

# ***A New Low Calorie Sweetener D-Tagatose from Lactose in Cheese Whey as a Nutraceutical Value-Added Product***

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## ***Abstract***

*In this manuscript content, a new low calorie sweetener D-tagatose from lactose in cheese whey has been described. These research results are showing progress in improving enzymes bioconversion rate, thermos ability in increasing the half-life of the immobilized enzyme, shorting enzyme reaction time and improving both bioconversion efficiency for the hydrolysis of lactose to lactose hydrolysate (D-glucose and D-galactose) and for isomerization of D-galactose to higher yield of D-tagatose.*

## ***1. Introduction***

*An excess of lactose is currently produced in North America dairy industry as a byproduct of cheese manufacture in the form of whey or whey permeate. These by-products are potential source of food for both human and animal consumption, plus there are technologies for whey utilization that are being investigated. One of these investigated methods is to convert the D-galactose in the disaccharide lactose in whey or whey permeate into the low calorie sweetener D-tagatose via isomerization.*

*D-tagatose is the keto sugar of D-galactose. It has a sweetness equivalent to*

*sucrose but it is poorly digested. It is non-cariogenic, reduces insulin demand and can be used as a reduced calorie food sweetening and bulking agent. D-tagatose is also useful as an intermediate chemical for the synthesis of other optically active compounds and as an additive in detergent, cosmetic and pharmaceutical formulations.*

*The disaccharide lactose in cheese whey can be hydrolyzed by acid or by the enzyme lactase to prepare a mixture comprising of D-galactose and D-glucose at the ratio of 1:1. D-galactose from lactose hydrolysate is the substrate for the production of D-tagatose and it can be separated from D-glucose in lactose hydrolysate by column separation or by relatively converting D glucose in lactose hydrolysate to ethanol by microbial fermentation and distillation.*

*The separated D-galactose can be isomerized into the low calorie sweetener D-tagatose via chemical method or by enzymatic method. Chemical method is based on using a metal hydroxide such as calcium hydroxide in the presence of an inorganic salt catalyst to form an intermediate metal hydroxide-D-tagatose complex. This intermediate complex is then neutralized with acid to yield D-tagatose (Beadle et.al.,1990). Enzymatic method is based on employing the microbial enzyme L-arabinose isomerase*

for isomerization of D-galactose into D-tagatose (Ibrahim and Stradlin,2000).

Currently D-tagatose is generally recognized as safe by North America, Europe, Australian and Asia and these two manufacturing methods of D-tagatose from the substrate D-galactose have been evaluated by worldwide researches for future manufacturing D-tagatose on a large scale.

Researcher's activities are mainly focusing on enzymatic method to improve L-arabinose isomerase properties, activity, and stability in order to improve manufacturing process for cost reduction. Reducing D-tagatose production cost is the key factor for marketing D-tagatose as and an affordable low calorie sweetener for applications in foods, cosmetics and pharmaceuticals industries.

## **2. D-Tagatose**

D-tagatose is a keto hexose, an epimer of D-fructose (Bertelsen et.al.,1999) isomerized at the fourth carbon. The chemical structure of D-tagatose differs from the chemical structure of D-fructose (Bueman et.al.,1998), only in the position of hydroxyl group (OH) on the fourth carbon (Figure 1).

D-tagatose has approximately half the calorie of sucrose (1.5 kcal) and has a sweetness equivalent to sucrose thus it can

be used as a reduced calorie food sweetening and baking agent. Also it is useful as intermediate chemical for the synthesis of other biologically active chemical compounds and it can be used as additive in detergents, cosmetics and pharmaceutical formulations (Levin et.al.,1995).

Functional uses of D-tagatose as food additives are as sweetener, texturizer, stabilizer, humectant, formulation aid, and provides several health benefits to consumers such as attenuate a glycemic response (Armstrong et.al.,2009) and preventing tooth decay.

D-tagatose received a GRAS status (Generally Recognized As Safe) in United States, Australia, New Zealand, South Korea, Brazil, South Africa, the European Union and other parts of the worlds (Lina and Kuper,2002) due to its history of use naturally in foods with no reported adverse effects (Adachi,1958) and for Its naturally existing at a low concentration in human milk, sterilized and powdered cow's milk (Levin et.al.,1995), fermented dairy products such as yogurt (Hirst et.al.,1949) and it is also, present as a component in plants cell structure such as gum exudate of the cacao tree (*Sterculia setigera*) (Elin and Olarsdottir,2001) and one of sugars component in the chemical structure of the oligosaccharide in lichens of the *Rocella* species (Marshall and Kooi,1957)

### **3. Methods of D-Tagatose**

#### **Manufacturing:**

*The substrate for the production of the keto sugar D-tagatose is the aldehyde sugar D-galactose (Figure 2)*

