Int.J.Curr.Microbiol.App.Sci (2016) *5*(11): 721-732

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 11 (2016) pp. 721-732**
Journal homepage: http://www.ijcmas.com

Review Article

http://dx.doi.org/10.20546/ijcmas.2016.511.083

Lactulose: Significance in Milk and Milk Products

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Keywords

Epimer, Isomerization, Prebiotic, Endotoxemia.

Article Info

Accepted:
26 October 2016
Available Online:
10 November 2016

A B S T R A C T

Many kinds of lactose derivatives can be obtained using various methods, including epimerization, oxidation and reduction. Lactulose is not only utilized as an indicator substance for milk heat treatment but it also represents one of the most valuable lactose derivatives with therapeutical applications dating back to the middle of the last century. Lactulose is a non-caloric, synthetic disaccharide formed from one molecule each of fructose and galactose. Technologically, lactulose can be produced by the isomerization of lactose molecule in which a new ketose sugar from aldose by regrouping the glucose residue to the fructose molecule. It is widely used in pharmaceutical and food industries because of its beneficial effects on human health. In particular, lactulose, galactooligosaccharide and lactitol are used in foodstuffs and pharmaceuticals, and new lactose derivatives such as epilactose and tagatose have also recently received attention.

Introduction

Lactose, a unique disaccharide, occurring exclusively in the mammalian milk plays an important role in nutrition. Most of the lactose that is manufactured on an industrial scale is produced from whey derived from the production of cheese, casein or paneer using crystallization and purification technologies. Lactose can be converted to various derivatives like lactitol, lactobionic acid, galacto-oligosaccharide, epilactose etc., using laboratory or industrial processes. They are widely used in food and in pharmaceutical fields due to their special characteristics. Lactulose is a derivative of lactose obtained by Isomerization of glucose moiety of lactose to fructose (Claeys *et al.*, 2001).

Chemistry and Properties of Lactulose

Lactulose (4-O-β-D-Galactopyranosyl-D-fructofuranose), the isomerized lactose, is a disaccharide consisting of galactose and fructose with a molecular weight 342. It belongs to the few carbohydrates other than lactose that play an important role in the dairy fields (Gaenzle *et al.*, 2008). Although Lactulose was first systhesized in 1929 (Montgomery and Hudson, 1930) physically, its significance was not recognized until 1957 when Petuely discovered that it act as a growth factor for Bifidobacteria (Petuely, 1957).

It is more soluble in water than lactose and about half as sweet as sucrose and does not crystallize easily even in concentrated

solutions. Lactulose is much less stable in solution than lactose and may subsequently degrade via process of β-elimination to give galactose, tagatose and saccharinic acids and other low molecular weight products (Olano and Martinez-Castro, 1981). Subsequently, lactulose may epimerize via a 2,3-enediol to form epilactose (4-O-β-D-galactopyranosyl-D-mannose).It reduces Fehling's solution on heating.

As per Speck (1958) two main pathways should exist since the pH range of milk systems permits the possibilities of transformation in two ways. The first path is theLobry de Bruyn-Alberda van Ekenstein (LA) transformation. This is in essence an alkaline isomerization. The second theory of the mechanism of lactulose formation assumes the formation of lactosylamine which undergoes Amadori rearrangement to lactulose by hydrolytic degradation.

Formation in Milk during Heat Treatment

Temperatures above 100°C as applied in the production of sterilized and UHT milk enhanced product stability, but also induce several chemical reactions of milk components, such as the isomerization of lactose into lactulose. In the case of lactulose formation, the glucosemoiety of lactose is isomerized into its keto-analogue fructose, a process which is favoured by heat and alkaline conditions. Observed lactulose concentrations vary between 0.3 g/l for UHT milk and 1.6 g/l for sterilized milk (Mendoza *et al.,* 2005).

Increasing the content of lactulose in milk by extending heating time or temperature is not possible due to a heat-induced drop of pH and a simultaneous thermal degradation of lactulose.

Production of lactulos Chemical methods

Industrial production of lactulose is exclusively carried out by chemical isomerization of lactose via the Lobry de Bruyn-Alberda van Ekenstein (LA) rearrangement. Three different methods namely alkalising catalysts, complexing agents and ion-mediated process are employed for lactulose production by chemical methods.

Alkalising catalysts

Alkaline catalysts like triethylamine, sodium hydroxide, magnesium oxide and sulfites, provoking a pH of 10-12 in the reaction mixture, have been successfully employed for the isomerization of lactose (Montgomery and Hudson, 1930). A new group of catalysts was introduced with calcium carbonate-based by-products like egg and oyster shells (Montilla *et al.,* 2004; Villamiel *et al.,* 2002).

Complexing agents

The employed catalysts are borate and aluminate which form an insoluble complex with ketose sugars like lactulose under alkaline conditions (Hicks and Parrish, 1980; Kozempel and Kurantz, 1994). Zokaee *et al*. (2002) reported that under similar reaction conditions $Y_{lactu/lacto}$ was about 80% for borate, whereas with aluminate less lactulose was formed ($Y_{lactu/lacto}$ ¼ 70%) and its degradation was increased (Hicks *et al*., 1984).

Ion-mediated processes

Alkalisation of whey was achieved by circulation over strong ion exchange resins activated with hydroxide ions. The exchange of ions like chloride and the subsequent release of hydroxide ions from the resin

increased the pH of whey and induced the LA-transformation of lactose into lactulose (Khramtcov *et al*., 2004). Ion-mediated processes have not been established in industrial processes yet. Evdokimov and Alieva (2004) suggested using the alkaline fraction of electrochemically activated water or lactose solutions obtained by electro-membranous methods for the production of lactulose.

Enzymatic Method

Enzymatic synthesis of lactulose is commonly carried out with classes of enzyme β-galactosidase and glycosidase. β-galactosidase is a well-known biocatalyst for transgalactosylation reaction and for the synthesis of lactose based derivatives including galacto-oligosaccharides (Panesar *et al*., 2006; 2010).

Using free enzymes

Mayer *et al*.(2004)reported the production of lactulose by enzymatic transgalactosylation from lactose to fructose by using β-galactosidase from *Aspergillus oryzae* and the hyperthermostable β- glycosidase from *Pyrococcus furiosus.*Further, gene encoding a thermostable β-galactosidase from *Sulfolobus solfataricus* has been cloned and expressed in *Escherichia coli* for lactulose production (Kim *et al*., 2006).Enzymatic synthesis of lactulose from whey permeate was affected by β-galactosidase preparation, substrate concentration and by theratio of lactose and fructose (Adamczak *et al*.,2009).

Using immobilized enzymes

Lactulose has also been successfully synthesized by dual enzymatic consisting of immobilized lactase and immobilized glucose isomerase. Immobilized lactase is prepared by cross-linking the free lactase into Fe_3O_4-chitosan magnetic microspheres (Hua *et al*., 2010). Mayer *et al*., (2010) developed a continuous enzymatic process for the production of lactulose through transgalactosylation using free and immobilized β-glycosidase from *Pyrococcus furiosus*.

Using whole cells

Whole cells used as biocatalyst for the production of lactulose often causes very low reaction rates due to permeability barrier of the cell envelope for substrates and products. The permeabilized *Kluyveromyces marxianus* cells as a source of β-D-galactosidase were used to overcome this problem. Ethanol permeabilization of yeast cells has been shown to be an economical, easy, convenient and safe (Lee *et al*., 2004).

Extraction and Purification of Lactulose

During lactulose production, the reaction mixture is generally not pure, and usually contains appreciable quantities of other substances such as lactose, glucose, galactose, epilactose etc. The recovery of lactulose during downstream processing has been found to be an important reaction because the amount of purified lactose was affected by both physical and chemical treatments. A strong acidification of obtained solution has been carried out at temperature in order to release lactulose and to induce precipitation of borate as boric acid and sodium aluminate as gel like aluminium hydroxide (Kozempel *et al*., 1995). Moreover, separation steps have been evolved for the removal of borate by the crystallization of boric acid of 50% in a solution with 20% (w/w) carbohydrates and 15% (w/w) borate (Kozempel *et al*., 1995). But, in case of heterogeneous catalysts (like powdered egg shells), centrifugation method has been used for recovery of lactulose and the obtained sugar solution has been

decolourized using activated carbon without decreasing the concentration of recovered lactulose (Montilla *et al*., 2005). The purification of lactulose from a mixture with lactose has also been carried out by using pressurized liquid extraction (PLE) at 1500 psi for 30 min and the recovery of lactulose reached up to 84.4% with a purity of over 90% (Ruiz-Matute *et al*., 2007).

Analysis

A range of methodologies have been reported to determine the lactulose including, gas-liquid chromatography (GC), thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC), capillary electrophoresis, differential pH methods and flow analysis methods. A high pressure liquid chromatography was also used for the separation of galactose, tagatose, lactose and lactulose with a commercial carbohydrate analysis column (Parrish *et al*., 1980).

Khan *et al*., (2006) developed a method based on hydrolysis of lactulose under acidic conditions followed by reaction of the hydrolysed product with resorcinol, giving absorption peaks at 398 and 480 nm. A simple and rapid flow system was developed for the determination of lactulose in milk samples, which is based on the hydrolysis of lactulose to galactose and fructose by the enzyme β-galactosidase immobilized in a reactor (Moscone *et al*., 1999).

Amine *et al*. (2000) developed an enzymatic spectrophotometric assay for the determination of lactulose in milk samples by the hydrolysis of lactulose to fructose and galactose, and fructose dehydrogenase reacts with fructose in presence of a tetrazolium salt giving a coloured compound which can be detected spectrophotometrically at 570 nm.

Physiological Effects of Lactulose

The β-glycosidic linkage of the disaccharide lactulose is not hydrolyzed by mammalian digestive enzymes and ingested lactulose passes the stomach and small intestine without degradation. This selective metabolism of lactulose alters the microbial balance and the biochemical composition of caecal contents. Several *in-vivo* studies have demonstrated that lactulose favours the growth of grampositive cocci and rods mostly belonging to the genera *Bifidobacterium* and *Lactobacillus* (Bouhnik *et al*., 2004), while bacterial counts of galactosidase negative microorganisms like subspecies of the genera *Clostridium* and *Bacteroides* have been shown to decrease (Mizota *et al*., 2002). Lactulose is now a commonly used drug worldwide, and is listed in the US Pharmacopeia, European Pharmacopoeia and Japanese Pharmacopoeia.

Fermentation by colonic bacteria

Sahota *et al*., (1982) screened sixty-four bacteria cultured under anaerobic conditions in lactulose containing media to assess their ability to ferment lactulose. Some organisms were unable to metabolize the disaccharide, while others, e.g. *clostridia* and *lactobacilli*, metabolized lactulose extensively. Quantitative analyses of the fermentation products indicated that the major non-gaseous metabolites were acetic, lactic and butyric acids and hydrogen and carbon dioxide were the only gases detected.

Treatment of constipation and hepatic encephalopathy

Lactulose is widely established as a laxative agent in the treatment of constipation (Schumann, 2002).Theextent of purgative action depends on various facts, including

the health status, age, weight, gender and diet of the concerned person. Oku and Okazaki (1998) determined the laxative threshold concentration of lactulose to be 0.26 g per kg (body weight) in 20 Japanese females. Assuming an average body weight of 55 kg, a healthy female may consume up to14.3 g lactulose in a single dose without suffering diarrhea. Studies confirmed that lactulose cured the neuropsychiatric symptoms of hepatic encephalopathy, although the mode of its action remained unclear (Schumann, 2002). Minimal hepatic encephalopathy (MHE) could be treated with 30-60 ml lactulose (adult) (approx. 20-40 g lactulose), thus clearly improving of the health related quality of life of cirrhotic patients (Sharma *et al.*, 2008).

Enhancement of mineral absorption

The improved absorption of minerals has been generally described for prebiotics and is presumably mediated by an increased permeability of intestinal mucosa and an enhanced solubility of minerals in the colon at low pH (MacFarlane *et al*., 2006). Studies by Seki *et al.*, (2007) and Van den Heuvel *et al.* (1999) showed Ca and Mg absorption can be improved by lactulose administration.

Prebiotic action

The prebiotic action of lactulose has been documented throughout the last five decades. According to Bovee-Oudenhoven *et al.* (2003) lactulose inhibited the colonisation of Salmonella in rats. Lactulose leads to a sharp drop of the colonic pH, which makes *Salmonella* difficult to survive. Lactulose therapy clears faecal *Salmonella* and *Shigella* species and reduces the prevalence of urinary-tract infection and respiratory tract infections (Liao *et al*., 1994).The application of lactulose as a beneficial, prebiotic nutrient is restricted to low doses as a higher intake would probably cause frequent bowel movements or diarrhea. A survey of studies focussing the dose-response of lactulose indicates that single doses of lactulose exert prebiotic action from a daily intake of 4 to 10 g in adult (Mizota *et al*., 2002).

In the work carried out by Oliveira *et al*. (2011) use of lactulose in fermented milk improved the quality of skim milk fermented by *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus bulgaricus* and *Bifidobacterium lactis* in co-culture with *Streptococcus thermophilus.* Compared to control fermentations without lactulose, the addition of such a prebiotic in skim milk increased the counts of all probiotics, with particular concern to*B.lactis*.

Treatment of colon carcinogenesis

Lactulose appear to reduce the risk of colon cancer (Schumann, 2002). Challa *et al.* (1997) investigated the influence of *Bifidobacterium longum* and lactulose on the chemical induction of aberrant crypt foci (ACF), a precursor of colonic adenoma, in rats. They showed that lactulose decreased the incidence of ACF, a phenomenon that was partly ascribed to the lowering of caecal pH.

Anti-endotoxin effects

Lactulose treatment before operation can prevent endotoxindependent complications such as renal dysfunction (Özçelik *et al*., 1997; Pain *et al*., 1991). The anti-endotoxin effect of lactulose has also important medical applications in metabolic diseases like the hepatorenal syndrome (Kramer, 1988), exocrine pancreatic dysfunction (Mack *et al*., 1992), diabetes mellitus (Yelich *et al*., 1992) and hypercholesterolemia (Liao and Florin, 1995).

Blood glucose and insulin

Lactulose has also an important application in lowering blood glucose level (Bianchi *et al*., 1994). Cornell (1985) also suggested that endotoxin reduces the pancreatic insulin production and thus lactulose shows anti-diabetic effect.

Tumour prevention and immunology

Bifidobacteria play an important role in tumour prevention. The antitumour and immunologic effects of *Bifidobacteria* can be enhanced by intake of lactulose (Schumann, 1997).

Inflammatory bowel disease

Oral administration of lactulose abolishes and prevents systemic endotoxemia of gut origin. Obstructive jaundice is often accompanied by bacterial translocation and subsequent sepsis and the administration of lactulose may prevent systemic endotoxaemia and the subsequent inflammatory response in an experimental model of obstructive jaundice (Koutelidak *et al.*, 2003). Fermentation of lactulose by gastrointestinal tract bacteria, can produce a considerable amount of mobilized endogenous hydrogen, which is protective for DSS-induced colitis as a unique antioxidant, which can reduce oxidative stress and ameliorate symptoms of inflammatory bowel disease in human beings (Chen *et al.*, 2011).

Applications

Food Applications

The use of lactulose as a food ingredient was introduced in 1957 by Petuely (Petuly, 1957) who was the first to favour the growth of the bifidus flora in infants by adding lactulose to diet. Its high thermostability under acidic conditions is considered one of its most advantageous characteristics and allows it to be utilized in acidic foods such as fruit juice. Incorporation of 0.5% lactulose in the infant formula is considered adequate to stimulate *Bifidobacteria* flora to the extent observed in breast-fed babies, while the presence of lactulose at 1% level in the formula may also provide laxative effect (Nagendra *et al.*, 1995). Adriana *et al*. (2002) found that lactulose contents of infant formulas containing lactose were between 29 and 108 mg/l of reconstituted formulas based on milk and 97 and 312 mg/l of reconstituted formulas based on milk enriched with whey. Although no limit to lactulose content in infant formulas has been established, the lactulose contents in these formulas were well below this limit suggested by IDF (1992) and EU commission (1992) for commercial UHT milk. The addition of lactulose reduced the incubation period in the manufacture of bio-yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* and caused a significant increase in the cell counts of *B.bifidum* during cold storage (<6°C, 14 days) (Ozer *et al.*, 2005).Pham and Shah (2008) observed that the addition of 0.5% (w/v) of lactulose to soy milk appeared to favour the growth of *B. longum* and *B. Animalis* which in turn enables transformation of isoflavone glycosides to biologically active isoflavone aglycones.

An Indicator for Heat Treated Milk

Assuming that only minimal lactulose degradation occurs within traditional milk heating, lactulose can be used to characterize the heat load and to distinguish between differently processed batches of milk. Concentration of lactulose in sterilized milk differs significantly from UHT milk, which itself can be assigned to either direct or indirect heating by the amount of formed

lactulose (Olano *et al*., 1989). Lactulose was proposed by the International Dairy Federation (IDF) and the European Union (EU) as a parameter capable of differentiating between UHT milk and in-container sterilized milk (EU Commission, 1992; IDF, 1992, 1993) with 600 mg/l of lactulose as a marker for distinguishing between two milk types.

With regard to pasteurized, microfiltered and pasteurised, high temperature pasteurized milk and reconstituted powered milk, upper limits (threshold) of lactulose concentration cannot be proposed since the methods available for lactulose determination are neither sufficiently sensitive (level of detection 50mg/l) (Moscone *et al*., 1999). Marconi *et al.* (2004) derived a sensitive enzymatic assay method to distinguish lactulose content, not only in-container sterilized milk, indirect and direct UHT, but also UHT milk produced by mild technologies such as milk treated by infusion, high temperature pasteurized milk and low temperature pasteurized milk. Cho *et al*. (2012) reported that lactulose and furosine concentration ratio is a suitable milk quality indicators of heat damage and for demonstrating improper addition of reconstituted milk powder.

Diagnostic Applications

Lactulose is applied in the diagnosis of colonic disorders by means of the breath hydrogen test. This test consists of the oral administration of a single dose of lactulose and the determination of exhaled hydrogen formed by means of bacterial metabolism of lactulose in the intestine. The amount and the time of hydrogen production and exhalation conclude the colonic transit time and microbial colonization (Simren and Stotzer, 2006). The lactulose breath test is also applied for the diagnosis of bacterial overgrowth in the small intestine (Nucera *et al*., 2005).

Safety and Regulatory Status

European Food Safety Authority (2010) released a "scientific opinion on the substantiation of health claims related to lactulose and decreasing potentially pathogenic gastro-intestinal microorganisms (ID 806) and reduction in intestinal transit time (ID 807)". The European chemical Substances Information System (ESIS) provides a detailed description of chemical and pharmacological properties of lactulose. Included data on the acute oral toxicity of commercial lactulose preparations summarize various animal studies with values for the median lethal dose LD_{50} ranging from 25 to 65 g per kg body weight. Drugbank, a Canadian database operated by the University of Alberta additionally specifies that pure lactulose could provoke slight irritations in case of skin or eye contact. According to the Anatomical Therapeutical Chemical Classification System for pharmaceutical drugs established by the World Health Organization, lactulose is an osmotically acting laxative substance (ATC Code ATC06AD11). For this purpose the recommended administration of lactulose, expressed as the defined daily dose (DDD) is indicated with 6.7 g. But apart from its status as a pharmaceutical drug, lactulose is regarded as a functional food additive. The Japanese Ministry of Health and Welfare has acknowledged the nutritional benefits of lactulose by including it in the list of functional foods with the official label FOSHU (Food of Specified Health Use). In the European Union, the labelling of food containing lactulosewith thehealthrelated claims "prebiotic" and "lowers colonic transit time" has been proposed for products with a minimum dose of 2.5 g lactulose.

In conclusion, lactulose is a disaccharides and produced by the isomerization of lactose. Lactulose can be generated by either alkaline isomerization, enzyme-catalyzed synthesis or by membrane technology. Lactulose can be used as an indicator of heat treatments. The physiological action of lactulose has been focussed by clinical trials for over five decades, leading to its application as pharmaceutical as well as a prebiotic food ingredient.

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How to cite this article:

Sonali L. Parekh, Smitha Balakrishnan, Subrota Hati and K.D. Aparnathi. 2016. Lactulose: Significance in Milk and Milk Products. *Int.J.Curr.Microbiol.App.Sci.* 5(11): 721-732.
doi: http://dx.doi.org/10.20546/ijcmas.2016.511.083