Review Article

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Production of D-tagatose: A review with emphasis on subcritical fluid treatment

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ABSTRACT

D-Tagatose is gaining much interests as a sugar substitute and a functional ingredient. Development of tagatose production process has been investigated by researcher around the world for more than 30 years. Chemical and enzymatic processes are currently employed for commercial scale production but the cost is still an obstacle. In this review, all potential methods for producing tagatose were summarized with the recent publications. In addition, a novel method, subcritical fluid treatment was also introduced and discussed for its potential.

Keywords: D-Tagatose production; Chemical reaction; Enzymatic reaction; Subcritical aqueous ethanol

1. INTRODUCTION

Sugar reduction has been a very popular issue in food industries for decades because of several possible adverse health effects of high sugar consumption (Stanhope, 2016). For example, in USA, food and beverage products with low-sugar, no-added sugar, and sugar-free claims were about 3.1% in 2010 and increased to 4.5% in 2014 (Williams, 2015). Reduction of sugar consumption is also an important health promoting policy in Thailand. In many countries around the world, sugar tax policy has been already implemented. The trend, therefore, has attracted researchers around the world to search for alternative substances, especially natural ones, to replace commonly used high-calorie sweeteners such as sucrose, fructose, and glucose and permitted artificial high-intensity sweeteners such as saccharin, acesulfame potassium, aspartame, neotame, and sucralose.

Among several compounds, tagatose is one of the natural sweeteners that has shown a strong potential for the purpose. In fact, tagatose is not new in sweetener industry because it has been proposed for using as a low-calorie sweetener since 1990s (Levin et al., 1995). Review articles and book chapters on properties and health benefits of tagatose have been periodically published (Bertelsen et al., 1999; Levin, 2002; Skytte, 2006; Espinosa and Fogelfeld, 2010; Vera and Illanes, 2016; Jayamuthunagai et al., 2017a; Guerrero-Wyss et al., 2018). Even though tagatose has been accepted for use as food additive in several countries, high cost of production is a main obstacle for the success of the application of this sugar and therefore suitable technology for producing tagatose at lower cost is still needed. In recent years, several studies have been focused on producing of tagatose at higher efficiency. In this review, production technologies for manufacturing tagatose from published research articles and patents

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were summarized and discussed. A new process, subcritical fluid treatment was then introduced and emphasized for its strong potential.

2. CHEMICAL AND ITS USES

Tagatose or specifically D-tagatose is a ketohexose sugar of an aldohexose galactose and an epimer of the well-known fructose (Figure 1). Tagatose can be naturally found in some fruits and dairy products at the concentrations varying from 0.05 to 35 g/kg (Skytte, 2006). It was also reported that tagatose is formed during the heat treatment of milk at high temperatures such as sterilization conditions (Adachi, 1958; Troyano et al., 1992).

Due to its limited amount in nature, tagatose has also been classified as rare sugar (Beerens et al., 2012) which can be used as a low-caloric sweetener because it provides only 1.5-2.4 kcal/g and low glycemic index with 92% of sweetness comparing to sucrose. Tagatose also exhibits prebiotic properties and showed several functional properties which beneficial in food industries such as flavor enhancing properties. Important properties of tagatose are shown in Table 1. Currently, tagatose is approved to be used as a food ingredient in many countries. Tagatose has been permitted in USA as a Generally Recognized As Safe (GRAS) dietary ingredient (GRN No. 78 for Arla Foods Ingredients, Denmark) since 2001 and in 2011 (GRN No. 352 for CJ Cheiljedang, Korea). It was approved as a Novel Food by Food Standards Australia New Zealand (FSANZ) in 2004 and approved as a Novel Food Ingredient for marketing in EU by the UK Food Standards Agency in 2005. It is also approved as health food (Functional grade II) by Korean Food & Drug Administration (KFDA) (Park and Lee, 2013).

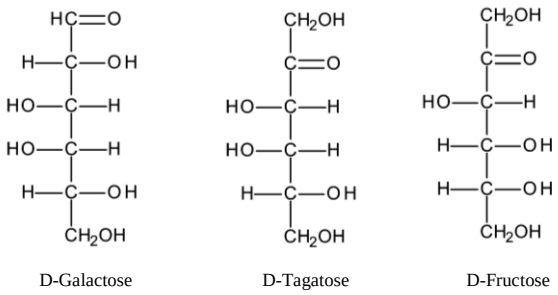

Figure 1 Chemical structures of D-tagatose, D-galactose, and D-fructose.