*The conversion of D-galactose into D-tagatose is known by the name isomerization and this conversion process can be practice by chemical isomerization using calcium hydroxide at pH12, or by enzymatic isomerization using the enzyme L-arabinose isomerase.*

*These two processes for manufacturing D-tagatose can be summarized.*

#### **3.1. Chemical Isomerization Method**

*The D-galactose at a concentration of 30% is isomerized to D-tagatose under alkaline conditions at pH 12 for 2-4 hours using calcium hydroxide in the presence of an inorganic salt as catalyst to form an intermediate “calcium hydroxide-D-tagatose complex”. Treatment the precipitated intermediate calcium hydroxide- D-tagatose complex with sulfuric acid forming calcium sulfate and liberates D-tagatose solution. The filtered D-tagatose solution is demineralized in a cation and anion exchanger. The demineralized D-tagatose solution is concentrated and purified by chromatographic fractionation using a*

*cation exchanger. The purified D -tagatose fraction is crystallized (Ibrahim and Spradlin,2000), separated by centrifugation, washed by spraying distilled water and dried in drum dryer (Ibrahim and Spradlin,2000; Beadle et.al,1992)*

#### **9.3.2. Enzymatic Method**

*Enzymes are the catalysts of biological process that bring the reaction catalyzed to its equilibrium with more specific reaction in short time with less energy than the chemical method. Chemical method is nonspecific and require an extra steps for purification and decolonization to remove generated impurities and color (Ibrahim and Spradlin,2000).*

*Converting an aldose or aldose derivatives to a ketose or ketose derivatives are well known. For example, the enzymatic conversion of D-glucose to D-fructose, using the enzyme xylose isomerase (EC 5.3.1.5) is widely practiced on a commercial scale for the production of high fructose corn syrup (Nakamatu and Yamanaka,1969). However, the enzymatic method for converting D-galactose to D-tagatose, using the enzyme L-arabinose isomerase (EC 5.3.1.4) (Ibrahim and Spradlin,2000) was not been developed until the last fifteen years as described in*

*the granted patent for manufacturing of D-tagatose (Ibrahim and Spradlin,2000).*

*Immobilized free enzyme of L-arabinose isomerase or cell producing intracellular L-arabinose isomerase can be operated in a continuous, semi continuous or batch operation, consistent with the enzyme system employed the isomerization of 20-60 % D-galactose at PH range of 5.0-7.0 in the presence of metallic ion as an activator such as manganese chloride (5mM) and optimum enzyme temperature range of 50-80 °C (Patrick and Lee,1975). The optimum pH and temperature of the enzyme depends on the microbial source of the enzyme L-*

*arabinose isomerase. L-arabinose isomerase from thermophilic microorganisms produce thermostable enzyme with higher optimum temperature (Sang-Jae et.al.,2005) comparing to L-arabinose isomerase produced from mesophilic microorganisms. Thermostable L-arabinose isomerase has advantages over mesophilic L-arabinose isomerase in shifting the isomerization equilibrium toward the end product (D-tagatose) and also, preventing the potential microbial contamination in the case of continuous immobilization system.*

*Isomerization of D-galactose into D-tagatose can be completed in 4-8 hours depending on the enzyme activity and properties. Plus, high yield of D-tagatose*

*in the isomerization process depends on the concentration of D-galactose as a substrate. Isomerization end products are a mixture of D-tagatose and D-galactose. Separation of D-tagatose from D-galactose in the mixture can be performed by chromatographic fractionation using a cation exchanger column separation methods. Separated and purified D-tagatose can be crystallized, centrifuged, washed and dried in drum dryer. The separated D-galactose fraction from D-tagatose / D-galactose mixture can be recycled for enzyme isomerization to D-tagatose.*

### **3.2.1. L-arabinose Isomerase**

*L-arabinose isomerase (EC 5.3.1.4) is inducible enzyme (Patrick and Lee, 1975) by the pentose sugar L-arabinose and is produced as an intracellular enzyme. The enzyme can be derived from various microorganisms (Sang-Jae et.al.,2005) include but not limited to Lactobacillus pentosus, Lactobacillus brevis, Lactobacillus pentoaceticus , Lactobacillus fermentum ( previously known Lactobacillus gayonii), Aerobacter aerogenes, Bacillus amyloliquefaciens, Bacillus subtilis, Candida utilis, Clostridium acetobutylicum Escherichia coli, Erwinia cativosa , Pediococcus spp, Arthrothacter spp.,and Mycobacterium smegmatis. Many of these microorganisms*

are Generally Recognized As Safe (GRAS) and are suitable source of L-arabinose isomerase for the enzymatic method to isomerize D-galactose to D-tagatose.

