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Quantification of total phenols of *Cymbopogon citratus and Gay orellana*,and their use in meat (August 2021)

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ABSTRACT - During the last years the consumption of chemical additives in food has decreased as the health damage they generate is evident, and as a result, the interest in researching the use of extracts of plants, which are rich in phenolic compounds responsible for antioxidant, antimicrobial, dye, condiment properties among others; allowing it to be used to maintain sensory quality and prolong the shelf life of food, without compromising the health of the consumer. The objectives of this research were to determine the content of total phenols and the optimal extraction time of extracts obtained by maceration of *Cymbopogon citratus and Bixa orellana* for which used the method of Folin & Ciocalteumethod, and a kinetics of solid-liquid extraction wasconstructed using ethanol as a solvent, the second objective was to carry out a revision that allow to highlight the application of plants to increase the shelf life in meat foods, for this a bibliographic review of articles generated in databases of: pubMed,SCIELO, DOAJ and THE SELVIER. As results it was determined that the optimal time of extractionwas 25 and 30 minutes, with an amount of total phenols of 5,615 GAE/g dry sample and 21,305 GAE/g dry sample for *Cymbopogon citratus and Bixa orellana* respectively.

Keywords: Phenolic compounds, Salmonella typhimurium, Cymbopogon citratus and Bixa orellana.

I. INTRODUCTION

Currently, consumption habits have changed, more and more people are looking for foods with excellent quality, which have an extended shelf life, but, without making use of chemical preservatives which have adverse effects on human health, to the contain carcinogenic components, such as sodium benzoate, nitrates and BHT [3]. Among the foods most consumed within the human diet are meat products as an important source of nutrients, such as vitamins (thiamine, niacin, riboflavin, B12 and B6), minerals (iron, magnesium, phosphorus, potassium and zinc), and amino acids [4]. However, these foods are exposed to bactean contaminationandoxidation [4].

In relation to the above, one of the meat foods with the greatest bacterial contamination and a short shelf life is poultry meat, since they are a product Highly perishable food that provides an almost perfect means for deterioration by the growth of

Pathogenic microorganisms, one of these microorganisms, is the so-called *Salmonella typhimurium,* which has been identified as one of the main etiological agents responsible for outbreaks of foodborne diseases [1].

By this reason many Research in the Current events focus on the search for alternatives in which plant extracts are used how additives What Allow maintain the characteristics Organoleptic of the foods meat, with low presence of etiological agents [2]. However, endemic and autochthonous plants present in the región nariñense, have properties Antimicrobial What sound scarcely Used y little known, several of them are in danger of extinction, therefore, the scarcity of documentation y the lack of diffusion y disclosure of the knowledge Traditional scientists y Technological Available Carry a his scarce use [3].

Among these plants, the lemongrass *(Cymbopogon citratus)* stands out, which is a perennial herb, belonging to the poaceae family. It is recognized as oneof the most used medicinalplants in Latin America, for its multiple antimicrobial uses and its pleasant lemon aroma [5] in the lemongrass leaf, some antioxidant compounds have been identified among which are: isoorientina 2-O-ramnoside, isoorientia and swerorriajaponina the latter with greater antioxidant capacity another plant also known for these antimicrobial properties is the achiote *(Bixa orellana),* which belongs to the Bixaeaefamily, of genus Bixa and species: *orellana,*is a tree or shrub of a maximum height of 3 to 8 meters, has smooth and pointed ovate or heart-shaped leaves with a hermaphrodite flower and fruit in a thorny capsule, which encloses the seeds inside and which are of r ojizo color, in the leaves of this plant is found flavonoids such as; (apigenin, hypoaltine and cosmosiine), diterpenes (farnesilacetone, garanil geraniol and geranyl format), and a sesquiterpenederivative, alkaloids, asteroids, phenols, pyrogallic tannins, anthraquinones, fixed coumarins, essential oils and gallic acid [6].

Taking into account the above context, in this research was focused on determining the content of total phenols and the optimal extraction time of the imoncillo*(Cymbopogon citratus)* and achiote *(Bixa orellana)*obtained by maceration and finally, perform a review on the use of plant extracts to increase shelf life in meat foods.

