CHAPTER 4

GENERAL DISCUSSION AND FUTURE WORK

1. General discussion

The maltodextrin complex medium in this study had similar components to that of Hehre and Hamilton (1951) containing 5 g l^{-1} yeast extract and 33 g l^{-1} alcohol-precipitated, soluble 'bacteriological' dextrin made from corn-starch. In this study, a culture-based method and a cell suspension method gave oligodextran yields ranging from 24.67-30.41% and 19.64-24.57%, respectively compared to dextran yields of 1.21-41.69% reported by Mountzouris *et al*. (1999) using a cell suspension method. A wide range of dextran yields (16-79%) from maltodextrins with DP 3-85 were reported (Sims *et al*., 2001). Variation in yield may due to the conditions (i.e. type of substrate and production method) as well as analytical techniques used. Defloor and co-workers (1998) determined product yield by ethanol precipitation from culture broth followed by gel chromatography. This method, however, overestimated dextran yield due to co-precipitation of maltodextrin substrate (Defloor *et al*., 1998) whereas in this study yield was measured by HPSEC with no prior ethanol precipitation.

Oligodextran yield and the ratio of 1-4 to 1-6 linkages largely depended on substrate type and its concentration, cell concentration and temperature. The molecular weight was 7.8-65.6 kDa, whereas Mountzouris *et al*. (1999) reported of 6.6-38 kDa and Goulas *et al*. (2004) reported of 1.8-10.8 kDa for oligodextran derived by hydrolysis of native dextran. However, the product contained both fractions of oligodextran and maltodextrin substrate and they cannot be separated using HPSEC due to them having the same range of molecular weight. Thus, the product contained a mixture of oligodextran product and maltodextrin substrate with α-1,4 linkages originating in the product. The molecular weight of oligodextrans could be controlled by enzymatic hydrolysis of dextran with endodextranase in membrane bioreactors. The product comprised of 63% oligomers of chain-length 2-10 and 36% > chain-length 11 (Mountzouris, 1999). In addition, batch syntheses of isomalto-

oligosaccharides (IMO) from sucrose, using the enzymes dextransucrase and dextranase achieved giving the product oligosaccharides mixtures (IMO and oligodextrans) up to 36% with DP varying between 10 to 60 (Goulas *et al*., 2004). This low molecular weight oligodextran was thought to have potential for application as a prebiotic. Low molecular weight oligodextran obtained by hydrolysis of commercial dextran could be fermented by the human colonic microflora and generated high level of butyrate, which is believed to be protective against colon cancer (Olano-Martin *et al*., 2000).

The method of synthesis of oligodextrans in this study was similar to the production of commercial prebiotic IMO using starch as substrate (Crittenden and Playne, 1996) using different enzymes. Synthesis of oligodextran from maltodextrin required only one enzyme (DDase) whereas production of IMO required at least three enzymes to complete these two steps. Unfortunately, the activity of DDase did not be determined in this study due to the previous study by colleagues of the research group reported that the activity of this enzyme was not easy to measure. Non-digestible oligosaccharides manufactured by transglucosylation reactions include GOS, lactosucrose, glucosylsucrose, FOS, IMO, gentio-oligosaccharides, palatinose and lactulose (Voragen, 1998). Oligodextran can be synthesized by the action of dextrin-dextranase (DDase) which acts by hydrolysis of linear α-1,4 linkages of maltodextrin followed by transglucosylation to add the residue glucose giving branching α-1,6 linkages. Meanwhile IMO was constructed by hydrolysis of starch using α- and β-amylase to give glucose residues and followed by transglucosylation to add the glucose residue giving α-1,6 linkages by action of α-glucosidases (Crittenden and Playne, 1996). Composition of IMO depended on the production conditions, i.e. IMO with DE 42 consisted of 9.82% isomaltose, 3.64% isopanose, 6.13% branched tetraose, 1.21% branched pentaose. Thus the end product contains the same type of linkages but in different ratio. This method of IMO production gave higher yields when immobilized enzymes were used, whereas the purified DDase enzyme gave low yields due to the low stability of the purified DDase. This study has identified an alternative method to produce oligodextrans using either the culture medium method or the cell suspension method where higher purity product could be obtained compared to the culture-based method.