L-arabinose isomerase is mainly catalyzes the isomerization of the pentose -sugar L-arabinose to keto-sugar L-ribulose. This enzyme is also demonstrated its capability to isomerize the hexose sugar D-galactose at high concentration into D-tagatose (Manzo et.al.,2013) (Figure 3). The high concentration of D-galactose as a substrate for the enzyme L-arabinose isomerase is due to the Km value for the substrate D-galactose. (Km value expresses the binding efficiency of the enzyme to its substrate).

### **3.2.2. Enzyme Immobilization**

Isomerization of D-galactose to D-tagatose can only be made economically feasible by immobilizing thermostable L-arabinose isomerase, at high reaction temperature to achieve a higher conversion rate of D-galactose to D-tagatose and yield (Yan et.al.,2014) of D-tagatose.

Immobilized enzymes are defined as enzymes physically confined or localized in a certain region of space with retention of their catalytic activities, which can be used repeatedly and continually. This definition is applicable to the immobilization of free enzymes (extract), or intracellular enzymes

encapsulated in its microbial, plant or mammalian cells. There are various immobilization techniques that are available for L-arabinose isomerase immobilization. The principle of these techniques are illustrated in (Figure 4).

### **3.2.3. Methods of Enzymes**

#### **Immobilization**

**Cross linking:** Several chemicals serve as reagent for intermolecular are cross linking of enzymes. Glutaraldehyde (Kim et.al.,2001) is the most popular cross-linked reagent for both free and cellular enzymes.

**Covalent binding:** enzyme amino acid residues that are not part of the enzyme active site or substrate binding site can be used for covalent binding with the enzyme support.

**Entrapment:** Entrapped enzymes are classified into: **Lattice types** (gel matrices), **Microcapsule type** (synthetic polymer), **Liposome type** (phospholipids), **Hollow fiber type** (hollow fiber), and **Membrane type** (ultrafiltration membrane).

**Encapsulation:** Incorporate, enzymes or microbial cells in a micro metric scale. Microcapsules have pores with diameters between a few micrometers and a few millimeters: to allow the interaction between the enzyme and the substrate.

**Adsorption:** Binding enzyme to carrier by physical interaction such as hydrogen bonding, hydrophobic interaction or van der waal's forces.

#### **4. Cheese Whey for the Production of D-Tagatose**

A total of over 90 billion pounds of whey are generated in United States as a byproduct of cheese manufacturing, comprises about over 84 billion pounds sweet whey and a 6 billion pounds acid whey at the ratio of 9:1 (Charles Ling,2008). The average composition of whey is approximately 0.3 % butterfat, 0.8 % whey protein, 4.9 % lactose and 0.5% minerals (USA Wisconsin Center for Dairy Research). Cumulatively, there are over 4.1 billion pounds of lactose in cheese whey that can be utilized for the production of D-tagatose. Currently, there are about 726 million pounds of lactose per year are recovered in United States from whey.

Whey is the major byproduct from cheese processing industry and it is estimated that for the production of one pound of cheese it will generate a nine pounds of whey. The current lactose crystal/ powder price in United States is in the range of 20 cents per pound. The chemical structure of lactose ( $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-glucose) is a

disaccharide aldehyde sugars composed of D-galactose and D-glucose with  $\beta$  1--4 glycosidic bond (Figure 5). This glycoside bond that bound D-galactose to D-galactose can be hydrolyzed to produce lactose hydrolysate (a mixture of D-glucose and D-galactose at the ratio of (1:1) by acid hydrolysis method using strong acids such as sulfuric acid or by enzyme hydrolysis method Lactase enzyme (E.C.3.2.1.23) is a beta-D galactoside-galactohydrolase enzyme produced by many microorganisms. Lactase enzyme is intracellular enzyme and can be extracted commercially from yeasts such as Kluyveromyces fragilis and Kluyveromyces lactis or from molds, such as Aspergillus niger and Aspergillus oryzae. The commercial enzyme lactase has an optimum temperature of over 48 °C and an optimum pH of 6.5.

The hydrolysis of lactose can be summarized as follow; 20-40 % lactose powder in water is subject for acid or enzymatic hydrolysis. Acid hydrolysis method (Douglas et.al.,2001) can be carried out using 0.1% w/w of sulfuric acid and incubation at 100<sup>0</sup>C for 1-2 hours followed by neutralization to PH 5.5-7.0. . Enzymatic hydrolysis method (Demaimay et.al.,1978) can be carried out using Lactase enzyme by conventional means (free enzyme in batch process) or a continuous (immobilization process), at

optimum temperature 40-60<sup>0</sup>C and optimum PH 4-6 (the enzyme optimum conditions depends on the microbial source and the properties of lactase enzyme being used).

The end product after lactose hydrolysis by either method (acid or enzyme) is lactose hydrolysate which is a mixture of D-galactose and D-glucose at an equal quantities (Figure 6).

