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Deacidification of cranberry juice protects against disruption of *in-vitro* intestinal cell barrier integrity

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ABSTRACT

Cranberry juice is a well-known functional juice that has many beneficial effects on human health. However, it also has a high concentration of organic acids which may cause gastrointestinal discomfort. Hence, the organic acid content in cranberry juice was reduced to different levels of deacidification (0%, 19%, 37%, 50%, and 77%) by electrodialysis to study the impact of the deacidification rate on intestinal cell integrity. Before *in vitro* tests on Caco-2 cells, all samples underwent three steps of *in vitro* digestion: oral, gastric and intestinal. Digested and deacidified cranberry juices were applied to Caco-2 cells and the transepithelial electrical resistance (TEER) was measured after 24 hours of contact to evaluate the resulting cell integrity. In the presence of deacidified cranberry juice, the integrity of caco-2 cell monolayers measured by the ΔTEER was increased by 56% in comparison with raw cranberry juice, but a minimal deacidification rate of 37% was necessary to reach this level of protection.

1. Introduction

Cranberry (*Vaccinium macrocarpon*) is a typical North American berry. In comparison with other fruits, it has a high concentration of polyphenolic compounds, especially anthocyanins and proanthocyanidins (Neto, 2007). These molecules confer several beneficial effects for human health such as prevention of cardiovascular diseases and carcinogenesis, reduction of urinary tract infection, and protection against cavities (Khoo & Falk, 2014; Raz, Chazan, & Dan, 2004; Vasileiou, Katsargyris, Theocharis, & Giaginis, 2013). For its health properties, cranberry juice is well recognized as a functional food.

Unfortunately, after consumption of cranberry juice, many clinical trials noted that approximately 40% of drop-outs were due to undesirable side effects such as diarrhoea, vomiting and

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Abbreviations: ED, electrodialysis; TEER, Transepithelial Electrical Resistance; EDBM, Electrodialysis with Bipolar Membrane; HBSS, Hank's Balanced Salt Solution; RPMI, Roswell Park Memorial Institute medium; PACs, proanthocyanidins; TA, titratable acidity
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bloating (McMurdo, Bissett, Price, Phillips, & Crombie, 2005; Vasileiou et al., 2013; Wing, Rumney, Preslicka, & Chung, 2008). This intestinal inflammation is mainly due to the high content of organic acids in cranberry juice since the pH (pH around 2.4) is neutralized after gastric digestion. The main organic acids responsible for the high acidity of cranberry juice are citric, malic, quinic and succinic acids (Bazinet, Brianceau, Dubé, & Desjardins, 2012). Many authors report that high titratable acidity is a stimulus for potential inflammation of the gastrointestinal tract (Holzer, 2015; Kress & Waldmann, 2006; Steen, Steen, & Reeh, 1995; Wemmie, Price, & Welsh, 2006). Inflammation is a complex biological response involving a protective defence by immune cells, mediator molecules and blood vessels to a variety of hostile agents (Davies & Hagen, 1997; Fiocchi, 2003). In the intestinal system, inflammation is characterized by the infiltration of inflammatory cells into the intestinal mucosa (Ferguson, Shelling, Browning, Huebner, & Petermann, 2007; Waldner & Neurath, 2009). Caco-2 cells are generally used to measure the integrity of gut epithelium (Daugherty & Mrsny, 1999). Indeed, Caco-2 cells differentiate spontaneously into epithelial cells after 21 days of culture and create a monolayer (Vachon & Beaulieu, 1992) which is an ideal model for intestinal intake (Sun, Chow, Liu, Du, & Pang, 2008; Vermeulen, de Jong, Vaessen, van Leeuwen, & Houdijk, 2011; Zimnicka, Ivy, & Kaplan, 2011).

