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In vitro colonic fermentation of ultrasonicated blackberry (Rubus fruticosus) residues cv. Tupy

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Abstract

The objective of present study was evaluated non fermented residue (NFR), disappeared organic matter (DOM), bacteria acid lactic (LAB), short-chain fatty acids (SCFA), flavonoids and the microstructure during *in vitro* colonic fermentation of ultrasonicated blackberry residues (pulp, peel and seeds). Results showed that ultrasonicated blackberry residues (US-BR) presented low NFR (6.30 ± 1.27 mg) and a high percentage of DOM (93.70 ± 1.27%) in comparison with blackberry residues (BR), indicating high fermentability of the sample. The same behavior in the other parameters was observed, since had an increase in LAB (6.97 ± 0.08 log CFU/mL), total SCFA (409.49 mg/L), individual fatty acids such as acetic and propionic acid (387.65 ± 3.60 and 20.17 ± 0.15 mg/L, respectively). Also total flavonoids were high in US-BR (1.3 mg/100 g) where flavonoids myricetin and quercetin were identified. The microstructure of the fermented US-BR had a reduction of particle size and less fibrous matrix compared with the fermented BR. The use of ultrasound improves the properties of the fibrous matrix of blackberry residues available for intestinal microbiota, obtaining final products of fermentation with possible health benefits.

Introduction

Blackberry (*Rubus fruticosus*) is a fruit that can be consumed raw or after processing into jams, wine, tea and juices. When the juices are elaborated, fruit residues as peel, stem, pulp and seeds are obtained [1,2]; and these residues could be harnessed as a source of proteins, flavonoids and dietary fiber in the design of healthy foods [3]. The dietary fiber (mainly soluble fiber) is fermented in the large intestine by intestinal microbiota producing short chain fatty acids (SCFA) as acetic, propionic and butyric [4]. SCFA are absorbed by the colonocytes (colonic epithelial cell) and metabolized in the large intestine and other cells of different tissues providing health benefits [5]. The butyric acid exerts protection in the colon due this is the principal source of energy for colonocytes and prevents cancer of the colon [6].

Technologies used to preserve the food components are responsible for structural modification of the food matrix affecting nutritional, physical and rheological characteristics [7]. One of these technologies is the ultrasound, that after their application causes structural changes due to the depolymerization of dietary fiber by the presence of free hydroxyl radicals or mechanical degradation; both modifications are due to the cavitation that generates the ultrasound [8]. The depolymerization of fiber allow major availability of substrate for colonic microbiota, which impact on the production of SCFA, increase of beneficial bacteria over pathogens, lowering the pH between others [9]. Recent studies evaluated the effect on *in vitro* colonic fermentation of ultrasonicated plantago and blackberry whole [10,11]. However, there are no studies of ultrasonicated blackberry residues (pulp, peel and seeds) during *in vitro* colonic fermentation. Therefore, the aim of the present study was to evaluate the effect on *in vitro* colonic fermentation (non fermented residue, disappeared organic matter, bacteria acid lactic, short chain fatty acids, flavonoids and their microstructure) of ultrasonicated blackberry residues.

Materials And Methods

This section is presented as supplementary material.

Results And Discussion

Non Fermented Residue and Disappeared Organic Matter. Non-fermentable residue (NFR), which is the fraction that was not utilized by the gut microbiota and therefore excreted, while the disappeared organic matter (DOM) is the production of secondary metabolites [12]. These determinations were performed after *in vitro* colonic fermentation of blackberry residues (BR), ultrasonicated blackberry residues (US-BR) and lactulose (control) as a point of comparison and the results are presented in Table 1.

