Research Article

Assessment of the Accelerated Shelf Life of Human Milk Dehydrated by Aspersión and Treated by UV, High Pressures, and Pasteurization

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Human milk banks pasteurize and freeze the milk in order to conserve it, but thawing and prolonged storage cause loss of nutritional components. The aim of this work was to evaluate the effect of pasteurization, high hydrostatic pressures, UV radiation, and spray drying in human milk packed and stored at 25 to 40°C by a period of 14 weeks, using an accelerated shelf life method with Arrhenius model. Effectiveness of packaging, microbiological viability, and deterioration of carbohydrates, lipids, and proteins was evaluated. The results showed that proteins and carbohydrates in powdered human milk with different treatments did not show significant changes during storage at 25 to 40°C and without the growth of microorganisms. However, 33.3% deterioration of lipid oxidation was observed up to 40°C. We predict with the applied model that, at 18°C, the human milk powder will be preserved for approximately one year without significant changes in its composition.

1. Introduction

Human milk bank not only promotes, protects, and supports breastfeeding but is also responsible for collecting, pasteurizing, and distributing milk to feed premature and lactating newborn with nutritional disorders and allergies to heterologous proteins [1]. Despite knowing the benefits that provide human milk for the development of infants, Mexico shows a low rate of breastfeeding practices. According to the Ministry of Health, the average exclusive breastfeeding practice in Mexico during the first 6 months of the infant's life is 14.4%, which represents that 85% of the mothers feed their children with formula milk [2]. Solís-Pacheco et al. [3] developed a spray drying process for LH, obtaining a harmless dehydrated human milk, without preservatives or additives, achieving a 95% retention of the nutritional

content (proteins, lipids, and carbohydrates) and 70 to 80% immunoglobulins and lactoferrin. These authors describe that powdered human milk has several advantages over pasteurized and frozen raw milk in breast milk banks since powdered human milk is easy to store at room temperature and easy to transport and handle; in addition to its low water activity, microorganisms are inhibited and do not grow.

On the other hand, it is known that dry powder products have a long shelf life, conferred due to the low water content, which prevents the development of microorganisms and undesirable enzymatic reactions. Besides, the product can be stored in small spaces at room temperature, without the need to refrigerate and/or freeze it, resisting warm temperatures for longer periods [4]. To estimate the shelf life of powdered products, quality attributes (nutritional content and microbiological content) that vary during shelf life or through

accelerated tests can be evaluated [5]. Accelerated life tests (ASLTs) allow to measure shelf life in shorter periods of time by using more severe conditions (usually temperature rise, oxygen pressure rise, humidity increase, and combined), so it is used more frequently to predict the expiration dates of food in a more economical way [6]. The application of kinetic models has been a useful tool to predict changes that induce deterioration in food; these models are based on the kinetics of chemical reactions. However, food is a complex system in which different reactions can occur, and thus, it is applied to a characteristic that reflects these reactions [7]. Emerging processes for food preservation such as high hydrostatic pressures and UV radiation, in combination with other processes, such as spray drying, have been effective in human milk preservation.