II. CONTENT DEVELOPMENT

A. Collection, cleaning and drying of plant material

The plants of *Cymbopogon citratus* and *Bixa orellana,* were used as raw material for the quantification of total phenols, drying these plants at a temperature of 45 ° C, to preserve the quality of phenolic compounds.

B. Obtaining the extract

The extracts were obtained with the SHAKER model GS-30 equipment. The parameters used were: temperature at 40°C, solvent plant material ratio 1:40, at a constant agitation of 400 rpm, and the solvent was a hydroalcoholic mixture 50% V/V.

C. Optimal extraction time

Extraction kinetics was performed, where muestras was taken every 5 minutes for three hours, the extracts obtained were centrifuged and filtered, then stored in a freezing medium at a temperature of -18°C. Prior to the determination, the extracts were thawed, and thetotal phenols were quantified. The data obtained were used to graph the concentration curve (mg GAE/L vs time).

D. Quantification of total *phenols*

For each extract, the total phenol content was evaluated using the method published by Folin and Ciocalteu (1997), the reaction was performed with 0.1 ml of the extract, 0.5 ml of the Folin - Ciocalteu reagent and 1.5 ml of an aqueous solution of sodium carbonate (Na_2 CO_3) 20 % W/V; aforando to 10 ml with water

Distilled. After two hours absorbance reading was taken at 765 nm in the spectrophotometer, then an interpolation of the results was performed with the gallic acid curve and the total phenol content was determined.

E. Preparation of the inoculum

After obtaining and preserving the strain of *Salmonella Typhimurium*, before performing each test se reactivated the strain grown in trypticase soy broth for 18 to 24 hours at 37 ° C +/- 2 °C. An inoculum was taken from the broth and sown in Hagar Müller Hinton. With this it was possible to always obtain fresh bacteria and free of contamination. Subsequently, to achieve a standardization of the inoculum, the Mc Farland scale was used, to achieve a number of colonies representing 3x10 8 CFU/ml.

F. Preparation of chicken meat cutting

Chicken meat samples were immersed in boiling water for three min, in order to reduce the number of microorganisms adhered to the surface. The cooked surface was removed with sterile knives in aseptic conditions and subsequentlyand crushed.

G. Incubation of Salmonella spp in chicken meat cutting

25 grams of the minced chicken meat sample sterilized in stomacher bags were used and inoculated with the Salmonella typhimurium strain, which were homogenized for a time of two minutes at room temperature, additionally 1.25, 1.5 and 2.5 ml of each extract were added. per 100 grams of chicken meat. The samples were then incubated in petri dishes and analyzed over a period of 72 hours, recording data each as pH and color every six hours.

III. RESULTS

A. Total phenols of *Cymbopogon citratus and Bixa orellana*

Tabla I

Gallic acid calibration curve

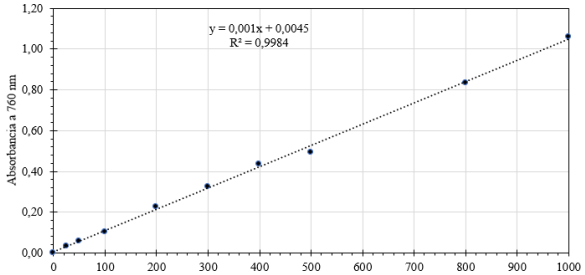
Concentración de ácido gálico (mg/L)	Absorbancia promedio	
0	0,000 ±	0
25	0,033 ±	0,025
50	0,058 ±	0,013
100	0,105 ±	0,004
200	0,226 ±	0,020
300	0,324 ±	0,033
400	0,437 ±	0,019
500	0,493 ±	0,022
800	0,833 ±	0,013
1000	1,061 ±	0,044

Source: Preparation of this research.

Average absorbance data from the gallic acid calibration curve.

Table 1 shows the mean absorbance data (three replicas) for the construction of the gallic acid calibration curve which is observed in Figure 1, with an R 2 of 0.9984 which allowed to find the Ec. [1] that allows to relate the gallic acid with the total phenols of each sample, being used as a reference standard for the quantification of these compounds.

