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Sensory and texture properties of “chipá”: Influence of ingredients and storage conditions of batter

Ana Ávalos¹, Pamela Goytiño¹, Paula A. Conforti¹,² and Cecilia E. Lupano¹*

Abstract: “Chipá” is a gluten-free bread made with cassava starch and cheese. Usually, the batter is baked immediately and the product is eaten warm. In this study, the effect of refrigeration and freezing of batter, and the role of cheese upon “chipás” prepared with cassava or corn starch were analyzed. This may offer alternatives to the storage of batter, and contribute to the knowledge of the effect of refrigeration and freezing of batter in gluten-free baking products. Texture and colour were analyzed in batter and baked products. Volume, sensory quality and in vitro starch digestibility of “chipás” were also determined. The refrigeration or freezing of batter turned darker mainly the baked products containing cheese, probably due to the Maillard reactions between proteins and products of lipid oxidation. The “chipá” made with cheese and cassava starch was bigger, softer and gummier than that prepared with corn starch, and these properties did not change when the batter was frozen. No differences were found in the in vitro starch digestibility of “chipás” due to the batter storage conditions. Cassava starch was more digestible than corn starch. Freezing would be a good alternative to storage the batter of cassava starch and cheese.

Subjects: Food Science & Technology; Food Additives & Ingredients; Food Chemistry; Food Engineering

Keywords: gluten-free product; cassava starch; corn starch; cheese; refrigeration; freezing

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PUBLIC INTEREST STATEMENT

“Chipá” is a gluten-free bread of Guaraní origin consumed in South America, made with cassava starch and cheese. It represents an alternative to celiac individuals. Usually, the batter is baked immediately and the product is eaten warm. The loss of freshness of the “chipás” has a negative influence on product quality and consumer acceptance. This may be solved by cooling or freezing the batter and baking it before consumption. This procedure allows to transport the batter to the selling point, where it is baked. Freezing allows batter storage for long periods of time, and refrigeration could be an alternative in areas where “chipá” is consumed daily. The study of the effect of refrigeration or freezing of batter, and the role of cheese and type of starch on the properties of these products may offer alternatives to the batter storage, and contribute to the knowledge of gluten-free baking products.
1. Introduction
Recent epidemiological studies have shown that celiac disease is one of the most common lifelong disorders; the estimated prevalence is about 1% of the general population (Capriles & Arêas, 2014). The gluten matrix, which encloses the starch granules and fibre fragments, is a major determinant of the properties of dough (extensibility, resistance to stretching, gas holding ability). The absence of gluten can result in low quality baked products. The formulation of gluten-free bakery products presents a formidable challenge to both cereal technologists and bakers. In view of the current increasing incidence of celiac sufferers (due to improved diagnostic procedures), there is a major need for more research and development in the area of gluten-free cereal based products. In recent years, several studies were made on gluten-free products, including the use of rice and buckwheat flour (Ronda, Pérez-Quirce, Angioloni, & Collar, 2013; Torbica, Hadnađev, & Dapčević, 2010; Wronkowska, Haros, & Soral-Śmietana, 2013), chestnut flour (Demirkesen, Mert, Sumnu, & Sahin, 2010), carob germ and starch (Smith, Bean, Herald, & Aramouni, 2012), amaranth flour (Schoenlechner, Mandala, Kiskini, Kostaropoulos, & Berghofer, 2010), cassava, soy, chia, quinoa, potato (Capriles & Arêas, 2014) and hydrocolloids (Lorenzo, Zaritzyk, & Califano, 2009). It was found that both the type and the lipid content affect the texture properties of non-fermented gluten-free dough (Lorenzo et al., 2009).

On the other hand, the nutritional quality of baking products can be enhanced by including specific ingredients into the recipes. The requirement for dietary calcium is one of the most difficult to meet in developing countries. This mineral can be incorporated in bakery products including milk or cheese in the formulation. “Chipá” or “pão de queijo” is a traditional gluten-free bread usually made by cassava starch and consumed in the north of Argentina, Paraguay and south of Brazil. It is a guarani culture recipe. “Chipá”s is an alternative recipe to celiac individuals. Even when there are some studies on gluten-free cheese bread (Rodriguez-Sandoval, Franco, & Manjarres-Pinzon, 2014), very little research has been found in literature concerning the “chipá”. In order to study the effect of cheese and cassava starch on the properties of “chipá”, products without cheese or with corn starch instead of cassava starch were analyzed, but we will call “chipá” only the products containing cheese.