Concerning this, Malinowska-Pańczyk et al. [8] evaluated microwave heat treatment application to preserve human milk through pasteurization, and the authors showed that total pasteurization can be achieved in a shorter time than by using the traditional method. The use of microwave heating allows inactivating all bacterial strains inoculated to human milk and its microbiota. On the other hand, Salcedo et al. [9] evaluated lyophilization and freezing as a combined method to ensure microbiological reduction for storing human milk. Lyophilization and storage at -80°C significantly reduced the content of mesophilic aerobic microorganisms and *Staphylococcus epidermidis* when compared with storage at -20°C , being a feasible alternative for human milk banks. High-pressure carbon dioxide (CO_2) processing is an emerging technology that has been studied as a promising alternative for the reduction of microorganisms in different liquid food. Berenhauser et al. [10] assessed the impact of high-pressure carbon dioxide processing on the microbial inactivation of aerobic mesophilic bacteria and *E. coli* ATCC 25922 inoculated in human milk. The variables studied were the effect of the ratio between sample mass and CO_2 (1:0.2, 1:0.6, and 1:1 m/m), depressurization rate (1, 5.5, and 10 MPa/min), and pressure cycling (1, 3, and 5). The best log reductions in aerobic mesophilic bacteria as well as in *E. coli* (>6.0 and >5.0 log, respectively) were obtained with a 1:1 ratio, a depressurization rate of 10 MPa/min, and one cycle of pressurization/depressurization. The results found by this group showed that depressurization rate is an important variable in the inactivation process and suggests that high-pressure carbon dioxide processing can be applied to human milk as a safe alternative to the pasteurization used in human milk banks. Therefore, the aim of this work was to evaluate the shelf life of a human milk powder product, treated with high hydrostatic pressures and UV radiation, in order to verify the nutritional content and sanitary quality of the powder product during storage.

2. Materials and Methods

2.1. Human Milk Treatment. Human milk was collected from healthy donors of the Human Milk Bank of the Hospital Civil of Guadalajara “Fray Antonio Alcalde” in Mexico. This study was approved by the Ethical Research Committee in May 2015 of the same hospital. The volunteer

donors signed the informed consent about the donation of the excess of their human milk, and they accepted to participate voluntarily and use their milk for this research. The human milk was extracted by an electric extractor with automatic pumps of Lansinoh brand and kept in sterile bags, following the hygiene guidelines established by the Human Milk Bank of Hospital Civil de Guadalajara “Fray Antonio Alcalde”. The bags (50 to 100 mL of human milk) were mixed and homogenized in a single batch and then divided into 1 L portions to use the milk for the different treatments (Figure 1).

For high hydrostatic pressure treatment, raw milk was deposited in 250 mL sterile plastic bags FoodSaver® vacuum-sealed and treated at pressures of 300 megapascals (MPa) for 10 min, at 45°C . For drying the milk, a cone-based laboratory scale spray dryer (BÜCHI Mini Spray Dryer, B-29) was used, in which previously homogenized human milk was fed at a flow of 2 mL/min, an air inlet temperature of 165°C , and an air outlet temperature of 110°C . For pasteurization, human milk was placed at a temperature of 85°C for 5 min, using a water bath (Heidolph Laborota 4000, USA), and immediately cooled in a cold-water bath ($2-4^{\circ}\text{C}$). Finally, for UV irradiation, human milk previously dried was exposed under an INDELAB 30/70 RV® Series UV irradiation lamp for 2 hours. The milk was placed on a sheet of aluminum foil and spread over its entire length, ensuring that a layer approximately 1 cm thick remained.

2.2. Accelerated Shelf Life. Accelerated shelf life was evaluated in human milk after spray drying (previously subjected to pasteurization, high hydrostatic pressure, and ultraviolet light). The treatments were identified as HMWT (human milk without treatment), HMUV (human milk with ultraviolet light treatment), HMHP (human milk with high hydrostatic pressure treatment), and HMP (human milk pasteurized); all treatments were performed as follows.

5 g of powdered human milk was packaged individually in trilaminated bags to perform microbiological analysis and protein, lipid, and carbohydrate assays. After, the air inside the bags was manually removed and immediately sealed with heat using a 25 cm manual sealer (Alpha toner). Additionally, the existence of leaks in the packed material was avoided. The human milk package was stored at 25 and 40°C to a relative humidity of 32.8 and 74.7 (± 0.2), respectively, and the Arrhenius model was used to determine the optimal storage time.

The packaging used for this study is made of a metalized PET sheet (Alfipa Brand). The original size of the pouch bag was 25.4×25.4 cm and was cut to a size of 4×4 cm, to manually pack human milk powder and then manually sealed.

