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Bioaccessibility of curcuminoids in buttermilk in simulated gastrointestinal digestion models

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ABSTRACT

In vitro gastrointestinal digestion models were used to investigate bioaccessibility of curcuminoids delivered with buttermilk. The percentage of solubilised curcuminoids that partitioned into the micelle in aqueous phase was determined. In fasted states (0–2.5 mg bile extract/mL sample), the bioaccessibility of curcuminoids (2% v/v ethanol) ranged from 16.3% to 26.7% in buttermilk, and from 11.4% to 18.7% with neat curcuminoids. In fed states (10–40 mg bile extract/mL sample), the bioaccessibility of curcuminoids in buttermilk was 21.3% (no ethanol) and ranged from 37.1% to 69.2% (2% v/v ethanol), while for neat curcuminoids bioaccessibility was 14.1% (no ethanol), ranging from 45.6% to 79.6% (2% v/v ethanol). The *in vitro* bioaccessibility of curcuminoids was influenced by the presence of the carrier (buttermilk) and ethanol, and increased significantly with increasing amount of bile extract. Curcuminoids did not markedly influence the digestibility of protein or lipids. These findings demonstrated that buttermilk could be used as a carrier for curcuminoids especially if delivered with food.

1. Introduction

Curcuminoids are derived from turmeric roots (*Curcuma longa*) and consist of curcumin (70–80%), demethoxycurcumin (15–25%) and bis-demethoxycurcumin (3–10%), all of which are considered as bioactive polyphenols with beneficial health properties (Chainani-Wu, Collins, & Silverman, 2012; Quiles et al., 2002). The low bioavailability of curcuminoids is a limitation for using these bioactives in clinical treatments and general healthcare. The poor aqueous solubility and stability of curcuminoids at intestinal pH are contributing factors for their low intestinal absorption and bioavailability (Anand, Kunnumakkara, Newman, & Aggarwal, 2007). Additionally, it is known that polyphenols are also transformed into bioactive products by the microflora in the colon (Del Rio, Costa, Lean, & Crozier, 2010). Intestinal absorption of water insoluble compounds, such as curcuminoids, is dependent on their solubilisation in the aqueous intestinal environment *via* the emulsifying action of the bile salts. The bile salts can trap lipophilic compounds in mixed micelles or vehicles, carry them through intestinal cells barriers, and transport them into the blood circulation (Porter, Trevaskis, & Charman, 2007).

Buttermilk is a by-product of dairy processing. There are many types of buttermilk including buttermilk from sweet and sour cream and also from whey. The major components of buttermilk from sweet cream include protein (31.5–33.1% w/w dry weight), fats (5.7–13.1% w/w dry weight) and lactose (48.7–53.8%, w/w dry weight) (Sodini, Morin, Olabi, & Jiménez-Flores, 2006). The potential for utilisation of buttermilk as a carrier for curcuminoids has been suggested, as buttermilk increases curcuminoid solubility and stability at neutral pH (Fu et al., 2014). Although the majority of curcuminoids are associated with proteins in buttermilk, some of the curcuminoids are partitioned into the fat phase of buttermilk (Fu et al., 2014). Indeed, adding lipophilic bioactive compounds to formulas with high fat content has been considered to be an effective way to optimise bioaccessibility, as lipids can promote the solubility of lipophilic compounds in aqueous environment (Fernández-García, Carvajal-Lérida, & Pérez-Gálvez, 2009). However, some *in vivo* studies have shown that milk, milk cream and coconut cream delay and/or compromise polyphenols absorption in the gastrointestinal tract (Mullen, Edwards, Serafini, & Crozier, 2008; Mullen et al., 2009; Vitaglione et al., 2012). The reasons for these *in vivo* phenomena may be related to the interaction between dietary polyphenols and β-lactoglobulin (milk whey protein). Binding of polyphenols to β-lactoglobulin decreased the susceptibility of the protein to digestion and consequently hindered the release of polyphenols from milk and cream matrix (Stojadinovic et al., 2013). Understanding whether buttermilk alters the intestinal absorption of curcuminoids in the gastrointestinal tract and the digestibility of buttermilk in the presence of curcuminoids can

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provide an insight into the utility of using buttermilk as a delivery matrix for curcuminoids.

The bioaccessibility of bioactives is a prerequisite for their bioavailability. It is based on evaluating the percentage of solubilised substance after gastrointestinal digestion and assumes that the solubilised substance may have a high potential to be absorbed by the small intestine (Etcheverry, Grusak, & Fleige, 2012; Fernández-García et al., 2009). Several formulations of curcuminoids using an oil-in-water emulsion and a nanoemulsion (Ahmed, Li, McClements, & Xiao, 2012), an oil-in-water organogel (Yu & Huang, 2012) and nanostructured lipid carriers (Aditya et al., 2013) have been evaluated *in vitro* for bioaccessibility using simulated gastrointestinal digestion models.

The objectives of this study were to assess the bioaccessibility of curcuminoids in buttermilk in simulated gastrointestinal digestion models. The simulated digestion model was designed with the fasted state (0 and 2.5 mg bile extract/mL digested sample) to simulate a digestion condition before meals and the fed state (10 and 40 mg bile extract/mL digested sample) to simulate the condition after meals. The curcuminoids were added directly or solubilised in ethanol prior to the addition to buttermilk, and the influence of ethanol (2%, v/v) present in the buttermilk–curcuminoid formulation on the bioaccessibility of curcuminoids was also determined. This study also evaluated the effect of curcuminoids on the digestibility of proteins and lipids in buttermilk. Additionally, the stability of curcuminoids in buttermilk during simulated gastrointestinal digestion was measured.

