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Cocoa polyphenols (*Theobroma cacao*) as natural Amazonian antioxidant in sausage fresh.

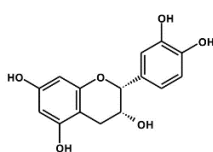
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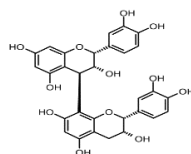
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Graphical Abstract**Cocoa (*Theobroma Cacao*)**

Polyphenols (*Theobroma cacao*) are used as natural



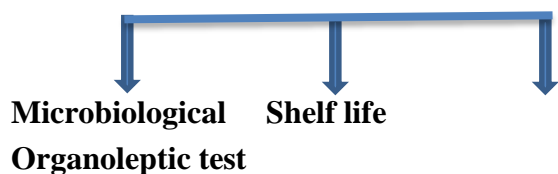
Epicatechin



Procianidin



Sausage prepared

ANALYSIS**Abstract**

Ecuador is considered one of the richest countries regarding biodiversity and its Amazon region guests several flora and fauna species. Cacao (*Theobroma Cacao*) is a tropical fruit with high commercial and biological importance due to the presence of polyphenols with antioxidant activity. In the present work cocoa polyphenols (*Theobroma cacao*) are used as natural Amazonian antioxidant in sausage fresh. Experimental units of sausage samples with different percentages (0, 2, 4, 6%) of cocoa polyphenols in the formulation with an experimental size of 5 kg were made; once the sausage was prepared, samples of 100 g were taken to perform sensory analysis and 100 g of samples destined for microbiological analysis. The microbiological analysis was performed on each sample of the formulated product, analyzes on the newly elaborated samples (1 day of elaboration) and an analysis with samples was developed after an estimated time (30 days) to assess the differences and active action of the antioxidant as a natural Amazonian preservative were performed. 15 people evaluated sensory characteristics to the sausage samples through a tasting test. As main results the natural antioxidants use allows prolonging the product shelf life, which results in an increase in color stability, since it prevents the transition from myoglobin to metamyoglobin, as well as maintaining its organoleptic conditions unalterable, slowing down oxidative phenomena such as product rancidity or increasing resistance to bacterial growth, since antioxidants of polyphenolic nature have antimicrobial activity.

Keywords: Polyphenols, antioxidant, functional foods, fresh sausage.

Introduction

For years, various strategies have been developed to prevent oxidative deterioration in products of meat origin through the use of antioxidants (Rostamzad *et al.*, 2011). Most of these strategies have focused on limiting oxygen access to meat components susceptible to oxidation phenomena such as lipids and proteins. At the same time, new storage methods have been developed, such as vacuum packaging or packaging in a modified atmosphere in order to prevent the appearance of oxidation phenomena in the final product (Armenteros *et al.*, 2012).

One way to reduce the occurrence of oxidation phenomena in meat and / or meat products is the use of antioxidants. The term antioxidant is generally attributed to any substance that is present at low concentrations, with respect to those of an oxidizable substrate and retards or prevents the oxidation of that substrate (Halliwell and Gutteridge, 1990). When antioxidant reacting with the free radical, it gives an electron oxidizing in turn and becoming a weak radical, with little or no toxic effects. In recent years it has been shown that a diet rich in plant polyphenols can improve health and decrease the incidence of cardiovascular diseases (Quiñones *et al.*, 2012). In the present work cocoa polyphenols (*Theobroma cacao*) are used as natural Amazonian antioxidant in sausage fresh.

Materials and methods

Location and duration of the experiment

The present research was carried out in the Agroindustry's Laboratory, located in the Amazon State University, Km. 2 1/2 via Puyo to Tena (Paso Lateral), province of Pastaza, between coordinates 0° 59 '1 "S and at a length of 77° 49' 0" W, it is found in the Amazonian Region of Ecuador in the west of the province of Pastaza, at about 924 m.a.s.l. Temperature 18 to 24 °C.

Experimental units

Experimental units were formed for each sample of chorizo with different percentages of *Theobroma cacao* polyphenols in the formulation (0, 2, 4, 6%) and an experimental size of 5 kg of prepared dough. Once the sausage was prepared, samples of 100 g of each replicate sample were taken to perform sensory analysis and 100g of samples destined for microbiological analysis.

Microbiological analysis.

The microbiological analysis was performed on each sample of formulated product, analyzes were performed on the newly elaborated samples (1 day of elaboration) and an analysis with samples was developed after an estimated time (30 days) to assess the differences and active action of the antioxidant as a natural Amazonian preservative.

Sensory analysis and shelf life.

Through a tasting test, 15 people evaluated sensory sausage samples. An evaluation the product to know the shelf life in 30 days was made.

Results and discussion

Sensorial analysis

40% of the evaluated peoples that correspond to 6 persons, likes sausage samples without any addition of natural antioxidant; 9 from 15 people like the product with 2% natural antioxidant addition, 7 people who represent 47% have similarity in the sausage with 4% natural antioxidant incorporated, finally, 6 people equivalent to 40% like it and they like the product with 6% antioxidants. It should be noted that 4 people (27%) like the product a lot when they sensually find it optimal when 4% natural antioxidant is added (table 1).

Table 1. Percentage (%) of antioxidant in sausage samples.