Ultrafiltration (UF) for separation of molecules with molecular weight between 1000-100,000 Daltons has been applied in various fields of industrial practice such as water treatment, the food industry and various biological processes. Recently, UF was applied to the optimization of bioconversions for separation of polysaccharides from smaller molecules. This study applied UF to separate the oligodextran product (7.8-65.6 kDa) from maltodextrin substrate (1 kDa). This system succeeded in separating oligodextran produced by enzymatic hydrolysis from native dextran (Mountzouris, 1999), oligosaccharides from oligosaccharide mixtures (Goulas *et al*., 2003), oligosaccharides from steamed soybean wastewater (Matsubara *et al*., 1996) and oligosaccharides from defatted soybean meal (Kim *et al*., 2003).

In a preliminary study, some parameters affecting UF performance such as molecular weight cut off of the membrane, recycling time of the permeate or number of diafiltration steps were investigated. Oligodextran mixtures and dextran had different in membrane rejection characteristics. Dextran solutions are considered to be non-fouling thus avoiding the effects of adsorption on the rejection characteristics (Zeman, 1983). The rejection characteristics will depend on the type of solute, the operating conditions and the membrane configuration. Oligodextran mixtures in this study are viscous resulting in fouling. Separation of oligodextran mixtures required a volume concentration ratio (VCR = initial volume/ volume of retentate) of at least three, whereas the most effective VCR for protein removal and recovery of oligosaccharides from defatted soybean meal was found to be 5 (Kim *et al*., 2003). Fractionation of oligosaccharides (high-panose syrup, branched oligosaccharide, GOS) by NF membrane (1 kDa cut off) showed clearly that no irreversible fouling occurred during the filtration of sugar solutions, which was in accordance with this study. Permeate flux decreased with the increase of VCR and gave a flux of 120 and 70 l m^{-2} h^{-1} at VCR of 1.2 and 6, respectively. This was also found in this study, but permeate flux was much lower (13.2 – 30.6 l m^{-2} h^{-1}) at VCR of 3. This suggested that the permeate flux depends (apart from the filtration parameters such as pressure and temperature) on the total sugar concentration of the solution. Thus oligodextran mixtures in this study has slightly lower sugar content since it had slightly lower viscosity. Recovery yields of oligosaccharide from high-panose syrup, branched oligosaccharides and GOS by NF were 46, 43 and 39%, respectively (Goulas *et al*.,

2003). It was suggested that NF gave better purification of the initial feed solution as far as removal of the monosaccharides was concerned, but a high proportion of the oligosaccharide content of the mixture was lost in the permeate (Goulas *et al*., 2003).

Measuring digestion of any substance in the stomach and small bowel *in vivo* is difficult. One useful model is the ileostomy patient, with an alternative approach being the aspiration of residual digesta from the ileum using intubation. At least 88% of inulin and oligofructose reach the colon, using both the ileostomy patient model and the intubation model (Cummings and Macfarlane, 2002) and at least 60% of oligodextrans in this study reach the colon using an *in vitro* technique.

Typically, dextran was resistant to amylase digestion and acidic conditions in the human digestive tract and similar results were seen in this *in vitro* study. However, a previous study (Debnam *et al*., 1998) showed that chronic exposure of dextran to rat intestinal mucosa resulted in a higher degree of hydrolysis of dextran. This is because both rat and human intestines have low levels of activity of isomaltase towards α-1,6 glucose linkages. However, isomaltase has about 10% activity on dextrans compared to isomaltose (Dahlqvist, 1961; 1963). Thus, the transit time will affect the degree of hydrolysis *in vivo*. This study indicated that at least 60% of oligodextran would reach the colon, whereas 54% of resistant starch was estimated to reach the colon in *in vivo* human studies. The other fraction of 46% is digestible and absorbable carbohydrate that will contribute to daily carbohydrate intake (Brouns *et al*., 2002). This observation implied that consumption of oligodextran will reduce caloric intake as well as insulinaemia compared to the consumption of normal starch. Accordingly, the inclusion of oligodextran in food supplements may be suitable for a wide variety of food products designed for overweight individuals and diabetic prevention. One way to achieve higher amount of oligodextran reaching the colon is encapsulation of the oligodextran. Maltodextrin, but not dextran, is hydrolysable by α-glucosidases within minutes (Grimble *et al*., 1992) so do not reach the colon and no fermentation occurs in the colon.

