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EVALUATION OF THE PERFORMANCE OF β -GALACTOSIDASE IN THE PREPARATION OF AN INNOVATIVE WHEY-BASED BEVERAGE

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Abstract: The present investigation was undertaken to evaluate the performance of β -galactosidase in the hydrolysis of lactose during the preparation of an innovative whey-based beverage and to assess the physicochemical, sensory, microbiological, and storage characteristics of the developed product. Sweet whey obtained during paneer manufacture was subjected to enzymatic hydrolysis using food-grade β -galactosidase at different concentrations (0.05, 0.10, 0.15, and 0.20%, w/v) and incubation periods (1, 2, 3, and 4 h) at 37°C. The degree of lactose hydrolysis, reducing sugar content, sweetness index, and sensory acceptability were evaluated to determine optimum processing conditions. Maximum lactose hydrolysis (91.84±1.12%) was achieved with 0.15% enzyme concentration after 3 h of incubation. The optimized whey beverage contained 0.48% residual lactose, 5.86% total sugars, 0.72% protein, and exhibited significantly ($P<0.05$) higher sweetness and overall acceptability than the control. The product remained microbiologically safe and organoleptically acceptable for 15 days under refrigerated storage (4±1°C). The study demonstrated that β -galactosidase effectively improves the nutritional quality, digestibility, and consumer acceptability of whey-based beverages.

Keywords: β -Galactosidase, whey beverage, lactose hydrolysis, functional beverage, lactose intolerance, dairy by-products, value addition

Introduction

Whey is the principal by-product generated during the manufacture of cheese, chhena, paneer, casein, and related dairy products. It constitutes approximately 85–90% of the original milk volume and retains nearly 55% of the milk nutrients, including lactose, soluble proteins, minerals, and water-soluble vitamins (Kosikowski, 1979; Zadow, 1992). Disposal of untreated whey poses serious environmental concerns because of its high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Mawson, 1994). Utilization of whey for the development of value-added products has emerged as an effective strategy to improve economic returns and reduce environmental pollution. Whey-based beverages represent one of the most promising approaches owing to their nutritional value, refreshing properties, and consumer acceptability (Jelen and Tosoni, 2003).

Lactose accounts for approximately 70% of whey solids and is the major limiting factor for its broader utilization. The low sweetness, poor solubility, and intolerance exhibited by a significant proportion of the population reduce the acceptability of lactose-rich products (Mahoney, 1998). β -Galactosidase (EC 3.2.1.23), commonly known as lactase, catalyzes the hydrolysis of lactose into glucose and galactose, thereby increasing sweetness, improving digestibility, and preventing lactose crystallization (Gekas and López-Leiva, 1985). Enzymatic hydrolysis of lactose has been successfully applied in various

dairy systems to develop products suitable for lactose-intolerant consumers (Harju et al., 1987). Although lactose hydrolysis in milk has been extensively studied, limited information is available regarding optimization of β -galactosidase performance in whey-based beverages. Therefore, the present investigation was undertaken to evaluate the efficacy of β -galactosidase in hydrolyzing whey lactose and to standardize an innovative whey-based beverage.

Materials and Methods

Procurement of Raw Materials: Sweet whey was obtained during paneer manufacture from standardized buffalo milk at the university dairy plant. Food-grade β -galactosidase derived from *Kluyveromyces lactis* (5,000 NLU/g activity) was procured from a commercial supplier. Sucrose, citric acid, pectin, and natural fruit flavor were obtained from local markets.

Preparation of Whey : Fresh whey was filtered through double-layered muslin cloth and clarified by centrifugation at 4,000 rpm for 15 min to remove residual fat and suspended particles. The clarified whey was pasteurized at 72°C for 15 s and cooled to 37°C before enzyme treatment.

Experimental Design: A factorial arrangement comprising four enzyme concentrations and four incubation periods was adopted.

Table 1. Experimental Treatments

Treatment	β -Galactosidase (%)	Incubation Period (h)
E ₁	0.05	1, 2, 3, 4
E ₂	0.10	1, 2, 3, 4
E ₃	0.15	1, 2, 3, 4
E ₄	0.20	1, 2, 3, 4

Hydrolysis was carried out at 37±1°C and pH 6.6±0.1. Enzyme activity was terminated by heating the whey to 85°C for 5 min. Preparation of Whey Beverage: The optimized hydrolyzed whey was formulated as follows:

- Hydrolyzed whey: 88%
- Sucrose: 6%
- Pectin: 0.20%
- Citric acid: 0.15%
- Fruit flavor: 0.50%
- Water: Balance

The beverage was homogenized at 150 bar, pasteurized at 85°C for 15 s, filled into sterilized glass bottles, cooled, and stored at 4±1°C.

