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Research Article

Assessment and Relationship between Chemical Composition and Microbial Load in Corn Tortillas Sampled from Different Vending Points in Two Regions of Mexico

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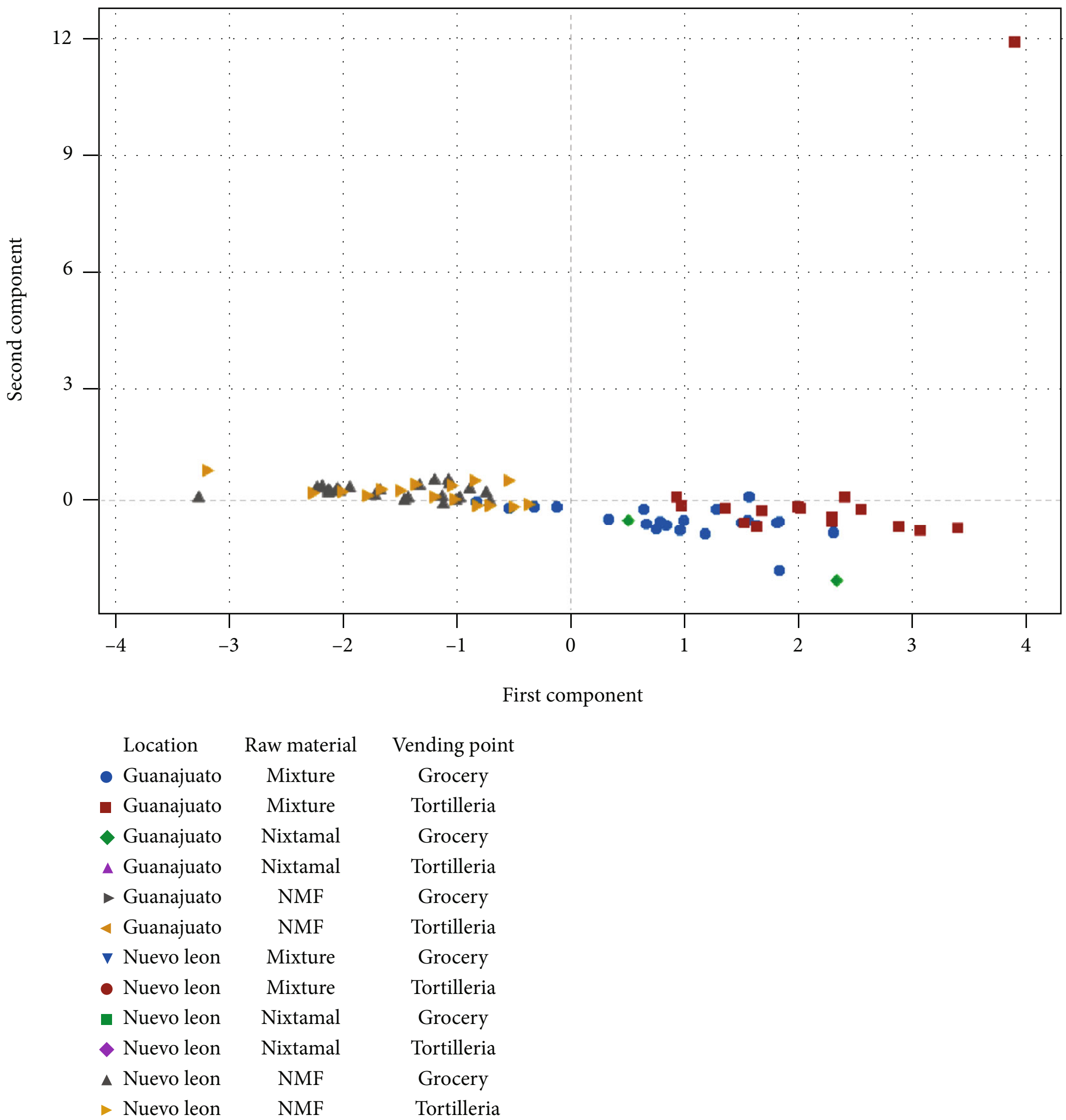
During tortilla production, different heating stages help to ensure low bacterial loads that contribute to their safety. However, there is an increasing concern about these products' stability. This work is aimed at analyzing corn tortillas collected at different vending points from two regions of the country, Nuevo Leon and Guanajuato. A total of 80 samples were analyzed, and the bacterial presence was related to the origin of the raw materials. Guanajuato region resulted with the highest concentration (6.04 ± 0.07 Log UFC/g). Tortillas made with nixtamalized corn masa flour (NMF) had significantly lower microbial loads ($p < 0.05$) than those made with 100% nixtamal masa or mixtures of NMF/nixtamal masa. In all samples, there were no pathogenic bacteria. On 28 selected samples, through PCA analysis, a strong influence of the microbial loads (which include foodborne bacteria) and their changes in chemical composition being reflected in the free sugars, free amino nitrogen (FAN), and peroxides contents, respectively, could be related with the vending point good manufacturing practices.

