

Determination of the Shelf-life of Arepa with Egg through Accelerated Testing

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Abstract

The objective of this research was to determinate the shelf-life of arepa with egg through accelerated tests. The shelf-life of the arepa was estimated taking into account the increase in the product's peroxide index (IP) under accelerated conditions with the aid of the factor Q10. For this purpose, a zero-order model for kinetic degradation was used, in addition to physical-chemical and microbiological analyses. The results indicated an increase in IP at higher temperatures and also during the first frying period. IP tended to increase basically with temperature and frying time, this may have been due to the accumulation of less stable primary oxidative compounds. A value of Q10 of 1.93 was obtained, which indicates that the speed is accelerated those times for every 10°C increase in temperature.

Keyword: accelerated testing; peroxide value, physicochemical characteristics, microbiological analysis

1. Introduction

Oxidation indicated by free radicals and mediated by chain reactions is one of the main causes of degradation of oil quality and oily foods [1, 2]. Frying oil is quickly

heated to high temperatures (158 – 185°C) in the presence of oxygen, moisture, pro-oxidants and antioxidants from frying foods, resulting in oxidation of lipids. This can lead to impaired sensory and nutritional characteristics of fried foods. Even with careful control of all aspects of frying and storage after use, the frying oil is becoming increasingly oxidized with repeated heating. Particular case is in Colombia, where the same food is usually fried in large quantities for several days, the oil under these conditions must be removed and replaced periodically to maintain the quality of the oil. Therefore, it is recommended to have guidelines for the use of oil, including oil temperature, fryer selection, frying time, oil cooling and storage. Indeed, lipid oxidation products exert cytotoxic and genotoxic effects, repeated consumption of oxidized fat with diet could represent a chronic threat to the health of consumers. Lipid oxidation is not a single reaction, but a series of reactions. Once oxidized, a series of primary oxidation products are produced, such as free fatty acids, conjugated diene or triene conjugate and peroxides; secondary products such as alcohol, aldehydes and subsequent ketones [3].

Primary oxidation processes in oil form hydroperoxides (ROOH), which are reflected in the peroxide value. In general, it is a measure in which the oil has suffered primary oxidation. However, this value reaches a measure and then decreases as peroxides decompose in secondary oxidation products, particularly carbonyl compounds [4], provides the initial evidence of rancidity in unsaturated fats and oils and gives a measure of the measurement of oil in which an oil sample has suffered primary oxidation, especially during storage. Newly refined oils have a peroxide value of less than 1 mEq kg⁻¹ oil, while a peroxide value of more than 10 mEq kg⁻¹ oil, the oil is considered oxidized [5]. Oxidation development is the critical event that determines the shelf life of oils/fats, therefore a reliable life assessment is crucial to verify how long the product will last before it oxidizes to unacceptable levels.

The peroxide index (IP) is probably one of the most common methods used to measure the initial oxidation phases of oils and fats. PI is often carried out using a titration-based method to determine the level of iodine released from potassium iodide by the oxidized species. Samples of oils and fats can be analysed directly using the IP method. However, food and finished products need to be removed to recover fat for the determination of peroxide value. This extraction should be carried out with great care to avoid further oxidation, but also to ensure that the fat is sufficiently recovered from the finished product. The peroxide value measures hydroperoxide that is produced in the early stages of the oxidation process. Care should be taken when interpreting the peroxide value as hydroperoxides degrade easily, so samples with a low peroxide value may have been subjected to significant oxidation. The objective of this research was to determine the shelf-life life of arepa with egg using accelerated testing.

2. Methodology

2.1 Shelf-life of arepa with egg

The determination of shelf-life of the product was determined taking into account the increase in the peroxide value of the product under accelerated conditions. For this purpose, the samples were packed in plastic-coated aluminium bags, since it is the packaging that is conventionally used for fried products. This shelf-life study was applied to products classified as better and to control.

2.2 Study of shelf-life

The determination of the shelf-life of the final products was made taking into account the increase in the product's peroxide value under accelerated conditions with the aid of factor Q₁₀.