Figure 1
Gallic acid calibration curve



Source: Preparation of this research.

$$[\text{Gallic acid}]=\frac{\text{Absorbance}-0.0045}{0.0010}$$

Ec. 1

B. Total phenols of *Cymbopogon citratus and Bixa orellana*

Table 2 and 3 show the results of the quantification of total phenols of the extracts of

by the method of Folin-Ciocalteu, whose results herself express in mg/L and GAE/g dry sample, (mg/g), as can be evidenced in the hydro extract etanólico of *Gay Orellana* Presented one elder quantity of Phenols Total What *Cymbopogon citratus*.

Cymbopogon citratus had a maximum recovered value of 5.79 GAE/g dry sample which correspondsto the research carried out by [7], who mention that they found this phenolic content in a hot extraction in a range of 2.6 to 7.3 mgGAE/g dw which corresponds to the results found by this research.

The final value of the total phenols for *Bixa orellana* was 21.44 GAE/g dry sample which was lower than reported for this reason many factors arose among the which are the edaphoclimatic characteristics which vary depending on the place of cultivation [8], as well as the solvents used that according to the research carried out by [8], which use methanol formic acid solvents such solvents as methanol or ethanol have a significantly lower polarity compared to water and this favors the solubility and diffusion of the compounds, however, use these solvents with high Purity can cause a collapse in the cells such as the denaturation of proteins, making it difficult to extract phenols [8], meanwhile, also these compounds are affected by the preparation such as drying and particle reduction. since for an effective extraction to occur, the
Solvent must come into contact with the target analytes so particle size and drying are determining factors.

Table 2
Total phenols of *Cymbopogon citratus* by extraction kinetics (40 °C, 400 rpm).

Time (min)	***Cymbopogon citratus***	
	Phenolic concentration mg/L)	**Total phenols (mg/g dry sample)**
5	5,31 ± 0,000	0,17 ± 0,000
10	7,86 ± 0,070	0,23 ± 0,070
15	166,92 ± 0,003	5,23 ± 0,003
20	168,51 ± 0,003	5,26 ± 0,003
25	179,05 ± 0,003	5,59 ± 0,003
30	179,69 ± 0,000	5,62 ± 0,000
45	179,69 ± 0,000	5,62 ± 0,000
60	180,33 ± 0,003	5,65 ± 0,003
90	183,84 ± 0,003	5,74 ± 0,003

Source: Preparation of this research.

Table 3
Total phenols of *Bixa Orellana* by extraction kinetics (40 °C, 400 rpm)

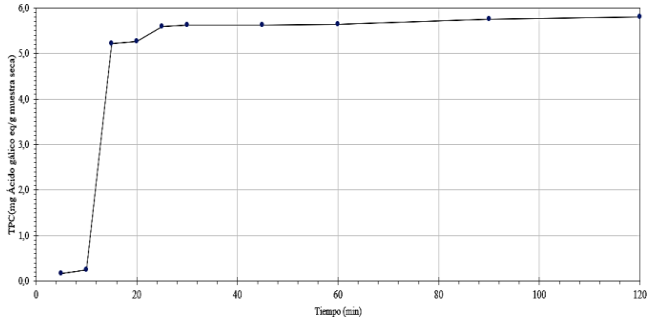
Time (min)	***Orellana*** Bixa	
	Phenolic concentration mg/L)	**Total phenols (mg/g dry sample)**
5	264,65 ± 0,004	8,27 ± 0,004
10	288,92 ± 0,003	9,03 ± 0,003
15	478,96 ± 0,001	14,97 ± 0,001
20	673,78 ± 0,001	21,06 ± 0,001
25	680,81 ± 0,000	21,28 ± 0,000
30	682,09 ± 0,001	21,32 ± 0,001
45	681,77 ± 0,000	21,31 ± 0,000
60	683,05 ± 0,001	21,35 ± 0,001
90	684,00 ± 0,001	21,38 ± 0,001
120	686,24 ± 0,002	21,44 ± 0,002

Source: Preparation of this research.

