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Effects of ciceritol from chickpeas on human colonic microflora and the production of short chain fatty acids by *in vitro* fermentation

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ABSTRACT

The aim of this study was to evaluate the effects of ciceritol on human colonic microflora and the production of short chain fatty acids (SCFAs). Ciceritol was extracted from chickpeas by using 50% ethanol-water solvent with a ratio of 1:10, and the extract was purified by chromatography of charcoal-Celite column and gel chromatography of Biogel P-2 column. Bacterial population and the concentration of SCFAs during *in vitro* anaerobic fermentation were investigated to evaluate the effect of ciceritol on human colonic microflora. The results indicated that the addition of ciceritol could significantly enhance the growth of *Lactobacillus–Enterococcus* group (8.26 compared to 7.71 $\log_{10}$ cells/mL of control group) and *Bifidobacterium* spp. (10.43 compared to 9.45 $\log_{10}$ cells/mL of control group), and inhibit the growth of *Bacteroides–Prevotella*, *Clostridium histolyticum* and *Eubacterium–Clostridium* groups. Besides, the production of SCFAs was significantly improved by addition of ciceritol that the content was twice of the control group. Accordingly, we conclude that ciceritol can behave as a potential prebiotics by optimizing the microflora of human colon and promoting the production of SCFAs, which will benefit to human health.

1. Introduction

Chickpea (*Cicer arietinum* L.) is the third most essential pulses in the world with a production of 13.3 million tons in 2013 and grown in over fifty countries across Asia, North Africa, South Europe, America and Australia. Recently, the 68th United Nations General Assembly declared 2016 as the International Year of Pulses (Foyer et al., 2016). Like other legumes, its seeds are not only considered as a good source of protein, but also rich in polyphenols, high-content dietary fibres and complex carbohydrates (Asif, Rooney, Ali, & Riaz, 2013; Morales et al., 2015; Ruiz et al., 1996; Singh, Subrahmanyam, & Kumar, 1991). For carbohydrates of chickpeas, mono-, di-, oligo- and polysaccharides are all included (Chavan, Kadam, & Salunkhe, 1986; Sun et al., 2014). Generally, oligosaccharides are composed by 2–10 monosaccharide units, and the amount of oligosaccharides (based on dry mass) is around 10.4–17.0% for different species of chickpeas (Saini & Knights, 1984; Xiang et al., 2008). Specifically, chickpea grains are a good source of α-galactooligosaccharide (α-GOS), mainly ciceritol, raffinose, stachyose and verbascose. Furthermore, ciceritol, *O*-α-D-galactopyranosyl-(1 → 6)-α-D-galactopyranosyl-(1 → 2)-1D-4-*O*-methyl-*chiro*-inositol (Fig. 1A), is a characteristic trisaccharide in chickpea (Bernabe et al., 1993; Quemener & Brillouet, 1983; Sánchez-Mata, Penuela-Teruel, Camara-Hurtado, Diez-Marques, & Torija-Isasa, 1998; Xiang et al., 2008).

Due to the specific properties, oligosaccharides play an important role on human's health, for example, the sweetness of oligosaccharides is relatively low, so they can be used as a sweeter for low calories foods (Crittenden & Playne, 1996). Besides, non-digestible oligosaccharides which usually have fructose, glucose and xylose units (Mussatto & Mancilha, 2007) could not be digested due to the lack of enzymes in human body, so they can be utilized by microflora as substrate in the colon (Gibson & Roberfroid, 1995). It has been reported that non-digestible oligosaccharides can modify the gut microflora through improving the growth of beneficial bacteria (such as bifidobacteria and lactobacilli) and inhibiting the growth of pathogenic and putrefactive bacteria (Delzenne & Roberfroid, 1994; Fernando et al., 2010; Johnson, Thavarajah, Combs, & Thavarajah, 2013; Younes, Demigné, & Rémésy, 1996). In addition, they are fermented by colonic flora to produce a mixture of short-chain fatty acids (SCFAs, mainly acetic,

