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Commercial limes (calcium hydroxide) in corn tortilla production: Changes in pH, color, sensory characteristics, and shelf life

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Abstract

Lime, an essential component of the nixtamalization process, influences several aspects of tortilla quality. This study evaluated the effect of six commercial limes of different purities on the quality of tortillas made from white and blue corn. The $Ca(OH)_2$ and heavy metal content in lime, color, pH, and calcium content in tortillas, acceptability by attributes, and shelf life were determined. Limes with lower levels of $Ca(OH)_2$ fell below the standard specifications and exceeded the limits for heavy metals such as arsenic (6.6 and 7.3 mg kg^{-1}) and lead (2.4 mg kg^{-1}). Tortillas made with the highest purity lime had a higher pH (white > 8.1, blue > 7.3), saturation index (white > 21.6, blue > 5.7), calcium content (white > 200, blue > 176 mg 100 g^{-1}), and lower luminosity. Moreover, these limes imparted better organoleptic characteristics to the tortillas, which led to a higher preference among the panelists. The purity of lime is a key factor in improving the quality and safety of the tortilla; thus, countries that adopt the nixtamalization process using lime should pay particular attention to the purity of the lime used in their processes, as it modifies the characteristics of the final product. In addition, manufacturers of food-grade lime must guarantee high purity to obtain a safe, high-quality food product.

Keywords: fungal contamination; heavy metals; lime purity; sensory attributes of tortillas

Introduction

The tortilla market has experienced remarkable growth, expanding its consumption to the United States, Europe, and some Asian countries (Báez-Aguilar *et al.*, 2022). According to Future Market Insights, the tortilla market is estimated to reach $45,163.75 million by 2024, with a growth rate of 5.2%. This increase is attributed to the popularity of Mexican and Latin American cuisine, as consumers perceive tortillas as a healthier alternative to traditional bread. The growing demand for gluten-free products has also contributed to this growth (Serna-Saldivar, 2021).

In Mexico, tortillas represent the most important staple food, with an annual consumption of approximately 12 million tons (Directorio Estadístico Nacional de Unidades Económicas, 2023). The production of tortillas involves over 110,118 tortilla shops (Directorio Estadístico Nacional de Unidades Económicas, 2023). These tortilla shops use fresh masa, nixtamalized flour, or a combination of both to make tortillas, which contain lime (calcium hydroxide), either food-grade ($Ca[OH]_2 \geq 90\%$) or with lower purity. NOM-187-SSA1/SCFI-2002 states that calcium hydroxide or calcium oxide may be used as the alkaline agent for nixtamalization, provided that they have a minimum purity of 90%, a maximum content of 5% magnesium hydroxide, and lead, fluorine and arsenic contents not exceeding 8, 40, and 3 mg kg^{-1}, respectively (Secretaría de Salud, 2003). Nevertheless, there is limited information available regarding the adherence to this regulation.

The first step in obtaining tortillas is alkaline cooking or nixtamalization, which involves cooking maize with lime, where the lime concentration can vary from 0.5 to 5.0% based on the weight of the maize grain (Gutiérrez-Llanos *et al.*, 2023; Valderrama-Bravo *et al.*, 2021). The combination of heat treatment and lime facilitates the hydrolysis of the pericarp during cooking (approximately 92°C), controls microbial activity, and contributes to the texture, flavor, aroma, color, shelf life, and nutritional value of the final product (Serna-Saldivar and Chuck-Hernandez, 2019). According to animal trials, the calcium absorbed by nixtamal and present in the tortillas is highly bioavailable (Serna-Saldivar *et al.*, 1991). Moreover, nixtamalization has been suggested as a means to reduce aflatoxin content by 30–90% (Odukoya *et al.*, 2021).

The use of lime in Mesoamerican cooking dates back to the Preclassic era and has persisted to the present day, primarily due to nixtamalization, which is the essential process for obtaining tortillas and over 300 other products ingrained in Mexican culture and diet (Serna-Saldivar and Chuck-Hernandez, 2019). Lime is used in different settings, from rural households where artisanal nixtamalization is practiced, to small- and medium-scale tortilla factories, to large industrial plants that process nixtamalized flour and corn snacks. However, the quality of lime employed varies by sector. While large industries and some tortilla shops prefer food-grade lime (>90% purity), construction lime is common in certain urban and rural areas. Limited knowledge or access to food-grade lime contributes to its limited use, which poses potential risks to public health, as construction lime often contains mineral additives and impurities, including heavy metals.

Research has explored the use of alternative calcium sources to replace lime during nixtamalization, such as calcium chloride, calcium sulfate, calcium carbonate, and calcium lactate, to reduce pollutant effluents (Escalante-Aburto *et al.*, 2020). However, the use of these additives is not yet regulated in Mexico, as NOM-187-SSA1/SCFI-2002 (Secretaría de Salud, 2003) stipulates that only $Ca(OH)_2$ or CaO is permitted. Furthermore, little attention has been paid to the impact of other sources of calcium on public health, tortilla shelf life, or sensory quality. Calcium hydroxide (lime) has recently been reported to be superior to other salts (sodium hydroxide, potassium hydroxide, and calcium chloride) in reducing fumonisin levels during the nixtamalization process (Odukoya *et al.*, 2021).

Calcium hydroxide, classified with the International Numbering System INS 526, serves as a food additive in various international dishes and products. It falls under the functional categories of firming agents and acidity regulators, providing corresponding technological functions. This classification, established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), is referenced in Mexican health regulations outlined in the "Agreement determining the additives and coadjuvants in foods, beverages, and food supplements, their use and health provisions" (Secretaría de Gobernación, 2012). In this context, calcium hydroxide functions as a pH and acidity regulator and a firming agent. Despite its recognition in Mexican health standards, there is a concerning absence of reported lime usage in prepackaged tortillas, which conflicts with consumer rights regulations (Mier Sainz-Trapaga *et al.*, 2022). Little research has been conducted on the quality of lime for safe use as a food additive. In this regard, Galvan-Ruiz *et al.* (2007) analyzed the samples of CaO and $Ca(OH)_2$ and found that they were within safe limits for human consumption.

The objective of this study was to evaluate the quality of six commercial limes in terms of heavy metal content and purity, and their effects on alkalinity (pH), color, calcium content, shelf life, and consumer acceptance of white and blue corn tortillas, to determine whether the evaluated limes meet the criteria to be classified as food additives.

Materials and Methods

Two corn types, white and blue, sourced from a producer in León, Guanajuato, Mexico, were used. Clean grains were conditioned to 13% moisture for 48 h before the nixtamalization process.

