



Colorimetric Characterization and Storage-Related Color Changes in Pasteurized and Sterilized Lactose-Hydrolyzed Flavored Milk

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Abstract - Color is an important quality attribute of flavored milk and can indirectly indicate physicochemical changes during processing and storage. The present study investigated the color characteristics of pasteurized and sterilized flavored milk prepared from lactose-hydrolyzed milk using immobilized β -D-galactosidase and compared them with untreated control flavored milk. Lactose hydrolysis was achieved using β -D-galactosidase immobilized in calcium alginate beads, with fresh and reused beads used to prepare lactose-hydrolyzed milk. Color was evaluated instrumentally using Hunter color parameters, namely L^* (lightness), a^* (redness/greenness), and b^* (yellowness/blueness), during refrigerated storage of pasteurized flavored milk for 7 days and storage of sterilized flavored milk at 37 °C for 90 days. Pasteurized lactose-hydrolyzed flavored milk showed significantly lower L^* and b^* values and higher a^* values than the control during storage ($p < 0.05$), indicating a gradual loss of whiteness and a comparatively darker, slightly reddish-brown appearance. In contrast, the differences in L^* , a^* , and b^* values between sterilized lactose-hydrolyzed and control flavored milk were generally nonsignificant ($p > 0.05$), although progressive darkening and changes in redness and yellowness were observed during extended storage. The greater color changes in lactose-hydrolyzed products are plausibly associated with increased availability of glucose and galactose from lactose hydrolysis, which provide more reactive reducing sugars for Maillard reactions.

Keywords- lactose-hydrolyzed milk; flavored milk; β -D-galactosidase; Hunter colorimeter; L^* value; a^* value; b^* value; storage stability

I. INTRODUCTION

Lactose hydrolysis is an established approach for developing dairy products suitable for consumers with lactose malabsorption while simultaneously modifying the functional and technological properties of milk. Enzymatic hydrolysis of lactose using β -D-galactosidase converts lactose primarily into glucose and galactose. Although this treatment improves lactose digestibility, it also changes the chemical reactivity of the milk system because glucose and galactose are considerably more reactive reducing sugars than lactose.



The increased concentration of reducing monosaccharides in lactose-hydrolyzed milk can influence several quality characteristics during subsequent thermal processing and storage. In particular, glucose and galactose can participate in Maillard reactions with amino groups of milk proteins, forming intermediate compounds and, eventually, colored melanoidins. Consequently, lactose-hydrolyzed milk is generally more susceptible to heat-induced and storage-related browning than conventional milk. Previous investigations have demonstrated enhanced Maillard-related changes in lactose-hydrolyzed milk during ambient storage compared with conventional milk.

Color is one of the most readily measurable indicators of such changes. Instrumental color measurement provides objective information on product appearance through the L^* , a^* , and b^* parameters. In the Hunter color system, L^* represents lightness, with higher values indicating a lighter or whiter product; positive a^* values indicate redness, whereas negative values indicate greenness; and positive b^* values represent yellowness, while negative values indicate blueness. Changes in these parameters can reflect browning, pigment modification, lipid oxidation, and other physicochemical reactions occurring during processing and storage. Previous research on sterilized milk beverages has demonstrated that increased a^* values can be associated with greater browning intensity, while storage and heat treatment can produce systematic changes in L^* , a^* , and b^* values. The objective of the present study was therefore to characterize the instrumental color of pasteurized and sterilized lactose-hydrolyzed flavored milk and to determine the changes in L^* , a^* , and b^* values during storage. Particular emphasis was placed on comparing flavored milk prepared using fresh and reutilized immobilized enzyme beads with untreated control flavored milk.

II. MATERIALS AND METHODS

1. Preparation of lactose-hydrolyzed milk

Milk was subjected to enzymatic lactose hydrolysis using β -D-galactosidase immobilized in calcium alginate beads. The immobilized enzyme beads had an approximate diameter of 2 mm. Lactose hydrolysis was carried out under the optimized conditions established in the study. Following the first hydrolysis cycle, the immobilized enzyme beads were recovered and reutilized for a subsequent hydrolysis cycle. Accordingly, two lactose-hydrolyzed milk treatments were considered:

- LHFB: flavored milk prepared from lactose-hydrolyzed milk using fresh immobilized enzyme beads.
- LHRB: flavored milk prepared from lactose-hydrolyzed milk using reutilized immobilized enzyme beads.
- Control: flavored milk prepared from untreated/non-hydrolyzed milk.