Table 1. Non-fermentable residue (NFR) and disappeared organic matter (DOM) after *in vitro* colonic fermentation

	Lactulose*	BR	US-BR
NFR (mg)	0.00 ± 0.00 ^{a}	61.45 ± 0.21 ^{c}	6.30 ± 1.27 ^{b}
DOM (%)	100.00 ± 0.00 ^{c}	38.55 ± 0.21 ^{a}	93.70 ± 1.27 ^{b}

$^{a\text{-}c}$ Indicates significant difference (p<0.05) between the samples. *Lactulose (control)

It was observed that the lactulose substrate did not present NFR, while that US-BR showed an amount low (6.30 ± 1.27 mg) with respect to BR (61.45 ± 0.21 mg). The latter indicates that the gut microbiota used almost 10 times more to US-BR as substrate than BR. In the present study, lactulose was used completely by gut microbiota since this is a substrate fully fermentable and so it was used as control [13]. Respect to the study sample, probably the microbiota did not consume the BR in percentage similar to US-BR, due to their native chemical structure of the polysaccharides and physical form, both can influence the rate and the fermentation end-products [14].

In the disappeared organic matter, the lactulose had the 100% of DOM, followed by US-BR (93.70 ± 1.27%). Although lactulose and US-BR were statistically different, US-BR also is a highly fermentable sample. The DOM results in US-BR were similar to a study performed in dietary fiber of beer (98%) [15], slightly high in comparison with plantago (87%), higher than blends fiber (fruit fiber, wheat bran and guar gum) (50%) [12] and white grape peel and seed residues (31%) [13].

This is important because the gut microbiota use all substrate and therefore produce more metabolites such as short chain fatty acids (acetic, propionic and butyric acid) [4] with positive effects in the health e.g. anti-inflammatory and anti-proliferative [16]. The sonication allowed the breakdown of substrate cell walls, reducing the particle size, generating the depolymerization and carbohydrates of low molecular weight [17]. This behavior could favor the substrate being used almost entirely by the gut microbiota. Dou et al. [18] applied ultrasound treatment to the whole blackberry and observed that it decreased the size particle and molecular weight of polysaccharides contained in the fruit.

pH and Lactic Acid Bacteria during *In vitro* Colonic Fermentation. Table 2 shows pH and lactic acid bacteria (LAB) results of BR and US-BR samples. Before *in vitro* colonic fermentation (hour 0), the BR sample had significantly ($p<0.05$) high pH in comparison with lactulose and US-BR.

Table 2. pH and lactic acid bacteria (LAB) count to 24 h of *in vitro* colonic fermentation of blackberry residues (BR) and ultrasonicated blackberry residues (US-BR)

Samples	0 h	24 h
	pH	
Lactulose [A]	7.84 ± 0.30 [a, *]	6.84 ± 0.08 [a]
BR	8.66 ± 0.08 [b, *]	7.15 ± 0.03 [b]
US-BR	7.85 ± 0.03 [a, *]	7.02 ± 0.11 [ab]
	LAB (log CFU/mL)	
Lactulose	5.27 ± 0.04 [b, *]	7.07 ± 0.11 [b]
BR	4.00 ± 0.02 [a, *]	6.54 ± 0.04 [a]
US-BR	4.01 ± 0.02 [a, *]	6.97 ± 0.08 [b]

[a-c] Indicates significant difference ($p<0.05$) between samples from the same column. *Indicates significant difference ($p<0.05$) between the rows (0 and 24 h). [A] Lactulose: positive blank.

In all samples the pH significantly was reduced at 24 h of *in vitro* fermentation, in relation at hour 0, and lactulose had value low respect to the BR sample. Similar to the behavior of the present study, in cactus pear and pineapple peel there was a decrease at the end of *in vitro* fermentation [19]. The decrease of pH was due to the presence of short-chain fatty acids produced by the gut microbiota [4] (results will be shown later). Before *in vitro* colonic fermentation, the samples started between 4 and 5.27 log CFU/mL of LAB, being the lactulose with more LAB ($p<0.05$) in comparison with blackberry residues (Table 2). The behavior of LAB at the end of fermentation was the following: lactulose > US-BR > BR. In all samples LAB increased ($p<0.05$) in relation to the start of fermentation (time 0). According to Saura-Calixto et al. [15] the increase in bacterial mass is considered as one of the products of the colonic fermentation process. US-BR had a similar LAB count than lactulose, which is related to fermentability of the substrate and as mentioned above, US-BR presented a high DOM (Table 1). However, for their fermentation it is necessary to consider the molecular weight, size, the sugars of molecules, the number and bonds of monosaccharides of dietary fiber [4].

