Journal of Functional Foods 24 (2016) 351–358

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Hypocholesterolaemic and prebiotic effect of partially hydrolysed psyllium husk supplemented yoghurt

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ARTICLE INFO

Article history:
Received 13 May 2015
Received in revised form 21 April 2016
Accepted 25 April 2016
Available online 4 May 2016

Keywords:
Psyllium husk
Partially hydrolysed psyllium husk
Yoghurt
Hypocholesterolaemic effect
Prebiotic effect

ABSTRACT

Wistar rats fed with partially hydrolysed psyllium husk (PHPH) supplemented yoghurt for 60 days showed a significant ($P < 0.05$) decrease of 10.6% in triacylglycerol level (TGs), 16.2% in total cholesterol (TC), 53.01% in low density lipoprotein (LDL)-cholesterol and an increase of 25.49% in high density lipoprotein (HDL)-cholesterol in their blood. In addition, short chain fatty acid (SCFA) concentration and lactobacilli count in the faecal matter of PHPH supplemented yoghurt fed rat group were high compared to the faecal matter of the native psyllium husk supplemented yoghurt fed rat group and other control groups (positive, negative and test control). Results showed that psyllium husk retained its hypocholesterolaemic property on partial hydrolysis besides the increase in its prebiotic effect. Therefore, the biofunctional effect of PHPH on cardiovascular and gut health (by qualitatively changing the gut microbiota) can be accounted for hypocholesterolaemic and prebiotic effects, respectively.

1. Introduction

Psyllium husk (Isabgol) (*Plantago ovate*) is popularly used in the traditional Indian medicine system (*Ayurveda*) for prevention of skin irritations, haemorrhoids, constipation as well as diarrhoea. It is a gel forming mucilage known for its laxative effect due to its high-water holding capacity, which is approximately 80 times of its weight. The seed husk contains about 78% soluble fibres and 13% insoluble fibres (Yu, Lutterodt, & Cheng, 2008; Yu & Perret, 2003). It has a scientifically proven use for treatment of constipation (Bouchoucha, Faye, Savarieau, & Arsac, 2004; Ramkumar & Rao, 2005), diarrhoea (Washington, Harris, Mussellwhite, & Spiller, 1998), inflammatory bowel disease-ulcerative colitis (Fernandez-Banares et al., 1999), obesity in children and adolescents (Pittler & Ernst, 2004), reducing high LDL-cholesterol (Anderson, Allgood, Turner, Oeltgen, & Daggy, 1999; Anderson et al., 1995, 2000; Rodriguez-Moran, Guerrero-Romero, & Lazcano-Burciaga, 1998; Romero, West, Zern, & Fernandez, 2002), reducing hyperglycaemia, reducing risk of colon cancer, and weight management, among others (Singh, 2007). Psyllium husk fibre appeared to be one of the most effective with the least adverse effects (Bell, Hectome, Reynolds, Balm, & Hunninghake, 1989; Bell, Hectorn, Reynolds, & Hunninghake, 1990). In previous studies it has been found that psyllium husk is poorly fermented in the colon, but still it was

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http://dx.doi.org/10.1016/j.jff.2016.04.028
1756-4646/

able to produce a moderate concentration of SCFA (Campbell, 1997) and thus promote the growth of probiotic microbes (Elli, Cattivelli, Soldi, Bonatti, & Morelli, 2008). Psyllium has been approved by the United States Food and Drug Administration (USFDA) in 1998 for lowering blood cholesterol, thus preventing the risk of coronary heart disease, with 2.4 g of psyllium husk in a single serving of food, i.e. 228 g/234 ml (FDA, 1998). The USFDA further determined that 4 servings of it are likely to provide the effective daily dose. Psyllium has also received Food for Specific Health Use (FOSHU) approval from the Japanese Ministry of Health, Labour and Welfare as a dietary fibre food to modify gastrointestinal conditions and reduce cholesterol and triacylglycerols.

The high water absorbing capacity of native psyllium husk leads to gel formation and thus limits its use in dairy products like yoghurt for the supplementation in the amount recommended by USFDA. To overcome this problem, the native psyllium husk was partially hydrolysed at a condition optimized for maximum bile binding capacity and the prebiotic effect by response surface methodology (RSM) using Viscozyme L. It reduces psyllium's water absorbing capacity and enables supplementation in yoghurt in the amount recommended for claiming health benefits.