The deacidification of fruit juices to increase pH and/or decrease the high content of organic acids in food products was successfully performed by a green electrochemical process called electrodialysis with bipolar membrane (EDBM). This eco-friendly technology was applied to fruit juice deacidification, wine stabilization, molecule purification and whey demineralization (Husson, Araya-Farias, Gagné, & Bazinet, 2013; Mikhaylin, Nikonenko, Pourcelly, & Bazinet, 2014; Rozoy, Boudesocque, & Bazinet, 2015; Vera et al., 2007). A recent study by Serre, Rozoy, Pedneault, Lacour, and Bazinet (2016) demonstrated, on a laboratory scale, the feasibility of deacidifying cranberry juice, without altering beneficial compounds, by up to 80% after six hours of EDBM treatment. However, these studies did not determine the impact of deacidification on gut epithelium integrity or on potential inflammation of the gastrointestinal tract, which is a major concern for this functional beverage and its consumption.

In this context, the objectives of the current study are 1) to compare the composition of cranberry juice deacidified by EDBM at different final rates before and after gastrointestinal digestion, and 2) to evaluate the impact of the EDBM deacidification rate on Caco-2 cell monolayer integrity.

2. Materials and methods

2.1. *Cranberry juice*

Treatments were carried out on a pasteurized and clarified cranberry juice produced by Fruit d'Or (Notre-Dame-de-Lourdes, QC, Canada). This raw juice was diluted to obtain a value of 8°Brix, then stored at –20 °C and thawed at 4 °C before each experiment. The physicochemical characteristics of the cranberry juice used in this experiment are presented in Table 1.

Table 1 – Physicochemical characteristics of the raw cranberry juice.

pH	2.35 ± 0.03
Conductivity (mS/cm)	2.7 ± 0.5
Titratable acidity (g/L of citric acid monohydrate equivalents)	9.1 ± 0.6
Total soluble solids (° Brix)	7.2 ± 0.5
Total proanthocyanidins (mg/L)	210.7 ± 7.3
Total polyphenols (mg/L of gallic acid equivalents)	1 001 ± 101
Anthocyanins (mg/L)	
Cyanidin-3-galactose	38.2 ± 0.2
Cyanidin-3-glucose	1.2 ± 0.0
Cyanidin-3-arabinose	37.9 ± 0.2
Peonidin-3-galactose	53.7 ± 0.3
Peonidin-3-glucose	3.8 ± 0.0
Peonidin-3-arabinose	28.6 ± 0.2
Organic acids (mg/L)	
Quinic acid	12 167 ± 1 676
Citric acid	21 451 ± 2 383
Malic acid	13 794 ± 1 837
Succinic acid	3 021 ± 686

2.2. *Deacidification treatment*

The cell configuration and the electrodialytic protocol were similar to those used by Serre et al. (2016) but at a pilot scale. Cranberry juice with different final deacidification rates was produced on a pilot scale after 0, 20, 40, 60 and 80 min of treatment.

2.3. In vitro *digestion*

According to Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005), an *in vitro* digestion process was simulated on treated and untreated cranberry juice. Briefly, samples (12 g) were mixed with simulated saliva (6 mL at pH 6.8 ± 0.2) and incubated at 37 °C for 5 min without agitation. Gastric juice (6 mL at pH 1.3 ± 0.1) was added and each sample was rotated for 2 h at 37 °C. During this period, the pH of each sample was adjusted to 2.4 ± 0.1 with 5 M HCl. Finally, intestinal juice (12 mL at pH 8.1 ± 0.2), bile juice (6 mL at pH 8.2 ± 0.2) and bicarbonate solution (2 mL) were added and agitated for 2 h at 37 °C. In duodenal juice, lipase was not added since it is already present in porcine pancreatin (Sigma-Aldrich, Saint Louis, MO, USA). Three replicates of each sample were *in vitro* digested, frozen at –20 °C and thawed at 4 °C before analyses of organic acid, proanthocyanidin, anthocyanin, total polyphenol content and oxygen radical absorbance capacity. The *in vitro* digested cranberry juice was centrifuged at 3250 × *g* for 15 min and filtered using a 0.22 µm filter prior to use.

2.4. *Co-culture system (Fig. 1)*

The Caco-2 cells and the human leukaemia monocytic cell line (THP-1) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Caco-2 cells were seeded at 3×10^5 cells/well onto Transwell insert plates (12 mm diameter, 0.4 µm pore size; Costar Corp., Cambridge, MA, USA) and grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum, 1% non-essential amino acid solution, 1 mM sodium pyruvate, 100 µg/mL streptomycin,