For the production of D-tagatose, the substrate D-galactose can be separated from D-glucose by a column separation using a cation exchange resin or by selective fermentation of the D-glucose to ethanol using bacteria or yeast strains (Ibrahim and Spradlin,2000). In the case of D-glucose fermentation to ethanol, microorganisms for ethanol production consumes first the sugar D-glucose for the production of ethanol and the fermentation should be terminated as soon as the sugar D-glucose is completely consumed into ethanol. The ethanol produced from sugar D-glucose can be separated from the remaining D-galactose by distillation and the remaining D-galactose is concentrated to 40-70 % to be used as a substrate for the isomerization process into D-tagatose by chemical or enzymatic method (Figure 7). The D-tagatose production yield varies from 20-30%

depends on the initial concentration of the substrate D-galactose.

### **5. D-Tagatose Properties**

D-tagatose is virtually odorless, white or almost white, non-hygroscopic crystals and it has almost the same sweetness as sucrose with less than half the calories of sucrose (Figure 8). It naturally exists as a monosaccharide keto sugar with unique properties. The standard specification of D-tagatose is illustrated in (Table 1).

D-tagatose provides health benefits to consumers such as having prebiotic function, attenuating glycemic response (Donner et al.,1999), does not promote tooth decay (Levin, 2002; Livesey and Brown,1996) and has poor absorption in GI and low metabolizing energy. These properties of D-tagatose have gained international attention as a low calorie sweetener for diabetics, obese and for weight-conscious populations.

D-tagatose is stable in food products during processing and storage. It is stable at both room and cold temperatures. D-tagatose undergoes Maillard browning reactions with amino acids that give brown color to baked goods (FAO 2004)

## **6. D-Tagatose Metabolism**

Only about 20% of tagatose is absorbed in the small intestine, the rest passes to the large intestine where it is fermented by the beneficial microflora in the colon into short chain fatty acids such as butyric acid which helps in preventing from colon cancer (Johansen and Jensen,1997). These short chain fatty acid are absorbed into blood stream from the Intestinal tract (Bruemann et.al.,1998), as a source of energy. Also, D-tagatose blocks the digestion of sucrose, maltose, and other carbohydrates causing a small or no increase of glucose level in blood stream after meals (Lu et.al.,2008).

The metabolism of D-tagatose, which is a stereoisomer of D-fructose is in the liver as fructose metabolism (Buemann et.al.,2000). It convert into glucose which stored in the form of glycogen or broken down to produce energy. In addition D-tagatose promote the metabolism of Glucose-6- Phosphate which stimulates and maintain the storage of glucose in the form of glycogens (Espinosa and Fogelfeld,2010) stored in the liver.

It is hypothesized that D-tagatose is metabolized in the liver like fructose with similar pathway, but at a lower rate. .The first step in the metabolism of D-tagatose in the liver as illustrated in (Figure 9) is the phosphorylation of tagatose to tagatose -1- phosphate by the enzyme fructokinase.

This enzyme fructokinase is the same enzyme that phosphorylate fructose in fructose metabolism into fructose-1-phosphate.

Similar to fructose -1- phosphate, the tagatose -1-phosphate is an inhibitor of the enzyme glycogen phosphorylase causing glycogen to remain stored in the liver as a mechanism of action for glycemic control. However, the cleavage of tagatose -1-phosphate is slower than fructose -1-phosphate, therefore D-tagatose ingestion may cause a longer lasting reduction in inorganic phosphate (Pi) and adenosine triphosphate (ATP) levels in the liver comparing to D-fructose metabolism (Bar,2000).

Tagatose -1-phosphate in the tagatose pathway is then undergoes hydrolysis by the enzyme tagatose-1 phosphate aldolase to form dihydroxyacetone phosphate (DHAP) and glyceraldehyde. The formed dihydroxyacetone phosphate (DHAP) is isomerized to glyceraldehyde 3-phosphate by the enzyme triose phosphate isomerase and the formed glyceraldehyde is phosphorylated to glyceraldehyde 3-phosphate by the enzyme glyceraldehyde kinase. The two similar metabolites of glyceraldehyde -3- phosphate from one molecule of D-tagatose are the intermediate in glycolysis pathway that leads to:



- 1 Glycogen synthesis,
- 2 Oxidized to pyruvate pathway.
- 3 Reduced to lactate.
- 4 Acetyl CoA for the anaerobic citric acid cycle in the mitochondria, for energy generation in the form of ATP.