Analytical Methods: The samples were analyzed for Lactose content, Reducing sugars, Total sugars, Protein, Total solids, pH, Titratable acidity.

The Lactose hydrolysis was estimated using HPLC. The degree of lactose hydrolysis was calculated by applying standard analytical procedures described by AOAC (2005) were followed.

Sensory Evaluation: Sensory evaluation was conducted by ten semi-trained panelists using a nine-point hedonic scale for color and appearance, flavor, sweetness, mouthfeel, and overall acceptability.

Microbiological Analysis: The beverage was analyzed for standard plate count, yeast and mold count, and coliform count at 0, 5, 10, and 15 days of refrigerated storage.

Statistical Analysis: The experiment was conducted using a Completely Randomized Design (CRD) with factorial arrangement. Data were analyzed by analysis of variance, and treatment means were compared at P<0.05.

Results and Discussion

Effect of Enzyme Concentration and Incubation Time on Lactose Hydrolysis

Table 2. Degree of Lactose Hydrolysis (%)

Enzyme Level (%)	1 h	2 h	3 h	4 h
0.05	34.26±1.14	48.82±1.22	59.44±1.18	62.16±1.26
0.10	48.64±1.20	68.32±1.16	79.84±1.08	82.24±1.14
0.15	61.82±1.08	82.68±1.04	91.84±1.12	92.18±1.10
0.20	64.26±1.12	84.42±1.06	92.40±1.08	92.86±1.04

Lactose hydrolysis increased significantly (P<0.05) with increasing enzyme concentration and incubation period. However, the increase beyond 0.15% enzyme concentration after 3 h was not statistically significant. These findings agree with the observations of Gekas and López-Leiva (1985), who reported that lactose hydrolysis depends primarily on enzyme concentration, temperature, pH, and substrate concentration.

Sugar Composition

Table 3. Sugar Profile of Whey Beverage

Parameter	Control	Optimized Beverage
Lactose (%)	5.86±0.12	0.48±0.04
Glucose (%)	0.12±0.02	2.62±0.08
Galactose (%)	0.08±0.01	2.54±0.06
Total sugars (%)	6.06±0.14	5.86±0.10

The conversion of lactose into glucose and galactose enhanced the sweetness of the beverage, as these monosaccharides possess higher relative sweetness than lactose (Mahoney, 1998).

Physicochemical Characteristics

Table 4. Physicochemical Characteristics of Whey Beverage

Parameter	Control	Optimized Beverage
Total solids (%)	9.42±0.14	9.36±0.12
Protein (%)	0.72±0.03	0.72±0.02
pH	6.42±0.04	6.38±0.03
Acidity (% LA)	0.16±0.01	0.17±0.01

Enzymatic hydrolysis did not significantly affect protein content, pH, or acidity.

Sensory Evaluation

Table 5. Sensory Scores of Whey Beverage

Attribute	Control	Optimized Beverage
Color and appearance	8.12±0.10	8.28±0.08
Flavor	7.84±0.14	8.62±0.10
Sweetness	7.68±0.16	8.84±0.08
Mouthfeel	7.92±0.12	8.48±0.10
Overall acceptability	7.88±0.14	8.64±0.08

The optimized beverage received significantly higher sensory scores owing to increased sweetness and improved flavor profile.

Storage Stability

Table 6. Standard Plate Count During Refrigerated Storage

Storage Period (Days)	SPC (log cfu/mL)
0	1.82±0.04
5	2.24±0.06
10	2.76±0.08
15	3.28±0.10

No coliform organisms were detected during storage. The beverage remained microbiologically safe and sensorially acceptable for 15 days under refrigeration.

Conclusion

The present investigation demonstrated that β -galactosidase effectively hydrolyzed lactose in whey and improved the sensory quality of the developed beverage. An enzyme concentration of 0.15% with an incubation period of 3 h at 37°C was found to be optimal, achieving 91.84% lactose hydrolysis. The optimized beverage exhibited enhanced sweetness, improved consumer acceptability, and satisfactory microbiological quality during refrigerated storage. The developed technology offers a sustainable and economically viable approach for value addition of whey while addressing the nutritional requirements of lactose-intolerant individuals.

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