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In vitro fermentation of anthocyanins encapsulated with cyclodextrins: Release, metabolism and influence on gut microbiota growth

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ABSTRACT

Anthocyanins are thought to exert protective influences on chronic gut disorders such as colon cancer and inflammatory bowel disease. They may also positively modulate intestinal bacterial populations. However, their bioavailability in the gastrointestinal tract is an important determinant factor for their *in vivo* activity. In this study, we attempted to increase their stability by encapsulation with cyclodextrins. From anaerobic batch-culture fermentation experiments with gut bacteria, release of anthocyanins and the formation of phenolic microbial metabolites were quantified. Despite a rapid release of anthocyanins observed within the first 30 min, encapsulation allowed anthocyanin degradation to be slowed down. As a consequence of anthocyanin degradation phenolic acids were formed. Quantitative analysis of bacterial populations revealed that there was a significant growth of members of the domain *Bacteria* in vessels with malvidin-3-glucoside, compared to the negative control, and there was inhibition of the *Clostridium histolyticum* group in those vessels where delphinidin-3-glucoside or cyanidin-3-glucoside was added. These results illustrate the ability of encapsulation to increase bioavailability of anthocyanins, allowing them to be released in the colon and to exert their potential health benefits.

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Chemical compounds: Cyanidin-3-glucoside (PubChem CID: 12303203); Delphinidin-3-glucoside (PubChem CID: 443650); Malvidin-3-glucoside (PubChem CID: 443652); Ferulic acid (PubChem CID: 445858); Gallic acid (PubChem CID: 370); Syringic acid (PubChem CID: 10742).

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1. Introduction

Interest in anthocyanin health benefits has increased over the last several years. *In vitro* and *in vivo* studies have shown that anthocyanins may exert a wide range of biological activities such as antioxidant capacity, cardioprotective effects, anti-inflammatory properties, reduction in the risk of diabetes and inhibition of tumour cell growth, especially those in the colon (He & Giusti, 2010; Norberto et al., 2013; Zafra-Stone et al., 2007). Some researchers have also proposed that anthocyanins can influence health by modulating gut microbial community composition (Forester & Waterhouse, 2010; Hidalgo et al., 2012). In this regard, microbial activities have been related to different disease outcomes (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012).

However, despite their health promoting effects, the use of anthocyanins has been hindered by their low chemical stability under physicochemical conditions they are exposed to after oral consumption by humans (Fleschhut, Kratzer, Rechkemmer, & Kulling, 2006). Limited available experimental evidence indicates that in the acidic conditions that prevail in the gastric compartment, anthocyanins occur under the red-coloured flavylium cations form (pH ~2). When they pass to the intestine and the pH shifts from acidic to close to neutral or mildly alkaline, anthocyanins are converted to an unstable form, blue quinoidal base, by the loss of protons (Woodward, Kroon, Cassidy, & Kay, 2009). As a consequence, such transformations contribute towards a low bioavailability of anthocyanins (Vitaglione et al., 2007).

Encapsulation may provide a robust means to stabilize anthocyanins and thus increase availability in the intestine. Different procedures have been used for food product encapsulation (Aceituno-Medina, Mendoza, Rodríguez, Lagaron, & López-Rubio, 2015; Desai & Park, 2005; Martín, Mattea, Gutierrez, Miguel, & Cocero, 2007). In particular, several materials may be considered as capsule matrices for anthocyanins, including maltodextrin, cyclodextrins (CDs), pullulan, glucan gel, curdlan, sodium alginate and pectin (Fernandes, Sousa, Azevedo, Mateus, & de Freitas, 2013; Ferreira, Faria, Grosso, & Mercadante, 2009; Tonon, Brabet, & Hubinger, 2010).

Among the materials used to encapsulate anthocyanins, CDs offer some advantages. They have the capacity to protect bioactive food components from the deleterious conditions in the stomach and upper small bowel, allowing them to be liberated in the colon (Kosaraju, 2005) and thus boost their beneficial effects, e.g. inhibition of the growth of tumour cells (Tsukahara & Murakami-Murofushi, 2012). CDs possess macrocycles that present a torus-shaped structure with an adaptable hydrophobic cavity, which gives them the ability to form reversible inclusion complexes with a wide variety of organic compounds (often phenolic substances). Concerning anthocyanins, they include in their structure hydrophobic aromatic moieties and hydrophilic polar groups like hydroxyl groups. This amphiphilic character makes anthocyanins a good candidate for molecular inclusion with cyclodextrins (Dangles, Wigand, & Brouillard, 1992).

However, despite the potential benefits of encapsulation to increase bioavailability, its use has not been widely examined by food and nutritional researchers. In this line, most of the studies found in the literature evaluate anthocyanin encapsulation techniques mainly to protect them from thermal or pH degradation for use as natural food colourants (Ferreira et al., 2009). The vast majority of knowledge about targeted release of encapsulated molecules in the gut has been obtained from research concerning delivery of drugs (Kosaraju, 2005).

The present study aimed to evaluate the influence of the human gut microbiota on the degradation of CDs coverage and the subsequent release of anthocyanins. In addition, it includes an evaluation of bacteria–anthocyanin interactions using *in vitro* batch culture systems modelling the human colon. Changes in the faecal microbiota were evaluated using 16S rRNA-based fluorescence *in situ* hybridization (FISH), whereas the potential biological effects of anthocyanin intervention metabolic end products were assessed by short chain fatty acid (SCFA) analysis. Changes in anthocyanins and phenolic microbial metabolites were also monitored by HPLC and LC-MS analysis.

2. Materials and methods

2.1. Chemicals

Standards of gallic acid and syringic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, Spain), ferulic acid was obtained from Koch-Light Laboratorie Ltd. (Colnbrook, Bucks, England) and cyanidin-3-glucoside, delphinidin-3-glucoside and malvidin-3-glucoside were supplied by Extrasynthèse (Genay, France). Formic, lactic, acetic, propionic and butyric acids were provided by Sigma-Aldrich Co. Ltd (Poole, Dorset, UK). β-CDs were supplied by Fluka (Madrid, Spain).

General chemicals and reagents were obtained from Sigma-Aldrich Co. Ltd. (Poole, Dorset, UK) or Fisher (Loughborough, Leics, UK). Bacteriological growth media supplements were purchased from Oxoid Ltd. (Basingstoke, Hants, UK). Raftilose P95 fructooligosaccharide was supplied from Beneo (Tienen, Belgium). Isopore (0.22 μm) membrane filters were obtained from Millipore Corp. (Watford, Hertfordshire, UK).

All the nucleotide probes used for fluorescent *in situ* hybridization (FISH) were commercially synthesized and labelled with the fluorescent dye Cy3 at the 5′ end (Sigma Aldrich Co. Ltd., Spain). Sterilization of media and instruments was carried out by autoclaving at 121 °C for 15 min.

2.2. Encapsulation of anthocyanins with CDs

A stock solution of each anthocyanin (1.0×10^{-4} M) was prepared using 0.1 M HCl. The low pH ensured that most of the compounds were in the flavilium form and thus prevents pigment degradation. Different concentrations of β-CD (4×10^{-4}, 2.5×10^{-3} and 5×10^{-3} M) were dissolved at 40–45 °C with agitation in deionized water. Then, the solution was cooled and each one of the anthocyanins in solution was added. The reaction was carried out for 24 h at 25 °C, with stirring under a nitrogen atmosphere. Water was removed by freeze-drying. Absorbance of the encapsulates was monitored at 517 nm on a microplate reader (Biotek Instruments, Winooski, VT, USA).