1. Introduction

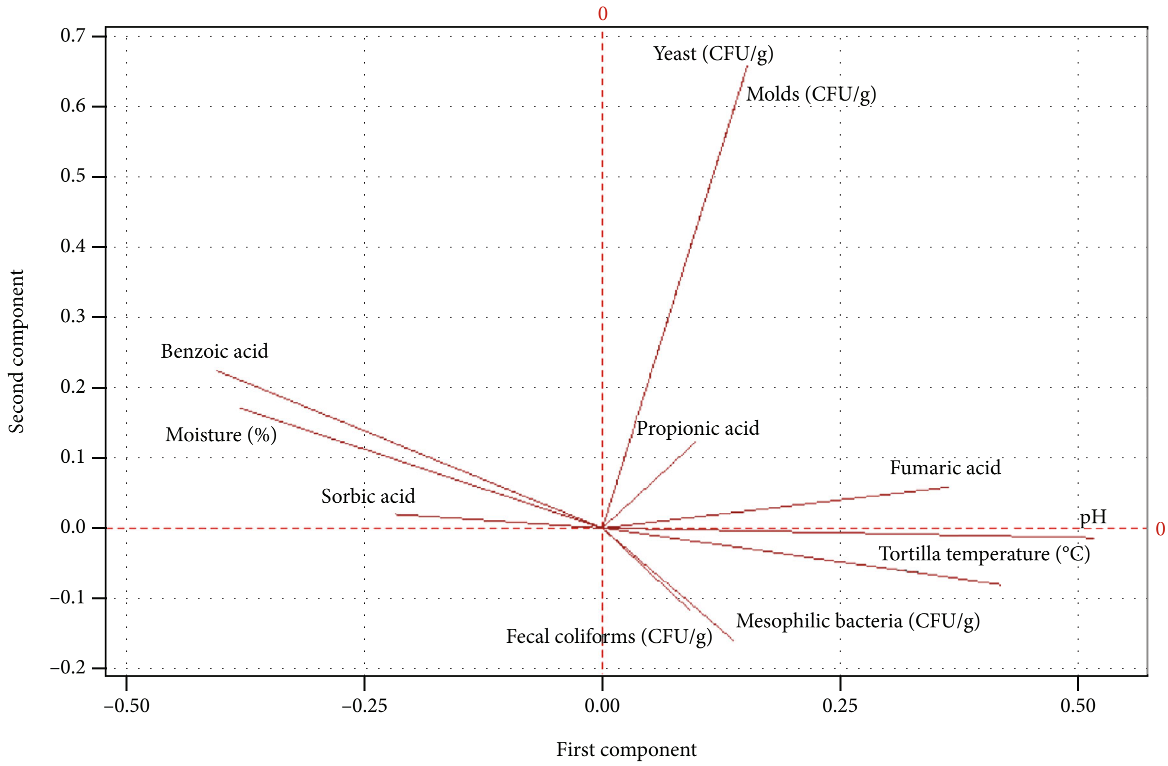
Maize is Mexico's most consumed staple grain, with up to 12 million tons annually processed for the manufacturing of direct foods [1]. It is processed and consumed in many varied presentations, which contributes to preserving the population's gastronomic heritage while providing reliable access to sufficient quantities of affordable and nutritious food. Undoubtedly, maize tortillas are the culinary preparation that is most consumed, with a *per capita* annual estimated consumption of 79.5 kg in rural and 56.7 kg in urban areas [2]. In Mexico, tortillas represent an important part of our cultural identity and the major contribution of calories to the diet. During tortilla production, mature corn kernels are alkali-cooked and steeped and then ground and formed into thin circles in preparation for a high temperature and relatively short baking. During these sequential steps, many molecular and structural changes promote interactions relevant to tortillas' functional, nutritional, and microbiological characteristics [3].

In the last decades, the industrialization of maize kernels has diversified into different categories aimed to produce various foods with different colors, textures, microbial shelf life, and flavor. Tortillas are prepared from masa of nixtamalized maize, either fresh or flours (NMF), that in both cases are manufactured from corn kernels that undergo alkaline cooking. Nixtamalization is a hydrothermal process that promotes the necessary molecular interactions to achieve the tortillas' desired physicochemical and functional characteristics. This process improves the bioavailability of the kernel nutrients, particularly proteins and carbohydrates [4, 5]. Besides the three basic ingredients, maize, water, and food-grade lime, in recent years, tortilla producers have employed different additives with different functions, including texture, flavor, and microbiological control, to guarantee shelf-life expectations and to meet the customer needs and the market conditions. The dry masa flour industry enriches flours with vitamins and minerals to improve the nutritional profile and to comply with current government regulations [6, 7].



(a)

Figure 1: Continued.



(b)

Figure 1: Principal component analysis of 80 tortilla samples collected in two regions of México. (a) Scatterplot and (b) loading plot.

In tortilla shops (Tortillerias), tortillas are produced under a general understanding of good manufacturing practices (GMPs), promoted and ruled by the governmental health authority (NOM-251-SSA1-2009). Traditionally, this product is manufactured using masa, obtained by *in situ* wet grinding of nixtamalized corn. In other cases, the masa is supplied by specialized shops dedicated solely to producing masa for distribution. This occurs in most rural areas or small towns of the country, where strong consumption habits are rooted. The product's physicochemical and textural characteristics depend on the masa producers' processing methods. In these cases, tortilla producers adopt local practices for product manufacturing that appeal to the consumer flavor, texture, and color preferences; this is achieved by using 100% nixtamal masa or by preparing mixtures with NMF [4]. Such mixtures or local variations present chemical, functional, and organoleptic differences and even product dimensions that are noticeable by the consumers [5].

Currently, in urban areas, consumers can either buy NMF or RTE product that is commonly offered in supermarkets. Despite this, the vast majority of tortilla consumption is either in Tortillerias or in convenience stores, where the product is offered in individual packages, often of 1 kg, wrapped in kraft paper and in a plastic bag stored inside cooling boxes that keep tortillas at a temperature of ≈30°C.

In this regard, there is a growing concern about the preservation of these products since the rise of the working population decreases *in situ* purchases directly from the Tortillerias; for this, their sales in convenience stores have become a common practice. These changes in consumption have promoted the interest in the microbial changes and susceptibility of spoilage during its commercialization [4]. Due to their composition, corn tortillas are prone to bacterial spoilage since they contain high moisture or Aw and are a rich source of nutrients that can be utilized as substrate by diverse microorganisms, such as bacteria, molds, and yeasts, that are prevalent in open environments [5].

The ability of these organisms to attack many foods is due mainly to their relatively versatile environmental requirements. Although most yeasts and molds are obligate aerobes (which require free oxygen for growth), their acidity/alkalinity requirement for development is quite broad, ranging from a pH of 2 to above pH 9. Their temperature range (10-35°C) is also broad, with a few species capable of growth below or above this range. Moisture requirements of foodborne molds are relatively low; most species can grow at a water activity (aw) of 0.85 or less, although yeasts generally require a higher water activity. Both yeasts and molds can invade and grow virtually any food at any time; they invade crops such as grains, nuts, beans, and fruits in fields before harvesting and storage [8]. They also grow on processed foods and food mixtures, although their detectability in or on foods depends on the food type and the bioavailability of nutrients that, once contaminated, could produce decomposition, being visible by rot spots of various sizes and colors, unsightly scabs, slime, white cottony mycelium, or highly colored sporulating mold; abnormal flavors and odors may also be produced [9]. Occasionally, a food

Table 1: Proximal analysis of selected tortilla samples obtained in two regions of Mexico.

