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Evaluation of the Shelf Life at Room Temperature and in the Refrigerator of Infant Flour Made from Senescent Plantain, Soybean, Maize and Fish

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Abstract | **Original Research Article**

Determining the shelf life and best preservation strategy for food products is crucial to guaranteeing their safety and nutritional value. This research aimed to assess the duration of post-weaning food made from senescent plantains, soybeans, maize, and fish may be stored, as well as the most effective means of preserving them. The approach included storing the pre-prepared instant flours at two different conditions: room temperature (30 ±2 °C) and in the refrigerator (4 °C) for a duration of six months. Physicochemical, biochemical, and microbiological studies were conducted on the flours at the time of their production, followed by two-month intervals until the end of the sixth month. The findings indicated that the ash content remained constant throughout the duration of the investigation. Nevertheless, no notable alterations were detected in the pH, titrable acidity, dry matter, protein, and fat composition. There was a clear discrepancy between the start of production and the end of the shelf-life investigation. However, the levels observed at the end of the trial were in line with the established standards for post-weaning baby food. Both during manufacture and at the end of the storage trial, the flours demonstrated good microbiological quality. Flours kept at room temperature had the most nutritional value, but those stored in the refrigerator were devoid of GAM, yeast, and mold starting from the fourth month of preservation. Flours stored in this way maintained their nutritional qualities and were appropriate for newborn nourishment even after six months of storage.

Keywords: Infant flour, food preservation, room temperature, refrigerator, nutritional quality, microbiological quality.

1. INTRODUCTION

According to experts and government agencies, it is recommended to use local agricultural resources to produce highly nutritious infant foods (Gillepsie and Bold, 2017; Martín-Rodríguez *et al*., 2022). This approach aims to promote optimal health and combat infant malnutrition. Therefore, a range of agricultural products are used in food formulations to produce infant flours that have dietary properties that match the nutritional needs of infants aged 6 to 36 months (Assoumou *et al*., 2022, Kouadio *et al*., 2022; Kra *et al*., 2022).

Nevertheless, it is evident that communities are progressively encountering instances of foodborne illnesses that impact both children (Diakite *et al*., 2018) and adults (Guehi *et al*., 2022). Given this circumstance, customers are progressively displaying higher expectations about the quality of the food they are presented with (Houssou *et al*., 2016). According to Sylla *et al*., (2014), the ingestion of tainted food and unclean water leads to around 700,000 fatalities annually in African nations. International institutions have clear standards that state food products must adhere to stringent health, safety, and quality regulations in order to be eligible for entry into the most profitable markets (Humphrey, 2017; Togan, 2024). Producers are recommended to exert maximum effort in guaranteeing the hygienic and commercial excellence of the food products they manufacture. Additionally, they should focus on maintaining the stability of these products, allowing them to be stored for extended periods without

compromising their nutritional, hygienic, and sensory attributes (Houssou *et al*., 2016). Food stability is defined as the length of time it can be kept without deteriorating and becoming unsuitable for eating or use. The maximum suggested duration for storing items while maintaining acceptable quality under predicted circumstances of distribution, storage, and exposure is referred to as the shelf life (Gyesley, 1991; Calligaris *et al*., 2019). A food product that adheres to its designated shelf life must be both safe for consumption and maintain its look, color, texture, taste, and nutritional claims.

Every kind of food undergoes degradation as time passes, but the speed of this process varies across different diets. Food quality may be influenced by several factors, including the development of microorganisms like bacteria, yeasts, and molds, as well as non-microbial spoilage caused by factors like protein, fat, and sugar content. Additionally, product-related deterioration, such as pH and dry matter content, can also impact food quality (Fontana, 2008; Sivasankar, 2010). The storage conditions, whether at ambient temperature or in a refrigerated environment, significantly influence the longevity of food items.

The objective of this research is to determine the length of time and the optimal technique of storing a ready-to-eat infant flour formulated from senescent plantains, soybeans, maize, and fish at ambient temperature (30 ±2 °C) and in the refrigerator (4 °C).

2. MATERIAL AND METHODS

2.1. Material

The material used in this study consisted of senescent plantain (*Musa spp*), soybean (*Glycine max*), maize (*Zea mays*) and horse mackerel fish (*Trachurus trachurus)*.

