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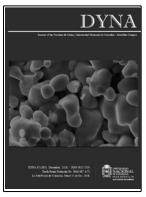

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Evaluation of the antimicrobial capacity of Hass avocado seed extract *(Persea americana)* for potential application in the meat industry

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Abstract
$NaNO_2$ is used in meat products to inhibit pathogenic microorganisms; its use is limited, and it forms carcinogenic N-nitroso compounds. There is currently a great demand for natural products. The Hass avocado seed extract produces an antimicrobial reaction against bacteria such as *Staphylococcus aureus*. After the seed of the Hass avocado (AS) had been dehydrated at 50°C for 10 hours, we undertook a quality and analysis of the moisture and microbiological test.
The extract was obtained in hot water and in solvents to perform an antimicrobial sensitivity test, which is an inhibition halo test using the strain Staphylococcus aureus as microorganisms. A minimum capacity inhibition test was also carried out. The concentration of the extract by solvents was 7 mg/mL, and it presented an inhibition halo of 1.8mm.The combination of AS and nitrites caused oxidation and darkening in the halos. The compounds that were extracted from the Hass avocado seeds with the methods used are not effective against *S. aureus*.

Key words: antimicrobial capacity; avocado variety Hass; by-products; microorganisms.

Evaluación de la capacidad antimicrobiana del extracto de semilla de aguacate Hass (*Persea americana*) con potencial aplicación en la industria cárnica

Resumen
El $NaNO_2$ es utilizado en productos cárnicos para inhibir microorganismos patógenos, su uso es limitado ya que forma compuestos N-nitrosos carcinógenos. Actualmente hay gran demanda de productos naturales. El extracto de semilla de aguacate Hass tiene actividad antimicrobiana contra bacterias como *Staphylococcus aureus*. A la semilla de aguacate (SA) Hass deshidratada a 50°C por 10 horas se le realizó análisis de humedad y calidad microbiológica; se obtuvo el extracto en agua caliente y solventes para hacer la prueba de halo de inhibición frente a la cepa *Staphylococcus aureus*, además de una prueba de capacidad mínima inhibitoria. La concentración del extracto por solventes fue de 7mg/mLy presentóun halo de inhibición de 1.8mm; la combinación extracto de SA y nitritos causó una oxidación y oscurecimiento en los halos. Los compuestos que se extrajeron de la semilla de aguacate Hass con los métodos empleados no son efectivos contra *S.aureus.*

Palabras clave: capacidad antimicrobiana; aguacate variedad Hass; subproductos; microorganismos.

1. Introduction

Currently, the avocado is grown in 59 countries, both in subtropical and tropical regions. More than 60% of avocado plantations are in America [1]. In 2016, the FAO estimated an overall production of 5.57 million tons; Mexico was the largest grower, and Colombia occupied fourth place [2]. According to the National Agricultural Survey, ENA (DANE, 2016), during 2015, Colombia produced 274,330 tons of avocado fruit [3]. According to statistics gathered by Asohofrucol, at the end of 2016, in the country 78547 tons of Hass avocado were grown in an area that measured

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approximately 14,084 hectares. The departments with the highest production potential are Antioquia (3,500 hectares), Caldas (2,597 hectares) and Tolima (1,325 hectares) [4].

The by product of this large industrial production of avocado fruit is the generation of waste from husks and seeds. The most widely-produced and used avocado in Europe and the U.S.A is the Hass variety, advertised as a "ready to eat" fruit [5]. The peel: seed: pulp ratio is 8.5: 11.5: 72%, respectively [3]. The amount of waste produced by the food industry, in addition to being a huge loss of valuable materials, also poses serious management problems for both the economy and the environment [6].