### **7. D-Tagatose Applications**

*D-tagatose has a potential multiple applications in foods such as cereals, diet soft drinks, confectionary, hard and soft confectionaries, baked goods, ice cream, chewing gum, ready-to-eat breakfast, etc., as illustrated in (Table 2). In addition to the above foods use. D-tagatose has the potentially for use as a low calorie oral prescription drugs additive to mask unpleasant taste.*

### **8. D-Tagatose Safety**

*D-tagatose is Generally Recognized As Safe (GRAS) by the US Food and Drug Administration (FDA) (Bar,2000). Plus, the World Health Organization's Joint Expert Committee on Food Additives (JECFA) has left Acceptable Daily Intake (ADI) for tagatose "unspecified," which means that high intake of D-tagatose does not have any expected long-term toxic effects (FDA,2003).*

*In sensitive individual, D-tagatose when is taken in doses over 10-15 gm / day could cause mild digestion problems such*

*as nausea, flatulence and diarrhea. In addition, since D-tagatose is metabolized the same way as D-fructose, it is probably not safe to use by individuals with hereditary fructose intolerance (HFI). However accidental intake of small amount of D-tagatose is not likely harmful. In addition, D-tagatose does not likely trigger allergic reaction in individuals with milk allergy (lactose intolerance) and in compare to D-fructose it is slightly increases uric acid levels, but there is no evidence that it could increases the risk of gout (Bar,2004).*

### **9. D-Tagatose and Health Benefits**

*Consumption of D-tagatose provides the following multiple health benefits along with antioxidant property, the treatment of type 2 diabetes and control obesity:*

1. **Antidiabetic potential:** promoting glycogen synthesis and decrease glycogen utilization. Also , attenuate intestinal glucose absorption
2. **Weight loss:** has net zero value
3. **Increase in HDL:** helps rise the good cholesterol (HDL) and may prevent heart attack.
4. **Prebiotic effect:** only 20 % of ingested D-tagatose absorbed into bloodstream while the remaining 80% promote the growth of beneficial bacteria in the colon.

5. **Dental Health:** non-fermentable and it does not promote tooth decay.

## **10. Conclusions**

*D tagatose is a rare natural monosaccharide which can be manufactured by the chemical or enzymatic isomerization of D-galactose. D-galactose can be obtained by acid or enzymatic hydrolysis of milk disaccharide sugar lactose, into lactose hydrolysate (D-galactose and D-glucose at ratio 1:1), The D-galactose is the substrate for D-tagatose manufacturing and can be separated from D-glucose by column separation or by selectively converting D-glucose to ethanol. Ethanol can be removed via distillation as a valuable byproduct. The separated aldehyde hexose sugar D-galactose can be isomerized to keto hexose sugar D-tagatose by chemical or enzymatic isomerization. . Enzymatic isomerization of D-galactose to D-tagatose is by the enzyme L-arabinose. Isomerase. Enzyme isomerization is the most promising future process for the large scale production of D-tagatose.*

*Enzymatic methods for the isomerization of aldose to ketose are widely practiced on a commercial scale for converting aldehyde sugar D-glucose from starch to keto sugar D- fructose, however this enzymatic method for converting aldehyde sugar D-galactose from cheese*

*why to keto sugar D-tagatose have not been developed on a large bench scale until recently (Ibrahim and Spradlin,2000)..*

*An estimated of over 90 billion pounds of cheese whey are generated in United States as a byproduct of cheese production and relatively small volume of cheese whey are converted into products with various processes and technologies such as condensed whey, dry whey, dried modified whey, whey protein concentrate , whey protein isolate, lactose permeate and crystalized or dried lactose. The rest of unprocessed cheese whey are used for animal feed rand for land spreading.*

*Crystalized or powder lactose is the best pure feedstock for the two steps processes in D-tagatose manufacturing from whey. These two steps for D-tagatose manufacturing from whey are lactose hydrolysis and D-galactose isomerization. In addition to pure lactose crystal or powder as a feedstock for D-tagatose manufacturing ,the concentrated lactose permeate after the removal of whey protein and other solids can be used as a feedstock in these two steps process for D-tagatose manufacturing . Concentrated lactose permeate consists mainly of 65-85 % lactose and salts. Utilization of lactose permeate is the cheapest feedstock than lactose crystal or powder for D-tagatose production but required an extra step in*

*the process for salts removal by desalination process (Ibrahim and Spradlin,2000) using reverse osmosis or ultrafiltration technology. Concentrated lactose permeate contain over 7% ashes.*

*D-tagatose is listed as a GRAS ingredient in United States., Europe, Australia and Asia, and in the year 2003 the United States Food and Drug Administration (U.S .FDA) has allowed a health claim for D-tagatose and the risk reduction of dental caries. In addition, the European Union (EU) has been formally approved D-tagatose as a “**novel food ingredient**) without any restriction on usages. Plus the joint FAO /WHO Expert Committee on Food Additives (JECFA) concluded that D-tagatose was-not genotoxic, embryo-toxic or teratogenic, and identified a NOEL of 075 gm / kg body weight (45 gm. D-tagatose/ 60 kg body weight) for human intake.*

