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Microbiological safety of artisanal corn doughs sold in Puerto La Cruz, Venezuela

Inocuidad microbiológica de las masas artesanales de maíz expendidas en Puerto La Cruz, Venezuela

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Abstract

Recently, artisanal doughs of pilar corn have become a food for dough consumption in Venezuela, without adequate sanitary control in terms of production and sale. There are very few internationally referenced studies and none recently locally, regarding this product; for this reason, the objective of this research has focused on its microbiological evaluation with samples obtained in the municipal market of Puerto La Cruz, which are the main point of sale of the same in the main Venezuelan cities. The frequencies of total coliforms, fecal coliforms, presence of Escherichia coli, aerobic mesophiles and Staphylococcus aureus were determined as indicators of inadequate manipulation; The presence of molds and yeasts was also considered as microorganisms associated with cereals. It is concluded that all the samples of pillared corn doughs present a high level of these indicator microorganisms and that their consumption without proper cooking could carry significant health risks.

Keyword: corn, dough, flour, microbiological evaluation, FBD.

Resumen

Recientemente las masas artesanales de maíz pilado han pasado a ser un alimento de consumo masivo en Venezuela, sin un control sanitario adecuado en materia de elaboración y expendio. Existen muy pocos estudios referenciados internacionalmente, y ninguno reciente a nivel local, al respecto de este producto; por ese motivo el objetivo de esta investigación se ha enfocado su evaluación microbiológica con muestras obtenidas en el mercado municipal de Puerto La Cruz, que son el principal punto de expendio de las mismas en las principales ciudades venezolanas. Se determinaron las frecuencias de coliformes totales, coliformes fecales, presencia de Escherichia coli, aerobios mesófilos y Staphylococcus aureus como indicadores de una manipulación inadecuada; también se consideró la presencia de mohos y levaduras como microorganismo asociados a cereales. Se concluye que la totalidad de las muestras de masas de maíz pilado presentan un elevado nivel de estos microorganismos indicadores y que su consumo sin la cocción adecuada podría conllevar riesgos importantes para la salud.

Palabras clave: maíz, masa, harina, evaluación microbiológica, ETA.

Introduction

One of the most ingrained food customs of the Venezuelan is the acquisition and consumption of artisan products, ignoring whether they are really safe chemically and microbiologically. The current social reality has exacerbated this behavior, given that Venezuela is going through several years of a difficult political and economic crisis, where according to the most recent official figures issued by the Central Bank of Venezuela, its Gross Domestic Product experienced a fall of approximately 5.7%. (1); and characterized by an accentuated shortage of various foods, medicines and other items in the basic market basket (2).

Due to this general situation, aspects related to the availability of food, especially its regular distribution and sale channels, have been significantly altered by generating alternative systems for the production and handling of raw materials in the form of artisan products, absolutely far from the controls. established by the state to ensure food safety. This precariousness may be linked to the regular notification of outbreaks of Foodborne diseases (FBD) carried out weekly by the health authorities, as well as that of hepatitis, amoebiasis and diarrhea, which are the most important mandatory epidemiological reporting events within the causes. of medical consultation in Venezuela (3).

At the center of this problem is the consumption of corn, since the price of its precooked flour has increased, making access difficult for the regular consumer. This represents a real food security problem, given that all consumption frequency studies have shown that it is the food with the highest purchase intention and availability in households (4,5); In addition, since 1983 it has been the main vehicle of the official enrichment program, adding with mandatory character, certain amounts of vitamin A, thiamine, riboflavin, niacin and iron to provide 25% of the recommended daily

nutrients, taking into account the losses caused by the cooking (6).

In the current context, several formulas have emerged to replace precooked corn flour using oats, banana, cassava, sweet potato, but mainly for the original corn dough, which is generally sold freshly ground (7,8) but, as it is not subject to sanitary controls for its artisanal elaboration and supervised sale, it has generated an important public and scientific debate about its safety (9).

