beef burger where the 100% of fat content was replaced with BWC.

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burgers were cooked on a grill until reaching an internal temperature of 72 °C, approximately 4 min for each side. Stock-digested solutions (oral, gastric, and intestinal) were prepared with the same saline concentration and pH as those indicated in the protocol described by Minekus *et al*.[19] To start the GID process, the burger samples were homogenized to obtain a paste and 5 g was weighed into a tube. Then, 5 mL of oral stock solution was added to the sample and vortex for 5 s; later the samples were incubated in an agitation bath at 37 °C, 30 rpm for 2 min. For the gastric phase, 7.5 mL of gastric stock solution and 200 μL of HCl (2 mol L^{-1}) was added to the oral digested sample, when the pH reached 3.00 ± 0.05, 0.5 mL of pepsin (2000 U mL^{-1}), 0.005 mL of $CaCl_2$, and water (6.795 mL) was added to de mixture. Then the samples were left in the agitation bath (30 rpm) at 37 °C for 2 h. In simulated intestinal conditions, NaOH (2 mol L^{-1}) was added drop by drop until a pH of 7.00 ± 0.05 was achieved and 16 mL of intestinal stock solution with pancreatin (2000 UL mL^{-1}), 2.5 mL of bile solution (10 mmol L^{-1}), and 0.075 mL of $CaCl_2$ was added to the gastric sample. Water was added until a final volume of 40 mL was achieved. Then the samples were incubated in the agitation bath (30 rpm) at 37 °C for 2 h. A blank for the oral, gastric, and intestinal phases was made by replacing the beef burger sample with distilled water. To assess the polyphenolic and methylxanthine compounds, fatty acid stability, and lipid oxidation after *in vitro* gastrointestinal digestion, three independent digestion processes for each burger formulation were carried out.

Extraction and determination of bound and free polyphenolic compounds in beef burgers

Extraction of bound and free polyphenolic compounds

Free and bound fractions of polyphenolic compounds were studied in undigested and digested beef burger samples. In the case of undigested samples, to obtain the free polyphenolic compounds from the cooked burgers, the methodology reported by Genskowsky *et al*.[21] was used. To extract bound polyphenolic compounds, the methodology described by Mpofu *et al*.[22] was used, utilizing the pellet remaining after the free polyphenolic extraction.

After the intestinal phase of GID, the digested beef burger samples were placed in ice for 5 min and then they were centrifuged at 4 °C and 4500 *x g* for 10 min. For free polyphenolic compounds, the supernatant was passed through a C-18 Sep-Pak cartridge previously activated. Compounds were extracted following the methodology reported by Mpofu *et al*.[22] to obtain the bound polyphenolic fraction.

Analysis of bound and free polyphenolic compounds

High-performance liquid chromatography (HPLC) was used to determine the polyphenolic profiles of free and bound fractions obtained from undigested and digested beef burgers following the procedure described by Genskowsky *et al*.[21] Retention time and UV spectra were used to identify the polyphenols in samples by comparing them with the standard. A calibrate curve of each mentioned standard was used to quantify polyphenols in samples.

Analysis of bound and free methylxanthines in beef burgers

The HPLC methodology proposed by Grillo *et al*.[23] was used to assess the methylxanthines present in the extracts obtained to determine polyphenolic compounds. Caffeine and theobromine were quantified according to the peak area measurements, which were reported in the calibration curves of the corresponding authentic standards.

Bioaccessibility of polyphenolic and methylxanthine compounds

The release of polyphenolic compounds and methylxanthines was analyzed by determining the bioaccessibility index and the CAI (Eqns (1) and (2) respectively):

$$bioaccessibility\ index\ (\%) = \frac{BCS_i}{BCP_s} x\ 100 \quad (1)$$

where BCS_i refers to the polyphenolic compounds or methylxanthines in the soluble fraction after the intestinal phase; BCP_s refers to the total polyphenolic compounds or methylxanthines in undigested samples;

$$colon\ available\ index\ (\%) = \frac{BCF_i}{BCB_s} x\ 100 \quad (2)$$

where BCF_i refers to the polyphenolic compounds or methylxanthines in the bound fraction after the intestinal phase; BCB_s refers to the total bound polyphenolic compounds or methylxanthines in undigested samples.

