The application of local Dahlia tuber (*Dahlia pinnata* L) as prebiotics for improving viability of probiotics *Bifidobacterium bifidum* in yoghurt[1]

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ABSTRACT: The objective of this recent study was to examine the potential application of inulin extracts obtained from local dahlia tubers (*Dahlia pinnata* L). Inulin was extracted from dahlia tubers and this defined as inulin extracts which contain total proteins 3.7%, crude fats 2.8% and total fibres 1.3% of the dry weight basis. The supplementation of inulin extracts at 40 mg/g total solid showed the optimum level to support the growth of probiotics *Bifidobacterium bifidum* in vitro. The same concentration of inulin extracts in yoghurt fermentation accelerated the growth of probiotic as shown with higher concentration of *Bifidobacterium bifidum* (log 8.8 cfu/ml) than in control (log 8.6 cfu/ml). Supplementation of inulin extracts in yoghurt fermentation also increased the level of lactose degradation (59%) as compared to control (26%), and accumulated a higher level of acidity (1%) than in control (0.68%). As conclusion, inulin extracts harvested from dahlia tubers was potental prebiotics as seen in promoting the growth of probiotics and accelerating lactic acid production.

Key words: inulin, prebiotics, probiotics, dahlia tubers, yoghurt

INTRODUCTION

Lactic Acid Bacteria (LAB) are group of microorganisms which is known as starter cultures in many fermented dairy products, such as cheeses, yoghurt, sour milks, kefirs, and nowadays it is popular as probiotics. The roles of LAB in dairy fermentation is associated to the ability to convert lactose to lactic acid, to degrade casein, to produce antibiotics-like substances and are generally regarded as safe bacteria (GRAS). LAB is Gram-positive and generally catalase-negative without spore-forming and grows under microaerophilic to strictly anaerobic conditions. According to Klein et al. (1998), the most important genera of LAB are *Lactococcus, Lactobacillus, Enterococcus, Pediococcus, Weissella, Carnobacterium, Tetragenococcus, Bifidobacterium, Streptococcus* and *Leuconostoc*. From all of these genera, *Lactobacillus* and *Bifidobacterium* are commonly used as probiotics (Roberfroid, 2000). These two genera are typically chemoorganotrophic and ferment carbohydrate with lactic acid as a major end product. Their resistance to low pH and bile salts as well as their ability to survive gastrointestinal conditions is examples of physiological characteristics for their function as probiotics (Fuller, 1989). Probiotics are defined as live microorganisms that provide beneficial effects on human health (Havenaar and Veld, 1992 *cit* Kailasapathy et al., 2000). The ability to support human health is associated with their function to reduce lactose intolerance, to inhibit the growth of pathogens, to prevent gastrointestinal diseases, to induce body's immunity, to decrease blood cholesterol level and to minimize growth of cancer and tumor cells (Havenaar and Veld, 1992; Ouwehand et al., 1999).

Prebiotics are defined as non-digestible food components which are selectively consumed by gut probiotics resulting in beneficial effects to the host (Roberfroid, 2000). Non-digestible oligosaccharides, such as fructo-oligosaccharides, are main utilized prebiotics which support the growth of endogenous bifidobacteria in the human gut (Roberfroid, 200b). Other oligosaccharides are also known as prebiotics, and this includes galacto-oligosaccharides and inulin. Fermentation of these carbohydrates resulted in the generation of short-chain fatty acids, e.g. acetic acid, butyric acid and

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propionic acid, which function as energy sources (Grajek et al., 1995). Dahlia tubers contain water (90%) and the rest (10%) is total solid, in which 85% of the solid are inulin and other cellulolytic compounds (Rohdiana, 2006). The objective of this recent study was to explore potential function of inulin extracted from dahlia tubers as prebiotics to support the growth of probiotic *bifidobacterium bifidum* in yoghurt products.