The microorganisms present in corn flour are relatively scarce, but once rehydrated, bacteria, molds and yeasts grow, since the conditions of aqueous activity become favorable for them. In the case of cereal doughs, they can not only be accessed from the air, water, soil or the warehouse environment, but are particularly incorporated into the handling and processing phase (10). Among the bacteria used as indicators of food quality, the coliform group is perhaps the most important international criterion for assessing contamination and the sanitary quality of a sample. Coliforms provide important information on the type and source of contamination present, and include genera such as Escherichia, Citrobacter, Enterobacter, and Klebsiella (11). The presence of coliforms indicates that food could be contaminated with human or animal feces, representing these pathogens a health risk, especially in the case of children and people with severely compromised immune systems (12).

Additionally, in Venezuela the analysis of corn grain samples have shown high levels of fungal incidence and its consequent contamination by mycotoxins (8, 13, 14), even in precooked corn flour molds and yeasts have been detected (15); they grow in almost any food, at low aw and acidic pH values, while most bacteria grow at high aw and pH close to neutrality (10).

Thus, in the absence of recent local analyzes and the few international studies related to this product, this study was framed in the search and contribution of data on the microbiological safety of the artisan dough of chopped corn, through a sampling in the municipal market of Puerto La Cruz, Anzoátegui State, which allowed: to characterize the environmental conditions of sale, to characterize the product microbiologically and physically, to compare the experimental values with microbiological criteria, as well as to relate the presence of indicator microorganisms with humidity, temperature and acidity of the doughs there expended to the public.

Material and methods

Sample selection criteria: the research consisted of a descriptive exploratory observation of an epidemiological nature, which was carried out in the facilities of the municipal market of Puerto La Cruz, randomly evaluating 5 out of a total of 8 regular corn dough of shelled corn. From there, 5 doughs of shelled corn were selected in accordance with the Venezuelan Standard COVENIN 2135-1996 (16), which were wrapped in closed polyethylene plastic bags, of approximately 1 Kg and were treated according to the Venezuelan Standard COVENIN 1126-89 (17).

Transfer and reception of samples: the samples were labeled and transferred to the laboratory in refrigerated cellars with ice (0 ° C). In the laboratory they were kept refrigerated between 0 ° C and -4 ° C for a period of less than 24 hours. Vending conditions were recorded in a structured way for each stall, such as ambient temperature, time, exposure conditions (light / shadow), storage (refrigeration / environment), clothing, handling.

In situ temperature measurement of the dough samples: the samples were measured with a ShT thermometer, model LX-001, range -20 ° C - 110 ° C, and appreciation 1 ° C, previously disinfected with isopropyl alcohol at 70 % v / v, introducing the bulb into the sample for one minute.

Measurement of humidity in dough samples: it was carried out according to the Venezuelan Standard COVENIN 1553-80 (18). A Sartorius brand analytical balance, model BL210S, was used. 5 g of sample were placed in aluminum capsules and left in an oven at 130 ° C for one hour. They were allowed to cool in a desiccator for one hour and the mass was determined. The procedure was repeated until a constant dough was reached.

Measurement of acidity in dough samples: it was carried out according to the Venezuelan Standard COVENIN 1787-81 (19). 5 g of the sample were taken and left to stand with 25 ml of neutralized 90% ethyl alcohol for 24 hours. A 10 ml aliquot of the supernatant was taken and titrated with 0.05 N sodium hydroxide (NaOH),

using phenolphthalein as indicator. Acidity is expressed in terms of% sulfuric acid, and is determined by the equation:

$$\% \text{ Acidez} = \left[\frac{V \times N \times 0{,}049 \times V_1}{M \times V_2}\right] \times 100$$

Where: V = Volume of the sodium hydroxide solution used in the titration (ml)
N = Normality of the sodium hydroxide solution.
V1 = Volume of neutralized alcohol added to the sample (ml)
V2 = Volume of the aliquot taken for the titration (ml)
M = Mass of the sample (g)

Microbiological analysis of the shelled corn dough

Pretreatment of the samples: the plastic packages were disinfected prior to opening with a sponge impregnated with isopropyl alcohol solution at 70% v / v. 10 g of sample was weighed on a Sartorius brand analytical balance, model BL210S and placed in sterilized polyethylene bags with a hermetic seal. 90 ml of 0.1% peptone water was added and stirred in a Stomacher 80 brand homogenizer at 8000 r.p.m. for 1 minute.