A survey was conducted among tortilla makers across the country to identify the brands of lime they used, and six were selected as the most used. The commercial lime brands were coded as CH01-71, CH02-79, CH03-95, CH04-95, CH06-93, and CH07-93; the control treatment was identified as C_{wl} (the control consisted of grain cooked without lime). The abbreviation CH stands for calcium hydroxide, followed by numbers indicating the assigned order (01, 02, 03, 04, 06, and 07) and the calcium hydroxide content in the lime (71, 79, 95, and 93%). The quantification of heavy metals (arsenic and lead) was determined by inductively coupled plasma mass spectrometry. The sample was prepared according to the method recommended by the Environmental Protection Agency (USEPA 3050B, 1996). Briefly, 1 g of sample was subjected to acid digestion with 10 mL HNO_3 for 45 min and allowed to cool. 10 mL 30% H_2O_2 was added to the mixture and heated. 10 mL 37% HCl was added, and the sample was brought to a boil for 15 min and allowed to cool. The resultant was finally filtered and diluted to 100 mL with deionized water. Analysis of heavy metals was performed using an ICP-MS 7900 instrument (Agilent, Santa Clara, California) and quantified using standard curves for each element. The contents of available calcium hydroxide, magnesium hydroxide, and sulfur were determined using volumetry according to the American Society for Testing and Materials C25-11 method (ASTM, 2001), and fluorine was determined by X-ray fluorescence in an ARL^{TM} 9900 Simultaneous-Sequential XRF series (Thermo Fisher Scientific, Waltham, MA, USA). The results were compared against the physicochemical specifications outlined in the Mexican standard governing the production of corn tortillas, NOM-187-SSA1/SCFI-2002 (Secretaría de Salud, 2003), and the Food Chemical Codex (United States Pharmacopeial Convention, 2010).

Tortillas were produced according to the traditional nixtamalization method (grain-masa-tortilla) described by Vázquez-Carrillo *et al.* (2016), which consisted of boiling 1.8 kg of grain with 3.6 L of water and 18 g of lime (1% to grain weight). The nixtamalization time ranged from 36 to 55 min. It was previously determined based on two parameters: 1) 40% moisture in the nixtamal and 2) the detachment of the pericarp when the nixtamal was rubbed between the index finger and the thumb. The nixtamal was left to rest for 16 h, after which the grain (nixtamal) was separated from the cooking liquor (nejayote), washed with distilled water, and ground in a volcanic stone mill. The dough was conditioned, stamped, and cooked in a mechanical tortilla machine (V-14 CR, Villamex, Jalisco, MEX). The tortillas were baked on hot metallic plates at 270°C for 20 s on each side. The tortillas were divided into three batches: 1) for testing color (in fresh tortillas and those stored at 4°C for 24 h), pH, and calcium content; 2) for microbiological testing (those stored at room temperature and 2°C), and 3) for sensory evaluation.

pH and color determinations in tortillas

The pH values of the nejayote, masa, and tortilla were measured using a pH meter (Corning Glass Works, Model 7, Corning, NY), following Method 02-52.01 by the AACC (AACC Approved Methods of Analysis, 2020). For color, three tortillas from each treatment were used for evaluation, focusing on the back of the tortilla blister. Measurements were performed using a colorimeter (HunterLab MiniScan, Reston, Virginia, USA). The variables' luminosity, a* and b* generated by the equipment, were used to calculate the saturation index ($SI = [a^2 + b^2]^{1/2}$) (Sant'Anna *et al.*, 2013).

Calcium analysis in tortillas

The quantification of calcium (Ca) content in grain and tortillas was conducted using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Optima 8300DV; Perkin-Elmer, USA) following the method described by Palacios-Rojas (2018).

Determination of fungi and yeasts during the storage period

Twenty-five grams of tortillas were collected at specified intervals during storage at 2 ± 1°C and 22 ± 2°C. After homogenization with 225 mL of 1% sterile peptone water, serial dilutions were prepared, and the 10^{-4} dilution was used. 200 μL of each sample was plated onto potato dextrose agar and incubated at 28°C for 5 days. Fungal colonies were counted and their concentrations were determined, with results expressed as colony-forming units per gram (CFU/g) of sample (BAM, 2001). Three experimental units, each consisting of a package of 20 tortillas, were used for each treatment. Fungal identification involved colony purification and microscopic observation using morphological keys.

Sensory acceptability test

This test was conducted in two sessions to avoid fatigue among the panelists; those who evaluated the white and blue corn tortillas were not the same. The white and blue corn tortillas were evaluated by panels consisting of 80 (45 women and 35 men, aged 18–60 years) and 35 people (22 women and 13 men, aged 19–75 years), respectively, all regular consumers of tortillas. In both cases, the panel was not trained. The attributes evaluated were rollability, color, aroma, flavor, taste, and texture. A five-point hedonic scale (1 = I dislike it very much; 5 = I like it very much) was used for the evaluation (Meilgaard *et al.*, 2016). Sensory analyses were performed following the Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods (Institute of Food Science & Technology, 2021). All panelists gave verbal consent to participate in the sensory evaluation.

Data analysis

Instrumental variables were analyzed using one-way analysis of variance in a completely randomized design, with Tukey's post hoc test ($P \leq 0.05$). Sensory evaluation variables were analyzed using the Kruskal–Wallis test ($P \leq 0.05$), and mean ranks were compared using the Mann–Whitney test ($P \leq 0.05$). In addition, principal component analysis was performed, incorporating both the sensory analysis and instrumental variables (color, pH, and calcium content). Data were analyzed using SPSS for Windows and RStudio.

Results and Discussion

Calcium hydroxide as a pH regulator

The highest purity limes (CH03-95 and CH04-95) met the specifications of NOM-187-SSA1/SCFI-2002 and Food Chemical Codex, with over 90% $Ca(OH)_2$ and acceptable levels, as well as traces of heavy metals (Table 1). In limes CH06-93, CH07-93, and CH02-79, elevated arsenic contents were found (Table 1), and in lime CH01-71, high levels of lead were detected and sulfur, which, although compliant with NOM-187-SSA1/SCFI-2002 (Pb 8 mg kg^{-1} maximum), did not meet the FCC standards.

The lime manufacturing process involves direct contact between the rock and the fuel, which in some cases is petroleum coke, which can cause contamination with various types of heavy metals (Galvan-Ruiz *et al.*, 2007). Since lime is a key component in nixtamalization, a process that produces different types of foods that are part of the diet in Mexico and several Central American countries, it is important to monitor its quality and heavy metal content to ensure public health safety.