*The D-tagatose safety is also due to its long history of safe use as one of the components in natural foods that demonstrated no indications of any significant adverse effects related to D-tagatose was published in the publically available literature.*

*Health professional and consumers believe that low calorie sweeteners including D-tagatose are effective for weight maintenance, weight reduction, managements of diabetes, reduction of*

*risks associated with obesity, and reduction of dental caries.*

*Reported health benefits associated with of low calorie sweeteners resulted in the increasing worldwide demands for such sweeteners in foods and beverages, and the 2016 global market analysis for low calorie food products and beverages is reaching to over \$ 9.0 billion and the majority of these products containing high intense zero calorie artificial sweeteners such as aspartame, and sucralose, or low calorie sugar alcohols such as sorbitol and xylitol. Up- to- date the natural low calorie sweetener D-tagatose is failed to compete in this market share of low calorie products due to its production cost that estimated to be in the range of \$4-6 per pound and the estimated market price is over \$10 per pound. This high market price of D-tagatose making difficulties for the application of D-tagatose in low calories food products and beverage*

*There is a potential in reducing the production cost of D-tagatose as a result of the ongoing research in fields of molecular biology, enzyme engineering and bioprocessing. These ongoing research is aimed to improve the two enzymes lactase (B-D- galactosidase) and L-arabinose isomerase expression (Bueman et.al.,1998), activities, specificities (Marta and Jozef,2012) and thermostability (Yan et.al.,2014) for the two steps process for*

*the production of D-tagatose from lactose in whey.*

*These research results are showing progress in improving enzymes bioconversion rate, thermos ability in increasing the half-life of the immobilized enzyme, shorting enzyme reaction time and improving both bioconversion efficiency for the hydrolysis of lactose to lactose hydrolysate (D-glucose and D-galactose) and for isomerization of D-galactose to higher yield of D-tagatose. These researches progress will reduce the D-tagatose production cost (Kim et.al.,2001) and might bring hope for the future marketing this natural low calorie sweetener (D-tagatose) with health benefits into the market and potential applications in flow calories products (Marta and Jozef,2012).*

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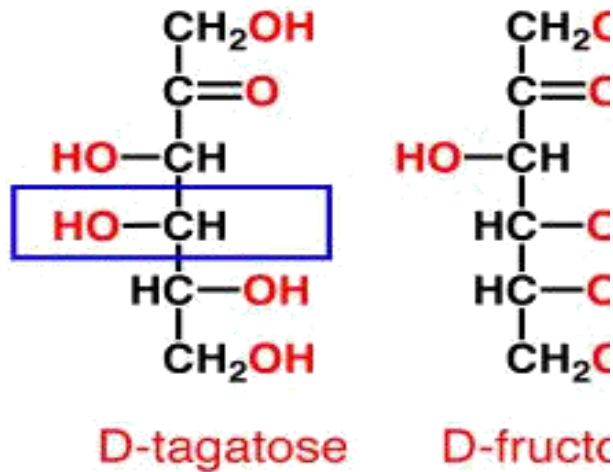
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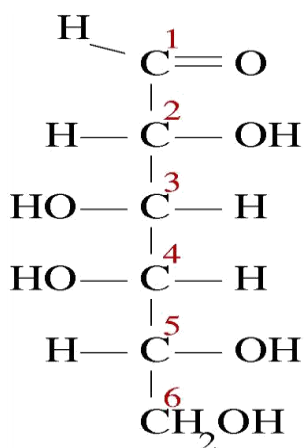
**A new low calorie sweetener  
D-tagatose from lactose in  
cheese whey as a nutraceutical  
value-added product**

**FIGURES**



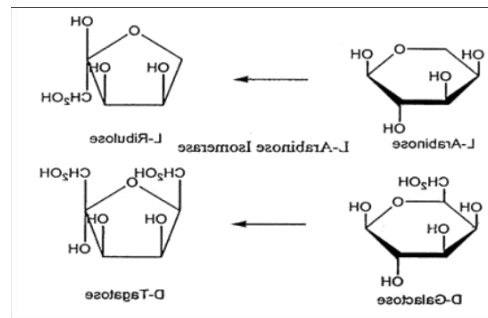
**Figure (1):** D-tagatose vs, D-fructose

[D-tagatose differs from D-fructose only in the position of hydroxyl group (OH) on 4<sup>th</sup> carbon]



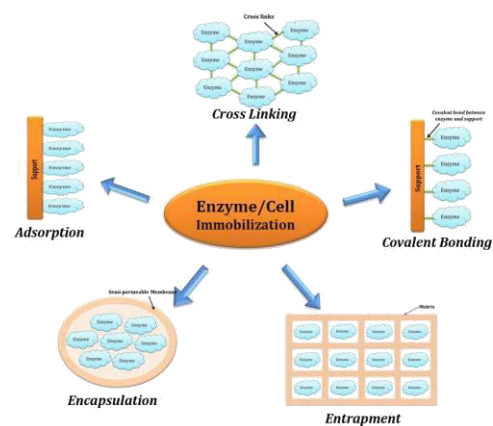
**Figure (2):** D-galactose chemical structure