The result obtained by HPSEC of oligodextran before and after hydrolysis by amylase in this study confirmed that amylases acted on oligodextran and released mostly lower molecular weight products (approx. 1 kDa). The percentage of hydrolysis of oligodextran by amylases obtained by either HPSEC or reducing sugar

analysis gave similar results. Molecular weight determination by HPSEC depends on the degree of similarity between the unknown solute and the molecular weight standards. The molecular weights of 7.8-65.6 kDa obtained in this study are anticipated to be accurate since the standards used are wide range molecular weight dextrans of similar structure to the unknown oligodextrans.

The optimal pH for hydrolysis of oligodextran was found to be neutral to slightly alkaline. This was similar to the pH of human saliva in which up to 50% of α-1,4 linked glucose is hydrolyzed within 30 min, however, oligodextran in this study was only 15% hydrolyzed. Pancreatic amylase continues the digestion of starch and glycogen in the small intestine. Pancreatic amylase is produced in larger amounts than salivary amylase, however, their catalytic properties appear to be identical. However, this study suggested human pancreatic amylase was more active on digestion of oligodextran than human salivary amylase. Brush-border enzymes, i.e. isomaltase, glucoamylase, maltase, sucrase and lactase hydrolyse α-1,4- and α-1,6 linked gluco-saccharides present in the small intestine and yield monosaccharides as end products (Smith and Morton, 2001). However, this study did not investigate the digestion of oligodextrans with brush-border enzymes, so the percentage of oligodextran reaching the colon might be less than the expected 60%.

Among the intensive studies to investigate new substances having prebiotic properties, this is the first study on the *in vitro* fermentability of oligodextran produced by *G. oxydans* NCIMB 4943 by the human colonic microflora. Typically, prebiotic substances are low molecular weight oligosaccharides which easily fermented by certain components of the microflora, especially bifidobacteria and/or lactobacilli. Gibson (2004) suggested that if the molecules of dietary carbohydrate are too large they will not be selective towards bifidobacteria and lactobacilli. For example inulin is much more slowly fermented than the lower molecular weight FOS. This was confirmed by recently published data using 5% fructans with different DP in Wistar rats (Biedrzycka and Bielecka, 2004). The results confirmed the *in vitro* observation of chain-length dependent *Bifidobacterium* growth stimulation. Low-DP FOS (DP 2-4) increased bifidobacteria whereas FOS with DP≥9, or highly polymerized FOS (DP≥ 22) was not active. Highly polymerized inulins are not degraded by bifidobacteria. The authors suggested that bifidobacteria utilize first short chain FOS, whereas

Bacteroides start fermentation of fructan of higher DP (Laere *et al*., 1999; Biedrzycka and Bielecka, 2004).

However, some higher molecular weight carbohydrates had prebiotic effects included this study. For example, polyglucose and polyfructan contained in a water-soluble polysaccharide (WSP) preparation from wheat and rye were metabolized by bifidobacteria (Korakli *et al*., 2002). Large quantities of acetate and butyrate were detected on fermentation of G19 oligodextran, suggesting the main producers were bacteroides, bifidobacteria, eubacteria and lactobacilli since large numbers of these genera were detected. Butyrate could be converted from acetate according to the pathway proposed in Fig. 1.6.

In vitro fermentation of inulin revealed that molecules with a chain length (DP) > 10 are fermented on average half as quickly as molecules with a DP < 10 but no difference in fermentation rate was seen between GF_n and F_n-type β-fructans (Roberfroid *et al*., 1998). This study showed that fermentation of oligodextrans with the same molecular weight (7.8-65.6 kDa) but different ratios of α-1,6 and α-1,4 linkages gave similar fermentation rates and was completed within 48 h. This was similar to the previous pure culture study of Palframan and co-workers (2003) in that almost every strain from nine bifidobacteria species preferred glucose as carbon and energy source except *B. catenulatum* which preferred xylose over glucose and XOS over FOS (Palframan *et al*., 2003). This suggested different strains had different pathway to metabolize these substrates.