In the nixtamalization process, the control treatments without lime recorded the lowest pH values in nejayote, masa, and tortillas (Table 2). This can be explained by the absence of calcium hydroxide, especially the $(OH)^-$ ions, which increase the alkalinity. Santiago-Ramos *et al.* (2018) reported higher pH values (6.5) in the cooking water of a lime-free control and corn nixtamalized with 1% lime (11.0). The difference in values with regard to what was obtained in the present study is attributed to the type of corn, water, and lime used by these authors. Sefa-Dedeh *et al.* (2004) reported a pH value of 6.68 in the cooking water of a lime-free control, which decreased to 4.98 after resting, a reduction attributed to fermentation during resting. Meanwhile, in nejayote with 1% lime, the initial pH was 11.81, which slightly increased to 11.95 after resting for 24 h.

In the treatments nixtamalized with $Ca(OH)_2$, the average pH values increased by over 100% compared to the lime-free control, due to the dissociation of $Ca(OH)_2$ in the cooking water (hot) into calcium (Ca^{2+}) and $(OH)^-$ ions. The latter is responsible for hydrolyzing hemicellulose (Muñoz-Hernández *et al.*, 1999), a component of the pericarp. Some Ca^{2+} and $(OH)^-$ ions become trapped in the hydrolyzed pericarp, while the other portion enters the maize grain. The $(OH)^-$ ions are responsible for increasing the pH of the masa and tortillas (Roque-Maciel *et al.*, 2016). It was found that in masa and tortillas prepared from blue corn, the pH values decreased slightly compared to those of white corn, which may be due to a higher pericarp detachment in the former (81.1% vs 70.8% in white corn, data not shown), contributing to the presence of a higher number of $(OH)^-$ ions in the

Table 1. Chemical composition of commercial limes and their compliance with NOM-187-SSA1/SCFI-2002 and Food Chemical Codex (FCC) requirements.

	Calcium hydroxide	Arsenic	Lead	Fluorine	Magnesium hydroxide	Sulfur
	%	(mg kg^{-1})			(%)	
NOM-187	90 min	3 max	8 max	40 max	5 max	
FCC	95 min	3 max	2 max	50 max	4.8 max	
CH03-95	94.61 ± 0.0^{ab}	0.89 ± 0.21^{c}	0.35 ± 0.22^{b}	19.20 ± 0.25^{e}	0.97 ± 0.02^{d}	0.07 ± 0.004^{a}
CH04-95	95.14 ± 0.37^{a}	1.07 ± 0.24^{bc}	0.32 ± 0.17^{b}	29.40 ± 0.12^{d}	0.66 ± 0.01^{e}	0.01 ± 0.0001^{a}
CH06-93	92.63 ± 0.18^{c}	2.87± 0.48^{b}	0.79 ± 0.27^{b}	36.50 ± 0.05^{b}	1.74 ± 0.10^{b}	0.02 ± 0.001^{a}
CH07-93	93.16 ± 0.19^{bc}	6.62 ± 0.13^{a}	0.22 ± 0.08^{b}	35.00 ± 0.11^{c}	0.61 ± 0.01^{e}	0.01 ± 0.001^{a}
CH01-71	70.69 ± 0.19^{d}	1.05 ± 0.01^{bc}	2.43 ± 0.35^{a}	39.20 ± 0.30^{a}	2.81 ± 0.04^{a}	0.55 ± 0.003^{a}
CH02-79	79.1 ± 0.74^{e}	7.27 ± 0.98^{a}	0.44 ± 0.28^{b}	37.20 ± 0.41^{b}	1.62 ± 0.04^{c}	0.02 ± 0.001^{a}
HSD	1.69	1.88	0.96	0.96	0.104	0.69

Means with the same letter between columns are not statistically different (Tukey, 0.05). HSD: honest significant difference.

Table 2. Effect of calcium hydroxide (CH) purity on the pH of nejayote, masa, and nixtamalized white and blue corn tortillas.

Lime	pH nejayote		pH masa		pH tortillas	
	Cwhite	Cblue	Cwhite	Cblue	Cwhite	Cblue
CH03-95	12.68 ± 0.01^{c}	12.76 ± 0.01^{ab}	8.64 ± 0.05^{a}	7.97 ± 0.03^{a}	8.31 ± 0.06^{a}	8.03 ± 0.05^{a}
CH04-95	12.76 ± 0.01^{b}	12.77 ± 0.0^{ab}	8.20 ± 0.01^{c}	8.01 ± 0.04^{a}	8.22± 0.03^{ab}	7.71 ± 0.11^{ab}
CH06-93	12.80 ± 0.01^{a}	12.79 ± 0.03^{a}	8.03 ± 0.01^{d}	7.79 ± 0.04^{a}	8.00 ± 0.01^{bc}	7.68 ± 0.04^{ab}
CH07-93	12.70 ± 0.01^{c}	12.69 ± 0.04^{b}	8.34 ± 0.01^{b}	7.26 ± 0.04^{bc}	8.15 ± 0.01^{abc}	7.28 ± 0.18^{c}
CH01-71	12.24 ± 0.01^{e}	12.31 ± 0.02^{c}	7.84 ± 0.01^{e}	7.18 ± 0.04^{c}	7.68 ± 0.04^{d}	7.13 ± 0.04^{c}
CH02-79	12.65 ± 0.01^{d}	12.68 ± 0.02^{b}	7.97 ± 0.01^{d}	7.47 ± 0.01^{b}	7.91 ± 0.0^{cd}	7.40 ± 0.04^{bc}
C_{wl}	5.76 ± 0.01^{f}	5.24 ± 0.03^{d}	6.02 ± 0.04^{f}	6.1 ± 0.01^{d}	6.14 ± 0.14^{e}	6.16 ± 0.02^{d}
HSD	0.032	0.092	0.097	0.253	0.242	0.366

Means with the same letter between columns are not statistically different (Tukey, 0.05). C: corn; C_{wl}: control without lime; HSD: honest significant difference.

nejayote, resulting in a reduction in pH in masa and tortillas (Sefa-Dedeh *et al.*, 2004).

According to Roque-Maciel *et al.* (2016), nejayote initially exhibits a high pH, which decreases by an average of 0.2 units following alkaline cooking. This reduction is attributed to the absorption of $(OH)^-$ and Ca^{2+} ions by the grain (Fernández-Muñoz *et al.*, 2004), resulting in a decline in the pH of the nejayote. This trend is also influenced by the higher concentration of available H^+ ions in the nejayote (Roque-Maciel *et al.*, 2016). The decrease in alkalinity can further be attributed to the leaching of hemicellulose components (Ruiz-Gutiérrez *et al.*, 2010), including uronic and phenolic acids (Gonzalez *et al.*, 2005), leading to a neutralization reaction of $Ca(OH)_2$. Additionally, Bartolo-Perez *et al.* (1999) suggest that Ca^{2+} ions penetrate and integrate into the pericarp as microcrystals of $CaCO_3$.