All packages had a size of 4×4 cm containing 5 g of milk and with size inside the seals of 3.5×3.5 cm. The packaging has a thickness of 0.112 mm and is made of aluminum and polyethylene terephthalate (PET-met) made up of a layer PET, aluminum, OPA, and CPP, with a thickness of 12 μm , 9 μm , 15 μm , and 76 μm , respectively.

FIGURE 1: Methodological diagram of different treatments for human milk preservation.

All packages had a barrier to oxygen of 90% RH: 2 cm/ (m²*day) @ 38°C and a H₂O vapor barrier of 0% RH: 1 g/ (m²*day) @ 23°C and with a measure in each layer of 12 μm PET, 9 μm aluminum, 15 μm OPA, and 76 μm CPP.

2.3. Permeability of Package. To assess the permeability of package, 1 g of CaCl₂ was weighed and packed in trilaminated bags of 4 × 4 cm, same as the milk samples of each treatment. Later, it was placed in an incubator at 25 and 40°C for 98 days. Weight gain in CaCl₂ was recorded in an analytical balance, and the water vapor transmission denoted by WTA (*b/A*) was obtained through a graph of water gain as a function of time, which is the ratio between the slope of the graph and the transfer area. The permeability of the package was calculated by the following expression:

$$B = \frac{(b/A) * \Delta x}{(P_o/100)(HR_1 - HR_2)}, \quad (1)$$

where *b* is the slope, *A* is the area arranged by the water vapor transfer gasket, Δx is the thickness of the laminate, P_o is the vapor pressure of the pure water at the temperature at which the experiment is conducted, and HR_1 and HR_2 are the relative humidity outside and inside the package.

2.4. Microorganism Quantification. Quantification of microorganisms was made as a part of the monitoring of the shelf life of powdered human milk. Mold and yeasts, total coliforms, and lactic acid bacteria were determined according to the Mexican standards for the determination of microorganisms (NOM-111-SSA1-1994, NOM-113-SSA1-1994, and NOM-184-SSA1-2002) [11–13]. For this analysis, we employed Petrifilm plates (3M®). For mold and yeast count, the plates were incubated for 3 and 5 days at 25°C, and

for total coliforms and lactic acid bacteria, the plates were incubated at 37°C for 24 and 48 hours.

2.5. Determination of Total Proteins and Total Carbohydrates. The determinations of total proteins and total carbohydrates were made to evaluate possible changes concerning the storage time in powdered human milk and in this way determine the shelf life of the packed milk. The determination of total proteins was performed using the method of Lowry et al. [1], and the total carbohydrates were made using the sulfuric phenol method of Dubois et al. [14].

2.6. Determination of the Percentage of Free Fatty Acids. This method is based on the titration of free fatty acids with an alkali. This was determined by following the Mexican Standard NMX-F-101-SCFI-2012 [15, 16]. Briefly, 0.85 g of powdered milk was mixed with 1.65 mL of BDI reagent with a high-speed homogenizer. Once the samples were homogenized, the tubes were placed in a hot water bath at 57°C and stirred for 20 min inside the bath. The tubes were removed from the bath, and 1.65 mL of aqueous methyl alcohol was added to extract the fat. Immediately, the tubes were centrifuged for 2 min at 4500 rpm and placed again in the water bath at 57°C for 15 min. The fat resulting from incubation with hot water was recovered and transferred to a 125 mL Erlenmeyer flask using a Pasteur pipette, recording the weight of the fat recovered, as accurately as possible. The heavy fat was dissolved in 5 mL of petroleum ether and *n*-propanol with a ratio of 4:1. Five drops of phenolphthalein were added, to subsequently titrate the sample with 0.02 N KOH, stirring until a pink color was observed.

The result is expressed in milligrams of potassium hydroxide according to the following expression:

$$\text{acidity index} = \frac{56.1 \times N \times V}{P}, \quad (2)$$

where 56.1 is the chemical equivalent of potash; N is the normality of the potassium hydroxide solution; V is the volume of titrated solution of potassium hydroxide spent on the titration of the sample; and P is the mass of the sample in grams.