MATERIALS AND METHODS

Bacterial Strains, growth media and materials utilized

Bacterial strains used in this study were *Streptococcus thermophilus* FNCC041, *Lactobacillus bulgaricus* FNCC040 and *Bifidobacterium bifidum* ATCC 29521. All strains were grown on MRS agar or MRS broth (Oxoid), and were sub-cultured at two weeks intervals and stored at 4^0C. For culturing *Bifidobacterium bifidum*, the growth media was added with bile salts 0.15% and was incubated at a microaerophilic conditions with the addition of L-*cystein* HCl 0.05%. Other materials utilized were ethanol 30% and 70%, skim milk powder, distilled water, tomato juice, Kalium Iodida (KI) 10%, HCl 2 N, NaCl 0.86%, glycerol, sucrose, NaH_3, H_2SO_4, $Na_2S_2O_3$, $ZnSO_4$, NaOH 0.75 N, NaOH 0.1 N, NaOH 50%, methylene blue 0.02% and Chloramine-T.

Inulin extraction from Dahlia tubers

Inulin was extracted from dahlia tubers according to Allais et al. (1987). Dahlia tubers were washed, speeled, cut and then blended followed by boiling at temperatures 80 to 90ºC for about 30 minute. After cooling, filtrat were collected and filtered, and subsequently were mixed with ethanol 30%. The mix solution was then kept frozen at about -10ºC for 18 hours, followed by defrozen for 2 hours and centrifuged at 1500 rpm for 15 minute. The sediment obtained was then mixed with a double volume of water (1:2) and then heated at 70ºC for 30 minute with the addition of active carbon of about 1 to 2% of the total volume. Upon completion of heating, the solution were filtered and added with ethanol 30% of about 40% of the total volume and then kept frozen for 18 hours. The samples were then defrozen and centrifuged at 1500 rpm for 15 minutes. Upon centrifugation, white sediment were collected and heated at 50 to 60ºC for 6 to 7 hours and harvested as inulin exract. To investigate the potential application as prebiotics in supporting the growth of probiotics, inulin extract was supplemented, namely at 20, 40 and 60 mg/g total solid, into MRS broth followed by plating and counting total viable cell of *Bifidobacterium bifidum*.

Preliminary analysis of inulin extracts

Preliminary analysis was carried out to investigate the chemical composition of the extract. This includes protein, fat, fibre, and ash content which were analysed according to AOAC (1975). The analysis of Neutral Detergent Fiber (NDF), Neutral Detergent Solution (NDS), Acid Detergent Fiber (ADF) dan hemicellulose was carried out according to Van Soest (1982).

Yoghurt fermentation and probiotics viability evaluation

Yoghurt fermentation was performed according to Dave and Shah (1997) and Lankahputra and Shah (1997). The media employed consist of fresh milk added with skim milk powder to obtain total solid of 18%. This media was pasteurized at 85°C for 30 minutes followed by cooling to obtain temperature ready for fermentation at 40°C. The addition of starters was initiated with the *Bifidobacterium bifidum* for 2 hours at temperature 41°C. After two hours of incubation, yoghurt starters *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were added, and the incubation temperatures were increased at 42°C for 6 hours. The starter cultures were added at the proportion of 2:2:2 (v/v).

RESULTS AND DISCUSSION

Chemical composition of inulin extracts

Preliminary analysis is required to investigate the chemical composition of the inulin extracts obtained from dahlia tubers. The ideal analysis should be be able to determine inulin content qualitative and quantitatively. However, as both quantitative and qualitative analysis of inulin requires sensitive and accurate technologies and this takes time, yet it could not be presented here. The analysis data presented here focus on general chemical composition of extracts without specific information on inulin content.

Table1. Chemical composition of inulin extracts obtained from dahlia tubers

	Concentration (%)	
Composition	Dahlia tubers	Extracts of dahlia tubers
Water	90.260	10.577
Dry matter	9.740	89.423
Total proteins	2.104	3.672
Crude fats	0.134	2.789
Ashes	0.932	0.987
Dietary fibres		
1) NDF	nd	0.648
2) NDS	nd	99.352
3) ADF	nd	0.396
4) Hemicellulose	nd	0.252

Nd= not determined, NDF, NDS and ADF

Table 1 showed high level of dry matter content of inulin extract from dahlia tubers as it contains 89.4%. The inulin extract also contained higher level of proteins at 3.7% compared to 2.1% proteins in the tubers. Interestingly, level of dietary fibres which was not detected in tubers was detectable in the extracts. Level of NDF, NDS, ADF and hemicellulose was 0.6%; 99.3%; 0.4% and 0.3% respectively based on dry weight basis. To unravel the role of inulin extract in supporting the growth of probiotics *Bifidobacterium bifidum*, different level of inulin extracts was added into the media, namely at 20, 40 and 60 mg/g total solid, followed by measurements of total viable cell of *Bifidobacterium bifidum*. From those different levels, the addition of 40 mg/g total solid resulted in the optimum growth of probiotics (data not shown). From this preliminary analysis, the addition of 40 mg/g total solid was applied for next the experiments.