2.2. Preparation of different flours and infant flour

The soybean and maize grains underwent sorting and washing were thereafter exposed to oven drying at a temperature of 60 °C for a duration of 48 hours. Subsequently, the dehydrated grains were subjected to the process of roasting and pulverization in order to generate flour. Afterward, every variety of flour underwent a sifting procedure and was then placed in airtight glass containers at an ambient temperature of 30 ±2 °C. Then, the fish were deboned and diced into tiny fragments, and then arranged evenly on a platter. Afterwards, the whole specimen underwent a 24-hour drying procedure in an oven. Following this, it was crushed into flour using a blender.

The formulation was prepared by combining 75% of pre-ground senescent plantain pulp with 5% maize flour, 2.5% fish flour, and 17.5% soybean flour in a sequential way. Afterwards, the combination underwent fermentation. Next, the fermented dough was separated into 150-gram pieces and then cooked in an oven for a duration of 30 minutes. The cooked sections were then sliced, dehydrated in the oven at 60 °C for 48 hours, and subsequently transformed into flour using a blender. The flours obtained were subsequently placed in sterile bags in 20-gram quantities for further examination using aseptic techniques.

2.3. Flour preservation procedure

The physicochemical and microbiological properties of infant flours were monitored over a six-month period in the storage study. The flours were kept at two different conditions: room temperature in the laboratory (30 ± 2 °C) and in the refrigerator (4 °C), both in sterile plastic bags. To keep the sterile sachets at room temperature, they were placed on a tray on a bench. The aging test was performed following the AFNOR NF V01003 (2004) for a duration of six months. The purpose was to observe the natural flora in the food and determine its behavior over time in order to provide an appropriate expiration date for the flours. The research specifically examined mesophilic aerobic bacteria, yeasts, molds, and sulfite-reducing anaerobic microorganisms. Additional microorganisms, such as total and fecal coliforms, *Escherichia coli*, Enterobacteria, *Staphylococcus aureus*, and *Salmonella*, were only examined on the first day (day 0), right after flour production. Simultaneously with the aging test, the physico-chemical properties of each sample were assessed just after manufacture and then at two-month intervals. This was conducted to evaluate the metabolic activity of microorganisms that contaminate, namely those that cause spoiling, as well as the natural variation of the product over time and based on storage circumstances.

2.4. Physicochemical and biochemical analysis

The method used to determine water and dry matter content was described by AOAC (2005). pH and titratable acidity were determined using the AOAC (2005) method. Total and reducing sugars were determined from a 5% water-soluble extract prepared in distilled water heated to 60 °C. Total sugars were determined by Dubois *et al*., (2005) using sulfuric phenol, and reducing sugars by Bernfeld (1955) using 3,5-dinitrosalicylic acid (DNS). Fat content was determined by the BIPEA (1976) method using Soxhlet and hexane. The protein content of the samples was determined from the total nitrogen content of the sample according to the Kjedahl method described by AOAC (2005). The ash content was determined according to the AOAC (2005) method using a muffle furnace set at 550 °C.

2.5. Microbiological germ testing protocol

According to ISO 21527-2 (2008), yeast and mold were enumerated on yeast extract glucose agar. Neutral red crystal violet bile lactose agar (VRBL agar) was used to enumerate total coliforms according to ISO 4832 (2006). According to Kornacki and Johnson (2001), neutral red crystal violet bile lactose agar (VRBL agar) was used to enumerate fecal coliforms. Escherichia

coli were detected on McConkey medium with crystal violet and Levine EMB medium according to ISO 21150 (2015). Enterobacteriaceae enumeration was performed according to ISO 21528-2 (2017) using VRBG (Violet-Red-Bile-Glucose) medium. The total aerobic mesophilic flora was enumerated using PCA (Plate Count Agar) in accordance with ISO 4833-1 (2013). Staphylococcus aureus enumeration was performed according to ISO 6888-1 (1999) using Chapman agar. Anaerobic sulfite-reducing (ASR) flora were enumerated according to ISO 15213 (2003) using the deep incorporation technique. The process of finding Salmonella in flour followed the steps outlined in ISO 65791-1 (2017). These steps were a pre-enrichment step in peptone water, an enrichment step on Rappaport-Vassiliadis Soja (RVS) medium, and an isolation step on XLD selective medium.