In both white and blue corns, nejayote with the highest pH was obtained from limes with the highest purity (CH03-95, CH04-95, CH06-93, and CH07-93). This was attributed to the higher concentration of $Ca(OH)_2$, which resulted in greater quantity of $(OH)^-$ ions. When nixtamalizing the same corn with a lime of lower purity (CH02-79), slightly lower pH values were recorded (12.65). The lowest pH value in the nejayote was observed with the lime of the lowest purity (CH01-71). Santiago-Ramos *et al.* (2018) reported lower pH values in nejayote (11) when 1% lime was used to nixtamalize white corn; whereas in blue corn, the pH was 12.1, which indicated that higher pH values were achieved with higher purity limes.

In the masa, a parallel trend to that observed in the nejayote was evident (Table 2). Limes with higher purity (CH03-95, CH04-95, CH06-93, and CH07-93) demonstrated elevated average pH levels (8.03–8.64), indicating the presence of $(OH)^-$ ions in the masa, particularly when contrasted with control values (Table 2). In particular, blue corn exhibited lower pH values (7.26–8.01). It is worth mentioning that white corn treatments retained a higher percentage of pericarp (29.2%), while blue corn treatments retained an average of 18.9%, potentially leading to greater leaching of $(OH)^-$ ions into the nejayote in the latter. Lastly, limes with lower purity (CH02-79 and CH01-71) yielded the lowest pH values in the masa for both corn varieties (Table 2). The pH values reported for fresh white corn masa vary between 8.6 and 11.2; however, the pH in masa depends on many factors, such as the percentage of lime used in nixtamalization and the number of times the nixtamal is washed; in some tortilla factories, it is a common practice to add small amounts of lime to the masa to increase its alkalinity and shelf life (López-Espíndola *et al.*, 2020).

Tortilla pH is strongly influenced by the amount of lime used during nixtamalization. The pH of tortilla increases with the increase in the amount of lime (Gutiérrez-Llanos *et al.*, 2023), and as evidenced in this study, tortilla pH also increases according to the purity of the calcium hydroxide; purer limes resulted in higher tortilla pH (Table 2). Serna-Saldivar and Chuck-Hernandez (2019) note that pH can also vary depending on the process employed, including traditional methods, nixtamalized flour, blends, nejayote rinsing, and resting time. This study observed the highest pH value in white tortillas (8.1), while it was 7.68 for blue tortillas. With both corn types, tortillas made with the purest limes (CH03-95, CH04-95, CH06-93, and CH07-93) recorded the highest pH, whereas those made with less pure limes (HC02-79 and HC01-71) exhibited lower pH values (Table 2). These values were lower than those reported by Vázquez-Carrillo *et al.* (2011) for tortillas prepared with fresh masa and nixtamalized flour, and for nixtamalized corn flour tortillas (Báez-Aguilar *et al.*, 2022). With blue corn tortillas, a pH of 7.9 was reported (Gutiérrez-Llanos *et al.*, 2023).

Calcium hydroxide alters the color of masa and tortillas

In the nixtamalized treatments, the luminosity value was lower in both types of corn (73.6 and 52.3%) compared to the control (Table 3), with a greater reduction observed during storage (2.6 and 4.2% in white and blue tortillas, respectively). It was also found that higher purity limes (93–95% $Ca[OH]_2$) caused a greater reduction in luminosity of both types of tortillas (Table 3); these changes suggest significant ($P \leq 0.05$) color changes induced by nixtamalization. White tortillas made with higher purity limes had higher saturation indexes (21.6–26.4) compared to lower purity limes (19.0–20.1) and the C_{wl}, indicating better color definition in tortillas made with higher purity limes. Tortillas made with blue corn showed an inverse behavior, with the highest saturation index in the C_{lw} treatment (8.0), followed by the CH03-95 and CH04-95 limes. After 24 h of storage, the luminosity values decreased in both types of tortillas; however, the most drastic changes occurred in the saturation index of the blue tortillas (Table 3). This is because anthocyanins, the pigment that gives blue corn its color, are highly sensitive to the alkaline pH generated during nixtamalization (López-Vásquez *et al.*, 2020). The appearance of white and blue tortillas is shown in Figure 1, where it can be seen that the color differences caused by the use of limes of different purity are more pronounced in tortillas made with blue corn.

According to Muñoz-Hernández *et al.* (1999), the color change of white corn during nixtamalization is attributed to flavonoid-O-glucosidic pigments. Due to their flavone structure, these pigments are colorless in neutral or acidic solutions. Under alkaline conditions, the hydroxyl group on the phenyl or benzopyrone ring is substituted, resulting in their flavonol structure and exhibition of a yellow hue. With increasing pH, the flavonoid acquires the characteristic red color of this pigment under alkaline conditions, and lime has been identified as the agent responsible for these changes, as demonstrated by Muñoz-Hernández *et al.* (1999). Color changes in tortillas have also been linked to starch gelatinization during cooking and formation of the starch-calcium complex (Valderrama-Bravo *et al.*, 2021). In addition, Salinas-Moreno *et al.* (2007) reported color changes in masa and tortillas due to oxidation of phenolic compounds in corn. Significant differences ($P \leq 0.05$) in color variables were observed in freshly made (0.5 h) and stored (24 h at 2°C) tortillas, indicating the impact of lime type on color. White control tortillas (C_{wl}) exhibited the highest luminosity and the lowest saturation index (Table 3), attributed to their slightly acidic pH (6.01), preserving pericarp flavonoids' color (Muñoz-Hernández *et al.*, 1999). In contrast, tortillas made with high-purity limes

Table 3. Effect of calcium hydroxide purity on color variables in white and blue corn tortillas, freshly made (0.5 h) and stored at 2°C (24 h).