C. Determination of the optimal extraction time

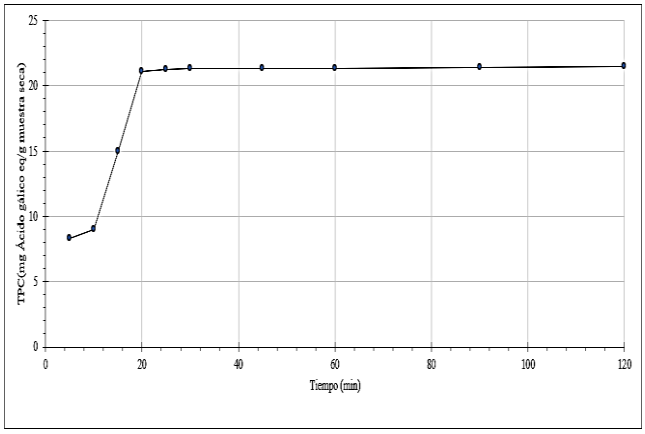
Figure 1 and 2 shows the extraction kinetics constructed for *Cymbopogon citratus and Bixa orellana* where it can be seen that the concentration gradient of Total phenols for the first extract were standardized at 25 minutes and for the second at 30 minutes, determining that these are the optimal extraction times.

Figure 1
Extraction kinetics of Cymbopogon citratus



Source: Preparation of this research

Figure 2
Extraction kinetics of Bixa orellana



Source: Preparation of this research

using solvent at 50% ethanol, plant material-solvent ratio 1:30 and temperature of 40°C.

In the previous graphs an increasing trend was identified during the first 20 minutes, where the total phenolic content was subsequently standardized, this is presented since, within 10 min to 25.67 min, there is the process commonly as "washing" in which most phenol compounds can be extracted. After this time the content of phenols begins to decrease and when it is already greater than 60 min there is a degradation of these compounds.

Plant extracts in meat foods

Meat foods are among the most consumed, however, they are very prone to deterioration [8] given by factors such as the type of animal species, ante and post-mortemhandling, hygiene during handling, MeatpH, room temperature, and packaging. In this way, the proliferation of lipolytic and / or proteolytic microorganisms alter the composition of the meat and can generate oxidation products, and microbial metabolites causing undesirable reactions that deteriorate the taste, smell, color, properties sensory and texture [8].

In relation to this, the rise of firm movements in search of naturalness is shown, promoting the use of plant extracts, as they have antimicrobial and antioxidant properties.

Antioxidant properties of plant extracts

Among the studies carried out in the last years herself Has lent much attention to the use of extracts y oils how antioxidants y antimicrobials Natural in the meat y the products meat, including the regulatory aspects of the substitution of antioxidants Synthetic by ingredients Natural in this sense the efficiency demonstrated antioxynatural dantes, either in pure extract form or in mixture, to retard the lipid oxidation, color and taste deterioration in meat products has stimulated a wide range interest [8].

Several compounds with anti-oxidant activity have been identified in plant extracts, in which phenolic compounds are considered to be the main active group. Generally, that is attributed to the natural extracts being very susceptible to being oxidized, and this is why they prevent metals from catalyzing oxidation reactions due to the various groups. hydroxyls present in the aromatic ring(s) being important antioxidant agents.

Antimicrobial properties of plants

More and more research confirms the use of these extracts as natural antimicrobials Generally, the mechanism of action of these in microorganisms asit happens with other phenolic compounds, it consists of disturbing the cytoplasmic membrane and altering the motive force of the p roton, interfering with active transport and coagulation of the cellular contents.

In this sense it can be seen in **Table 1.** several plant extracts that when added benefit meat foods.

