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Shelf-Life Assessment of Pre-Fried and Unfried Corn Empanadas Cold Stored

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Abstract

Around 12 million corn empanadas (a type of fried patty) are eaten per day in Colombia (South America); nevertheless, there is no record of the shelf-life of this product under cold storage (refrigeration). Due to their high humidity and high content of carbohydrates, empanadas are susceptible to deterioration by microorganisms. Two hundred and ten samples of pre-fried and not-fried empanadas were evaluated for three weeks. The behavior of the water activity (a_w), pH, and the mesophilic aerobic microorganisms, molds, and yeasts were evaluated during storage time. The probability of survival was calculated by a Kaplan-Meier estimator to determine the microbiological stability of the empanadas. The main results show a microbiological shelf-life estimated in three days for a non-fried empanada and six days for a pre-fried empanada. The deterioration caused was mainly associated with the mesophilic aerobic microorganisms, which also impacted the reduction of pH.

Keywords: corn dough, maize, survival analysis, conservation methods, *Zea mays*

1 Introduction

Corn (*Zea mays*) is cultivated in most countries of the world, being the third most important crop after wheat and rice. The intake in Latin-American countries has increased due to its nutritional properties, carbohydrate contents, proteins, and lipids, which perform an essential function in human nutrition [10]. For these characteristics, corn becomes a primary raw material for the preparation of several products in Latin-America such as tortillas, arepas, humitas, alcoholic drinks, and

empanadas. Corn empanada is a Colombian snack-type food, made from corn dough obtained from wet grinding of maize, stuffed with protein or non-protein filler, which is frying at high temperatures [13]. Because it is a product with high humidity, it is susceptible to deterioration by microorganisms [12]. The deterioration processes in food are generated by physical, chemical, or microbiological factors that impact the product's shelf-life and food safety. The World Health Organization (WHO) points out that in underdeveloped countries, foodborne illnesses represent a leading cause of illness and death, and is an impediment to socioeconomic development [24]. According to reports provided by the National Institute of Health of Colombia until week 51 of 2018, 11502 cases were notified, including 881 reported outbreaks, 49.8% more than in 2017 [17]. The corn empanada is a food of high consumption in Colombia, therefore maintaining its safety is key to avoiding foodborne disease in consumers.

Refrigeration is usually the most suitable preservation method to increase the shelf-life of this type of product without affecting the food's characteristic properties [19]. Currently, there are few studies on this type of Colombian food. Hernández, Castañeda Peláez and Castro-Ríos [13] conducted a study to determine the impact of refrigeration on the texture of unfried corn dough, unfried empanada, fried empanada, and pre-fried empanada, stored in freezing, ultra-freezing, and refrigeration; it was observed that the texture increases with frying time and decrease with the storage time. Also, there is research that evaluates the effect of the vacuum frying (temperature and frying times) on the bromatological and sensory properties of corn empanadas stuffed with meat, concluding that the best temperature was 120 °C for sensory properties and 140 °C for bromatological characteristics [7]. Also in the same product, was evaluated the effect of vacuum frying on the oil absorption of corn empanadas, it was observed that a longer time (120 s) and lower frying temperature (115 °C) increase oil absorption, impacting food quality [1]. There are studies regarding the determination of the shelf-life of the egg-stuffed arepa (similar corn product) through accelerated tests and by increasing the peroxide index, indicating that it increases with the temperature and the frying time due to the accumulation of primary oxidant compounds [2]. These work developed have mainly focused on the effect of frying on different parameters, but does not establish the impact of cold storage on the quality of the empanada, for this reason, the objective of this work was to evaluate the effect of refrigeration on the microbiological survival estimation probability of the unfried and pre-fried Colombian corn empanada.