Viability of Bifidobacterium

Viability of Bifidobacterium bifidum

Viability of *Bifidobacterium bifidum* was measured on MRS agar supplemented with bile salts 0.15% and L-*cystein* HCl 0.05% for creating microaerophilic conditions. The addition of bile salts and growth condition on microaerophilic was intended to inhibit the growth of yoghurt bacteria (*Streptococcus thermophillus* and *Lactobacillus bulgaricus*). Viability of *Bifidobacterium bifidum* in yoghurt with addition of 40 mg/g inulin extracts and in control, before and after fermentation is presented in Table 2.

Statistical analysis showed that the addition of inulin extracts of dahlia tubers (40 mg/g total solid) significantly affects the growth of probiotics *Bifidobacterium bifidum* ($P<0.05$). During fermentation there was an increase on total probiotics both in control and in supplemented media with higher level of probiotics was obtained on inulin extracts-supplemented media ($P<0.05$) than in control. Overall, total *Bifidaobacterium bifidum* in yoghurt control and with inulin extracts supplementation was 8.6 and 8.8 log cfu/ml, respectively. Level of total probiotics in yoghurt presented this study is in agreement with previous finding of Oliveira et al. (2009) who reported viability of *Bifidobacterium*

lactis at 8.9 log cfu/ml in yoghurt supplemented with inulin (Beneo^{TM}) at 4 g/100 g total solid. Bruno et al. (2002) also reported the viability of *Bifidobacterium pseudolongum* at 8.6 log cfu/ml in yoghurt supplemented with 5% (w/v) of inulin (Orafti^{TM}) from chicory roots. The stimulation effects of inulin and lactulose for *Bifidobacterium bifidum* BB-02 was previously reported by Ozer et al. (2005). The data presented here suggests that addition of inulin extracts from dahlia tubers accelerates the growth of probiotic *Bifidobacterium bifidum* during yoghurt fermentation. This indicates that inulin extracts from dahlia tubers is prebiotics for *Bifidobacterium bifidum* with unknown mechanisms. The most possible mechanism is that *Bifidobacterium bifidum* consumed and fermented inulin extracts using specific enzymes that still unknown. The increase of the growth of bifidobacteria after the addition of inulin was previously reported by Muir (1999). Meanwhile Mc Kellar dan Modler (1989) reported that dairy fermentation was more efficient in lactose degrading when bifidobacteria starter was added.

Table 2. Total *Bifidobacterium bifidum* (log cfu/ml) in yoghurt

Yoghurt	Time of sampling		Average (*n*=3)
	Before fermentation	After fermentation	
Control	7,12	10,22	8,67 [a]
Inulin extract at 40 mg/g	7,11	10,51	8,81 [b]
Average	7,12 [a]	10,37 [b]	

[a, b] Different superscript at the same rows and columns was statistically significant (P<0.05)

In general, fermentation significantly increased total probiotics (P<0.05) from 7.1 log cfu/ml before fermentation to 10.4 log cfu/ml after fermentation. Total probiotics obtained in this experiment is within the standards of required viability of probiotics for functional that is at 10^7 cell/ml or 7 log cfu/ml products (Kailasapathy et al., 2000; Homayouni et al., 2008). Meanwhile Lourens-Hattingh and Viljoen (2001) proposed that level of probiotics in yoghurt should be at 10^8 cell/ml or 8 log cfu/ml products. A lower level of probiotic viability for functional food wad proposed by Kurmann and Rasic (1991) that is at 10^6 cell/ml or 6 log cfu/ml products.