Lime	Luminosity (0.5 h)		Saturation index (0.5 h)		Luminosity (24 h)		Saturation index (24 h)	
	Cwhite	Cblue	Cwhite	Cblue	Cwhite	Cblue	Cwhite	Cblue
CH03-95	72.6^{cd}	51.4^{de}	26.4^{a}	$6.1b^{c}$	69.2^{e}	47.5^{d}	28.5^{a}	6.1^{b}
	(0.4)	(0.5)	(0.1)	(0.04)	(0.1)	(0.3)	(0.1)	(0.4)
CH04-95	73.2^{c}	50.6^{e}	24.5^{b}	6.1^{b}	70.1^{c}	47.2^{d}	25.6^{b}	5.8^{bc}
	(0.2)	(0.04)	(0.2)	(0.04)	(0.1)	(0.08)	(0.1)	(0.09)
CH06-93	73.0^{cd}	52.8^{cd}	21.6^{c}	5.9^{bc}	70.1^{cd}	48.7^{c}	24.4^{c}	5.9^{b}
	(0.1)	(0.01)	(0.2)	(0.02)	(0.2)	(0.2)	(0.2)	(0.4)
CH07-93	72.2^{d}	50.1^{e}	23.4^{b}	5.1^{e}	69.5^{de}	44.4^{e}	25.4^{b}	4.9^{c}
	(0.3)	(0.0)	(0.2)	(0.02)	(0.5)	(0.41)	(0.5)	(0.17)
CH01-71	75.2^{b}	55.9^{b}	19.1^{de}	5.6^{d}	73.2^{b}	52.2^{b}	22.6^{d}	5.9^{b}
	(0.2)	(0.9)	(0.1)	(0.05)	(0.3)	(0.11)	(0.3)	(0.03)
CH02-79	75.5^{b}	53.3^{c}	20.1^{d}	5.9^{c}	73.4^{b}	48.7^{c}	21.9^{d}	5.6^{bc}
	(0.2)	(0.01)	(0.7)	(0.04)	(0.1)	(0.01)	(0.1)	(0.16)
C_{wl}	79.3^{a}	60.5^{a}	18.1^{e}	8.0^{a}	77.3^{a}	58.8^{a}	18.2^{e}	9.6^{a}
	(0.1)	(0.03)	(0.1)	(0.1)	(0.1)	(0.01)	(0.00)	(0.26)
HSD	0.96	1.51	1.16	0.21	0.69	0.88	0.927	0.95

Means with the same letter in the column are not statistically different (Tukey, 0.05). Values between parentheses are the standard deviations.
C: corn; C_{wl}: control without lime; HSD: honest significant difference.

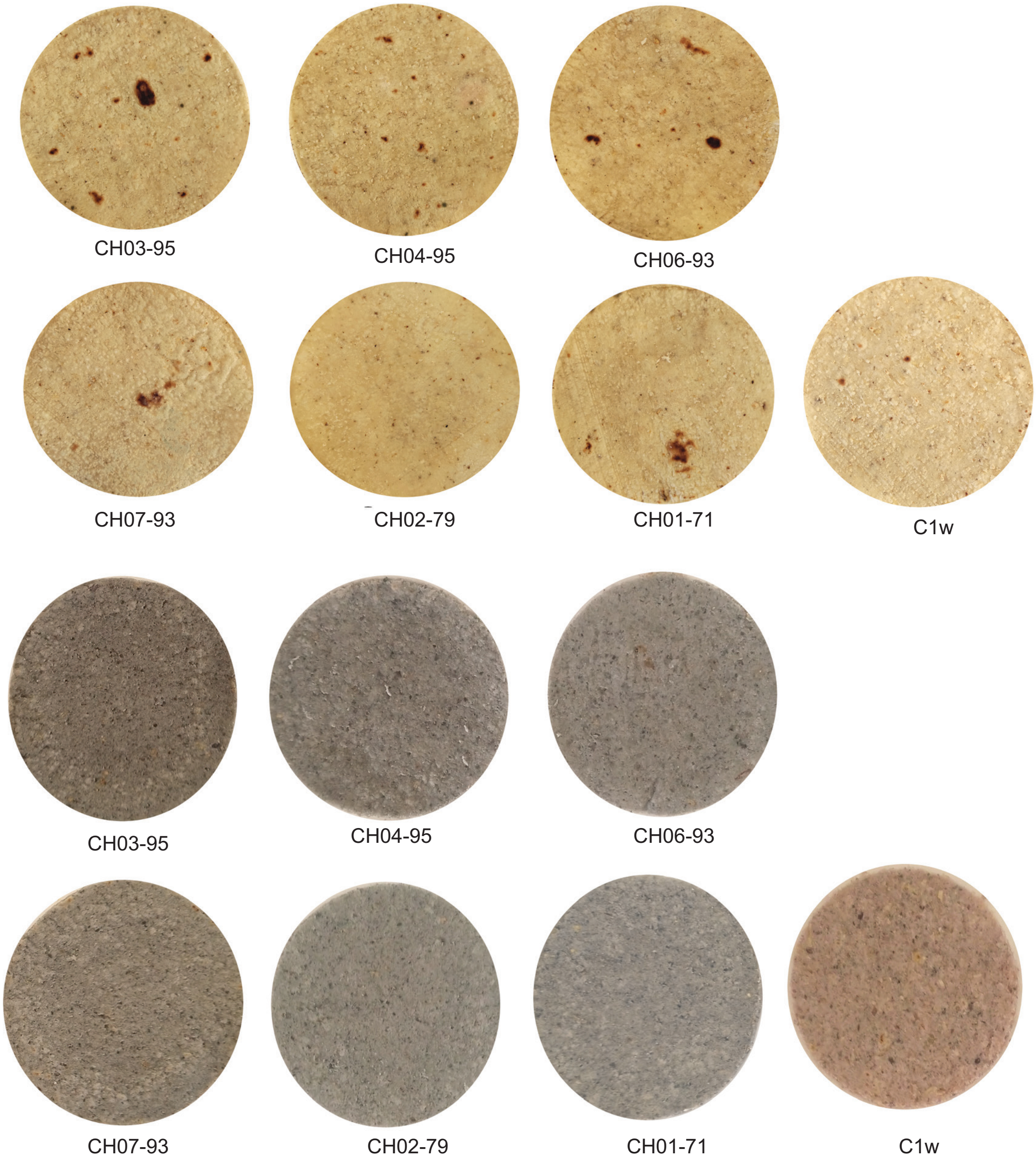
CH03-95 CH04-95 CH06-93
CH07-93 CH02-79 CH01-71 C1w
CH03-95 CH04-95 CH06-93
CH07-93 CH02-79 CH01-71 C1w

Figure 1. Effect of the use of limes with different degrees of purity on the appearance of tortillas made with white and blue corn.

(CH03-95 and CH04-95) were less luminous with a higher saturation index (SI), showcasing creamier, slightly reddish tones (Table 3). Conversely, tortillas made with lower purity limes were brighter with lower SI and less alkalinity (Table 2), consistent with the findings by Báez-Aguilar *et al.* (2022).

In the case of blue corn tortillas, the behavior was different. The anthocyanins, responsible for the color (Vázquez-Carrillo *et al.*, 2025), show structural changes depending on the pH values at which they are found; an intense red color is observed at pH 2, a red-pink color at pH 4–6, purple at pH 7–9, and green at pH 12 (Ahmed *et al.*, 2024). This study observed that the higher purity limes resulted in higher pH values, producing purple-colored tortillas, while the control treatment resulted in a pH value of 6, imparting a pink color (Figure 1). Gutiérrez-Llanos *et al.* (2023) reported similar results in tortillas made with blue corn, under different lime concentrations and higher $Ca(OH)_2$ contents. The tortillas were pink at low lime concentrations and had a luminosity of 47%, which decreased drastically as the lime concentration increased.

