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Evaluation of a colorant and oil extracted from avocado waste as functional components of a liquid soap formulation

Evaluación de un colorante y aceite extraídos de residuos de aguacate como componentes funcionales de una formulación de jabón líquido

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ABSTRACT

The present research evaluated the antioxidant, antimicrobial and *in vitro* coloring capacity of extracts with different polarity obtained from avocado seeds (*Persea americana* Mill cv. Lorena). Besides, avocado oil was extracted from the residual mesocarps of *P. americana* Mill Hass cultivar by Soxhlet methodology, and the physicochemical properties of the extracted oil, as well as its fatty acid composition, were evaluated. Both the colorant and the avocado oil were used as supplies for a liquid soap type formulation. The antioxidant activity of the colorant extracts was determined by DPPH whereby water extracts showed the highest activity among the treatments. None of the extracts showed antimicrobial activity against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. The iodine value (177.52 cg I_2 g^{-1}) indicated that the avocado oil obtained has a high degree of unsaturation, and the Saponification index had a value of 190.74 mg KOH g^{-1}. The colorant extracted with NaOH (L*=0.15, a*=0.05, and b*=-0.44) from the seeds was completely stable in a liquid soap matrix at pH 6.2 during one month of storage. This analysis suggests that it has high opportunities in the soap and cosmetic industry.

Keywords:
Byproducts
Food wastes
Persea americana
Value-added product

RESUMEN

El presente trabajo evaluó la capacidad antioxidante, antimicrobiana y de colorante *in vitro* de extractos de diferente polaridad obtenidos a partir de la semilla de aguacate (*Persea americana* Mill cultivar Lorena). Además, se extrajo aceite de aguacate a partir de mesocarpios residuales de *P. americana* Mill. cultivar Hass por medio de la metodología soxhlet y se evaluó las propiedades fisicoquímicas del aceite y su composición de ácidos grasos. Tanto el colorante como el aceite de aguacate fueron usados como insumos en la formulación de un jabón líquido. La actividad antioxidante de los extractos de colorante fue analizada mediante DPPH y mostró que los extractos con agua destilada tienen la más alta actividad entre los tratamientos. No se presentó ninguna actividad antimicrobiana en los extractos evaluados ante *Staphylococcus aureus* ATCC 29213 y *Escherichia coli* ATCC 25922. El índice de yodo (177,52 cg I_2 g^{-1}) revela que el aceite obtenido tiene un alto grado de insaturaciones y el índice de saponificación fue de 190,74 mg KOH g^{-1}. El colorante extraído con NaOH (L*= 0,15 a* = 0,05 y b*= -0,44) a partir de la semilla de aguacate variedad Lorena, es completamente estable en una matriz de jabón líquido con un pH de 6,2 durante un mes de almacenamiento. Estos análisis indican que los productos obtenidos tienen altas oportunidades en las industrias de jabones y cosmética.

Palabras clave:
Subproductos
Desechos alimentarios
Persea americana
Productos de valor agregado

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The use of agro-industrial wastes has proved to be an alternative solution for obtaining bioactive compounds because most of the wastes still contains interesting compounds which can be extracted and used in different industry sectors (Palomino García *et al*., 2015). The problem of food waste is currently increasing, involving all sectors of waste management from collection to disposal (Girotto *et al*., 2015). Colombia is a large producer of avocado; it is the fifth largest avocado producer in the world after Mexico (2,029,890 t), Dominican Republic (637,690 t) and Indonesia (363,160 t) (Statista, 2017). The national production in 2017 was around 335,000 t with a planted area of 40,000 ha (Agronet, 2017).

The avocado agroindustry generates waste with great potential to be harnessed since the seeds (12-16% of the total weight of the fruit) is a source of dietary fiber, fatty acids, polyphenols, and antioxidants (Ayala-Zavala *et al*., 2011; Hiwot, 2017). On the other hand, the mesocarp or pulp has high unsaturated fatty acids content, and other valuables bioactive phytochemicals such as carotenoids, tocopherols, phytosterols, lutein, and vitamins (Dávila *et al*., 2017; López-Cobo *et al*., 2016). The previous compounds have a high biological value and can be used for new products development. According to the biorefinery concept, the processing avocado is an attractive opportunity for integrated processing of the fruit into a series of valuable products, using the waste of pulp, peel and the seed of the fruit (Dávila *et al*., 2017).

