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Evaluation of the Effect of Ultra-High Temperature (UHT) Treatment on Color, Lysine, Furosine, and Vitamin C in Liquid Infant Formula

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ABSTRACT

Ready-to-feed liquid infant formula (LIF) is used for preterm infants when human milk is unavailable. However, sterilization of liquid infant milk using ultra-high temperature (UHT) treatment leads to the formation of Maillard reaction products (MRPs), which may negatively impact the immune system and kidney development. In this study, infant liquid milk, after being prepared at the Pegah Tehran pilot plant, was subjected to UHT treatment (137°C for 3 seconds). The color indices, nutritional content, and initial Maillard reaction products were examined before and after the UHT process. The findings revealed that there were significant differences in the numerical values of L*, a*, and b* indices before and after thermal processing ($p<0.05$). The available lysine content before and after UHT treatment was 2.30±0.23 and 1.۹۹±0.05 % in dry matter, respectively (13.48% reduction).

The amount of Furosine (early-stage indicator) before and after UHT treatment was reported as 60.64±0.91 and 178.57±1.51 mg per 100 grams of protein, respectively. Vitamin C levels, another nutritional index, decreased during the UHT process (before and after UHT treatment were 147.15±0.49 and 128.29±1.65 mg per kilogram, respectively). Overall, while UHT treatment has benefits for food safety, it significantly reduces the nutritional value of infant liquid milk through Maillard reaction products.

1-Introduction

Infant formula is used to meet the nutritional needs of infants, especially during the first six months of life. This product is primarily based on cow's milk or soy milk (to cater to infants who are sensitive to cow's milk proteins or gluten). The goal of infant formula is to mimic and replicate the nutrients found in breast milk, and in cases where healthy and sufficient breast milk is unavailable, it is the only nutritionally approved food for children under one year of age by the medical community [1, 2].

Based on the classification of physical state, there are three types of milk available in the market for infant nutrition. The most common type is in powder form, which is the least expensive form of infant formula and must be mixed with water before feeding. The second type is in liquid concentrate form, produced at a high concentration and must be mixed with safe water before consumption to achieve the required concentration as defined by standards. The third type is the most expensive compared to the others and does not require mixing or preparation; it is referred to as ready-to-feed. This product is packaged under aseptic conditions after processing and requires no preparation for consumption [3].

Among the limitations of using infant formula are errors during preparation and failure to adhere to the defined nutritional proportions, which may jeopardize the infant's health. The preparation method for infant formula is printed on the product can, but errors can still occur. Additionally, the lack of access to safe water for preparing infant formula poses a potential risk. If safe water is unavailable or secondary contamination occurs during preparation, it may lead to serious and irreversible health damages to the infant.

Thermal methods are the most common techniques for sanitizing milk, including processes such as pasteurization and ultra-high temperature (UHT) sterilization [4]. In the UHT thermal process, milk is subjected to heat treatment at temperatures ranging from 130 to 150 degrees Celsius for a duration of 1 to 10 seconds, resulting in commercially sterile products [5].

Processing and supplying infant milk using the UHT method (chemical compositions can be seen in Table 1) is a suitable and safe option, as it does not require preparation before consumption. Furthermore, ready-to-use liquid infant milk is the primary choice for feeding premature, low-weight, or immunocompromised infants [6].

One of the limitations of milk processing, particularly for infant milk, is the non-enzymatic browning reaction (Maillard reaction), which is attributed to the high levels of lactose and protein in the product. Non-enzymatic browning is a complex reaction resulting from the interaction between reducing sugars and amino groups. Furfurals are one of the indicators of the formation of primary Maillard compounds and serve as a criterion for detecting thermal damage to milk [7].

Table ۱ -Liquid Infant Formula Ingredients

Composition	gr per 100gr
Carbohydrate	**7.18**
Fat	**3.21**
Protein	**1.14**
Ash	**0.21**

Lysine is one of the essential amino acids that reacts with reducing sugars during the Maillard reaction, becoming unavailable to the body. Lysine that is blocked in milk is found in the form of Amadori compounds, which are not absorbable by the body. Infants require significantly more lysine compared to adults (103 mg per kg of body weight per day for infants; 12 mg per kg of body weight per day for adults), making them more susceptible to the adverse effects of its unavailability. Additionally, lysine deficiency can lead to symptoms such as nausea, loss of appetite, fatigue, slow growth, kidney stones, anemia, and other physiological disorders [8].

