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INFLUENCE OF PAPRIKA (*Capsicum annuum L*) ON QUALITY PARAMETERS AND BIOGENIC AMINES PRODUCTION OF A RIPENED MEAT PRODUCT (*chorizo*)

INFLUENCIA DE LA PAPRIKA (*Capsicum annuum L*) SOBRE LOS PARÁMETROS DE CALIDAD Y LA PRODUCCIÓN DE AMINAS BIÓGENAS EN UN PRODUCTO CÁRNICO MADURADO (*chorizo*)

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Abstract

Paprika (*Capsicum annuum L*) is the main spice used in the elaboration of *chorizo* contributing to both color and flavor, but until now there have not been any studies evaluating its role in the sausage quality parameters. Therefore, our aims were: to know the effect of paprika on the quality parameters during *chorizo* ripening estimating an optimal time and, simultaneously, exploring the evolution of biogenic amines during this operation. Two types of *chorizo*, one with paprika (CH) and the other without paprika (CWS), ripened for 33 days, were prepared for this study. Physico-chemical, texture and microbiological analyses were carried out. Additionally, the biogenic amines: tyramine, cadaverine and putrescine were analyzed by high-performance thin-layer chromatography (HPTLC) at 0, 12, 19 and 33 days. At day 19, the sample CH obtained the pH and a_w values of 4.9 and 0.90, respectively, the highest growth of lactic acid bacteria, and a decrease in the coliform bacteria group and *Enterobacteriaceae* family. The total content of biogenic amines also decreased, mainly cadaverine, while tyramine content was lower than 100 mg/kg. In general, paprika favored the development and quality parameters of *chorizo* after 19 days of ripening and preserved the product for a longer time.

Keywords: *chorizo*, paprika, quality indicators, biogenic amines, HPTLC.

Resumen

Paprika (*Capsicum annuum L*) es la principal especie utilizada en la elaboración del *chorizo* aportando color y sabor; sin embargo, no hay estudios sobre el papel que desempeñe sobre los parámetros de calidad del producto. Los objetivos fueron: conocer el efecto de la paprika sobre los parámetros de calidad durante la maduración de un *chorizo*, estimando un tiempo óptimo, y explorar la evolución de aminas biógenas durante esta etapa. Se elaboraron dos tipos de *chorizo*; con paprika (CH) y sin paprika (CWS), madurados por 33 días. Se realizaron análisis físico-químicos, textura, microbiológicos y cuantificación de aminas biógenas: tiramina, cadaverina y putrescina, usando cromatografía en capa-fina de alta-resolución (HPTLC) a los 0, 12, 19 y 33 días. La muestra con paprika a los 19 días obtuvo un pH de 4.9, a_w de 0.90, mayor crecimiento de bacterias acido lácticas y una reducción de bacterias coliformes y *Enterobacteriaceae*. También se observó una disminución del contenido total de aminas biógenas, principalmente de cadaverina, y la cantidad de tiramina fue menor de 100 mg/kg.En general, la paprika favoreció el desarrollo de los parámetros adecuados de calidad del *chorizo* en 19 días de maduración y mantuvo por más tiempo la estabilidad del producto.

Palabras clave: *chorizo*, paprika, parámetros de calidad, aminas biogénicas, HPTLC.

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1 Introduction

Chorizo is a type of fermented sausage prepared with both pork meat and fat, with different spices, salt, curing salts; then the mix is stuffed, fermented and ripened (Lorenzo *et al*., 2013). It is one of the most commonly consumed types of sausages and the most representative of Spain, where almost every region has its own version, creating a wide diversity of Spanish *chorizo* recipes. It is also consumed in other countries, where different types and amounts of chili peppers are added as part of its ingredients. In Mexico, a mixture of chile ancho (*Capsicum annuum L. var. annum*) and chile pasilla (*Capsicum annuum L. var. annuum*) is used. In the south of Mexico and other American countries the dried chili peppers are replaced either with annatto (achiote, *Bixa Orellana*) or aji (*Capsicum chinense*, mainly for Central and South America), expanding the variety of types of *chorizo*, each one with different sensory characteristics. Traditional sausages, such as Spanish *chorizo*, undergo a spontaneous fermentation without the addition of a starter culture, therefore the microorganisms present in these sausages derive from the raw materials or the environment, called "house flora". The initial microbiota is composed of lactic acid bacteria (LAB), coagulase-negative cocci, yeast, and moulds, but may also have spoilage microorganisms such as enterobacteria, enterococci and *Pseudomonas* (Juárez-Castelán *et al*., 2019; Leroy, Verluyten, & De Vuyst, 2006; Talon, Leroy, & Lebert, 2007). LAB are the main microorganisms responsible for fermentation and the generation of a variety of organic acids that decrease the pH. During the ripening process, the water diffusion from the product's surface to the environment, due to the difference in water activity (a_w) between them, generates the decrease in moisture and a_w parameters. These parameters at low values enable the reduction of pathogens and spoilage microorganisms, and they also promote the development of color, flavor and texture in the fermented meat products. Traditional fermented products may have variability in their quality depending on many factors,such as the hygienic quality of raw materials, ingredients, type of indigenous microbiota, temperature and time of ripening (Talon *et al*., 2007). The time of ripening of *chorizo* or any fermented meat product has not been well established; González-Tenorio *et al*. (2013) mentioned that the Mexican-style *chorizo* is stored from hours to weeks, and the Spanish-type *chorizo* is ripened from one to several months. Although sensory characteristics depend on the ripening time, it is also important to consider the quality parameters that guarantee a safe product.

Shelf life stability and the hygienic quality of these products are mainly determined by a combination of pH and a_w. A dry fermented sausage has a pH value of 5.2-5.8, moisture of about 30% or lower, and an a_w range from 0.85 to 0.91(Toldrá, 2008). The content of *Enterobacteriaceae* has also been used to determine the quality of the product (Bover-Cid *et al*., 2001; Ruiz-Capillas *et al*., 2007; Suzzi & Gardini, 2003). Other indicators of quality are the biogenic amines that are present in several foods; tyramine, cadaverine and putrescine are the major biogenic amines present in fermented meat products (Latorre-Moratalla, Bover-Cid, Veciana-Nogués, & Vidal-Carou, 2012; Latorre-Moratalla, Comas-Basté, Bover-Cid, & Vidal-Carou, 2017; Signorini & Guerrero-Legarreta, 2009; Suzzi & Gardini, 2003).

Biogenic amines are produced by the microbial decarboxylation of amino acids, and their content constitutes a quality index due to the fact that high levels of biogenic amines are undesirable because of their possible toxic effects. Tyramine can cause poisoning, with symptoms such as migraine, gastric and intestinal problems, hypertension, and psychological depression. Cadaverine and putrescine can increase the toxicity of tyramine and histamine, and are also both precursors of carcinogenic nitrosamine (Costantini *et al*., 2013; Van Ba *et al*., 2016). Biogenic amines are also related to the growth of *Enterobacteriaceae*, micrococcus and *Pseudomonas*, as well of some pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Salmonella paratyphi* (Komprda *et al*., 2008; Kongkiattikajorn, 2015; Ruiz-Capillas *et al*., 2007; Kuley *et al*., 2011). Additionally, a high content of them can have negative effects on sensory properties (Alvarez & Moreno-Arribas, 2014; Gökoğlu *et al*., 2004; Kongkiattikajorn, 2015). Several studies exist about the content of biogenic amines in *chorizo*(González-Fernández *et al*., 2003; González-Tenorio *et al*., 2013; Miguélez-Arrizado *et al*., 2006; Ruiz-Capillas *et al*., 2007, 2012) and other fermented sausages (Laranjo *et al*., 2017; Latorre-Moratalla *et al*., 2010, 2008; Lu *et al*., 2010; Van Ba *et al*., 2016). Most of the studies of biogenic amines were focused on the effect of different factors such as the starter

culture, salt content, high pressure, chilled storage, and diameter of casing, but there are few studies about the effect of spice content.