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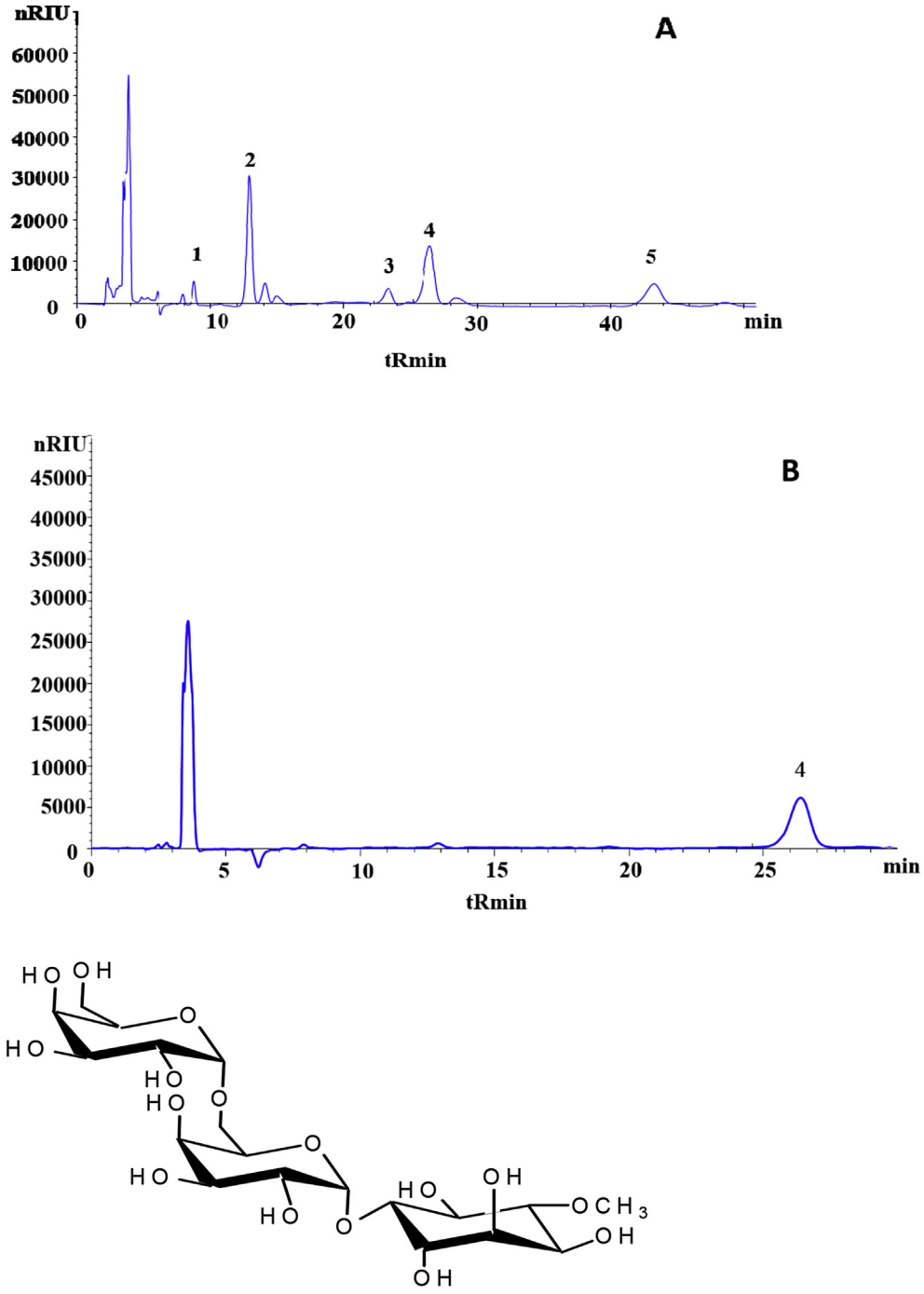

Fig. 1. The chemical structure of ciceritol, chromatographic profile of sugars in ethanol extraction of chickpeas (A: 1, frucose; 2, surcose; 3, raffinose; 4, ciceritol; 5, stachyose) and purified ciceritol (B: 4, ciceritol).

propionic and butyric acids.

In terms of gut microflora, they are distributed in three regions, stomach, small intestine and colon. The population of microflora in colon is the heaviest, about 10^{11}–10^{12} CFU/mL of contents (Cummings, Gibson, & Macfarlane, 1988). Strict anaerobic microflora are the predominant species in colon, such as *Bacteroides* spp. *Eubacteriu* spp. and *Bifidobacterium* spp. the amount of facultative anaerobes (such as lactobacilli, enterococci and streptococci) is 1000 fold smaller (Gibson & Roberfroid, 1995). Colonic microflora can produce antimicrobials to protect human body, and antimicrobials are the microbial barrier to infection, for example, the antimicrobials created by bifidobacteria are able to inhibit Gram-positive and negative organisms (Gibson & Wang, 1994). Further, beneficial bacteria in colon can compete with pathogens for space and nutrients, which will exclude pathogens (Rastall, 2004). The balance of bacteria in colon is necessary for their significant benefit to the host. Consequently, it is important to keep the balance of microflora in colon (Fooks, Fuller, & Gibson, 1999). Normally, prebiotics have effect on simulating the growth of beneficial bacteria in colon, they can alter the composition of colonic bacteria to a healthier profile (Fooks et al., 1999). Oligosaccharides as prebiotics have been reported that they are able to change the composition of