Recent studies indicate that the Maillard reaction is an important pathway in the development of undesirable flavors during thermal processing and serves as a control point for improving and developing strategies related to the flavor of UHT milk and milk-based beverages. The effects of the Maillard reaction on flavor, color, toxicity, and nutritional value of products have been identified in recent research. Additionally, its negative effects on biological systems, diabetes, and age-related cardiovascular diseases have been established [4, 9].

Zhang et al. (2021) [10] investigated the effects of various thermal processes on the formation of Maillard reaction products. The findings revealed that the lowest amount of furfural (3.9 mg per 100 grams of protein) was found in raw milk. As the thermal treatment temperature increased to 105 degrees Celsius, the amount of furfural rose to 20-40 mg per 100 grams of protein. Furthermore, the level of furfural showed a direct relationship with temperature, with the highest amount of furfural (19.70 ± 30.255 mg per 100 grams of protein) being formed at 135 degrees Celsius.

Stephanie et al. (2015) [11] examined the effects of continuous heating using direct steam injection on the nutritional properties of liquid infant milk as part of their research. The findings indicated that UHT thermal treatment significantly increased thermal damage to liquid infant milk. As the temperature increased, the changes in color indices intensified, resulting in a darker sample, and the amount of furfural increased to 0.1 ± 2.14 mg per 100 grams of protein.

Gova et al. (2023) [12] investigated the changes in Maillard reaction products, volatile compounds, and active proteins in goat milk under the influence of various thermal processes. The results showed that the content of furfural and 5-hydroxymethylfurfural increased with rising temperature and duration of thermal treatment. The amounts of furfural and 5-hydroxymethylfurfural in UHT-processed goat milk were 0.15 ± 22.130 mg per 100 grams of protein and 0.03 ± 1.31 mg per liter, respectively. The findings of this study indicated that the application of UHT treatment increases the intensity of the Maillard reaction in goat milk.

Sanders et al. (2018) [5] investigated the progression of the Maillard reaction in UHT milk during storage at different temperature levels. The results showed that the concentration of furfural (a marker of primary Maillard reaction products) significantly increased during the storage of UHT milk at a constant temperature of 20 degrees Celsius and above. Browning in UHT milk stored at temperatures of 10 and 20 degrees Celsius did not show a significant increase (with a color change of 1.2 units over 24 weeks), whereas UHT milk stored at 30 and 40 degrees Celsius showed color change indices of 2.3 and a significant change after 24 weeks and 6 weeks, respectively.

With the industrialization of societies and the limited access to safe water resources, food processing technology is shifting towards the production of ready-to-eat foods, and products for infants are no exception. According to a report by the Food and Agriculture Organization (FAO), the production of liquid infant milk in the United States reached 350 tons in 2020 [13]. In response to the growing market for this product, companies producing infant formula are adding liquid infant milk to their product lines. Liquid infant milk is highly susceptible to the Maillard reaction due to the presence of reducing sugars and the amino acid lysine. The nutritional properties of this milk are altered by the UHT thermal process, which may impact the health of infants.

In this study, the effect of applying UHT treatment (137 degrees Celsius for 3 seconds) on the levels of furfural, changes in color indices (L*, a*, and b*), the availability of lysine, and vitamin C was investigated. The aim was to assess the color changes and the progression of the Maillard reaction, as well as the reduction of nutritional properties in liquid infant milk.

2- Materials and Methods

Chemicals and Equipment Used

The raw materials and chemical compounds required for this research included the initial mixture of infant formula (Nestlé Iran), sodium acetate (Sigma Aldrich), propionic anhydride (Merck), orange-12 acid (Merck), hydrochloric acid 37% (Merck), acetonitrile (Merck), and formic acid (Merck), which were purchased from reputable companies and suppliers.