It should be expressed as a percentage of oleic, palmitic, or lauric acid by applying the following expression, using the meq of the reference fatty acid:

$$\% \text{ free fatty acids} = \frac{\text{meq} \times N \times V}{P} \times 100, \quad (3)$$

where meq is the chemical milliequivalent of the reference fatty acid; N is the normality of the potassium hydroxide solution; V is the volume of titrated solution of potassium hydroxide spent on the titration of the sample; and P is the weight of the sample in grams.

2.7. Statistical Analysis. A one-way analysis of variance with a statistical Tukey test with a confidence level of 95% ($p < 0.05$) was used to analyze the variations among the treatments evaluated at different storage temperatures.

3. Results and Discussion

3.1. Packaging Effectiveness. To assess the permeability of the trilaminated packages that contained powdered human milk, water vapor transmission was measured as previously described in the methodology section. Results on the permeability of trilaminated packages showed a significant difference in relation to the WTA in both storage temperatures (25 and 40°C). The effectiveness of a package can be determined by shelf life tests or by combining information from breakpoint tests (keeping humid), as well as having knowledge of the moisture and permeability of the container material.

In Figure 2, it is observed that as the temperature increases and time passes, there is a greater effect of water vapor transmission inside the trilaminar bag.

It has been described that the main purpose of the packaging is to protect the powdered food from the moisture that enters the interior and preserve the characteristics of the product since when the moisture enters, the powdered becomes lumpy or pasty. In addition, moisture can lead to harmful effects, such as changes in its composition, and activate enzymatic reactions, causing oxidation or Maillard reaction [17]. Uppu [18] reported that the moisture or water vapor ingress into powdered milk package, in combination with light, O_2 , and an elevated temperature, can cause texture loss and caking due to crystallization of lactose, causing microbial deterioration and fat oxidation.

In this work, trilaminar packaging offers better resistance to temperature and the passage of water, being less permeable to water vapor compared to packet laminar, since it has more laminar layers providing a protective barrier against the environment.

FIGURE 2: Analysis of the water vapor transmission by the trilaminar packet across days at 25°C and 40°C.

3.2. Determination of Proteins and Total Carbohydrates. Regarding the results obtained on the protein and carbohydrate contents in powdered human milk treated with the different preservation processes, there were no significant changes during storage time (98 days) in both temperatures evaluated (25 and 40°C) (Figure 3). Díaz et al. [19] mention the behavior of proteins against a heat treatment and explain that casein micelles are remarkably stable at temperatures up to 140°C. On the contrary, whey proteins are relatively thermolabile, undergoing intense denaturation at 80°C, only a slight color change was noted in milk stored at 40°C, probably due to the Maillard reaction that could have been carried out at a high temperature (40°C) and prolonged storage of the milk samples. These results are in agreement with those obtained by Davis et al. [20], where they indicated that the temperature and storage time (4 and 22°C for 0, 2, and 4 months) in powdered goat milk showed no effect on the levels of proteins, carbohydrates, and lipids.

3.3. Determination of the Percentage of Free Fatty Acids. According to Roos [2], one of the important factors increasing the rate of self-oxidation in powdered milk is the increase in A_w . The results obtained in this work regarding the oxidation of lipids in human milk powder treated with the different preservation processes showed a significant increase in lipid oxidation, from the 14 days of storage in both temperatures evaluated (Figure 4), an increase in A_w from 0.3 to 0.7 during the 98 days of storage. The pasteurized milk with subsequent drying showed the highest oxidation of lipids, probably due to the process of temperature and time to which the milk was subjected before drying. Research conducted by Borgo et al. [21] explains that slow or rapid pasteurization in human milk is not a factor that activates lipid oxidation in milk. However, the results found in our work demonstrate the opposite, since the pasteurization of human milk, before spray drying, showed the highest oxidation of lipids. On the other hand, Weber et al. [4] describe that the lack of homogeneity in milk can affect the fat content, and we believe that this could be another factor in the evolution of lipolysis in the analyzed milk.