Balzan, et al. (2017) used purified phenols from olive oil wastewater in raw sausages and cooked sausages, resulting in a significant reduction of various oxidation markers, thus maintaining general sensory acceptance. When combinations with other compounds, expected results have also been demonstrated such as that made by [8] who developed a mixture of chitosan and mint extracts, which together imparted antioxidant and antimicrobial properties, extending the shelf life of meat and meat products

Regarding the antimicrobial activity, some research stands out, such as [8] that the addition of oregano essential oils at a concentration of 0.7% provided an antimicrobial activity in minced sheep meat against S. enteritidis . In vitro tests detected the antibacterial activity of oregano oil against S. enteritidis in foods such as traditional salted fish and cod. Research has also been conducted to discover the antimicrobial activity of clove oil against L. monocytogenes in chopped lamb. Thyme thymol essential oil in an eleventretion of 250-750 mg is used in fresh minced meat in combination with modified atmosphere packaging against different microorganisms and also increases the shelf life of beef [4].

As for chicken meat there is much research focused on evaluating the effect of plants on the shelf life of this food such as that of Zhang, Wu, Guo,(2016) that evaluated the antioxidant and antimicrobial effects of clove or rosemary extracts, alone or combined, in freshchicken pechuga on pH, microbiological analysis, colour, thiobabaiturate acid reactive substances (TBARS) and sensory analysis during storage at 4°C. The TBARS values of the T-RO-CL samples were the lowest among the samples. Finding as a result that spice extracts are highly effective against microbial growth and lipid oxidation and show potential as a natural antioxidant in raw chicken meats.

They compared grape seed extracts and green tea with sodium ascorbate in cooked pork meatballs during refrigerated storage (4°C in aerobic containers for 0, 4, 8, 12 and 16 days). The total viable count indicated irregular behaviour during storage time, however, they exhibited significant antimicrobial activity [10].

The antimicrobial effect of oregano extract(Origanum vulgare), thyme (Thymus vulgaris), garlic (Allium sativum), grapefruit (Citrus paradisi)and vinegar (acetic) on the growth of Salmonella enteritidis (SE) in different types of meat. 1 cm3 cuts of chicken, beef and pork were immersed for 30 s in solution with $10E^6$ CFU/mL of SE, suggesting the authors that the use of natural antimicrobial agents on chicken, beef and pork meat could extend their shelf life due to its inhibiting effect on the growth of SE.

On the other hand, also sage essential oil is used in a concentration of 0.3% in minced meat in combination with soy protein. Rosemary or Chinese mahogany (500, 1000 and 1500 ppm) is used to increase fresh chicken sausage [7].

Likewise, Chan, et al. (2014) reported that the deodorized aqueous extract of cinnamon(CinDAE) contained a total phenolic and flavonoid content of 315.3 ± 35.4 mg GAE/g and 99.3 ± 9.6 mg of rutin equivalents (RE)/g, respectively. Compared tothe control samples, they reported that cooked chicken balls containing CinDAE had an increased period of induction and redness, while their peroxide and TBARS were significantly lower during thestorageperiod at 8 °C. In addition, CinDAE did not adversely affect the sensory acceptability of the food product and its antioxidant activity was found to be comparable to that of ascorbic acid. BHA and BHT (Chan et al., 2014).

The feasibility of using pomegranate extract (PE) as a preservative in ready-to-use meat products was evaluated. Compared to the control sample, PE prevented growth in meat by 4.1 log CFU/g for 46 days at 4°
C. The reduction reached 9.2 log CFU / g on day 18 of storage, they also determined that at a higher temperature inhibition decreased.

IV Conclusions

The studies and research reviewed, allowed to identify that among the methods with more advantages to obtain plant extracts, are the methods of ultrasound and hot maceration, on the one hand, the first method, allows to obtain a higher yield, this given by the cavitational force presented in plant molecules, allowing a greater recovery of compounds, and on the other hand, the hot maceration method combines variables such as agitation and temperature allowing a good recovery of phenolic compounds these two methods compared to microwaves need less energy, are easy to handle and lower cost, however, if the effect is to reduce time the most effective method is microwave-assisted extraction.
Making a comparison between the two plants studied, the total phenolic content recovered for Bixa orellana, was greater than Cymbopogon citratus,and in turn thelatter presented a shorter optimal extraction time, observing a great potential to be able to relate with its phenolic content with different beneficial properties of these plants, in addition to this it is mentioned that, parameters such as temperature, extraction method, drying, edaphoclimatic conditions, among others; they must be taken into store in account since they influence the amount of phenols recovered.

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