Sample Preparation

To prepare the samples, the method of Zhu et al. (2020)[14] was used with some modifications. Skim milk powder and grade one lactose (MR1), mineral compounds, galacto-oligosaccharides, fructo-oligosaccharides, whey protein isolate (WPI), vitamin C, and micronutrient compounds containing essential amino acids were combined according to the preparation instructions of the initial mixture and mixed with RO water at a temperature of 55 degrees Celsius (the final dry matter was 10.52). The resulting solution stirred with an electric mixer for 5 minutes and was kept at room temperature for 20 minutes to hydrate. Then, for UHT treatment (137 degrees Celsius for 3 seconds), it was added to the inlet chamber of the sterilizer and underwent sterilization and homogenization (under pressure of 50-150 bar). After the process was completed, it filled into sterilized one-liter Tetra Pak containers under hygienic conditions (Figure 1) and stored in the refrigerator (3 degrees Celsius) until the tests conducted.

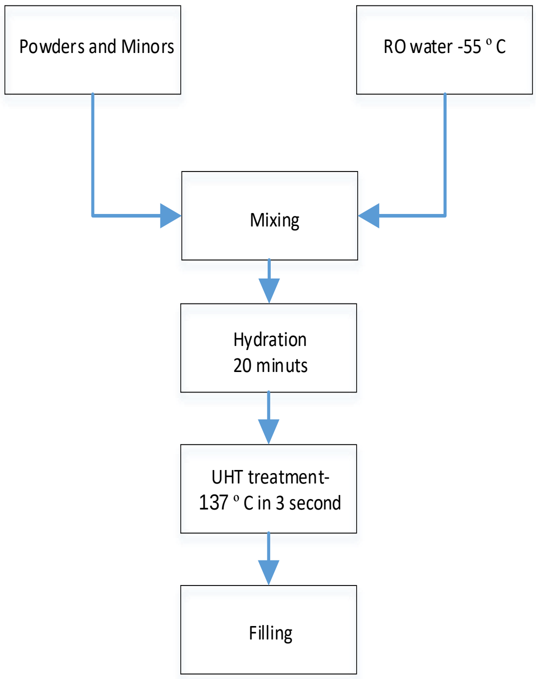

Figure ١ -Flow diagram of sample preparations

Physicochemical Tests of Milk

To measure moisture content, fat-free dry matter, protein, fat, density, acidity, and pH, the following methods were used respectively: AACC (44-15A), Iranian National Standard No. 637, AOAC (991.20), Iranian National Standard No. 366, Iranian National Standard No. 366, Iranian National Standard No. 638, and Iranian National Standard No. 2852.

Colorimetry Test for Measuring Maillard Reaction Final Products

The formation of melanoidins was measured by assessing color change according to the method of Al-Sadi and Dith (2008) [15]. The colorimetry test was performed directly on the fresh sample using the HunterLab system, measuring the color indices L*, a*, and b*. The color difference ΔE for each sample compared to the control sample was calculated using the following equation

$$\Delta E = \sqrt{(\Delta a^2 + \Delta b^2 + \Delta L^2)}$$

Measurement of Available Lysine

The amount of available lysine was measured using the method of Alavi et al. (2016) [16]. The concentration of available lysine was calculated using a standard color curve in buffer, and the results were expressed as a percentage of available lysine based on dry matter.

Measurement of Furosine (Primary Metabolites of the Maillard Reaction)

To measure furosine, the method of Carisio et al. [17] was used. One milliliter of the sample was hydrolyzed with 7 milliliters of 0.08 M HCl at 120 degrees Celsius for 23 hours. High-purity nitrogen was bubbled through the solution for 2 minutes, and then the hydrolyzed sample was filtered using filter paper .

٠.٥ milliliters of the filtered portion was added to a Sep-Pak C18 cartridge along with 5 milliliters of methanol and 10 milliliters of deionized water. It was then washed with 3 milliliters of 3 M HCl and evaporated under vacuum. The dried sample was dissolved in a mixture of water, acetonitrile, and formic acid (in the ratio of 95, 5, and 0.2, respectively), and 50 microliters of the resulting solution was analyzed by HPLC.

Measurement of Vitamin C

Vitamin C was measured to assess the effect of thermal treatment and as an indicator of the nutritional quality of the product. This was done using a two-step titration method as described in Iranian National Standard No. 2685.

