AREPAS LISTAS PARA COCER DE LARGA VIDA ÚTIL A TEMPERATURA AMBIENTE. ESTUDIO DE RETO MICROBIOLÓGICO

READY TO COOK AREPAS OF LONG SHELF-LIFE AT ROOM TEMPERATURE. II. MICROBIOLOGICAL CHALLENGE STUDY

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RESUMEN

Las arepas son una parte importante de la dieta de venezolanos y colombianos. La introducción en el mercado de una arepa lista para cocinar de larga duración a temperatura ambiente seria de gran ventaja. Se realizaron estudios para determinar la vida útil de una arepa lista para cocinar desarrollada en el laboratorio. Se llevó a cabo un estudio de reto microbiano para determinar la estabilidad microbiológica de las arepas listas para cocción. Para ello, se evaluaron después de siete días de almacenamiento a temperatura ambiente, muestras de arepas inoculadas con flora bacteriana nativa previamente aislada de la masa almacenada durante 30 días a 28 ± 2 °C tratadas con tres barreras antimicrobianas (tratamiento térmico, tipo y concentración de aditivo y empacado al vacío). Como resultado se obtuvo un prototipo de arepas listas para cocción, con posibilidad de almacenamiento de siete días a temperatura ambiente y quince bajo refrigeración, similares a las elaboradas en casa con una completa caracterización microbiológica, química y de aceptabilidad sensorial.

Palabras clave: *Arepas, listo para cocción, vida de anaquel, estable, reto microbiano.*

SUMMARY

Arepa is an important part of the Venezuelan and Colombian diet. The introduction in the market of a ready-to-cook-arepa of long lasting life on the market shelves at room temperature, or refrigeration, will be of great convenience. Studies were conducted to determine the sensory shelf-life of a ready-to-cook arepa developed in the laboratory. Microbial challenge tests were conducted with natural flora isolated from corn dough, stored and spoiled after 30 days at room temperature. The selected formulation, a prototype of long shelf life ready-to-cook arepa was obtained. These arepas have the same taste as any homemade arepa. A complete characterization and its microbial, chemical and sensorial stability were evaluated during seven days at room temperature and beyond fifteen days in refrigeration.

Key words: *Arepas, ready-to-cook, stable shelf life, microbial challenge study.*

INTRODUCTION

Arepas the traditional food for Venezuelan consumers have usually been prepared by two methods time-consuming. The introduction in the market of a ready-to-cook-arepa of good keeping qualities at room temperature, or refrigeration, will be of great advantage. The consumer could take the ready-to-cook-arepa from the shelf to the stove, saving time and decreasing the risks of microbial contamination. In order to produce arepas with extended shelf life, the knowledge of the native microbial flora and its suppression from corn dough is necessary. The objective of this work was to determine the process for obtaining ready-to- cook-arepas of extended shelf-life at 28 ±2 ºC (room temperature) through a microbial challenge approach. This test is performed in order to evaluate the stability of a product in presence of known, generally massive, microbial inoculums. Physical, proximate analysis and microbiological stability of the final products, kept seven days at room temperature were performed.

MATERIAL AND METHODS

Materials

Pre-gelatinized corn flour, vacuum packaging polyethylene bag, GRAS additives: pH-dependent antifungal and acetic and citric acids.

Methods

Design of the procedure at laboratory scale.

Arepas were prepared by slowly mixing with continuous agitation 1,760 parts of potable salted (0,75%) water with 1 part of commercial pregelatinized corn flour in a KitchenAid (Model K5SS, Michigan, USA.) during 10 minutes. The dough was left to proof 5 minutes and mixed again for 15 minutes. Two pre-cooking methods selected on previous assays were used: 7 minutes in microwave oven (method A) or 5 minutes in boiling water (method B). The subjective mechanical resistances to the touch of the arepas and solids losses were the main criterion to choose each parameter. When using the method B, a significant loss of solid was observed in the boiling water after cooking, and the arepas were, by touch, less resistent to the mechanical treatment. The microwave method (A) was chosen, since it was easier to control, the arepas had more mechanical stability, or less deformity when handling, and also there were less loses of solids than in the arepas precooked in boiling water. Consequently, the microwave exposure time (method A), the type of packing (thermoplastic polyethylene) and the parameters of vacuum packaging, using a vaccum packaging machine (VacMaster, Mod: SVP 20, USA, at V=2; G=3 and S=4) were selected after several assays. The arepa size was determined to fit the "tostiarepa" (Oster de Venezuela, S.A. Caracas, Venezuela) molds.

Isolation of the dough native microbial flora

Three batches of samples (each package of 6 arepas) were elaborated by using method A, and stored during 30 days in a room with temperature control at 28 ± 2 ºC. The stored samples were investigated for microbiological aerobic count (on plate count agar) as well as yeast and mold count (on potato dextrose agar + tartaric acid) using the pour plate method (Deibel and Swanson, 2001, APHA, 1992, FDA, 1992). The more concurrent and representatives colonies observed on the plates were aseptically picked out and transferred into 10 mL of fresh sterile nutrient broth. The inoculated broths were incubated at 30° C with agitation during 24 hrs (this should yield approximately 5x109 cells) and stored to be used in the microbial challenge study.