Fatty acid profile

Fat was extracted from undigested beef burgers following the procedure described by Folch *et al*.,[24] while in digested beef burgers, after the intestinal phase, the fat sample was extracted as recommended by Brodkorb *et al*.[25] After that, all the samples were transmethylated as described by Golay and Moulin.[26] The fatty acid methyl esters (FAMEs) were identified in an HP-6890 gas chromatographer equipped with a flame ionization detector (FID) and a Suprawax 280 capillary column (30 m × 0.25 μm film thickness × 0.25 mm i.d). The chromatographic conditions used were those reported by Pellegrini *et al*.[27] The results were expressed as mg fatty acid/g of fat.

Lipid oxidation

Lipid oxidation values of undigested and digested (after the intestinal phase) beef burgers were assessed following the thiobarbituric acid reactive substances (TBARs) methodology described by Sobral *et al*.[28] Malondialdehyde quantification was made using a standard curve with 1,1,3,3-tetramethoxypropane, and the results were expressed as a μmol malondialdehyde (MDA) kg^{-1} sample.

Statistical analysis

The full process (burger manufacture and simulated gastrointestinal digestion) was replicated three times (three independent batches). Each repetition was realized on a different manufacturing day and each batch was assessed in triplicate. The data obtained, when variables followed a normal distribution, were analyzed with a one-way ANOVA, and a Tukey post-hoc test was performed at the 5% significance level using XLSTAT for Windows, version 2016.02.

RESULTS AND DISCUSSION

Analysis of polyphenolic compounds in undigested and digested burgers

Figure 1(A), (B) shows the free and bound polyphenolic compounds detected in undigested and digested beef burgers where

