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Use of *Hibiscus sabdariffa* extract as a natural antioxidant in sausages

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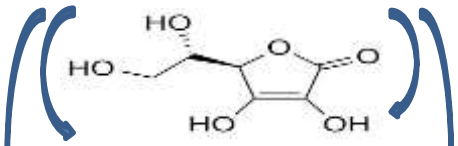
Jamaican (*Hibiscus sabdariffa*)



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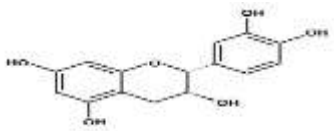

Ácido polifenólico

Chorizo normal

Chorizo funcional (plantas amazónicas)

No information about *Hibiscus sadariffa*

Abstract.

The use of Jamaican extract (*Hibiscus sabdariffa*) in different concentrations (2, 4 and 6%) as a natural antioxidant in the preparation of sausages allows us to offer healthy alternatives as a sausage preservative. Due to its composition, meat and fat present several alteration phenomena, among which the oxidation or rancidity produced by exposure to air stands out, which causes losses in trade and industrialization. The proper treatment of *Hibiscus sabdariffa* extract was determined by bromatological, sensory and evaluation of antioxidant activity over time. The sausage was made with pork meat and fat, mainly as raw materials to be ground and mixed into a thick paste along with the other ingredients. The results determined that the 4% addition of *Hibiscus sabdariffa* extract provides acceptable bromatological and sensory characteristics. The evaluation of antioxidant activity determined that the 4% incorporated product provides better conservation qualities, a result that corroborates that of the sensory evaluation in which the product presents stable appearance and acceptable coloring with the same treatment. The bromatological analysis presents variations in the concentrations of protein, fat, water, carbohydrates, minerals, when compared the results of day 1 versus day 30 showed that the time traveled directly influences the results with a reduction of each component by dehydration in the elapsed time.

Keywords: *Hibiscus sabdariffa*, antioxidant activity, sausage.

Meat is an important food for the human diet, its great nutritional richness is formed by the high content of high value biological proteins, but, on the other hand, is one of the most perishable foods due to its high content in water, composition and pH, which favors the alteration and microbial contamination, may constitute a risk to health (Tiburcio, 2018). Due to its nutritional value and composition, different agents easily alter meat. Among the usual problems is the oxidation of adipose and muscular tissue, a phenomenon that alters the biochemical composition of meat and presents colour changes reducing its industrial importance. Pérez (2000) points out that colour is the factor that most affects the appearance of meat and meat products during storage and the one that

most influences customer preference, so colour alteration may well be the most important cause that defines the durability of meat products.

For many reasons, meat products are exceptionally suitable foods for introducing various bioactive compounds into the diet without changing consumption habits. In recent years, there has been a notable development of meat products designed as potentially functional, beneficial to health (Olmedilla et al., 2014). Industries have employed various techniques to prevent oxidation of meats; additives such as industrial and natural antioxidants play an important role in the meat industry. Valenzuela (2016) mentions that natural antioxidants are substances that are added to foods of meat origin to avoid "rancidity", a problem that causes discoloration, unpleasant taste and produces elements harmful to health. The antioxidant defense system is made up of a group of substances that, being present in low concentrations with respect to the oxidable substrate, significantly delay or prevent its oxidation (Pace, 2010).

The jamaica plant (*Hibiscus sabdariffa*) contains several compounds. These include alkaloids, ascorbic acid, anisaldehyde, anthocyanins, b-carotene, citric acid, malic acid, galactose, pectin, stearic acid (Hirunpanich et al., 2005). The extracts obtained from these have been attributed with various mediational properties such as diuretic effects, choleretic effects, reduction of blood pressure, reduction of cholesterol levels (Duke et al., 2003). Due to the antioxidant activity of their anthocyanins, they can also help in the prevention and treatment of some types of cancer (Chen et al., 2003).

Salazar et al. (2012) obtained liquid extracts of dried Jamaican flower calyxes (*Hibiscus sabdariffa*) using ethanol:water (in ratios of 50:50 and 70:30%, v/v), water, ethanol:HCl 1.5 N (85:25%, v/v) and ethanol (96%), to evaluate some antioxidant characteristics and color parameters by high-performance liquid chromatography (HPLC), and total monomeric anthocyanins, by the differential pH method. These authors found antioxidant capacity in a range of 3.11±0.50 (in ethanol) to 8.04±0.22 mmoles of trolox 100 g^{-1} of dried Jamaican flower calyxes.