Short chain glucose-based dietary carbohydrate is degraded approximately 90% within 24 h by faecal flora whilst long chain polymer such as starch need longer fermentation time (Lebet *et al*., 1997) thus this study needed for 48 h to complete the fermentation of oligodextrans. Bifidobacteria have been found to be able to consume β-gluco-oligomers, xylo-oligomers and raffinose to a degree of 3.6, 79.3 and 72.7%, respectively (Jaskari *et al*., 1998). Lactobacilli consumed β-gluco-oligomers, xylo-oligomers and raffinose to 43.7, 0, 10.4%, respectively. Bacteroides consumed β-gluco-oligomers, xylo-oligomers and raffinose to 3.5, 17.8 and 12.4%, respectively. The fermentation results suggested that the oligodextrans in this study would not be complete metabolized by a single intestinal strain but would need a consortium of

mixed cultures to complete the fermentation and it is thought that bacteroides might be involved in the fermentation of long chain glucose contained in the oligodextran.

The end products, SCFA, by fermentation of oligodextran supported the pathway of carbohydrate fermentation in the large intestine (Fig. 1.6) proposed by Gottschalk (1979). Lactate and formate appeared in short period and disappeared thereafter. This is because they are intermediates of the pathway and converted to propionate and acetate, respectively. Oligodextran is fermented to give phosphoenol pyruvate (PEP) then converted to pyruvate, lactate (by lactate dehydrogenase) and to propionate, respectively. Formate is formed from pyruvate then it might be converted to methane or acetate. Acetate is formed from acetyl CoA (by acetate kinase) whereas butyrate is produced either from acetate by butyryl CoA: acetyl CoA transferase or via butyryl CoA by butyrate kinase (Pryde *et al*., 2002). High level of butyrate in this study was proposed to be converted from acetate that was produced by bifidobacteria and/or lactobacilli because large numbers of bifidobacteria and lactobacilli and also high level of acetate were detected in this study.

The current paradigm for prebiotic action is that probiotic, especially bifidobacteria and lactobacilli, possess cell-associated glycosidases which hydrolyse oligosaccharides prior to uptake of the monosaccharide (Perrin *et al*., 2001). However, some bacterial flora such as *B. bifidum* and *B. longum* suggested using a specific oligosaccharide transport mechanism thus oligosaccharides were being fermented internally (Palframan *et al*., 2003). However, oligodextrans in this study are consisted of only glucose monomers so that it might need a simple transport mechanism. This mechanism involves the cleavage of the oligosaccharides externally and uptake of the subsequent monomers as described by Perrin *et al*. (2001). Monomer compositions of known prebiotic are arabinose, xylose, mannose, galactose and glucose (Cummings *et al*., 2001). The exact mechanism for metabolism of oligodextran needs to be clarified in further study.

The changes of bacterial populations by fermentation of commercial prebiotics (FOS, inulin, IMO, GOS, lactulose) in stirred pH-controlled batch culture was previously reported (Palframan *et al*., 2002). They concluded that FOS and inulin demonstrated the greatest bifidogenic effect at pH 6.8 and 1% (w/v) carbohydrates, whereas GOS, IMO and lactulose demonstrated their greatest bifidogenic effect at pH

6 and 2% (w/v) carbohydrates. This indicated that various prebiotics demonstrated differing bifidogenic effects at different conditions *in vitro*. In this study the commercial prebiotic inulin showed higher selective fermentation than the carbohydrates tested, particularly maltodextrins. Oligosaccharides, on the other hand, have more selective fermentation properties than the higher molecular weight pectin (Olano-Martin *et al*., 2003). The similar result was obtained by fermentation of FOS (Raftilose P95) which gave higher selectivity on bifidobacteria than inulin (Raftiline HP). Bifidobacteria number increased from baseline level 1.9×10^5 to 3.7×10^7 and 1.2×10^7 on FOS and inulin, respectively. In pure culture studies, large proportion of inulin-HP was consumed by *Bifidobacterium adolescentis* ATCC 15703 but not by *Bifidobacterium cuniculi* MB280. These suggested that the fermentation of FOS and inulin by faecal population resulted always in the complete consumption (Corradini *et al*., 2004). Large increase in bifidobacteria was always seen if start with a low level of bifidobacteria, high response to intake of a prebiotic is more enhanced than when start with higher levels (Gibson, 2004).

Fermentation of 1% (w/v) pectin and pectic oligosaccharides by mixed faecal in a pH (6.8) controlled batch culture was conducted at 37°C for 48 h**.** Bifidobacteria increased significantly ($P<0.01$) both in the presence of pectin and pectic oligosaccharides whereas no significant changes in other bacteria. Oligodextran, having high molecular weight (7.8-65.6 kDa), demonstrated higher prebiotic properties compared to the commercial prebiotic inulin.