The loss of freshness of “chipá”s has a negative influence on product quality and consumer acceptance. This problem may be solved by refrigerating or freezing the batter and baking it before consumption. This procedure allows the transport of the batter to the selling point, where it is baked. Freezing allows batter storage for long periods of time. Refrigeration of the batter could be an alternative in areas where “chipá” is a product of daily consumption, and do not require prolonged storage. However, the refrigeration or freezing of batter may have adverse effects on the structure and functional properties of the finished product (Lorenzo et al., 2009). Several studies have been performed on the effect of freezing on the properties of wheat batter (Inoue & Bushuk, 1996; Nemeth, Paulley, & Preston, 1996; Slade, Levine, & Finley, 1989; Yi & Kerr, 2009). The formulation of batter plays an important role in its properties and behaviour after freezing (Rouillé, Le Bail, & Courcoux, 2000). Some authors have analyzed the effects of freezing and frozen storage conditions on the rheological properties of gluten-free bread batter formulated with corn flour, starch, hydrocolloids, oil, water, and sometimes yeast (Leray, Oliete, Mezaíze, Chevallier, & de Lamballerie, 2010; Lorenzo et al., 2009; Mezaíze, Chevallier, Le-Bail, & de Lamballerie, 2010). However, very little information was found concerning the effect of freezing on gluten-free batter with cheese.

The aim of this study was to analyze the effect of refrigeration and freezing of batter, and the role of cheese upon “chipá” prepared with cassava or corn starch. This may offer alternatives to the storage of batter, and contribute to the knowledge of the effect of refrigeration and freezing of batter in gluten-free baking products.

2. Materials and methods

2.1. Materials
Ingredients include corn starch (Maizena, Unilever, Argentina S.A., Buenos Aires), cassava starch (Yin Yang, Dietética Científica S.A.C.I.F.I., Ciudad Autónoma de Buenos Aires, Argentina), butter (La
Serenísima, Mastellone Hnos. S.A., General Rodríguez, Argentina), skim milk powder (Sancor, Sunchales, Santa Fe, Argentina), salt (Dos Anclas, Salinas Grandes, Hidalgo, La Pampa, Argentina), fresh eggs, and cheese (medium-hard, 30% fat, Tregar, Gobernador Crespo, Santa Fe, Argentina).

2.2. Batter preparation
A traditional recipe was prepared, with the following ingredients: 200 g cassava or corn starch, 30 g butter, 200 g ground cheese (particle size about 1 mm), 20 g skim milk powder, 2 g salt, and egg (1 unit). The cheese was ground with a food processor (Philips Cuccina, type HR7633 Brazil). Control batter without cheese was also performed. The ingredients were mixed by hand, 50 mL of tap water were gradually added, and the mixture was remixed. Batter was left at room temperature for at least 15 min in a polypropylene bag before sheeting with a rolling pin to a thickness of 1 cm. The sheet was left again for 15 min covered with a polypropylene film to avoid desiccation. After this time, discs of 3.0 cm diameter (for sensory analysis, colour and batter texture) or rectangles of 5 cm × 2.5 cm (for “chipá” texture) were cut.

2.3. Storage conditions of batter
Discs or rectangles of batter were placed on aluminum foils into a polyethylene bag, and were storage in closed plastic containers three days at 4°C (refrigerated batter) or one month at −20°C (frozen batter).

Fresh and stored discs and rectangles of batter were removed from the plastic container, let reach room temperature, and cooked in an oven (Ariston type F9 M, Italy) at 170°C during 15 min. Fresh, refrigerated and frozen batter prepared with corn or cassava starch were baked simultaneously. Assays were performed in duplicate.