Table 1 Some properties of tagatose

Properties	Value	Citation
Energy (kcal/g)	1.5 – 2.4	Lamothe et al. (2017)
	3.0	Dominique et al. (2016)
Glycemic index (Glucose = 100)	3±1	Atkinson et al. (2008)
Sweetness (% of sucrose)	92	Bertelsen et al. (1999)
Solubility in water (g/100 g water at 20°C)	58	Skytte (2006)
Solubility in aqueous ethanol (g/100 g solvent at 20°C)		Gao et al. (2015b)
20 (wt %)	9.5	
40 (wt %)	7.4	
60 (wt %)	3.9	
80 (wt %)	0.9	

Commercially, tagatose is available from only a few numbers of manufacturers. Damhert Nutrition (The Netherlands) is producing and marketing tagatose as a sweetener containing tagatose (24%), sucralose (11%) and lactose and also used in the company's several products e.g. biscuits, jams, and chocolate spread under the brand "Tagatesse". CJ Cheil Jedang, a Korean food ingredient company is also manufacturing tagatose using their own technology under the brand "CJ Beksul". The price of tagatose in the market is very high comparing with ordinary table sugar. NuNaturals (Eugene, OR, USA), a distributor in USA is selling tagatose at 19.49 USD/1 lb (454 g) while refined sugar is normally sold at grocery stores around 0.6 USD/1 lb.

3. PRODUCTION OF TAGATOSE

Even though tagatose has been considered as rare sugar, it can be industrially produced at relatively high amount at present. In this review, all possible processes for producing tagatose were discussed. The possible manufacturing process of tagatose can be divided into 3 methods: chemical, enzymatic, and subcritical fluid treatment processes. The latter is a main focus method in this article and was discussed in details because it is relatively new.

3.1 Chemical process

Tagatose was originally produced by isomerization of galactose under alkaline conditions which is well-known as Lobry de Bruyn-Alberda van Ekenstein transformation (Pigman and Horton, 1972). This is similar to isomerization of glucose into fructose and mannose in alkaline solution (Belitz et al., 2009). In mild alkaline solution, galactose will be isomerized to tagatose and talose via enolization reaction through 1, 2-enediol (Figure 2). However, alkaline solution gave limited amount of tagatose due to the equilibrium constant of the reaction. El Khadem et al. (1989) reported that in aqueous KOH at pH 11.5, 25°C, 18% of tagatose was produced from galactose at equilibrium after 14 days while very small amount of talose, an epimeric aldose of galactose, was formed.

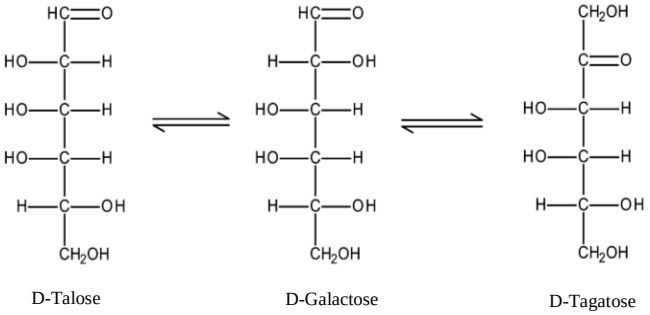

Figure 2 Isomerization of D-galactose to D-tagatose and D-talose in alkaline solution.

A patented process of tagatose manufacturing by Beadle et al. (1989) which is owned by Arla Foods Ingredients (Denmark) is a chemical isomerization of galactose which can be directly obtained by enzymatic hydrolysis of lactose. It should be noted that lactose was a preferred starting raw material for commercialization because it is much cheaper and more abundant than galactose. The process described in the patent comprises hydrolysis of lactose into galactose and glucose by lactase at 50°C for 4-6 h and the obtained glucose is separated by chromatography. Galactose is isomerized under alkaline conditions using $Ca(OH)_2$ and $CaCl_2$ at 25°C for 2 h. Calcium hydroxide-tagatose complex is then neutralized with acid, e.g. bubbling with carbon dioxide, to give tagatose. The yield of tagatose from this method was around 72%.

Chemocatalytic isomerization of aldose into ketose was reviewed recently by Irina and Regina (2016). Sn in dealuminated β zeolites (Sn-β) have been showed to promote isomerization of galactose (5 mL of 10% sugar with 100 mg of catalyst) at 110°C for 2 h and found to increase the yield of tagatose from galactose to 25% with the total monosaccharide yield of 89.5% (Dijkmans et al., 2013). This result was confirmed by Drabo and Delidovich (2018) with the

highest yield of tagatose of 26% under the reaction at 100°C for 2 h or 24% under the reaction at 110°C for 1 h. The latter work also showed that both soluble (NaH_2PO_4 + Na_2HPO_4) and solid base catalysts (Mg-Al hydrotalcite) gave low efficiency for production of tagatose from galactose with the maximum yield of 16%.