Short Chain Fatty Acids (SCFA). The production of fatty acids after *in vitro* fermentation of samples was as follows: acetic acid > propionic acid > butyric acid. Total production of SCFA was 190.45 and 409.49 mg/L for BR and US-BR, respectively. The US-BR presented high ($p<0.05$) concentrations of acetic and propionic acid, while both samples showed no significant differences ($p>0.05$) for butyric acid (Table 3).

In general, the most produced compound was acetic acid, reaching 93.5% for BR and 94.6% for US-BR, while the least was butyric acid (0.87% and 0.40%, respectively). The obtained results have relation with the behavior of non-fermentable residue and disappeared organic matter (Table 1), due to the US-BR sample had less non-fermentable residue and major organic matter disappeared, indicating high production of end metabolites (short chain fatty acids).

Table 3. Quantification of short chain fatty acids (SCFA) after *in vitro* colonic fermentation (mg/L) in blackberry residues (BR) and ultrasonicated blackberry residues (US-BR)

Sample	Acetic acid	Propionic acid	Butyric acid	Total SCFA
BR	178.16 ± 3.06*	10.62 ± 0.04*	1.67 ± 0.04	190.45*
US-BR	387.65 ± 3.60	20.17 ± 0.15	1.67 ± 0.03	409.49

*Indicates significant difference (p<0.05) between samples.

The results of the present study was agree with in a study of orange and fruit passion wastes from different origins, since the major production of SCFA was acetic acid (between 60-70% and 50-60%, respectively) [20]. Tejada-Ortigoza et al. [21], obtained the percentage of SCFA in orange, mango and prickly pear peel after *in vitro* colonic fermentation and observed that acetic (71.5-76.6%), propionic (13.4-15.7%) and butyric acid (7.7-11.4%) were produced in the same decreasing order with respect to BR and US-BR samples, although presented different percentages. These differences could depend on the fiber type present contained in the food matrix e.g. cellulose, arabinoxylan, β-glucan, inulin and fructooligosaccharides (FOS) [22,23].

On the other hand, the ultrasound application in blackberry fruit and seeds of *Plantago asiatica* L. produced high concentrations of acetic and propionic acid in comparison with the sample without treatment [18,10]. Similar behavior was observed in the present study, due to US-BR presenting two times more the amount for acetic and propionic acid with respect to BR. The ultrasound treatment allows the microbiota improved the fermentation of dietary fiber, which may break glycosidic bonds, facilitating their utilization [10]. SCFA such as acetic, propionic and butyric acid are important in human health, due to acidify the medium, promoting the growth of beneficial bacteria and the inhibition of pathogens, as well as intervening in the development of the immune system of the intestine [16]. In particular, each short-chain fatty acid participates in metabolism. For example, acetic acid intervenes in the processes of lipogenesis and gluconeogenesis, also is used by the hepatocytes and peripheral cells as a source of energy [24]. Propionic acid has a hypocholesterolemic effect and it is involved in glucagon secretion and gluconeogenesis, which contributes to decreased appetite [25]. While the butyric acid is the main source of energy for colonocytes, prevents colon cancer due to feeds to the colonic mucosa, also intervenes in the apoptosis of cancer cells [5]. **Quantification of Flavonoids.** The flavonoids quantified after *in vitro* colonic fermentation were myricetin and quercetin. In BR was quercetin (0.55 ± 0.05 mg/100 g DM), while for US-BR was myricetin and quercetin with 0.85 ± 0.02 and 0.45 ± 0.02 mg/100 g DM, respectively. The concentration of total flavonoids was high in the US-BR (1.3 mg/100 g DM) in comparison with BR (0.55