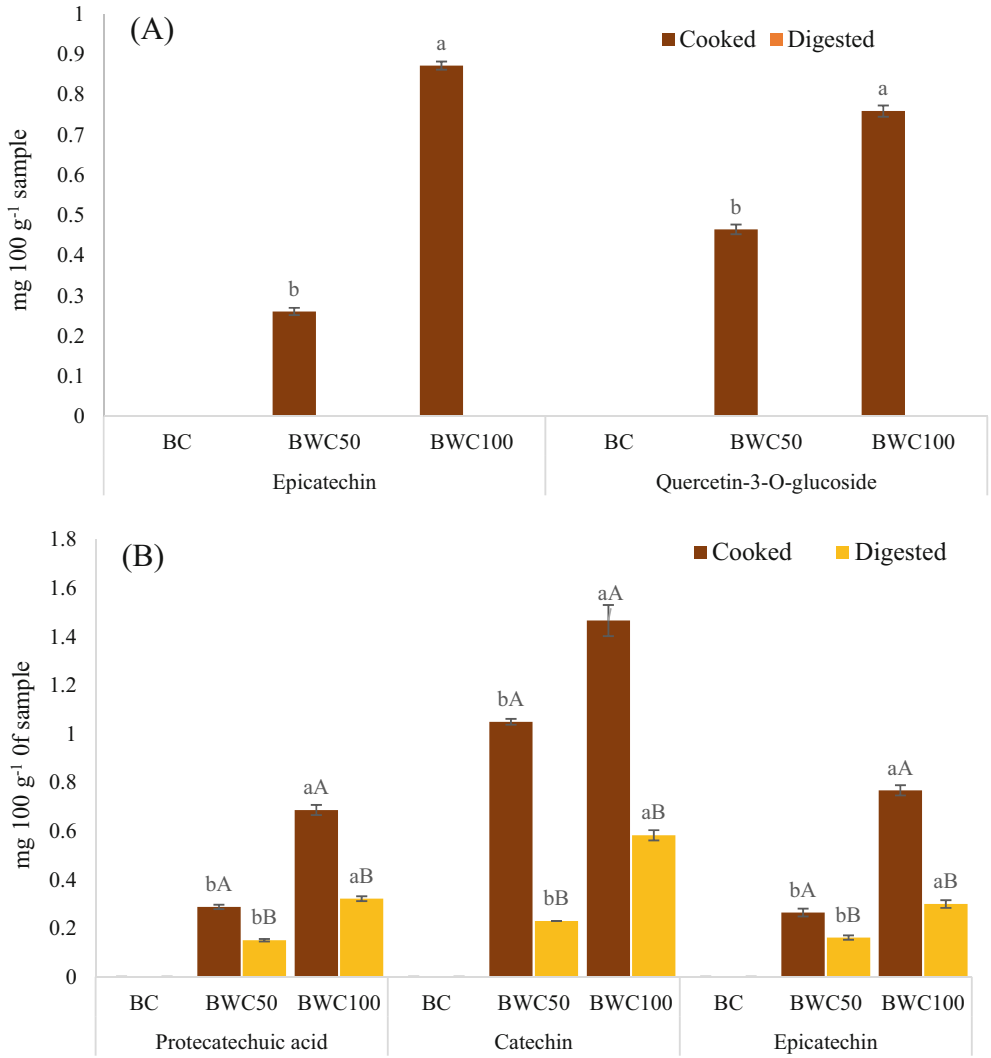

Figure 1. (A) Free polyphenolic compounds. (B) Bound polyphenolic compounds detected in undigested and digested cooked beef burgers (control and reformulated). Values expressed as mg100 g^{-1} of sample. BC, burger control; BWC50, Beef burger in which 50% of the fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil; BWC100, beef burger where 100% of the fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil. For the same polyphenolic compounds, columns with different small letters indicate significant differences ($P < 0.05$) according to Tukey's multiple range test. For the same polyphenolic compounds and the same fat replacement (BWC50 or BWC100), columns with a different capital letter indicate significant differences ($P < 0.05$) according to Tukey's multiple range test.

the fat was partially or totally replaced with a gelled emulsion elaborated with cocoa bean shell flour and walnut oil. Only one flavan-3-ol (epicatechin) and one flavonol (quercetin-3-*O*-glucoside) were found in free polyphenolic compounds in cooked samples, whereas the total decomposition of epicatechin and quercetin-3-*O*-glucoside was observed during simulated *in vitro* gastrointestinal digestion; thus, no free polyphenolic compounds were detected in the soluble fraction. These compounds, together with catechin, are characteristic of the cocoa bean and its co-products.[29] For both epicatechin and quercetin-3-*O*-glucoside, the BWC100 sample showed higher values ($P < 0.05$) than the BWC50 sample. The results were in agreement with those reported in the scientific literature, which related to the low availability of flavan-3-ols due to their instability in the gastrointestinal environment.[30] An alternative explanation could be that the occurrence of macronutrients deeply changes the bioaccessibility of flavan-3-ols due to these compounds having high affinity with proteins, and they can link more strongly or preferentially to them.[31]

As far as bound polyphenolic compounds were concerned (Fig. 1(B)) only one phenolic (protocatechuic acid) and two flavan-3-ols (epicatechin and catechin) were detected in both cooked and digested BWC50 and BWC100 samples. For all bound polyphenolic compounds, the BWC100 had higher values ($P < 0.05$) than the BWC50 in both cooked and digested samples. In cooked samples, catechin with values of 14.8 and 10.5 mg kg^{-1} for BWC100 and BWC50 respectively, and epicatechin with values of 7.70 and 2.90 mg kg^{-1} for BWC100 and BWC50 respectively, were the principal polyphenolic compounds; protocatechuic acid was found in lower concentrations (6.90 and 2.64 mg kg^{-1} for BWC100 and BWC50, respectively). These results agreed with Ramos-Escudero *et al.*[32] and Cantele *et al.*[33] who reported that these compounds were predominant in cocoa bean shells.

In digested samples, the same polyphenolic compounds were found that were in undigested samples (protocatechuic acid, epicatechin, and catechin). However, gastrointestinal digestion had a strong impact on the concentration of these compounds. In the BWC50 samples, for the protocatechuic acid, catechin, and

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epicatechin there were reductions of 47.57, 78.01, and 38.87%, respectively, in comparison with the undigested sample. Similarly, in BWC100 the reductions obtained in comparison with the undigested sample were 53.12, 60.26, and 60.95% for protocatechuic acid, catechin, and epicatechin, respectively. These results were in accordance with Cañas *et al*.[34] who found a reduction in the bound polyphenolic fraction of cacao bean shell flour in the last phase of gastrointestinal digestion. Several factors including the mechanical break, the composition of the food matrix, the time remaining under the different gastrointestinal conditions and the enzymatic activity may contribute to the physico-chemical release of the polyphenolic compounds.[35]