2.6. Statistical analysis

Windows Excel 2013 software was used to calculate means, standard deviations, and plots. Three copies of each experiment were done, and descriptive analysis was done on the data using Duncan's multiple test (DMRT) and Student's t and z tests for two separate samples with Addinsoft software (X LStat version 2016). For both tests, the differences were significant at the P < 5% probability level. Hierarchical Ascending Classification (HAC) and Principal Component Analysis (PCA) were also used to determine the best flour storage method.

3. RESULTS

3.1 Physical characteristics of flour during storage

Figure 1 depicts the changes in the pH, titrable acidity, and dry matter of flours made from senescent plantains, soybeans, maize, and fish over time when stored at ambient temperature and in the refrigerator. The statistical study indicated that the pH values of the flours held for a duration of six months varied between 5.30 and 5.50 at ambient temperature and between 5.33 and 5.50 while stored in the refrigerator (Figure 1a). In addition, the titrable acidity levels of the flours held for six months varied from 60.47 to 24.00 meq/100 g DM at ambient temperature and from 38 to 48.67 meq/100 g D.M. in the refrigerator (Figure 1b). In this study, the pH and titrable acidity values were found to be higher than those reported in the flour production step. Specifically, the pH value was 5.15, and the titrable acidity content was 34.47 meq/100 g D.M. Moreover, these values exhibited significant variations over the storage period at ambient temperature and in the refrigerator. In terms of the heating process, the statistical analysis revealed that there was no significant variation in the pH values of the flours kept between the second and fourth months. However, after six months, the flours kept in the refrigerator showed a reduced acidity level of 5.40, in contrast to the flours held at ambient temperature, which had an acidity level of 5.30. Concerning the titratable acidity, flours that were kept at ambient temperature had elevated titratable acidity levels throughout the second and fourth months, but those stored in the refrigerator showed the greatest values during the sixth month.

Statistical analysis revealed that there was no significant difference (P > 5%) in the dry matter content of the flours held at room temperature (Figure 1c). The composition ranged from 88.53% to 92% over the period from manufacture to the sixth month. However, there was a notable disparity (P < 5%) in the dry matter content of the flours that were kept in the refrigerator. The percentages for the flours from the day of production to the second, fourth, and sixth months of storage were 88.53%, 85.00%, 93.20%, and 93.40%, respectively. It is important to mention that the dry matter contents in the fourth and sixth months are larger compared to the second month.