2 Methodology

2.1 Samples preparation

A quantitative experimental study was developed for the determination of the microbiological shelf-life of cold storage corn empanada. These were made in the CM06B machine of Maquiempanadas company located Manizales (Colombia), with white corn dough and with a protein-free filling, composed of potato, onion,

salt, vinegar, and soybean oil. The pre-fried empanadas were fried in soy oil at a temperature of 180 °C for 3 min. They were subsequently cooled at room temperature for 10 min and packed in Ziploc-type low-density polyethylene bags (Figure 1). The storage temperature was 5.19 ± 0.79 °C. All the physicochemical and microbiological analyses were performed at the same time. For the physicochemical analysis, 3 units of unfried and pre-fried empanada were used for a total of 14 samples. For the microbiological analysis, 15 units of unfried and pre-fried empanada, for a total of 210 samples. The analysis was performed every three days for three weeks in a row.

Figure 1. Pre-fried corn empanadas.

2.2 Microbiological and physicochemical analyses

A plate count method was used according to the NTC 4491-1 [14] criteria, 10 gr of each sample was taken in 90 mL of peptone water (Oxoid), then it was homogenized in a stomacher (Seward 400, Lab System). Serial dilutions were made by transferring 1 mL aliquots to test tubes with 9 mL of peptone water solvent (Scharlau) until obtaining the required consecutive dilutions according to the conditions of the product. After that, 1 mL of each dilution was spread in sterile Petri dishes adding the culture medium at 45 °C. The Plate Count agar (Scharlau) was used for the count of aerobic mesophilic bacteria, and the Rose Bengal agar with chloramphenicol (Scharlau) for the enumeration of mold and yeast. The microbiological samples were incubated at 35 ± 2 ºC for 48 h for aerobic mesophilic bacteria analysis and 30 ± 2 ºC for 5 days for fungi analysis. When the incubation time elapsed, the colonies of aerobic mesophilic bacteria, molds, and yeast were counted following the standards criteria [16]. The Potentiometric technique was employed to determine the pH of the samples (SIAnalytics Lab 855 pH). The water activity (a_w) of the food was quantified through an a_w meter (Schaller 3115, mod RH2 + AW- Wert).

2.3 Statistical Analysis

The data obtained were analyzed through the statistical package SPSS version 24. A comparison of t-student means was used to indicate differences among the count of microorganisms in storage time. Through non-parametric statistical analysis, the survival probability was calculated using a Kaplan-Meier estimator for the occurrence time of the determined phenomena. The error bars in graphics represent standard deviation (SD) of the mean values.

3 Results and discussion

Based on the analysis performed on the unfried and pre-fried corn empanada, during the storage stage under refrigeration at an average temperature of 5.19 ± 0.79 °C, the microorganism population and the physicochemical properties were established throughout the three weeks (18 days). Statistical differences ($p<0.05$) were identified among the count of microorganisms analyzed for the unfried and pre-fried product.

In figure 2, the effect of the storage under refrigeration over the mesophilic bacteria population growth can be observed; this was higher in the unfried than in the pre-fried corn empanada. This reduction is related to the thermal destruction of the microorganisms and the reduction of the a_w on the surface of the empanada [18]. It can be seen that the number of mesophilic bacteria for day 1 was 6.75 Log_{10} CFU/g and 9.46 Log_{10} CFU/g in the day 18 of cold storage, almost two times of the obtained in the pre-fried empanada. The results were similar to those obtained in a study performed on nixtamalized corn dough, where counts of $3.5x10^4$ y $8.3x10^7$ CFU/g, were obtained, considering that this type of food has acidic pH values, the microbial profile is mainly composed by lactic acid bacteria and non-lactic mesophilic bacteria, and mold and yeast count [3]. Moreover, pre-fried empanada presented a constant increase in the mesophilic bacteria behavior, with values oscillating between 3.39 Log_{10} CFU/g on the first day to 5.68 Log_{10} CFU/g in the 18 days of cold storage, without showing differences statistically significant ($p >0.05$). These results show that the unfried empanada is more susceptible to have a higher microbial load, which will quickly increase during the storage under refrigeration, in comparison with the pre-fried empanadas. The high temperatures used in the frying process (between 160 and 190 °C), can inactivate some of microorganisms and enzymes, due to the fast heating of the food in its external part (around 100 °C) and its internal part, reaching temperatures higher than 80 °C [9]. Additionally, the thermal stress affects the expression of relevant genes in the primary and secondary metabolism processes, as well as in the ones associated with the osmotic protection and proteins that favor the resistance to oxidative stress, representing a microbial survival predominant factor [20].