The above results highlight that lime enhances the color of corn tortillas. This function aligns with the category of color retention agents in the Additive Agreement (Secretaría de Gobernación, 2012).

Calcium hydroxide as a component in tortillas

Tortillas serve as the main source of calcium for a significant portion of the population in Mexico and Central America. They help promote bone health, especially among communities that rely on corn-based diets. The traditional nixtamalization method, where lime is one of the main ingredients, is an effective means of increasing available calcium intake; without this treatment, tortillas would not be a substantial source of calcium. In addition, calcium improves the textural characteristics of the masa by providing elasticity and cohesiveness (Topete-Betancourt *et al.*, 2020).

In this study, the calcium content in tortillas prepared with the different commercial limes ranged from 161 to 213.4 mg 100 g^{-1} (with a mean value of 232.06 mg 100 g^{-1}) (Figure 2), which is consistent with previously reported ranges for traditional nixtamalization (Salinas-Moreno *et al.*, 2024). Tortillas made with limes CH04-95, CH03-95, CH06-93, and CH07-93 had higher calcium content compared to limes CH01-71 and CH02-79, suggesting that higher purity lime enhances calcium absorption in the tortilla. Furthermore, white corn tortillas contain a higher calcium content than the blue variety. These differences could be due to the difference in the nixtamalization time, since white corn was nixtamalized for an average of 42.5 min as it is very hard, and blue corn was nixtamalized for only 35 min (data not shown). Thus, longer exposure to thermal and alkaline conditions during cooking could have favored the entry of more calcium, since the nixtamalization of both corn was done at the same temperature. Argun and Doğan (2017) observed a similar behavior, where different types of corn were nixtamalized at the same temperature but with two nixtamalization times (30 and 45 min). They found that out of the three of the five corns tested, after 9 h of steeping, those with longer nixtamalization time had higher ash content, which indicated a higher amount of calcium in the kernel.

A significant difference was observed in the calcium content between tortillas made with and without calcium hydroxide. The values in white tortillas ranged from 20.9 to 213.4 mg 100 g^{-1} and in blue tortillas from 26.4 to 187.4 mg 100 g^{-1} (Figure 2). The results confirm that calcium hydroxide in the nixtamalization process causes

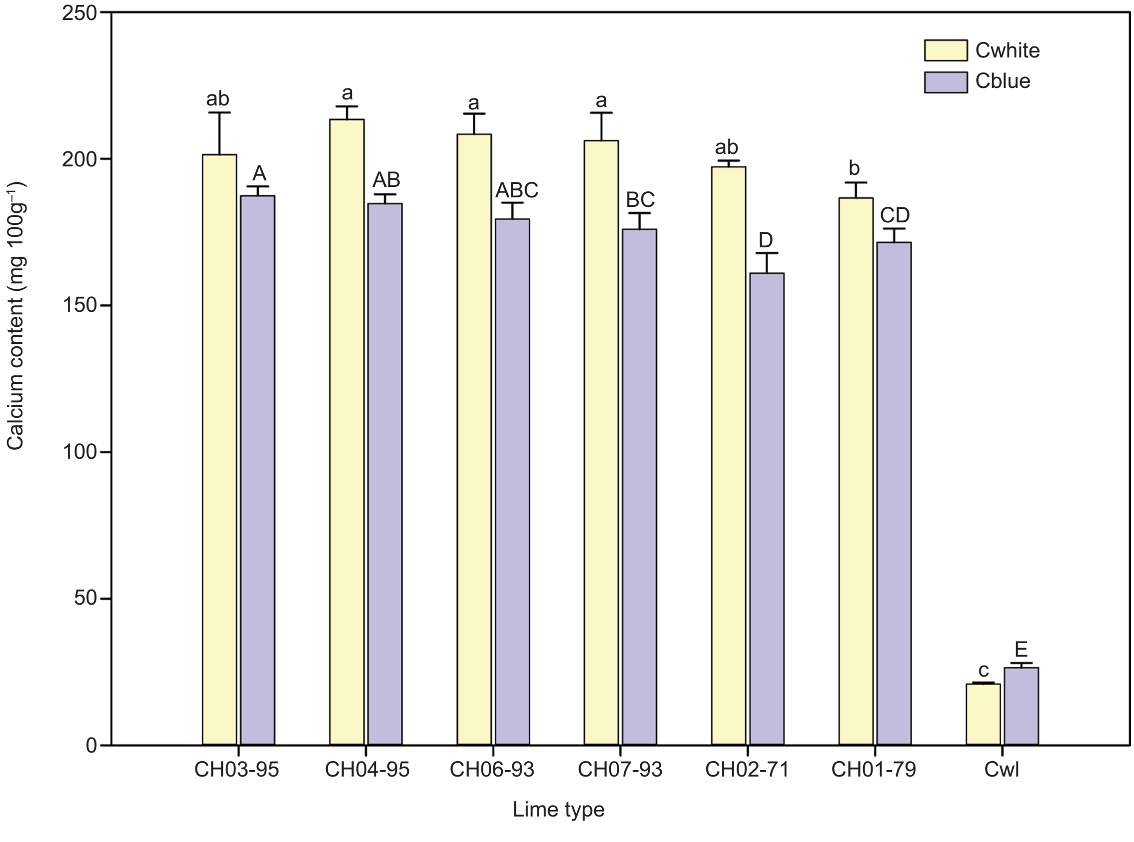

Figure 2. Calcium content of white and blue corn tortillas made with limes of different degrees of purity. Lower case letters compare white corn tortilla, capital letters compare blue corn tortilla. Means with the same letter are not statistically significant (Tukey, 0.05). C_{wl}: control without lime.

the added calcium to become a component of the tortilla as a final product.

Calcium hydroxide as a fungistatic agent in tortillas

The plate count method for white tortillas stored at room temperature (22°C) revealed significant differences ($P \leq 0.05$) among treatments (Table 4). Initially, tortillas from the control treatment without lime (C_{wl}) exhibited the highest colony-forming units (CFUs), while those nixtamalized with lime showed either minimal CFUs or remained within the NOM-187-SSA1/SCFI-2002 limits (<300 CFUs/g), indicating the role of lime in restricting microbial growth. By the third day, all treatments showed colony development, with the CH03-95 treatment demonstrating the lowest CFUs. Previous studies of commercial tortillas without preservatives have reported a shelf life of 3 days (Tellez-Giron *et al.*, 1988). By the second day, tortillas from the control group and CH01-71 treatment displayed stickiness and a sour odor, although visually devoid of colony development.