Paprika comes from different varieties of *Capsicum annuum L.* (Pascual-Pineda *et al*., 2018), and is the principal spice used in Spanish *chorizo* manufacture, providing flavor, the characteristic color and a preservative effect (Toldrá, 2008). Studies about the effect of paprika have been reported by Revilla & Quintana, (2005), who studied the effect of both the amount and the type of paprika on texture, color and physico-chemical parameters during the ripening process of *chorizo*, but they did not evaluate the effect on the microbial population and biogenic amine formation. On the other hand, other studies investigated the effect of mixed spices (Komprda *et al*., 2004, 2009), and the ethanol extract of spices such as garlic (*Allium sativum*), cinnamon (*Cinnamomumverum*), clove (*Syzygiumaromaticum*) and anise (*Pimpinellaanisum*) (Mah, *et al*., 2009; Sun, *et al*., 2018) on biogenic amine content in a dry fermented sausage. Nevertheless, no other study exists about the effect of paprika powder on the biogenic amines content. High-performance liquid chromatography (HPLC) has been the most generally used technique in the determination of biogenic amines and other active compounds in many types of foods (Bedia Erim, 2013). Recently, another chromatography method has been developed; the high-performance thin-layer chromatography (HPTLC) method is an improvement on thin layer chromatography (TLC). Its advantages are the speed of the method, the small amount required for the mobile phase and the possibility of analyzing several samples simultaneously, reducing time and cost per analysis (Gomathi *et al*., 2012).This method has been used to obtain fingerprints of the active compounds of plants (Gomathi *et al*., 2012), *Agave* fructans (Alvarado, Camacho, Cejas, & Rodríguez, 2014) and other compounds, but is less used to both detect and quantify biogenic amines in food (Wang *et al*., 2018).

The objectives of this study were: 1) to investigate the effect of paprika on the quality parameters during the ripening of *chorizo* 2) to determine the time of ripening required to achieve the quality parameters, 3) to determine biogenic amines in *chorizo* proposing the HPTLC method and 4) to investigate the relationship between biogenic amine content and physico-chemical, textural and microbiological parameters in *chorizo*.

2 Materials and methods

2.1 Sausage manufacture

Chorizo samples were manufactured and analyzed in the pilot plant of the Centro de Investigación y Asistencia enTecnología y Diseño del Estado de Jalisco (CIATEJ). The base for the experimental samples (24 kg) was prepared with 20% of pork back fat and 80% of lean pork, obtained from a local market in Guadalajara, Mexico. Lean and back fat were ground through a 5 mm diameter mincing plate (Torrey CI-22-1 L9N2E, Nuevo Leon, Mexico) on grinder (Torrey Mod. M-22RW, Nuevo Leon, Mexico). Then they were mixed with the basic ingredients (g/kg): 20 of salt, 10 of glucose, 0.5 of sodium ascorbate, 0.15 of sodium nitrate (Fabpsa®, CDMX, Mexico) and 40 mL of water. When all ingredients were mixed, the total batter was separated into two batches. One batch, 12 kg, was for *chorizo* without paprika (CWS), and the other batch had paprika added (CH) as (g/kg): 22 of paprika (*Capsicum annuum L*), 3 of garlic (*Allium sativum*), and 1.5 of pepper (*Piper nigrum*). All spices were obtained in a local market in Guadalajara, Mexico. Both batches were maintained at 4ºC for 24 h and then were stuffed into synthetic casings with a diameter of 35-38 mm, tied in small pieces of approximately 15 cm and transferred to a dry-ripening chamber where they were kept for 5 days at 6-8ºC and at the relative humidity of the environment of Guadalajara, Jalisco in May (50-80%). Finally, the temperature was increased to 10-12ºC and kept for 28 more days for ripening.

A sample of five pieces of *chorizo* was randomly taken at 0, 12, 19 and 33 days of ripening for subsequent analysis. Physico-chemical and microbiological analyses were performed the first three days. For biogenic amine determination, the *chorizo* samples were vacuum-packaged and stored at -20ºC for not more than 3 months until respective analysis.

2.2 Physico-chemical analysis

The moisture content of the samples was determined by dehydration at 105ºC of 5 g of *chorizo* until constant weight (Bover-Cid *et al*., 2001). For pH, 10 g of *chorizo* in 100 mL of distilled water was used, measured witha pH meter Orion 3 STAR (Thermo Scientific, Massachusetts, USA). Water activity (a_w) was determined using Aqualab 4TE

(Decagon Devices, Inc., Washington, USA) at 25ºC (Ruiz-Capillas *et al*., 2007). Three samples of CWS and CH, from each day of ripening, were analyzed.

2.3 Microbiological analysis

The microbiological analysis consists in determining the population of lactic acid bacteria (LAB), aerobic mesophilic bacteria (AMB), coliform bacteria (COL), *Enterobacteriaceae* (ENT), moulds (ML) and yeasts (YS).

Two duplicated *chorizo* portions of 10 g, of CWS and CH for all sampling times, were transferred to sterile plastic bags and homogenized in a stomacher machine for 2 min with peptone water (BD Difco, New Jersey, USA). The solution was used to prepare decimal dilutions. The microbial growths were in 3M^{TM} Petrifilm ^{TM}(3M, Minnesota, USA) count plates, except LAB. The AMB (cat. 6400), COL (cat. 6410) and ENT (cat. 6420) were incubated for 24 h at 37ºC. The ML and YS (cat. 6407) were incubated for 5 days at 25ºC. LAB population was obtained on plates of Man Rogose Sharpe (MRS) agar (BD Difco, New Jersey, USA) at 32ºC for two days. Microbiological data was transformed into logarithms of colony-forming units (log CFU/g).

2.4 Texture analysis

The texture profile analysis (TPA) was according to Gómez & Lorenzo (2013), using a texture analyzer (TA-XT.plus, Stable Micro Systems, Vienna Court, UK). Six blocks of 1.5 cm^3 of each *chorizo* samples (CWS and CH) in the four times of ripening were used for TPA. Each block was cut in a meat slicer (Torrey). Textural parameters were measured by compressing 60% with a compression probe of 50 mm x 100 mm (diameter x high), speed of pre and post test was 5.00 mm/s. Force-time curves were recorded at a crosshead speed of 3.33 mm/s. Hardness (N), cohesiveness, springiness and chewiness (N x mm) were obtained using computer software (Exponent 6,1,12,0; 2016, Stable Micro Systems, Vienna Court, UK).

2.5 Biogenic amine analysis

The biogenic amines tyramine, putrescine and cadaverine were determined and quantified by HPTLC using a system CAMAG (Switzerland).

2.5.1 Sample preparation

Five g of each *chorizo* sample, without casing and small pieces, were weighed into a test tube, with 10 mL of 5% trichloroacetic acid (TCA) added and then homogenized in a homogenizer T18 digital ULTRA-TURRAX (IKA-Werke GmbH & Co, Germany) for 1 min. The homogenized sample was centrifuged (Multifuge X3R, Thermo Scientific) 1100 g for 20 min at 4ºC. The aqueous layer was filtered through a paper filter No. 4 (Whatman, GE Healthcare, United Kingdom) and the solid residue was extracted again as described above. Both aqueous extracts were collected and made up 25 mL with TCA. Two aqueous solutions were obtained by CWS and two solutions by CH, each one in the four times of ripening.

A sufficient amount of tyramine, putrescine and cadaverine (their respective hydrochlorides were used as standards, 98% of purity, Sigma-Aldrich, Germany) was weighed and dissolved in 1 mL of water for HPLC (Chromasolv Sigma-Aldrich) to obtain a final concentration of 50 mg/mL of each amine.