Statistical Analysis

In this study, the effect of ultra-high-temperature (UHT) processing on liquid infant milk was investigated. A General Full Factorial design was used with two replications for the experimental design. The data were analyzed using Minitab v.16 software, and analysis of variance (ANOVA) was conducted using the GLM method, with mean comparisons performed using Tukey's method at a 95% confidence level. Excel 2013 was used for graphing the results

3-Result and discussion

Effect of UHT Treatment on Changes in Color Indices

The color evaluation of the samples conducted using the HunterLab colorimeter. The L* index indicates lightness (0 = black and 100 = white), the a* index indicates redness (positive) and greenness (negative), and the b* index indicates yellowness (positive) and blueness (negative).

Color is one of the most important parameters that affects consumers' perception of food products. Since the visual perception of color is a combination of different dimensions of the color space, examining parameters that represent various aspects of color provides a better indication of color changes, which are reflected in the variations of L* (lightness), a* (redness), and b* (greenness) values. The color indices of the liquid infant milk samples measured before and after thermal treatment (Table 1).

Table٢ - The effect of the UHT heat treatment process on changes in color indices

Sampling time	L*	a*	b*	ΔE
Before UHT treatment	97.86±0.0123[a]	-1.82±0.0003[a]	7.95±0.0011[a]	-
After UHT treatment	80.23±0.0113[b]	-1.2±0.0058[b]	6.87±0.0014[b]	**6.99±0.0005**

The data presented in the table are in the form of mean ± standard deviation of two replications. Different letters indicate a significant difference (p<0.05) in the mean values.

The results obtained from the measurement of color indices showed that the thermal process caused changes in the color indices of liquid infant milk. As shown in Table 2, the L* index decreased after the thermal process, and data analysis using the Tukey method ($p < 0.05$) indicated that there was a significant difference between the samples before (86.97 ± 0.0123) and after the UHT thermal process (80.23 ± 0.0113). The numerical value of a* index increased after the thermal process, and data analysis with the Tukey method ($p < 0.05$) showed that there was a significant difference between the samples before (-1.82±0.0003) and after the UHT thermal process (-1.2±0.0058).

The numerical value of the b* index also increased after the thermal process, and data analysis using the Tukey method ($p < 0.05$) indicated a significant difference between the samples before (7.95±0.0011) and after the UHT thermal process (6.87±0.0014).

Additionally, the total color change was 6.99±0.0005, which showed with the ΔE index in Table 2. The results obtained were consistent with the study by Schamberger et al. in 2007[18]. The examination of raw milk samples before and after the sterilization thermal process indicated a decrease in the L* index and an increase in a* and b* indices.

Findings from various studies [19-21] indicated that several factors, including non-enzymatic browning reactions, denaturation of proteins (denaturation of casein and whey proteins causes changes in the L* index). The main reasons are Caramelization of lactose (which significantly affects the b* index), oxidation of fats (leading to an increase in the b* index and intensifying yellow color), and degradation of pigments present in milk (the degradation of naturally occurring pigments in milk, such as chlorophylls and carotenoids, causes this color change).

Effect of UHT Thermal Treatment on Available Lysine

The thermal process is significant from the perspective of enhancing food safety, improving sensory quality, eliminating microorganisms, and increasing the shelf life of food products. However, it may have adverse effects on the nutritional quality of protein-containing foods due to chemical changes and the production of certain undesirable compounds. Lysinoalanine (2-amino-2-carboxylysine) is an unnatural compound formed from the thermal or alkaline treatment of protein-containing foods. The reaction that leads to the formation of lysinoalanine (LAL) begins with the removal of the beta hydroxyl group from serine, -O-phosphoryl serine, -O-glycosyl serine, or the disulfide group from cysteine. The final product of this removal is dehydroalanine (2-aminoacrylic acid or DHA). This highly reactive compound is an intermediate product in the formation of lysinoalanine and other xenobiotic amino acids and can react with various amine-containing compounds, such as amino acids and biogenic amines. LAL is formed from the reaction of DHA with the amino acid lysine.