mg/100 g DM) (data not shown). In a previous study, the same samples were analyzed after *in vitro* bioaccessibility and were not detected the flavonols (myricetin and quercetin) in the dialyzed fraction [2]. This may indicate that were not released from the matrix fibrous in the small intestine but if by action of the microbiota of colon and some compounds can remain intact; the remaining unidentified flavonoids in this section of the gastrointestinal tract were probably transformed by the colonic bacterias to smaller compounds as phenylacetic, phenylpropionic and phenylvaleric acid [26]. Chait et al. [27] quantified the flavonoids in carob pulp powder and after *in vitro* colonic fermentation the values of myricetin were high in comparison with US-BR.

Scanning Electron Microscopy (SEM). Scanning electron microscopy has been used as an effective tool to observe the changes in the morphology of the food surface related to the treatments which they were subjected to. In order to describe and evaluate the changes in BR and US-BR due to *in vitro* colonic fermentation, these samples were analyzed by means of SEM (Fig. 1). The blackberry residue (BR) tissues show a partly damaged cell structure, collapsed and broken cell wall can be observed as big flake-like lamellas, this damage could be due to mechanical changes during the juice process and grinding (Fig. 1A, B). The structure of residue blackberry was modified by the ultrasound process (US-BR) and can be seen in the Fig. 1C and D, this was evidenced by the reduction of the size of the cell wall fragments (flake-like structure). This behavior where the integrity of cell structure was reduced with the ultrasound treatment is similar with other reports for fruits treated by ultrasound [18,10].

Fig. 1C and D (US-BR) show lighter regions compared with Fig. 1A and B (BR), this may be due to the release of organic materials [28]. The colonic fermentation changed the microstructural of the BR and US-BR; this can be observed in Fig. 1E, F, G and H. A major reduction of particle size and less content of the fibrous matrix in the fermented US-BR (Fig. 1G, H) compared with the fermented BR (Fig. 1E, F) is observed. This last is relationed with the results of disappeared organic matter mentioned above (Table 1). The cavitation effect by the ultrasound process reduced the particle size and probably the intestinal microbiota releases enzymes that degrade fiber allowing the depolymerization and utilization during the colonic fermentation process [29,10]. The US-BR samples show greater brightness (Fig. 1G, H), this may be due to the fact that a greater amount of organic compounds have been released compared to BR (Fig. 1E, F), being an indication of a greater use of these compounds during colonic fermentation.

Conclusion

The application of ultrasound on blackberry residues (pulp, peel and seeds) was effective on colonic fermentation. The ultrasound allowed fibrous matrix blackberry residues to be available for intestinal microbiota. After *in vitro* colonic fermentation, US-BR (ultrasonicated blackberry residues) sample presented minor non-fermentable residues, major organic matter disappearance, high lactic acid bacteria amount, as well as metabolites production, such as high concentration of short chain fatty acids (acetic and propionic acid) and flavonoids (myricetin). Therefore, blackberry residues with ultrasound applied could be used as an ingredient in foods, which their consumption would be beneficial to health.

Declarations

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Data Availability. Authors declare that database are available on request.

Authors' contributions. Q.Z. Developed the project, wrote the main manuscript text and revising it critically for important intellectual content; N.C. Designed the study and final approval of the version to be published; A.C. Interpretation of data for the work; J.A. Supported the determination of short-chain fatty acids; H.H. Supported in the analysis of the microstructure; L.D. Supported the determination of flavonoids; E.O. Analyzed the data; E.R. Coordinated the research project and final approval of the version to be published. All authors reviewed the manuscript.

Ethics approval. Not applicable.

Consent to participate. Not applicable.