2.4. Texture characteristics of batter
The texture of batter was evaluated by texture profile analysis (TPA) using a texture analyzer (TA-XT2i, Stable Micro Systems Ltd, Godalming, UK). Firmness, consistency and cohesiveness of batter discs were measured, by two compression cycles (50% strain) with a cylindrical probe (7.5 cm diameter) at a displacement speed of 0.5 mm/s. (Conforti & Lupano, 2004). Firmness was defined as the peak force (Newton) exerted during the first compression cycle. Cohesiveness was calculated as the ratio between the positive force area of the second compression and that of the first compression (A2/A1) (Bourne, 1978; Conforti & Lupano, 2004; Yamul & Lupano, 2003). Consistency was taken as the sum of A1 + A2 (Conforti & Lupano, 2004). For each type of batter, the average of ten determinations was calculated.

2.5. Differential scanning calorimetry
A differential scanning calorimeter (DSC Q100 Thermal Analysis Instruments, New Castle, Delaware, USA) calibrated with indium was used. Samples of 4.1–7.8 mg of cheese or batter, prepared with cheese and cassava or corn starch, were placed into aluminium Differential scanning calorimetry (DSC) hermetic pans. Samples of cassava or corn starch with distilled water to reach the same water content as batter were also assayed. An empty pan was used as reference. Sample and reference were heated between 20 and 120°C at a heating rate of 10°C/min. The peak temperatures (Tp) were computed from the endothermic peaks. Determinations were performed at least in duplicate.

2.6. Colour of batter and baked products
Five discs of each group of batter were simultaneously baked and then allowed to cool at room temperature. The superficial colour of batter and “chipá” was measured with a Chroma meter CR-400 Minolta (Osaka, Japan). Two series of each type of “chipá” were analyzed. The Hunter parameters L* (L* = 0 [black], L* = 100 [white]), a* (−a* = green, +a* = red), and b* (−b* = blue, +b* = yellow) were determined. The total difference of colour (ΔE) between the batter and the baked product, and the browning index (BI), were calculated as follows (Maskan, 2001):

\[
\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2}
\]
where $L^*$, $a^*$ and $b^*$ are the Hunter parameters of baked products and $L_0^*$, $a_0^*$ and $b_0^*$ are the Hunter parameters of batter.

### 2.7. Size and texture of baked products

Rectangles of batter ($5 \times 2.5 \times 1$ cm) were baked and let at room temperature. The fracture properties were studied by a three-point bending test with a TA.XT2i texture analyzer (Stable Micro Systems, Godalming, UK). Span length was 3.0 cm and compression speed was set at 0.5 mm/s. The size of each baked sample ($d$: width, $l$: length and $b$: thickness) was determined with a vernier calliper before placed on supports with their top surface down. The volume of each chipá was calculated as $d \times b \times l$.

The texture was measured over a 30 min period immediately after baking. Two series of each type of “chipá” (five pieces each) were analyzed. The force (N) needed to break the sample, and the distance before rupture were determined (Baltsavias, Jurgens, & Vliet, 1997). The fracture stress ($\sigma$) (kNm$^{-2}$), the fracture strain ($\varepsilon$) and the Young's modulus ($E$) (kNm$^{-2}$) were calculated as follows:

\[
\sigma = \frac{3FL}{2db^2} \quad (4)
\]

\[
\varepsilon = \frac{6bY}{L^2} \quad (5)
\]

\[
E = \frac{L^3s}{4db^3} \quad (6)
\]

where $F$ is the force (N), $L$ is the distance between supports (m), $d$ is the width of the test-piece (m), $b$ is the thickness of the test-piece (m) (Baltsavias et al., 1997), $Y$ is the deflection (m) or distance before rupture: the lower the value, the more brittle the product (Gaines, Kassuba, & Finney, 1992), and $s$ is the tangent of the linear region of the curve force vs deformation (Baltsavias et al., 1997).

### 2.8. Magnifying glass observation of “chipás”

The structure of “chipás” prepared with cheese and cassava or corn starch was observed with a magnifying glass (Zeiss, Oberkochen, Germany) with 12 × magnification.