Total coliforms: it was carried out by the Standard Methods SM-9221-B. For each sample, 1 ml was inoculated in 3 Durham tubes with Lauryl Sulfate Tryptose and incubated at 35 ° C for 24 hours. Subsequently, a confirmatory test was performed by loop inoculation in Durham tubes with Brilliant Green Bile broth. They were incubated for 24-48 hours. A Memmert brand natural convection incubator was used.

Fecal coliforms: it was performed by the Standard Methods SM-9221-E. For each positive test for Lauryl Sulfate Tryptose (total coliforms), 3 Durham tubes were loop inoculated with coliform enrichment (EC) broth and incubated at 45 ° C for 24 hours. A Memmert brand natural convection incubator was used.

Escherichia coli: it was performed by the Venezuelan COVENIN 1104-96 method (20). For each tube with a positive result in the fecal coliform test, 2 seeds (replicas) of 1 ml each were prepared in tubes with 9 ml of Enrichment Broth (EC) by applying a hoe. All replicas were incubated in an oven at 45 ° C for 24 hours. After the incubation, for each tube with a positive result in the EC test, 2 seeds (replicas) were prepared on plates of Eosin Blue Methylene Agar (Levine), which were incubated in an oven at 35 ° C for 24 hours. A Memmert brand natural convection incubator was used.

Determination of Staphylococcus aureus: it was carried out by the Venezuelan Standard COVENIN 1292-89 method (21). For the 10-1 dilution of the Stock Solution, 3 seeds (replicas) were prepared in Baird-Parker Agar plates, and incubated at 37 ° C for 24 hours.

Determination of molds and yeasts: it was carried out by the Venezuelan Standard COVENIN 1337-90 method (22). For each of the dilutions (10-1, 10-2 and 10-3) of the stock solution, a seed (replica) is prepared in plates with by pouring 0.1 ml of the dilution onto the center of the plates. and the subsequent addition of Papa-Dextrose Agar. After solidification, the plates were incubated in an oven at 25 ° C for 5 days with daily observation.

Determination of Mesophilic Aerobes: it was carried out by the Venezuelan Standard COVENIN method 902-1987 (23). For each of the dilutions (10-1, 10-2 and 10-3) of the stock solution a seeding is prepared (replica) in plates with by pouring 0.1 ml of the dilution onto the center of the plates and the subsequent addition of the Plate Counting Agar (PCA). After solidification, the plates were incubated in an oven at 25 ° C for 5 days with daily observation.

Data processing and analysis.
In the absence of a Venezuelan standard referring specifically to the study product and a general lack of other studies that allow comparison, the standards of Spanish legislation were established as comparative references between the experimental data and the reference values (24,10) , and Sanitary Norm N ° 615-2003 "Sanitary Norm that establishes the microbiological criteria of sanitary quality and safety for food and beverages for human consumption" of the General Directorate of Environmental Health of the Peruvian Ministry of Health (DIGENSA) (25) :

Microbiological criteria for fresh pasta and precooked dough
Sanitary Standard N ° 615-2003. General Directorate of Environmental Health.
Peruvian Ministry of Health

Microbial Agent	Category	Class	n	c	Limit per g	
					m	M
Mesophilic aerobes cfu / g	2	3	5	2	104	105
Total coliforms	5	3	5	2	10	102
Staphylococcus aureus	8	3	5	1	102	103
Escherichia coli	6	3	5	1	10	102
Fungi and mold	5	3	5	2	103	104
Yeasts	2	3	5	2	103	104

Components of the sampling plan:

"n" (lowercase): Number of sample units required to perform the analysis, which are chosen separately and independently, according to national or international standards regarding food and beverages appropriate for microbiological purposes.