Analysis of methylxanthines in undigested and digested burgers

The predominant methylxanthine alkaloids found in cocoa bean shells are theobromine and caffeine.[36] These compounds are widely recognized for showing several beneficial health effects including neurostimulator, vasodilator, diuretic, anti-inflammatory, and anticarcinogenic besides cardiovascular protection.[37] Both methylxanthines were detected in undigested and digested BWC50 and BWC100 samples where the fat was partially or totally replaced with a gelled emulsion elaborated with cocoa bean shell flour and walnut oil (Table 2). In undigested samples, BWC100 had higher values ($P < 0.05$) for free theobromine and caffeine than BWC50. This was repeated in the undigested samples for the bound theobromine where the BWC100 showed higher values ($P < 0.05$) than BWC50 but the bound caffeine was not detected in any of the samples analyzed. The *in vitro* gastrointestinal digestion elicited a decrease ($P < 0.05$) in free methylxanthine content (theobromine and caffeine), with respect to undigested samples. Thus, in BWC50 the theobromine content reduction was 68.61%, whereas in BWC100 the reduction achieved was 48.42%. For caffeine, the content reduction in comparison with undigested samples was 96.47% and 97.96%. Contradictory results may be found in the scientific literature; thus, Cantele *et al*.,[33] who digested a cocoa beverage, and Rojo-Poveda *et al*.,[38] whose samples were cocoa biscuits, reported that theobromine and caffeine were highly stable under gastrointestinal conditions because these compounds were not degraded by the enzymes or by the pH conditions.

With regard to bound methylxanthines, for theobromine, the gastrointestinal digestion process increased the values in both BWC50 and BWC100 in comparison with undigested samples. These results could be explained by several bonds that might have been produced among the food matrix, mainly proteins and dietary fiber, and theobromine.

Bioaccessibility index and CAI

There is a growing body of evidence that bioactive compounds (polyphenolic compounds and methylxanthines) found in cocoa and cocoa co-products are beneficial to human health.[39] However, these health effects depend very much on the bioaccessibility and bioavailability of these bioactive compounds in the digestive tract as well as in the circulatory system. The bioactive compounds found in the bound fraction that are not released in the intestinal phase may also reach the colon intact and will be metabolized by the intestinal microbiome.[20,40]

In the case of polyphenolic compounds in digested beef burgers where the fat was partially or totally replaced with a gelled emulsion elaborated with cocoa bean shell flour and walnut oil, no bioaccessibility values were obtained. Thus, no polyphenolic compounds present in undigested samples were detected in digested samples. This fact could be due to the low concentration of this compound in the sample, which could cause them to be lost in their entirety during the digestion process due to the pH or enzymatic conditions. These results contradict the findings reported by Paz-Yépez *et al*.[35] who observed that the bioaccessibility, after *in vitro* gastrointestinal digestion, of polyphenolic compounds found in chocolate (dark and white) was significantly higher than the original values of undigested chocolate.

On the other hand, Fig. 2(A) shows the CAI of polyphenolic compounds of digested beef burgers where the fat was partially or totally replaced with a gelled emulsion elaborated with cocoa bean shell flour and walnut oil. In BWC50, epicatechin had the highest CAI ($P < 0.05$) (61.13%) followed by protocatechuic acid, catechin being the polyphenolic compound that showed the lowest CAI ($P < 0.05$) (21.90%). In the case of BWC100, no statistically significant differences ($P > 0.05$) in the CAI were found between epicatechin and catechin (39.74 and 39.06%, respectively), the compound with the highest CAI ($P < 0.05$) being protocatechuic acid. For catechin and protocatechuic acid, the CAI obtained

Table 2. Bound and free methylxanthines compounds detected in undigested and digested cooked beef burgers (control and reformulated)

		Theobromine		Caffeine	
Treatment	State	Free	Bound	Free	Bound
BC	Cooked	nd	nd	nd	nd
	Digested	nd	nd	nd	nd
BWC50	Cooked	15.58 ± 1.05^{aB}	0.07 ± 0.03^{bB}	2.27 ± 0.45^{aB}	nd
	Digested	4.89 ± 0.95^{bB}	0.13 ± 0.02^{aB}	0.08 ± 0.02^{bA}	nd
BWC100	Cooked	19.35 ± 0.68^{aA}	0.22 ± 0.03^{bA}	5.39 ± 0.15^{aA}	nd
	Digested	9.98 ± 0.86^{bA}	0.35 ± 0.06^{aA}	0.11 ± 0.06^{bA}	nd

Note: Values expressed as mg 100 g^{-1} of sample. For the same treatment (BWC50 and BWC100), values with different small letters in the same column indicate significant differences between states ($P < 0.05$) with Tukey's multiple range test. For the same state (cooked and digested) values with different capital letters in the same column indicate significant differences between treatments ($P < 0.05$) with Tukey's multiple range test.
Abbreviations: BC, burger control; BWC100, beef burger where the 100% of fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil; BWC50, beef burger where the 50% of fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil; nd, not detected.