Studies have shown that LAL has nephrotoxic effects in some animals and can lead to nephrosclerosis, microscopic injury to kidney cells, increased nucleoprotein levels, and consequently, disruption in DNA synthesis and mitotic division. Due to its chelating ability, LAL can inactivate enzymes containing metal ions, primarily divalent copper and zinc ions, known as metalloenzymes (metalloproteins). On the other hand, the formation of LAL results in reduced availability of the essential amino acid lysine and, consequently, a decrease in the nutritional quality of the protein source. In fact, by measuring the available lysine content, the effect of the thermal process on the nutritional quality of lysine can be examined, indirectly interpreting and assessing its role in the reduction of the nutritional quality of liquid infant milk. To evaluate the onset of the Maillard reaction, available lysine may provide reliable information. Generally, the Maillard reaction is divided into three stages, with the interaction of lactose with lysine and the formation of Amadori products considered as the initial stage of the reaction [22].

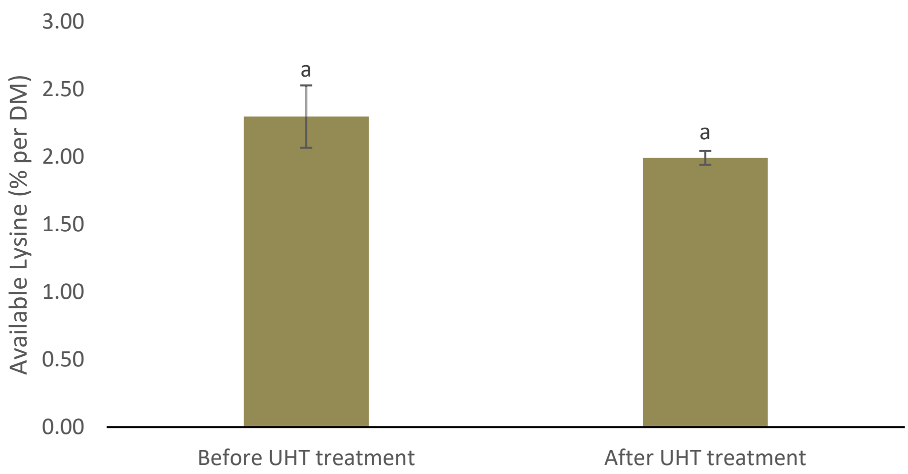

Figure۲ -Impact of the UHT thermal process on Available Lysine (% per DM); Means with different superscript indicate significant differences in column (P<0.05)

The examination of infant liquid milk samples before and after the UHT thermal process showed that applying the UHT thermal treatment reduces the amount of available lysine in infant liquid milk. As shown in Figure 2, the amount of available lysine before and after applying the UHT thermal treatment was 2.30 ± 0.23 and 1.99 ± 0.05 percent in dry matter (respectively 13.48% reduction), and data analysis using the Tukey method ($p<0.05$) indicated that there is no significant difference in the amount of available lysine before and after the UHT thermal process.

The results obtained were consistent with the study by Prestl et al. in 2020 [23]. They reported that the amount of lysine in UHT-treated milk is lower than in untreated samples. It has been well established that the thermal process can lead to the loss of lysine due to the blocking of active sites of amino acids and also the presence of glucose before the thermal process, which can facilitate the Maillard reaction.

As previously mentioned, the Maillard reaction occurs between lactose or its hydrolysis products and lysine, leading to a significant reduction in available lysine through the formation of furfural. The nutritional requirement for the amino acid lysine is reported to be about 1070 milligrams per day for infants weighing 10 kg [24]. The study by Aktaş et al. in 2019 [8] showed that 1516.8 milligrams/serving per day of available lysine was analyzed in infant milks, and a positive correlation between the formation of furfural and the loss of lysine may lead to serious diseases through the accumulation of Amadori products and loss of micronutrients in dairy products.

Furosine (milligrams per 100 grams of protein)

Furosine is one of the indicators of the formation of initial compounds in the Maillard reaction and serves as a criterion for detecting thermal damage to milk. The measurement of Furosine in milk samples directly indicates the amount of initial compounds in the Maillard reaction, Amadori compounds, and lactolysine.

