Lactose derivatives: turning waste into functional foods

Introduction

While cheese production steadily increases and discharge of whey permeate solids to effluent treatment is increasingly accepted as wasteful, dairy manufacturers face a choice of seven options to utilise their permeate streams from milk and whey (sweet and acid): drying, fermentation into various products (ethanol, lactic acid, bacteriocins), lactose production, protein standardisation of milk powders (with milk permeate or lactose derived from whey permeate), lactose hydrolysis, recovery of minor components and synthesis of derivatives from a lactose-rich feedstock. As whey permeate is currently underutilised (Gänzle *et al.* 2008), significant research and development focus on permeate and lactose-derived ingredients (galacto-oligosaccharides, lactulose, lactosucrose, lactitol, lactobionic acid, tagatose and sialyllactose). Many oligosaccharides are already on the market, lactulose is an established pharmaceutical product, and commodities such as lactose and permeate powder still make up the most significant part of the lactose business (Affertsholt-Allen 2007). However, high value-added lactose derivatives ($5000/t to $10,000/t) are showing interesting new application opportunities and significant annual growth rates (5 to 20%). The objective of this review is to highlight recent research related to the manufacture or the applications of the lactose derived prebiotics as well as other derivatives attracting increasing interest because of their various health and functional benefits. As noted by Peters (2005), the identification of the different avenues for permeate utilisation is important because future sustainable economic gains from whey products will most likely be built on the lactose derivatives rather than the protein streams.

Prebiotics

According to the updated concept of Gibson *et al.* (2004), prebiotics are selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microflora that confer benefits upon host wellbeing and health. The main applications for prebiotics currently are in infant, clinical and geriatric nutrition and in food segments such as beverages, dairy products (yogurts especially) and bakery products, although they could potentially be incorporated in many other food products for both human and animal consumption (Crittenden and Playne 1996; Gibson *et al.* 2004). Two out of the three carbohydrates which completely fulfil the criteria for prebiotic classification are lactose derivatives (galacto-oligosaccharides and lactulose), the third one being the inulin/fructo- oligosaccharides (Goulas *et al.* 2007). A larger variety of ingredients are produced and marketed worldwide as prebiotics; they are already accepted by consumers but convincing scientific evidence about their non-digestibility, fermentation and selectivity is still lacking, as is the case for lactosucrose (Gibson *et al.* 2004). The properties and applications of the main commercial dairy prebiotics are presented in Table 1.

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Abstract

Whey permeate-derived ingredients such as galacto-oligosaccharides, lactulose, lactosucrose, lactitol, lactobionic acid, tagatose and sialyllactose have been the focus of intense research investigating their health and functional properties for the past 20 years. Despite the research, those derivatives are not always considered by dairy manufacturers to carry enough added value to alter the focus on commodities such as permeate powder and edible grade lactose which still represent the principal outlets for permeate solids utilisation. However, the review presented in this paper summarises the recent research published in the last decade on lactose derived functional ingredients and shows that significant opportunities can arise from investing in added value ingredients issued from permeate streams, especially where market sizes are higher than 10,000 tonnes per annum and annual growth is stronger than 5%. Galacto-oligosaccharides and lactobionic acid are two examples of promising functional ingredients coming from otherwise wasted whey permeate.

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Galacto-oligosaccharides

Human milk oligosaccharides (HMO) are important components of breast milk representing, at peak, 27% of the total carbohydrates in colostrum (Darragh 2003). The conviction that HMO play vital roles in the development of the human infant has coincided with a surge in research. HMO are numerous, more than 130 identified compounds, diverse with respect to saccharide residues and glycosidic bond and variable with time for an individual and between different individuals (Kunz and Rudloff 2006). Compared to HMO, galacto-oligosaccharides (GOS) synthesised from permeate and other lactose-rich streams by a transgalactosylation reaction catalysed by β-galactosidase, are usually simple mixtures of tri- and tetra-saccharides made up of glucose and galactose molecules along with residual lactose, glucose and traces of galactose. The enzyme, β-galactosidase, which normally favours hydrolysis, can be used at high temperatures and high lactose concentrations, to form a mixture of galactosides. Some enzymes favour 1,4-links while others favour 1,6-links between the monosaccharide residues. As GOS composition will change with the source of the enzyme used, GOS products from different manufacturers are expected to vary. Other points of difference between products relate to their degree of purification and their (undesirable) mineral content. GOS are available as powders or as syrups, typically with about 75% solids, of which actual oligosaccharides comprise about 55-60% (Goulas *et al.* 2007).