3.2 Enzymatic process

The possible bio-conversion processes for producing tagatose using several kinds of enzymes have been reviewed recently (Beerens et al., 2012; Jayamuthunagai et al., 2017a). It has been shown that D-tagatose 3-epimerase, aldose isomerase, aldose reductase, and oxidoreductase enzymes can be systematically used for producing several rare sugars including tagatose (Granström et al., 2004). Enzymatic process has a great advantage on substrate and product specificities and consequently reduced undesirable by-products. As shown in Table 2, there are several possible pathways for producing tagatose by enzymatic method. The most common pathway is isomerization of galactose by L-arabinose isomerase. Different sources of L-arabinose isomerase were investigated for their efficiencies (Oh, 2007; Xu et al., 2018). According to the document submitted to US FDA by CJ CheilJedang (GRN No.352), tagatose is produced as follows. First, lactose was hydrolyzed into galactose and glucose using sulfuric acid and then after neutralization, the mixture was subjected to enzymatic isomerization using L-arabinose isomerase (in immobilized *Corynebacterium glutumicum*) for 4-8 h.

Besides the isomerization of galactose, other enzymatic reactions with different substrates are also possible. Lee et al. (2017) used a three-step enzymatic cascade reaction including phosphorylation of fructose by hexokinase, epimerization of fructose-6-phosphate to tagatose-6-phosphate by fructose-1,6-biphosphate aldolase, and dephosphorylation of tagatose-6-phosphate by phytase to convert fructose to tagatose with a yield of 77% without any by-products. In addition, after a simple recrystallization with ethanol, tagatose with 99.9% purity could be obtained. Therefore, it is also possible to use starch which is very cheap raw material for producing tagatose since fructose has been commonly produced from starch by enzymatic process. Epimerase enzyme is also a convenient pathway to produce tagatose from fructose. CJ CheilJedang also patented the process of using hexuronate C4-epimerase to catalyze the direct epimerization of fructose to tagatose (Yang et al., 2013; Yang et al., 2018). The major drawback of enzymatic reaction might be the cost of enzyme production which is normally high and difficulties of operation at industrial scale.

Table 2 Various enzymatic processes for producing tagatose

Substrate	Enzyme/Reaction	Yield (reaction time)	Citation
Galactose	L-Arabinose isomerase	16.2% (48 h)	Jayamuthunagai et al. (2017b)
	L-Arabinose isomerase	22.3% (14 h)	Patel et al. (2017)
	L-Arabinose isomerase	79.7% (28 h)	Guo et al. (2018)
	D-Galactose isomerase	55% (3 h)	Shin et al. (2016)

Table 2 Various enzymatic processes for producing tagatose (Continued)

Substrate	Enzyme/Reaction	Yield (reaction time)	Citation
Fructose	Phosphorylation of fructose to fructose-6-phosphate by hexokinase, epimerization of fructose-6-phosphate to tagatose-6-phosphate by fructose-1,6-biphosphate aldolase, and dephosphorylation of tagatose-6-phosphate to tagatose by phytase	96.3% (16 h)	Lee et al. (2017)
Fructose	Hexuronate C4-epimerase	30% (3 h)	Yang et al. (2013)
Galactitol	Polyol dehydrogenase	91% (15 h)	Sha et al. (2018)

3.3 Subcritical fluid treatment

Subcritical fluid is usually defined as solvent under temperature between its boiling and critical temperatures under pressurized conditions to keep it at liquid state. Therefore, the required pressure for subcritical condition is at least higher than the equilibrium vapor pressure at certain temperature. Water (critical temperature of 374°C, critical pressure of 22 MPa) is possibly the most widely investigated solvent under subcritical conditions. Subcritical water at temperatures in a range 100-300°C (Figure 3) has been often studied as a green solvent for valorizing underutilized agricultural by-products through extraction process (Wiboonsirikul and Adachi, 2008) and hydrolysis reaction (Khuwijitjaru et al., 2007; Khuwijitjaru, 2016).

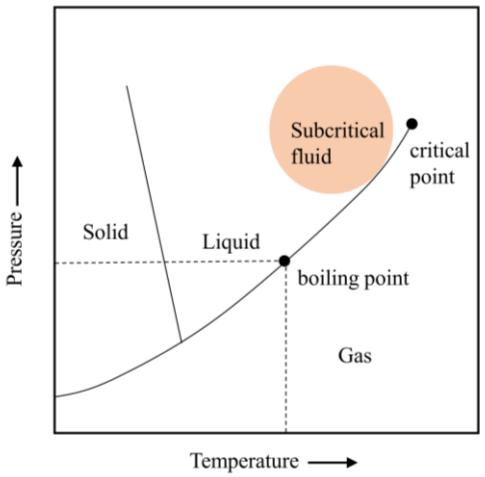

Figure 3 Schematic phase diagram of substance showing subcritical fluid area.