2.5.2 Derivatization and HPTLC method

The methodology for derivatization of biogenic amines was performed by a modification of the methods proposed by De Mey *et al*. (2012) and Smělá *et al*. (2003). One mL of an aqueous solution, or 50 μL for amine standard was mixed with 150 μL of 2 M NaOH (J.T. Baker, AVANTOR, USA), 150 μL of saturated Na_2CO_3 (J.T. Baker) and 1 mL of dansyl chloride (5 mg/mL acetone for HPLC;Sigma-Aldrich). The mixture was shaken for 1 min in a vortex mixer (Fisher Scientific Company LLC, Pittsburgh, USA). Derivatization proceeded for 1 h in the dark at 40ºC. After derivatization, 200 μL of ammonia was added; this was shaken for 1 min and kept for 30 min in the dark. The amine derivatives were extracted by 2 mL of diethyl ether (Purity of ≥99.9%, Sigma-Aldrich) (3 x 2 mL). The organic phase was evaporated to dryness in a water bath at 40ºC. The solid residue was dissolved in 3 mL of acetonitrile for HPLC (Purity of ≥99.9%, J.T. Baker) and made up 5 mL (derivatized solution) and kept at -40ºC, in amber flasks covered with aluminum foil, for no more than a month. One mL of the derivatized solution was filtered through a nylon membrane filter 0.45 μm (Millipore, Merck KGaA, Darmstadt, Germany) and was kept at -40ºC in amber flasks on previous days before the HPTLC analysis (Martuscelli *et al*., 2009). At the end we obtained 16 derivatized solutions, comprised of samples from the 4 times of ripening of the 2 *chorizo* samples (CWS and

CH), all of these in duplicate.

The samples were applied with Camag 100 μL syringe (Hamilton-Bonaduz, Switzerland), by a nitrogen gas stream, into bands of 8.0 mm on silica gel precoated aluminum plate 60 F_{254} (20 x 20 cm, Millipore) using a TLC sampler Linomat 5 (CAMAG, Switzerland). Each plate contained 10 tracks with position setting; 10 mm from lower edge, 17 mm initially from the left side and with 18 mm distance between tracks. The 12 plates were elaborated, six plates to CH samples and the other six to CWS samples. Tyramine, cadaverine and putrescine were the amines analyzed in different plates, all of these in duplicate (2 types of *chorizo* samples x 3 amines x 2). In general, one plate contained the four times of ripening of one type of *chorizo* (CWS or CH) and one amine at six different concentrations.

The plates were developed in a glass TLC chamber, which was saturated with the steam of the mobile phase for 30 min prior to analysis. The mobile phase was 5:1 bencen:ethyl acetate(Naguib *et al*., 1995) ascending up to 175 mm. The plates were scanned with TLC Scanner 3 (CAMAG, Switzerland) with lamp D2&W, wavelength 254 nm, slit dimension 6.00 x 0.20 mm (Micro), scanning speed 20 mm/s, data resolution 100 μm/step. Data acquisition and processing was done by CAMAG with CATS software version 1.4.4.6337. In addition, the plates were illuminated at a wavelength of 365 nm using a portable UV lamp (UVP, United Kingdom) in a dark room (Figure 1A). The identification of the three biogenic amines was confirmed by comparing the retardation factor (Rf) obtained from their respective standard. The Rf is a measure of the substance position on TLC plate.

2.6 Statistical analysis

Means and standard deviations were calculated for physico-chemical, microbiological, texture and amine biogenic data. Experimental design was considered as a factorial array with *chorizo* type and ripening time as factors; therefore, a two-way analysis of variance (ANOVA) was carried out to analyze their main factors and interaction effects on the response variables for physico-chemical, microbiological and texture. Biogenic amines were analyzed by three-way ANOVA; the factors were ripening time, the type of *chorizo*, method repeatability, and their respective interactions. The method repeatability factor evaluated the effect to quantify the amines content of one type of *chorizo* sample in three different plates. Mean significant differences were determined by Duncan test for the ripening time and interactions factors. Finally, Principal Component Analysis (PCA) and Pearson's correlation were made with all variables. All analyses were performed in XLSTAT, version 2016,18.07 (Addinsoft, New York, USA).

3 Results and discussions

3.1 Physico-chemical parameters

The ripening time had a significant effect ($p<0.001$) on the three physico-chemical parameters: moisture content, a_w and pH, Figure 1A, 1B and 1C, respectively. The type of *chorizo* samples (CH and CWS) had a significant effect ($p<0.05$) on moisture content and pH, while the interaction was only significant for pH.

The moisture content and a_w decreased ($p<0.05$) during ripening time in both *chorizo* samples, CH and CWS, the values of these parameters at the beginning, day 0, were different from the rest of the days of ripening Figure 1A and 1B. Also, this figure shows no differences between both types of *chorizo* samples from day 0 up to day 12 in both parameters. During the first 19 days of ripening, the *chorizo* samples lost nearly 50% of their water content, from 62.5%to 34.3% for CH and from 61% to 38.7% for CWS, and then in the subsequent days the decrease was lower. The moisture loss was mainly due to the migration of water to the surface, where it evaporates as a result of the difference between the a_w of the product and the environment. There was also a gradual reduction of the meat water holding capacity as pH decreased during the fermenting and ripening process (Toldrá & Hui, 2007). In general, we observed that the water loss was slower in the *chorizo* sample CWS. Although the interaction factor was not significant ($p>0.05$) in both parameters, the moisture content of CWS at day 19 and 33 was similar to that of CH at day 12 and 19, respectively. The sample CH had a lower ($p<0.05$) moisture content and a_w than CWS, the a_w values of CH and CWS on this day were different ($p<0.05$) from the rest of the days of ripening. This fact is related to the pH value of this sample. The pH value of CH was below the isoelectric point (pH 5.1), Fig 1C, and the water-holding capacity of the meat protein was reduced, due to the fact that at this point positive and negative charges are balanced, and minimum repulsion between meat protein is reached (González-

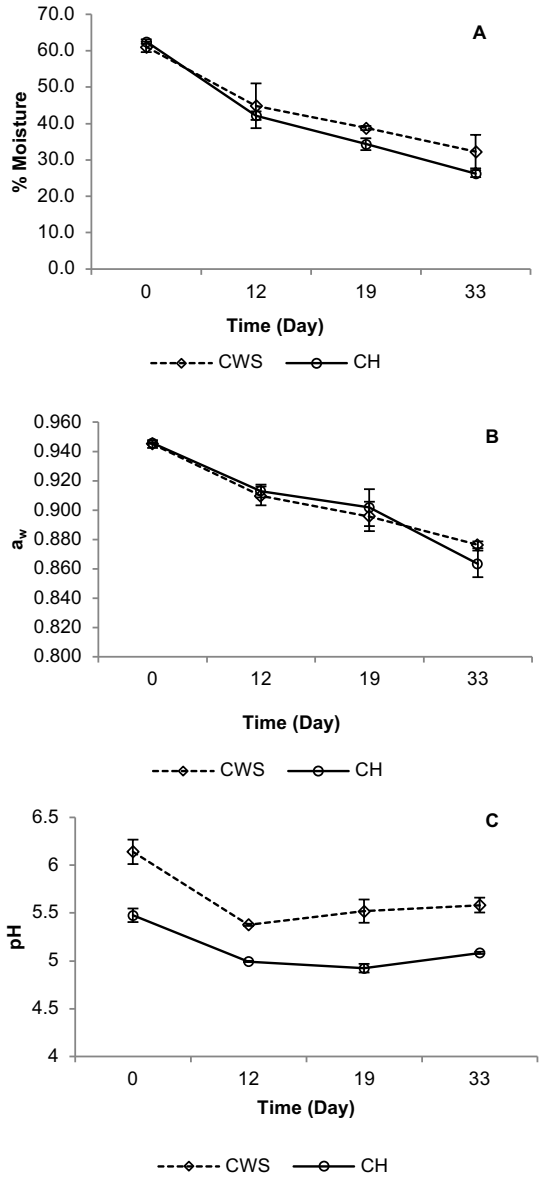

Fig. 1. Physico-chemical changes A. Percent moisture content, B. Water activity (a_w) and C. pH in chorizo samples without paprika (CWS) and with paprika (CH) during 33 days of ripening.