The application of a natural colorant from avocado seeds can be commercially significant; therefore, its high phenol content and other functional attributes should be explored (Dabas *et al*., 2011). Natural colorants production is an interesting alternative for the use of waste with tinting characteristics. However, the main limitation of most of these dyes for commercial applications is their chemical instability and low colorant strength. On the other side, by obtaining the avocado oil from *Persea americana* Mill. cv Hass arises as an alternative to strengthen the fruit productive chain and counteract the losses of Colombian producers due to overproduction (Serpa *et al*., 2014).

The liquid soap industry uses colorants as additives for improving its appearance (Hilgert Valderrama, 2012). The addition of fatty acids in mixes of anionic surfactants can play an important role in foam stability and ink removal from cellulose fibers (Theander and Pugh, 2003). Mixtures of synthetic surfactants, avocado, olive, mineral and castor oils have been used to improve the moisturizing properties of the liquid soaps and to prevent skin dryness (Glenn, 1996). Therefore, exploring the use of avocado waste pulps and seeds in liquid soap-like ingredients in a formulation becomes an option to generate added value to avocado agroindustry.

MATERIALS AND METHODS

Colorant extraction

Avocado seeds were harvested and classified at La Fortuna farm municipality of Mariquita, Tolima (5.255607 N, -74.998586 W). Size reduction was made with flake cuts of 3 mm and the material was dried at 55 °C during 14 h in a Memmert UF 55 oven, then they were ground and filtered with a 500-micron mesh. For colorant extraction, three solvents were used independently: distilled water, an aqueous solution of NaOH (0.5%) (Devia Pineda and Saldarriaga, 2005) and a mixture of distilled water and ethanol (1:1). A ratio of 0.05 milled seed-solvent, as well as the reflux system at a temperature of 45 °C and the extraction time of 120 min, remained constant. Extraction yields were calculated using a moisture determinant XM 60 HR.

Antioxidant activity evaluation

The antioxidant activity was measured using the DPPH method applied by Brand-Williams *et al.* (1995), which is based on the absorbance reduction of the DPPH 1, 1-diphenyl-2-picrylhydrazyl radical, measured at 515 nm.

To determine the antioxidant activity, 200 µL of each extract at different concentrations and 2800 µL of DPPH solution (0.1 mM) were mixed and taken to a dark chamber during 30 minutes at room temperature, and the absorption was measured at 518 nm in a Jenway 7305 spectrophotometer. The 2-carboxylic-6-hydroxy-2, 5, 7, 8 tetramethylchroman acid (Trolox) was used as a control. Tests were replicated three times.

The percentage of antioxidant inhibition from the extracts at different concentrations (expressed in ppm) was evaluated. The pH value of the NaOH aqueous extract

(pH 9.3), water extract (pH 5.1) and ethanol extract (pH 5.8) was neutralized to pH 7.0 The percentage of inhibition was calculated according to equation 1.

$$\%\ of\ inhibition = \frac{A - A_1}{A} x\ 100 \qquad (1)$$

A: Blank absorbance
A_1: Sample absorbance

The results were expressed as the maximum concentration of the inhibitory mean (IC_{50}), defined as the amount of antioxidant necessary to reduce the initial concentration of DPPH to 50%.