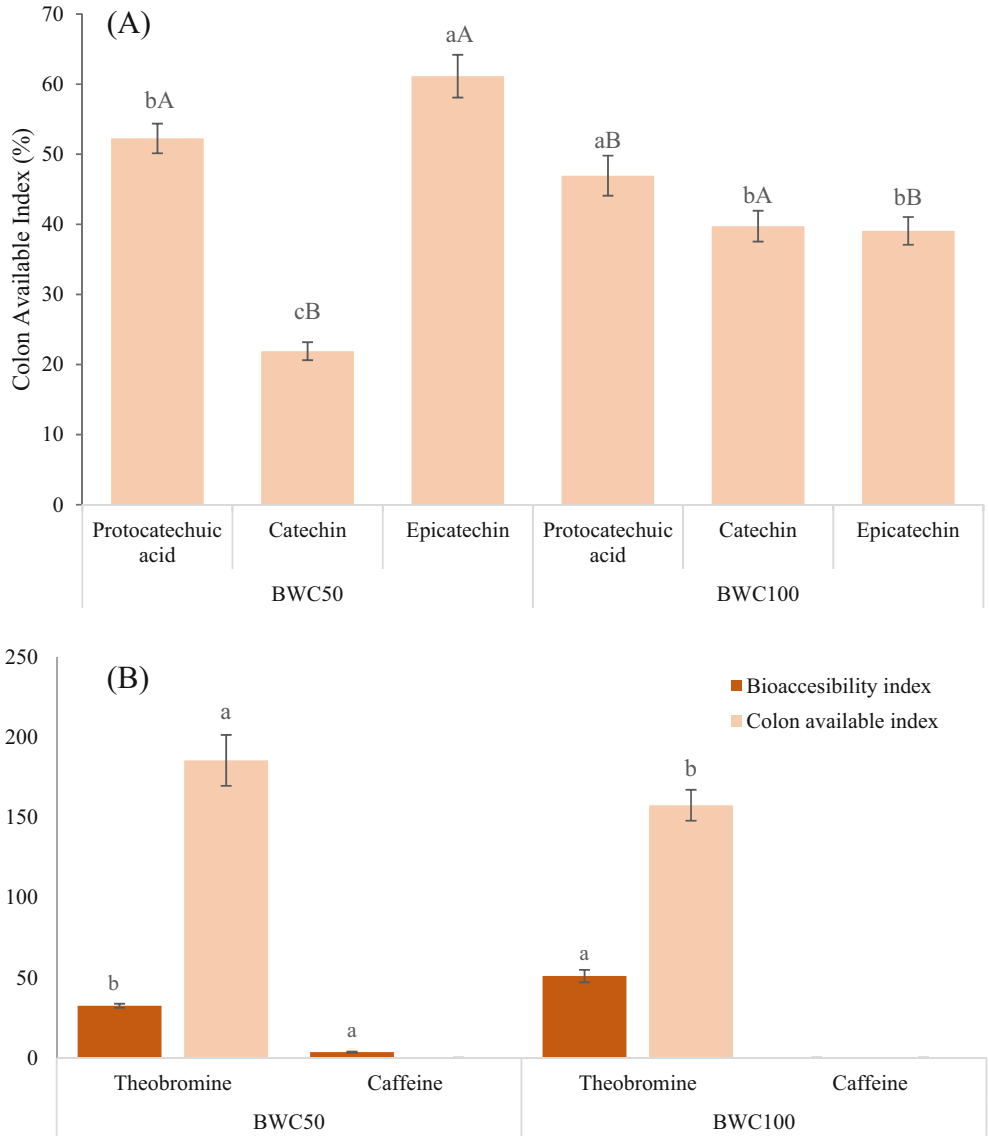

Figure 2. (A) Colon available index of polyphenolic compounds. (B) Bioaccessibility and colon available index of methylxanthines present in digested cooked beef burgers (reformulated). BC, burger control; BWC50, beef burger in which 50% of fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil; BWC100, beef burger where the 100% of fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil. For the same sample (BWC50 or BWC100) columns with a different small letter indicate significant differences ($P < 0.05$) according to Tukey's multiple range test. For the same polyphenolic compounds and the same sample (BWC50 or BWC100) columns with a different capital letter indicates significant differences ($P < 0.05$) according to Tukey's multiple range test.