2.3 Peroxide index (PI)

The peroxide index (PI) is the amount (expressed in milliequivalents of active oxygen per kg fat) of peroxides in the sample that cause oxidation of potassium iodide under the described working conditions [6, 7]. The test sample, dissolved in acetic acid and chloroform, was treated with potassium iodide solution. The released iodine was evaluated with sodium thiosulfate solution according to the International Standard ISO 3960 method of IP determination technique under the guidelines of A.O.A.C. 965.33 [8]. The assays were carried out in triplicate. Finally the PI was calculated using Equation (1).

$$IP = \frac{1000(V - V_0)C}{m} \quad (1)$$

Where PI (meq de O₂/kg) is the peroxide index in oil, V (cm³) is the volume of sodium thiosulphate solution used for the determination, V₀ (cm³) is the volume of sodium thiosulfate solution used in the standard sample, C (mol L⁻¹) is the concentration of the thiosulfate solution and m (g) is the mass of the sample.

2.4 Periodicity of analysis

The minimum number of temperatures required to conduct a shelf-life study using accelerated testing is three. For this study temperatures of 20 °C, 30 °C and 40 °C were established. Temperatures were chosen to establish a difference of 10°C and to calculate the value of Q₁₀ that represents the ratio of the speed constants of reaction to the temperatures mentioned [6]. The following is the analysis carried out on samples of arepa with egg at temperatures of 20°C, 30°C and 40°C (Table 1).

Table 1. Periodicity of peroxide index analysis

| Storage temperature (°C) | Measurement period (d) | Maximum storage time (d) | Sampling (d) |
|--------------------------|------------------------|--------------------------|--------------------------------|
| 20°C | 15 | 105 | 0, 15, 30, 45, 60, 75, 90, 105 |
| 30°C | 7 | 49 | 0, 7, 14, 21, 28, 35, 42, 49 |
| 40°C | 3 | 21 | 0, 3, 6, 9, 12, 15, 18, 21 |

2.5 Model for Kinetic Degradation

For its simplicity and good results in the reported research [9, 10, 11], this study worked with a zero-order model such as Equation 2. Integrating Equation (2) and rearranging, it is obtained the Equation (3) of a straight line with a slope k ; k being the specific constant of reaction and whose value depends on temperature.

$$-\frac{\partial X}{\partial t} = k \quad (2)$$

$$X_f = X_0 - kt \quad (3)$$

Since the reaction velocity constant is temperature dependent, this dependence is described by the Arrhenius equation. The Arrhenius model describes the relationship of the reaction speed constant with temperature according to Equation (4).

$$k = Ae^{\left(\frac{-E_a}{RT}\right)} \quad (4)$$

By applying logarithms on both sides of Equation (4) the equation of a straight line with an $E_a R^{-1}$ slope, as expressed in Equation (5). The term E_a can be evaluated to determine the value of the activation energy. Where k is constant of reaction speed; A is the frequency factor, E_a is the activation energy, R is the ideal gas constant ($8.314472 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$) and T is the absolute temperature (K).

$$\ln k = \frac{E_a}{R} \cdot \frac{1}{T} + \ln A \quad (5)$$

2.6 Calculation of parameter Q_{10}

Parameter Q_{10} is defined as the ratio between the velocity constant at one temperature (T) and the velocity constant at another temperature ($T + 10 \text{ }^\circ\text{C}$). This is not constant but depends on E_a and absolute temperature T . Parameter Q_{10} is

calculated according to Equation (6). Where k_T is the reaction constant at T_1 and k_{T+10} is the constant at $T_2 = T_1 + 10^\circ\text{C}$. V_{UT} is the shelf-life of the product at T_1 and V_{UT+10} is the shelf-life at $T_2 = T_1 + 10^\circ\text{C}$.

2.7 Microbiological analysis

11 g of the samples were taken and added to 99 mL of peptone water, followed by consecutive serial dilutions and finally the bacterial concentration of: Mesophilic Aerobes, *Staphylococcus aureus*, *Salmonella* sp, Total and fecal coliforms.

3. Results

3.1 Shelf-life of the samples classified as best and the control sample under accelerated conditions and with the aid of factor Q_{10} .