Three-stage continuous system used in this study had been validated and designed to reproduce spatial, temporal, nutritional, and physicochemical characteristics of the microbiota in the proximal (vessel 1), transverse (vessel 2) and distal colon (vessel 3) (Macfarlane *et al*., 1998). Unfortunately, the system used in this study was designed for differentiation of luminal flora but not for the gut wall-associated microflora. Changing the luminal flora is advantageous because it is going to change certain products, i.e. SCFA that will have health consequence (Gibson, 2004). Gibson (2004) tried to put human colonocytes in the model in some sort of semi-permeable membrane where the colonocytes need oxygen to thrive and the system is anaerobic so there is a difficulty there. They have done films of agarose or mucins, which have tried to stimulate extra wall growth. Generally, majority of

carbohydrate was fermented in vessel 1 where the residence of saccharolytic bacteria existed (Macfarlane *et al*., 1996) and generated high concentration of short-chain fatty acids. However, in this study, majority of SCFAs particularly butyrate were produced in transverse (V2) and distal colon (V3) which were the sites of the most common cancer. This may due to the carbohydrates tested had higher molecular weight than the typical prebiotic oligosaccharides, hence, needed longer fermentation time.

Fermentation of dietary carbohydrate in continuous system suggested competition between strains of microflora. Bifidobacteria and lactobacilli are able to use FOS and lactobacilli can out-compete bifidobacteria in continuous culture at pH 5.2-5.4 when FOS is the primary carbon and energy source. Moreover, bifidobacteria can grow faster on FOS than lactobacilli under controlled conditions (Sghir *et al*., 1998). However, no out-compete of bifidobacteria and lactobacilli was observed in this study. This is because the different type of carbon and energy source and also the conditions used.

Wiele *et al*. (2004) reported the prebiotic effect of native chicory inulin (92% FOS with average DP of 10) in the simulator of the human intestinal microbial ecosystem (SHIME). The SHIME is a dynamic model of the human gastrointestinal tract similar to the three-stage continuous system used in this study but that model consisted of two more vessels represented stomach and small intestine. Wiele and co-workers (2004) found that inulin addition (2.5 g d^{-1}, which was equivalent to a human dose of 5 g d^{-1}) selected for a higher SCFA production with shifts towards propionic and butyric acid. Concentrations of total SCFA in the vessels represented of ascending, transverse and distal colon were 53.3, 70.7 and 77.6 μmol ml^{-1}, respectively whereas propionic acids were 17.6, 21.3 and 21.6 μmol ml^{-1}, respectively and butyric acids were 14.3, 19.3 and 19.3 μmol ml^{-1}, respectively. These concentrations, particularly butyric acid, were less than obtained from this study suggested a novel G19 oligodextran had higher butyrogenic effect than the commercial inulin. Increase in bifidobacteria was seen from the proximal to distal colon which was similar to this study and suggested bifidogenic effect. Bifidobacteria numbers in ascending, transverse and distal colon were 8.05, 8.46 and 8.01 log CFU ml^{-1}, respectively obtained by quantitative PCR technique (Requena *et al*., 2002)

whereas bifidobacteria numbers in ascending, transverse and distal colon of this study were 8.59, 8.84 and 8.92 log CFU ml^{-1}, respectively obtained by FISH technique.

This study used genus-specific probes for detection of microbial population thus the results obtained is a rough change in genus level of population. To see what happen in the species level in the further study, particular *Bifidobacterium* species, had been achieved by PCR and DGGE technique and mostly three species were detected in adult such as *B. adolescentis*, *B. longum* and *B. bifidum* (Requena *et al*., 2002).

Total bacterial counts of faecal donor, from this study, of 1.11×10^{11} cells (g wet weight of faces)$^{-1}$ was lower than those reported of 2.72×10^{11} cells (g wet weight of faeces)$^{-1}$ by Suau *et al*. (1999) and 2.71×10^{11} cells (g wet weight of faeces)$^{-1}$ by Langendijk *et al*. (1995). They determined the populations by DAPI similar to this study whereas it was 5×10^{11} cells (g wet weight of faeces)$^{-1}$ determined by direct microscopic clump counting (Moore and Holdeman, 1974). The variation of microbial populations in the faecal donor may due to their different in diet intake, i.e. previous studies used European population whereas this study used Asian population as faecal donor. However, the population who has similar diet intake resulted in similar microflora numbers. For example, total anaerobes of UK faecal pool and Italian faecal pool were 10.4 and 10.6 $\log_{10}$ cfu g^{-1}, respectively (Silvi *et al*., 1999).