Sample	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)	Carbohydrates (%)
Low count					
Nuevo Leon 11	36.42 ± 0.37^{a}	8.92 ± 0.13^{a}	2.60 ± 0.54^{a}	5.93 ± 0.62^{a}	82.55 ± 0.23^{a}
Nuevo Leon 12	36.06 ± 0.38^{a}	8.55 ± 0.50^{a}	2.38 ± 0.30^{a}	6.09 ± 0.48^{a}	82.97 ± 0.16^{a}
Guanajuato 17	35.78 ± 0.42^{a}	9.22 ± 0.51^{a}	2.92 ± 0.33^{a}	5.79 ± 0.31^{a}	82.07 ± 0.57^{a}
Guanajuato 18	36.40 ± 0.51^{a}	8.90 ± 0.51^{a}	3.26 ± 0.14^{a}	5.91 ± 0.37^{a}	81.93 ± 0.38^{a}
High count					
Nuevo Leon 3	36.47 ± 0.47^{a}	8.85 ± 0.45^{a}	2.76 ± 0.48^{a}	5.50 ± 0.12^{a}	82.89 ± 0.68^{a}
Nuevo Leon 6	36.31 ± 0.48^{a}	8.71 ± 0.48^{a}	2.53 ± 0.36^{a}	5.94 ± 0.68^{a}	82.83 ± 0.68^{a}
Nuevo Leon 8	36.22 ± 0.67^{a}	8.78 ± 0.59^{a}	2.84 ± 0.38^{a}	5.66 ± 0.60^{a}	82.73 ± 0.52^{a}
Nuevo Leon 11	36.53 ± 0.58^{a}	9.28 ± 0.37^{a}	2.62 ± 0.35^{a}	6.22 ± 0.14^{a}	81.88 ± 0.30^{a}
Nuevo Leon 14	36.22 ± 0.59^{a}	8.78 ± 0.59^{a}	2.52 ± 0.26^{a}	5.36 ± 0.17^{a}	83.34 ± 0.61^{a}
Nuevo Leon 16	36.00 ± 0.30^{a}	8.55 ± 0.61^{a}	3.22 ± 0.37^{a}	5.87 ± 0.54^{a}	82.35 ± 0.11^{a}
Nuevo Leon 18	36.14 ± 0.53^{a}	8.62 ± 0.41^{a}	3.08 ± 0.67^{a}	5.69 ± 0.61^{a}	82.61 ± 0.16^{a}
Nuevo León 22	36.41 ± 0.62^{a}	9.35 ± 0.43^{a}	3.26 ± 0.56^{a}	5.48 ± 0.38^{a}	81.92 ± 0.19^{a}
Nuevo Leon 26	36.56 ± 0.14^{a}	9.33 ± 0.45^{a}	3.18 ± 0.37^{a}	5.38 ± 0.13^{a}	82.10 ± 0.33^{a}
Nuevo Leon 28	36.24 ± 0.26^{a}	8.86 ± 0.29^{a}	3.14 ± 0.36^{a}	6.22 ± 0.16^{a}	81.78 ± 0.47^{a}
Nuevo Leon 33	35.75 ± 0.61^{a}	8.54 ± 0.60^{a}	2.33 ± 0.59^{a}	5.57 ± 0.56^{a}	83.56 ± 0.11^{a}
Nuevo Leon 36	36.02 ± 0.31^{a}	8.79 ± 0.59^{a}	3.00 ± 0.24^{a}	5.27 ± 0.59^{a}	82.94 ± 0.38^{a}
Guanajuato 2	35.69 ± 0.26^{a}	8.41 ± 0.20^{a}	3.04 ± 0.40^{a}	5.91 ± 0.56^{a}	82.64 ± 0.42^{a}
Guanajuato 20	36.16 ± 0.54^{a}	8.42 ± 0.43^{a}	2.48 ± 0.12^{a}	5.90 ± 0.40^{a}	83.21 ± 0.62^{a}
Guanajuato 25	35.90 ± 0.27^{a}	8.85 ± 0.39^{a}	2.79 ± 0.58^{a}	5.81 ± 0.23^{a}	82.54 ± 0.56^{a}
Guanajuato 26	36.15 ± 0.14^{a}	9.35 ± 0.64^{a}	2.39 ± 0.22^{a}	5.77 ± 0.51^{a}	82.49 ± 0.66^{a}
Guanajuato 27	36.08 ± 0.18^{a}	8.95 ± 0.17^{a}	2.54 ± 0.47^{a}	5.90 ± 0.23^{a}	82.60 ± 0.61^{a}
Guanajuato 29	36.11 ± 0.30^{a}	8.48 ± 0.35^{a}	3.03 ± 0.20^{a}	5.72 ± 0.41^{a}	82.76 ± 0.19^{a}
Guanajuato 33	36.59 ± 0.50^{a}	8.77 ± 0.69^{a}	2.38 ± 0.38^{a}	5.43 ± 0.21^{a}	83.41 ± 0.66^{a}
Guanajuato 34	36.56 ± 0.21^{a}	8.76 ± 0.42^{a}	2.84 ± 0.33^{a}	5.88 ± 0.67^{a}	82.52 ± 0.14^{a}
Guanajuato 37	35.96 ± 0.67^{a}	8.57 ± 0.49^{a}	2.40 ± 0.39^{a}	6.24 ± 0.67^{a}	82.78 ± 0.39^{a}
Guanajuato 38	35.99 ± 0.22^{a}	8.87 ± 0.38^{a}	3.23 ± 0.47^{a}	5.32 ± 0.40^{a}	82.59 ± 0.40^{a}
Guanajuato 39	36.15 ± 0.22^{a}	9.34 ± 0.12^{a}	3.15 ± 0.38^{a}	6.25 ± 0.53^{a}	81.26 ± 0.24^{a}
Guanajuato 40	35.70 ± 0.14^{a}	9.15 ± 0.51^{a}	3.08 ± 0.31^{a}	6.14 ± 0.39^{a}	81.64 ± 0.21^{a}

The results are the average of three replicates ± std. deviation. Different letters in the same column reflect significant differences ($p > 0.05$).

appears mold-free but is found upon mycological examination to be contaminated. Contamination of foods by yeasts and molds can result in substantial economic losses to producers, processors, and consumers and significantly impact the family economy.

Consumers nowadays expect simple nutrition or low prices when purchasing foods. Understanding preferences and the traditional preparation and process of the corn tortilla will help improve the commercial production processes to effectively offer consumers good products with the authenticity of sensory quality.

For decades, different efforts to improve the nutritional value of nixtamalized products have been made, often enhancing the bioavailability of macromolecules. Although food security policies aimed to protect the health and well-being of the consumer do not always accompany such strategies, in developing countries, they become a public health concern, such as the mandatory fortification of micronutrients in nixtamalized corn masa flour, established in the government regulation NOM-247-SSA1-2008.

The objective of this work was to analyze corn tortillas obtained in tortilla shops (Tortillerias) or at external points of sale in two regions of Mexico, Nuevo León (Monterrey metropolitan area) and Guanajuato (Silao and its surroundings), to understand the factors that influence the microbial growth and its relationship with the product physicochemical characteristics.