Figure 1, 2 and 3 show the result of the analysis carried out on samples of arepas with eggs stored at temperatures of 20°C , 30°C and 40°C . The results indicated that the peroxide index (PI) increased at higher temperatures, and also during the first frying period. PI tended to increase basically with temperature and frying time, this may be due to the accumulation of less stable primary oxidative compounds. This was reported by Park and Kim [12] who obtained a peroxide number of 6.88 during the first two days of frying with 10 bumps/day. The quality of the oils depends on their chemical composition, such as the percentage of the degree of unsaturation, and the value of the peroxide depends on the temperature, time and light, it measures the degree of primary oxidation of the oils (rancidification). The rancidity of oils can produce potentially toxic compounds associated with long-term health effects with neurological disorders. Oils with a high degree of unsaturation are highly susceptible to microorganisms, in which bacteria and yeasts use their enzymes to break down the chemical structures of the oil, leading to the production of unwanted odours and flavours.

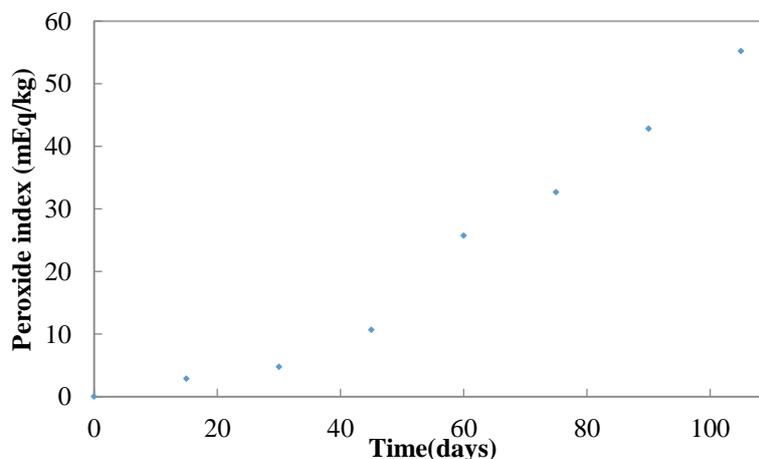


Figure 1. Peroxide index of arepa with egg store at 20°C .

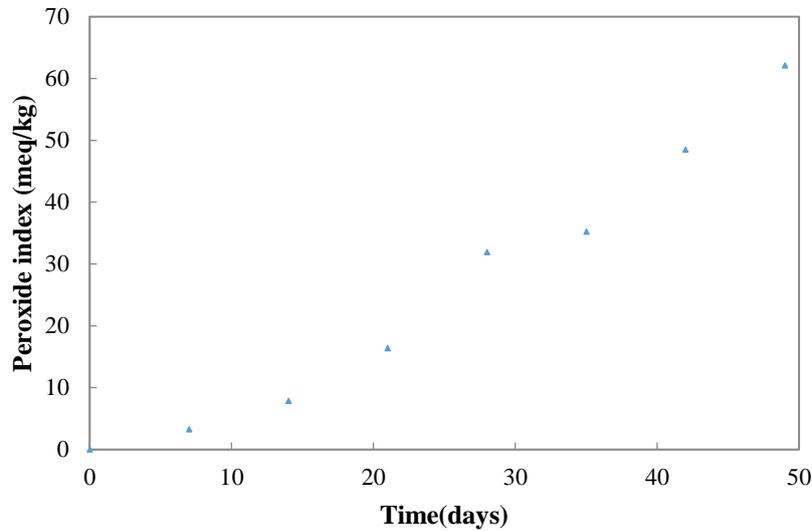


Figure 2. Peroxide index of arepa with egg stored at 30 °C.

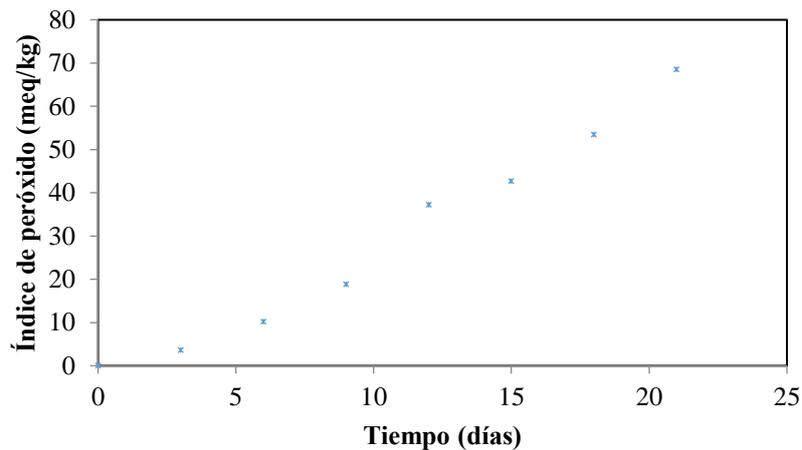


Figure 3. Peroxide index of arepa with egg stored at 40 °C.

