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# Effect of Starter Cultures and Stuffing Time in the Quality of "Chorizo Ambateno"

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**Abstract:** Chorizo Ambateno is a fresh raw product which is made in the central region of Ecuador. It is made from a mixture of chopped meat (pork and beef), salt, additives (nitrite and antioxidants) and spices. In its traditional form, no starter cultures are added. The effect of the incorporation of starter cultures Bactoferm™ LHP (*Pediococcus acidilactici* and *Pediococcus pentosaceus*), Bactoferm™ F-RM-52 (*Lactobacillus curvatus* and *Staphylococcus carnosus*), Bactoferm™ F-LC (*Pediococcus acidilactici*, *Lactobacillus curvatus* and *Staphylococus xylosus*) and dairy culture SLB 953 (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) combined with different stuffing times were evaluated. The results showed that the sample which contains Bactoferm™ F-RM-52 with 24 h of fermentation has the best sensorial and rheological characteristics. Microbiological analysis of the product showed safety due to the absence of microorganisms such as *Escherichia coli, Salmonella, Staphylococcus aureus* and *Clostridium perfingens*. The matured sausage has a 14.10% of protein, 27.47% of fat, 35.98% of moisture and 5.95% of ash. These results could allow that the chorizo Ambateno offer a tasty variation from the original without losing its traditional characteristics.

**Key words:** Matured sausage, starter culture, fermentation, performance, sample, microorganisms

## INTRODUCTION

Modern food production processes are dependent on a wide range of conservation technologies which are responsible to ensure the quality and acceptability of food from production to consumption (Ross *et al*., 2002). Fermentation is probably one of the oldest biotechnological processes and it has been used since prehistory for food preservation for extended periods of time (Bourdichon *et al*., 2012). Of course, these processes were artisanal and obviously could not have considered the role of microorganisms in the biochemical and sensory modifications that were produced (Caplice and Fitzgerald, 1999; Lucke, 1994; Molly *et al*., 1997). The origin of the processing of matured products probably refers to the time of Babylon when methods of preserving food such as drying, salting or fermenting meat were already used (Leistner, 1992; Ross *et al*., 2002). Today, ripened products are renowned for their low water activity values, a suitable pH, characteristic aroma, typical red colour, consistency, cohesion when cutting and a long shelf life without refrigeration (Bartkiene *et al*., 2017; Scetar *et al*., 2013). The production technology of meat and matured meat products allows many variations as long as basic concepts such as reduction of pH and water activity are taken into account. Throughout the world there is a great variety of matured fermented products that are available for commercialization (Fanco *et al*., 2002; Zeuthen and Bogh-Sorensen, 2003).

The addition of starter cultures to meat and meat products have four different purposes to improve food safety by pathogen inactivation to increase stability and consequent extension of shelf life by means of inhibition of undesirable changes produced by sporulated microorganisms or abiotic reactions to provide diversity by modification of raw materials to obtain new sensory properties and to provide health benefits through positive effects on the intestinal flora (Bacus, 1984; Leistner, 1992; Lucke, 2000; Mata, 1999). Depending on the main action, starter cultures can be categorized as acidifying cultures, cultures for colour and taste formation, surface cover crops or cultures for bio protection to suppress the growth of pathogenic and food spoilage bacteria (Bartkiene *et al*., 2017; Leroy and Vuyst, 2004; Roman *et al*., 2006).

Chorizo Ambateno is a traditional meat product made in the central Andean region of Ecuador (Ambato). It is a high on demand product by consumers in the local

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markets. However, this product does not have the uniformity and consistency of quality demanded by the modern market which limits its potential for expansion to new markets. An option for this type of product is the inclusion of starter cultures which could contribute to the development of acceptable biochemical and organoleptic characteristics. The objective of the study was to evaluate the effect of the incorporation of starter cultures and stuffing time in the manufacture of Chorizo Ambateno.

## MATERIALS AND METHODS

**Preparation of Chorizo Ambateno:** Chorizo was prepared from beef (53%, w/w), pork (22% w/w), fat (18% w/w) and spices (8% w/w) which were purchased at a local supermarket. To this mix, 0.125 grams of a starter microbial culture per each kg of meat were added. The starter cultures used were Bactoferm™ LHP (*Pediococcus acidilactici* and *Pediococcus pentosaceus*), Bactoferm™ F-RM-52 (*Lactobacillus curvatus* and *Staphylococcus carnosus*), Bactoferm™ F-LC (*Pediococcus acidilactici, Lactobacillus curvatus* and *Staphylococcus xylosus*) and dairy culture SLB 953 (*Lactobacillus bulgaricus* and *Streptococcus thermophillus*). Table 1 shows the formulations used in the present study which combine a specific starter culture and different stuffing times.