Acidity level as lactic acid

Level of acidity in yoghurt and other dairy fermented products is relevant with lactic acid production during fermentation (Hui, 1993). As the production of lactic acid increases, level of acidity also increases but the pH decreases. This results in micelle casein destabilization, dairy coagulation and the formation of curd. Production of lactic acid corresponds with enzyme β-galactosidase produced by yoghurt starter cultures and probiotics added in the media. Table 3 presents level of acidity in yoghurt with and without the addition of inulin extracts before and after fermentation.

Table 3. Level of acidity (%) in yoghurt control and supplemented with inulin extracts

Yoghurt	Time of sampling		Average (*n*=3)
	Before fermentation	After fermentation	
Control	0.37	0.99	0.68[a]
Inulin extract 40 mg/g	0.36	1.64	1.00[b]
Average	0.37[a]	1.32[b]	

[a, b] Different superscript at the same rows and columns was statistically significant (P<0.05)

Statistical analysis showed that either inulin addition or fermentation time significantly increase the level of acidity (P<0.05). The average of acidity level in yoghurt control was 0.68% and in yoghurt supplemented with inulin extracts was 1.00% (P<0.05). The higher level of acidity in yoghurt supplemented with inulin extracts is associated with higher level of *Bifidobacterium bifidum* suggesting the potential roles of this probiotics in lactic acid production. This also suggests that inulin supplementation increases lactic acid production in yoghurt fermentation. As lactic acid production is associated with lactose degradation, the supplementation of inulin extracts in yoghurt fermentation possibly increase the synthesis of β-galactosidase enzymes during fermentation. Tsai and Luedecke (1989) proposed that the increase in acidity level always associated with the increase in metabolisms.

Level of acidity reported here is in agreement with previous study by Murti et al. (1993) who reported level of acidity at 0.8% in yoghurt fermented with triple starters of *Lactobacillus bulgaricus, Streptococcus thermophillus* and *Bifidobacterium*. According to Standar Industri Indonesia (SII-0717-1990), accepted level of acidity in yoghurt must fall between 0.5 to 2% suggesting that level of acidity in this study is within SII standards. As the acidity level increases, accordingly pH of yoghurt decreases (data not shown). This contributes for inhibition of growth of spoilage and pathogenic microorganisms in the products.

Lactose content

Lactose is the main carbon source available in fresh milk, and this is fermented to produce lactic acid. This disaccharide is hydrolyzed to glucose and galactose by enzyme β-D-galactosidase (lactase). As such, the presence of starter cultures for dairy fermentation with enzyme lactase helps to ferment lactose to more readily available monosaccharide. As the lactose content decreases, lactic acid increases and level of acidity also increases. Table 4 presents lactose content in yoghurt control and supplemented with inulin extracts before and after fermentation.

Table 4. Lactose content (%) in yoghurt control and supplemented with inulin extracts

Lactose	Time of sampling		Average (*n*=3)
	Before fermentation	After fermentation	
Control	4.86	3.56	4.21[a]
Inulin extracts 40 mg/g	4.87	2.87	3.87[b]
Average	4.87[a]	3.22[b]	

[a, b] Different superscript at the same rows and columns was statistically significant (P<0.05)

Statistical analysis showed that either inulin addition or fermentation time significantly decrease lactose content (P<0.05). The average of lactose content in yoghurt control was 4.21% and in yoghurt supplemented with inulin extracts was 3.87% (P<0.05). The lower level of lactose content in yoghurt supplemented with inulin extracts is associated with higher level of lactic acid production (Table 3). This suggests that inulin supplementation increase lactose degradation (59%) compared to 26% in control, resulting in a higher lactic acid production in during fermentation. According to van den Berg (1988), 15 to 40% lactose was fermented depends on bacterial employed while 60 to 85% lactose was intact upon fermentation. As lactose degradation is always associated with level of β-galactosidase enzymes, the inulin addition during fermentation proposed to accelerate the synthesis of such enzymes. The degradation of lactose and lower level of lactose in dairy products bring to beneficial effects due to decreasing the number of lactose intolerance cases.

CONCLUSION

The supplementation of inulin extracts of dahlia tubers at 40 mg/g total solid was able to improve the growth of probiotics *Bifidobacterium bifidum* in yoghurt, and to increase lactose degradation and to accelerate lactic acid production.

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