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Figures

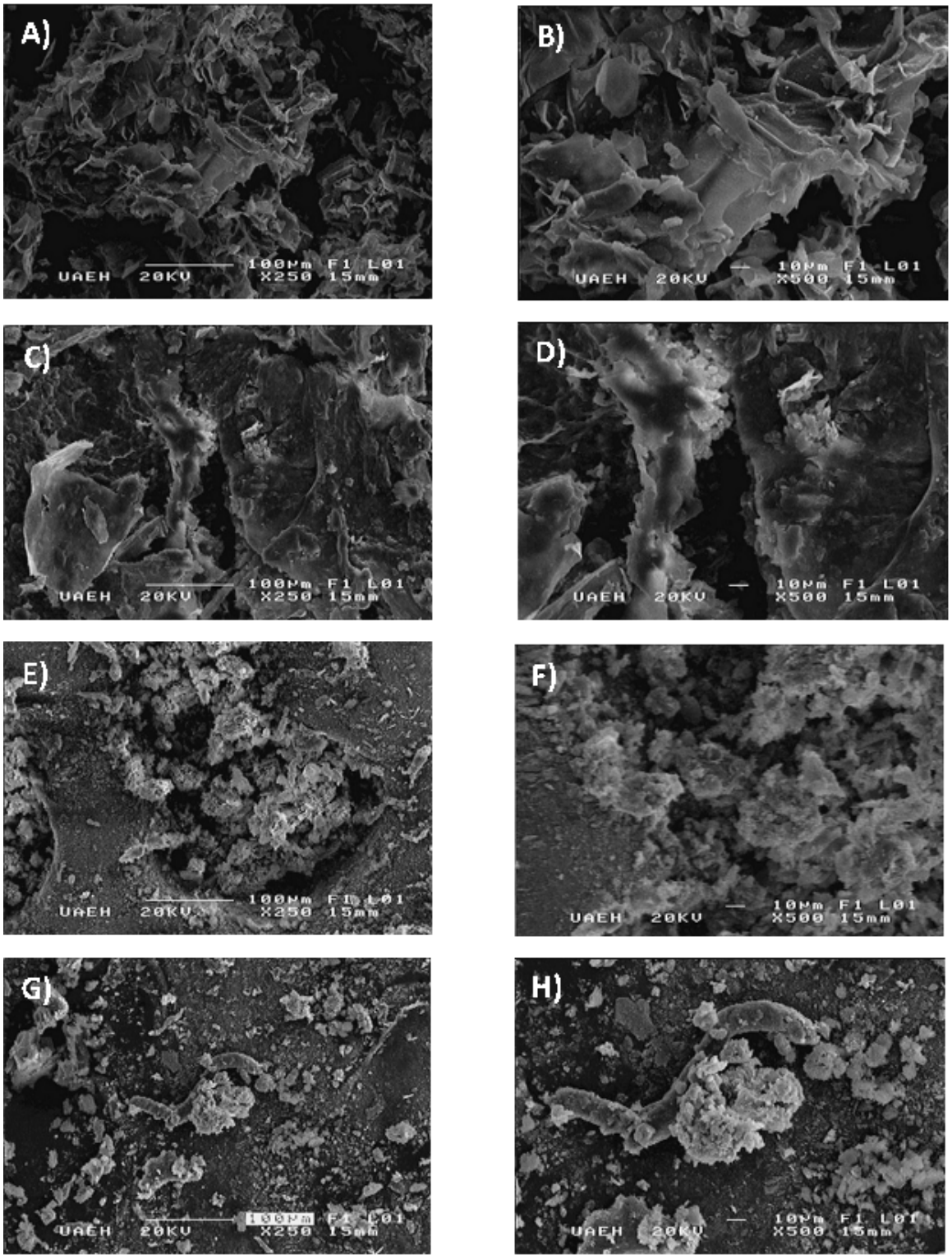

Figure 1

Scanning Electron Microscopy of blackberry residues (BR) and ultrasonicated blackberry residues (US-BR) before and after *in vitro* colonic fermentation. A) and B) BR at 250x and 500x, respectively; C) and D) US-BR at 250x and 500x, respectively; E) and F), BR after *in vitro* colonic fermentation at 250x and 500x, respectively; G) and H) US-BR after *in vitro* colonic fermentation at 250x and 500x, respectively.

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