Fernández *et al*., 2006). Similar results have been obtained by other authors (Komprda *et al*., 2004; Revilla & Quintana, 2005; Sun *et al*., 2018), who reported that the water activity was lower in sausages with the highest content of red pepper, paprika or spice extracts.

The pH, Figure 1C, decreased ($p<0.05$) during ripening and had an effect ($p<0.001$) on the type of *chorizo* and the interaction with maturation time ($p<0.05$); the pH decrease of CWS was slower than CH; the values of CWS on days 12, 19 and 33 of maturation showed no significant difference ($p> 0.05$) to CH on day 0, and this behavior explains the interaction of the two factors. In general, the CH sample had the major decrease in pH ($p<0.05$) during all the ripening time, which was probably due to the paprika content in this sample. In general, the spices stimulate LAB activity (Revilla & Quintana, 2005; Toldrá, 2008) that explains that an increase in LAB counts in CH samples, Table 2, generated a slight decrease in pH. Paprika also had a variety of acids, mainly acetic acid (Martín *et al*., 2017); so this and other acids in the ingredients also contributed to a decrease in the pH values. Sun *et al*. (2018) found a similar effect of other spices on pH; they found that the sausages with cinnamon had the lowest pH. The subsequent decrease in pH during ripening time is due to the production of organic acids such as lactic acid, acetic acid, formic acid and propionic acid by carbohydrate fermentation (Toldrá & Hui, 2007). The lowest ($p<0.05$) pH values were at day 12 and 19 for CH and at day 12 for CWS; their pH values were 4.9 and 5.4, respectively. This is due to the difference of LAB count, Table 2B, the microorganisms responsible for the production of diverse acids. Those days showed the highest LAB counts for each type of *chorizo* samples. On subsequent days, the pH values of CWS and CH increased to 5.6 and 5.1, respectively, by the end of ripening. This increase was probably due to the accumulation of nitrogen-containing compounds such as peptides, amino acids and ammonia from proteolysis associated with yeast growth, mostly *Debaryomyceshansenii* (Ammor& Mayo, 2007; Toldrá & Hui, 2007), see Figure 2E, to observe this we could consider quantifying the protein content in a future study.

González-Fernández *et al*.(2006) indicated that 30-37% moisture content and a_w values 0.86-0.90 are considered the normal range for these types of products. By 33 days of ripening, both types of *chorizo* samples were within a_w range and only CWS obtained final moisture content inside of this range. Although a_w is an important safety index in fermented meat products (Sun *et al*., 2018), the stability and safety of a variety of the fermented products, such as ripened *chorizo*, are determined mainly by the combination of the acidification of the product, pH, and low water activity. In general, the limits of these parameters to maintain the quality and safety are pH ≤ 5.3 and a_w ≤ 0.95 and moisture content ≤ 30% (Toldrá, 2008).

Table 1. Evolution of the textural properties in the *chorizo* samples without paprika (CWS) and *chorizo* samples with paprika (CH) (means values ± standard deviation of six replicates).

Parameter	Type of *chorizo*	Days of ripening				Significance		
		0	12	19	33	S	D	SxD
Hardness (N)	CWS	16.13±4.35^{d}	139.94±30.1^{c}	194.18±40.29^{ab}	192.09±46.33^{b}	*	***	n.s
	CH	19.19±4.30^{d}	145.51±17.74^{c}	220.15±23.20^{ab}	227.38±17.04^{a}			
Springiness	CWS	0.59±0.11^{a}	0.58±0.13^{a}	0.56±0.06^{a}	0.57±0.6^{a}	n.s	n.s	n.s
	CH	0.52±0.12^{a}	0.57±0.13^{a}	0.59±0.06^{a}	0.65±0.01^{a}			
Cohesiveness	CWS	0.34±0.03^{e}	0.48±0.04^{cd}	0.53±0.05^{bc}	0.59±0.04^{a}	**	***	***
	CH	0.34±0.00^{e}	0.47±0.06^{d}	0.52±0.03^{bc}	0.45±0.03^{ab}			
Gumminess	CWS	5.42±1.27^{c}	68.59±18.96^{b}	103.88±25.08^{a}	112.79±27.53^{a}	n.s	***	n.s
	CH	6.58±1.47^{c}	68.96±15.94^{b}	115.94±17.0^{a}	123.46±4.34^{a}			
Chewiness	CWS	3.16±0.84^{d}	39.38±9.97^{c}	58.11±16.33^{b}	64.99±20.49^{ab}	n.s	***	n.s
	CH	3.42±1.13^{d}	38.93±11.36^{c}	66.66±4.63^{ab}	72.82±8.15^{a}			

CWS. *Chorizo* without paprika; CH *Chorizo* with paprika.

S. Chorizo type effect; D: Ripening time effect; SxD: Interaction of type of chorizo samples and ripening time. ***p<0.001; **p<0.01; *p<0.05; n.s: not significate.

$^{a-e}$Means with different letter of each texture parameter (double row) differ significantly (p<0.05) between days of ripening and type of *chorizo*.

Considering these parameters, only the *chorizo* sample CH was inside the safety parameters from day 19 up to the end of ripening.

The results of TPAfor each type of *chorizo* samples followed during ripening are given in Table 1. The ripening time had a significant effect (p<0.001) on the parameters of hardness, cohesiveness, gumminess and chewiness. The major texture change was from day 0 to day 12, so these parameters increased close to ten times more than at the beginning, except in cohesiveness, because at day 12 the pH and a_w declines, Fig 1, and the solubilized myofibrillar proteins coagulate, releasing the water and forming a strong gel that binds the fat and meat closely together. González-Fernández *et al*. (2006) and Lorenzo *et al*. (2013) suggested that the major changes in fermented sausage structure took place during fermentation, but the drying process also affected these parameters. During the drying process, the water was continuously removed and the endogenous or microbial enzymes acted on proteins, creating a dense structure; therefore, these parameters increased in subsequent days (Toldrá & Hui, 2007). The sample CH had the highest (p<0.05) cohesiveness, gumminess and chewiness from day 19, and hardness at day 33. Also, Table 1 shows one significant interaction for cohesiveness, so we observed that at day 33 the cohesiveness of CH had a slight decrease when compared to day 19, behavior that explains the factors interaction. The decrease of cohesiveness in CH and hardness in CWS at day 33 were probably due to the excess of proteolysis, related to the growth of moulds and yeast (Toldrá, 2008). In general, the *chorizo* sample with paprika, CH, was harder than CWS. Similar results were reported by Revilla & Quintana (2005): the dry sausage with a greater proportion of paprika obtained higher hardness and lower cohesiveness after 21 days of ripening. The texture is one of the most important sensory parameters of quality in fermented meat products. The usual textural descriptors applied to dry sausage are hardness, chewiness and cohesiveness (Zdolec, 2016). So, our results showed that paprika is an important ingredient in developing the texture of the *chorizo* samples. Nevertheless, a balance of firmness and softness is required. Lorenzo *et al*. (2013) obtained a low textural acceptability in the dry sausages with the highest hardness and cohesiveness.

3.2 Microbiological analysis

The statistical analysis (ANOVA), showed differences (p<0.05) for the six groups of microorganisms during ripening time, between types of *chorizo* and their interactions, except in moulds (Figure 2). All microorganisms increased their counts during the first twelve days of ripening, except in COL and ENT in CH sample.