Figure 3 shows that the mold population in the unfried empanada increased during storage time, with a variation of 1.77 Log_{10} CFU/g during the first day to 4.77 Log_{10} CFU/g during the last day of cold storage, while in the pre-fried empanada, the molds remained stable in storage with an average of 1.05 ± 0.23 Log_{10} CFU/g. There were no statistically significant differences ($p >0.05$) for the microbial count at cold storage between the days of analysis. Although the number of molds was low, they may remain viable in the food, which has been previously processed for a long time due to the formation of spores or toxins that subsist in hostile environments. Nevertheless, the role of fermentation must be considered an influence in the inhibition of this type of microorganisms' growth and can impact the deterioration processes [22]. The spores of some environmental fungi can also pollute the product during the cooling and packaging process, among which some

resistant structures can survive to determined thermal processes [11].

In figure 4 it can be observed that the population of yeast in the unfried empanada, was 1.15 Log_{10} CFU/g at day 1 and 5.95 Log_{10} CFU/g at day 18 of cold storage, with a statistically significant difference ($p < 0.05$) for the microbial count between the days of analysis, these results can be compared with research in cornflour microbiological profile, where the yeast count was 3.91 and 3.62 Log_{10} CFU/g [22]. In contrast, in the pre-fried empanada, the initial number of yeast was similar to that obtained in the unfried empanada, with an average value of 1.38 ± 0.76 Log_{10} CFU/g. There were only differences statistically significant ($p<0.05$) between the first and last day of cold storage analysis; this behavior could be related to water activity and enzyme destruction at adverse conditions.

Regarding the pH, both corn empanadas showed a decrease of values, between 5.6 and 4.8 for unfried empanadas and 5.9 and 5.4 for pre-fried empanadas, being more pronounced on the unfried empanada, associated with corn spontaneous fermentation processes. In the case of unfried empanada, a reduction of pH, an increase of microorganisms, and a white layer with an unpleasant odor during refrigeration storage time was observed. These can be related to the corn spontaneously fermentation processes, the enzymatic activities of the diverse microbial consortium that occur, and can be related to microorganisms like *Lactobacillus* spp., acetic bacteria, and yeasts that reduce quality and originate off-flavors in the corn dough [8].

The a_w for both types of empanadas increased at the end of cold storage; however, during the first 6 days, the a_w increased in the pre-fried empanada (0.79 to 0.94) and decreased in the unfried empanada (0.70 to 0.61). The pre-fried empanada has a crust that promotes the exchange of humidity to the interior and the crust, increasing the a_w inside the empanada; however, the a_w stabilized with time at an average value of 0.85 ± 0.06. The unfried empanada does not have a crust generated by the frying process; therefore there is a direct interchange between the package, the refrigerator and the interior of the corn empanada that promotes the dehydration reflected in the reduction of a_w during the first days of storage, with a posterior stabilization with an average value of 0.70 ± 0.07. The results suggest the importance of stabilization of a_w between the crust and interior of the empanada [4] and the permeability of the packaging film [25].

The microbiological criteria for survival analysis selected were the highest microbiological values considered according to the national standard for refrigerated corn arepas since they are the only standard in Colombia similar to the tested product [15]. The highest values are 3 Log_{10} CFU/g for molds and yeasts and 4 Log_{10} CFU/g for mesophilic aerobic microorganisms.

