**Research Article**

**PRODUCTION OF LOW CALORIE SUGAR MANNITOL BY LACTIC ACID BACTERIA DURING PRODUCTION OF COW MILK CURD AND SOY MILK CURD**

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DOI: http://dx.doi.org/10.24327/ijrsrc.2020.1106.5407

**ABSTRACT**

Low-calorie sweetener mannitol is a carbohydrate, made from fructose and hydrogen. It is poorly absorbed in the small intestine, and travels to the large intestine where beneficial intestinal bacteria ferment into gases and short-chain fatty acids (SCFAs), which can be absorbed and provide energy.

In this study, we have observed mannitol production by *Lactobacillus* sp. and *Streptococcus thermophilus* in fermented cow milk and soy milk curd. It was found that, maximum production of mannitol was observed in the soy milk curd inoculated with 1:1 ratio of *Lactobacillus plantarum* and *Streptococcus thermophilus* of 0.9 % compared to cow milk curd.

**INTRODUCTION**

Low or reduced calorie sugar free foods and beverages are extremely popular in United States. According to the survey conducted in 2004 by the Calorie Control Council, a trade organization, 180 million adult Americans used these products. Consumers often select these foods and beverages because they want to taste sweetness without added calories or because they want to reduce the risk of tooth decay. The dietary options that such products provide may be especially helpful in the management of obesity or diabetes mellitus.

It has been difficult to establish a general term to refer to this type of sweetener. Among professionals, the term ‘high-potency sweetener’ is widely used and well understood; nut consumers sometimes find this term confusing because it suggests that containing these ingredients are extremely sweet. The term ‘alternative sweetener’ or ‘sugar substitute’ are sometimes used nut they can be confusing because, they also refer to other types of sweeteners. We prefer the term ‘low calorie sweetener’ it indicates the purpose for which these ingredients are used and suggests the types of products in which they can be found.

There are another group of sweeteners consisting of ingredients that can substitute for both the physical bulk and sweetness of sugar. Products of this type, sometimes called ‘bulk sweetness’, include the sugar alcohols (also called polyols) mannitol, sorbitol, xylitol, lactitol, hydrogenated starch hydrolysates, and hydrogenated glucose syrups.

**Dietary importance**

Obesity is a growing problem in Western countries. Therefore, special diets and dietary ingredients for body weight control are of major interest to the food industry. Belonging to the family of low-calorie sugars, polyols such as mannitol and sorbitol are nonmetabolized sugar alcohols that can replace sucrose or lactose in food products, with a nearly equivalent sweetness and taste [1, 2].

Moreover, these compounds have a stabilizing effect on food by partially mimicking fat [3]. The range of potential applications of polyols goes far beyond their use as low-calorie...
sweeteners or texturing agents. Taking into consideration health benefits and industrial applications, the development of novel dairy products naturally enriched in polyols during fermentation processes offers interesting perspectives [4].

Mannitol is a polyol (sugar alcohol) widely used in the food and pharmaceutical industries because of its unique functional properties. It is about 50 percent as sweet as sucrose and has a desirable cooling effect often used to mask bitter tastes. Mannitol is non-cariogenic and has a low caloric content. Mannitol is suitable for ingestion and has been used safely around the world for over 60 years [5].

Mannitol is found in abundance in nature, particularly in exudates from trees, and in marine algae and fresh mushrooms. It is an isomer of sorbitol and is typically produced today by the hydrogenation of specialty glucose syrups. Mannitol is converted to fructose after absorption. It is not metabolized as well as sorbitol and provides only 2 Kcal/g of energy [6]. Mannitol is commercially available in variety of powder and granular forms. In the United States, mannitol is provided by a number of manufacturers, including Cargill, Roquette America, and SPI Polyols.

Functional Advantages
Polyols, such as mannitol, are resistant to metabolism by oral bacteria and do not increase the acidity of the mouth after ingestion. This means that they will not lead to cavities or erode tooth enamel. The usefulness of polyols (including mannitol) as alternatives to sugars and as part of a comprehensive program including proper dental hygiene has been recognized by numerous authorities, including the American Dental Association [7]. Unlike sorbitol, a polyol often used for its humectants properties, mannitol is nonhygroscopic (that does not pick up moisture). For this reason, it is often used as a dusting powder for chewing gum to prevent the gum from sticking to manufacturing equipment and wrappers. Due to its high melting point (165-169 °C), mannitol is also used in chocolate-flavored coating agents for ice cream and confections. It has a pleasant taste, is very stable to moisture pickup and does not discolor at high temperatures, which makes mannitol ideal for use in pharmaceuticals and nutritional tablets. Mannitol is also beneficial to people with diabetes. The control of blood glucose, lipids and body weight are three major goals in diabetes management. Mannitol is slowly absorbed from the intestinal tract. Therefore, when mannitol is used, the rise in blood glucose and demand for insulin is much less than would be experienced after sucrose ingestion. The reduced caloric value of mannitol compared to sucrose (1.6 vs. 4.0 calories/g) is consistent with the objective to control caloric intake and body weight in people with diabetes. Products sweetened with mannitol in place of sugar may be useful in providing a wider variety of reduced calorie and sugar-free choices to people with diabetes [8]. Recognizing that diabetes is complex and requirements for its management may vary between individuals, the usefulness of mannitol should be discussed between individuals and their health care providers. Foods sweetened with mannitol may contain other ingredients that also contribute calories and other nutrients. These must be considered in meal planning.