The husks and seeds that are rich in bioactive substances such as polyphenols and chlorophylls have been found to produce antioxidant activity [7]. The AS contains various kinds of natural products such as phytosterols and triterpenes, fatty acids with olefinic, acetylenic, furanoic acid, oligomeric proanthocyanidin flavonole dimers, 8-hydroxyabscysic acid β-d-glucoside and epi-dihydro-d-glucoside acid [8]. The characterization of phenolic compounds has made it possible to identify the presence of 3-O-caffeoylquinic acid, 3-O-p-coumaroylquinic acid, and procyanidintrimers [9]. Seed and exocarp have higher total antioxidant levels than mesocarp [10], predominantly in ascorbic acid AS and total phenolic compounds [11]. AS has a phytochemical composition of saponins 19.21, tannins 0.24, flavonoids 1.9, alkaloid 0.72, and phenols 6.14: all of which are measured in mg/100 g AS. These may function as antioxidants as a result of the interaction between them [12].

Antimicrobial activity of the Hass avocado seed extract has been discovered to work against the bacteria *Salmonella Enteritidis, Citrobacterfreundii, Pseudomonas aeruginosa, Entero bacteraerogenes, Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, and the yeast Zygo saccharomyces bailii*. It could potentially be used as a byproduct and as a food additive [13].

The Colombian market presents growing demand for both sausage meat products and foods with natural ingredients that preserve culinary and artisanal tradition; for example, sausages in the department of Antioquia. Our goal is to prepare a Chorizo type meat sausage, which incorporates dehydrated avocado seed, with the intention of partially or totally replacing the nitrite content in the standard formulation, evaluating the antimicrobial effect exerted by this substitute, preventing the growth of the strain of Staphylococcus aureus. As such, the bioactive compounds present in the previously dehydrated seed should be extracted and the antimicrobial effect produced by nitrites must be compared to the standard sausage. The purpose of this research is to find ways to make use of these avocado byproducts.

2. Materials and methods

2.1. Obtaining dehydrated Hass avocado seeds

2.1.1. The Hass avocado seed sample

The Hass avocado units were obtained as by-products while developing the royalties project called "Development and supply of certified avocado materials for Antioquia with genetic, physiological, and sanitary quality: the Sustainable Hass avocado production systems". The project was based in Eastern Antioquia and undertaken by the Sensory Analysis Research Group, which is part of the University of Antioquia.

The AS from mature avocados was frozen in polyethylene bags for approximately three months. The cleaning, disinfection, peeling, and color measurement was undertaken using the Pantone chart, and they were subjected to a five-minute blanching pre-treatment at a temperature of 80 ± 2 ° C. The seeds were then crushed using a Kalley cross-sectional food processor.

2.1.2. Preparation of dehydrated avocado seed

The AS was dried at 50°C for ten hours and then processed in a mill (Hamilton Beach) and packed in a polyethylene bag.

2.2. Physicochemical analysis

The samples were milled into approximately 1 mm particles in a spray mill.

Humidity: usinga vacuum stove, 3 ± 0.04g were taken at 70 ± 1 °C for five hours until weight was constant in the previously tarred capsules, and the humidity was controlled using a desiccator.

2.3. Microbiologic analysis

Coliform count methods were performed with the most probable number (ISO 4831: 2006). Additionally, we used a traditional Mesophilic aerobic method of cultivation (AOAC 966.23).

2.4. Extraction processes

2.4.1. Extraction in hot water

10 mL of distilled boiling water was added to 1.5g of seed and filtered after ten minutes.

2.4.2. Extraction with solvents

The scalded and dehydrated AS extracts (ground with a less-than 8%percentage of humidity) were obtained using the method described by Contreras-Calderon et al. (2011) [14] with slight modifications. 0.5 g of dehydrated ground AS was placed in a centrifuge tube, 6 mL of methanol-acid water (50:50 v/v pH 2) was added, it was then manually stirred at room temperature and put in a shaker for one hour in a Heidolphunimax 1010 Shaker, which protected it from the light. The tube was centrifuged at 6000 rpm/8 min (Hettich® EBA 20 centrifuge), and the supernatant was recovered by filtration in a 25 mL volumetric flask, which was then stored in the dark. 6 mL of acetone-water was added to the residue in the tube, it was stirred for one hour and centrifuged. The new supernatant was collected in the 25 mL volumetric flask and was brought to capacity. The sample was rotated (in a Rotavapor® R-300) to remove the solvents and then subjected

Table 1.
Combinations of AS extract with nitrites for the diffusion method.