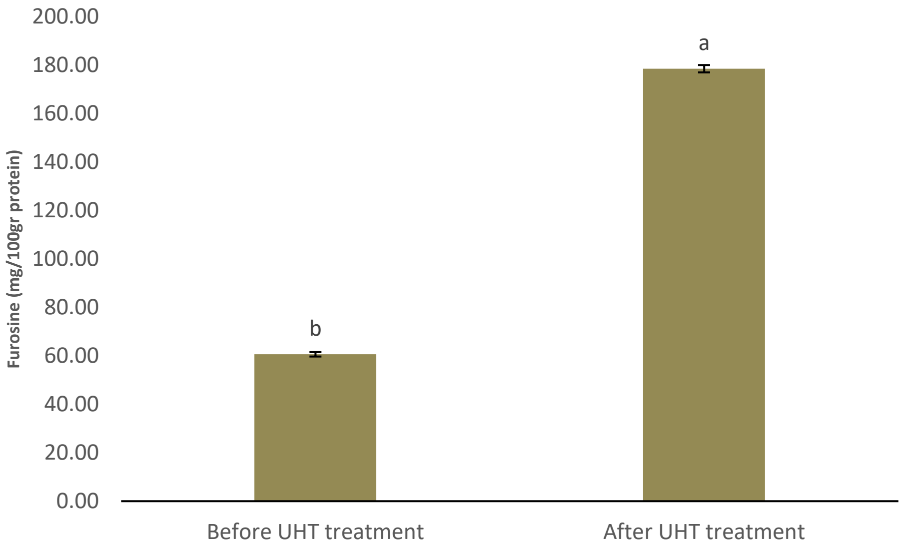

Figure٣ -Content of Furosine (milligrams of Furosine per 100 grams of protein) ;Means with different superscript indicate significant differences in column (P<0.05)

The measurement of Furosine levels indicated that applying the UHT thermal treatment increases Furosine in infant liquid milk. As shown in Figure 3, the amount of Furosine before and after applying the UHT thermal treatment was 60.64 ± 0.90 and 178.57 ± 1.51 milligrams per 100 grams of protein, respectively. Data analysis using the Tukey method ($p<0.05$) showed that the samples before and after the UHT thermal process have a significant difference.

The results obtained were consistent with the study by Zhao et al. in 2023 [25], which reported that the formation of Furosine in camel milk is directly related to the temperature and duration of the thermal treatment, with increasing temperature and time intensifying the Maillard reaction.

Recent studies [8, 22] have shown that the Furosine content in milk treated with different thermal processes varies significantly, and with the intensification of the thermal process, the Furosine content increases as an indicator of the initial compounds in the Maillard reaction.

Measurement of Vitamin C (milligrams per kilogram)

Breast milk has a higher level of Vitamin C (L-ascorbic acid) compared to commercial infant formulas. However, as mentioned at the beginning of this article, the use of commercial formulas as a substitute for breast milk is sometimes unavoidable. Vitamin C is very unstable against heat, oxygen, light, pH, and moisture content.

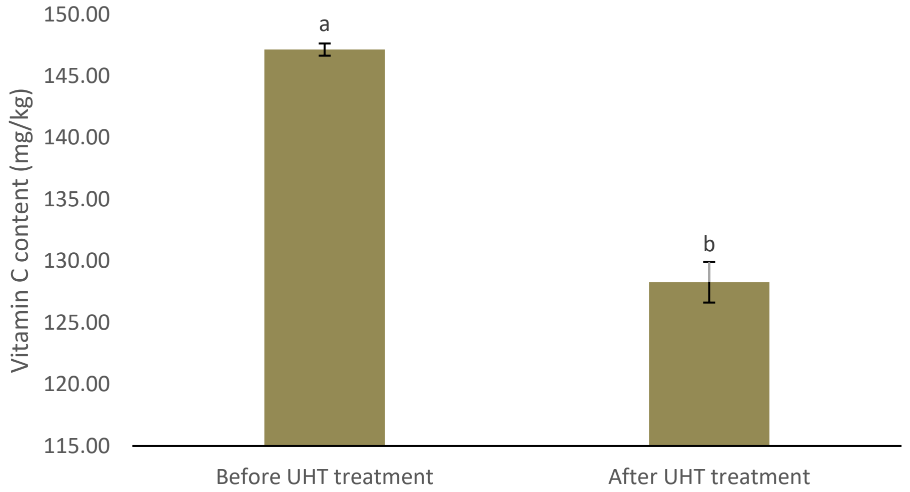

Figure۴ -Vitamin C content (mg/kg) ;Means with different superscript indicate significant differences in column (P<0.05)

The UHT thermal treatment resulted in a decrease in Vitamin C levels in infant liquid milk. As shown in Figure 4, the amount of Vitamin C before and after applying the UHT thermal treatment was 147.15 ± 0.49 and 128.29 ± 1.65** milligrams per kilogram, respectively (a reduction of **12.82%**). Data analysis using the Tukey method (p<0.05) indicated that there is a significant difference between the samples before and after the UHT thermal process.