Preparation of flavored milk

Flavored milk was prepared as per the method given by De, 1980, from control milk and lactose-hydrolyzed milk obtained using fresh and reutilized immobilized β -D-galactosidase beads. The formulation and processing conditions were maintained constant among treatments to enable direct comparison.

C: Control flavored milk

LHFB: Lactose-hydrolyzed flavored milk prepared using fresh beads

LHRB: Lactose-hydrolyzed flavored milk prepared using reutilized beads

3. Pasteurization and storage

The prepared flavored milk samples were pasteurized according to the standardized processing conditions adopted for the study. The samples were subsequently stored under refrigerated conditions and evaluated periodically over a 7-day storage period. Color measurements were performed at predetermined storage intervals, including day 0 and subsequent storage intervals.



4. Sterilization and storage

A separate portion of the flavored milk samples was sterilized using the standardized sterilization process adopted in the study. The sterilized samples were stored at approximately 37 °C and evaluated periodically up to 90 days.

Instrumental color measurement

Color was measured using a Hunter colorimeter/Hunter Color Lab instrument. The color parameters were expressed as L^* , a^* , and b^* values.

The parameters were interpreted as follows:

- L^* : lightness/whiteness; higher values indicate greater lightness.
- a^* : redness–greenness coordinate; increasing positive values indicate increasing redness.
- b^* : yellowness–blueness coordinate; increasing positive values indicate increasing yellowness.

All measurements were performed under standardized instrumental conditions.

Statistical Analysis

The experimental data were analyzed using the procedure adopted for the study. Differences among treatments and/or storage periods were evaluated at a significance level of $p < 0.05$. Values with $p < 0.05$ were considered statistically significant, whereas differences with $p > 0.05$ were considered statistically nonsignificant.

III. RESULTS AND DISCUSSION

Color characteristics of pasteurized flavored milk during refrigerated storage

1. Instrumental color measurements showed clear differences between control and lactose-hydrolyzed flavored milk during refrigerated storage. The L^* , a^* , and b^* values are presented in Fig. 1
2. On day 0, control flavored milk exhibited significantly higher L^* values ($p < 0.05$) than flavored milk prepared from lactose-hydrolyzed milk using either fresh immobilized beads (LHFB) or reutilized beads (LHRB). The control's higher L^* value indicates a lighter, whiter appearance. During storage, the L^* values of all flavored milk samples declined gradually. This reduction indicates a progressive loss of lightness during storage. However, the control retained greater lightness than the LHFB and LHRB treatments throughout storage. The lower L^* values observed in lactose-hydrolyzed flavored milk indicate a greater tendency toward darkening.
3. The reduction in lightness of lactose-hydrolyzed milk products may be associated with the increased reactivity of glucose and galactose formed during lactose hydrolysis. These reducing sugars provide favorable substrates for Maillard reactions involving milk proteins, ultimately contributing to the formation of brown-colored compounds. Previous studies have reported that lactose-hydrolyzed milk is more susceptible to Maillard-related changes.
4. The a^* values showed an opposite tendency. Lactose-hydrolyzed flavored milk generally showed higher positive a^* values than the control, indicating greater redness and/or reddish-brown coloration. The increase was more evident during storage, particularly in the hydrolyzed treatments. Such an increase in a^* is consistent with progressive browning reactions.
5. The b^* values also differed among treatments. The control generally retained comparatively greater yellowness during storage, whereas the lactose-hydrolyzed samples showed lower b^*



values toward the end of storage. Thus, the combined reduction in L^* and b^* values with an increase in a^* values suggests a transition from a lighter, more yellowish appearance to a comparatively darker, reddish-brown appearance in lactose-hydrolyzed flavored milk.

6. Similar relationships between increased a^* values and browning intensity have been reported in sterilized milk beverages.

Effect of fresh and reutilized immobilized beads on color

Both LHFB and LHRB showed broadly similar color trends during storage. Although minor differences were observed between flavored milk prepared using fresh and reutilized beads, the overall direction of change was comparable.

The slightly more intense color observed in the lactose-hydrolyzed treatments may be attributed primarily to the formation of glucose and galactose during enzymatic hydrolysis. These monosaccharides have greater reducing activity than lactose and can therefore accelerate non-enzymatic browning during subsequent processing and storage.

Reusing the immobilized beads did not produce a fundamentally different color-development pattern. This observation is technologically relevant because it indicates that reusing the immobilized enzyme system can produce lactose-hydrolyzed flavored milk with a color response broadly comparable to that obtained with fresh beads.