Fungi identified in tortillas made with different limes and stored at room temperature included *Fusarium oxysporum*, *Aspergillus flavus*, and *Rhizopus* spp. for CH01-71; *Penicillium viridicatum* for CH02-79; *Fusarium oxysporum* for CH04-95; *Aspergillus niger* for CH06-93; *Fusarium verticilloides* for CH07-93; and *Aspergillus flavus* for the control group without lime.

After 3 days of refrigeration, the treatment C_{wl} exceeded the CFU thresholds outlined in NOM-187-SSA1/SCFI-20021 (Table 4). Similarly, Heredia-Sandoval *et al.* (2021) reported that tortillas made with nixtamalized flour and no preservatives, stored at 2°C, had a shelf life of only 3 days. By the 11th day, all treatments with lime purities exceeding NOM-187-SSA1/SCFI-20021 standards had lower CFU content, demonstrating compliance with regulatory guidelines (Table 4). Conversely, treatments with lower lime purity (CH01-71 and CH02-79) exhibited high CFU counts.

The fungi identified during the refrigerated shelf-life evaluations were as follows: *Penicillium italicum* (CH04-95 and CH06-93), Aspergillus glaucus (Cwl, CH01-71, CH04-95, CH06-93, CH07-93), *Aspergillus oryzae* (Cwl, CH06-93, CH04-95, CH03-95), *Aspergillus niger* (CH03-95, CH06-93), *Aspergillus parasiticus* (CH01-71, CH06-93), and *Aspergillus flavus* (Cwl, CH02-79, CH03-95).

Most fungi found on tortillas produce mycotoxins, but contamination mainly occurs under specific conditions that combine high relative humidity with high temperature, grain moisture > 14% at harvest, and inadequate storage (Asghar *et al.*, 2020). In this sense, fungi growing on tortillas during storage can produce aflatoxins, however, their content could be low due to the lack of optimal conditions for their production. Ventura-Aguilar *et al.* (2022) reported aflatoxin levels between 2.4–3.2 $\mu g\ kg^{-1}$ in tortillas stored for 21 days at 4°C with up to 50% incidence of *Aspergillus flavus*, values below the limit set by Codex Alimentarius (2019) (5 $\mu g\ kg^{-1}$).

The difference in CFU counts observed between corn tortillas subjected to different treatments with or without lime highlights the role of calcium hydroxide as a food additive that performs the technological function of acting as a preservative with strong antifungal properties. These results show that lime preserves the masa and tortillas by increasing the pH, thus reducing the development of microorganisms and extending shelf life.

Sensory evaluation

In the sensory evaluation of both types of tortillas (white and blue), it was found that tortillas made with the highest purity limes (93 and 95 %) presented the highest values in all the attributes evaluated (Table 5). This trend suggests that lime purity significantly influences the sensory properties of tortillas, probably due to its higher alkalinizing effect, which improves pericarp solubility and starch gelatinization, thereby providing better tortilla characteristics.

Table 4. Effect of lime purity on the number of CFU*/g in white tortillas stored at 22 and 2°C.

Type of lime	Days of storage at 22°C		Days of storage at 2°C				
	0	3	3	6	9	11	15
CH03-95	ND	5.7×10^3	ND	ND	2.5×10^2	2.5×10^2	2.5×10^2
CH04-95	ND	1.0×10^4	ND	ND	2.5×10^2	2.5×10^2	2.5×10^2
CH06-93	ND	3.3×10^4	ND	ND	2.5×10^2	2.5×10^2	8.5×10^4
CH07-93	2.5×10^2	8.0×10^3	ND	ND	2.5×10^2	2.5×10^2	3.5×10^4
CH01-71	2.5×10^2	2.2×10^4	ND	ND	5.0×10^2	5.0×10^2	8.2×10^4
CH02-79	2.5×10^2	1.5×10^4	ND	ND	7.5×10^2	1.5×10^3	8.2×10^4
Cwl	5.0×10^2	2.7×10^4	5.0×10^2	7.5×10^2	1.5×10^3	1.5×10^3	9.3×10^4

*Colony-forming units. C_{wl}: control without lime; ND: not detected.

However, according to the statistical analysis, in the case of white tortillas, the best evaluated tortillas were those made with CH06-93 and CH07-93 limes, while in blue tortillas, the panelists found fewer differences.

Despite receiving lower ratings in all attributes, the control without lime stood out in the panelists' comments because its aroma reminded them of corn flavor, contrary to the previous findings by Cuevas-Martínez *et al.* (2010), who mentioned that if the amount of lime used in the nixtamalization process is not sufficient to give the tortilla its flavor characteristics, it will be rejected by consumers. On the other hand, the highest ratings in the color attribute were due to a higher intensity of yellow, as expressed by the panelists. In this sense, Cuevas-Martínez *et al.* (2010) indicate that the higher the lime concentration, the more intense the yellow color of the tortillas.

In the principal component analysis, the first two components explained 85 and 91.5% of the variability in the data for white and blue corn tortillas, respectively (Figure 3). This analysis revealed that tortillas made with the highest purity limes had the highest calcium content, elevated pH values, and the best sensory characteristics. This suggests that calcium content significantly influences the pH of the tortillas, and consequently, their color acceptance by the panelists. Conversely, treatments with lower lime purity did not exhibit strong characteristics across the evaluated variables, indicating that lime purity affects both the technological properties of the tortillas and their sensory perception. The control treatment was located next to the luminosity vector and had the highest value for this parameter, since no agent modified the color of the tortillas. The differences observed in the rollability, taste, aroma, and texture of corn tortillas among the different lime treatments, as perceived by the panelists, affirm that lime fulfills the technological functions of a masa conditioning agent, and a flavor enhancer and modifier.

Conclusions

Commercial calcium hydroxides of 93 and 95% purities available in Mexico meet the specifications of NOM-187-SSA1/SCFI-2002. In addition, limes of higher purity had the highest pH in nejayote, masa, and tortillas, as well as

Table 5. Comparison of sensory attributes of corn tortillas made with commercial limes of different purity grades based on the Kruskal–Wallis test.