2. Materials and Methods

For this study, 80 samples of corn tortillas were collected in two Municipalities in Mexico. The selection criteria were random in tortilla shops (Tortillerias) and external points of sale (grocery stores). A total 40 samples were acquired in each location, Nuevo León and Guanajuato, of which 16 samples were from Tortillerias and 24 were from external selling points in each location. Records of the establishments were collected but not displayed in this study, along with municipality, raw material type, either nixtamalized corn masa flour (NMF), mixtures (NMF/nixtamal masa), and

Table 2: Carbohydrate fraction analysis of selected tortilla samples obtained in two regions of Mexico.

Sample	Starch (%)	Amylose (%)	Amylopectin (%)	SF (%)	IF (%)	TDF (%)
Clean						
Nuevo Leon 11	75.80 ± 0.20^{a}	21.43 ± 0.27^{h}	78.57 ± 0.20^{a}	0.51 ± 0.39^{a}	3.40 ± 0.19^{a}	3.90 ± 0.10^{a}
Nuevo Leon 12	75.88 ± 0.54^{a}	22.44 ± 0.40^{h}	77.56 ± 0.26^{a}	0.51 ± 0.49^{a}	3.38 ± 0.48^{a}	3.89 ± 0.64^{a}
Guanajuato 17	74.81 ± 0.69^{a}	21.64 ± 0.53^{h}	78.36 ± 0.32^{a}	0.56 ± 0.11^{a}	3.76 ± 0.31^{a}	4.32 ± 0.23^{a}
Guanajuato 18	74.48 ± 0.28^{a}	21.37 ± 0.63^{h}	78.63 ± 0.40^{a}	0.54 ± 0.60^{a}	3.59 ± 0.69^{a}	4.13 ± 0.67^{a}
Not clean						
Nuevo Leon 3	72.02 ± 0.37^{b}	24.36 ± 0.24^{g}	75.64 ± 0.43^{b}	0.55 ± 0.28^{a}	3.69 ± 0.68^{a}	4.25 ± 0.51^{a}
Nuevo Leon 6	71.78 ± 0.65^{b}	25.22 ± 0.19^{f}	74.78 ± 0.28^{c}	0.47 ± 0.38^{a}	3.16 ± 0.14^{a}	3.64 ± 0.49^{a}
Nuevo Leon 8	70.17 ± 0.66^{b}	27.78 ± 0.12^{c}	72.22 ± 0.23^{d}	0.54 ± 0.30^{a}	3.62 ± 0.29^{a}	4.16 ± 0.37^{a}
Nuevo Leon 11	72.98 ± 0.17^{b}	27.83 ± 0.42^{c}	72.17 ± 0.18^{d}	0.50 ± 0.12^{a}	3.37 ± 0.42^{a}	3.88 ± 0.53^{a}
Nuevo Leon 14	71.74 ± 0.12^{b}	28.07 ± 0.69^{c}	71.93 ± 0.51^{e}	0.53 ± 0.53^{a}	3.53 ± 0.64^{a}	4.05 ± 0.57^{a}
Nuevo Leon 16	72.41 ± 0.20^{b}	29.26 ± 0.51^{b}	70.74 ± 0.52^{f}	0.48 ± 0.52^{a}	3.24 ± 0.11^{a}	3.72 ± 0.66^{a}
Nuevo Leon 18	71.63 ± 0.32^{b}	27.29 ± 0.48^{c}	72.71 ± 0.18^{d}	0.52 ± 0.19^{a}	3.48 ± 0.66^{a}	4.00 ± 0.68^{a}
Nuevo León 22	70.70 ± 0.33^{b}	27.35 ± 0.14^{c}	72.65 ± 0.39^{d}	0.54 ± 0.36^{a}	3.62 ± 0.43^{a}	4.16 ± 0.46^{a}
Nuevo Leon 26	72.32 ± 0.21^{b}	28.26 ± 0.31^{c}	71.74 ± 0.33^{e}	0.53 ± 0.33^{a}	3.53 ± 0.57^{a}	4.06 ± 0.25^{a}
Nuevo Leon 28	70.60 ± 0.23^{b}	28.20 ± 0.48^{c}	71.80 ± 0.32^{e}	0.49 ± 0.22^{a}	3.30 ± 0.43^{a}	3.79 ± 0.41^{a}
Nuevo Leon 33	71.43 ± 0.62^{b}	26.61 ± 0.56^{d}	73.39 ± 0.55^{c}	0.52 ± 0.11^{a}	3.48 ± 0.21^{a}	3.99 ± 0.47^{a}
Nuevo Leon 36	70.83 ± 0.28^{b}	29.58 ± 0.25^{b}	70.42 ± 0.56^{f}	0.50 ± 0.18^{a}	3.37 ± 0.12^{a}	3.87 ± 0.14^{a}
Guanajuato 2	69.44 ± 0.46^{c}	26.64 ± 0.33^{d}	73.36 ± 0.39^{c}	0.58 ± 0.64^{a}	3.85 ± 0.61^{a}	4.43 ± 0.54^{a}
Guanajuato 20	71.24 ± 0.54^{b}	24.80 ± 0.54^{g}	75.20 ± 0.67^{b}	0.56 ± 0.16^{a}	3.72 ± 0.11^{a}	4.28 ± 0.31^{a}
Guanajuato 25	72.44 ± 0.56^{b}	27.86 ± 0.28^{c}	72.14 ± 0.47^{d}	0.48 ± 0.35^{a}	3.21 ± 0.50^{a}	3.69 ± 0.41^{a}
Guanajuato 26	72.42 ± 0.67^{b}	27.79 ± 0.34^{c}	72.21 ± 0.17^{d}	0.48 ± 0.33^{a}	3.23 ± 0.60^{a}	3.71 ± 0.40^{a}
Guanajuato 27	71.32 ± 0.30^{b}	29.85 ± 0.20^{b}	70.15 ± 0.69^{f}	0.56 ± 0.11^{a}	3.72 ± 0.28^{a}	4.27 ± 0.27^{a}
Guanajuato 29	70.23 ± 0.65^{b}	29.68 ± 0.55^{b}	70.32 ± 0.49^{f}	0.49 ± 0.20^{a}	3.25 ± 0.46^{a}	3.74 ± 0.52^{a}
Guanajuato 33	71.16 ± 0.43^{b}	31.89 ± 0.24^{a}	68.11 ± 0.64^{g}	0.52 ± 0.34^{a}	3.50 ± 0.59^{a}	4.02 ± 0.41^{a}
Guanajuato 34	71.51 ± 0.11^{b}	28.90 ± 0.59^{c}	71.10 ± 0.11^{e}	0.55 ± 0.37^{a}	3.67 ± 0.58^{a}	4.22 ± 0.15^{a}
Guanajuato 37	72.03 ± 0.38^{b}	28.67 ± 0.14^{c}	71.33 ± 0.29^{e}	0.53 ± 0.42^{a}	3.56 ± 0.41^{a}	4.10 ± 0.61^{a}
Guanajuato 38	70.70 ± 0.48^{b}	28.97 ± 0.59^{c}	71.03 ± 0.42^{e}	0.58 ± 0.61^{a}	3.90 ± 0.32^{a}	4.48 ± 0.10^{a}
Guanajuato 39	71.04 ± 0.16^{b}	30.46 ± 0.23^{a}	69.54 ± 0.42^{g}	0.46 ± 0.37^{a}	3.09 ± 0.20^{a}	3.55 ± 0.13^{a}
Guanajuato 40	70.78 ± 0.62^{b}	28.26 ± 0.38^{c}	71.74 ± 0.50^{e}	0.53 ± 0.16^{a}	3.52 ± 0.46^{a}	4.04 ± 0.68^{a}