Polydextrose (PD) is a randomly bound polymer of glucose containing various 1,6-, 1,4-, 1,3-, 1,2-linked glucose and small amount of sorbitol and citric acid. PD polymer prevents mammalian digestive enzymes thus reach the colon quantitatively (Murphy, 2001). Since PD consisted of more complex linked glucose than oligodextran, so it is possible to use more complex metabolism route than oligodextran.

Prebiotic oligosaccharides (FOS), inulin, lactulose, XOS, GOS, SOS and IMO were found to increase the number of bifidobacteria and most decreased clostridia (Rycroft *et al*., 2001b). Samples used in this study contained a mixture of low molecular weight (maltodextrin residues, average DP 5.5) which was first non selectively fermented at the first 24 h and high molecular weight fraction (oligodextran) which was high selectively fermented by human colonic flora after the first fraction depleted (at 48 h). The compositions of maltodextrin with DE of 22 were 3.92% glucose, 12.84% maltose, 15.48% maltotriose, >67.74% maltotetraose (Kwon

et al., 1999). Fermentation properties of gentio-oligosaccharides (GEOS) as compared to FOS and maltodextrin in mixed faecal culture were previously reported (Rycroft *et al*., 2001a). GEOS gave the higher increase in bifidobacteria, lactobacilli and total bacterial numbers. However, FOS appeared to be a more selective prebiotic compared to GEOS and maltodextrin. This study showed similar results to the work of Rycroft and co-workers (2001a) that maltodextrin was non-selectively stimulated the growth of microflora as it increased bifidobacteria, lactobacilli, clostridia and bacteroides. SCFA production by fermentation of G12 maltodextrin in this study (mmol l^{-1}; lactate, 0; acetate, 81.59; propionate, 19.4; butyrate, 2.05) was similar to that of Rycroft and co-workers (2001a) (mmol l^{-1}; lactate, 21.86; acetate, 36.49; propionate, 7.73; butyrate, 2.05), but much higher acetate level and lower lactate level.

Maltodextrin has been reported enhance the effect of probiotics in the small intestine but not in the colon (Bomba *et al*., 2002), moreover produce diarrheal toxin (Rowan and Anderson, 1997). This study succeeded to convert maltodextrin to oligodextran which having potential use as prebiotic rather promoting toxin producing bacteria in the human colon.

Human trial (32 healthy subjects) on the high molecular weight dietary carbohydrate, wheat germ preparation Viogerm®PB1 (54.1% total carbohydrate consisted of 30.6% starch, 21.1% dietary fibre, 17.5% raffinose). The result suggested the decrease in coliform and pH and increase the numbers of lactobacilli and bifidobacteria. These results showed that the product Viogerm®PB1 possesses a prebiotic effect and had potential to improve host's health (Matteuzzi *et al*., 2004).

Maltodextrin in the presence of *L. paracasei* decreased the number of *E. coli* colonising the jejunal mucosa of gnotobiotic piglets (Bomba *et al*., 2002). This means maltodextrin had benefit when use as synbiotic but not for use as prebiotic solely. Prebiotic index (PI) of several prebiotic carbohydrates (Palframan *et al*., 2003) indicated that low molecular weight carbohydrate exhibited prebiotic properties in short time (≤24 h) of fermentation than higher molecular weight carbohydrate (48 h). They reported PI of pectin fermentation was 0.11 at 8 h and 2.14 at 48 h whereas PI of pectic oligosaccharides was 1.79 at 8 h and 2.77 at 48 h. PI of FOS, inulin, XOS, lactulose, IMO, GOS and SOS were –0.95, 0.36, -0.05, 1.34, 1.46, 1.39 and 1.47, respectively at 5 h whereas PI were 2.31, 1.82, 2.19, 4.90, 3.95, 3.76 and 4.36,

respectively at 24 h. More accurate prediction on prebiotic effect had been recently proposed in the terms of prebiotic index (PI_m) and measure of the prebiotic effect (MPE). PI_m and MPE of 1% IMO, SOS, FOS and TOS were 0.1 and 0.1, 0.1 and 0.3, 0.2 and 0.4, 0.8 and 1.0, respectively in the presence of human faecal bacteria in pH-controlled batch culture (Vulevic *et al*., 2004). Oligodextran in this study showed similar prebiotic properties to lactulose, IMO, GOS and SOS using PI as indicator.