However, because color is only one component of product quality, the suitability of bead reutilization should also be assessed in relation to hydrolysis efficiency, sensory quality, physicochemical stability, enzyme activity retention, and shelf life.

Color changes in sterilized flavored milk during storage

The color behavior of sterilized flavored milk differed from that of pasteurized flavored milk because of the more severe thermal treatment and extended storage period. The initial L^* value of control flavored milk was higher than that of flavored milk prepared from lactose-hydrolyzed milk using fresh and reutilized beads. This indicates that the control product initially appeared lighter.

During storage at 37 °C, L^* values changed progressively in the control, LHFB, and LHRB treatments. The lactose-hydrolyzed treatments generally exhibited greater visual darkening than the control. However, the statistical results showed no significant differences among treatments for L^* , a^* , and b^* at the specified comparison points ($p > 0.05$).

At later storage stages, particularly around 60 and 75 days, the hydrolyzed samples showed higher a^* values and lower b^* values. The higher a^* values suggest increased redness and development of brown coloration, while the decrease in b^* indicates reduced yellowness.

The observed browning is consistent with the well-established higher susceptibility of lactose-hydrolyzed milk to Maillard reactions. Lactose hydrolysis produces glucose and galactose, which react more readily with amino groups than lactose. During heat processing and prolonged storage, these reducing sugars can participate in a cascade of Maillard reactions and subsequent reactions leading to colored melanoidins. Studies on lactose-hydrolyzed UHT milk have similarly shown that storage temperature strongly affects Maillard-related chemical changes and browning. At elevated temperatures, Maillard reactions and lipid oxidation can act together to modify milk color.

Comparison of pasteurized and sterilized lactose-hydrolyzed flavored milk

The color measurements indicate that processing intensity and storage conditions play important roles in determining the visual stability of lactose-hydrolyzed flavored milk. In pasteurized flavored milk stored under refrigeration for 7 days, differences between the control and lactose-hydrolyzed treatments were statistically significant for the measured color parameters. In contrast, the sterilized



products stored at approximately 37 °C for 90 days showed comparatively smaller statistical differences among treatments, despite observable trends toward darkening and increased redness in the lactose-hydrolyzed products. The apparent difference between statistical significance and visual color development may arise from the combined effects of treatment variation, storage duration, sample variability, and the kinetics of individual chemical reactions. Extended storage does not necessarily result in statistically significant treatment differences at every sampling point.

Nevertheless, the direction of the color changes supports the hypothesis that lactose hydrolysis increases flavored milk's chemical susceptibility to browning. Similar observations have been reported for lactose-hydrolyzed milk, where hydrolysis was associated with increased formation of Maillard reaction products during storage. These findings also align with previous research showing enhanced browning in sterilized milk products during prolonged storage.

IV. CONCLUSION

Instrumental Hunter color analysis demonstrated that lactose hydrolysis influenced the color stability of flavored milk during both refrigerated and elevated-temperature storage. Pasteurized flavored milk prepared from lactose-hydrolyzed milk using fresh and reutilized immobilized β -D-galactosidase beads exhibited lower L^* values and higher a^* values than the untreated control, indicating reduced lightness and a tendency toward reddish-brown coloration during storage.

The sterilized lactose-hydrolyzed flavored milk also showed progressive changes in L^* , a^* , and b^* values during 90 days of storage at approximately 37 °C. Although several treatment differences were statistically nonsignificant ($p > 0.05$), the overall color trajectory suggested greater browning in lactose-hydrolyzed samples than in the control. This behavior can reasonably be associated with the increased availability of glucose and galactose following lactose hydrolysis and their greater participation in Maillard-type reactions during thermal processing and storage.

The comparable color trends observed for products prepared using fresh and reutilized immobilized beads suggest that bead reutilization may be technologically feasible without producing a fundamentally different color profile. Nevertheless, optimizing thermal processing and storage conditions will be important to minimize browning and maintain a consumer-acceptable appearance. Overall, Hunter colorimetry provides a useful objective tool for monitoring the visual stability of lactose-hydrolyzed flavored milk. Future studies should combine instrumental color measurement with direct determination of Maillard reaction markers such as 5-hydroxymethylfurfural, furosine, advanced glycation end products, and sensory evaluation to establish a stronger mechanistic relationship between lactose hydrolysis, browning, and shelf-life stability.

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