The results are the average of three replicates ± std. deviation. Different letters in the same column reflect significant differences ($p > 0.05$). SF: soluble fiber; IF: insoluble fiber; TDF: total dietary fiber.

100% nixtamal masa. In the Tortillerias, samples were taken on two different days, in both cases, immediately at the countertop of the tortilla machine, wrapped in paper, and stored in polyethylene bags until they arrived at the laboratory for the microbiological analysis. While in grocery stores, tortillas were taken from the storing containers (insulated coolers, aimed to preserve the temperature, often in 1/2 kg or 1 kg packages, always paper wrapped, and often inside plastic bags). In both cases, the collection temperature was recorded with an infrared thermometer. Upon arrival at the laboratory, triplicate samples of 100 gr were pH measured using a Mettler Toledo pH Metter (Columbus, Ohio, USA) and processed for microbiological analysis. The rest was immediately frozen in liquid nitrogen by submersion for 2 minutes, subsequently being lyophilized for the chemical analysis.

2.1. Microbiological Analyses. In all samples, mesophilic bacteria, molds, and yeast counts were measured/evaluated according to the FDA-BAM manual procedures [10] using the Plate Count Agar (M124, Standard Methods). Briefly, the media contained tryptone, yeast extract, dextrose, and agar dissolved in distilled water. After, they were autoclaved for 15 min at 121°C; the final pH was 7.0 ± 0.2. For viable mesophilic bacteria, yeasts, and molds, 25 ml portions were poured into sterile 15×100 mm Petri dishes. Mesophilic bacteria were incubated at 35°C for 48 h; yeast and mold were incubated at 25°C for 72 h. The total coliform count assessment was grown with the Coliform ChromoSelect Agar (Sigma-Aldrich) and following the requirements of NOM-113-SSA1-1994. Pathogenic bacteria such as *Listeria monocytogenes* and *Salmonella* were evaluated by molecular bioluminescence kits (MDA2LMO96, MDA2SAL96, 3 M) and the presence of *E. coli* was assessed with Petrifilm ™ Rapid E. coli/Coliform Count (REC) that were incubated at 35°C for 48 h.

2.2. Presence of Additives. The contents of benzoic, sorbic, propionic, and fumaric acids were estimated following the

Table 3: Physicochemical analyses as an indicator of bacterial degradation of selected tortilla samples obtained in two regions of Mexico.

Sample	Free sugars (%)	PV (meq/kg)	FAN (mg/100 g^{-1})
Clean			
Nuevo Leon 11	2.85 ± 0.11^{d}	0.66 ± 0.50^{b}	88.11 ± 0.14^{o}
Nuevo Leon 12	3.21 ± 0.20^{d}	0.61 ± 0.43^{b}	87.05 ± 0.42^{o}
Guanajuato 17	2.94 ± 0.28^{d}	0.44 ± 0.21^{b}	87.38 ± 0.43^{o}
Guanajuato 18	3.33 ± 0.33^{d}	0.53 ± 0.61^{b}	87.86 ± 0.60^{o}
Not clean			
Nuevo Leon 3	6.62 ± 0.59^{c}	2.33 ± 0.64^{a}	287.87 ± 0.26^{j}
Nuevo Leon 6	7.41 ± 0.43^{b}	2.18 ± 0.58^{a}	287.97 ± 0.69^{j}
Nuevo Leon 8	8.40 ± 0.19^{a}	2.36 ± 0.48^{a}	287.60 ± 0.42^{j}
Nuevo Leon 11	5.02 ± 0.58^{c}	2.13 ± 0.47^{a}	136.67 ± 0.55^{k}
Nuevo Leon 14	7.55 ± 0.32^{b}	2.11 ± 0.65^{a}	133.50 ± 0.52^{l}
Nuevo Leon 16	6.22 ± 0.49^{c}	2.54 ± 0.32^{a}	116.79 ± 0.60^{n}
Nuevo Leon 18	6.98 ± 0.24^{c}	2.67 ± 0.65^{a}	118.31 ± 0.25^{n}
Nuevo León 22	7.05 ± 0.64^{b}	2.71 ± 0.66^{a}	117.77 ± 0.60^{n}
Nuevo Leon 26	5.72 ± 0.30^{c}	2.76 ± 0.36^{a}	121.51 ± 0.19^{m}
Nuevo Leon 28	7.39 ± 0.22^{b}	3.03 ± 0.23^{a}	336.63 ± 0.39^{e}
Nuevo Leon 33	8.14 ± 0.11^{a}	3.42 ± 0.31^{a}	387.80 ± 0.12^{a}
Nuevo Leon 36	8.23 ± 0.61^{a}	3.11 ± 0.62^{a}	386.39 ± 0.31^{b}
Guanajuato 2	8.77 ± 0.18^{a}	3.19 ± 0.13^{a}	386.58 ± 0.40^{b}
Guanajuato 20	7.70 ± 0.56^{b}	2.57 ± 0.48^{a}	312.36 ± 0.19^{h}
Guanajuato 25	6.41 ± 0.44^{c}	2.54 ± 0.15^{a}	317.54 ± 0.66^{g}
Guanajuato 26	6.36 ± 0.60^{c}	2.63 ± 0.24^{a}	337.72 ± 0.20^{e}
Guanajuato 27	7.01 ± 0.59^{b}	2.59 ± 0.22^{a}	317.84 ± 0.34^{g}
Guanajuato 29	8.80 ± 0.44^{a}	2.71 ± 0.63^{a}	307.98 ± 0.53^{i}
Guanajuato 33	8.23 ± 0.15^{a}	2.78 ± 0.37^{a}	388.34 ± 0.40^{a}
Guanajuato 34	6.79 ± 0.13^{c}	2.28 ± 0.18^{a}	387.83 ± 0.60^{a}
Guanajuato 37	6.65 ± 0.58^{c}	3.41 ± 0.30^{a}	375.47 ± 0.67^{c}
Guanajuato 38	7.41 ± 0.16^{b}	3.39 ± 0.33^{a}	358.14 ± 0.50^{d}
Guanajuato 39	6.67 ± 0.21^{c}	3.43 ± 0.31^{a}	327.06 ± 0.66^{f}
Guanajuato 40	6.81 ± 0.19^{c}	3.42 ± 0.38^{a}	338.25 ± 0.34^{e}

The results are the average of three replicates ± std. deviation. Different letters in the same column reflect significant differences ($p > 0.05$). PV: peroxide value; FAN: free amino nitrogen.