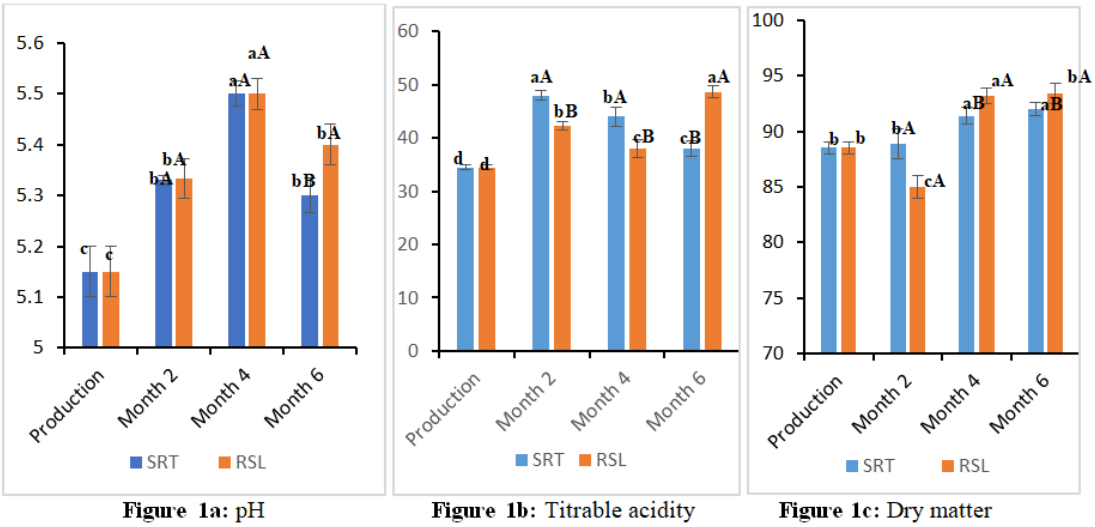

Figure 1a: pH **Figure 1b:** Titrable acidity **Figure 1c:** Dry matter

Figure 1: Physical characteristics of flours stored at room temperature and in the refrigerator. Histograms with the same letter (a, b, c, etc.) in lower case are not significantly different P>5% for the same parameter. Also, those with the same letter (A, B, C, etc.) in capital letters on the same month are not significantly different P>5% for cooking modes

SRT: Shelf-life at Room Temperature; RSL: Refrigerator Shelf-Life

3.2. Biochemical characteristics of flours during storage

Table 1 presents the biochemical properties of flours stored at ambient temperature and in refrigerator conditions. Statistical analysis indicates that there was no significant difference (P > 5%) in the ash contents of the room temperature and refrigerated flours over the whole storage period. Their statistical measurements were indistinguishable at the start and end of the storage test (4.00 ±0.69%). The ash contents of the flours held at room temperature were 4.20 ±0.20%, 4.20 ±0.40%, and 4.27 ±0.12% during the second, third, and fourth months of storage, respectively. The ash contents of the flours held in the refrigerator were 4.20±0.40%, 4.00±0.20%, and 3.73±0.12% during the second, third, and fourth months of storage, respectively. The ash content of flour held at ambient temperature was shown to be much greater than that of flour stored in a refrigerator. Over the six-month studies, there was no significant difference in the fat content of the different flours, regardless of the storage technique used. The flours' fat content varied from 14.00 ±1.73% to 11.00 ±0.33% over the six-month period of storage at room temperature, starting from the date of manufacturing. The flours held in the refrigerator showed a variation in their contents, ranging from 14.00 ±1.73% to 11.17 ±0.44%, over the period from the date of the production to the sixth month of storage. The statistical analysis reveals a notable disparity in protein content across different months for the various flours maintained at ambient temperature and in the refrigerator. Nevertheless, the flours kept at ambient temperature consistently displayed the highest amounts during the entire storage period, as opposed to those stored in the refrigerator. The amounts of reducing and total sugars displayed significant variations throughout the trial, from the initial preparation stage to the storage periods at room temperature and in the refrigerator. However, the flours kept at ambient temperature consistently had the highest amounts of reducing sugars throughout the trial, in contrast to the flours placed in the refrigerator.

Table 1: Biochemical properties of flours stored at ambient temperature and in the refrigerator

Storage time / Parameters		Production	Month 2	Month 4	Month 6
Ash	**SRT**	4.00 ±0.69^{a}	4.20 ±0.20^{aA}	4.20 ±0.40^{aA}	4.27 ±0.12^{aA}
	RSL	4.00 ±0.69^{a}	4.20 ±0.40^{aA}	4.00 ±0.20^{aB}	3.73 ±0.12^{aB}
Fat	**SRT**	14.00 ±1.73^{a}	12.83 ±0.58^{bA}	11.67 ±0.58^{cA}	11.00 ±0.33^{cA}
	RSL	14.00 ±1.73^{a}	12.67 ±0.15^{bA}	13.00 ±0.34^{aA}	11.17 ±0.44^{cA}
Protein	**SRT**	19.68 ±0.04^{d}	22.22 ±0.57^{bA}	21.00 ±0.70^{cA}	23.33 ±0.46^{aA}
	RSL	19.68 ±0.04^{d}	22.94 ±0.02^{aA}	20.94 ±0.02^{cA}	21.64 ±0.52^{bB}
Total sugars	**SRT**	64.49 ±0.80^{b}	63.43 ±0.46^{bA}	46.66 ±0.24^{cA}	68.21 ±0.09^{aA}
	RSL	64.49 ±0.80^{a}	36.09 ±0.93^{cB}	35.22 ±0.20^{cB}	61.20 ±0.09^{bB}
Reducing sugars	**SRT**	74.53 ±0.63^{a}	35.44 ±0.89^{bA}	32.41 ±0.05^{cdB}	32.87 ±0.04^{cA}
	RSL	74.53 ±0.63^{a}	27.62 ±0.49^{cB}	33.48 ±0.05^{bA}	21.75 ±0.05^{dB}

Values with the same letter (a, b, c, etc.) in lower case are not significantly different P>5% for the same parameter. Also, those with the same letter (A, B, C, etc.) in capital letters on the same month are not significantly different P>5% for cooking modes. SRT: Shelf-life at Room Temperature; RSL: Refrigerator Shelf-Life.