Diffusion method	
Concentration haloes of inhibition	
% Nitrite	% AS
37.5	62.5
0	100
12.5	87.5
25	75
100	0

Source: The author

to lyophilization in order to extract the added water. The product obtained was filled with distilled water; thus, a 7mg/mL concentration was obtained.

2.4. Analysis of the antimicrobial activity

2.4.1. The broadcast method

The strain of *Staphylococcus aureus* was activated 24 hours earlier in incubation at 37 °C; the antimicrobial activity of the extracts was obtained by hot extraction. Using solvents, the strain of *Staphylococcus aureus* was analyzed, and then the AS extracts were combined with nitrites (seethe statistical design in Table 1). They were grown in a Hilton Pier, and the size of the inhibition halo was measured.

2.4.2. MIC Dilution method

Dilution in an agar or broth was the method used to test microbial susceptibility so as we could determine the minimum inhibitory concentration (MIC), which is defined as the lowest concentration of substance that can inhibit the visible growth of a microorganism after being incubated for 24 hours. Microplates were used (microdilution) that contained increasing concentrations of the AS extract obtained by solvents in (10, 20, 30, 50, 80, 100% v/v). The extract was then inoculated with 100 µL of the strain of Staphylococcus aureus: 0.5 McFarland standard (1.5 * 10^8 UFC/mL).

3. Results

Fig. 1 shows the cleaning and disinfection process with the characterization of the AS that was stored at freezing after being removed from the fully mature fruits.

3.1. Microbiological analysis

The analyses of a good level of microbiological quality for the elaborated infusions (Table 2).

Table 2.
Microbiological Quality of dehydrated AS.

NMP Fecal Coliforms(NMP/g)	NMP Coliforms totals (NMP/g)	Mesophiles (UFC/mL)
m<3 NMP/g	m<3 NMP/g	m<10 UFC/mL
<3	<3	<10

M = Maximum index to indicate the acceptable quality level
m = Maximum allowable index to identify level of good quality

Source: The author

Figure 1. The process to obtain the dehydrated AS.
Source: The author

3.2. Analysis of antimicrobial activity

Fig. 2.b shows that there was no inhibition of the extract obtained in hot AS water. After analyzing the plates, we found that there was possible contamination from another microorganism, and, due to their characteristic smell, we intuited that pseudomonas were present, which limited the veracity of the result. In Fig. 2.b, haloes of inhibition are observed in 100%, 80%, and 25%concentrations of AS extract obtained by solvents. They are approximately 1.8 mm thick at the edge; therefore, the extract is not effective.

In the 80% extract, a greater inhibition halo was observed, however, with other microorganisms, there was also external contamination of unknown microorganisms (Fig. 2.c). Fig. 2.d shows the MIC result, which was negative, and no antimicrobial activity was found for *Staphylococcus aureus* (1.5 x 10^8 CFU / mL).

4. Discussion

The count of mesophilic microorganisms, total, and fecal coliforms of the dehydrated AS was in compliance with what is established by Colombian Technical Standard 2698:there is acceptable microbiological quality for use and incorporation in food. The humidity of the samples was found to be6.108 ± 0.131%.