2. Materials and methods

2.1. Materials

A turmeric extract (Bio-curcumin®) was kindly provided by Arjuna Natural Extracts Limited (Alwaye, Kerala, India). The curcuminoid content in this Bio-curcumin® was reported to be 88% (w/w), of which 70% was curcumin, 16% demethoxycucumin and 2% bisdemethoxycucmin (Fu et al., 2014). Buttermilk powder was kindly provided by Warrnambool Cheese and Butter Factory (Allansford, Victoria, Australia). According to the manufacturer's specification, the buttermilk powder contained 32.5% protein, 8.4% fat, 50.1% lactose and 2.9% moisture.

Pepsin from porcine gastric mucosa (P7000, Lot.019K1146, 453 units/mg solid and 1079 units/mg protein), pancreatin from porcine pancreas (P7545, Lot.064K1451, 8 × U.S.P specification), bile extract porcine (B8631, Lot.037K0196) and HPLC grade 2-propanol, tetrahydrofuran (THF), heptanes and hexane were all purchased from Sigma Aldrich (St. Louis, MO). HPLC grade acetonitrile, methanol and chloroform were purchased from Merck (Darmstadt, Germany). An internal standard, methyl tricosanoate, (purity > 99.0%) and standard milk fat CRM164 for lipids analysis were also purchased from Sigma Aldrich, as were sodium dodecyl sulphate (SDS) (purity > 99%), EDTA (purity > 99%), DL-dithiothreitol (purity > 99%) and bromophenol blue used for electrophoresis. Glycerol (EMSURE®) was from Merck and food-grade ethanol was from Wilmar BioEthanol (Victoria, Australia).

2.2. Preparation of buttermilk dispersion and Bio-curcumin® stock solution

Buttermilk dispersion (5% total solids, TS, w/w) was reconstituted by mixing buttermilk powder with MilliQ-water and stirring at 45 °C for 30 min using a water-bath. The pH of the freshly reconstituted buttermilk was 6.8. The reconstituted buttermilk dispersion was stored at 4 °C for up to 5 days before use. Bio-curcumin® stock solution (10 mg/mL) was prepared by dissolving 100 mg of Bio-curcumin® in 10 mL of food-grade ethanol. Ultrasonication for 2 min was applied to disperse the curcuminoids.

2.3. Preparation of buttermilk–curcuminoids and neat curcuminoids with and without ethanol

2.3.1. Buttermilk–curcuminoids and neat curcuminoids containing 2% ethanol (v/v)

An aliquot (2 mL) of Bio-curcumin® stock solution (10 mg/mL) was mixed with buttermilk dispersion (5% TS, w/w) to obtain 100 mL of buttermilk–curcuminoid mixture. The same procedures were used to prepare the neat curcuminoid dispersion, except using 5 mM phosphate buffer solution (PBS) (0.011% NaH_2PO_4 and 0.058% Na_2HPO_4, pH 6.8) instead of buttermilk dispersion. All the above mentioned prepared mixtures contained 20 mg of Bio-curcumin® and 2% (v/v) food-grade ethanol. The mixtures were prepared freshly before use. It was previously shown that Bio-curcumin® powder contains 88% curcuminoids (Fu et al., 2014).

2.3.2. Buttermilk–curcuminoids and neat curcuminoids without ethanol

Bio-curcumin® powder (1.0 mg) was added to 5 mL of buttermilk dispersion (5%, TS, w/w) or phosphate buffer (5 mM PBS, pH 6.8), respectively, to prepare buttermilk–curcuminoid and neat curcuminoid samples in absence of ethanol. The mixtures were prepared freshly before use.

2.4. In vitro simulated gastrointestinal digestion of buttermilk, buttermilk–curcuminoids and neat curcuminoids

2.4.1. Preparation of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)

The SGF solution was prepared according to the U.S. Pharmacopeia Convention (United States Pharmacopeial Convention, 2009). Sodium chloride (2 ± 0.01 g) and 7 mL of HCl (37%, w/v) were dissolved in 800 mL of Milli-Q water and the pH was adjusted to 1.2 using 1 M NaOH. Pepsin (6.4 mg/mL) was added to the SGF solution and stirred for 15 min before use.

The SIF solution was prepared following the procedures of Li, Hu, and McClements (2011), and Salvia-Trujillo, Qian, Martín-Belloso, and McClements (2013) with some modifications. The SIF contained 5 mM PBS, 0.4 M NaCl and15 mM $CaCl_2$. The pH of SIF was adjusted to 6.80 ± 0.05 by using 1 M NaOH and 1 M HCl. The bile extract (0, 12, 47 and 188 mg/mL) and pancreatin (10 mg/mL) were freshly mixed in the SIF solution before use. The final concentration of bile extract used to simulate the fasted and the fed state were selected according to the published literature (Holm, Müllertz, & Mu, 2013; Porter et al., 2007). These authors suggested that in human, the bile salts concentrations ranged from 2 to 6.4 mM, and from 0.5 to 37 mM in the fasted and fed states, respectively. Consequently, the equivalent selected bile salts concentrations in the current study were 0 and 2.5 mg/mL in the fasted state, and 10 and 40 mg/mL in the fed state.

2.4.2. Simulated gastric digestion

Aliquots of 5 mL of buttermilk–curcuminoid or neat curcuminoid samples were combined with 5 mL of SGF containing pepsin (6.4 mg/mL). Gastric digestion was performed in a shaking water-bath at 37 °C and 100 rpm (SW23, Julabo, Seelbach, Germany) for 2 h. Digestion was ceased by adjusting pH to 6.8 at which pepsin was denatured and lost its activity (Piper & Fenton, 1956).

2.4.3. Simulated intestinal digestion in fasted and fed states

Digestion in the fasted and fed states were performed under the same conditions, with the exception of bile salts concentrations,