was higher ($P < 0.05$) in BWC50 than in BWC100. This result was not expected because the greater the concentration of cocoa flour in samples, the greater should be the concentration of polyphenolic compounds collected in the bound fraction. This fact, could be explained by interactions of these compounds with the food matrix, which probably made it impossible to extract them. The results were in agreement with those reported by Juániz *et al.*[41] and Swetha *et al.*[42] who found CAIs of bound polyphenolic compounds in raw and cooked green pepper and *Moringa oleifera* seed flour that were similar to those obtained in this work. At the end of gastrointestinal digestion, a considerable concentration of polyphenolic compounds remained linked to several cell-wall structures and, as previously mentioned, could be metabolized by the intestine microbiome.[40]

The bioaccessibility index and CAI of methylxanthines of digested beef burgers, where the fat was partially or totally replaced with a gelled emulsion elaborated with cocoa bean shell flour and walnut oil, were reported in Fig. 2(B). After the intestinal phase, the bioaccessibility indices of the main methylxanthines present in reformulated BWC50 burgers were 32.49% and 33.52% for theobromine and caffeine, respectively, whereas in reformulated BWC100 burgers, the bioaccessibility index was 50.99% for theobromine ($P < 0.05$), whilst caffeine was not detected. These values represent the concentration of soluble and accessible methylxanthines that may be absorbed but also to hypothetically exert their functions at the intestinal level such as the ability to inhibit the alpha-glucosidase enzyme and antioxidant properties as reported by Cantele *et al.*[33] These results contradicted those reported by Nieto-Figueroa *et al.*[43] who found bioaccessibility values of theobromine from cocoa and its coproducts lower than 3%. With reference to the CAI of methylxanthines (Fig. 2(B)), for caffeine, no CAI values were obtained. In the case of

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Table 3. Fatty acid profile of undigested and digested in undigested and digested cooked beef burgers (control and reformulated)

		Fatty acid profile									
		C14:0	C16:0	C16:1n-9	C18:0	C18:1n-9	C18:2n-6	C18:3n-3	ΣSFA	ΣUFA	ΣPUFA
BC	Cooked	15.53 ± 0.39^{aF}	242.20 ± 5.97^{aB}	33.24 ± 0.03^{aE}	119.57 ± 1.14^{aC}	453.27 ± 16.53^{aA}	83.94 ± 1.77^{bD}	nd	373.30 ± 24.96	486.51 ± 24.96	83.94 ± 1.77
	Digested	15.44 ± 0.72^{aF}	227.37 ± 4.58^{bB}	32.97 ± 0.09^{aE}	118.54 ± 1.42^{aC}	427.17 ± 10.94^{bA}	91.92 ± 1.98^{aD}	nd	361.35 ± 24.96	460.14 ± 15.39	91.92 ± 1.98
BWC50	Cooked	9.28 ± 0.19^{aG}	164.27 ± 3.92^{aC}	19.74 ± 0.05^{aF}	76.39 ± 1.93^{aD}	334.26 ± 8.67^{aA}	304.58 ± 7.54^{aB}	52.44 ± 1.08^{aE}	249.94 ± 1.82	354.00 ± 2.33	357.02 ± 2.50
	Digested	9.63 ± 0.25^{aG}	163.16 ± 1.84^{aC}	19.91 ± 0.04^{aF}	71.23 ± 1.37^{bD}	323.43 ± 6.67^{bA}	310.06 ± 5.29^{aB}	58.72 ± 1.04^{aE}	244.02 ± 1.12	343.34 ± 3.96	368.78 ± 6.69
BWC100	Cooked	9.00 ± 0.44^{aF}	144.03 ± 8.77^{aC}	12.37 ± 0.04^{aE}	77.55 ± 3.22^{aD}	228.92 ± 3.38^{aB}	413.35 ± 14.17^{aA}	82.35 ± 2.15^{aD}	230.59 ± 10.96	241.29 ± 3.79	495.70 ± 16.89
	Digested	8.12 ± 0.62^{aF}	127.94 ± 5.54^{bC}	12.34 ± 0.06^{a}	64.67 ± 3.97^{bE}	218.31 ± 2.24^{bB}	434.34 ± 12.22^{aA}	86.36 ± 2.71^{aD}	200.73 ± 9.75	230.65 ± 4.98	520.70 ± 13.63

Note: Values expressed as mg g^{-1} of fat. Values followed by the same small letter within the same column are not significantly different ($P > 0.05$) with Tukey's multiple range test. Values followed by the same capital letter within the same row are not significantly different ($P > 0.05$) with Tukey's multiple range test.
Abbreviations: BC, burger control; BWC100, Beef burger where the 100% of fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil. For the same sample (BC, BWC50, and BWC100); BWC50, Beef burger where the 50% of fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil; nd, not detected.