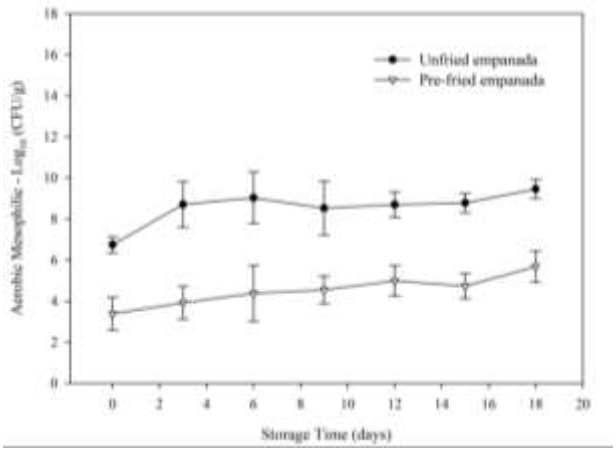

Figure 2. Aerobic mesophilic behavior under refrigeration of an unfried and pre-fried corn empanada.

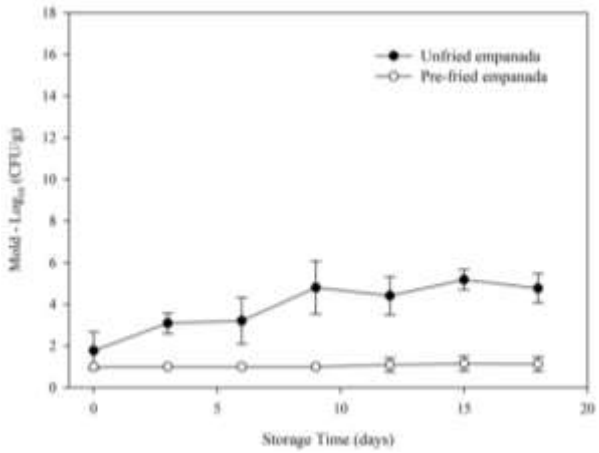

Figure 3. Mold behavior under refrigeration of the unfried and pre-fried corn empanada.

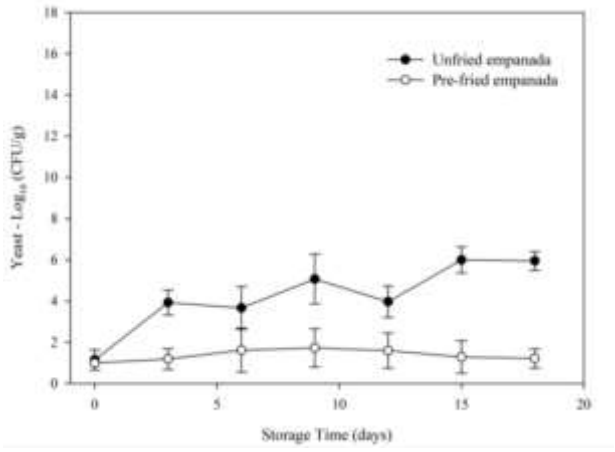

Figure 4. Yeast behavior under refrigeration of the unfried and pre-fried corn empanada.

As seen in figure 5, the survival curve shows that there is a 90% probability that the unfried empanada "survives" or "does not fail" during storage under refrigeration until the third day for a mesophilic aerobic population ≥ 6 Log_{10}, for the case of molds and yeasts (data not shown), the estimated fault time for a molds and yeasts population ≥ 2 Log_{10}, would be 3 days with a 90% probability. It is important to remark that the shelf-life of the product can be influenced not only by the type of contaminant population but also by the existing thermic variability even among fungal species which belong to the same gender, as demonstrated with molds of the gender *Byssochlamys*, which can be differentiated regarding the formation of ascospores, which are inactivated in a lower degree by thermal sub-lethal treatment [23].