**Physicochemical analysis:** The pH of samples was measured using a digital pH-meter (HANNA HI 9126, Rhode Island, USA). The acidity was determined by titration with NaOH 0.1N using phenolphthalein as an indicator according to the methodology described in AOAC.

**Weight loss:** Weight losses were determined applying Eq. 1 by weighing samples before (initial weight) and after (final weight) the fermentation step:

$$\text{Weight loss}(\%) = \frac{100\times(\text{Initial weight-Final weight})}{\text{Initial weight}} \quad (1)$$

**Sensory evaluation:** The sensory characteristics were evaluated using an incomplete block design with 15 semi-trained judges. Attributes as colour, fragrancy, flavour and general acceptability were considered. Three tastings sessions with a structured hedonic scale of 5 points were performed.

**Proximal analysis:** Moisture, ash, protein and fat content were evaluated following the official methods AOAC 19 927.05, AOAC 923.03, AOAC 2001.11 and AOAC 2033.06, respectively. Carbohydrate contents were estimated by difference. All determinations were performed in triplicate.

Table 1: Formulations used to produce "Chorizo Ambateno"

| Sample | Culture | Stuffing time (h) |
|---|---|---|
| LHP 16 | Bactoferm™ LHP | 16 |
| LHP 24 | Bactoferm™ LHP | 24 |
| FRM 16 | Bactoferm™ F-RM-52 | 16 |
| FRM 24 | Bactoferm™ F-RM-52 | 24 |
| FLC 16 | Bactoferm™ F-LC | 16 |
| FLC 24 | Bactoferm™ F-LC | 24 |
| SLB 16 | Dairy culture SLB 953 | 16 |
| SLB 24 | Dairy culture SLB 953 | 24 |

**Slicing:** The slicing process was performed using an automatic cutter until obtain 0.5 cm thickness slices (Dimitrakopoulou *et al*., 2005). The slicing was evaluated applying Eq. 2, considering the weight loss before and after cutting:

$$\text{Slicing}(\%) = \frac{100\times(\text{Initial weight-Final weight})}{\text{Initial weight}} \quad (2)$$

**Microbiological analysis and starters cultures growing:** In all samples, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp. and *Clostridium perfringens* were evaluated following the official methods AOAC, 991, 2003, 2003 and 976, respectively. The best "chorizo" formulation was selected by pH and sensory evaluations and a growth curve of *Staphylococcus carnosus* and *Lactobacillus curvatus* as a function of time was developed using the agar dilution method.

**Statistical analysis:** A two-way ANOVA was obtained using the GraphPad Prism 5.0 program (GraphPad Software, San Diego, California, USA). Additionally, statistical comparisons of the mean values of the data were carried out using the Tukey test with a significance level of $p \leq 0.05$.

## RESULTS AND DISCUSSION

**Physicochemical properties:** The pH values of all samples for the first 24 h of the stuffing time is shown in Fig. 1a. During this time is evident that the pH decreased from ~6.85-5.14. The lowest pH value (pH 5.14) was observed in the sample inoculated with Bactoferm™ FRM-52 while the dairy cultures SLB 953, Bactoferm™ F-LC and Bactoferm™ LHP have an average pH of 5.25, 5.71 and 5.73, respectively. These values are significantly higher when compared to the evaluation parameter. Differences in pH values could be explained by the production of one or more active metabolites. It is well known that organic acids such as lactic, acetic, formic, propionic and butyric acids, produced as consequence of growth of starter cultures can influence the pH values (Casaburi *et al*., 2016). Usually, fermented meat products have a low pH value (4.6-5.9). These characteristics inhibit the growth of pathogenic and spoilage bacteria and