[Galactose is a monosaccharide sugar that is less sweet than glucose and fructose. It is a C-4 epimer of glucose]



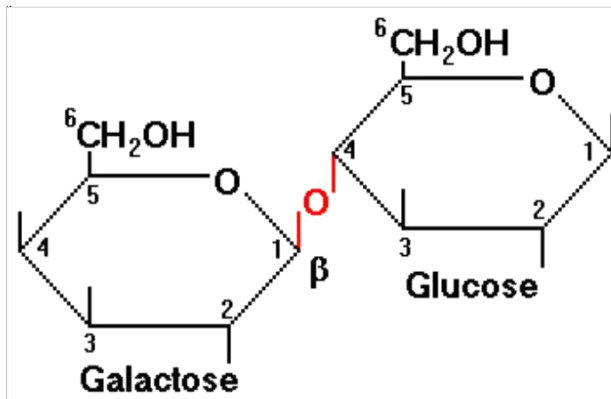
**Figure (3):** L-arabinose isomerase mechanism of action

[Isomerization of L-arabinose to L-rapulose and D-galactose to D-tagatose]



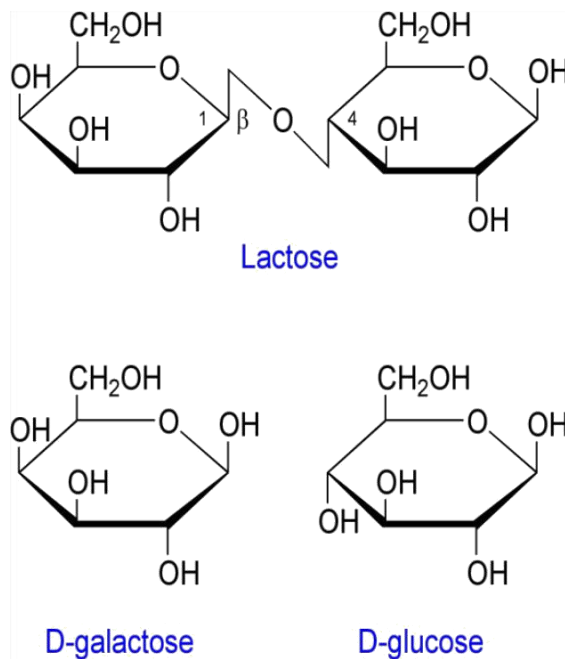
**Figure (4):** Enzymes Immobilization methods

[Cross linking, covalent binding, entrapment encapsulation and adsorption],



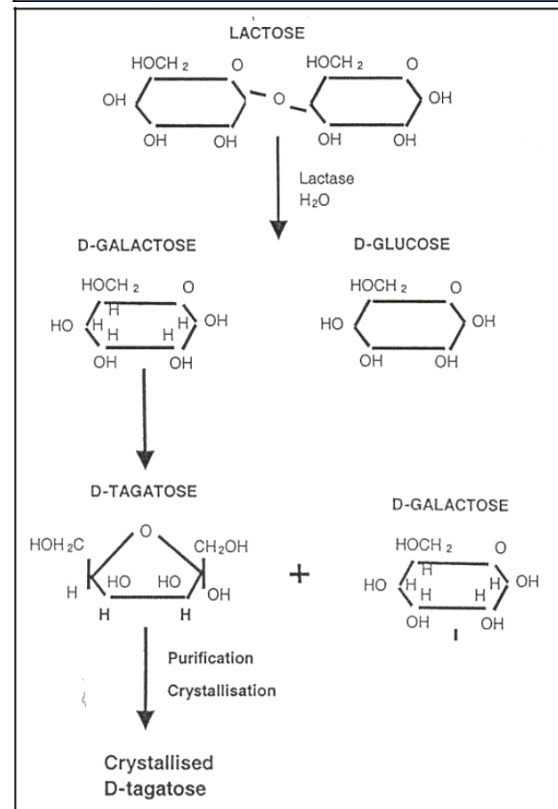
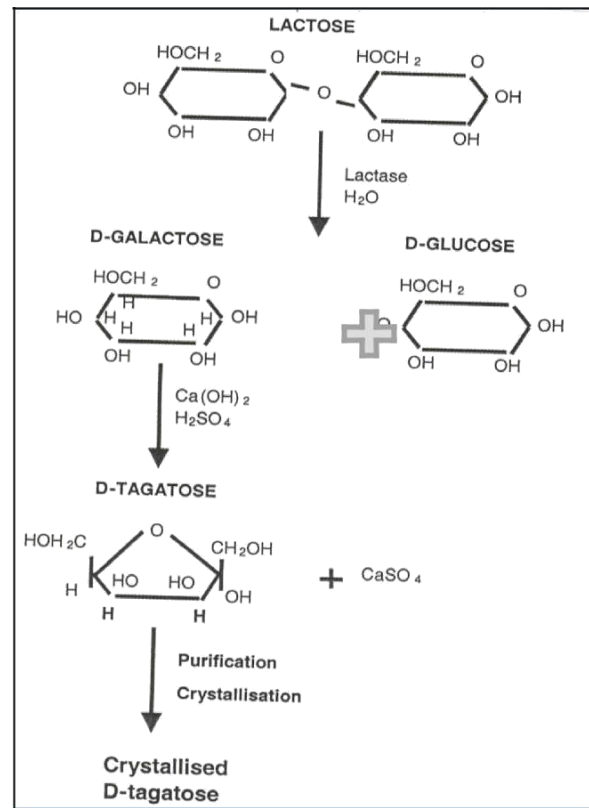
**Figure (5):** Milk sugar lactose