Most antioxidants are found in plant foods, which explains why including fruits, vegetables or whole grains in our diet is so beneficial. It is a group of vitamins, minerals, natural colouring agents and other compounds of plants and enzymes. According to the above information, it may be possible to use natural antioxidants of *Hibiscus sabdariffa* for the conservation and nutritional improvement of sausage in the conditions of the Ecuadorian Amazon.

The aim of the work was to quantify the level of *Hibiscus sabdariffa* extract as a natural antioxidant in the sausage meat product.

Materials and Methods

Location and duration of the experiment

The experimental work was carried out in the biology and environmental chemistry laboratories of the Amazonian State University located at Km 2 ½ via Napo (lateral pass), in the city of Puyo, Pastaza province. The environmental conditions that the Puyo possesses are altitude of 954 m.a.s.l, its coordinates are 1°28′02″S 77°59′50″O, the relative humidity that characterizes the area is 85%, its temperature is 25.9 °C and rainfall is 45000 mm / year. The study time was 8 months.

The antioxidant extracts of *Hibiscus sabdariffa* were obtained according to Abreu et al. (2018). While fresh sausage was prepared according to Silva et al. (2018) on polyphenols from *Theobroma cacao* L. as a natural antioxidant in fresh sausage. In the study, samples of each treatment were taken on day 1 and after 30 days, the results of sensory, bromatological and antioxidant activity evaluations were analyzed and compared.
The sensorial analysis was carried out with 20 tasters who determined the degree of acceptability of the sausages according to Sánchez Albarracín (2010). The treatments were coded and placed alternately.

The method to determine antioxidant activity was the stabilization of 2,2 - diphenyl-1 - picrylhydrazil (DPPH) which is based on the measurement of the ability of antioxidant compounds to reduce DPPH from the decrease in reaction absorbance as a function of time (Prior et al., 2005).

Bromatological analysis

The bromatological analysis was carried out to evaluate the physical-chemical content of the product, variables such as protein, fat, water and minerals, important when it comes to nutritional information (AOAC, 1999).

Data processing

The results obtained from the design were evaluated by ANOVA (analysis of variance) at 5% level of significance (Tukey $p<0.05$). Design generation and statistical analysis were performed using Software Design Expert version 10.0.3 (Stat-Ease, USA).

Results and Discussion *(optional), no page limit*

Antioxidant activity

Results of antioxidant activity are presented in table 1. Superior values of antioxidant activity ($p<0.05$) are observed in treatments with hydroalcoholic extract of *Hibiscus sabdariffa* flowers. Increases in antioxidant activity values are mainly due to the antioxidant activity of phenolic compounds and their redox properties, which play an important role in the adsorption and neutralization of free radicals that appear with the presence of oxygen and as time passes (Murthy et al., 1998).

Coronado et al. (2015) highlighted aspects of antioxidants and health. These authors conducted a literature search and report that antioxidants can neutralize excess free radicals during the oxidative activity of the body. Different metabolic pathways regulate the production of free radicals, a natural event, because they represent the first line of defense of living beings. However, although they are relevant to maintaining health, the imbalance between endogenous antioxidants and free radicals (oxidative stress) is associated with different diseases or with human aging.

Healthy eating has been based on combinations of foods, nutrients and the addition of ingredients that provide certain conditions for health, designed for different physiological situations. Based on the above, a definition has been studied and developed to refer to these types of products, called "Functional Foods". Meat-based foods with ingredients that provide health benefits are becoming increasingly popular and offer an alternative in the consumption of foods that maintain and even improve the quality of life (Ospina et al., 2011). This work is a contribution to functional nutrition through the incorporation of a hydroalcoholic extract of flowers from Hibiscus sabdariffa to sausage.