Brouns *et al*. (2002) reported high concentration of butyrate produced by fermentation of resistant starch (30 mmol l^{-1}) whereas wheat bran and pectin (20 mmol l^{-1}) gave lower butyrate. The levels of butyrate produced by fermentation of oligodextrans in batch culture (14.52 mmol l^{-1}) in this study showed high comparable levels to wheat bran (15 mmol l^{-1}) and resistant starch in three-stage continuous system (27.53 mmol l^{-1}). Although maltodextrin showed prebiotic effect, it could be hydrolyzed by upper gut digestive system and very small amount of maltodextrin reached the colon where fermentation occurred. In addition, maltodextrin was poor prebiotic since it generated large amount of gas (8 ml) compared to oligofructose (7 ml) and GOS (7 ml) (Probert and Gibson, 2002).

Tuohy *et al*. (2001) reported consumption 8 g d^{-1} of maltodextrin for 14 days followed by 8 g d^{-1} inulin-HP for 14 days. Generally, the population changes for consumption of maltodextrin was similar to this study, however total bacterial numbers in this study was slightly lower (1×10^9 cell ml^{-1}). Inulin-HP confirmed stimulation on bifidobacteria and clostridial numbers similar to inulin-ST used in this study however the change of bacteroides and lactobacilli were different. *In vitro* faecal slurry fermentations showed that inulin-HP was metabolized twice as slowly as lower DP oligofructose (Roberfroid *et al*., 1998; Gibson and Wang, 1994). However, not much difference on the changes of microbial population between inulin-HP and inulin-ST was observed in this study. They reported no change in both populations contrasted to the increase in this study. The actual prebiotic effect could be further investigated *in vivo* by human trial to confirm its prebiotic properties.

Butyrate is important for the energy metabolism and normal development of colonic epithelial cells for protecting colon cancer. However, the optimum concentration of butyrate is complex. Cells cultured *in vitro* showed growth arrest at the concentrations of 1-10 mM (Csordas, 1996). In this study, high butyrate

concentration (> 10 mM) was produced from G12 and G19 oligodextrans comparable to a commercial inulin-ST, moderate-butyrate concentration (5-10 mM) was produced from G20, G29 and G37 oligodextrans comparable to a commercial inulin-HP. Low butyrate concentration (<5 mM) was produced from a commercial levan. Thus oligodextrans produced in this study provides enough energy for the growth of epithelial cells and the most potent for butyrogenic effect is G12 and G19 oligodextran.

Although direct supply of butyrate, or of butyrate 'carriers' such as tributyrin, has been considered via oral (Wachtershauser and Stein, 2000; Tulea *et al*., 2001), or rectal enema (Hove *et al*., 1995) routes as treatment for ulcerative colitis. Alternatively, consumption of prebiotic targeted stimulation of native butyrate-producing bacteria (i.e. *Clostridium*, *Eubacterium*, and *Fusobacterium*) provides an obvious approach for delivering butyrate to its site of utilization at the colonic mucosa. Lowering hydrolysis of maltodextrin by conversion to oligodextran in the small intestine enhances butyrate production by delivering more oligodextran to the large intestine and can shift the site of fermentation distally down the intestine.

The minimum dose for consumption of prebiotic depended on their type such as lactulose, lactosucrose and XOS were effective at low doses (1-3 g d^{-1}) whereas inulin, FOS, SOS and GOS were required in larger amount (> 10 g d^{-1}) (Rycroft, 2001). Consumption of oligodextran was estimated to be between 10 and 20 g d^{-1} in which only a half on this carbohydrate reach the colon. However, excessive intake of prebiotics will give an additional side effects such as extra gas generation (particular CO_2 and H_2), emesis, and lack of selective fermentation (Gibson, 2004).