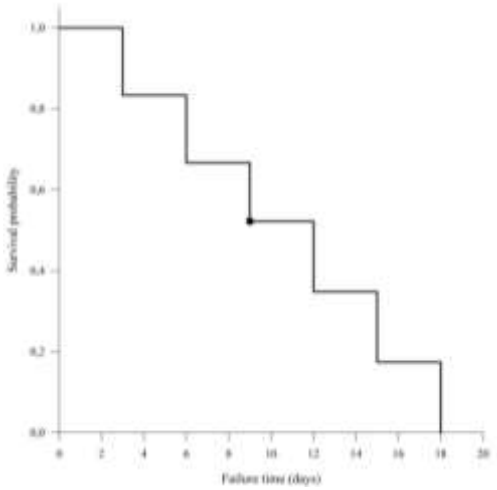

Figure 5. Survival curve of unfried corn empanadas in the presence of mesophilic aerobic bacteria (6 Log_{10}).

As observed in figure 6, the survival curve indicates that there is a 90% probability that pre-fried corn empanadas "survives" or "does not fail" during the storage under refrigeration in day 6 for a population of mesophilic aerobic ≥ 5 Log_{10}. For molds and yeasts (data not shown), the estimated shelf-life for a population of molds and yeasts ≥ 2 Log_{10}, would be 18 days and 8 days accordingly. Considering that the risk to health is high, the recommendation for storage under this study's conditions would be 6 days under refrigeration for pre-fried empanadas.

Refrigeration reduces the development of microorganisms and delays some reactions involved in food deterioration [5]. However, it does not avoid the growth of microorganisms; because it provides necessary conditions to favor the preservation of innocuous conditions of the corn empanada in short storage times for the consumer. The frying process has an effect on the partial destruction of the microorganisms and inactivates some enzymes; nevertheless, the effect on the shelf-life will be limited by the internal humidity after the frying process [9].

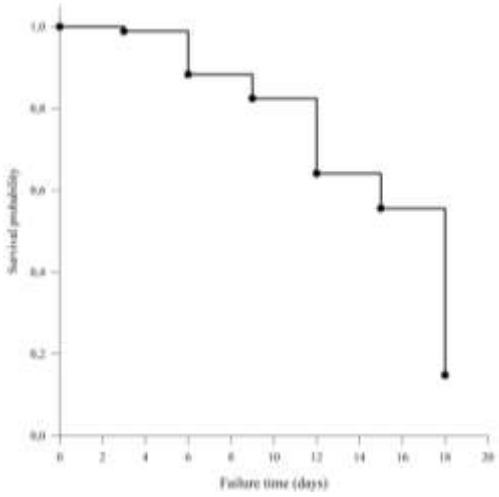

Figure 6. Survival curve of pre-fried corn empanadas in the presence of mesophilic aerobic microorganisms (5 Log_{10}).

Corn dough of empanadas have high humidity (greater than 60%) [12]; this aspect limits the shelf-life of the cold stored product unless it is complemented with a different chemical or physical preservation method. Similar results were observed in a study performed within a fried corn dough stuffed with egg; the shelf-life was estimated within 0.9 to 1.6 days, using the accelerated method [2]. Other studies in a corn dough (arepa), showed that their microbiological stability could be improved to 30 days by the effect of the ascorbic acid and the refrigeration storage [6]. Likewise, the use of a modified atmosphere packaging and a polymeric packaging allows a shelf-life of 9 days at a temperature of 25 °C and 55 days under refrigeration at 5 °C [21].

4 Conclusion

The cold storage with refrigeration of pre-fried empanadas increases their shelf-life up to 6 days, compared with the unfried empanadas, which had a shelf-life of 3 days. The refrigeration process, along with the frying process, improves the microbiological preservation of corn empanada. During storage, the pH decreased for the unfried and pre-fried empanada, which is an indicator of the empanada degradation, nevertheless it was slower for the pre-fried empanada due to the reduction in the number of microorganisms after the fried process.

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