Antimicrobial activity evaluation

The antimicrobial activity was evaluated using the Kirby Bauer disk diffusion method (Hudzicki, 2009). Strains of *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25992 were used for this purpose. Those strains were isolated in EMB and Baird Parker agar respectively. Three to five colonies of each microorganism were placed in a saline solution (5 ml, 0.85%) and the concentration was adjusted to the 0.5 McFarland tube (1.5×10^6 CFU mL^{-1}). Once the suspension was adjusted, it was massively seeded with a sterile brush in Mueller Hinton agar. The sterile filter paper discs (10 mm in diameter), used for the test, were previously impregnated with 0.1 mL of the extract at 5000 ppm and dried in a closed petri dish. The treatments were classified in A1 (aqueous extract; 5000 ppm and pH 5.4), A2 (NaOH aqueous extract; 5000 ppm and pH 8.2), A3 (water-ethanol extract; 5000 ppm and pH 5.5). A paper disc with chloramphenicol (10 mg mL^{-1}) was used as the positive control (A4). All experimental units were incubated at 37 °C for 24 h (Casana *et al.*, 2016). Then inhibition halos were read.

Avocado oil characterization

Matured Hass avocado was reduced in size and dried in a Memmert UF 55 plus oven at 50 °C for 12 h. Subsequently, the free fat total extraction was carried out using the Soxhlet method for 4 h with hexane (grade HPLC, Scientific). The fatty acids profile was analyzed by means of FID gas chromatography in a Thermoscientific Trace 1310 chromatographer with an Rtx-5 Restek Corporation column of 30 m long, 0.32 mm internal diameter and a film thickness of 1µm under the following conditions: injection volume 1.0 L, injector temperature 230 °C, detector temperature 250 °C, column pressure 23.04 psi, hydrogen flow in the detector 45 mL min^{-1}, air flow in the detector 450 mL min^{-1}, makeup gas flow (N_2) 45 mL min^{-1}, split flow 70.2 mL min^{-1}, split ratio 40:1.

Temperature ramp: The initial column temperature was 190 °C (during 12 min) and rose up to 220 °C with a ratio of 2.0 °C per 4.0 min. The fatty acids composition was found by comparing the peaks retention times obtained with the Fatty Acids Methyl Esters (FAMEs) standards.

Physicochemical parameters such as density was determined following the NTC 336 (ICONTEC, 2002a), Iodine index following the NTC 283 (ICONTEC, 1998a), peroxide index following the NTC 236 (ICONTEC, 1998b), refractive index following the NTC 289 (ICONTEC, 2002b), acidity index following the NTC 218 (ICONTEC, 1999), and saponification index following the NTC 335 (ICONTEC, 1998c). Tests were measured in duplicate.

Color evaluation

The extract with the highest yield was used to calculate the color parameters by using the CIELab space coordinates (L*, a*, b*) in a Konica Minolta Cr-5 colorimeter. Different concentrations of liquid dye avocado were tested (1%, 2%, and 3%) in the liquid soap matrix. The soup emulsion was stored at room temperature and exposed to light for one month. The color difference between the samples was expressed in ΔE* (Hikita *et al.*, 2001). The color variation can be estimated through the ΔE* which is determined through the L*, a*, and b* parameters (Manayay *et al.*, 2013) (Equation 2).

$$\Delta E^* = \sqrt{(L_0 - L)^2 + (a_0 - a_i)^2 + (b_0 - b_i)^2} \qquad (2)$$

ΔE*: Color variation or alteration
ΔL*: Luminosity variation between the measurements
Δa*: Variation from green to red between measurements
Δb*: Variation from blue to yellow between measurements

Liquid soap formulation, stability assessment and BOD calculation

Texapon 40 Sodium Lauryl Ether Sulphate (SLES) and distilled water were mixed and placed on a magnetic stirring plate at 200 rpm. Then sodium benzoate, cocamidopropyl betaine, glycerine, colorant, alcohol,

and salt were homogenously added in a strict sequential order. Different formulations are shown in Table 1.

Color behavior was evaluated by using the CIELab space coordinates (L*, a*, b*) in a Konica Minolta Cr-5 colorimeter (with liquid analysis accessory). The pH variation was measured with a Lovibond SD 300 potentiometer. Soap samples were stored at room temperature for one month and exposed to light.

An airtight vial was overflowed with a soap sample and incubated for 5 d to determine the biochemical oxygen demand. Dissolved oxygen was measured before and after the incubation phase and the BOD calculation results from the difference between the initial and final values of dissolved oxygen (Gender Cevallos and Arnao Ramirez, 2005). Commercial liquid soap and a sample soap (formula 4) were used to determine the BOD.