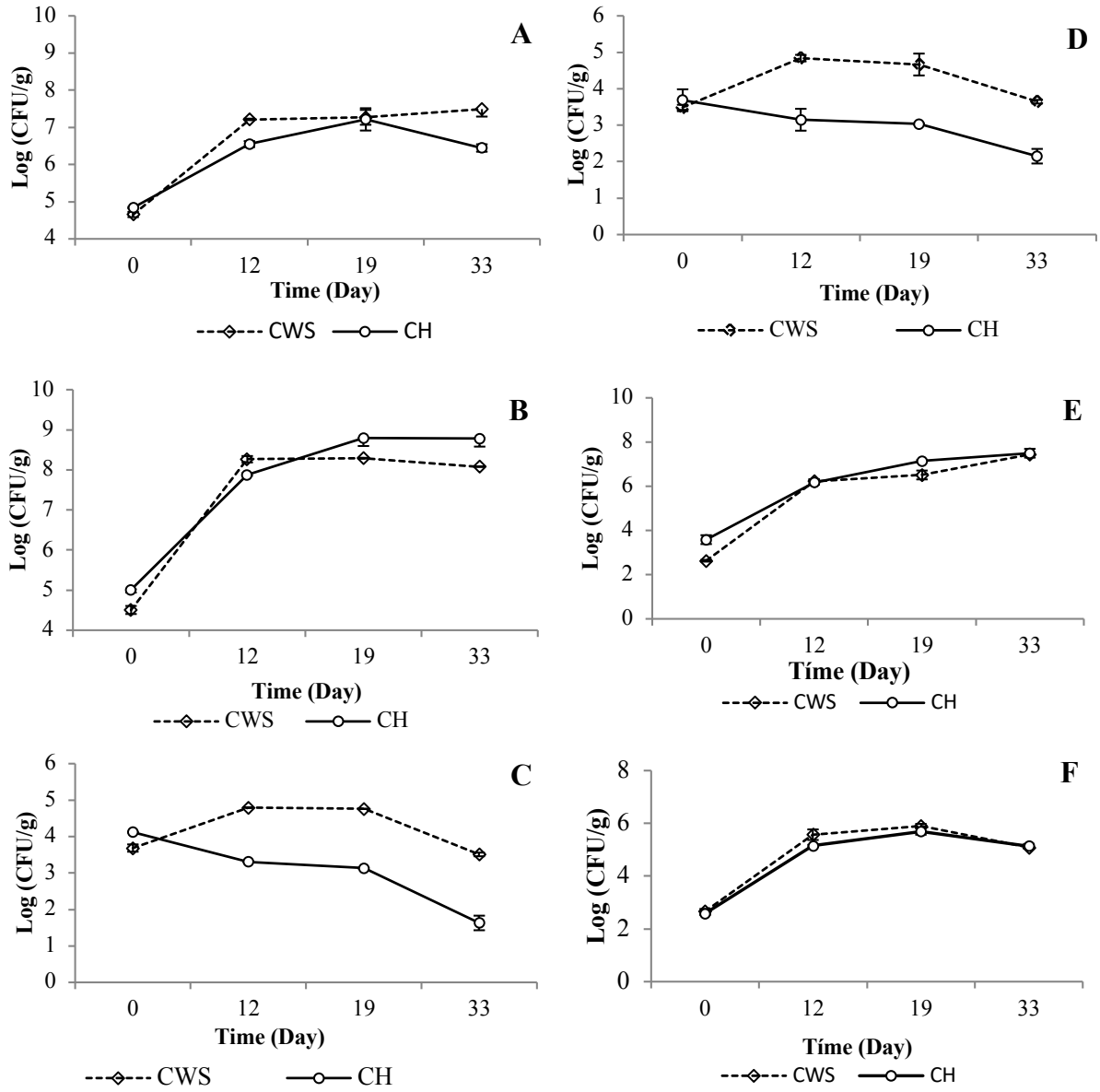

Fig. 2. Microbial growth of (A) mesophilic bacteria, (B) lactic acid bacteria, (C) coliform bacteria, (D) *Enterobacteriaceae*, (E) yeast and (F) moulds during 33 days of ripening in sample of *chorizo* with paprika (CH) and without paprika (CWS).

The AMB count increased ($p<0.05$) during ripening in both types of *chorizo* samples, Figure 2A. The sample CWS had the major increase ($p<0.05$) at day 12, from 4.7 to 7.2 log UFC/g, then the AMB count remained stable during the rest of the days of ripening. This could be related to the a_w, as after day 12 this parameter dropped (Figure 1B); the lower the values of a_w, the slower the growth of AMB (Sun *et al*., 2018). The AMB growth was slow in the *chorizo* sample CH, the highest count ($p< 0.05$) was at day 19 and then decreased ($p<0.05$) at day 33, maybe due to low pH, and also related to the increase of LAB. The accumulation of diverse compounds, such as acetic, lactic acid, diacetyl and bacteriocins may have delayed or inhibited the growth of some microorganisms in these samples (Figueroa-González *et al*., 2010).

Furthermore, the paprika present in the sample CH, just as the other ingredients, contain different groups of compounds that have an antimicrobial activity, such as the terpenes, terpenoids and others (Gottardi, Bukvicki, Prasad, & Tyagi, 2016). Possibly, these compounds contributed to the slow growth of AMB and their decrease at the end of ripening. Similar results were obtained by Sun *et al*. (2018) and Cai *et al*. (2015) who found a lower count of total aerobic bacteria in sausage samples with spices or spice extract than in sausages without them.

The LAB population was essential for fermentation and the generation of a variety of acids (lactic acid, acetic acid, propionic acid and others) that decrease pH and contribute to both odor and flavor. LAB was the group of microorganisms with the highest counts ($p<0.005$) in the *chorizo* sample CH at day 0, Figure 2B. This may be explained as LAB had the ability to grow at 4-6 ºC, when preparing the batter, and they possess fast growth rates, which allow 0.55 generations to be produced per hour at 15 ºC (Ammor & Mayo, 2007); in addition, the paprika content favors their growth. LAB were the main microorganisms during ripening in both *chorizo* samples, CH and CWS, and showed a marked growth from 5 to 7.9 log CFU/g and 4.5 to 8.3 log UFC/g, respectively, during the first 12 days of ripening, Figure 2B. The CWS had a higher ($p<0.05$) growth of LAB than CH at day 12; this difference was probably associated with pH (Fig 1). The low value of pH, as in the case of CH, causes a slower growth rate in some LAB. For CWS, the LAB count remained the same until day 19 and then decreased at day 33. In contrast, in the sample CH, the LAB growth continued and their maximum amount was at day 19 and then remained constant. At the end of ripening, the LAB counts were the highest ($p<0.05$) in this *chorizo* sample. This result indicated that the paprika had a positive effect on LAB growth. Paprika has greater amounts of manganese, which stimulates the development of LAB (Revilla & Quintana, 2005; Toldrá & Hui, 2007). Opposite results were obtained by Lu *et al*. (2010), who did not find any effect of the spice extracts on LAB growth in sausages. Previous studies in *chorizo* (González-Fernández *et al*., 2003; González-Tenorio *et al*., 2013; Ruiz-Capillas *et al*., 2007) and other fermented products (Latorre-Moratalla *et al*., 2010) showed that the final count of LAB are around 8 log (UFC/g), which is similar to our results in both *chorizo* samples at day 33.

The antimicrobial effect of the spices was clearly observed in the growth of COL and ENT in both types of *chorizo*, Figure 2C and 2D, respectively. The samples CWS had a higher count of COL and ENT during ripening time, except at the beginning of the process. Even though spices, such as paprika, have an antimicrobial activity, this is also a source of external contamination of the product (Juárez-Castelán *et al*., 2019; Toldrá & Hui, 2007) and explained the high count of COL and ENT microorganism at day 0 in CH. *Escherichia*, *Klebsiella* and *Enterobacter* are the possible genera that grow in the COL plates, which are gram negative and have the ability to ferment lactose. The plates for ENT enumerated all coliforms gram negative with the ability to ferment glucose, which included the genera *Salmonella*, *Shigella* and *Yersinia* (Hervert *et al*., 2017). Bover-Cid *et al*. (2001) concluded that in the fermented meat products that are of good quality, their ENT count does not exceed 10^3 CFU/g. Table 2 showed both samples were within these limits at the beginning of the process, so the raw material was of good quality. In the sample CH, the growth of COL and ENT decreased, significantly ($p<0.05$), during ripening, from 4.1 to 1.6 log UFC/g and from 3.7 to 2.2 log UFC/g, respectively. This decrease was probably due to the decrease in pH and a_w that inhibits growth of COL (Toldrá, 2008), in addition to the antimicrobial activity of paprika. Similar results were obtained by Sun *et al*. (2018) in this group of microorganisms. Otherwise, in the *chorizo* samples CWS the counts of COL and ENT increased significantly ($p<0.05$) at day 12, and remained the same until day 19; then at day 33 both groups of microorganisms decreased with similar counts to those at the beginning of the process. The possible reason for the COL and ENT behavior in the CWS samples was associated with their pH and a_w, since these samples have higher pH values than CH, so the growth of these groups of microorganisms was favored, and their decrease was attributable to a decrease in moisture content and a_w. Ruiz-Capillas *et al*. (2007) and Latorre-Moratalla *et al*. (2010) considered that the ENT count 2 log or less was a normal count for Spanish *chorizo* and similar products, such as *fuet*, so the final count of ENT was considered normal only in the *chorizo* sample with paprika at day 33.