FIGURE 3: Protein (a) and carbohydrate (b) contents in human milk powder stored at 25°C and 40°C.

The oxidation of fats occurs in the presence of O_2 and moisture and can be catalyzed by light, the presence of antioxidants, A_w , and temperature, which influences the oxidation rate [22]. Powders that contain a high percentage of fats, especially unsaturated fats, are susceptible to sensory effects, which are the product of oxidative rancidity and changes in taste. In the case of saturated fatty acids, they oxidize slowly compared to unsaturated fatty acids [23]. Cavazos-Garduño et al. [24] analyzed the fat and lipid profile of spray-dried human milk, the same used in this work, in which it was found that from 2.08 g/mL of spray-dried milk fat, 41.41% belong to unsaturated fatty acids. It has been described that the presence of this type of fatty acids in the fat will increase oxidation and, the higher the level of establishment, the possibility of fat oxidation will be greater. Finally, the hydrolysis of lipids by microbial enzymes more resistant to the thermal process or by chemical processes (catalyzed by light) is discarded since in the microbiological analyses they did not show growth of them and the

trilaminar bags protected the human milk from the light in all the treatments evaluated.

3.4. Prediction of the Shelf Life of Human Milk Powder through the Arrhenius Model. The shelf life of food is defined as the period between production and the time when the food loses its state of safe and satisfactory quality in terms of nutritional value, microbial state, taste, texture, and appearance. Because the content of proteins and carbohydrates in the different milks analyzed did not present any significant change and also there was no growth of microorganisms that affected the composition of the milks, it was decided to use lipid oxidation as a response variable and as an indicator of quality over time and storage. With the data obtained in the linear regressions of the determinations of the content of free fatty acids, the useful life of human milk powder was calculated at 25 and 40°C, respectively.

Taking the equation of the straight line,

FIGURE 4: Percentage of free fatty acids in human milk powder stored at 25°C (a) and 40°C(b).

$$x = \frac{y - b}{m}. \quad (4)$$

Using 18% of reported free fatty acids as the permissible limit by the CODEX Alimentarius standard for powdered milks [23], and substituting the regression data in the equation, the days of useful life can be calculated for the powdered human milk. For example, the shelf life of human milk powder stored at 25°C would be 243.56 days:

$$x = \frac{(18 - 1.9248)}{0.066} = 243.56 \text{ days}. \quad (5)$$

Likewise, the days of useful life were calculated for all powdered human milk of the different processes at 25 and 40°C (Table 1). The useful life was also calculated using the Arrhenius equation, which allowed us to predict the useful life at different temperatures.

Figure 5 shows a graph of the natural logarithm of shelf life at 25 and 40°C ($\ln t$) against the inverse of the absolute temperature ($1/T$) for milks packed in trilaminar bags. This graph corresponds to the linearization of the Arrhenius model, which is represented by the following expression:

$$t_s = t_0 * e^{-(E_a/R) * [1/T_0 - T_0/T_s]}. \quad (6)$$

The slope and the intercept of the lines allow to calculate the values of the parameters EA and t_0 .