Adding organic solvent to subcritical water was found to be effective for adjusting the solvent power of subcritical fluid. Ethanol is acceptable in food-grade production and therefore adding ethanol into subcritical water has been reported for several purposes, particularly for reduction of polarity of water. As a pure solvent, Lu et al. (2002) found that as temperature increased ethanol under near and supercritical conditions become more nonpolar while it still possesses significant hydrogen-bond donating acidity but weaker hydrogen-bond accepting basicity.

To date, the potential of subcritical fluid treatment for isomerization of mono- and disaccharides to their rare isomers has been mainly investigated by our group at Kyoto University in Japan. Several water miscible solvents, e.g. methanol, ethanol, and acetonitrile, under subcritical conditions were tested for their promoting the isomerization reaction (Usuki et al., 2007; Gao et al., 2015f; Gao et al., 2015d; Gao et al., 2017) but ethanol seems to be the most effective (Gao et al., 2015a; Gao et al., 2015e; Gao et al., 2015c; Gao et al., 2016; Soisangwan et al., 2016; Soisangwan

et al., 2017a; Soisangwan et al., 2017b). Therefore, subcritical fluid treatment can be considered as a new process for preparing tagatose by enhancing of isomerization reaction of galactose at high temperatures.

In the proposed continuous process for producing tagatose (Gao et al., 2015a), galactose dissolved in aqueous ethanol (0.5% w/v) was heated under pressure (10 MPa) in a flow-type reactor (Figure 4) for a short reaction time without any catalyst. Using 80% w/v ethanol at 180°C, the highest yield of 24% was obtained at 500 s of reaction, which was very short compared to ordinary chemical or enzymatic reactions. The concentration of starting galactose can be raised to 8.5% w/v by using 60% ethanol and gave the highest tagatose yield of 13% and productivity of 80 g/(L•h). The process has advantages in that no catalyst is required and the operation is very fast and simple one step process. However, low solubility of galactose in high concentration of ethanol is a current limitation of this method (Gao et al., 2015b). In addition, degradation of galactose or tagatose via mainly dehydration reaction also occurred.

The mechanism of isomerization of galactose into tagatose in subcritical aqueous ethanol is still not clear but possibly follows the Lobry de Bruyn-Alberda van Ekenstein transformation as well. It is known that at high temperature, hydronium $[H_3O]+$ and hydroxide [OH]- ions of water increased due to the weakening of hydrogen bonds in water molecule and therefore several acid- and base-catalyzed reactions are promoted. However, in pure subcritical water, other acid- and base-catalyzed reactions of galactose progressed at much higher rate than isomerization and therefore only very small amount of tagatose could be found. Interestingly, the effect of ethanol concentration on alkaline isomerization of glucose to fructose was demonstrated (Vuorinen and Sjöström, 1982). Isomerization rate of glucose in 0.1 M sodium hydroxide increased with ethanol concentration and in 70% ethanol it was 2.4 times higher than in pure water. The authors also showed that the ionization of glucose increased with the ethanol concentration.

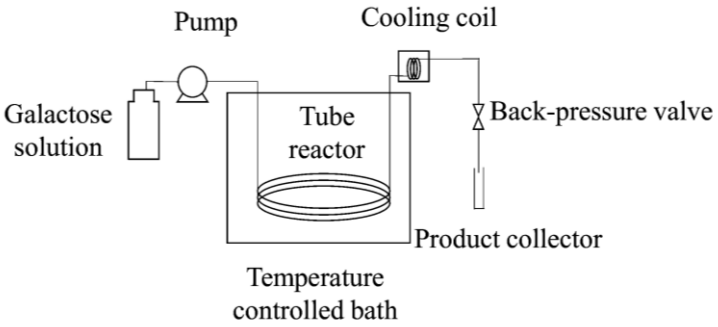

Figure 4 Schematic diagram of flow-type subcritical fluid treatment instrument for tagatose production.

4. CONCLUSION AND OUTLOOK

Chemical, enzymatic, and subcritical fluid methods for tagatose production were reviewed with an emphasis on the latter method. Subcritical fluid treatment has not yet developed into industry scale but its potential has been clearly demonstrated. In the authors' opinion, with the current fast development in science and technology, high competition among manufacturers and an emerging of start-up enterprise it is possible to find several new commercial processes for not only tagatose but other rare sugars in the very near future.

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