GOS are non-digestible prebiotics which promote a healthy bifidobacteria colonic microflora in humans and may protect against gut pathogens. They have been used for some time in foods in Japan where they are accepted as "foods for specified health use" (FOSHU) and were recently approved for use in infant foods by Food Standards Australia and New Zealand in 2008 (FSANZ 2008). Their main use is in infant formula but they are likely to spread to other foods with yoghurt being a likely candidate. The market price of GOS is about 10 to 12 times greater than edible lactose (Valero and Yang 2006). The current global market size is approximately 20,000 to 22,000 tonnes per annum with the fastest expected annual growth rate of all the lactose derivatives (10 to 20%) (Affertsholt-Allen 2007). While their prebiotic properties are widely accepted, GOS can also have varying health and technological benefits. They are non-cariogenic, have been found to have a positive effect on constipation and increase calcium absorption. They are half as sweet relative to sucrose and yield half the energy. They are heat and acid stable, have flavour enhancing properties and function as hygroscopic, water soluble, low molecular weight dietary fibre. The synthesis and uses of GOS have been reviewed extensively and most recently by Playne *et al.* (2003) and Gänzle *et al.* (2008).

Research issues focus on manipulating the enzymes and the lactose feed to achieve better specificity of the synthesis and higher yield (currently limited to about 50-60% maximum), as well as the separation and purification of the complex mixture of reaction products. Established methods use lactose as a feedstock rather than whey permeate as better yields are obtained and more efficient use of the enzyme is achieved, however any minerals remaining in the feed stream interfere with the attainment of high concentrations of lactose and complicate the purification of GOS from residual lactose and the monosaccharides formed in the competing hydrolysis reaction (Rustom *et al.* 1998). Durham *et al.* (1997) have developed separation processes for purifying lactose to the desired level which will also be applicable to separati[on] of the GOS reaction mixture. In addition, although GOS a[re] commercially produced by enzymatic manufacture in cell-fr[ee] systems containing β-galactosidase, they can also be formed [by] microbial fermentation. Research in this area has focused on t[he] use of lactic acid bacteria as producers of β-galactosidase enzym[es] from which GOS may be produced without costly downstrea[m] processing (Hung *et al.* 2001; Vasiljevic and Jelen 2003). [A] number of studies were conducted to improve the separation [of] the products, for instance by using nanofiltration (Goulas *et al.* 2003). Finally, despite a range of commercially available GO[S] products, mainly from Japan and the Netherlands, the curr[ent] generation of GOS was developed to provide low cost functio[nal] ingredients to the food industry rather than for targeted speci[fic] applications. Functionally enhanced prebiotic GOS could [be] produced to target specific group of bacteria (Rastall and Mai[tin] 2002) through a better understanding of the factors determini[ng] the prebiotic activity of a particular GOS and new developme[nts] in the manufacturing processes.

Lactulose

Lactulose is a semi-synthetic disaccharide made from lacto[se] by isomerisation catalysed by sodium hydroxide and boric ac[id]. Though not present in nature, small amount of lactulose a[re] formed in heat-treated milk products as a result of catalyst fr[ee] isomerisation of lactose. Since the 1950s, lactulose has be[en] used in humans for the treatment of specific medical conditi[ons] (constipation, chronic hepatic encephalopathy) and as a prebio[tic] (at that time named 'bifidus factor'), although the nomenclat[ure] of pro- and prebiotics has only come into use much later. For t[his] reason lactulose became known to science first as a medici[nal] product and not as a prebiotic food additive. This history of use a[nd] its semi-synthetic character explains the legal status of lactulo[se] which differs clearly from other prebiotics. These are usually [not] drugs but food components or additives. Lactulose is classifi[ed] both as a prescription and non-prescription drug depending on t[he] country. However, in Italy, Japan, and the Netherlands, lactulo[se]

Table 1: Properties and applications of lactose derivatives commercially used as prebiotics.

Compound	Reducing sugar	Maillard Browning	Relative sweetness	Prebiotic status	Other claimed health benefits	Potential segments	Manufacturers
GOS	yes	yes	0.3 – 0.6	yes	Protective against bowel cancer and constipation; Anticaries; Calcium absorption in gut Low energy	Infant formula; Beverages; Yogurts and dairy products; Bakery products; Sweeteners	Friesland Foods Domo; Snow Brand; Yakult; Nissin; New players such as Great Ocean Ingredients
Lactulose	yes	yes	0.6	yes	Laxative; Chronic hepatic encephalopathy; Reduced symptoms in inflammatory bowel disease; Increased antibiotics efficacy; Mineral absorption; Low energy	Medical uses; Diabetic food; Soft drinks and beverages; Infant formula; Yogurts; Pet food	Morinaga; Solvay; Inalco; Fresenius-Kabi; Relax Biofac; Danipharm; Chephasaar
Lactosucrose	no	no	0.3 – 0.6	More data needed	Anticaries; Calcium absorption in gut	Sweeteners; Dairy products; Beverages	Hayashibara; Ensuiko

can be sold as a food ingredient. This may also explain the lack of activity in the development of new applications for lactulose in the food industry. However, some activity has been seen in the medical field with a study showing antibiotics were more efficient when ingested with lactulose compared to when administered alone (Kiselev 2007). Recent research into lactulose production has evaluated selective fractionation of carbohydrate mixtures (lactose–lactulose) using supercritical carbon dioxide (Montanes *et al.* 2007). The market size was approximately 25,000 to 35,000 tonnes per annum in 2006/2007, with an annual growth rate of 2 to 4% (Affertsholt-Allen 2007).

