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Interactions of buttermilk with curcuminoids

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ABSTRACT

The ability of buttermilk to carry and stabilise a preparation of curcuminoids was examined. The quenching of intrinsic protein fluorescence confirmed that the curcuminoids interacted with proteins in buttermilk. The Stern-Volmer quenching constant was $\geqslant 9.4 \times 10^3$ M^{-1}. The apparent binding constant of curcuminoids to whole buttermilk was $\geqslant 2.2 \times 10^4$ M^{-1}. Centrifugation of buttermilk (5% total solids, TS) – curcuminoid mixtures demonstrated that curcuminoids were partitioned into the cream (18.0% w/w, 0.64% TS), milk serum (73.3% w/w, 2.86% TS) and the casein-rich precipitate (6.76% w/w, 1.87% TS) fractions in the ratio of 1:3.7:3.5. The interaction of curcuminoids with components in the buttermilk improved its stability, as evidenced by the faster degradation of curcuminoids in phosphate buffer (pH = 6.8) than in buttermilk. The ability of buttermilk to carry and stabilise curcuminoids has the potential to enable the delivery of these components into functional foods.

1. Introduction

Curcuminoids are major phenolic compounds that are isolated from turmeric (*Curcuma longa*) and contain about 70–80% of curcumin, 15–25% of demethoxycurcumin, and 3–10% of bisdemethoxycurcumin (Quitschke, 2012). Concentrated curcumin and curcuminoids have many therapeutic functions including anti-HIV, anti-inflammatory and anti-oxidative effects and are also protective against Alzheimer's disease, cystic fibrosis and colon cancer (Anand, Kunnumakkara, Newman, & Aggarwal, 2007; Nishikawa, Tsutsumi, & Kitani, 2013; Quitschke, Steinhauff, & Rooney, 2013). The compounds of curcuminoids have amphiphilic properties, relative high solubility in organic solvents such as ethanol, acetone and dimethyl sulfoxide (DMSO), but low solubility in aqueous solutions. The solubility of curcumin has been reported to be less than 0.03 μM in buffer at pH < 7 (Sahu, Kasoju, & Bora, 2008; Tonnesen, 2002). The low solubility of curcuminoids in aqueous solutions, its susceptibility to degradation in aqueous environments (Price & Buescher, 1997; Tonnesen & Karlsen, 1985) and low bioavailability (Anand et al., 2007) compromise its usefulness as a bioactive ingredient in functional foods.

Curcumin interacts with proteins and lipids by moving into the hydrophobic groups on these molecules (Gupta et al., 2011). The binding of curcumin to phospholipids in membranes and proteins, and entrapment of curcumin in hydrogels have been used to enhance the solubility and stability of curcumin in aqueous systems (Tapal & Tiku, 2012). Proteins, such as α-lactoglobulin, human serum albumin, fibrinogen, bovine serum albumin and soy proteins have been found to be effective carriers for curcumin (Bourassa, Kanakis, Tarantillis, Pollissiou, & Tajmir-Riahi, 2010; Leung & Kee, 2009; Sneharani, Karakkat, Singh, & Rao, 2010; Tapal & Tiku, 2012). Phospholipids have also been reported to have curcumin-carrying potential (Began, Sudharshan, Udaya, & Rao, 1999; Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2007).

Curcuminoids, which include curcumin, demethoxycurcumin and bisdemethoxycurcumin, are naturally present in concentrated or highly purified curcumin extracts. Curcumin, demethoxycurcumin and bisdemethoxycurcumin can all interact with proteins and cells (Anuchapreeda, Tima, Duangrat, & Limtrakul, 2008; Mohammadi, Bordbar, Mohammadi, Divsalar, & Saboury, 2010). The low solubility of curcuminoids in aqueous solution, their susceptibility to degradation and low bioavailability, which compromise their bioavailability and their application in functional foods, may potentially be overcome by using a carrier (Im, Ravi, Kumar, Kuttan, & Maliakel, 2012).