It should be considered that the relationship of antioxidant activity obtained in this work are similar to those obtained by Silva et al. (2018) in his study on polyphenols from *Theobroma cacao* L. almond as a natural antioxidant in fresh chorizo, where from the initial activity over time there is a relative increase of 0.216 to 0.388 (mg/kg), from 0.219 to 0.413 (mg/kg), 0.251 to 0.410, values obtained with 2, 4 and 6% respectively of incorporation of the natural antioxidant.

Table 1. Antioxidant activity (DPPH) in sausage with extracts of *Hibiscus sabdariffa* (mg/kg).

Sausages	Day 1	Day 30
0%	0.000^{a}	0.000^{c}
2%	0.200^{a}	0.280^{a}
4%	0.223^{a}	0.358^{b}
6%	0.239^{a}	0.365^{b}

^{a,b,c}Averages with different letters differ statistically (Tukey $p<0.05$).

Bromatological analysis

Table 2 shows the bromatological results of sausage with different levels of hydroalcoholic extracts from Hibiscus sabdariffa flowers. It can be observed a tendency to higher protein content in all treatments

with hydroalcoholic extracts, a different behavior is observed in the total fat content where treatments with hydroalcoholic extracts have a tendency to lower contents. The content of carbohydrates evidences superior contents in the treatments with the extract.

It is very important the decrease that exists in the contents of fat in sausage with the hydroalcoholic extract of flowers of *Hibiscus sabdariffa*. In recent years, some health problems have been associated with diets that have high fat content, especially saturated; this coupled with changes in consumer preferences, have led to extensive research and diversification of foods, some of them low in fat (Carrapiso, 2007; Yang et al., 2007).

The Food and Agriculture Organization of the United Nations (2017) points out that chorizo and longaniza are raw, heterogeneously textured sausages of Spanish origin made from minced pork scrambled with salt, spices and potassium nitrate. Being a thick paste product is difficult to obtain similar results from the same product and there are variations as shown in Figure 1 specifically in the protein contents.

Table 2. Bromatological analysis of sausage with extracts of *Hibiscus sabdariffa*.

Variable	Time	Treatments			
		0%	2%	4%	6%
Crude protein (g/100g)	Day 1	20,44	22,53	23,88	22,66
	Day 30	19,98	22,33	22,56	22,75
Total Fat (g/100g)	Day 1	29,27[a]	27,66[b]	26,33[b]	28,05[b]
	Day 30	27,63	25,00	24,02	25,60
Water g/100g	Day 1	40,76	39,44	39,24	37,08
	Day 30	36,25	35,56	33,56	32,22
Minerals g/100g	Day 1	4,55	4,73	4,24	4,75
	Day 30	4,44	4,32	4,56	4,33
Carbohidrates g/100g	Day 1	9,05	10,15	13,93	11,50
	Day 30	8,54	9,34	12,57	11,23

[a,b,c]Averages with different letters differ statistically (Tukey $p<0.05$).