Lactosucrose

The artificial sweetener lactosucrose is 30% as sweet as sucrose and is a trisaccharide consisting of glucose, galactose, and fructose. Lactosucrose can be obtained from lactose and sucrose or raffinose by a transfructosylation reaction catalysed by β-fructofuranosidase (Fujita *et al.* 1990) or by levansucrases (Park *et al.* 2005). Lactosucrose is mainly used as a prebiotic in Japan but has been found to also enhance calcium absorption (Kishino *et al.* 2006; Teramoto *et al.* 2006). It has been commercially produced in Japan since 1990 by the companies Hayashibara Biochemical laboratories and Ensuiko sugar refining. Current research has focused on improving the purity of the product and improving the process through better production and separation using simulated moving bed reactors, however yields are still low with a maximum of 70% (Pilgrim *et al.* 2005). Other studies relate to the health benefits of using lactosucrose as a functional food ingredient (sweetness, nondigestibility, noncariogenicity, bacteriostatic action, selective proliferation of bifidobacteria). The current market size is approximately 3,000 tonnes per year with an annual growth rate of 10% (Affertsholt-Allen 2007).

Other derivatives with health related benefits

The properties and applications of lactose derivatives with potential health benefits but not currently classified or used as prebiotics are presented in Table 2.

Lactitol

Lactitol is produced from the catalytic hydrogenation of lactose to produce the sugar alcohol. There are several recent patents describing the manufacture of crystalline lactitol (Heikkilae *et al.* 1998; Heikkilae and Nurmi 2003; Myers *et al.* 2005). Lactitol is used as a low calorie sweetener and as a laxative, it also acts as a dietary fibre, competing against sorbitol and maltitol. Not absorbed through the small intestine, lactitol does not raise blood glucose levels and is thus suitable for diabetic foods (Drakoularakou *et al.* 2007). The current market is approximately 10,000 tonnes per year with an annual growth rate of 2-4% (Affertsholt-Allen 2007).

Lactobionic acid

Lactobionic acid is obtained by oxidation of lactose either by electrolysis, patents for which can be traced back to the work of Isbell and others in the 1930s, or by the enzyme hexose oxidase. Its current commercial applications are in the chemical and pharmaceutical fields rather than in the functional foods area. The calcium chelating property of the lactobionate form is used in calcium supplements in pharmaceuticals and as an ion sequestrant in detergent solutions. Lactobionic acid is also used in the formulation of solutions for the cold storage transport of transplant organs. The current market size for lactobionic acid is approximately 15,000 to 17,000 tonnes per year and is expected to have an annual growth rate of 5% (Affertsholt-Allen 2007).

Two areas of research on lactobionic acid can be outlined from the literature. The first one focuses on achieving a beneficial change, such as acidification, in the food itself. The second one looks at the formation of lactobionic acid with a view to recovering it for use as a food ingredient either in the acid form or as a calcium salt. Many patents have been granted in the last ten years for isolation and use of hexose oxidase primarily for cross-linking proteins and/or phenolic groups in bread and biscuit doughs to achieve textural advantages in baked goods. Inevitably, the commercial availability of this enzyme has rekindled interest in its dairy applications explored 35 years ago by Rand (1972) for the enzymic acidification of milk to augment or accelerate

Table 2: Properties, health-related benefits and applications of non-prebiotic lactose derivatives.

Compound	Relative sweetness	Prebiotic status	Other claimed health benefits	Potential segments	Manufacturers
Lactitol	0.3	More data needed	Prevention of constipation; Low energy	Sweeteners; Laxative; Diabetic foods	Danisco; Purac; Towa; Nikken
Lactobionic acid	sour	More data needed	Cold storage of transplanted organs; Calcium fortification	Detergent and chemicals (calcium chelator, ion sequestrant); Dairy products (acidulant)	Solvay; Sandoz; US Dairy Ingredient Co. Friesland Foods
Syallyllactose	0.2 – 0.6	no	Cell adhesion; Protection against pathogenic attacks	Infant formula	NA
Hydrolysed lactose	0.6 – 0.9	no	Alleviation of lactose malabsorption	Lactose-hydrolysed dairy products, whey drinks; Feedstock for other transformations; Pet food; Concentrated dairy products (lactose crystallisation control)	Valio; Others

acidification by micro-organisms as in yoghurt or cheese. Recent patent activity has focused on the use of the enzyme for synthesis of lactobionic acid which is of interest as a food acidulant and, in the form of the calcium salt, for calcium fortification.