No effective antimicrobial activity was shown in any of the two diffusion and dilution tests. The diameter of the inhibition

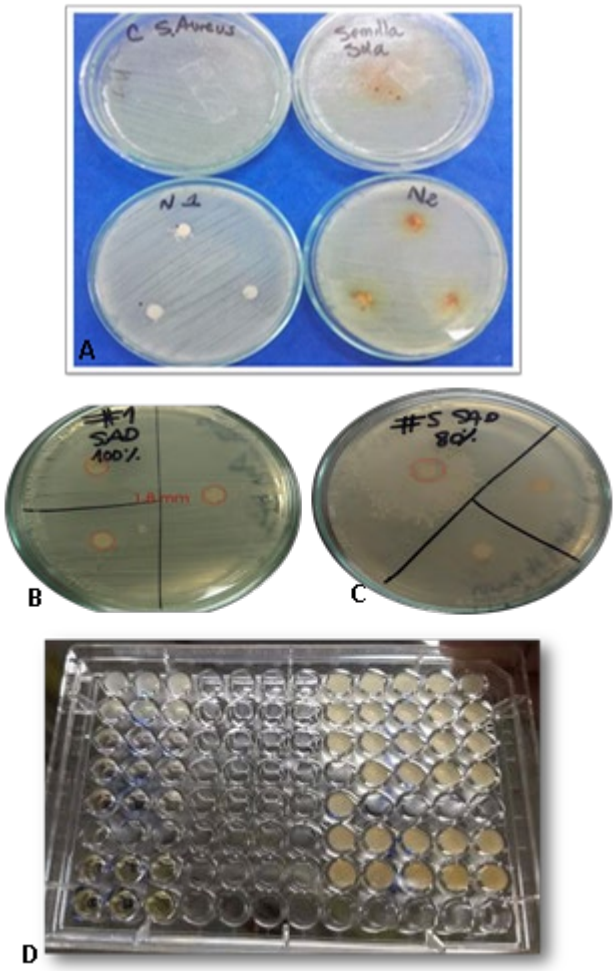

Figure 2. Growth of the Staphylococcus aureus strain. A) Hot extract; B) Extract with solvents; C) 80% extract contaminated with another microorganism; D) MIC test.
Source: The author

halo was approximately 1.8 mm, which is close to that reported by Raigoza (2016), where the halo was 1.1mm; therefore, the antimicrobial action for the *Staphylococcus aureus* strain is not effective [15].

A color change was found in the union of nitrites and the AS extract that was obtained both in hot and in solvents.This verifies that an oxidation reaction could possibly occur between these two; therefore, it is not possible to achieve a partial substitution of nitrites by joining this additive with the seed extract at the same moment of addiction since the expected effect would not be achieved. Conversely,the nitrite would be inactivated, resulting in a meat product with undesirable characteristics in appearance as well as taste. There would also be therisk of pathogenic microorganisms growing.

In Ceballos'(2013)study of the fiber present in the pulp, seed, and avocado peel, the author details considerable vitamin E, beta-carotene, and ascorbic acid content in the seed [8]. These compounds are currently used to prevent the formation of nitrosamines as toxic contaminants in food [16] because of their reaction with nitrites, especially with the residual nitrite present in meat products, which is caused by their excessive addiction during formulation [17]. As well as the effect that the addition of ascorbic acid showed in the elaboration of mortadella, thanks to the addition of ascorbic acid, there was a reduction in the concentration of residual nitrites and nitrates after the third day.

4. Conclusion

The hot-water extract of the dehydrated AS with a 6.108 ± 0.131% humidity does not show a halo of inhibition for the microorganism *Staphylococcus aureus* and neither does the combination of nitrite and AS extract.

There was visible formation of the dark brown and orange colors belonging to halos impregnated with the combination of the AS extract and nitrites.

The avocado seed extract with solvents that was then lyophilized showed a 1.8 mm inhibition halo. There was contamination by other microorganisms for which there was inhibition. In the MIC test of the diffusion method, all concentrations of the extract showed turbidity after incubation, which indicated that there was growth of *Staphylococcus aureus*. These results show that the extracted compounds of the AS dehydrated Hass variety in hot water and with solvents are not effective against strains of *Staphylococcusaureus.*

The concentration of 7mg/mL and the nature and origin of the solvents may not have been ideal for the experiment. In addition to the microbial strain, the two trials showed contamination from other microorganisms.

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