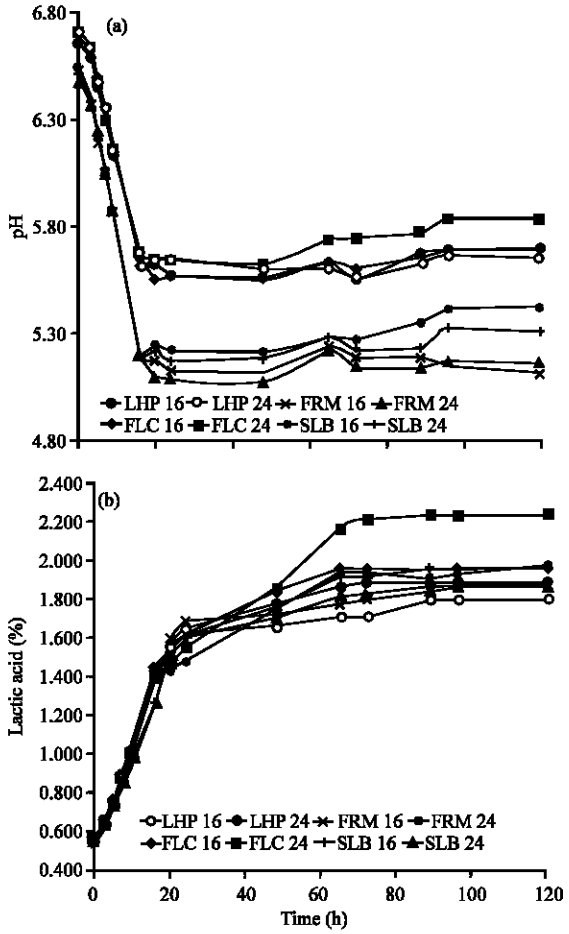


Fig. 1: a) Changes in pH during ripening and storage time and b) Lactic acid production of Chorizo Ambateno

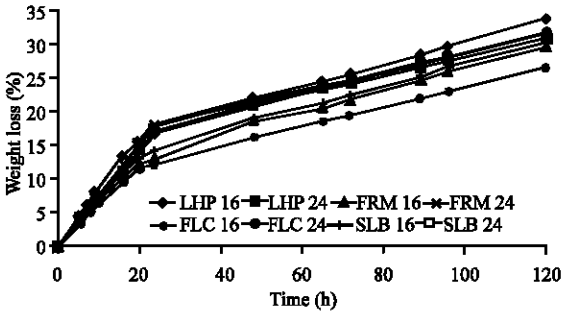


Fig. 2: "Chorizo" weight loss (%) as a function of maturation time (h)

contribute to a prolonged shelf-life (Leistner, 1992; Radulovic *et al*., 2011; Reis *et al*., 2012; Tsuda *et al*., 2012). Titratable acidity expressed as a percentage of lactic acid was evaluated at the end of maturation, values in the range of 1.78-2.22% were obtained. Bactoferm™ F-LC showed the highest production of lactic acid with an average of 2.08%. The other starter cultures that is Bactoferm™ LHP, Bactoferm™ F-RM-52 and dairy culture SLB 953 did not present significant statistical differences ($p>0.05$) in production of the lactic acid. The presence of *Pediococcus acidilactici* and *Lactobacillus curvatus* in the same sample could be the principal factor for the highest production of lactic acid in sample FRM24. It has been demonstrated that the lactic acid production is a major characteristic on bacterial groups such as *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Leuconostoc* and *Enterococcus* species (Alvarez *et al*., 2013; Reis *et al*., 2012). The increase of lactic acid content in all treatments as a consequence of the growth of the starter cultures (Fig. 1b) was in agreement with the pH values obtained (Fig. 1a).

**Weight loss:** During the first 24 h a significant weight loss was observed whereas at 120 h of maturation, most experimental treatments had weight loss values from 26.08-33.85% (Fig. 2). Based on the results, the drying process was considered as complete. It has been established that a mature meat product must have a weight loss of 30-35% (Frey, 1983).

**Sensory evaluation:** From the sensory evaluation of chorizo, it was possible to establish that at a 95% level of confidence there are not significant differences between treatments in attributes such as colour and smell. So that regardless of the starter culture and stuffing time the developing of colour and smell did not show a perceptible change by the judges. Likewise, the colour intensity ranged from red intense to very red intense while the smell was between perceivable to very perceivable. In the flavour attribute, significant differences were found ($p<0.05$). The multiple range test of Tukey HSD established the treatment FRM24 as the best treatment, finding that was corroborated by the acceptability evaluation which showed that the highest acceptability was obtained by treatment FRM 24 (Fig. 3).

**Proximal composition:** Nutritional characteristics were performed for the best treatment (FRM, 24) sensory analysis and pH were selected to determine the best treatment because pH is one of the most important parameters in production of fermented meat products. As a rule, meat and meat products must have lower than pH 5.9. At higher pH meat and meat products contain too little lactate and sugar for a safe fermentation (Caplice and Fitzgerald, 1999; Lucke, 1994). On the other hand, sensory analysis is a power tool in development of new products,