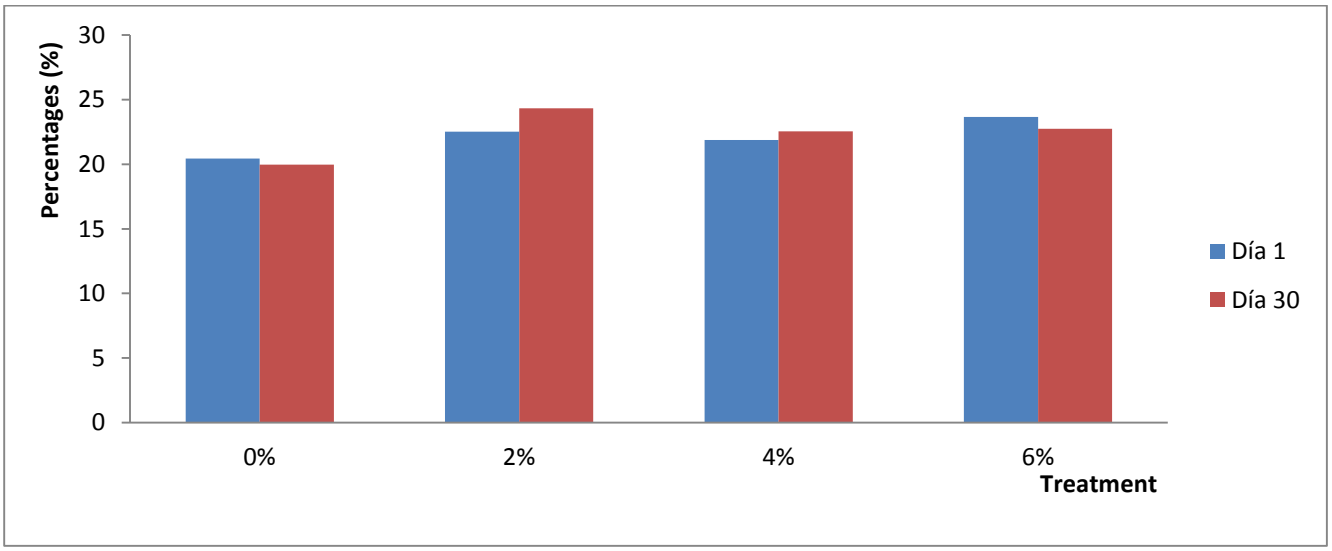

Figure 1. Protein content in sausage with extracts of *Hibiscus sabdariffa*.

Sensory analysis

Table 3 presents the sensorial analysis in sausage with three levels of hydroalcoholic extract of *Hibiscus sabdariffa* flowers on days 1 and 30, after production. It is observed that sausage with *Hibiscus sabdariffa* extract on day 1 was more popular with 4% addition of natural extract; similarly, on day 30 sausage with 4% treatment also presents favorable results.

The inclusion of natural antioxidant extracts in the preservation of meat is becoming increasingly important due to the need for processed foods with low chemical content, which are harmful to health (Lou, 2013). On the other hand, it is validod that they contain active principles of plants, such as antioxidants beneficial to human health, by contributing to the elimination of excess free radicals, which cause chronic diseases, with incidence in high mortality in today's world (Viada et al., 2017).

For Sánchez and Albarracín (2010), sensory interpretations in new foods can mark a new normalization that allows, by means of instrumental measures, to obtain an immediate assessment with a lower percentage of error, supported by sequential statistical methods that are easy to understand and use. On the other hand, the demands of the meat sector and its derivatives justify the importance of finding additives that maintain the nutritional properties of the food with less or no inclusion of chemicals adverse to health (Tofiño et al., 2017).

Table 3. Sensory analysis of sausage with *Hibiscus sabdariffa* extract.

Level of liking	Sausage with extract of *Hibiscus sabdariffa.*							
	Day 1				Day 30			
	0%	2%	4%	6%	0%	2%	4%	6%
I like it very much	6	7	9	8	2	6	7	5
I like	7	7	10	7	4	4	7	6
I don't like it or dislike it	5	5	2	3	4	5	4	8
I don't like	2	1	0	2	8	3	2	1
I don't like it very much	0	0	0	0	2	2	0	0
Total	20	20	20	20	20	20	20	20

Conclusions

Sausage made with 6% of the extract of *Hibiscus sabdariffa* presents an increase in antioxidant activity. The evaluation of protein presents a higher concentration is the treatment with 4% of the extract incorporated. The sensory evaluation determined that 4% is the treatment that best organoleptic characteristics gives the product to consumers. The use of the extract of *Hibiscus sabdariffa* as a natural antioxidant incorporated in formulate with 4% provides stable conservation properties, this percentage can be used in the elaboration of several products of meat origin.

References

Abreu‐Naranjo, Reinier; Arteaga‐Crespo, Yasiel, Bravo‐Sánchez, Luis Ramón; Pérez‐Quintana, Manuel Lázaro; García‐Quintana Yudel. (2018). Response surface methodology for optimisation of total polyphenol content and antioxidant activity of extracts from *Maytenus macrocarpa* bark by means of ultrasound‐assisted extraction. Wood Science and Technology. Wood Science and Technology https://doi.org/10.1007/s00226-018-1034-x. Published online: 07 July 2018.

AOAC. (1999). Association of Official Agricultural Chemists. Official methods of analysis. Cunning, P. (editor). 16th Ed., 5th Revision. Maryland, USA: AOAC International.

Carrapiso, A. 2007. Effect of fat content on flavor release from sausages. Food Chemistry 103(2): 396-403.