### 2.9. In vitro starch digestibility

In vitro starch digestibility was determined on “chipás” prepared with cheese and cassava or corn starch and with different storage conditions of batter. White bread crumbs were used as reference (van der Merwe, Erasmus, & Taylor, 2001). All samples were ground with a food processor (Philips Cuccina, type HR7633 Brazil) to pass through a 0.5 mm sieve. The digestibility was determined with pancreatic $\alpha$-amylase (Type VI-B from porcine pancreas, Sigma Chemical Co, St Louis MO, USA) according to the method described by Singh, Kherdekar, and Jambunathan (1982), with some modifications (Conforti & Lupano, 2008): about 40 mg of milled samples were weighed in six glass tubes, four of them for the reaction and two for the blank. The samples were dispersed in 3 mL of 0.2 M phosphate buffer, pH 6.9, and 0.5 mL of enzyme (pancreatic $\alpha$-amylase, 5.9 mg/mL, 57.8 IU) was added only to the four reaction tubes. To the blank tubes, 0.5 mL of inactivated enzyme (by placing in boiling water bath for 10 min) was added. The suspensions were incubated at 37°C, and mixed for 10 s with vortex every 15 min. Aliquots of 0.15 mL were removed from each tube at 30, 60 and 120 min and heated in a boiling water bath for 10 min to stop the reaction. The released
maltooligosaccharides were determined with 3,5-dinitrosalicylic acid. Maltose was used as a standard. Results were expressed as percent of maltose per milligram of dry sample with respect to the same measure for dry white bread.

2.10. Sensory evaluation

The sensory attributes flavour, texture, colour and overall acceptability of “chipás” prepared with cheese were evaluated by three different sensory tests with untrained panellists. In the first test, 18 panellists evaluated two samples that differed in the type of starch. In the second test (cassava starch “chipá”) and in the third test (corn starch “chipá”), 12 panellists evaluated three samples: “chipás” prepared with fresh, refrigerated or frozen batter. Samples were coded with a three digit number according to a randomized complete block design. Water was provided for mouth cleaning between samples. The order of presentation was balanced and randomised to eliminate contrast effect and positional bias. Samples were assayed within the first 20 min of baking because these products usually are consumed warm. All tests were performed between 10.00 and 11.00 am. The experimental environment was kept constant for all sessions and no outside influences were allowed to interfere with the subject’s assessments. Panellists were instructed to place a vertical line across a 10 cm horizontal line at the point that best describes their impression of each attribute of the sample. Each mark was then converted into a decimal numeric value from 0.0 to 10.0 by observing the scale length. The sensory scores for each sample were averaged and subjected to statistical analysis.

2.11. Statistical analysis

An analysis of variance (ANOVA) of the data was performed by using a Systat statistical computer program. A least significant difference (LSD) test with a confidence interval of 95% was used to compare the means.

3. Results and discussion

3.1. Observation of “chipás”

Photographs of “chipás” prepared with cheese and cassava or corn starch, with different storage conditions but baked simultaneously, were shown in Figure 1. The structure of “chipá” prepared with corn starch was more compact than the corresponding to cassava starch, which presented bigger round pores and a more expanded structure. The analysis of variance showed significant differences \( (p < 0.05) \) in the volume of “chipás” due to the batter composition: the “chipás” prepared with cassava starch were bigger than those prepared with corn starch. The apparent amylose content of cassava starch is 17.9%, whereas that of corn starch is 23.4% (Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005a). Tester and Morrison (1990) concluded that swelling was a property of the amyllopectin, and Srichuwong, Sunarti, Mishima, Isono, and Hisamatsu (2005b) observed that swelling power of starch granules increased with the ratio of the relative molar distribution of amyllopectin branch-chains with a degree of polymerization of 6–12 with respect to that of chains with a degree of polymerization of 6–24. This ratio was 0.489 and 0.392 for cassava and corn starch, respectively (Srichuwong et al., 2005b), which can explain the higher volume of the “chipás” made with cassava starch.