3.3 Microbiological properties of flour during the production

Table 2 displays the microbiological characteristics of the flours made from senescent plantains, soybeans, maize, and fish throughout the production process, before being stored at ambient temperature and in the refrigerator. The microbiological analysis revealed that the flours were devoid of *Enterobacteriaceae*, *total coliforms*, *Escherichia coli*, and *anaerobic sulfuto-reducers*. *Staphylococcus aureus* and *Salmonella typhimurium* were not present. The *Mesophilic Aerobic Germs* (MAG) were loaded with 5.36.102 ±0.05 CFU/g, according to the findings. The yeast and mold count was 636.36 ±0.06 CFU/g.

Table 2: Microbiological characteristics of infant flours during production stage

Germs	Load (CFU/g)	Microbiological criteria (CFU/g)*
Mesophilic aerobic germs (g)	$5.36.10^{2}$ ± 0.05	10^{5}
Yeasts and molds (g)	636.36 ± 0.06	10^{3}
Enterobacteriaceae (g)	00 ± 0.00	Trace
Total coliforms (g)	00 ± 0.00	Trace
Escherichia coli (g)	00 ± 0.00	10
Anaerobic sulfuto-reducers (g)	00 ± 0.00	Absence
Staphylococcus aureus (g)	Absence	Absence
Salmonella (g)	Absence	Absence

*****CODEX STAN, (1995)

3.4. Variation in mesophilic aerobic germs, yeasts, and molds in room-temperature and in refrigerator flours

Table 3 displays the fluctuations of GAM, yeasts, and molds in flours held under ambient conditions and in a refrigerator. The statistical analysis reveals that the GAM load remained consistent throughout the trial, irrespective of the storage technique, except during the second month, when the load was very high. The GAM loadings varied between 2.34 ±0.43 and 3.96 ±0.36 for flour held at ambient temperature and between 2.44 ±0.44 and 3.30 ±0.76 for flour stored in the refrigerator. According to the statistical study, in terms of storage mode, there is no significant difference in the loads of room temperature and refrigerated flours during months of production, month 2, and month 4. Nevertheless, the sixth month showed elevated levels of flour in the refrigerator.

Statistical analysis revealed that the yeast and mold loads were consistent for sprouts held at room temperature from month 0 to month 4, and for flours stored in the refrigerator from month 0 to month 2. Nevertheless, there were no signs of sprouting in month 6 for flour kept at ambient temperature and in months 4 and 6 for flour stored in the refrigerator.

Table 3: Variation in GAM, yeast and mold levels

Storage time / Parameters		Production	Month 2	Month 4	Month 6
GAM (Log(UFC))	**SRT**	2.73 ±0.16^{bA}	3.96 ±0.36^{aA}	2.93 ±0.50^{bA}	2.34 ±0.43^{bB}
	RSL	2.73 ±0.16^{aA}	3.30 ±0.76^{aA}	3.16 ±0.44^{aA}	2.44 ±0.44^{aA}
yeast and mold (Log(UFC))	**SRT**	2.80 ±0.36^{aA}	2.96 ±0.53^{aA}	2.66 ±0.66^{aA}	ND
	RSL	2.80 ±0.36^{aA}	2.66 ±0.37^{aB}	ND	ND

Values with the same letter (a, b, c, etc.) in lower case are not significantly different P>5% for the same parameter also, those with the same letter (A, B, C, etc.) in capital letters on the same month are not significantly different P>5% for cooking modes. SRT: Shelf-life at Room Temperature; RSL: Refrigerator Shelf-Life.