Safety aspect of mannitol
The use of mannitol in food is broadly permitted by FDA food additive regulations (21 CFR 180.25). The Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA) has reviewed the safety data and concluded that mannitol is safe. JECFA has allocated a temporary Acceptable Dietary Intake of 0-50mg/kg. Mannitol has monographs in the United States Pharmacopeia/National Formulary (USP/NF), as well as the various pharmacopoeias around the world. Mannitol is included in the Food Chemical Codex (FCC) [9]. In the context of polyol production, Lactobacillus plantarum possesses some relevant characteristics. It is a food-grade microorganism belonging to the group of lactic acid bacteria. L. plantarum is a normal member of the human intestinal microbiota and can also be isolated from the oral cavity (27, 32). It is largely found as the dominant species in the last step of natural food raw-material fermentation, including a variety of vegetables, meat, and milk (6, 14). Its sugar metabolism is dedicated to lactic acid production.

MATERIALS AND METHODS
Reagents
a. Periodic acid solution, 0.05 (N) as monobasic acid. 5.5 g of pur periodic acid was dissolved in distilled water and diluted to 500 ml. then the mixture was allowed to filter through a sintered glass funnel. The solution was then stored in a dark glass bottle with a glass stopper. This solution decreased slowly its oxidizing power and needed to be standardized daily.
b. Sodium thiosulphate solution, 0.2(N): 25 g of sodium thiosulphate (A.R.) was dissolved in 500 ml of freshly boiled distilled water, then it was cooled down. This solution was standardized with pure potassium iodate.
c. Starch indicator solution: 0.1 g of soluble starch was dissolved in a little water, and it was poured into 100 ml of boiling water by constant stirring. Then, the mixture was boiled for 1 min and was allowed to cool, and then 3 g of potassium iodide (KI) was added. Then, the solution was transferred to a stopper bottle.
d. Potassium iodide solution, 20%: 100 g of Potassium Iodide was dissolved in 400 ml of distilled water.
e. Procedure [10]
In order to ensure the necessary excess of periodic acid, the sodium thiosulphate solution consumed by the sample should not be greater than one fifth that required by the blank.
0.8 g of mannitol (MRECK) was weighing out accurately and dissolved in water and diluted to 250 ml of volumetric flask. 25 ml of the mannitol solution and 50 ml of periodic acid solution was used for each titration and allowed to stand for 80 min. a blank solution was prepared by 50 ml of periodic acid reagent. Then, the percentage of mannitol in the solution was calculated.

Calculation
If 1 ml of the polyhydric alcohol (here mannitol) requires Z mols of periodic acid, W g of the sample of molecular weight M will require
W X Z/M mols of periodic acid

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The experiment was carried out in triplicate. Soy milk contains lower calorie sugar mannitol compared to cow milk. Mannitol production depends on the different ratios of lactic cultures inoculated into the cow milk. Fig 1 shows that, 0.5:1.5 (v/v) ratio of L. plantarum and S. thermophilus produced maximum amount of mannitol, whereas, maximum amount of mannitol had been produced by the cow milk with addition to 1.5:0.5 (v/v) ratio of L. casei and S. thermophilus.

CONCLUSION
Low calorie sugar mannitol was present in a considerable amount in fermented dairy products i.e. both in cow milk curd and soy milk curd; and during fermentation lactic acid bacteria produce mannitol in adequate amount. From studies of other researchers, it was shown that, various metal ions viz. Ca⁺⁺ and Cu⁺⁺ increased the production of mannitol and they also had a synergistic effect when added together [11]. When both Ca⁺⁺ and Cu⁺⁺ were added together, the concentration of mannitol became 96.5 gL⁻¹. Therefore, such low calorie sweetener mannitol is very effective for human consumption; especially for the diabetic patients who are unable to consume sugar.

Acknowledgement
The research work was carried out by the UGC major research project in the Department of Food Technology and Biochemical Engineering, Jadavpur University, Jadavpur, Kolkata.

References

RESULTS AND DISCUSSIONS
Mannitol content of both the cow milk curd and soy milk curd were evaluated (Table 1). From the table value it had been shown that, maximum amount of low calorie sugar mannitol present in soy milk curd, inoculated with mixed culture of L. plantarum and S. thermophilus, which indicated that, during fermentation lactic acid bacteria produce adequate amount of low calorie polyol mannitol in soy curd; whereas in the cow milk curd, 0.6% mannitol had been found to be present.


How to cite this article:
DOI: http://dx.doi.org/10.24327/ijrsrc.2020.1106.5407

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