theobromine, the CAI obtained was higher ($P < 0.05$) in BWC50 than in BWC100. Thus, BWC50 had a CAI of 185.50%, the CAI for BWC100 being 157.53%. This increase in methylxanthines could be explained in terms of the release of the bound compounds from the food matrix as a consequence of the enzymatic action.[41]

Fatty acids profile

The fatty acid profiles of undigested and digested control burger and beef burgers where the fat was partially or totally replaced with a gelled emulsion elaborated with cocoa bean shell flour and walnut oil are shown in Table 3. As regards cooked undigested samples, for the control sample, the principal fatty acids found were monounsaturated, followed by saturated fatty acids, and a smaller amount of polyunsaturated fatty acids. Among monounsaturated fatty acids, oleic acid (C18:1n-9) represented the highest ($P < 0.05$) relative percentage and palmitic acid (C16:0) was the predominant acid ($P < 0.05$) within the saturated fatty acids. The substitution of animal fat by the gelled emulsion elaborated with walnut oil and cocoa bean shell flour improved the nutritional values of burgers because the principal fatty acids found in BWC50 and BWC100 were polyunsaturated fatty acids with values of 360.58 and 495.70 mg g^{-1} fat, respectively, due to the high linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3) content present in walnut oil.[44] In the same way, the saturated fatty acids were reduced with respect to the control sample, thus in BWC50 the reduction achieved was 33.75%, whereas in BWC100 the reduction of saturated fatty acid was 38.88%. The results obtained agreed with those reported in the scientific literature, which indicated that the substitution, in fresh and cooked meat products, of animal fat by gelled emulsion or oleogels elaborated with healthier vegetable oils improved the nutritional quality of this type of meat products.[6,45-47]

In reference to digested samples (Table 3), the fatty acid profile was not qualitatively changed after the *in vitro* gastrointestinal digestion process. In the control sample oleic acid was the predominant ($P < 0.05$) fatty acid followed by palmitic acid. For these two fatty acids, the digestion process produced a reduction with respect to undigested samples, whereas for the rest of the fatty acids no statistically significant differences ($P > 0.05$) were found between undigested and digested samples except for linoleic acid, which increased in concentration ($P < 0.05$) with respect to the undigested sample. This fact also occurred in the digested BWC50 and BWC100 samples. In both, BWC50 and BWC100 samples, stearic acid (C18:0) and oleic acid (C18:1n-9) in digested samples had lower values ($P < 0.05$) than in undigested samples, while for the rest of the fatty acids no statistically significant differences ($P > 0.05$) were found between undigested and digested samples. These results agreed with those reported by Lucas-Gonzalez *et al.*[20] who noticed a very similar fatty acid profile between undigested and digested pork liver pâte with added persimmon flour. However, Tormási and Abrankó[48] found that the fatty acid profile of digested baked fish had lower values for all fatty acids than the undigested sample.

The effects of *in vitro* gastrointestinal digestion conditions upon the lipid profile depend on several factors; thus, the position of fatty acids within triglyceride molecules could also influence the process of lipid hydrolysis of pancreas lipase in the gastrointestinal tract in *in vitro* digestion.[49] On the other hand, it is widely known that the occurrence of dietary fiber in the food matrix possibly influence lipid digestion, hindering the access of bile salts and digestive enzymes to the oil phase.[50]

Table 4. Lipid oxidation of undigested and digested cooked beef burgers (control and reformulated)

	Cooked	Digested
BC	3.43 ± 0.07^{cB}	9.46 ± 0.90^{cA}
BWC50	5.76 ± 0.69^{bB}	12.08 ± 0.68^{bA}
BWC100	8.44 ± 1.91^{aB}	15.33 ± 1.60^{aA}

Note: Values expressed as μmol MDA kg^{-1} sample. Values with different small letter in the same column indicate significant differences ($P < 0.05$) with Tukey's multiple range test. Values with different capital letters in the same row indicate significant differences ($P < 0.05$) with Tukey's multiple range test.
Abbreviations: BC, burger control; BWC100, Beef burger where the 100% of fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil; BWC50, Beef burger where the 50% of fat content was replaced with gelled emulsion elaborated with cocoa bean shell flour and walnut oil.