"c": Maximum allowed number of rejectable sample units in a 2-class sampling plan or provisionally acceptable sample units in a 3-class sampling plan. When a number of sample units greater than "c" is detected, the lot is rejected.

"m" (lowercase): Microbiological limit that separates the acceptable quality from the rejectable. In general, a value equal to or less than “m” represents an acceptable product and values greater than “m” indicate rejectable lots in a 2-class sampling plan.

"M" (capital letter): Microbial count values higher than "M" are unacceptable, the food represents a health risk.

The relationship between microbiological frequencies and physicochemical properties was established through a linear regression. The information processing was done based on the SPSS v 20 statistical packages, to tabulate, graph and analyze all the information.

Results

Characterization of the environmental conditions of the Puerto La Cruz market: in some areas direct exposure of food to the elements was observed, in direct contact with the consumer, and even without protection from sunlight. The food was sold in places near the sewage and sewage drainage, and the waste deposit. The sale of the ground corn dough did not escape these conditions, being sold together with vegetables, greens and tubers, which come directly from their growing areas, and therefore can be sources of cross contamination.

Microbiological characterization of the shelled corn dough: when making the comparison between samples for each microorganism analyzed, a high dispersion can be reflected in the relative standard deviation values for all microorganisms, except for the count for mesophilic aerobes, whichever is used to infer that there are factors in the process of obtaining and preserving the masses of ground corn that have been able to influence the microbial load of the product displayed for sale and that varies significantly between each vendor. In this case, they highlighted the high variability obtained for total and fecal coliforms, with a relative standard deviation greater than 100% (see Table 1).

Physicochemical characterization of the shelled corn dough: when comparing the moisture and acidity values between the refrigerated yellow corn masses and the non-refrigerated white corn mass, it was observed that there are practically no significant differences, although the values obtained for the corn mass white are slightly larger (see Table 1). The moisture value in the masses of ground corn (69.2%) is in the order of values referred to in cooked cereal foods, such as arepa, 5% broken grain rice, or corn-based infant cereals.

Tabla 1. Análisis microbiológico de las muestras de masa de maíz

Sample	Mesophilic aerobes cfu / g	Total coliforms NMP/g	Fecal coliforms NMP/g	*Staph. Aureus ufc/g*	Escherichia coli cfu/g	Fungi and mold cfu/g	Yeasts cfu/g
1	$2,97 \times 10^7$	$2,40 \times 10^7$	$1,60 \times 10^7$	$2,70 \times 10^2$	$4,50 \times 10^2$	Absent	$2,60 \times 10^2$
2	$3,91 \times 10^7$	$2,40 \times 10^5$	$1,60 \times 10^5$	$9,00 \times 10^2$	$3,96 \times 10^3$	Absent	$3,19 \times 10^3$
3	$5,40 \times 10^7$	$2,40 \times 10^6$	$1,60 \times 10^6$	$1,16 \times 10^3$	$2,32 \times 10^3$	Absent	$3,45 \times 10^3$
4	$3,52 \times 10^7$	$2,40 \times 10^6$	$1,60 \times 10^6$	$8,40 \times 10^2$	$2,56 \times 10^3$	Absent	$3,24 \times 10^3$
5	$6,75 \times 10^7$	$2,10x\ 10^5$	$1,20 \times 10^5$	$1,50x\ 10^2$	$3,60 \times 10^3$	Absent	$2,61 \times 10^3$
Average	$3,95 \times 10^7$	$7,26 \times 10^6$	$4,84 \times 10^6$	$7,93 \times 10^2$	$2,32 \times 10^3$	Absent	$2,53 \times 10^3$
Standard deviation	$1,04 \times 10^7$	$1,12 \times 10^7$	$7,47 \times 10^6$	$3,75 \times 10^2$	$1,44 \times 10^3$	Absent	$1,52 \times 10^3$
Relative Standard Deviation (%)	26,3	154,4	154,4	47,3	62,1	Absent	60,0
Technical norm	1×10^5	1×10^5	1×10^5	1×10^2	1×10	1×10^2	1×10^2