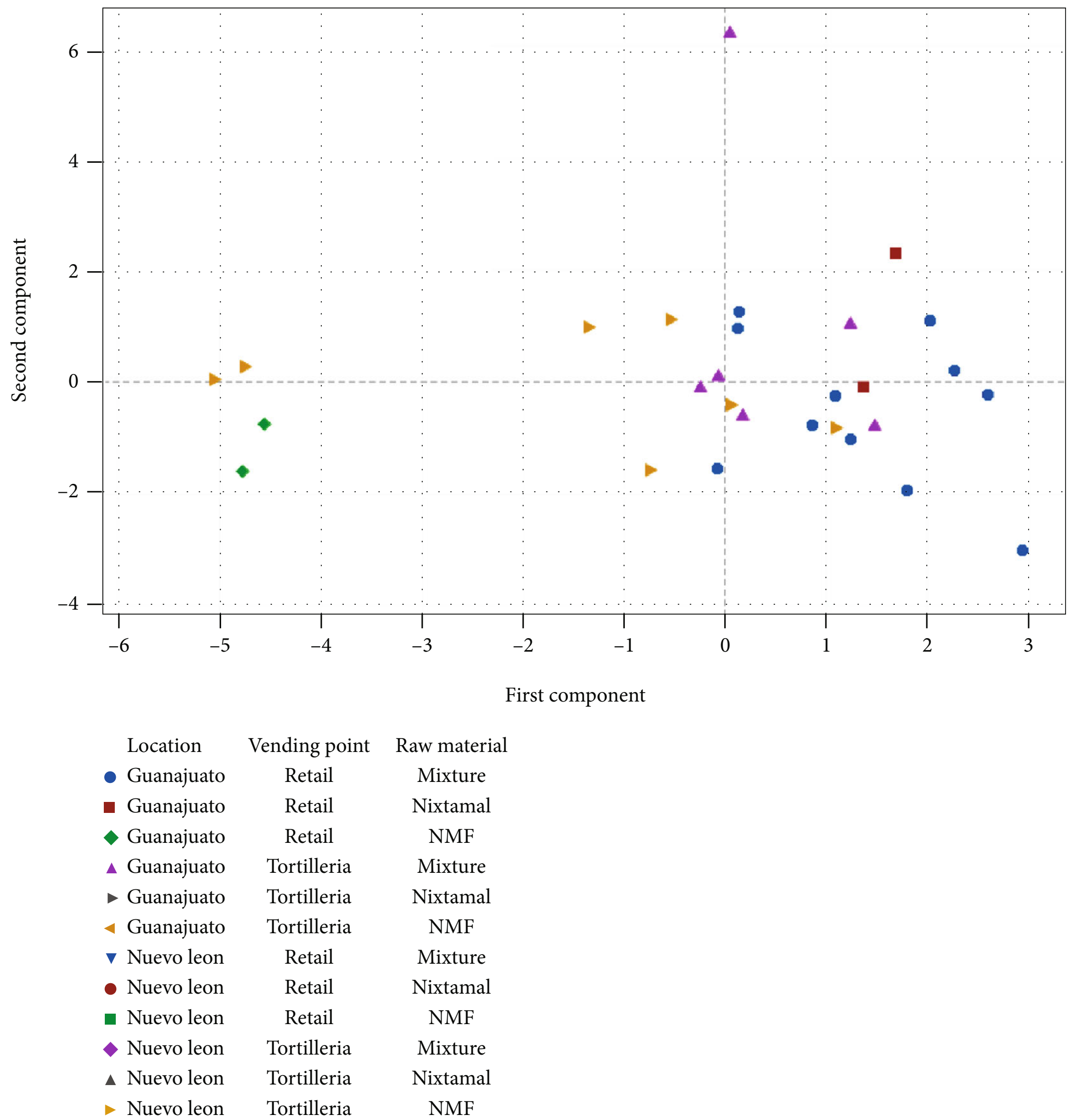
procedures by Xu et al. [11], using Sigma Aldrich reagent standards (≥95% purity); an e2695 series HPLC system (Waters, Milford, MA, USA) equipped with a 10 μL loop injector, UV detector, and an X-SELECT C18 column (150 × 2.1 mm, 5 μm) was used. The mobile phase consisted of an aqueous ammonium acetate buffer (pH = 4.2) and methanol (95: 5 v/v) with a flow rate of 1 mL min^{-1}. The injection volume was 10 μL, and the UV detector was set at 230 nm.

2.3. Chemical Composition and Starch and Fiber Characteristics of Selected Tortilla Samples. The moisture, protein, lipids, and ashes were performed using official methodologies of the AACC [12] and AOAC [10]. The carbohydrate fraction was characterized in terms of total starch content, amylose, amylopectin, fiber content, free sugars using commercial kits (Megazyme, Wicklow, Ireland), alpha-amino nitrogen, and peroxide index by official methodologies of the AOAC [10] as indicators of microbial growth. To delve into the chemical changes caused by this, 24 samples were selected, considering their microbiological load, 4 samples with a very low quantity, and the rest (20 samples) with the maximum loads recorded in aerobic mesophiles and fungi since the contents of pathogenic bacteria were below the minimum levels.

2.4. Statistical Analysis. All analyses were performed in triplicate and to establish significant differences, Tukey's comparison of means was used using a significance level of $p < 0.05$. Principal component analysis (PCA) was performed to understand the underlying causes of microbial presence.