Buttermilk is a by-product of butter manufacture and contains milk proteins (caseins and whey proteins), milkfat and the milkfat globule membrane (Sodini, Morin, Olabi, & Jiménez-Flores, 2006). The presence of proteins and lipids, including the phospholipids of the milkfat globule membrane in buttermilk, makes it an attractive carrier for curcuminoids. In this study, the potential of buttermilk as a carrier for curcuminoid was investigated using fluorescence spectroscopy. A turmeric extract (Bio-curcumin®) was used as a source of curcuminoids. Curcumin is the major component of turmeric extracts which also contains other curcuminoid

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complex such as demethoxycurcumin and bisdemethoxycurcumin. The Stern Volmer quenching constants and apparent binding constants for curcuminoids to buttermilk and its fractions were determined from fluorescence measurements. In addition, the stability of curcuminoids in phosphate buffer and buttermilk at pH 6.8 was investigated.

2. Materials and methods

2.1. Materials

A turmeric extract (Bio-curcumin®) was kindly donated by Arjuna Natural Extracts Limited (Alwaye, Kerala, India). According to the supplier's specification sheet, this turmeric extract contains 95.8% total curcuminoid complex which consists of curcumin, curcuminoids, and volatile oils from turmeric. A curcumin standard used for quantification, with a purity of 99% curcuminoids of which 93% was curcumin and 7% was demethoxycurcumin, was purchased from Sigma–Aldrich (Sydney, Australia). Buttermilk powder was obtained from Warrnambool Cheese and Butter Factory (Allansford, Victoria, Australia). According to the manufacturer's specification, the buttermilk powder contained 32.5% protein, 8.40% fat, 50.1% lactose and 2.9% moisture. Sodium phosphate dibasic (purity > 99%), sodium phosphate monobasic (purity > 99%) and HPLC grade ethanol were purchased from the Sigma–Aldrich (Sydney, Australia).

2.2. Characterisation of turmeric extract (Bio-curcumin®) using a HPLC–DAD assay

A curcumin standard stock solution (10 mM) was prepared in ethanol (100%). To facilitate dissolution of the curcumin, the mixture was ultrasonicated (WiseClean, WUC-A02H, Australia) for 2 min.

The HPLC method used was based on a reported method (Jayaprakasha, Rao, & Sakariah, 2002) with some modification. Briefly, the curcuminoids (curcumin, bis-demethoxycurcumin and demethoxycurcumin) in the turmeric extract (Bio-curcumin®) were separated using a guard column (4.6 mm × 5 mm, particle size 5 μm, Phenomenex, Luna™, USA) and a C_{18} silica saturator column (4.6 mm × 250 mm, particle size 5 μm, SUPELCO, USA) equipped with a Shimadzu HPLC System (Japan). The HPLC comprised a SCL-10A System Controller (version 5.42), a SIL 10 AD Auto-injector, a DGU-14A Degasser and a SPD-M10A Diode Array Detector. The mobile phase was composed of 2% acetic acid (v/v) in MilliQ water as Mobile phase A, and 2% acetic acid (v/v), and 10% methanol (v/v) in acetonitrile as Mobile phase B. The gradient program was as follows: 0–18 min, 45% B increasing to 65% B; 18–23 min, decreasing to 45% B. The column temperature was 40 °C and the sample temperature was at ~25 °C.