Table 1. Liquid soaps formulations.

Ingredients	Formulas' composition (%)			
	1	2	3	4
Water	54.9	53.9	52.9	52.7
Salt	4.0	4.0	4.0	4.0
Alcohol	2.0	2.0	2.0	2.0
Sodium benzoate	0.3	0.3	0.3	0.3
Texapon 40	35	35	35	35
Cocamidopropyl betaine	1.5	1.5	1.5	1.5
Glycerine	1.0	1.0	1.0	1.0
Boric acid	0.3	0.3	0.3	0.3
Avocado dye (liquid extract)	1.0	2.0	3.0	3.0
Essence	0.0	0.0	0.0	0.2
Avocado oil (waste mesocarps)	0.0	0.0	0.0	2.0

Statistical analysis

Statgraphics (Centurion version) was used to perform the analysis of variance of the color measurement, extraction performance, and antimicrobial activity. Simple linear regression was used to predict the percentage of antioxidant inhibition at different concentrations.

RESULTS AND DISCUSSION

Yields

The process of drying avocado seeds reported a yield of 27.90±0.99%. Sodium hydroxide showed the most efficient extraction with a percentage of total biomass extracted (weight/weight) of 35.72±0.43 and CIELab color coordinates of L*=0.15, a*=0.05 and b*=-0.44, followed by alcohol extraction (33.16 ±0.13 and CIELab color coordinates of L*=76.89, a*=15.49 and b*=66.74) and distilled water showed the lowest extraction yield (11.61±0.89 and CIELab color coordinates of L*=85.08, a*=5.05 and b*=50.25).

Antioxidant activity

Treatments with sodium hydroxide (T3 and T4, Table 2) showed the lowest percentage of inhibition. Samples extracted with NaOH at a concentration of 150 µg mL^{-1} and pH 9.3 showed a percentage of inhibition of 24.72%. However, at pH 7.0 the percentage of inhibition decreased to 14.03%.

Samples treated with water at pH 7.0 showed a decrease in the percentage of inhibition at all concentrations (reference value=150 µg mL^{-1}). T1 reached 51.69% (IC_{50} value=153.87 µg mL^{-1}) while T2 reached 38.96% (IC_{50} value 187.66 µg mL^{-1}). Both IC_{50} values were close to the Trolox control (84.10 µg mL^{-1}). Samples neutralized at pH 7.0 and treated with NaOH and water, showed less antioxidant activity.

Otherwise, treatments with a mixture of water and ethanol showed an increase in antioxidant activity when neutralized to pH 7.0. The ethanol extract at pH 5.8 (150 µg mL^{-1})

showed a lower percentage of inhibition (24.21%) than at pH 7 (35.01%). IC_{50} values from T5 (630.00 µg mL^{-1}) and T6 (265.67 µg mL^{-1}) were the closest to the control. Table 2 shows the results of the maximum concentration of the inhibitory mean (IC_{50}). The ANOVA (Analysis of Variance) indicates that there are statistically significant differences (P<0.05) between the treatment and the control.

Table 2. IC_{50} values of different avocado seed extracts (*Persea Americana* cv. Lorena) analyzed by the DPPH• radical scavenging method.

IC_{50} calculation of different extraction methods	µg mL^{-1}	R^2
Pattern: Trolox	84.10	98.59
T1: Water extract, Seed; pH 5.1	153.87	94.46
T2: Water extract, Seed; pH 7.0	187.66	92.39
T3: Water extract, NaOH, Seed; pH 9.3	1154.00	94.69
T4: Water extract, NaOH, Seed; pH 7.0	1284.00	95.47
T5: Water extract, Ethanol, Seed; pH 5.8	630.00	89.03
T6: Water extract, Ethanol, Seed; pH 7.0	265.67	93.78

Nagaraj *et al.* (2010) studied the antioxidant activity of methanol-water (4:1) extracts by the DPPH method and obtained a percentage of inhibition of 60.8%. In the present research, methanol was not used due to its incompatibility with the liquid soap matrix formulation; water was used instead. The aqueous extract showed 51.95% of inhibition while the Trolox control reached 80.76%. This difference is mainly due to the type of solvent used (Fu *et al.*, 2016).