Level of liking	0%	2%	4%	6%
I like very much	2	0	4	2
I like it	6	9	7	6
I do not like or dislike	5	6	3	4
I do not like	2	0	1	2
I dislike a lot	0	0	0	1
Total	15	15	15	15

Natural antioxidant and antimicrobial systems are set to become an important component in food preservation methodology. Wojciak et al. (2016) studied the effect of alternative natural preservatives (*Sinapis alba* L.-M, *Rosmarinus officinalis* L.-R, *Juniperus communis* L.-J) in combination with acid whey after the ripening period (21d) and over a prolonged storage period of sausage. An antioxidant activity of extracts exercise was performed. The antimicrobial, oxidative stability and sensory properties of these natural preservatives were compared to curing-control. Significantly lower rancid odor and rancid flavor were observed for R and M compared with the control sample. Incorporation of acid whey with rosemary extract will give the product a threefold effect: high quality (sensory acceptance), healthy benefit (elimination of nitrite and nitrate from meat products) and safety (improved microbiological and oxidative stability).

Marangoni and Moura (2011) determined sensory prolife of four samples of Italian salami using a methodology based on the Quantitative Descriptive Analysis. They select twelve individuals as judges and properly trained and used the following criteria: discriminating power, reproducibility, and individual consensus. The salami with coriander essential oil had lower rancid taste and rancid odor, whereas the control showed high values of these sensory attributes. Regarding brightness treated with coriander essential oil showed the best result.

By other hand, the addition of okra flour to an emulsified meat product (Frankfurter type sausage) was evaluated (Kitagawa *et al.* 2010) based on the physical, chemical, technological, and sensory characteristics of the final product. The results showed that the sausages containing okra flours A and B, as well as the control sausage, were accepted by the sensory panel. Moreover, there were no significant differences ($p \leq 0.05$) in the physical (color, objective texture, and emulsion stability) and chemical (pH and proximate composition) measurements of the sausages with and without the okra flour.

Microbiological analysis

The following table shows the significant reduction of microbial load in the stored sausages and that they contain polyphenols percentages incorporated in their formulation, while in the control product, since there is no protective agent, a microbiological growth can be observed (table 2). The use of natural antioxidants allows to prolong the useful life of the product which is in an increase of the stability of the color, since it avoids the transition from myoglobin to metamyoglobin, as well as maintains its

organoleptic conditions unalterable slowing down oxidative phenomena as the rancidity of the product or increasing resistance to bacterial growth, since antioxidants of polyphenolic nature have antimicrobial activity (Naveena *et al.*, 2008).

Consumers are becoming more aware of the toxicological implications of artificial additives in foods. Natural antioxidants, in addition to reducing the deleterious effects of lipid oxidation, are currently extremely highly valued. Santi *et al.* (2015) investigated the effect of addition of sun mushroom (*Agaricus blazei* Murrill) powder on the oxidative and microbiological stability of pork sausage during the shelf life. The results of the proximal composition and microbiological analysis for coagulase positive *Staphylococcus*, coliforms at 35 °C and 45 °C, *Salmonella sp* and sulfite-reducing Clostridium were consistent with those required by Brazilian legislation. The color of the products was of a decreased redness at the end of the storage period, on the 35th day, the TBARS values for the sausage with 4.0% powder was 0.509±0.12 mg MDA/kg sample and for the control was 1.131±0.12 mg MDA/kg sample. The sun mushroom powder had no effect on microbiological stability. It is concluded that sun mushroom was effective in terms of the oxidative stability of pork sausage when added in powdered form at concentrations of 1.0%, 2.0% and 4.0%.

Table 2. Total of microbial load in the stored sausages

General data			Indicators							
			Fresh samples				Sample with 30 days of preparation			
Type of sample	Code	Sample	Residual total coliforms	Mesophil bacteria count	Total coliforms	<i>E. coli</i>	Residual total coliforms	Mesophil bacteria count	Total coliforms	<i>E. coli</i>
Sausages	001	0%	<3	2x10 ⁴	579	NA	<3	2,2x10 ⁴	655	Nd
Sausages	002	2%	<3	2,6x10 ⁴	723	NA	<3	2,2x10 ⁴	567	Nd
Sausages	003	4%	<3	2,2x10 ⁴	564	NA	<3	1,8x10 ⁴	344	Nd
Sausages	004	6%	<3	2,8x10 ⁴	490	NA	<3	2,1x10 ⁴	302	Nd
Maximum Permissible Limits										
Total coliforms			Mesophil bacteria count			Total coliform		<i>E. coli</i>		
0,3 – 1 < 1/g			M ufc/g			<2 NMP/100 ml		<0 NMP/100 ml Absence		

Note:

ufc/g: colony forming unit per grams.

NMP/100ml: Most probable number of coliforms/100 milliliters of sample.

NA: Not Applicable

Conclusions

Cocoa polyphenols (*Theobroma cacao*) presents good qualities as an antioxidant in the use of sausages, allowing a better conservation and providing organoleptically unique qualities. Chorizo with 2% presents the best sensory characteristics and most welcome in the respondents. Meat products made with the addition of natural antioxidants from the Amazon provide, in addition to their basic nutritional properties, providing consumers with food that allows them to obtain better quality, health and life expectancy.

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