Treatment		White tortilla					Blue tortilla				
		Rollability	Color	Aroma	Taste	Texture	Rollability	Color	Aroma	Taste	Texture
CH03-95	Median	4.0	4.0	3.0	3.0	3.0	5	5	4	4	4
	Mode	4.0	4.0	3.0	3.0	4.0	5	5	4	4	5
	Mean rank	236.4^{b}	274.4^{a}		257.46^{abc}	240.7^{bc}	124.2^{ab}	163.1^{a}		138.1^{a}	134.4^{ab}
CH04-95	Median	4.0	4.0	3.0	4.0	4.0	5	4	4	4	4
	Mode	4.0	4.0	3.0	3.0	3.0	5	4	4	4	4
	Mean rank	257.9^{b}	285.7^{a}		260.49^{ab}	264.7^{abc}	143.6^{a}	131.5^{ab}		134.9^{a}	120.3^{ab}
CH06-93	Median	4.0	4.0	3.5	4.0	4.0	5	4	4	4	4
	Mode	5.0	4.0	3.0	4.0	4.0	5	4	4	4	4
	Mean rank	309.8^{a}	259.7^{ab}		276.92^{a}	304.4^{a}	144.9^{a}	121.3^{b}		124.3^{ab}	129.9^{ab}
CH07-93	Median	4.0	4.0	3.0	4.0	4.0	4	4	4	4	4
	Mode	4.0	4.0	3.0	4.0	4.0	4	5	4	4	5
	Mean rank	272.6^{ab}	268.0^{a}		280.93^{a}	288.0^{ab}	100.1^{b}	133.8^{ab}		121.5^{ab}	129.2^{ab}
CH01-71	Median	4.0	3.0	3.0	3.0	3.0	4	4	4	4	4
	Mode	4.0	3.0	3.0	3.0	4.0	5	5	4	3	5
	Mean rank	214.2^{bc}	204.8^{bc}		210.9^{bc}	230.1^{c}	108.7^{ab}	125.1^{ab}		113.6^{ab}	112.6^{ab}
CH02-79	Median	4.0	3.0	4.0	3.0	3.0	5	4	4	4	4
	Mode	5.0	3.0	4.0	3.0	4.0	5	4	5	4	4
	Mean rank	247.2^{b}	232.9^{bc}		229.29^{bc}	227.7^{c}	138.4^{ab}	116.3^{b}		141.9^{a}	144.3^{a}
C_{wl}	Median	3.0	3.0	3.0	3.0	2.0	4	3	4	3	3
	Mode	4.0	2.0	3.0	3.0	3.0	5	4	5	3	2
	Mean rank	179.8^{c}	192.6^{c}		202.84^{c}	162.8^{d}	100.8^{b}	69.8^{c}		86.5^{b}	90.1^{b}

Different letters in the same column indicate a significant statistical difference (Mann–Whitney 0.05). C_{wl}: control without lime.

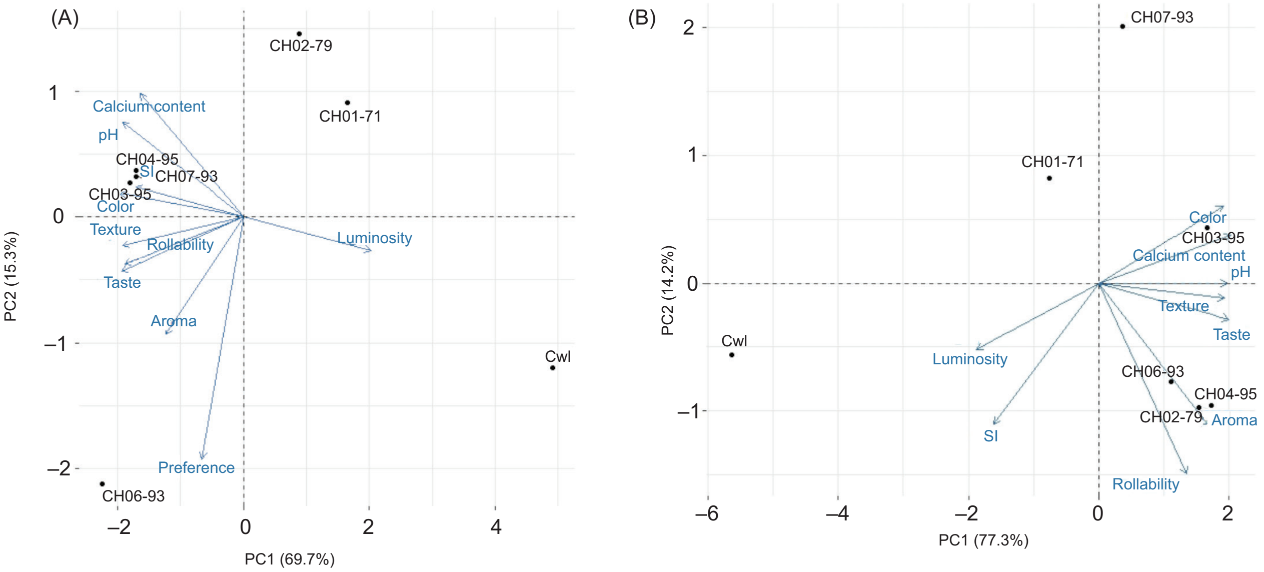

Figure 3. Principal components of sensory evaluation and instrumental variables measured in tortillas prepared using lime of different purity. (A) white corn tortilla, (B) blue corn tortilla. PC1 principal component 1, PC2 principal component 2.

the highest color intensity, increased calcium content in tortillas, and extended shelf life in refrigeration up to 15 days, and thus they could be considered as food additives in tortilla production. Conversely, limes with lower purity levels (71 and 76%) exhibit higher magnesium hydroxide, arsenic, lead, and fluorine contents, resulting in tortillas with lower pH, lighter color, and shorter shelf life.

According to the results, manufacturers of food-grade lime must ensure high purity to obtain safe and quality food. Thus, countries that adopt the process of nixtamalization with lime should pay special attention to the purity of the lime used in their processes, since it modifies the characteristics of the final product.

Ethical Approval

All sensory evaluation participants gave verbal consent before the start of the test and were allowed to withdraw from the study at any time without a reason. The products tested were safe for consumption.

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Authors Contributions

María Gricelda Vázquez-Carrillo contributed to the validation, formal analysis, investigation, resources, writing of the original draft, supervision, project administration, and funding acquisition. Natalia Palacios-Rojas was involved in the investigation, resources, review and editing of the draft manuscript, project administration, and funding acquisition. Rafael Mier-Sainz Trapaga contributed to the conceptualization, resources, review and editing of the draft manuscript, project administration, and funding acquisition. Leticia García-Cruz contributed to the methodology, formal analysis, investigation, writing, review, and editing of the original draft, and visualization. Gabriela Rosas-Zamora: Methodology, Formal analysis, Investigation. Aldo Rosales-Nolasco was involved in the methodology, formal analysis, investigation, and writing of the original draft. Lizeth Carolina Santillano-Gómez contributed to the study methodology and investigation.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study article.

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