Siallylactose

Siallylactose is recovered directly from whey permeate rather than being derived from lactose however its potential health benefit and market value are high. The family of compounds commonly called sialic acids play an important role in cellular interactions, particularly cell adhesion, from both beneficial and pathogenic points of view. They may protect against pathogenic attack by interfering with the binding of pathogenic micro-organisms to cells. In contrast to human milk, (where in the first week, sialic acid may comprise up to 1g per litre) cow's milk contains but a few percent of this level and several studies have suggested that breast fed infants achieve much higher tissue levels of sialic acid than those fed on either soy or bovine based formula. Interest has developed in concentrating and recovering sialic acids from whey, permeates and related sources so that they can be economically added to infant formula and other foods. Holst *et al.* (2007) have a process utilizing ultrafiltration and diafiltration on polyamide thin films to recover oligosaccharide bound sialic acid. Neose Pharmaceuticals have patents for recovery of sialic acid from whey streams. Roth *et al.* (2001) found ways of first separating the sialic acid from the much greater concentrations of mineral salts and lactose by enzymic hydrolysis while Spade *et al.* (2003) used phytase to decrease the phosphate content of streams containing sialic acid and Brian *et al.* (1998) recovered sialic acids as lithium salts following anion exchange of whey streams including mother liquor (delac). These inventors suggest that one kilogram of whey permeate could yield sialic acid worth more than $60,000 but at this value, it is hard to see how manufacturers could afford to add much to an infant formula.

Hydrolysed lactose

Lactose can be enzymically hydrolysed into glucose and galactose with β-galactosidase, producing a syrup which is more easily digested by those who are lactose intolerant. Many lactose-hydrolysed milks, milk powders and yoghurts are available around the world. Lactose intolerance has been reviewed by Savaiono's group (Hertzler *et al.* 1996; Savaiano *et al.* 2006). Hydrolysed lactose is sweeter and more soluble than lactose and can be used for sweetening syrups in ice creams, yoghurts and drinks without lactose crystallisation problems. Lactose-reduced milk products are commercially produced either by adding β-galactosidase directly into the product or by immobilisation of the enzyme on a resin such as the HYLA range produced by the Finnish company Valio. Jelen and Tossavainen (2004) have reviewed the processes and applications for lactose-free and low lactose dairy products. Cost and difficulty of storage both limit the production and uses of hydrolysed lactose syrup. However, Valio successfully produces lactose-free milk using a patented process based on removing most of the lactose by chromatography to avoid the sweetness caused by the release of glucose and galactose during hydrolysis, then hydrolysing the remaining lactose with a soluble enzyme (Harju 1987). More recently, a four-stage process has been described by Thomet *et al.* (2004), for manufacture of hydrolysed permeate employing pasteurisation, continuous lactose hydrolysis and two nanofiltration treatments. A process variation has been proposed by Temiz *et al.* (2004), for high fructose syrup from hydrolys[ed] permeate using glucose isomerase. The research led by Jele[n's] group on lactose hydrolysis by mechanically disrupted bacter[ia] was recently summarised by Gänzle *et al.* (2008).

Conclusion

Current and future research on manufacture of galact[o-] oligosaccharides (GOS) and the broader lactose derivatives [in] general, should be addressing the need to achieve continuo[us] and efficient production of the product in a sustainable mann[er] and to maintain a low cost while achieving higher yield a[nd] purity. The substantiation of health claims is another resear[ch] challenge facing industry. Galacto-oligosaccharides with target[ed] functional properties should be designed and their compositi[on] better controlled, especially chain length. Future economic gai[ns] in marketing of whey products should build on the "uniq[ue] biological and functional properties" of lactose, GOS and relat[ed] derivatives (Peters 2005). Research has already uncovered the unique functional properties and some of the processes to mak[e] good returns from lactose and derivatives. . The key to sustainab[ly] delivering those good returns on the market stage lies in using pu[re] lactose rather than edible grade lactose as a gateway for produ[ct] stream consistency. A purified lactose stream would bring great[er] flexibility to the ways in which manufacturers can use the lactos[e] in whey permeate whether it be in protein standardisation a[nd] other established uses, or liquid lactose or higher value lacto[se] derivatives. Two good candidates identified by this review alread[y] presenting sizable markets with high value and annual grow[th] rate above 5% would be galacto-oligosaccharides and lactobion[ic] acid.

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