HPLC analysis of curcuminoids showed three well separated peaks at retention times of 15.4, 14.4 and 13.3 min (curcumin, demethoxycurcumin and bis-demethoxycurcumin) respectively (chromatogram not shown). Curcumin and demethoxycurcumin in Bio-curcumin® were identified by the retention time of curcumin and demethoxycurcumin peaks obtained from curcumin standard. The contents of curcumin and demethoxycurcumin in samples were quantified by an external standard method using standard calibration plot of mass of compound (μg) versus peak area. As the external standard used did not contain bisdemethoxycurcumin, the content of bisdemethoxycurcumin was calculated based on the ratio of the peak area for bisdemethoxycurcumin to that of the other components. The limit of quantification (LOQ) of curcumin and demethoxycurcumin was 0.02 μM. The amount of curcumin and demethoxycurcumin in Bio-curcumin® was 70.3 ± 0.5% and 15.0 ± 0.2% respectively, calculated using external standard. The percentage of bisdemethoxycurcumin was estimated to be approximately 1.8%, by ratio of peaks at in Bio-curcumin®. These values were used to estimate the molar concentration of curcuminoids in all samples.

2.3. Preparation of buttermilk. Buttermilk fractions and Bio-curcumin® solutions

Buttermilk dispersions (5% TS, w/w) were reconstituted with MilliQ water using a water-bath at 45 °C with overhead stirring for 30 min. Buttermilk dispersions (0.5% and 0.005% TS, w/w) were also prepared by reconstituting with 10 mM phosphate buffer (pH = 6.8) and overhead stirring at 45 °C for 30 min. The pH of the fresh buttermilk dispersion was 6.8. All samples were stored at 4 °C and used over 5 days.

Buttermilk fractions were prepared by centrifugation of buttermilk dispersions (5% TS, w/w) at 30,000*g*, 60 min at 20 °C to obtain a cream fraction (top layer), a milk serum fraction (middle layer) and a casein-rich precipitate (bottom pellet layer). These fractions were used separately in the partitioning experiments. In addition, skimmed buttermilk sample was prepared by mixing the milk serum and casein pellet fractions at 45 °C for 30 mins and used for the quenching studies.

Stock solutions of Bio-curcumin® (1.0 mg/mL and 3.7 mg/mL) which correspond to preparations containing 2.4 and 9.0 mM curcuminoids were prepared in ethanol (100%). Ultrasonication (WiseClean, WUC-A02H, Australia) for 2 min was required to facilitate dissolution of stock solutions of Bio-curcumin®. The stock solution of Bio-curcumin® was diluted with 100% ethanol to obtain the working solutions, used for preparing mixtures of buttermilk or its fractions with curcuminoids.

Buttermilk (5% TS, w/w) – curcuminoid (0–18.4 μM) mixtures and fractions of buttermilk with curcuminoids (0–18.4 μM) in Milli-Q water were prepared by combining the required amounts of buttermilk or its fractions and Bio-curcumin® working solutions. Buttermilk (0.005% and 0.5% TS, w/w) – curcuminoid (9 μM) mixtures were also prepared in phosphate buffer. These mixtures were used for fluorescence experiments. The ethanol concentration in the final mixtures was maintained at 2% (v/v) in order to avoid any effect on protein structure. The mixture was stirred at ~22 °C for 30 min.

2.4. Fluorescence measurement

Fluorescence measurements were carried out using a Varioskan Flash Multimode Reader (Thermo Scientific, Australia). The fluorescence of curcuminoid dispersions (in phosphate buffer, 2% ethanol v/v) and buttermilk–curcuminoid mixtures (in 2% ethanol v/v) were measured at an excitation wavelength of 420 nm. The emission spectra were recorded from 450 nm to 700 nm. The fluorescence intensity was contributed by the presence of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the samples at excitation of 420 nm. The protein intrinsic fluorescence of buttermilk (containing 2% ethanol v/v) and buttermilk–curcuminoid mixtures (containing 2% ethanol v/v) were measured at an excitation of 280 nm. Emission spectra were recorded from 300 to 600 nm. The spectral resolution was 2 nm for emission.

2.5. Determination of quenching and apparent binding constants

The constant for protein quenching was calculated from Stern–Volmer equation (Liang, Tajmir-Riahi, & Subirade, 2008; Skrt, Benedik, Podlipnik, & Ulrih, 2012) from data obtained at an excitation wavelength λ_{280nm}.