Antimicrobial activity

None of the treatments showed antimicrobial activity against any of the evaluated strains; except for the chloramphenicol control. The extracts were not subjected to any type of bioactive compounds isolation or fractionation; A1 (aqueous extract; 5000 ppm and pH 5.4), A2 (NaOH aqueous extract; 5000 ppm and pH 8.2), A3 (water-ethanol extract; 5000 ppm and pH 5.5) and A4 (Chloramphenicol; 10 mg mL^{-1}). Although the use of sodium hydroxide as a solvent, showed to be the most efficient for the extraction of the dye and achieve greater dyeing power, this is not suitable for the extraction of compounds with antimicrobial activity. Therefore, in this case, the polarity of the solvent and variables such as temperature, extraction time, the nature of the matrix, the specific characteristics of the compounds and their location within the matrix must be taken into account for the optimization of the extraction of compounds with bioactive characteristics (Osorio-Tobón and Meireles, 2013).

Some studies have analyzed the antimicrobial activity from the avocado seed extracts and have defined its seeds as a good source of phytochemical components with high bioactivity (Dabas *et al.*, 2013; Nagaraj *et al.*, 2010). However, the solvent used in these studies was methanol in different conditions, and terpenoids and other bioactive compounds were fractionated.

Physicochemical characteristics of avocado oil

The drying performance of the avocado pulp was 47.41±1.22%. A paste with a rigid texture and dark green color was obtained afterward. The yield percentage of oil extraction from the dehydrated avocado pulp was 71.26±1.25%.

The iodine index is a measure of the degree of unsaturation of the fat components. There is a clear difference between the obtained index and what is established by the NTC 258 (ICONTEC, 2011). A value of 177.52 cg I_2 g^{-1} shows that the obtained oil has a high degree of unsaturation, different from the reported by Restrepo Duque *et al.* (2012) (77.85 cg I_2 g^{-1}) and Acosta Moreno (2011) (75-94 cg I_2 g^{-1}).

The peroxide index measures the fresh oil oxidation or its degree of rancidity at the time of the test (Lafont *et al.*, 2011). A value of 38.45 meq peroxide kg^{-1} reflects a high degree of rancidity. Similarly, a study that used

the same extraction method for the Hass cultivar oil reported a value of 31.66 meq peroxide kg^{-1}. However, the advanced state of ripeness and the prolonged heat treatment to which the pulp was subjected should be considered when comparing its value to olive oil with a maximum permitted limit of 20 meq peroxide kg^{-1}.

The saponification index of the avocado oil (190.74 mg KOH g^{-1}) was higher than the reported by Restrepo Duque *et al*. (2012) (175 mg KOH g^{-1}). This indicates a greater presence of low molecular weight fatty acids since the esters of this type of molecules require more KOH for saponification (Lafont *et al*., 2011). Soap and cosmetics industry demand a minimum value of 185 mg KOH g^{-1} (Lafont *et al*., 2011), this suggests that oil of this type can be used in such type of factories.

Chromatographic profile of fatty acids

The percentual sum of the oleic and linoleic fatty acids was 57.33 ± 0.33% (Table 3), a similar value was reported by Acosta Moreno (2011) (59.1%) for the Hass avocado cultivar (Acosta Moreno, 2011). This indicates that the obtained oil has a high degree of unsaturation; verified with the iodine value (177.52 cg I_2 g^{-1}). For palmitate and stearate, the sum was 24.27%. This value is higher than the reported by Acosta Moreno (2011) who obtained 16.99% for such saturated fatty acids. The difference is significant and may be due to the quality of the original raw material.

Table 3. Content of fatty acids in the avocado oil.

Fatty acid methyl esters (FAME)	Average percentage composition (%)
Laureate	0.02 ± 0.00
Myristate	0.04 ± 0.00
Palmitate	19.02 ± 0.02
Docosahexaenoic acid	0.02 ± 0.00
Linoleic	7.76 ± 0.29
Linoleic+Oleic	57.33 ± 0.32
Estearato	5.25 ± 0.04

Color and BOD assessment

The colorant obtained from the extraction with sodium hydroxide was added to the liquid soap since it showed the best performance and high colorant strength.