3.5. Impact of preservation method on flour shelf life

The impact of the storage method on the flours was assessed by hierarchical ascending classification (Figure 2) and principal component analysis (Figure 3). The examination of Figures 2a, 2b, and 2c reveals a notable disparity in the similarity threshold between flours maintained at ambient temperature and those stored in the refrigerator during the second, fourth, and sixth months. Furthermore, the quality of the flour held during the second, fourth and sixth months differs greatly from that of the freshly produced flours. The principal component analysis confirms a substantial disparity, as seen in Figures 3a, 3b, and 3c. These figures depict the distribution of variables based on axes 1 and 2, as well as the other research parameters. A biplot analysis reveals a positive correlation between storage at ambient temperature and the majority of the parameters examined, whereas refrigerated storage exhibits a negative correlation.

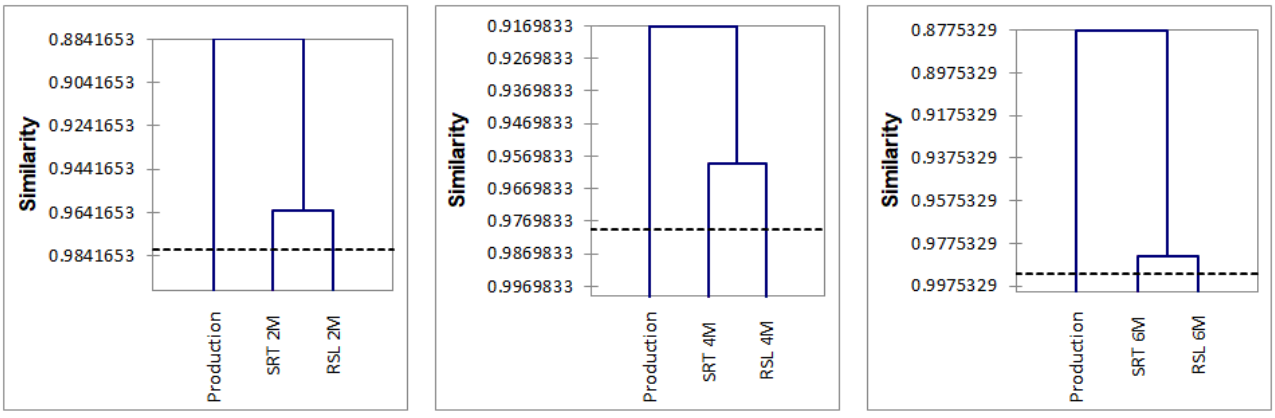

Figure 2a: Month 2 **Figure 2b**: Month 4 **Figure 2c**: Month 6

Figure 2: Similarity between preserving methods, production stage and physicochemical, biochemical and microbiological parameters

SRT: Shelf-life at Room Temperature; RSL: Refrigerator Shelf-Life.