Comparison of experimental values with the microbiological criteria: when comparing the values obtained for the mesophilic aerobes with the standards consulted, it was observed that for all the masses analyzed, regardless of whether or not they are sold under refrigeration, levels higher than those established as maximum of up to 2 orders of magnitude. The same situation was observed for total coliforms and fecal coliforms. In the case of Staphylococcus aureus, Escherichia coli and yeasts, the maximum value that the colony-forming units exceeded in relation to the reference values was one order of magnitude.

Relationship between the abundance of microorganisms and the humidity of the shelled corn dough: the humidity levels registered for the masses of pilled corn reached 70%, which is considered high for a food that can presumably be exposed for hours or days for the sale without proper preservation.

Comparison of refrigerated dough vs non-refrigerated dough: it was found that in the case of mesophilic aerobes there was a difference of approximately double cfu/ g, which is consistent with what was expected, but that in parameters such as total coliforms, fecal coliforms and Staphylococcus aureus, a difference cannot be established due to the variability obtained in the masses that were refrigerated. However, although these values are lower for the non-refrigerated dough, which is contrary to what could be expected, no relationship can be established due to the lack of information on the processing of the doughs. The greater presence of Escherichia coli in the non-refrigerated sample is also highlighted.

Relationship of the abundance of microorganisms and the titratable acidity: the levels of titratable acidity recorded for the masses of ground corn averaged 0.004% +/- 0.0005%, values that indicated a slightly acidic medium close to neutrality.

Discussion

Characterization of the environmental conditions of the Puerto La Cruz market: the market does not meet the appropriate conditions to guarantee the microbiological safety of food, which could entail significant risks for public health, also considering that it is the most important market not only for the city but the entire eastern part of the country and from which millions of people are supplied directly and indirectly. The incidence of FBD is a very current global issue and varies between different countries, but it is precisely the developing countries that bear most of the problem; although the episodes of these diseases continue to constitute a challenge for

public health in industrialized countries. Recent studies on the burden of disease in the Caribbean have reported that the incidence rate of acute gastrointestinal diseases associated with contaminated food ranges from 0.65 cases / person / year to 1.4 cases / person / year and estimates the total associated costs between $ 700,000 per year up to 19 million dollars (26,27), in Colombia and Paraguay they have established hygienic-locative evaluation of outlets as a microbiological guarantee for consumers (28, 29), as well as in Ghana they have reported (30) on the need to improve food safety to prevent the incidence of these diseases.

Microbiological characterization of the shelled corn dough: There are several factors after the processing of corn to obtain the masses that can significantly influence the microbiological activity, and for which detailed information is not available for the research. One is the time elapsed between preparing the dough and taking the sample. Another factor is the time and the temperature of refrigeration of the masses, if these are preserved before the sale. These factors would be variables of interest to be considered for future studies. Comparison of the microbiological indicators obtained for the non-refrigerated white corn dough with those obtained for the yellow dough that were refrigerated prior to sale, allows us to infer certain criteria related to the climatic factor. In the case of mesophilic aerobes, the value obtained for the non-refrigerated white corn dough exceeded all the values obtained for the refrigerated yellow corn doughs. This fact was consistent with what was expected for microorganisms that develop mostly at the average ambient temperature of the municipal market of Puerto la Cruz, which is around 30 ° C. However, the same pattern was not observed for total and fecal coliforms, where the values obtained for the chilled yellow dough were up to one or two orders of magnitude higher than those for the non-chilled white corn dough. For Staphylococcus aureus, Escherichia coli and yeasts, lower values are observed for unrefrigerated white corn dough, although remaining in the same order of magnitude, so there do not appear to be considerable differences. It is notable that there may be an incidence of some sanitary factor in the handling by each vendor, regardless of the type of corn and the conditions of preservation of the samples.