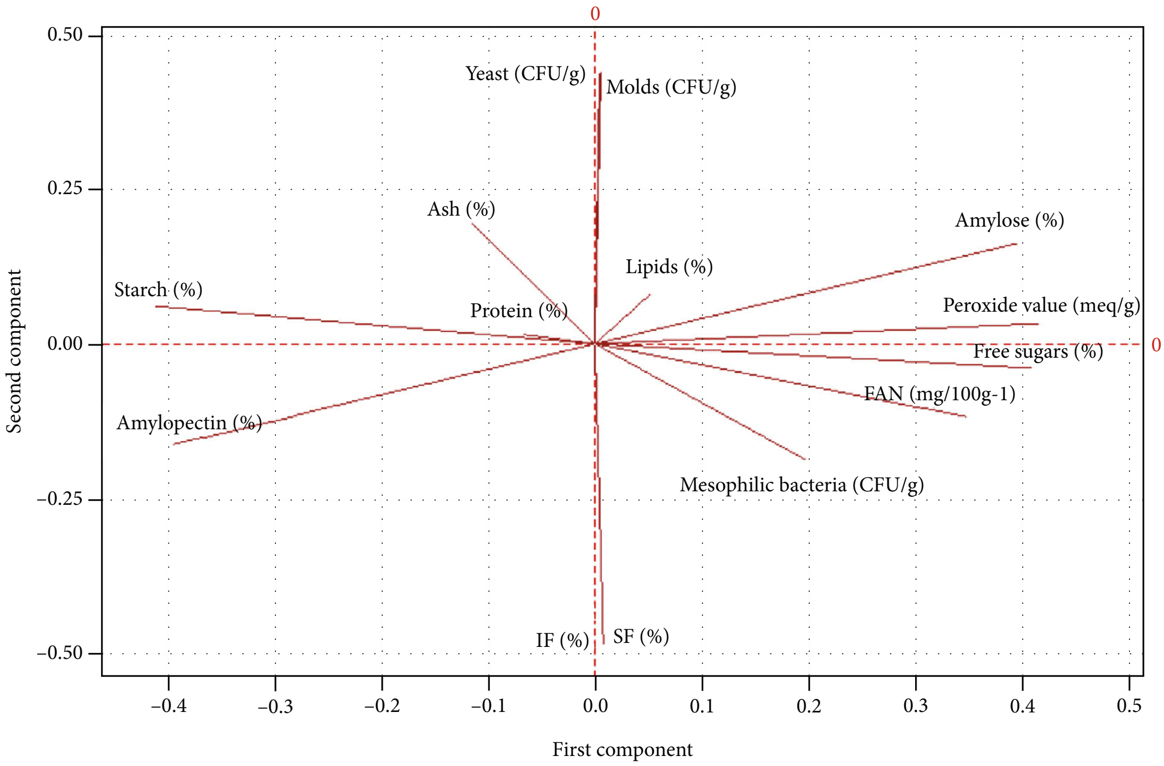
3. Results and Discussion

3.1. Physicochemical and Microbial Characteristics of Tortillas. The temperature profiles at the countertop of tortilla shops in the state the Nuevo Leon fluctuated from 37 to 45°C, while grocery stores showed lower values (33-39°C).



(a)

Figure 2: Continued.



(b)

Figure 2: Principal component analysis of selected tortilla samples collected in two regions of México. (a) Scatterplot and (b) loading plot.

In the case of Guanajuato, they ranged from 32 to 49°C, whereas for grocery stores from 30 to 35°C. Such temperature ranges are expected in Tortillerias because they were collected immediately after preparation. Similarly, grocery store temperatures favor microbial growth during storage time, increasing initial microbial loads (that include food born pathogens) when tortillas reach the consumer. Mexico's expected tortilla shelf life is often short due to this product's high consumption and rotation [13] (Table S1).

Tortillas showed similar moisture contents ranging from 41.87 to 53.15%, which is related to the manufacturing conditions of tortillas for retail. Similar values have been reported in other studies of freshly made products ([14, 15]). The tortilla moisture content is closely related to their textural and preference attributes and properties after reheating (Flores-[7, 16]). The pH range of NMF tortilla samples from Nuevo Leon region was 4.65 to 5.48, indicating a slight acid character that may influence and improve the tortilla's shelf life at room and refrigeration temperatures. While in Guanajuato, it ranged from 6.16 to 10.45, probably due to the alkalinization of tortillas with the addition of more lime after stone grinding, which is a common practice for the tortilla to acquire a stronger flavor and increased shelf life. These tortillas are usually made from nixtamal masa in this region of the country and are preferred by the general population, even though tortillas acquire harder textures and darker colorations due to these processing conditions. For this reason, in this region of Mexico, it is common to blend NMF with nixtamal masa to improve color and texture after reheating [9, 13, 17].

Regarding microbial loads (Table S2), samples varied widely in the mesophilic bacteria counts between regions regardless of the vending points, despite the absence of pathogenic bacterial load (Table S3). Tortillas produced and collected in Nuevo León showed one sample with 3,49 Log CFU/g; overall, the values were from 0.95 to 2.68 Log CFU/g. In the case of tortillas collected in Guanajuato, there was a wider variation because mesophilic counts varied from ≤0.95 to 6.04 Log CFU/g. In both regions, most samples showed ≤0.95 Log CFU/g in molds, yeast, and total coliforms, with only one sample with 2.55 Log CFU/g of molds. The counts of mesophilic bacteria could be related to the lack of good manufacturing practices that varied in each location; as for example, reusable fabric rags for cleaning, use of tap water for both tortilla manufacturing, and/or cleaning surfaces and contact of the operator with currency (coins and notes) during the production. Also, the surrounding environment of the Tortilleria's locations, that in most cases, is in high-traffic areas, under largely open spaces, where the consumers find them with relative ease [2, 16, 18]. In this regard, significant differences between regions occur ($p < 0.05$) that could be related to the local manufacturing practices.

Pathogenic bacteria were absent in all tortilla samples, likely due to the thermal treatment applied during tortilla baking (generally performed at 290°C for 1 minute), combined with the acidic or alkaline pH that is known to inhibit the growth of this group of microorganisms, that are known to thrive in pH environments between 5 and 7 (Table S3). Besides, good manufacturing practices, especially after baking, are recommended to ensure product safety [5, 7, 18, 19]. In another study, Castro-Rosas et al. [4] found *Salmonella* sp. contamination in tortilla samples collected in the central region of Mexico, which was attributed to poor manufacturing practices, but no further investigation was performed. Despite this finding, there is no other evidence or new studies about the concentration of pathogenic bacteria in tortillas and related products or the contamination sources.