In the present investigation, PHPH supplemented yoghurt was evaluated for hypocholesterolaemic effect and prebiotic effect *in-vivo* using hypercholesterolaemia-induced Wistar rats.

2. Materials and methods

2.1. *Psyllium husk*

Native psyllium husk (98% pure) was procured from the local market (Sat Isabgol, BG Telephone Brand, Sidhpur, Gujarat, India). The husk was grounded into powder (using electric Grinder) and sieved through 40 mesh sizes. The powder was stored in a plastic air-tight container until use.

2.2. *Viscozyme L*

The commercial food-grade multi-enzyme "Viscozyme L" was procured from Sigma-Aldrich, St. Louis, MO, USA. The multi-enzyme complex contains various carbohydrase activities, including cellulase, hemicellulase, xylanase, arabinase and β-glucanase activities. The specifications of multi-enzyme were: density of enzyme = 1.2 g/ml at 25 °C and enzyme activity = 100 fungal beta-glucanase (FBG)/ml. Thus, 8 µl is equal to 1 unit of enzyme. The enzyme was used for the preparation of partially hydrolysed psyllium husk (PHPH).

2.3. *Yoghurt starter culture*

Yoghurt starter culture was procured from the National Centre for Dairy Culture (NCDC), NDRI, Karnal, Haryana, India. It comprised *Lactobacillus bulgaricus* (NCDC–9) and *Streptococcus thermophilus* (NCDC–74).

2.4. *Partially hydrolysed psyllium husk (PHPH) preparation*

The native psyllium husk was hydrolysed in solid state condition, according to the methodology described by Yu et al. (2003). The hydrolysis conditions (enzyme concentration, time and temperature) were optimised using response surface methodology (RSM) (Liyana-Pathirana & Shahidi, 2005; Montgomery, 2001) for maximum hypocholesterolaemic and prebiotic effects *in-vitro*. Native psyllium husk powder (40 mesh size) was taken and Viscozyme L was added in a concentration of 20.0 units/gm of native psyllium husk powder. The mixture was kept at 42.5 °C for 12.0 hours. The reaction was stopped by microwave treatment for 2 min.

2.5. *Dispersible partially hydrolysed psyllium husk (PHPH) powder*

Dispersible PHPH powder was prepared as per the method described by Rudin (1985). As per the method, 100 g PHPH powder was added into a mixture of 86.67 g ethyl alcohol (Merck Ltd., Mumbai, Maharashtra, India) and 13.33 gm of Tween-60 (Merck Ltd., Mumbai, Maharashtra, India) and kept overnight. Finally, the mixture was dried in the air for 10–12h at 37 °C and the resulting dispersible PHPH powder was used to prepare yoghurt.

2.6. *Preparation of stirred yoghurt*

To prepare yoghurt, standardized skim milk with SNF 8.5% was used. To this, 8% sugar and 1% psyllium husk powder (native and partially hydrolysed) were added to prepare native psyllium husk supplemented yoghurt and PHPH supplemented yoghurt, respectively. The mixture was mixed well and pasteurized at 95 °C for 10 minutes. Then, cooled to 42 °C and 3–4% yoghurt culture (1:1 ratio of *L. bulgaricus* and *S. thermophilus*) was added under sterilized condition and incubated at 42 °C for 6 hours. Then, set yoghurt was blended with food grade strawberry flavour and food grade red colour and packaged and stored at 4 °C.

2.7. *Experimental animals*

Forty male albino rats of Wistar strain weighing 100–150 gm were procured from the Small Animal House of the NDRI (Karnal, Haryana, India) and approved by the Institute Animal Ethical Committee (IAEC) of NDRI, Karnal, Haryana (India). The animals were housed in polypropylene cages, which were well ventilated, and have air-conditioned room to maintain the relative humidity of 55.0 ± 5% at 22.0 ± 1 °C. A 12 hour light and 12 hour dark cycle was maintained. The animals were maintained in accordance with the guidelines of the IAEC of the institute.

2.8. *Grouping and their feeding schedule*

The rats were allowed a 7 day adaptation period to remove the effect of stress possibly experienced by the animals due to separation from the main stock. All groups were fed *ad libitum* on a synthetic diet (composition given in Table 1) and water. After adaptation to diet, rats were assigned to five groups consisting of 8 rats in each group on the basis of their body weight and age so that the mean body weight and mean age of four groups did not differ ($P > 0.01$) at the beginning of the experiment. Grouping of the rats was done as follows: