Lactobionic Acid: Significance and Application in Food and Pharmaceutical

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Abstract

Lactose has long been used as a precursor for the manufacture of high-value derivatives with emerging applications in the food and pharmaceutical industries. This review focuses on the main characteristics, manufacturing methods, physiological effects and applications of lactobionic acid. Lactobionic acid is a product obtained from lactose oxidation, with high potential applications as a bioactive compound. Recent advances in tissue engineering and application of nanotechnology in medical fields have also underlined the increased importance of this organic acid as an important biofunctional agent.

Keywords: Lactobionic acid, oxidation, physiological effects, applications

Carbohydrates have been used in the manufacture of bulk and fine chemicals, and are viewed as a renewable feedstock for the ‘green chemistry of the future. Lactose, a unique disaccharide, occurring extensively in the mammalian milk plays an important role in nutrition. Most of the lactose that is manufactured on an industrial scale is produced from the whey derived from the production of cheese, casein or paneer by using drying, crystallization and purification technologies.

However the use of lactose is limited in many applications, because of its low sweetness and solubility, as well as due to the intolerance of some population segments (Gutierrez et al., 2011). Lactose can be converted to various derivatives like lactobionic acid, lactulose, lactitol, galactooligosaccharides, epilactose etc., using laboratory or industrial processes. Lactobionic acid is produced by oxidation of lactose. It is widely used in food and in pharmaceutical field due to its excellent biocompatibility, biodegradability, nontoxicity, chelating, amphiphilic and antioxidant properties (Alonso et al., 2013).

CHEMISTRY

Lactobionic acid (4-0-β-D-galactopyranosyl-D-gluconic acid) belongs to the aldobionic family of acids (Pezzotti and Therisod, 2006). Chemically lactobionic acid comprises a galactose moiety linked with a gluconic acid molecule via an ether-like linkage and featured by the presence of multifunctional groups. Thus it acts as a metal ion chelator and can sequester calcium (Alonso et al., 2013). It has a molecular weight of 358.3 Da with pKa 3.6 (Armarego and Chai, 2009). From a nutritional point of view, this substance may be considered a low calorie sweetener which provides only 2 kcal/g (Schaafsma, 2008). Lactobionic acid appears as a white solid powder which is freely soluble in water and slightly soluble in anhydrous ethanol and methanol. It has a melting
point in the range of 128-130ºC (Alonso et al., 2013). Lactobionic acid can be dehydrated to a lactone. It is hygroscopic in nature. Its superior water-retention ability is valuable for cosmetic applications (Playne and Crittenden, 2009). Lactobionic acid and its mineral salts (mainly Na, Ca and K lactobionate) are commercially produced for industrial and medical applications, as well as for research purpose (Nakano et al., 2010).

PRODUCTION

The selective conversion of lactose to lactobionic acid consists of the oxidation of the free aldehyde group of glucose on the lactose molecule to the carboxylic group. Several processes including biocatalytic, electrochemical and heterogeneous catalytic oxidations have been investigated for the production of lactobionic acid.

Biocatalytic oxidation

The biocatalytic production of lactobionic acid comprises the oxidation of lactose by means of specific enzymes or by using microorganisms as biocatalysts. The general reaction mechanism, involves the formation of lactobiono-δ-lactone as an intermediate product, which is hydrolyzed to lactobionic acid (Nakano et al., 2010). The process is generally carried out at mild temperature conditions 25-50ºC, and the pH must be kept constant during reaction by adequate addition of a Na, Ca or K base, the product can be lactobionic acid or its salts. When lactobionate salts are produced, the solution can be passed through cation exchange resins to obtain a lactobionic acid solution, which can be further concentrated and crystallized to produce pure lactobionic acid.

Microbial production

Pseudomonas species are the main bionic producer microorganisms, lactobionic acid is formed via the lactose oxidation pathway, in which a membrane bound dehydrogenase enzyme initially catalyzes the oxidation of the lactose to a lactone intermediate (lactobiono-δ-lactone) which is subsequently hydrolyzed (its carbonyl group) by a lactonase into lactobionic acid (Alonso et al., 2012; Nishizuka and Hayaishi, 1962). While the production of lactobionic acid from lactose by using filamentous fungi, gives only 50% yield after 120 h, which suggests the presence of residual lactose oxidase activity in this fungus (Pedruzzi et al., 2011; Malvessi et al., 2013). In addition, the lactose-oxidizing ability has been also found in a red algae, which are capable of oxidizing diverse carbohydrates at an optimum pH of 5.0 (Murakami et al., 2003; Alonso et al., 2011). Oxidation of lactose by using Acetobacter orientalis gives 97 to 99% yield under resting cell conditions in nutrient rich media at the shake flask scale (Kiryu et al., 2012).

The oxidation of lactose to lactobionic acid by acetic acid bacteria, such as Gluconobacter cerinus UTBC-427 showed the highest lactose oxidizing activity of the screened microorganisms (Oe et al., 2008). This latter GRAS microorganism has been recently selected by Unitika Company in Japan as microbial platform for lactobionic acid industrial bio-production under resting cell conditions (Kimura, 2012).

Enzymatic synthesis

Enzymatic catalysis produces higher lactobionic acid, yields, and productivities than microbial fermentation, but enzymes are rather unstable under enzymatic production process conditions (Nordkvist et al., 2007). Lactose oxidizing enzymes are glucose-fructose dehydrogenase, cellobiose dehydrogenase (Van Hecke et al., 2009), and carbohydrate oxidase (Gutiérrez et al., 2012), have been used for lactobionic acid production, but technological challenges are many that require coenzyme regeneration and enzyme inactivation mostly by the formation of H₂O₂, which has to be reduced, by the addition of catalase (Hua et al., 2007) in addition, redox mediators required they may be incompatible with the intended use of the product (Ludwig et al., 2004).

Cellobiose dehydrogenase has been the mostly used enzyme for the synthesis lactobionic acid, it is an extracellular enzyme produced by several fungi,
like *Sclerotium rolfsii*, *Phanerochaete chrysosporium*, and *Trametes versicolor* (Zamocky et al., 2006; Kiryu et al., 2008). The enzyme requires a redox mediator like 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid and 2, 6-dichloroindophenol as efficient electron acceptors that can be regenerated by an additional enzyme, like laccase. Even though the system produces lactobionic acid from lactose at a high volumetric productivity, the system is complex involving two enzymes and a redox mediator (Murakami et al., 2008; Van Hecke et al., 2009).

In an industrial context, an enzymatic mixture called ‘LactoYIELD’ was launched onto the market in 2009 as a result of the joint venture in between the Danish companies and Novozymes A/S in 2002 (Novozymes, 2009). This standardized enzymatic mixture allows cheese manufacturers to convert lactose from cheese whey into lactobionic acid.

**Electrochemical oxidation**

Lactobionic acid has been also prepared by electrolytic oxidation processes. The studies carried out by Gutiérrez et al., (2012) reported that high yields (>90%) and selectivities (~100%) toward lactobionic acid can be obtained by means of the electro-catalytic oxidation of lactose on noble metal electrodes (platinum, platinum-modified and gold electrodes) in alkaline media. Gold electrodes are the best catalyst for the oxidation of sugars, and that lactone is the primary product of the electrochemical oxidation of lactose, which is hydrolyzed to lactobionic acid.

**Heterogeneous catalytic oxidation**

During this reaction, the desired lactobionic acid is normally obtained as the main product. In order to selectively produce lactobionic acid, the reaction should be carried out at atmospheric pressure in alkaline media at pH between 8.0 and 9.0, in the temperature range of 50-70°C, using air or oxygen as eco-friendly oxidizing agents (Gutiérrez et al., 2011). Lactobionic acid production by means of heterogeneous catalytic oxidation of lactose on palladium and bismuth-palladium supported catalysts was first successfully performed by Hendriks et al., (1990). To strip off the dissolved oxygen, and to maintain the overall reaction kinetics, Periodic bubbling of air and nitrogen has been successfully used (Belkacemi and Hamoudi, 2010). Gold catalysts have shown high stability against over oxidation and supported gold nanoparticles have shown an exceptional activity and selectivity in the oxidation of carbohydrates (Belkacemi et al., 2007; Murzina et al., 2008; Gutiérrez et al., 2012).

**EXTRACTION AND PURIFICATION**

In order to achieve a higher productivity of lactobionic acid, the enzymatic reaction can be interrupted after few hours of operation and unreacted substrates can be recycled after the separation of substrates from products (Silveira, 2003). Liquid chromatography is a high efficiency separation technique that could be useful in the current process since species can be recovered with high-purity (Pedruzzi et al., 2008).

By passing the solution of lactobionate ions through a series of ion-exchange resins, a batch of pure lactobionic acid solution with negligible amounts of calcium ions can be produced. Other techniques like ethanol precipitation (Armarego and Chai, 2009), evaporation and crystallization (Jones et al., 2002) and electrodialysis are also employed for the recovery of lactobionic acid (Peretti et al., 2009).

**PHYSIOLOGICAL EFFECT**

**Anticoagulant and Antithrombotic activity**

The most important physiological effect of lactobionic acid is the anticoagulant and antithrombotic activity of its sulfated derivative bislactobionic acid amides, which have been studied as potential anticoagulant and antithrombotic agents, without a risk of bleeding (Raake et al., 1989).

This compound, also known as Aprosulate sodium, stimulated HCII activity *in vitro* (Klauser, 1991) and has been found to delay the activation of factor X and prothrombin in plasma (Ofosu et al., 1992).
Antioxidant
Lactobionic acid has also revealed antioxidant properties in tissues, by inhibiting the production of hydroxyl radicals as a result of its iron chelating properties (Charloux et al., 1995). It also prevent the oxidation of other substances such as anthralin, hydroquinone and banana peel (Van et al., 2004).

Wound healing
Lactobionic acid also promotes wound healing, and it is useful for general care of skin, hair, nail, oral and vaginal mucosa, and oral and gum diseases (Yu and Van Scott, 2010). Lactobionic acid has been recently used in the fabrication of lactobionic modified materials for carrying drugs for the treatment of liver and breast cancer (Lu et al., 2010; Zhang et al., 2011).

Prebiotic action
Lactobionic acid also exerts potential prebiotic effects as a bioactive ingredient in functional foods, as this organic acid is resistant to digestive enzymes. It is poorly absorbed in the small intestine and can be eventually fermented by the gastrointestinal microflora (Saarela et al., 2003; Schaafsma, 2008). Lactobionic acid has also been described as an active bifidus promoter molecule for diverse functional foods and beverages (Kontula et al., 2000).

Enhancement of mineral absorption
Lactobionic acid is valuable for its chelating properties due to its capability to form complex structures with Mn, Cu, Fe and Ca (Shepherd et al., 1993; Oe et al., 2008). The incorporation of lactobionic acid into foods may stimulate intestinal Ca\(^{2+}\) or mineral absorption, therefore exerting a health-promoting influence (Baldwin et al., 2007).

COMMERCIAL APPLICATION

Food and dairy applications
The use of lactobionic acid as a food additive has also received growing attention from the food industry in recent years. Lactobionic acid can serve as an antioxidant, stabilizer or gelling agent in dessert products (FDA, 2011), an acidifier agent in fermented milk products (Faergemand et al., 2012), an aging inhibitor for bread (Oe and Kimura, 2011). Additionally, lactobionic acid has been proposed as a technological feed additive for laying hens to improve egg shell qualities by boosting calcium absorption (Kimura, 2006). Equally important in novel non-dairy beverages, milk-based beverages and cheeses containing calcium lactobionate have been recently developed to provide a valuable approach for calcium supplementation (Nielsen, 2007; Nielsen and Hoeier, 2009). Lactobionic acid containing functional milk, which may help to combat calcium deficiency, has been launched into the food market by ‘Megmilk Snow’, a well-known Japanese dairy company (Megmilk Snow, 2012).

The dairy industry has been particularly involved currently in the development and implementation of innovative manufacturing processes including lactobionic acid as a key ingredient in novel dairy making technologies (Novozymes, 2009; Merrill and Singh, 2011). In fact, adding lactobionic acid either directly (10% of the mix) or indirectly (an added lactose oxidase) enhanced production yields, lower processing times and cost savings in manufacturing of processed cheese (Bradbury et al., 2002).

Lactobionic acid provides extra functional properties and sensory attributes through the reduction of undesirable Maillard browning in cooking products (Merrill and Singh, 2011), and also used as a flavor enhancer for foods or beverages (Walter and Begli, 2011).

Baldwin et al., (2004) have also devised an antioxidant composition containing lactobionic acid and siderophores as key elements for retarding lipid oxidation in food products. Lactobionic acid act as a water holding capacity agent in meat products submitted to thawing and/or cooking processes has been recently reported for the first time, resulting in higher industrial product yields and water content after treating meat products with lactobionic acid (Nielsen, 2009).
Cosmetics field

The cosmetics industry is currently employing lactobionic acid as the key active component of novel antiaging and regenerative skin-care products due to its therapeutic efficacy. The use of lactobionic acid in cosmetics has grown three to five-folds since it was launched commercially. Lactobionic acid represents a major market niche as a novel and essential protective component of skin care formulations. The cosmetics company NeoStrata (USA) has invented a broad portfolio of skin care products based on the unique properties offered by lactobionic acid (West, 2004). As a cosmetic ingredient, lactobionic acid offers multiple benefits for the therapeutic treatment of dermatological pathologies such as atopic dermatitis and rosacea (Briden and Green, 2006) or can even be employed in antiacne treatments (Decker and Graber, 2012). Due to metal chelation property of lactobionic acid, it can act as an inhibitor of the breakdown of matrix metalloproteinase enzymes, thus reducing the appearance of photoageing and wrinkles (Grimes et al., 2004; Tasic-Kostov et al., 2010). In addition to lactobionic acid antioxidant role, it also exhibits strong moisturizing, exfoliative and humectant, showing antiaging effects, including skin plumping and smoothing of surface topography with diminished appearance of fine lines and wrinkles (Green et al., 2006; Tasic-Kostov et al., 2010). Which expand its commercial relevance within the cosmetics field. Some of the cosmetic products containing lactobionic acid are available in market such as Bionic eye cream, Bionic face cream, Intimate creamy wash, Lactobionic acid peel, Lactobionic acid Miceller Gel etc. (Yu and Van Scott, 2010; Hachem et al., 2010).

Chemical industry

Lactobionic acid is used in the chemical industry as a sugar based surfactant in biodegradable detergents. Its iron chelating and emulsifying properties have suggested its potential use in many industrial operations, use as an important starting chemical for manufacturing detergents (Bize et al., 2010). Numbers of lactobionic acid based surfactants has been currently developed as biodegradable surfactants with improved surface and performance properties (Oskarsson et al., 2007). Lactobionic acid has been proposed as a building block for the biocatalytic synthesis of novel polymers with possible industrial applications (Araki et al., 2006). Aside from the aforementioned applications, lactobionic acid can be used for the synthesis of innovative industrial systems such as functionalized carbon nanotubes with lactobionic acid amide amphiphile molecules which are capable of adsorbing proteins (Feng et al., 2011).

Drug delivery systems

Lactobionic acid offers unique properties such as biocompatibility, biodegradability, ion chelating ability and self assembly, in addition to their synergistic combination (Ortial et al., 2006). Owing to these unique properties, lactobionic acid provides an excellent platform for the synthesis of potentially biocompatible and targetable drug delivery vehicles, from DNA to bioactive molecules (Duan et al., 2011; Chen et al., 2012). In fact, lactobionic acid based drug delivery systems can successfully target hepatocytes due to the presence of asialoglycoprotein receptors (ASGPR) located on their surface, since lactobionic acid works as a ligand of these receptors (Lin et al., 2011).

Nanoparticle diagnosis

Bio-functionalized nanoparticles are used for biomedical applications such as biodetection, cancer therapies, magnetic resonance imaging or bio-labeling. Lactobionic acid has attracted increasing attention in recent years as a surface coating materials due to their enhanced properties (Feng et al., 2007; Knopp et al., 2009).

Tissue engineering

Although lactobionic acid does not contribute directly to the growth of any tissue or biomaterial, this organic acid may ease the attachment and entrapment of hepatocytes, as well as the establishment and construction of biocompatible scaffolds for liver tissue engineering purposes (Kim et al., 2008).
Galactosylated matrices through lactobionic acid have been proved to be effective in hepatocyte entrapment and attachment, which offer an efficient extracellular matrix for hepatocyte aggregation during liver tissue engineering approaches (Mi et al., 2006). Specifically, the incorporation of lactobionic acid in a synthetic polymer may provide a better microenvironment for cell aggregation along with nutrition and metabolite transfer (Wang et al., 2010).

SAFETY AND REGULATORY STATUS

The role of lactobionic acid as a food preservative is anticipated, its use as calcium lactobionate has already been approved in the USA by the FDA (FDA, 2011).

SUMMARY

Lactobionic acid, an oxidation product of lactose, is a relatively new lactose derivative with potential applications in the food, pharmaceutical and chemical industries, owing to its antioxidant, chelating, humectant and emulsifying properties. Lactobionic acid and its salts are attractive as new and value added food materials because of the good taste, the health-promoting functions, as well as the excellent solubility. Moreover, due to the remarkable growth of nanotechnology, the applications of lactobionic acid can be expanded as a functional molecule for carrying drugs. Lactobionic acid may undoubtedly play a fundamental role in functional and nutraceutical foods over the coming year. Efficient and safer processes for the production are therefore increasingly desired. More over long-term safety and clinical studies are required before recommendations can be made for clinical application.

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