The results of the present study are consistent with reports from other research [5, 26, 27]. Considering the nutritional importance of Vitamin C in the growth and development of infants, the reduction in its levels during product processing highlights the significance of fortifying infant food products with Vitamin C.

4-Conclusion

The findings of the present study confirm the adverse effects of the UHT thermal process on the nutritional properties of infant liquid milk. The use of UHT thermal treatment, despite its advantages in terms of food safety and industrial aspects, significantly influenced the nutritional indicators (lysine amino acid and Vitamin C) of this product. Additionally, the development of the Maillard reaction is evident, as indicated by the increase in Furosine levels and changes in color indices (decreased clarity and increased darkness, which may indicate the formation of melanoidins).

The increase in Furosine levels suggests a high potential for the vulnerability of infant liquid milk to the development of the Maillard reaction during storage. Considering the proven adverse effects of compounds resulting from the Maillard reaction on consumer health, and the sensitivity of the target consumer group (infants, especially preterm infants), further studies are needed, particularly to investigate methods for controlling and limiting the Maillard reaction during the processing of UHT infant liquid milk.

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مقاله علمی_پژوهشی

بررسی اثر تیمار حرارتی فرادما (UHT) بر تغییرات رنگی، لیزین، فوروزین و ویتامین ث شیر مایع نوزاد

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت:

تاریخ پذیرش:

کلمات کلیدی:

استرلیزاسیون،

شیر خشک نوزاد،

شیر مایع نوزاد،

میلارد،

UHT

چکیده

شیر مایع آماده برای تغذیه نوزادان (Ready-To-Feed Liquid Infant Formula) زمانی که شیر مادر در دسترس نباشد برای نوزادان نارس استفاده می‌شود. با این حال، استریلیزاسیون شیر مایع نوزاد با استفاده از تیمار فرادما (UHT) موجب تشکیل محصولات واکنش میلارد (MRPs) می‌شود که ممکن است بر عملکرد سیستم ایمنی و رشد کلیه‌ها اثر منفی بگذارد. در این تحقیق، شیر مایع نوزاد پس از آماده سازی در پایلوت شرکت پگاه تهران تحت تیمار حرارتی فرادما (۱۳۷ درجه سلسیوس به مدت ۳ ثانیه-UHT) قرار گرفت و شاخص‌های رنگی، تغذیه‌ای و ترکیبات اولیه واکنش میلارد قبل و پس از اعمال فرآیند UHT مورد بررسی قرار گرفت. یافته‌های این پژوهش نشان داد، مقدار عددی شاخص L^*، a^* و b^*، قبل و پس از فرایند حرارتی اختلاف معنی دار دارند ($p<۰/۰۵$)، همچنین مقدار لیزین در دسترس، قبل و پس از اعمال تیمار حرارتی UHT به ترتیب ۲/۳۰±۰/۲۳ و ۱/۹۹±۰/۰۵ درصد در ماده خشک بود (۱۳/٤۸ ٪ کاهش یافت). مقدار فوروزین (شاخص ترکیبات اولیه واکنش میلارد) قبل و پس از اعمال تیمار حرارتی UHT به ترتیب ٦۰/٦٤±۰/۹۱ و ۱۷۸/٥۷±۱/٥۱ میلی گرم در ۱۰۰ گرم پروتئین گزارش شد. میزان ویتامین ث به عنوان دیگر شاخص تغذیه‌ای، طی اعمال فرآیند UHT کاهش یافت (قبل و پس از اعمال تیمار حرارتی UHT به ترتیب ۱٤۷/۱٥±۰/٤۹ و ۱۲۸/۲۹±۱/٦٥ میلی گرم در کیلوگرم). بصورت کلی تیمار فرادما علی رغم وجود مزایایی از جنبه ایمنی موادغذایی، بصورت قابل ملاحظه ای ارزش تغذیه‌ای شیر مایع نوزاد را از طریق واکنش میلارد کاهش داد.

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