Lipid oxidation of beef burgers

One of the leading processes responsible for the degradation of the quality of meat and meat products is lipid oxidation. This negatively affected several parameters including (i) physicochemical (color and/or texture); (ii) nutritional, and (iii) sensory, including taste, aroma, and flavor due to rancidity.[51] These changes are compelling motives for consumer rejection. The lipid oxidation values of undigested and digested beef burgers where the fat was partially or totally replaced with a gelled emulsion elaborated with cocoa bean shell flour and walnut oil are shown in Table 4. In the undigested samples, the substitution of pork backfat by gelled emulsion elaborated with walnut oil led to an increase in the oxidation values ($P < 0.05$) and this occurred as a function of the degree of substitution. The use of gelled emulsions with high polyunsaturated fatty acid content as partial substitutes for animal fat in meat products could increase the oxidation predisposition of the reformulated meat product during the thermal treatment. Thus, the greater the degree of substitution of saturated fat for unsaturated, the greater the degree of oxidation. This fact is well documented in the scientific literature. In this regard, de Lima Guterres *et al.*[52] noticed that the partial replacement of animal fat with linseed oil and pea protein emulsion hydrogel increased lipid oxidation in pork burgers compared with the control. Similarly, Botella-Martinez *et al.*[46] reported that the lipid oxidation values of beef burgers where the fat content was replaced by gelled emulsions elaborated with chia or hemp oil had higher oxidation values than the control. In this study, the increase in lipid oxidation values was expected due to the high concentration of polyunsaturated fatty acids in walnut-gelled emulsion, which are more susceptible to oxidation due to their unstable double bonds. The increase in the lipid oxidation values could be explained by the effect of thermal treatment on the meat cellular structure, which permitted oxygen and other types of free radicals to react more easily with unsaturated fatty acids and develop peroxidation and consequently the development of reactive aldehydes.[53] The heat treatment may also release heme iron from the porphyrin ring, which causes an increase in the rate of oxidative reactions leading to deterioration.[54]

The same pattern was observed in the digested samples (Table 4). The control sample showed the lowest degree of oxidation ($P < 0.05$), while the sample where all the pork backfat was replaced with walnut-gelled emulsion (BWC100) had the highest lipid oxidation values ($P < 0.05$). Gastrointestinal digestion triggered the formation of TBARs and increased the oxidation values. Thus, the undigested samples had lower values ($P < 0.05$) than digested burgers. This increase in the lipid oxidation values of digested samples could be explained by the emulsification capacity of bile acids, which could provoke partially the rise of malondialdehyde concentration in the first phases of intestinal digestion as mentioned by Steppeler *et al.*[55] The bile emulsification will possibly increase the lipid droplets' surface area, which is susceptible to lipid peroxidation, increasing the generation of more aldehydes.[53] Several studies reported that the gastrointestinal digestion of foods with a moderate and high fat content led to the formation of toxic compounds, such as 4-hydroxy-trans-2-nonenal, 4-hydroxy-trans-2-hexenal, and malondialdehyde.[56,57] Martini *et al.*[58] reported that, in grilled turkey meat with extra virgin olive oil (10%) added, the concentrations of TBARs and hydroperoxides increased during *in vitro* gastrointestinal digestion.

CONCLUSIONS

The substitution of animal fat in beef burgers with gelled emulsion elaborated with cocoa bean shell flour and walnut oil is a good strategy, in this type of meat product, to increase the bioactive compound content. It was stable after *in vitro* gastrointestinal digestion. Cocoa bean shell flour is a good source of bound polyphenols and methylxanthines, mainly theobromine, which was also stable after gastrointestinal digestion. In the same way, few changes were obtained between the fatty acid profiles of undigested and digested beef burgers where the animal fat was replaced by gelled emulsion, highlighting an increase in polyunsaturated fatty acids in the digested reformulated samples in comparison with the undigested samples. On the other hand, lipid oxidation could be the critical factor due to this novel reformulation strategy strongly affecting this parameter mainly after the digestion process. In view of the results achieved, the total replacement of animal fat by gelled emulsion will be the best option.

Nonetheless, in-depth studies, both *in vitro* and *in vivo*, are necessary to fully understand the changes in bioactive compounds (polyphenolic and methylxanthines as well as polyunsaturated fatty acids) that occur during gastrointestinal digestion and to obtain a complete view of the health implications. This may be valuable when evaluating the suitability of meat products in which the animal fat was partial or totally replace by gelled emulsions elaborated with walnut oil and cocoa bean shell flour.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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