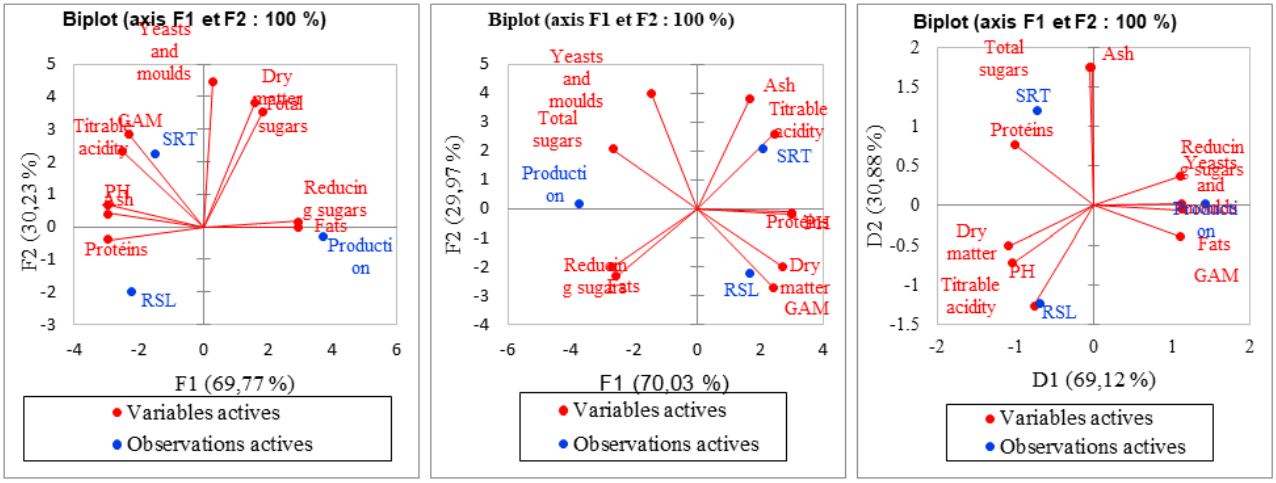

Figure 3a: Month 2 **Figure 3b**: Month 4 **Figure 3c**: Month 6

Figure 3: Principal component analysis of infant flours stored at room temperature and refrigerated for six months

SRT: Shelf-life at Room Temperature; RSL: Refrigerator Shelf-Life

DISCUSSION

The flours stored at room temperature and refrigerated for six months had a less acidic pH compared to their production pH. This variation in pH during storage could be explained by the fact that the flours underwent fermentation caused by their natural microflora and the preparation medium, on the one hand, and by chemical changes in the food, on the other hand (Sanogo *et al.*, 2022). According to Rong (2007) and Fontana (2008), pH is a factor that determines whether a food's microflora dies or stays alive. This is because an acidic pH does not help pathogens like Enterobacteriaceae and Aspergillus grow, which are linked to diarrheal diseases in children. The optimal pH for the growth of Enterobacteriaceae is between 6.0 and 8.0 (ICMSF, 1996). In addition, highly acidic infant food can lead to infant intolerance and facilitate the selection of acidophilic bacteria such as E. coli in the gut (Ahmia *et al.*, 2008). At the end of the study, the pH was 5.3 for flours stored at room temperature and 5.4 for those stored in the refrigerator. The relatively low pH of the samples at the end of the storage period means that microbial activities in the foods were reduced, which helped to extend the shelf life of the samples.

It was observed that the titrable acidity of the different samples increased during the six months of storage compared to their initial values at the time of production. These high titrable acidity levels could be explained by microorganisms using carbohydrates to produce large amounts of organic acids, such as citric, lactic, malic, etc., which are responsible for the flavor of the food (Tyl and Sadler, 2017). After six months, the flours stored in the refrigerator had the highest titrable acidity values. This high level suggests that these flours are the richest sources of organic acids. This high titrable acid content aids in understanding the inhibition of mesophilic aerobic germs, yeasts, and molds during the sixth month of flour storage, regardless of the storage mode.

Up to the sixth month of storage, the flours' dry matter content ranged from 85% to 94% of production. However, the amount of dry matter (93.40%) in the sixth month of refrigerated flours was nearly equivalent to 95%, which is the minimum standard recommended by the FAO/WHO (1991) for infant food preparations. This standard says that there should be a minimum content of 95% to stop the growth of microbes in post weaning foods and the loss of organoleptic qualities. As a result, the high dry matter content and relatively acidic pH value at the sixth month of storage in the refrigerator suggest that these two parameters may serve as an effective barrier against microbial proliferation and the preservation of the flours' organoleptic properties. Nevertheless, the relatively high dry matter content at the fourth and sixth months indicates that this parameter could be enhanced during the infant food production process to align with the standards recommended by the FAO/WHO (1991).

The ash content of a sample is a parameter that is positively correlated with the mineral content (Sika *et al.*, 2019). Thus, the higher the ash content, the higher the mineral content. As a result, it is necessary to determine the ash content of foods in order to assess whether they meet the body's mineral requirements. The findings indicate that the ash content of flours stored at room temperature and in the refrigerator showed no significant variation from the production stage to the end of the sixth month of storage. This lack of significant difference means that storing our flours for six months has no negative effect on their mineral content. Flours stored at room temperature had a higher ash content than those stored in the refrigerator. However, the ash content of these flours complied with the recommendations of the FAO/WHO (1991), whose maximum limit is 3%. As a result, flours preserved using these two methods remain excellent sources of minerals for the growth of children who consume them for six months.