The lipids of edible oils are susceptible to photo-oxidation and self-oxidation during storage and processing in the oil industry. Oxidation can produce undesirable flavours, break down nutritional quality and lead to the production of toxic compounds. Oil oxidation can be influenced by different factors such as the degree of unsaturation, heat, light, oil processing, antioxidants and transition metals. Self-oxidation, in which peroxide is the main product that gives rise to unpleasant tastes in foodstuffs, proceeds through the free radical chain reaction, where it attacks double binding at room temperature. Photooxidation is a much faster reaction that involves attacking the double bond, the rancidity of food products can be the result of auto and photooxidation, which are natural processes of oxidation and chemical degradation of edible oils, in which the fatty acid esters of oils are converted into free fatty acids. Oxidative stability of oils is defined as

resistance to oxidation during processing and storage [13], which can be expressed as the period of time required to reach the critical point of oxidation, depending on sensory change or sudden acceleration of the oxidative process. Sunisa et al., [14] evaluated the effect and frying time on changes in oil and fried chicken quality at 170 °C, 180 °C and 190 °C for 15 min, 18 min, and 21 min respectively. An increase in IP during the frying process indicated a decrease in unsaturated fatty acids due to oxidation, however peroxides are particularly unstable compounds under high temperature conditions; therefore, peroxides decompose to form carbonyl and aldehyde compounds that cause a decrease in the peroxide value.

The linear regressions obtained from these figures are presented in the peroxide index equations in Figure 4. With the three constants obtained, represented by the slopes of the equations mentioned above, for the three temperatures studied, the Arrhenius model was applied, as expressed in Equation (4) (figure of $\ln k$ as a function of $1/T$).

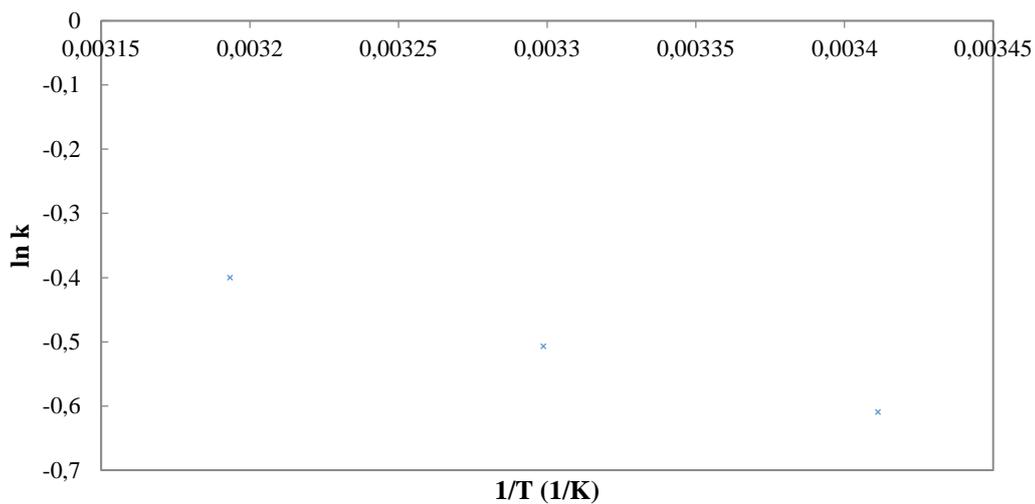


Figure 4. Linear Regression of the shelf-life.