The YS and ML, considered natural microflora in fermented sausage (Toldrá, 2008), were the minority group of microorganisms at day 0, but then they showed a constant increase during the ripening process, Figure 2E and 2F, respectively. The YS and ML are more resistant than bacteria to several food related stresses, such as slow pH and water

activity resulting from the fermented and ripening process, which modifies the environment that hinders bacterial growth and favors the development of natural competitors, such as these microorganisms (Toldrá, 2008). In Table 2, we observed that the rate of growth in ML was slow in the sample CH due to the fact that the paprika also has an effect on this group of microorganisms (Alves-Silva *et al*., 2013; Gottardi *et al*., 2016); the opposite effect was observed in YS; CH had a higher count of YS than CWS at the beginning of the process and at day 19. Previously, we mentioned that spices are a source of external contamination and it is possible the paprika powder had yeast counts and this could have contributed to the increased yeast count in CH.

3.3 Biogenic amine content by HPTLC

This study evaluated the three major biogenic amines: tyramine, cadaverine and putrescine, and the sum of these three amines was considered as the total biogenic amine content. In the example of the results obtained by HPTLC shown in Figure 3, in the chromatogram of the quantification of putrescine in CWS, we observed the three amines were clearly separated in the *chorizo* samples.

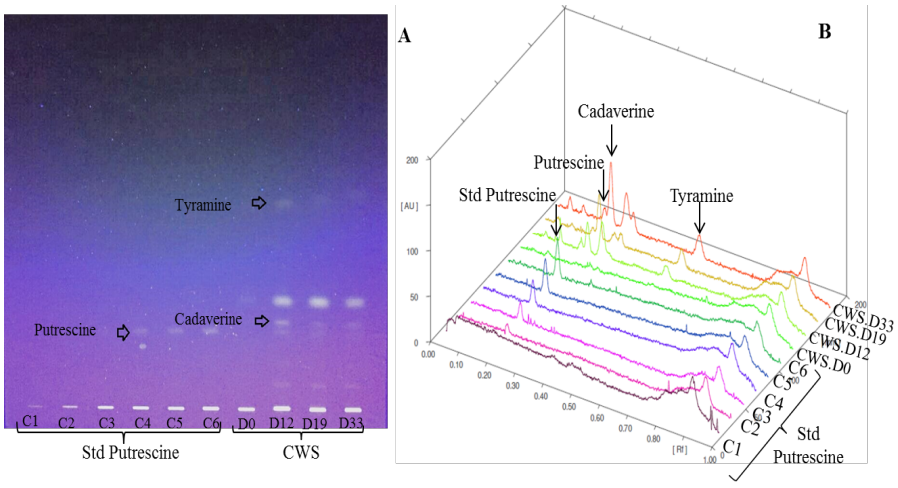

Fig. 3. Quantification of putrescine in chorizo without paprika (CWS) at day 0(D0), day 12 (D12), day 19 (D19) and D33 (D33) by HPTLC. A Image of TLC illuminated by a portable UV lamp at 365nm wavelength. B Chromatogram obtained by CAMAG TLC scanner. (Std) Standard of putrescine at six different concentrations (C1-C6).

Table 2. Content of biogenic amines in both types of *chorizo* samples during ripening, expressed in mg/kg (means ± standard deviation).

Biogenic amine	**Type of *chorizo***	**Days of ripening**				**Significance**			
		0	**12**	**19**	**33**	**R**	**S**	**D**	**SxD**
Tyramine	CWS	ND	44.7 ± 14.69^{c}	62.68 ± 8.84^{bc}	94.41 ± 13.42^{a}	n.s	*	***	ns
	CH	ND	63.45 ± 23.52^{bc}	88.14 ± 24.69^{ab}	98.15 ± 37.34^{a}				
Putrescine	CWS	ND	106.74 ± 45.05^{b}	142.74 ± 31.95^{b}	161.03 ± 17.26^{b}	n.s	**	***	ns
	CH	ND	150.89 ± 26.30^{b}	240± 61.16^{a}	224.28 ± 62.77^{a}				
Cadaverine	CWS	ND	145.22 ± 53.51^{c}	321.92± 46.42^{b}	389.70 ± 35.35^{a}	n.s	***	***	***
	CH	ND	157.13 ± 23.12^{c}	122.56 ± 26.70^{c}	170.76 ± 53.53^{c}				

CWS. *Chorizo* without paprika; CH *Chorizo* with paprika.

R: repeatability effect; S: Chorizo type effect; D: ripening time effect; SxD: Interaction of type of simple and ripening time. ***p<0.001; **p<0.01; *p<0.05; n.s: not significate.

$^{a-c}$Means with different letter of each biogenic amine (double row) differ significantly (p<0.05) between days of ripening and type of *chorizo*.

Putrescine and cadaverine eluted closely, their Rf maximum values were 0.20 ± 0.02 and 0.23 ± 0.01 respectively; while tyramine was the last amine observed in the chromatograms with its maximum Rf at 0.55 ± 0.02. The repeatability of HPTLC methodology was an important factor to know. The statistical analysis showed, Table 2, that the repeatability factor and its interactions (interactions not shown in Table 2), were not significant ($p>0.05$) for any of the three amines; therefore, the HPTLC is a repeatable and reliable methodology to detect and quantify biogenic amines in fermented meat products such as *chorizo*, even though this did not detect any amines at day 0, due to their low amount in the *chorizo* samples.

The concentration of each amine throughout the ripening time in *chorizo*, with and without paprika, is shown in Table 2. The ripening process had a significant effect ($p<0.05$) on the content of the three amines. This behavior is expected due to the fact that the formation of biogenic amines is favored by the increase in amino acids, the result of the proteolytic activity, an adequate condition for the growth of the microorganism with amino acids decarboxylase enzyme, and the optimum conditions for the enzyme activity. Their concentration increased in both *chorizo* samples, except for cadaverine that also had a significant interaction effect between *chorizo* type and ripening time ($p<0.05$). The amount of cadaverine was similar at day 12 in both types of *chorizo*. In the case of CWS, its concentration increased ($p<0.05$) in the subsequent days, being the major amine, followed by putrescine and tyramine, while for CH, cadaverine did not show significant changes during the maturation time, and always remained at lower levels than CWS, Table 2. The higher amount in CWS may be due to the highest count of ENT, as the activity of lysine carboxylase, responsible for generating the cadaverine, was associated with gram negative bacteria, especially ENT, such as *Enterobacter cloacae*, *Enterobacter aerogenes*, *Citrobacter freundii* and other bacteria from the genera *Klebsiella* and *Escherichia* (Bover-Cid *et al*., 2001; Bover-Cid & Holzapfel, 1999; Linares *et al*., 2012; Suzzi & Gardini, 2003). On the other hand, the lower levels of cadaverine in CH may be also due to the lowest count of ENT, which are decreased by the combination of low pH and high counts of LAB (Juárez-Castelán *et al*., 2019). Furthermore, the presence of some LAB has been reported to decrease the accumulation of cadaverine and other amines in silver carp sausages (Zhang, Lin, & Nie, 2013); however, some other studies have shown that LAB have the ability to produce amines, but mainly tyramine (Bover-Cid & Holzapfel, 1999). Although cadaverine could be considered as a good indicator to evaluate the hygienic condition for raw materials and/or the manufacturing process, due to association with the content of ENT (Bover-Cid & Holzapfel, 1999; Latorre-Moratalla *et al*., 2010), until now there does not exist a specific limit for this amine, and most of the studies focus on establishing the limit of the biogenic amines with toxic effects, such as tyramine and histamine (Latorre-Moratalla *et al*., 2017, 2008). According to Suzzi & Gardini, (2003) a high amount of cadaverine has been related to low quality; in this case the *chorizo* samples without paprika had the highest amount of cadaverine and could be considered a fermented product with low quality. This result shows that paprika improved the quality of the *chorizo*. Mah *et al*. (2009) and Sun *et al*. (2018) also found similar results: garlic and other spice extracts inhibit the accumulation of cadaverine. Nevertheless, the cadaverine content of both samples were within the range 200-600 mg/kg, reported previously in other fermented meat products, such as *chorizo* (González-Tenorio *et al*., 2013; Latorre-Moratalla *et al*., 2010; Suzzi & Gardini, 2003).