Comparison of experimental values with microbiological criteria: it was alarming that all the samples presented levels of total and fecal coliforms up to 2 orders of magnitude higher than the maximum limit for food, as well as the presence in all the analyzed samples of Escherichia coli in up to 100 times above the allowed value for foods for immediate consumption, and that must be completely absent in the case of cereals. However, the levels of Staphylococcus aureus were found at values slightly higher than the maximum limit, although in the same order of magnitude, but which are also indicators of inadequate procedures in the handling or packaging of the product, since it could reflect the use of materials not appropriate for preserving food safety. In the packaging process, direct contact of the inside of the package with the hands of the packer may occur, or when the walls of the bags are detached by rubbing and direct blowing of air with the mouth, causing contamination by not complying with the regulations of hygiene.

Relationship between the abundance of microorganisms and the humidity of the shelled corn dough: not all the water contained in the ground corn dough is chemically compromised, since part of the moisture is added during the milling process, so it could be inferring that the water activity must be high, at the level of values of 0.80 or even 0.90; this would promote the development of any type of microorganism (11). Bacteria grow at aw greater than 0.90; while yeasts do so in aw greater than 0.85 and fungi in aw greater than 0.80. Another factor to consider is the quality of the water added in the mill, which could be unfit for human consumption, and therefore, be a source of cross contamination. The absence of molds and fungi in the determinations, despite the high humidity levels recorded in the samples, led us to infer that the preparation of the doughs was recent, but that their exponential growth was easy to predict given the favorable humidity levels for Its reproduction. Not so in the case of yeasts, present in all the samples analyzed, up to an order of magnitude above the value referred to in the reference standard. The presence of yeasts could confirm high water activities in the corn dough. Mesophilic aerobes show a tendency to increase their formation as a function of the humidity presented in the dough. Although a formal mathematical relationship could not be established, not are values of water activity available for food, it is consistent with the fact

that bacteriological activity increases as humidity is higher. In the case of Staphylococcus aureus and yeasts, no definite trend can be asserted with respect to moisture. For Staphylococcus aureus values, ups and downs are observed that do not allow a defined mathematical relationship to be established, but in a certain region there is a tendency to increase the formation of colonies as humidity increases.

Relationship of the abundance of microorganisms and titratable acidity: in such conditions, microorganisms such as Staphylococcus aureus would present high activity because they grow at pH values between 4.5 and 9.3, with an optimum of 7.0 to 7.5, and therefore would generate enterotoxins associated with gastroenteritis. High variability in titratable acidity values was not reported, which makes it difficult to establish a clear trend between this parameter and the levels of microorganisms, however, for each of the microorganisms, maximums were observed at an acidity level of 0.004%. which may mean that this value would probably represent an optimal condition.

Conclusions

The artisanal dough of shelled corn sold in the municipal market of Puerto La Cruz presents a high level of indicator microorganisms with respect to the microbiological limits referred to as safe for human consumption, with statistically significant differences for mesophilic aerobes, Staphylococcus aureus and Escherichia coli, and critically significant for total coliforms and fecal coliforms. These risks associated with the safety of the product could be solved by improving dough preparation procedures where their acidity, humidity and temperature are controlled; as well as the general conditions of storage and handling for sale. The results of this research are addressed in the form of a proposal, both to consumers and to health authorities, as well as to academic and research instances in the food area, providing data that can be valuable in the necessary search for operational strategies that they manage to ensure the safety of the artisanal shelled corn dough, given its importance as a massive consumer product.

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Contribution of the authors

All authors participated in the entire research process.

Interest conflict

We declare that we have no conflict of interest.

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