Chen, C. Wang, S. Chiang, H. (2003). *Hibiscus sabdariffa* extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. Journal og Agriculture and Food Chemistry. 51, 5472-5477.

Coronado H Marta. Vega Salvador y Gutiérrez T. León Rey, Vázquez F. Claudia Marcela *(2015).* Antioxidants: the current outlook for human health. *Rev. Chil. Nutr.* [online]. 42(2): 206-212.

Duke, J. Ducellier, J. (2003). Handbook of medicinal spices. CRC Press LLC. New York, USA. 348 p.

Hirunpanich, V. Morales, N. Sato, H. Suthinsisang, C. (2005). Antioxidant effect of aqueous extracts from dried calyx of *Hibiscus sabdariffa* Linn. (Roselle) in vitro using rat lowdensity lipoprotein (LDL). Biological and Pharmaceutical Bulletin. 28(3). 481-484.

Lou-Arnal, Luis M. (2013). Impact of meat and fish processing on phosphorus intake in patients with chronic kidney disease. *Nefrología (Madr.)* [online]. 33(6): 797-807.

Murthy, B. Murch, S. Saxena, P. (1998). Thidiazuron: a potent regulator of *in vitro* plant morphogenis. In Vitro Cell Dev Biol Plant.34 (6):267-75.

Olmedilla-Alonso, Begoña y Jiménez-Colmenero, Francisco. Functional meat foods: development and evaluation of their health properties. *Nutr. Hosp.* [online]. 2014, vol.29, n.6 [citado 2019-01-08], pp.1197-1209.

Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO). (2017).

Ospina Meneses, Silvia Marcela; Restrepo Molina, Diego Alonso; López Vargas, Jairo Humberto. 2011. Meat Derivatives as Functional Foods Lasallista Research Magazine, 8(2): 163-172.

Pace, V. (2010). Universidad abierta interamericana. Antioxidantes naturales. p39.

Pérez, D. Andújar, G. (2000). Research Institute for the Food Industry. Changes in colouring of meat products. Rev. Cubana Aliment Nutr 2000;14(2):114-23.

Prior, R. We, X. Schiaich, K. (2005). Standarized methodos for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Jourdal of Agriculture and Food Chemistry. 53, 4290-4302.

Salazar-González, Claudia; Vergara-Balderas, Fidel T; Ortega-Regules, Ana E y Guerrero-Beltran, José Á. Antioxidant properties and color of *Hibiscus sabdariffa* extracts. *Cienc. Inv. Agr.* [online]. 2012, vol.39, n.1 [citado 2019-01-08], pp.79-90.

Sánchez Iván C, Albarracín William. (2010). Sensory analysis in meat. Revista Colombiana de Ciencias Pecuarias. Rev. Colomb. Cienc. 23:227-239.

Silva, L. Pérez, M. & Bravo, L. (2018). Olyphenols from the almond of *Theobroma cacao* L. 2, (2): 424-437. ISSN: 2602-8085.

Tiburcio, C. (2018). Economic zone. Characteristics of Meat as Food. Recuperado https://www.zonaeconomica.com/carne-alimento.

Tofiño-Rivera Adriana, Ortega-Cuadros Mailen, Katina Herrera-Hinojosa Belkis, Fragoso-Castilla, Pedro Pedraza-Claros Bertilda. (2017). Microbiological preservation of handmade meat sausage

with essential oils from *Eugenia caryophyllata* y *Thymus vulgaris.* Biotecnología en el Sector Agropecuario y Agroindustrial. Edición Especial No 2 · julio - diciembre 2017. 30-41.

Valenzuela, V. Carolina, C. y Pérez, M. (2016). Update on the use of natural antioxidants derived from fruits and vegetables to prolong the shelf life of meat and meat products. Rev. chil. nutr. [online]. 43,(2): 188-195. Missouri Bot Gard. In Press.

Viada Pupo, Esther; Gómez Robles, Lisvelt y Campana Marrero, Ibel Reyna. (2017). Oxidative stress. [online]. 21(1): 171-186.

Yang, H., S. Choi, J. Jeon, G. Park and S. Joo. 2007. Textural and sensory properties of low fat pork sausages with added hydrated oatmeal and tofu as texture modifying agents. Meat Science 75(2): 283–289.