For *chorizo* with paprika, CH, the major biogenic amine was putrescine, except at day 12, followed by cadaverine and tyramine, Table 2. Their content increases from 150.8mg/kg, at day 12, up to 240 mg/kg at day 19, and then remained constant. Komprda *et al*. (2004) found similar results; the putrescine was the major amine (247 mg/kg) in the sausage with a high content of spices. Other studies also found that putrescine and tyramine were quantitatively the principal amines in *chorizo* (González-Fernández *et al*., 2003; Ruiz-Capillas *et al*., 2007), which confirms that paprika favors their formation during ripening. Other studies in fermented meat products (González-Tenorio *et al*., 2013; Latorre-Moratalla *et al*., 2010; Ruiz-Capillas *et al*., 2007; Van Ba *et al*., 2016) have found that the putrescine levels are between 50- 450 mg/kg; therefore, both samples, CH and CWS, were within these ranges. Similar to cadaverine, the content of putrescine was related to the count of ENT (Signorini & Guerrero-Legarreta, 2009); however, the count of these microorganisms decreased during ripening in the sample CH. Bover-Cid *et al*. (2001), explaining that even though this family of microorganisms is usually found in low numbers, the enzyme which is released, and not the microbial cells, is the responsible for the biogenic amines'

accumulation. Another possible explanation for this fact is that the sample CH had the major count of yeast and LAB, and some authors found that some LAB belonging to the genera *Enterococcus*, *Lactobacillus* and *Lactococcus*, just like several yeasts, are potential producers of putrescine (Linares *et al*., 2012; Suzzi & Gardini, 2003). High levels of these two diamines, cadaverine and putrescine, may have an impact on sensory properties (Kongkiattikajorn, 2015). Alvarez & Moreno-Arribas (2014) reported that some extreme cases of a high content of these diamines can result in the formation of metallic, meaty or putrid aromas in wines. Additionally, Gökoğlu *et al*.(2004) found a significant correlation between both tyramine and cadaverine and the sensory properties in sardines.

In several studies, tyramine is reported as the main biogenic amine in fermented meat products (González-Fernández *et al*., 2003; González-Tenorio *et al*., 2013; Latorre-Moratalla *et al*., 2010, 2017; Ruiz-Capillas *et al*., 2007; Van Ba *et al*., 2016). Nonetheless, in this study we obtained the opposite result: tyramine was the biogenic amine with the lowest quantity during ripening time, in both samples (Table 2 and Figure 4). Suzzi & Gardini (2003) mentioned that there exists a great variability in biogenic amines content in fermented meat products, as their content depends on a complex interaction of factors, such as the quality of ra_w material, time, temperature, pH, and moisture content during ripening, and the content of salts, sugar, starter cultures, spices and others. Tyramine is, toxically, one of the most important biogenic amines formed by tyrosine decarboxylase; it is a potent vasoconstrictor and can induce hypertension, migraine, brain hemorrhages, and heart failure (Komprda *et al*., 2008). For this reason, several investigations have focused on establishing tyramine limits; these indicated that a tyramine concentration above 100 mg/kg of food represented a health hazard (Domínguez, Munekata *et al*., 2016; Latorre-Moratalla *et al*., 2017; Silla Santos, 1996). In addition, Eerola *et al*. (1998) proposed that the total vasoactive amines (tyramine, histamine, triptamine and 2-phenyletilamine) were a possible indicator of hygienic conditions; 200 mg/kg or less of total vasoactive amines indicated a good manufacturing practice. The content of tyramine in the samples CH and CWS throughout ripening was lower than 100 mg/kg and below 136 mg/kg, the mean tyramine values in European sausages (Domínguez *et al*., 2016). Therefore, the concentration of this amine in *chorizo* with and without paprika did not represent any risk to human health throughout the ripening time.

In general, at the beginning of ripening, the total biogenic amine content, Figure 4, of the CWS was lower than CH, 297 mg/kg and 371 mg/kg, respectively. The opposite behavior was found in the subsequent days; the sample CH had a lower amount of total biogenic amine content than CWS, for which the amounts at day 19 were, 451 mg/kg and 527 mg/kg, respectively. At day 33, their contents were 493 mg/kg and 645 mg/kg, respectively. According to Latorre-Moratalla *et al*. (2008), fermented sausages can be classified into five groups, considering the total biogenic amine content. The sample CWS at day 12 was considered to be in group "C", with a moderate content of total biogenic amines (150-350 mg/kg). CH at day 12, 19 and 33, and sample CW at day 19 of ripening, were within the range of "D", with a high content of total biogenic amines (350-550 mg/kg). The sample CW at day 33 was the sample with a very high content of the total amine biogenic, associated with "E" group. Although groups "C, D and E" are less desirable, due to a higher content of amines, the total biogenic amine content was lower than 1000 mg/kg, which has been considered dangerous for human health (Silla Santos, 1996), so the *chorizo* samples elaborated in this study did not represent any risk to health, considering the total biogenic amine content (tyramine, putrescine and cadaverine).

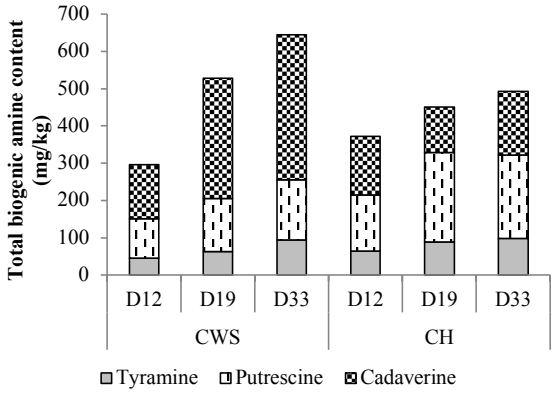

Fig. 4. Total biogenic amine content (Tyramine, Cadaverine and Putrescine) detected in the *chorizo* sample without paprika (CWS) and *chorizo* with paprika (CH) at day 12 (D12), day 19 (D19) and day 33 (D33) of ripening.