[Lactose;  $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-glucose is a disaccharide sugar composed of galactose and glucose that is found in milk]



**Figure (6):** Acid or enzymatic hydrolysis of lactose

[Lactose hydrolysis to D-galactose and D-glucose at ratio 1:1]



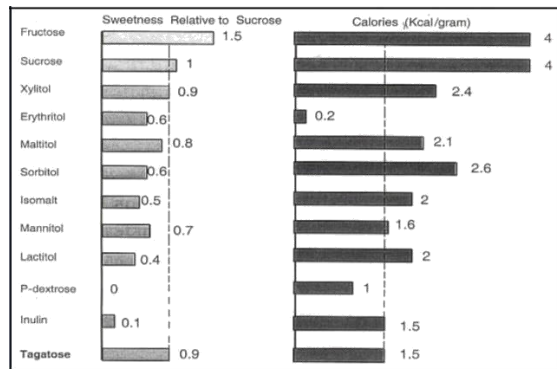


**Chemical method**  
**Enzymatic method**

**TABLES**

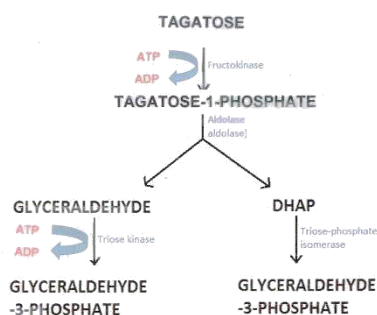
**Figure (7):** *D-tagatose manufacturing process*

[Diagrams for *D-tagatose manufacturing from lactose in whey by two methods*]



**Figure (8):** *D-tagatose sweetness and calorie comparing to others sugars*

[*D-tagatose is sweet as sucrose and has 1.5 kcal*]



**Figure (9):** *D-tagatose metabolism*

[*D-tagatose metabolism has a similar pathway as fructose*]

**Parameter Specifications**

*D-tagatose-*  
98.5% (wt. /wt.)

*D-galactose & other sugars*  
≤ 1% (wt. /wt.)

*Moisture*  
- ≤ 0.5% (wt. /wt.)

*Ash*  
≤ 0.1 % (wt. /wt.)

*Solubility in water: at 20*  
*°C* 160 mg / 100 ml.

*Solubility in ethanol: at 22°C*  
0.02 gm / 100 ml

*Melting point range*  
133 – 137 °C

*Total plate count*  
<10,000 CFU / g

*Coliform*  
Negative

*Staphylococcus aureus*  
Negative

*Heavy metals (Hg, Cd, As)*  
<0.1 ppm

*Lead*  
< 0.5 ppm

*Physical appearance*  
White Crystal

**Table 1:** D-tagatose finish product

[Standard specifications]

<u>Food Category</u>	<u>Maximum level of use (%)</u>
Ready-to eat Breakfast cereal	33
Diet soft drinks	2
Non-diet soft drinks	3.
Confectionary	25
Formulated diets for meal replacement	3.
Meal replacement drink mix (powder)	33
Cake, Pie	10.
Cake mixed powder	15.
Frostings	15.
Ice cream and frozen yogurt	7.5
Yogurt	7.5
Chewing gum, sugar free	60.
Jelly and pudding	7.5
Coffee mix powder	7.5

Biscuits  
10.

Cookies  
10.

Cereal bar  
10.

**Table 2:** The proposed food applications of D-tagatose and maximum level of use

[WHO's Joint Expert Committee on Food Additives (JECFA) has left Acceptable Daily Intake (ADI) for D-tagatose "unspecified," which means that high intake of D-tagatose does not have any expected long-term toxic effects]