3.2. Texture properties of batter and baked products

The texture of batter and baked products with different ingredients and different storage conditions of batter are shown in Figure 2. The analysis of variance showed significant differences \( (p < 0.05) \) in \( \sigma \), \( \varepsilon \), and \( E \) (Young’s modulus) due to batter composition: “chipás” prepared with cassava starch had higher \( \varepsilon \), and lower \( \sigma \) and \( E \) than “chipás” prepared with corn starch. The gumminess is inversely related with the Young’s modulus. These results reflect the characteristics expected on “chipás”: a soft and gummy texture, so “chipá” prepared from fresh batter containing cassava starch and cheese had the best texture. These differences are reflected in the photographs shown in the Figure 1, and could be attributed in part to the different composition of both starches. The other component that could affect the
properties of these products is cheese. As expected, most of the differences observed in different formulations of “chipás” were due to the presence of cheese. Cheese decreased the firmness and consistency, and increased the cohesiveness of batters ($p < 0.05$). Also, the volume of the baked products prepared with cheese was higher than the volume of those prepared without cheese ($p < 0.05$). Moreover, the values of $\sigma$ and Young ($E$) of “chipás” prepared with fresh batter with cheese were lower than those of the baked products without cheese (Figure 2). This means that the addition of cheese produced softer and gummier “chipás”. The gumminess of “chipás” containing cheese may be attributed to the presence of the casein matrix and to the high fat content (Bryant, Usyunol, & Steffe, 1995).

Figure 2 also shows the effect of the storage conditions of batter. The batter prepared with corn starch was more sensitive to refrigeration and freezing than those prepared with cassava starch; it presented changes in the firmness, consistency and cohesiveness, although the effect on firmness and consistency disappeared when cheese was incorporated to batter. The texture and volume of the “chipás” with cassava starch and cheese were the same, both in those prepared with fresh and frozen batter, whereas those prepared from refrigerated batter presented lower volume and $E$ values (Figure 2 and Table 1). All other formulations showed differences in the texture of the final product, according to the batter storage conditions.

In all cases, refrigeration of batter tended to decrease $\varepsilon$ and increase Young modulus; that is, turned the baked products more brittle and less gummy than those prepared with fresh batter.
Baked products made without cheese, prepared with frozen batter, showed $\sigma$ and $\varepsilon$ values lower than those prepared with fresh batter, which indicates that these products became softer and more brittle when batter was frozen before baking (Figure 2). Upon freezing, moisture in the food transforms into ice, often resulting in physical stress of the food matrix. When a frozen food is thawed for consumption, the moisture is readily separated from the matrix causing a softening of the texture (Lorenzo et al., 2009). This can explain the softening observed in these products.

**3.3. Differential scanning calorimetry (DSC)**

When cheese was heated in DSC equipment, two peaks corresponding to reversible transitions were observed between 20 and 40°C, which were attributed to the fat melting. At temperatures between 65 and 80°C, irreversible transitions were observed, which would correspond to the denaturation of cheese proteins (Figure 3). In agreement with the pasting temperature, reported by Srichuwong

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**Figure 2. Texture of batter and baked products with different storage conditions of batter.**

Notes: F: fresh; R: refrigerated during three days; Fr: frozen during one month. Dark gray columns: batter or baked products with cheese, light gray columns: batter or baked products without cheese. Different letters indicate that the mean values significantly differ (Fisher LSD, $\alpha = 0.05$).

**Table 1. Volume of baked products made from batter stored in different conditions**

| Starch | Storage condition | Volume (cm$^3$) | With cheese | Without cheese |
|--------|------------------|----------------|-------------|----------------|
| Cassava | Fresh           | 22,05 g        | 17,35 c     |
|        | Refrigerated     | 20,94 f        | 16,47 a,b   |
|        | Frozen           | 22,50 g        | 16,41 a,b   |
| Corn   | Fresh            | 20,54 f        | 18,58 c     |
|        | Refrigerated     | 19,65 e        | 16,09 a,b   |
|        | Frozen           | 19,66 e        | 15,85 a     |

Note: Values followed by the same letter were not significantly different at 5% level.
et al. (2005a), the heating of cassava and corn starch with water contents similar to that of batter showed two peaks of starch gelatinization between 60 and 90°C. Cassava starch presented gelatinization peaks at lower temperatures than corn starch (Figure 3). The thermograms of batter also showed the peaks corresponding to fat melting, and the peaks of protein denaturation superimposed with those of starch gelatinization. As expected, melting of fat takes place before protein denaturation and starch gelatinization.