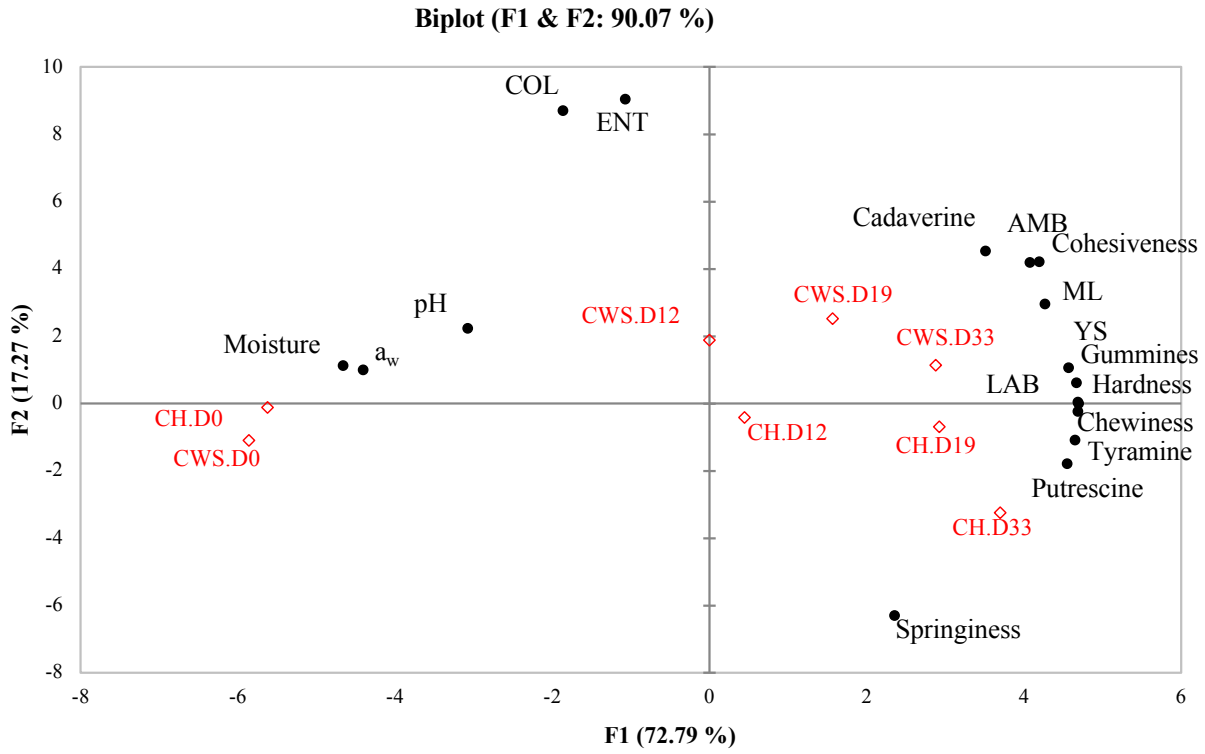

Fig. 5. Principal Component Analysis (PCA) of physico-chemical, textural, microbiological and biogenic amine content. (CWS) chorizo sample without paprika, (CH) chorizo with paprika; (.D0) day 0, (.D12) day 12 (.D19) day 19 (.D33) day 33 of ripening. (AMB) Aerobic mesophilic bacteria, (ML) moulds, (YS) yeast and (LAB) lactic acid bacteria (ENT) Enterobacteriaceae, (COL) Coliform bacteria.

3.4 Relationship between amines and physico-chemical and microbiological variables

The result of PCA is shown in Figure 5. PCA explained 90.06% of the variability in two dimensions. The first dimension separated the *chorizo* samples according to the length of ripening time. Both *chorizo* samples (CWS and CH) at D0 were on the negative side; the variables related to these were pH and a_w. The samples of the remaining days of ripening of the CH and CWS, were on the positive side. In all TPA variables, except springiness, LAB, AMB, ML, YS, cadaverine and tyramine were the parameters related to both *chorizo* samples at days 12, 19 and 33. The second dimension separated the *chorizo* samples with and without paprika. *chorizo* samples without paprika, CWS, were on the positive side and *chorizo* samples with paprika, CH, were on the negative side. Content of COL, ENT and cadaverine were the variables allowed to differentiate both types of *chorizo*. The *chorizo* without paprika was characterized by the highest content of these variables.

Correlation between biogenic amines with the physico-chemical, texture and microbial variables were in Table 3. Positive correlation was found between LAB, AMB, YS, ML, hardness, gumminess and chewiness, with the content of tyramine, cadaverine and putrescine. It means that the increase in these biogenic amines depends on the increase in these variables; the results confirm the relationship between the microbial growth and content of biogenic amines. Although the increase in biogenic amines content has been attributed to growth of LAB or ENT, statistical correlation between these microorganisms and biogenic amine content was not found by other authors (Komprda *et al*., 2009; Latorre-Moratalla *et al*., 2008). However, in this study we found a positive correlation between amines (putrescine and tyramine) and LAB, and both values were higher than 0.90, Table 3. We also found a positive correlation between the three amines and AMB.

Table 3. Pearson's correlation coefficient of biogenic amines, physico-chemical, textural and microbiological variables.

Variables\Amines	**Tyramine**	**Putrescine**	**Cadaverine**
pH	-0.638	-0.759	-0.139
a_w	-0.939	-0.852	-0.764
Moisture	-0.989	-0.953	-0.718
Lactic acid bacteria	0.911	0.928	0.671
Aerobic mesophilic bacteria	0.822	0.785	0.816
Coliform bacteria	-0.513	-0.526	0.051
Enterobacteriaceae	-0.361	-0.398	0.21
Moulds	0.82	0.844	0.706
Yeast	0.966	0.932	0.752
Hardness	0.967	0.968	0.714
Springiness	0.524	0.557	0.055
Cohesiveness	0.834	0.747	0.905
Gumminess	0.975	0.951	0.754
Chewiness	0.977	0.953	0.742

With respect to the relationship between physico-chemical parameters and biogenic amine content, opposite results were found by several authors. Van Ba *et al*. (2016) and González-Fernández *et al*. (2003) found a positive correlation between biogenic amines content and pH, moisture and a_w parameters, and Miguélez-Arrizado *et al*. (2006) and Latorre-Moratalla *et al*. (2010) found a negative correlation between these parameters and biogenic amines. Our results also showed a negative correlation between pH, a_w and moisture content and content of tyramine and putrescine. These negative correlations are explained by the increase in LAB microorganisms that produced a decrease in pH and a_w in the sample, and favored the increase of putrescine. Furthermore, cadaverine had a negative correlation with these parameters and a positive correlation, though not significant, with the ENT, so the *chorizo* samples without paprika, with the highest count of ENT, increased the content of cadaverine, while a_w decreased during ripening.

Conclusions

This study showed that paprika is not only an ingredient that contributes to the development of color, flavor and odor characteristics of the fermented meat, *chorizo*. Paprika allows the product to remain safe for a longer period of time. From 19 days of ripening, the *chorizo* sample with paprika had pH and a_w values within the range necessary to keep the quality safe, whereas the *chorizo* without paprika was only considered a safe product at day 12, but not afterwards. Also, at day 19 of ripening, the *chorizo* sample with paprika increased significantly in hardness, chewiness and cohesiveness, which are important textural characteristics in a dry fermented meat product. In terms of microbiological quality, paprika favored the decrease in coliform and *Enterobacteriaceae*, so that only the *chorizo* with paprika had a good microbiological quality throughout the ripening time.

HPTLC is a good alternative to detect and quantify biogenic amines in fermented meat products. With respect to this, it is impossible to eliminate the biogenic amines content in this type of product; therefore, it is important to reduce them. Although both samples were classified into the groups with the higher content of total biogenic amines, according to Latorre-Moratalla *et al*. (2008), the tyramine content, potentially toxic, was less than 100 mg/kg; therefore, both samples could be considered safe foods. In general, the total biogenic amine content was reduced with paprika; cadaverine was the main amine that was reduced after day 12 of ripening, while putrescine was the major amine after day 19 in this sample. High content of these two diamines, cadaverine and putrescine, could affect the sensory properties, so it is important to carry out a sensory study for future investigations.

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contribution to this work.

Nomenclature

LAB	lactic acid bacteria
a_w	water activity
HPLC	high performance liquid chromatography
HPTLC	high performance thin layer chromatography
TLC	thin layer chromatography
CWS	*chorizo* without paprika
CH	*chorizo* with paprika
AMB	aerobic mesophilic bacteria
COL	coliform bacteria
ENT	*Enterobacteriaceae*
ML	moulds
YS	yeast
CFU	colony-forming units
TPA	texture profile analysis
TCA	trichloroacetic acid
M	molar
NaOH	sodium hydroxide
Na_2CO_3	sodium carbonate
Rf	retardation factor
ANOVA	analysis of variance
PCA	principal component analysis

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