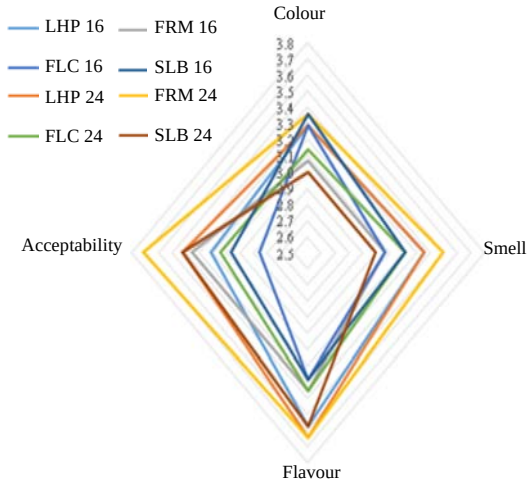


Fig. 3: Sensorial evaluation of "Chorizo Ambateno"

especially in traditional foods which are improved with different raw materials, spices and microorganisms. It is always important to know how customers perceive a new product as they are the ones that are going to purchase it, if it is better than the ones that are already available (Perez-Cacho *et al*., 2005). Protein, fat and carbohydrates contents were 14.10, 27.47 and 16.5%, respectively these values are in concordance with Ecuadorian food regulation. Moisture content was 35.98%. The moisture content of the chorizo can be categorized as a short maturation sausage (1-4 weeks) based on a humidity content between 30-40% and water activity between 0.92-0.94 (Lorenzo *et al*., 2012; Roca and Incze, 1990). On the other hand, the ash content of chorizo was 5.95%. This result could be explained by the addition of sodium chloride to the starting mixture. Consequently, the curing salts concentration will increase at the end of the drying process (Sidira *et al*., 2016).

**Slicing:** From the slicing process, the best sample was again FRM 24 with a 91.17% w/w. The slicing is related with the consistency and the formation of a matrix whose properties depend almost exclusively on the proteins of the meat used as raw material. The decrease of the pH values as consequence of the starter cultures metabolism could lead to the insolubilization and denaturation of proteins. This causes a decrease in the water retention capacity that allows the product to be desiccated. The progressive drying contributes to the consolidation of the consistency and texture of the sausage (Prpich *et al*., 2016; Roman *et al*., 2006).

**Microbiological quality:** The microbiological analysis establishes that the processed Chorizo Ambateno

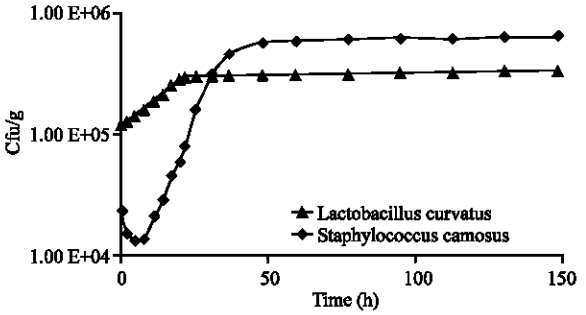


Fig. 4: Growth curve of *Staphylococcus carnosus* and *Lactobacillus curvatus* (cfu/g) as a function of time (h)

Table 2: Variables of the microbial growth

| Microorganisms | Growth models | n | g (h) | K ($h^{-1}$) |
|---|---|---|---|---|
| *Staphylococcus carnosus* | log (cfu/g) = 0.0177×(h)+5.0659 | 1.27 | 17.00 | 0.041 |
| *Lactobacillus curvatus* | log (cfu/g) = 0.0501×(h)+3.7606 | 5.10 | 6.01 | 0.115 |

n: Number of generations; g: generation time, K: constant speed

complies with the provisions by the INEN 1338:2012 for *Staphylococcus aureus* and *Clostridium perfringens* which must correspond to >10 cfu/g and absence for *Salmonella*. All the produce comply with the normative that request absence of harmful microorganisms to public health. The results of microbial growth of Bactoferm™ F-RM-52 (*Lactobacillus curvatus* and *Staphylococcus carnosus* Fig. 4) were used to establish the equations of growth kinetics, number of generations (n), generation time (g) and growth rate constant (k). All these variables are shown in Table 2.

## CONCLUSION

The research allowed to develop a fermented meat product as a variant to the classic chorizo ambateno. In addition, the best formulation was obtained using *Staphylococcus carnosus* and *Lactobacillus curvatus* (Bactoferm™ F-RM-52) at 24 h of stuffing time. The results obtained during the sensory evaluation establish that fermented chorizo ambateno FRM24 was accepted by judges and therefore, it could be accepted by potential customers. The pH of this product was 5.13 with a percentage of lactic acid of 1.78 at the end of the fermentation. The weight loss of the product ranges from 26.08-33.85% while the slicing was 91.17%. The matured sausage contains protein, fat, moisture and ashes according to the current regulations. Microbiological analysis made it possible to establish the absence of pathogenic microorganisms for public health.

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