During baking, the steam pressure of the trapped water, which increases as the temperature increases, induces bubble growth (Rodriguez-Sandoval et al., 2014). Fat crystals melt and can be incorporated into the surface of the bubble, allowing the bubbles to expand without rupturing (Brooker, 1996), and contribute to the increase in the volume observed in the baking products containing cheese (Figure 1). When temperature increases, the thermal transitions of proteins and starch fix the structure. Cassava starch “chipá” made with cheese had the highest volume (p < 0.05); the high swelling power of cassava starch would contribute to this increase.

3.4. Colour of batter and baked products
As expected, the batter and “chipá” made with cheese were darker and more yellow than those without cheese (data not shown).

The total difference of colour (ΔE) and the browning index (BI) of “chipás” are shown in Figure 4. The total colour difference ΔE, which is a combination of the parameters L*, a* and b*, is a colorimetric parameter extensively used to characterise the variation of colours in foods during processing, whereas browning index (BI) represents the purity of brown colour and is reported as an important parameter in drying processes where enzymatic and non-enzymatic browning take place (Maskan, 2001). The presence of cheese increased both ΔE and BI. Also, the storage conditions of batter affected mainly the colour of baked products containing cheese. The “chipás” made with refrigerated batter were darker than those prepared with fresh batter, and the “chipás” made with frozen batter...
were darker than those prepared with refrigerated batter. To explain this behaviour it must be taken into account that cheese has high lipid contents. A high level of ketones had been found in cheese made with frozen curd from pasteurized ewe milk (Alonso, Picon, Gaya, & Nuñez, 2013). Lipids can suffer oxidation during storage, and the products of lipid oxidation, as ketones, can react with proteins and produce Maillard compounds. This effect was observed previously in biscuits (Patrignani, Conforti, & Lupano, 2014). Thus, the higher ∆E values observed in the “chipás” with cheese prepared from the refrigerated or frozen batter could be due to Maillard browning.

3.5. In vitro starch digestibility
The results of in vitro starch digestibility of “chipás” are shown in Figure 5. The starch digestibility was affected by the composition (p < 0.05) but not by the storage conditions of batter (p > 0.05). The in vitro starch digestibility of “chipás” made with cassava starch was higher than that of corn starch, at all the times assayed. At 30 min of incubation, the amount of maltose released by the “chipás” made with cassava starch was near four times higher than that of
“chipás” prepared with corn starch. At higher incubation times, the difference was less important. This indicates that cassava starch was more accessible to the enzyme digestion than corn starch in these products.

3.6. Sensory evaluation

Figure 6 shows the results of the sensory evaluation. The first test, which was performed to evaluate the preference of the type of starch, showed that panelists preferred the “chipás” prepared with cassava starch (Figure 6(A)), which are the typical “chipá” consumed in the northeast of Argentina, Paraguay and southeast of Brazil. The “chipás” made with cassava starch had lower σ and E, and higher ε than the “chipás” prepared with corn starch (Figure 2), which was perceived as a softer, gummier and less compact structure. These attributes were preferred by the panelists, and were also reflected on the visual characteristics of “chipás” (Figure 1).

On the other hand, results of the second and third sensorial tests (Figure 6(B) and (C)), showed that the panelists had not preferences between the “chipás” made with batter stored in different conditions. These results suggest that refrigeration and freezing would be suitable alternatives to conserve the batter of “chipás” until baking.
4. Conclusions
Both cheese and cassava starch contribute to the volume and the desirable properties of “chipás”: expanded structures, with a soft and gummy texture.

The effect of refrigeration or freezing of the batter on the colour of “chipá” was important mainly in the batter containing cheese, which became darker, probably due to the Maillard reactions between the proteins and the products of lipid oxidation.

The texture and volume of “chipás” with cassava starch and cheese were the same, both in those prepared with fresh and frozen batter, whereas those prepared from the refrigerated batter presented lower volume and ε values. All other formulations showed differences in the texture of the final product, according to the batter storage conditions. However, the panellists had not show preferences between “chipás” made with batter stored in different conditions, even when in some cases, the colour, brittleness and gumminess presented some differences. These results suggest that refrigeration and freezing would be suitable alternatives to conserve the batter of “chipás” until baking.

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Competing Interests
The authors declare no competing interests.

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