The results show a decrease in fat content by the sixth month of 21.43% and 20.21% for storage at room temperature and in the refrigerator, respectively. This reduction in fat content, which resulted in significant differences between the different storage times, could be explained by fat oxidation, which leads to the formation of volatile odor compounds in preserved foods (Genot and Michalski, 2010). Despite this decrease, the fat content of flours stored for six months was consistent with the FAO/WHO's (1991) recommendation of between 10 and 25%. This compliance with the standard suggests that the consumption of these six-month-preserved flours can fully play the role predestined for them. The lower fat content of these flours observed over six months in no way alters their nutritional qualities, as fats are essential food components for maintaining the structure of cell membranes. They contribute enormously to the body's energy needs, the proper functioning of cellular reactions, and the intracellular transport of fat-soluble vitamins to the target organs.

With regard to protein content, the results showed significant variations, with very high levels in the sixth month. These levels are higher than the minimum recommended by FAO/WHO (1991), which is 15%. Protein is essential for normal growth, body development, and general tissue repair. Protein deficiency in children leads to poor growth, kwashiorkor, liver, and brain damage (Lawal and Adedeji, 2013). Therefore, a high amount of protein is needed during early childhood, as this is the period when protein is most required for rapid growth and maintenance of a healthy body.

Total sugars are carbohydrates that release reducing sugars during acid and heat hydrolysis. The results of our study show that flours preserved for six months are good sources of carbohydrates, as the content exceeds 64%, which is the minimum content recommended by FAO/WHO (1991) for infant food. According to recommended standards, the sugar contents of infant flours made from senescent plantains, soybeans, maize, and fish suggest that storage of flours for six months has no negative impact on the sugar content.

Mesophilic aerobic bacteria, yeasts, and molds were present in the flours during production, but their levels were below the recommended microbiological limits of 10^5 CFU/g for mesophilic aerobic bacteria and 10^3 CFU/g for yeasts and molds. This compliance could be explained by the relatively high dry matter content and low pH values. Total coliforms, Enterobacteriaceae, and *Escherichia coli* were not detected in any of the samples taken during flour production. Therefore, we can conclude that the hygienic conditions established during the production of infant food were beneficial. In addition, the flours are free of Salmonella, sulfite-reducing anaerobes, and *Staphylococcus aureus*. This leads to the conclusion that the flours are of satisfactory microbiological quality during production. In fact, flours must be prepared under hygienic conditions and then stored under hermetic conditions in a clean, dry room, protected from darkness, in order to protect them from contamination and prevent the growth of pathogenic germs.

Over the shelf life, monitoring of AMG, yeasts, and molds showed a decrease in their levels until they disappeared completely from infant flours. However, the levels of yeasts and molds are below the recommendations of CODEX STAN (1995). It is worth noting that the variation profile of GAM, yeasts, and molds during shelf life was positively correlated with variations in pH and temperature of the different flours. Thus, the disappearance of microorganisms could be explained by the pH and temperature conditions during storage. The results of this study are comparable to those of Houssou *et al.*, (2016), who observed a decrease in the load of AMGs and molds until their disappearance in their infant flours after 9 months of storage. The results also corroborate those of Adjadogbedji (2017), who obtained GAM loads consistent with our studies after 6 months of storage. The flours stored in the refrigerator showed excellent microbiological potential due to their absence of yeasts and molds from the fourth month and their GAM content in line with the Codex recommendations for couscous (CODEX STAN, 1995). The significant difference observed in the hierarchical ascending classification shows that room temperature and refrigerated storage have different effects on flours during storage. However, the positive correlation between ambient storage and the parameters studied suggests that this method should be considered for optimal infant flour storage.

CONCLUSION

This study aimed to evaluate the shelf life of infant flour stored at room temperature and in the refrigerator in order to determine the best before date. The results showed that storing the flour at room temperature and in the refrigerator for six months did not affect its physicochemical and biochemical characteristics. From a microbiological point of view, the stored flours were of satisfactory microbiological quality. Therefore, their consumption is safe for children aged between 6 and 36 months. The flours can therefore be consumed within six months of production. Storage at room temperature appears to be the best way to preserve infant food's physicochemical and biochemical characteristics. Once the sanitary quality has been verified and the duration and method of storage determined, we can proceed to another aspect of this study, namely the verification of the bioavailability of the nutrients, an important aspect for the body's assimilation.

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Conflict of interest: None

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