The color difference in all treatments can be considered small and inconspicuous since the ΔE^* values are less than 1.5 (Obón *et al*., 2009), meaning that the color given at different concentrations by the NaOH aqueous extract and avocado seed is stable. Also, the change in pH is not affected over time. According to the CIELab coordinates, the colorant has a tendency towards yellow; behavior that is linked to the concentration and reflected in the b* coordinate (where a positive number indicates yellow, and a negative number indicates blue). When the colorant concentration increased from 1% to 3%, the value of b* increased from 41.51 to 68.07. A slight tendency towards the range of red was observed in coordinate a* (where a positive number indicates red, and a negative number indicates green), this may also be closely related to the concentration since as colorant concentration increases from 1% to 3%, the a* value increases from 9.2 to 33.23. However, the L* value (where numbers between 0 - 50 indicate black or darkness and between 51 -100 indicates whiteness or clarity) decreased from 79.8 to 54.21 as the concentration increased from 1% to 3%.

In this sense, the colorant extracted with NaOH was completely stable in the liquid soap matrix (pH of 6.2) during a month of storage. The formulation containing oil at 2% also remains stable in color and pH during the same period. The ANOVA indicated that there are no statistically significant differences between the treatments for parameter ΔE^* (P>0.05).

The BOD of the natural colorant was 10.35 mg L^{-1}, higher than the value of the commercial soap (9.42 mg L^{-1}). This indicates that more oxygen is required to degrade the organic matter in the soap matrix by a microbial population.

Table 4. Color and pH assessment in the liquid soap for 30 days.

Liquid soap matrix treatment	Time (d)	CIELab coordinates				pH
		L*	a*	b*	ΔE*	
Soap + colorant 1%	1	79.80	9.20	41.51	0.00	6.20
	10	79.59	9.54	40.51	1.08	6.20
	20	79.14	9.37	40.08	0.65	6.21
	30	79.04	9.16	39.54	1.18	6.26
Soap + colorant 2%	1	70.23	17.95	53.92	0.00	6.20
	10	70.78	17.91	54.33	0.69	6.20
	20	70.56	17.68	53.92	0.52	6.20
	30	70.44	17.30	53.36	0.69	6.24
Soap + colorant 3%	1	54.21	33.23	68.07	0.0	6.20
	10	55.35	33.27	68.87	1.39	6.20
	20	54.80	32.67	67.78	1.36	6.20
	30	55.03	31.69	67.90	1.01	6.24
Soap + colorant 2% + avocado oil 2%	1	73.90	15.20	57.20	0.00	6.20
	10	72.83	15.20	56.47	1.30	6.20
	20	72.85	14.16	55.47	1.44	6.21
	30	73.07	13.96	54.95	0.60	6.21

CONCLUSIONS

None of the evaluated extracts from the avocado seed (Lorena cultivar) showed any antimicrobial activity against strains of *Staphylococcus aureus* ATCC 29213 or *Escherichia coli* ATCC 25922; therefore, it is necessary to evaluate the antimicrobial activity after fractionation of the avocado seed extracts and analyze the feasibility of its incorporation in liquid soap.

The dye obtained from the avocado seeds has a great potential to be used in the soap industry since it was shown to be able to confer an orange color range and is stable over time against factors such as light and pH. Additionally, this colorant presented has antioxidant characteristics.

The oil obtained from avocados that were not suitable for consumption has a significant value of unsaturated fatty acids (mainly oleic) which favors its nutraceutical and cosmetic characteristics. The avocado oil incorporated into the liquid soap presented a good behavior because parameters like the ΔE* and pH were kept constant; also no separation of the oil from the matrix was observed during the time evaluated. Therefore, this oil can have a synergistic effect on the evaluated matrix.

By using components with high biological value, it is possible to develop more environmentally friendly products that generate added value to the waste produced during the avocado processing.

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