For HMP:

$$\begin{aligned} E_{a\text{HMP}} &= 1264.3 * R = 1264.3 * 1.987207 \frac{\text{cal}}{\text{mol} * K} \\ &= 2512.42 \frac{\text{cal}}{\text{mol} * K}, \\ t_{0\text{HMP}} &= e^{-0.1552} = 0.85. \end{aligned} \quad (7)$$

With these values, we can predict the shelf life of the powdered human milk, in this case, packed in trilaminar bag at any storage temperature. A normal storage temperature could be set around 18°C, and for this temperature, the expected shelf life value for the powdered human milk can be calculated by replacing the activation energy values and the preexponential factor in equation (4):

$$\begin{aligned} t_{18^\circ\text{C}} &= 0.8562439116 * e^{(2512.42581/1.987207) * [1/273.15 + 18]} \\ &= 65.84 \text{ days}. \end{aligned} \quad (8)$$

For HMHP:

$$\begin{aligned} E_{a\text{HMHP}} &= 4085.2 * R = 4085.2 * 1.987207 \frac{\text{cal}}{\text{mol} * K} \\ &= 8118.13 \frac{\text{cal}}{\text{mol} * K}, \\ t_{0\text{HMHP}} &= e^{-8.6068} = 1.828581212 * 10^{-4}, \\ t_{18^\circ\text{C}} &= 8.497231223 * 10^{-4} * e^{(8118.138036/1.987207) * [1/273.15 + 18]} \\ &= 226.89 \text{ days}. \end{aligned} \quad (9)$$

TABLE 1: Days of useful life with respect to lipid oxidation in human milk powder without treatment (HMWT), treated with high hydrostatic pressures (HMHP), pasteurization (HMP), and UV radiation (HMUV), packed in trilaminar bags and stored at 25°C and 40°C.

Human milk powder	Temperature (°C)	Days of useful life
HMWT	25	243.56
	40	122.57
HMUV	25	277.09
	40	154.00
HMHP	25	163.21
	40	84.67
HMP	25	59.46
	40	48.53

FIGURE 5: Graph of $\ln t$ vs. $1/T$ in the evaluated milks stored at 25°C and 40°C.

For HMUV:

$$\begin{aligned}
 Ea_{\text{HMUV}} &= 3656 * R = 3656 * 1.987207 \frac{\text{cal}}{\text{mol} * K} \\
 &= 7265.22 \frac{\text{cal}}{\text{mol} * K}, \\
 t_{0\text{HMUV}} &= e^{-6.6378} = 1.31 * 10^{-3}, \\
 t_{18^\circ\text{C}} &= 3.218258491 * 10^{-4} * e^{((7265.228792/1.987207) * [1/273.15+18])} \\
 &= 372.15 \text{ days.}
 \end{aligned}$$

(10)

For HMWT:

$$\begin{aligned}
 Ea_{\text{HMWT}} &= 4274.5 * R = 4274.5 * 1.987207 \frac{\text{cal}}{\text{mol} * K} \\
 &= 8494.31 \frac{\text{cal}}{\text{mol} * K}, \\
 t_{0\text{HMWT}} &= e^{-8.8413} = 1.44 * 10^{-4}, \\
 t_{18^\circ\text{C}} &= 1.58590509 * 10^{-3} * e^{(8494.316322/1.987207) * [1/273.15+18]} \\
 &= 343.83 \text{ days.}
 \end{aligned}$$

(11)

The results show that the human milk powder with pasteurization treatment (HMP) has a shorter shelf life since, as can be seen in Figure 4, this milk showed the highest percentage of fat oxidation (lipolysis). These results are in agreement with the report made by Ajmal et al. [25], reporting that ultrahigh temperatures treatment induced significant changes in the triglyceride profile of milk cleaving the bond between the fatty acids and glycerol leading to the formation of free fatty acids and later oxidation.

4. Conclusions

From all the previous treatments used to preserve human milk powder, only pasteurization affected the percentage of free fatty acids, which means that this milk has a shorter shelf life when this was evaluated.

For microorganisms, protein, and carbohydrates, no changes were found in the powdered human milk evaluated at different temperatures of storage.

The shelf life for the powdered human milk could be estimated by evaluating the amount of free fatty acids as the quality attribute. Evaluating the effect of storage temperatures outside the refrigeration range is of vital importance for the powdered human milk, especially when it is required to transport this important food for children to distant places with little access, where sometimes there is no electricity to store and preserve human milk.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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