The activation energy was calculated from the equation in Fig. 1 as follows.

$$k = Ae^{(-E_a/RT)} \tag{6}$$

$$\ln k = \ln A - \left(\frac{E_a}{R}\right) \cdot \frac{1}{T} \tag{7}$$

$$m = \frac{E_a}{R} \tag{8}$$

$$m = 960.38 \tag{9}$$

$$E_a = 7985.05 \text{ J/mol.K} \quad (10)$$

Regarding activation energy, Torres et al., [15] stated that activation energy for lipid reactions is in the range of 41842 J mol^{-1} to $104605 \text{ J mol}^{-1}$, i. e. they were in the range established by the literature. Garcia and Molina [6] estimated the useful life of a mayonnaise using accelerated tests, these authors reported that the peroxide index in mayonnaise increases with respect to time, being its linear behavior, also obtained an activation energy of 80960 J mol^{-1} . For values of $7.96 \text{ meq O}_2/\text{kg}$ the judges considered that the product no longer had the qualities necessary for consumption. These authors estimated a product shelf life of 149 days, 40 days and 21 days at $21 \text{ }^\circ\text{C}$, $35 \text{ }^\circ\text{C}$ and $45 \text{ }^\circ\text{C}$. Guzmán et al., [7] studied the useful life of pork ham through accelerated tests. They indicated that the pig's ham peroxide index increased over time, behaving in a linear manner, which can be explained by the oxidation of fats, reporting an activation energy of $8405.93 \text{ J mol}^{-1}$.

Table 3 shows the average of the useful life analyzed, taking into account the acceptance or rejection of the sample according to the sensorial analysis carried out, this was used to determine the peroxide index of the arepas with egg at the three temperatures studied. Taking into account the above data, the lg of the sensory lifetime was plotted as a function of temperature in $^\circ\text{C}$ in order to obtain a straight line with a value of R^2 greater than 0.9 that would allow a data adjustment to be obtained.

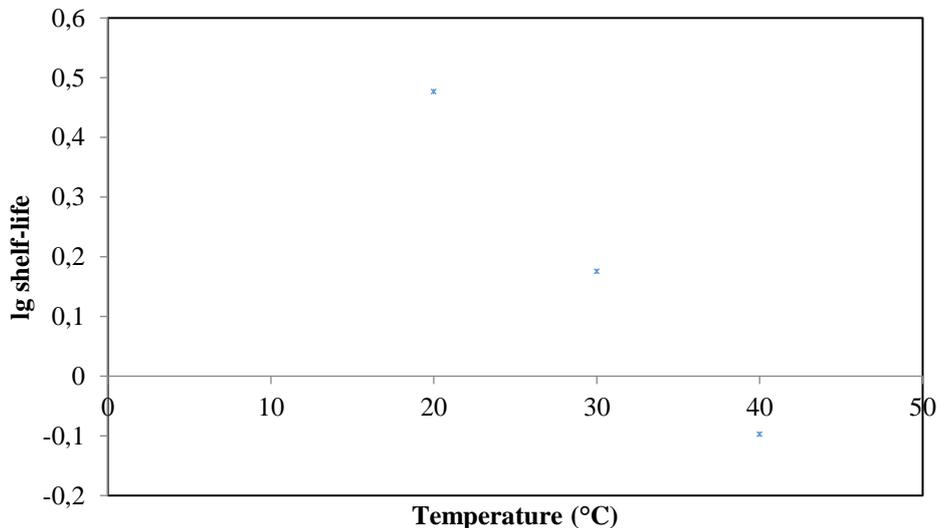


Figure 5. lg shelf-life vs Temperature $^\circ\text{C}$

Once Figure 5 was obtained, the model proposed by Labuza [16] was applied to calculate the Q_{10} . An alternative to expressing the temperature dependency of a reaction is the concept of using Q_{10} , which indicates how quickly the reaction will

occur if the temperature rises by 10 °C, and can therefore be used to predict the expected shelf life of the product. For example, if a food attribute is stable for 10 weeks at 30 °C, and has a Q₁₀ of 2, then its stability at 20 °C will be 2 x 10 weeks = 20 weeks [17].

$$Q_{10} = e^{(10^b)} \tag{11}$$

$$\ln Q_{10} = \frac{10E_a}{R(T)(R + 10)} \tag{12}$$

where

$$b = \frac{E_a}{R(T)(T + 10)} \tag{13}$$

$$Q_{10} = e^{(10^b)} \tag{14}$$

$$Q_{10} = e^{(10 \cdot 0.0661)} \tag{15}$$

$$Q_{10} = 1.937 \tag{16}$$

Table 2. Shelf-life of the arepa with egg

| Shelf-life (days) | Temperature (°C) | lg Shelf-life |
|-------------------|------------------|---------------|
| 3 | 20 | 0.477121255 |
| 1.5 | 30 | 0.176091259 |
| 0.8 | 40 | -0.096910013 |