This is confirmed that all the dietary carbohydrates tested, including oligomers of oligodextrans, maltodexrtrins and inulins as well as soluble polysaccharide of levan, are easily and completely fermentable. And its monomer composition mainly indicated their SCFA profiles. When conducted under well-defined conditions, using generally accepted methods, *in vitro* fermentations allow a realistic simulation of colonic events. Such experiments make it possible to follow the metabolism of substrates and formation of fermentation products, whereas kinetics cannot be determined *in vivo*. The interpretation of *in vitro* studies remain a very complex task, which involves the incorporation of many intrinsic chemical and physico-chemical

characteristics of substrates, in addition to all the parameters measured during fermentation experiments. The ultimate test for oligodextran functionality is the *in vivo* situation, in particular, well-controlled (placebo control and blind coded sample) human studies need to be clarified.

2. Future work

The production of oligodextran from maltodextrin by enzymatic synthesis (whole cells of *G. oxydnas*) using the culture medium method and the cell suspension method has been investigated in this study. However, the maximum oligodextran yield (approx 25%) seemed to be low for industrial production. To achieve higher oligodextran yields some alternatives are proposed. First, a synthetic approach using a combination of enzymes other than DDase such as hydrolase enzymes and glucosyltransferase enzymes, i.e. glucosidases, maltogenic amylase to give highly branched oligosaccharides and α-glucanotransferase. Second, genetic engineering of DDase to give better stability. Third, immobilization of DDase or *G. oxydans* cells, although immobilization of purified enzyme usually gives higher yields than whole cells, in this case, yields are limited by the low stability of purified DDase.

Oligodextrans produced in this study still contained residual maltodextrin having the same molecular weight as of the oligodextran product. Higher purity of oligodextran product could be achieved by the hydrolysis of a product mixture with α-amylase and glucoamylase to remove the remaining α-1,4 linkages residues and low molecular weight residues. The low molecular weight fraction, particular mono- and disaccharides in the oligosaccharide mixtures could be removed by yeast fermentation. Yeast would consume these low molecular weight residues contained in the mixtures thus giving higher content of oligosaccharide in the final product.

The oligodextrans demonstrating prebiotic potential in the present study should be further investigated. It should divided into two categories, one dealing with their application in food biotechnology and second one dealing with their physiological fate and their effects in the human host. The oligodextran in this study is maltodextrin (starch) origin so it might classify as food ingredient. The use of oligodextran might be similar to as of starch or maltodextrin. It is possible to partial substitute or replaces the starch or maltodextrin. However, their physical properties such as heat resistant,

sweetness, crystallization, solubility, solution stability, gelatinization, freezing point depression, retrogradation and viscosity need to be determined.

The functionality of oligodextran when they reach the colon and their effects on the gastrointestinal physiology and function have to be determined. It is expected that functionality properties are directly linked to the physicochemical composition of these products. Pure culture of selected gut bacterial genera on oligodextran is one way to provide information about their oligodextran degrading abilities.

The ultimate way to investigate prebiotic effect of these products would be performed in human studies. Large amount of oligodextrans could be obtained as described in pilot-scale production so that it possible however additional purification steps may require to get higher purity oligodextran before administration to a large number of volunteers, as part of both normal and controlled diet. Bacterial populations will be measured using FISH probes and PCR-DGGE as possible to detect the genera and species levels, respectively. The metabolites generated by fermentation of oligodextran by human colonic flora and enzyme activities of the bacterial population will be determined to obtain a fuller picture of metabolism route. The rate of gas production will also be measured from the subjects in sealed chambers and associated symptoms measured by means of questionnaires. The minimum effective dose and frequency for administration of oligodextran must also be determined.

There is currently an interest in designing new prebiotics with multi-functionality, possessing other properties in addition to prebiotic property. Oligodextran is possible to have anti-adhesive property to prevent pathogenic bacteria adhering to the intestinal epithelium, in addition to being prebiotic. Other desirable attributes include non-carcinogenicity, preventing tooth decay however the α-1,4 contents in oligodextran have to be removed prior use. Oligodextran was proven that resist to acid and amylases so it is possible to use as a low calorific substance. Oligodextran may be designed to be specific for a particular strain of bifidobacteria and/or lactobacilli. In addition, oligodextran may be designed for specific target populations such as microorganism those against strong anti-pathogenic activity. Thus *in vivo* prebiotic property of oligodextran has to be confirmed, particular in human volunteer. Use of oligodextran for specific purposes, i.e. targeting specific microorganism and use as food additives, need to be further investigated.