3.2. Principal Component Analysis of All Samples. By performing PCA analysis on the 80 samples, it was observed a strong relationship between the two first components, raw materials from which tortillas were manufactured and bacterial counts, that together explained 71% of the variability (Figure 1(a)); also, Figure 1(b) shows other interactions, standing out the influence of additives (benzoates, propionates, and sorbates) to control the growth of bacteria, molds, and yeast that have proven to extend the shelf life and assure product safety when used in adequate concentrations. However, it is also clear that pathogenic bacteria were not detected even when high amounts of microorganisms were present. This is coherent with other studies that have shown that tortilla processing (which occurs athigh temperatures $> 200 \circ C$) is enough to diminish bacterial load even in highly contaminated cooked maize kernels or nixtamal, and often it is not exposed during its manage to the risk of pathogen contamination (as E. coli or Salmonella), and its rapidly cooked, with very rare occasions of cross contamination, since in most cases, Tortillerias only process corn products and/or its derivates [4, 13, 17]. Likewise, other studies have found that despite the harsh thermal process, some spores from the reproductive phase of microorganisms could be present and viable. These spores can grow at room temperature storage conditions like those commonly used to merchandise tortillas. It is important to mention that tortillas have a high Aw (>0.98), which is adequate for microbial and mold growth, especially at temperatures around 32°C [4, 8, 19].

To this point, the results show interesting trends related to the raw material used to generate tortillas; however, it is crucial to understand the driving factors that may enhance or promote the growth of microorganisms in a substrate-rich environment such as tortillas [13, 14]. Due to the particular consumption habits of the Mexican population, fresh tortillas are conveniently sold in Tortillerias and grocery stores, where the combination of temperature, moisture content, initial bacterial load, and a rich source of a potentially available substrate may increase the risk of spoilage [5, 20].

Consequently, it is imperative to understand further the compositional factors that may enhance the growth of microorganisms. Therefore, we decided to evaluate representative samples of each studied region; the selection criteria were choosing those samples with the highest and lowest microbial loads from each region. This would help to determine whether the vending conditions or other compositional factors of the product affect the presence of microorganisms in tortillas.

3.3. Chemical and Compositional Characteristics of Selected Samples. In this regard, 28 samples were selected, 4 with the lowest bacterial concentration (two from each region) and 24 with the highest (12 from each region). These samples were characterized, given their potential influence on bacterial growth. The protein content expressed on a dry matter basis ranged from 8.41 to 9.35% and lipids from 2.34 to 3.26% (Table 1). The carbohydrate fraction was further analyzed in terms of total starch content, which ranged from 69.44 to 75.88%, with significant changes in amylose and amylopectin fractions that ranged from ≈21 and 78% in tortillas, with the lowest counts to <72 and <75% (amylose and amylopectin, respectively) in samples that had the highest contamination levels. These values could be related to the degradation of the amylopectin fraction. It is known that diverse populations of mesophilic bacteria possess amylolytic enzymes, particularly glucoamylases, which break 1-4 and 1-6 glycosidic linkages, and isoamylases, which break 1-6 glycosidic linkages; so, they can utilize complex carbohydrate sources as substrate.

Additionally, the soluble and insoluble fiber contents were estimated since they can also be used as a potential substrate for bacterial growth [5]. Both soluble and insoluble dietary values did not significantly change in all evaluated tortillas and were around 0.5 and 3.5%, respectively. The ranges found in this analysis were broader than those reported in other studies related to the chemical composition of tortilla products, implying molecular changes due to the presence of bacteria [7, 13, 19] (Table 2). For this, we decided to evaluate some characteristics that could help to understand what macromolecule group the primary substrate for bacterial growth was. The free amino nitrogen (FAN) was determined to understand the changes in tortillas' protein fraction since higher values are related to protein hydrolysis and thus reflect protein degradation by bacteria [8, 19]. The samples with the lowest bacterial count showed FAN contents ≈88 mg/100 g, while the rest varied widely from 116.79 to 388.34 mg/100 g (Table 3). The degradation of lipids due to the evaluated conditions and bacterial growth was estimated with the peroxide value, finding a similar trend; those samples with the lowest loads were around 0.5 meq/kg. On the other hand, the rest of the samples ranged from 2.11 to 3.43 meq/kg, which could be related to the degradation and oxidation of lipids. High peroxide values may promote tortilla rancidity and is related to off-flavors and aromas. Several authors reported that tortillas manufactured in rural regions with poor manufacturing practices had comparatively higher peroxide values [9, 17]. Other authors concluded that peroxide values are one of the leading indicators of spoilage in tortillas [5, 21]. Regarding the degradation of carbohydrates, tortillas with higher values had more microbial degradation. The sugar content of tortillas expressed on a dry matter basis ranged from 5.02 to 8.80%.

3.4. Principal Component Analysis of Selected Samples. A PCA statistical analysis was performed to understand the intricate relationships among samples better. The principal components of the 28 selected samples were the peroxide value (PC1) and the soluble fiber fraction (PC2), which accounted for 80% of the variations. The scatterplot

(Figure 2(a)) showed a clear separation of two samples from each region; the ones with the lowest bacterial load separated from the rest of the samples with the highest bacterial load were grouped closely. These relationships evidenced that the contamination influenced the tortilla composition regardless of the vending point [16, 18]. The analysis of tortilla samples produced from the different raw materials (nixtamal masa, NMF, and mixtures) indicated that tortillas made with NMF (lowest bacterial load) had lower degradation values compared to counterparts produced from nixtamal masa and mixtures (highest bacterial load). Figure 2(b) shows the close relationship of mesophilic bacteria counts with the FAN and free sugar contents that reflect the preference of this bacterial group for protein and starch substrates. This information can be helpful since it can help us describe the growth of these microorganisms and their effect on products during processing and could lead to further analysis of good manufacturing practices, handling, and changes during storage.

4. Conclusions

Overall, as observed in the PCA analysis, raw materials were an important factor that influenced the quantified concentrations of foodborne pathogen. All changes in chemical composition as result of microbial load were higher in those tortillas elaborated with nixtamal and were lower in those made with nixtamalized corn masa flour contained a lower microbial load than those made with nixtamal masa and mixtures. The free sugars and free amino nitrogen helped confirm the changes at the molecular level that occurred in the tortilla, exerted by the growth of microorganisms. The complementary analyses carried out on the 28 selected samples allow us to observe the influence of the raw material on the concentration of microorganisms regardless of the vending point. Further microbiological and chemical analysis are needed to understand the impact of good manufacturing and product handling practices, especially after tortilla baking.

Data Availability

Data is available upon request.

Conflicts of Interest

The author declares no conflicts of interest.

Supplementary Materials

The files describe all 80 samples analysis and are important to stablish the relationships among samples. Table S1: retail characteristics of tortillas in two regions of Mexico. Table S2: microbial load and additives present in tortillas obtained in two regions of Mexico. Table S3: pathogenic bacterial load in tortillas in two regions of Mexico. *(Supplementary Materials)*

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