The value Q₁₀ indicates that the speed is accelerated 1.937 times for every 10°C increase in temperature. The rate of chemical reactions is an important determinant of food quality. Chemical kinetics involves the study of the rates and mechanisms by which one chemical species becomes another, characterized by constant velocity and order of reaction. In zero-order reactions, brown colour formation in foods results as a result of Maillard's reaction. First-order reactions are frequent in the food reaction, including oxidation of lipids and rancidity, microbial growth, loss of vitamins in dry foods and loss of protein quality. Second-order reactions are relatively common, examples include amino acid changes involved in Maillard's reaction [17].

Table 3. Shelf-life at different temperatures

| | |
|-----------------------------|-------------------------|
| log shelf-life = | 1.0465 - 0.0287*T |
| Shelf-life = | Exp (1.0465 - 0.0287*T) |
| Shelf-life at 20 °C= | 1.603999183 |
| Shelf-life at 30 °C= | 1.2038202 |
| Shelf-life at 40 °C= | 0.903481179 |

3.2 Microbiological results

Table 4 shows the analyses carried out on the arepa with egg (final product and control sample). There is evidence that the sample showed a favorable indication according to the specifications of the standard. If the presence of *Salmonella* is analysed, it is evident that this micro-organism was absent from the analysed sample, i. e. recommendations for the preparation of this type of fried food were taken into account, with appropriate handling of the dough and other ingredients. Different results reported Durango et al., [18], who found contamination by *Salmonella* spp. In 10.5 % of the arepas with egg samples analyzed, they also observed that 97 % of the businesses where *Salmonella* spp. was isolated had poor sanitary conditions. They also identified that chorizo, arepa with egg and beef as the most contaminated foods, which are related to exaggerated handling and the various ingredients used in the preparation.

Table 4. Microbiological analysis of the arepa with egg.

| Microbiological analysis | Final product | Control sample | Specifications |
|--|---------------|----------------|----------------|
| <i>Salmonella</i> Detection 25 g ⁻¹ | Negative | Negative | Negative |
| Total Coliform Count CFU g ⁻¹ | <10 | <10 | <10 |
| E. Coli count UFC g ⁻¹ | <10 | <10 | <10 |
| Mesophilic aerobic microorganisms count | 8600 | 9400 | 10.000 Máx |
| <i>Staphylococcus aureus</i> coagulase positive count CFU g ⁻¹ | <100 | <100 | <100 |

Hanashiro et al., [19] analysed 40 samples of popular foods in São Paulo, Brazil; hot and cold sandwiches, acarajé (prepared with fried bean dough stuffed with shrimp) and coxinha (prepared with chicken breast wrapped in a fried dough). They reported that 35% of the samples studied on the street were unfit for consumption, when compared to the standard applied in their country. Regarding sanitary conditions, faecal Coliforms were detected in 22 samples (55 %) (<3 MPN g⁻¹), reaching values of 2.4 x 10⁵ MPN/g in cold sandwiches. The pH measured in the analysed samples was suitable for the growth of most microorganisms involved in foodborne diseases. The presence of faecal Coliforms (30%) indicated a high risk, probably from pathogenic organisms present in raw plants and lack of good hygiene practices. Bayona [20] analysed the microbiological quality of foods purchased on public roads in a sector in Bogotá. They reported that the maize arepas were not positive for *Salmonella* spp., but were positive for *Escherichia coli*, with a 25% finding. Carrera et al., [21] also

analysed food sold on the streets of all Cuban provinces in the period from January 1995 to June 1997, finding that 3.5 % of food sold on the streets was contaminated with *Salmonella* spp., possibly due to hygienic deficiencies and handling of cash and food at the same time.

4. Conclusions

The peroxide index (PI) basically increased with temperature and frying time. A value of Q_{10} of 1.93 was obtained, which indicates that the speed is accelerated those times for every 10°C increase in temperature. The shelf-life of the arepa with egg determined by accelerated tests was 1.6, 1.2 and 0.9 days at 20 °C, 30 °C and 40 °C respectively. Microbiological analyses carried out indicated that the arepa was made under cleanliness and disinfection conditions, complying with the Colombian standard.

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