Microbial challenge test

Previous to the microbial challenge test, several assays were performed using the two types of acids as acidulant and three concentrations of the antifungal pH-dependent additive (500, 1000 and 1500 ppm); in order to reach the volume of acid necessary to get the pH value of the dough close to the dissociation constant (*pKa*) of the additive, which is 4.75. The pH of the water, water plus acid and dough were evaluated by the method N° 02-52 described in the

AACC (2003). Afterwards, four batches of arepa dough with the allowed maximal concentration of the antifungal additive (Furia, 1972 and Sofos and Busta, 1981) and two acids types, with pH values close to dissociation constant of the antifungal pH-dependent additive, were inoculated with active cultures (titles of 10^3 to 10^5 CFU/g) of the native colonies selected (aerobic bacteria and yeast and molds) from the arepas elaborated using the scheme described in Figure 1.

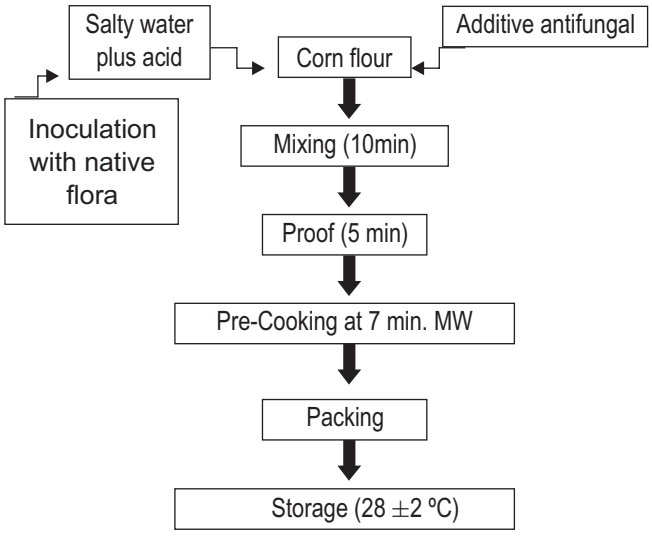

Figure 1 . Method for preparation of arepas in a microbial challenge approach

Hereafter, the arepas were aseptically molded, packed and stored for 7 days at room temperature (28±2 ºC). The stored samples were investigated for microbiological aerobic count; as well as for yeast and mold count, using the same methods described before.

Description of the dough formulation with the minimal antifungal additive concentration

Once the effectiveness of the pH dependent antifungal additives was corroborated, four batches of dough were elaborated by using method A: Three batches and one control were prepared using various concentrations of the antifungal additives, starting from the allowed maximal concentration according to the Venezuelan Official Standards (COVENIN), and decreasing it until the minimal level in terms of the microbial population inhibited. The three batches of inoculated arepas, stored during 7 days at (28 ±2 ºC) were assayed for microbiological aerobic count (on plate count agar) as well as yeast and mold count (on potato dextrose agar + tartaric acid) as previously described. The assays were performed three times.

Overall stability evaluation, proximal composition, and acceptability of the arepas elaborated with the minimal concentration of the selected GRAS additive.

Three batches of the samples of arepas were elaborated with the scheme previously chosen based on the minimal concentration of selected GRAS additives, microwave method, vacuum level and package type, [Scheme 1, without the phase of inoculation of native flora (red underlined)] and were stored during seven days at (28±2 ºC). After this time, the arepas were assayed for aerobic mesophilic count (plate count agar), total coliforms, yeast and mold count (potato dextrose agar/tartaric acid), *Staphylococcus aureus, Salmonella* sp., and additive concentration, according to Venezuelan official standards (COVENIN) moisture content, pH, titratable acidity (using methods N°s: 44-19, 02-52, 02-31 respectively) described by AACC, (2003), and aw using procedure described by Wilson et al. (2006) After a_w the microbial data were analyzed and having confirmed the microbial safety, the arepas were cooked and tasted with an affective laboratory panel that gave its opinion on sensory characteristics: taste, odor, and global acceptance. The test was performed using a hedonic scale of 7 points described by Pedrero et al. (1997).

RESULTS AND DISCUSSION

Table 1 shows the means of the results of the assay to select the volume and type of acid to be used in the final scheme. As can be seen, the amount of citric acid chosen was 120 mg and 2.5 ml for acetic acid. These amounts were incorporated to the recipe for dough elaboration at the microbial challenge test as is shown in Figure 1. Moreover, acetic acid was chosen to perform an assay to establish the adequate pH for the elaboration of the arepas with the minimal pH-dependent antifungal additive concentration that shall guarantee microbial quality. Acetic acid was

chosen due to the typical flavor contribution it offers to the product.

Table 1. Dough recipes based on amount of acid added, pH of the water and dough and final pH of the arepas.

Acid Type	Acid amount	Water´s pH	Dough´s pH	Arepas´s pH
	60 mg	3.9	5.5	5.5
Citric	120 mg	3.9	5.1	5.1
	0.35 ml	4.8	7.0	7.0
Acetic	2.5 ml	3.4	4.8	4.8

Table 2 is a summary of the pH found in the dough, when using acetic acid as acidulant at different concentrations of the GRAS additive. As can be seen in the table, three concentrations of the GRAS pH-dependent antifungal additive were used, varying them from the maximum allowed concentration of 1500 ppm to cero concentration used as control. At 500 ppm, the pH of dough is quite close to the additive dissociation constant (*pKa*) of the microbial additive (4.75). Table 2 also shows the mean of the results of the pH values of the acidulated water, dough and arepas. The concentration of the antifungal additive did not change the pH, guarantying the efficacy of the additive and the inhibition of microorganisms. The additives were incorporated to the recipe for dough elaboration, as shown in Figure 1.

Table 2. pH of the dough and arepas

	0 ppm	500ppm	1000ppm	1500ppm
Water´s pH	6.6	6.6	6.6	6.6
Water´s pH + acid	3.5	3.6	3.6	3.6
Dough pH	4.5	4.5	4.5	4.6

Table 3 summarizes the results of the microbial challenge test. As can be seen, both types of microbial population were suppressed by the antibacterial used. Despite the pH values are slightly above the *pKa* value, the test shows an effective inhibitory action of the additive on the microorganisms present in the dough.

Tabla 3 Results of the microbial challenge study performed on arepas. Samples were inoculated at pH close to *pKa* of the additive used.

Sample*[Inoculums]		Acid type	Dough pH	(FCU/g)	
				Aerobic mesophilic	yeast and mold
1	0	Acetic	5.03	<100	<100
2	0	Citric	5.48	1900	<100
4	10^3	Acetic	4.97	300	<100
5	10^3	Citric	5.44	<100	<100
7	10^5	Acetic	5.07	<100	<100
8	10^5	Citric	538	<100	<100

* Dilutions: -1, -2 y 3.

Table 4 shows that 500 ppm was good enough for the control of the microorganisms during 15 days. When concentrations less than 500 ppm were assayed, the results were very similar to microbial counts of control samples. Likewise, a slightly chemical taste in the product when the additive concentration was above 500 ppm was perceived. However, in order to be within microbial safety limits, expiration dates of 7 days at room temperature (28 ± 2°C) or 15 days at refrigeration temperatures (5 ± 2°C) are proposed.

Table 4. Results of the microbial challenge study performed on arepas. Samples were inoculated at pH close to *pKa* of the additive used and stored during 15 days.

Additive concentration (ppm)	(FCU/g) Aerobic Mesophilic	(FCU/g) Mold	(FCU/g) Yeast
0	3.0×10^5	1.2×10^3	2.1×10^4
500	2.4×10^3	<10	<10
1000	2.9×10^3	<10	<10
1500	5.0×10^3	<10	<10

* Dilutions: -1, -2 y 3.

The microbial evaluation, moisture content, a_w, pH and titratable acidity of the arepas that were elaborated with acetic acid and 500 ppm of the additive are shown in Table 5. The raw precooked arepas have a moisture content of 64.20 % with a_w value of 0.99. The pH was 4.71 and the titratable acidity 0.08 %. As can be seen, the results of the microbial evaluation indicate stability of the product

during seven days. Aerobic mesophilic counts of several dehydrated products are similar to the values found in this study. In a detailed study of the microbiology of dehydrated food products, Jay (1996) shows that these products have an aerobic plate count (APC) of less than 10.000/g. In addition, despite the acceptable high population of aerobic mesophilic (5.0×10^5), once the arepas have been cooked, the population is reduced as demonstrated in Table 1. All of the sensorial parameters (odor, taste, texture and color) were declared acceptable by the judges of the affective panel.

Table 5. Physical, chemical and microbiological characterization of arepas after performance of a microbial challenge study. Samples were inoculated at pH close to *pKa* of the additive used and stored during 7 days.

Parameters	
(FCU/g) *Aerobic Mesophilic*	5.0×10^5
(FCU/g) Total *Coliforms.*	<3
(25 g) Salmonella spp	–
(FCU/g) *Staphylococcus aureus*	<10
(FCU/g) *Mold*	<10
(FCU/g) *Yeast*	<10
Moisture content (%)	64.20
a_w	0.99
pH	4.90
Additive concentration	17.44 mg/100g
Titratable acidity (expressed as acetic acid)	0,08 g/100g

CONCLUSION

The feasibility of the elaboration of ready-to cook arepas using three antimicrobial barriers (additives, microwave, and vacuum packaging), stored at room temperature during seven days, was demonstrated using a microbial challenge test. As a result, a prototype of a long shelf life ready-to-cook arepas was obtained (Figures 1). These arepas have the same taste the same as any homemade arepa. The complete characterization and stability for seven days at room temperature and beyond fifteen days in refrigeration was performed corroborating these findings.

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