

ANTIOXIDANT CAPACITY AND OXIDATIVE STABILITY OF AN AVOCADO BY-PRODUCT PASTE WITH AND WITHOUT SEED

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ABSTRACT

During the harvest, processing, and distribution of avocado (*Persea americana* Mill.), a large amount of waste (peel, seed, and pulp) is produced, which has proven to have antioxidant properties and this by-product might be used as input within the food production chain. This study evaluates the lipid oxidation, antioxidant capacity and phenolic compounds of two pastes made with avocado by-products (with seed: PS and without seed: PWS) during 60 d stored under refrigeration. On the results obtained, an analysis of variance (ANOVA) was performed, followed by the Tukey's test ($p \leq 0.05$). The lipid oxidation was controlled in both pastes and was even reduced during storage. In the PS, the antioxidant capacity declined, although it remained high and very similar to the PWS towards the end of the storage. The concentration of phenolic acids increased during most of the time of storage. Although PWS displayed better results with two of the analyses performed, both pastes were considered to have important antioxidant characteristics, which remained during the refrigerated storage. Due to this, its potential use in the production of foods is inferred, particularly for animals such as cattle, pigs, in aquaculture, or even in the cosmetics industry.

Keywords: avocado, *Persea americana*, by-product, antioxidant activity, oxidative stability.

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INTRODUCTION

In Mexico, the production of avocado (*Persea americana* Mill.) stands out with the variety "Hass" as the most popular in the international market. Mexico is the main producer in the world with over 2 300 889 Megagrams (Mg). The state of Nayarit is the fourth largest producer in the country, with a production of 67 059 Mg (SIAP,

2020). Along with the fresh fruit, avocado is industrialized and transformed to other products such as guacamole and oil, mainly for industrial, gastronomic, medicinal, and cosmetic purposes.

In general, avocado peel and seeds contain catechins, procyanidins and hydroxycinnamic acids, which gives them an antioxidant potential, and the pulp is rich in hydroxybenzoic and hydroxycinnamic acids and procyanidins. This content of phenolic compounds in the avocado fruits used to obtain by-products can be affected by diverse factors such as the variety, agronomic conditions, postharvest handling, or the state of maturity and storage conditions, mainly (Goulao and Oliveira, 2008; Rodríguez-Carpena *et al.*, 2011). Avocado by-products obtained from the peel and seed of the fruit have been reported to present better antioxidant properties than pulp due to their higher concentration of phenolic compounds and can therefore be used for gastronomic and pharmaceutical purposes (Wang *et al.*, 2010; Rodríguez-Carpena *et al.*, 2011). Despite this, the edible pulp contains antioxidant properties as well as providing, when included in by-products, considerable amounts of fatty acids and essential amino acids (Gupta *et al.*, 2018).

During production and processing, avocado can undergo physical damage by compression, friction, or blows, which reduces the quality of the fruit and stops it from complying with the specifications desired by the consumer. Packaging companies select the fruit and separate those that do not satisfy the established requirements for sale or export. The discarded fruits occasionally remain near the plantation and cause contamination; when avocados arrive in factories, are selected once more and another percentage is discarded. Once the fruit is industrially processed, between 21 and 30 % of total weight is left behind as waste (Rodríguez-Carpena *et al.*, 2011). Recent reports by the FAO inform of wastes of around 54 % of the national production (FAO, 2015). This situation promotes the implementation and refining of techniques or methods to use these residues. There is even research on animal feed that has used avocado residues, particularly on pigs, and an improvement was found in the composition, as well as protection against oxidation of muscle in storage and of loin chops taken from these pigs (Hernández-López *et al.*, 2016 a, b).

Based to the above, since the residues of this fruit have displayed antioxidant properties, which are important for food production, the aim of this study was to evaluate the oxidative stability and antioxidant activity of two pastes made from avocado by-products, with and without seeds, during their storage under refrigeration, as an alternative for use in the animal feed or cosmetic industries.

MATERIALS AND METHODS

Production of avocado paste

The avocado of the variety "Hass" was acquired from packaging companies in the municipal area of Xalisco, Nayarit, Mexico. The avocado was sliced during physiological maturity and kept at room temperature until it reached maturity for consumption, which was determined by the colour of their surface (turned black at

100 % maturity). These selected fruits were not adequate for marketing due to their inadequate size, deformities, spots, grazes, or lesions in the epithelium. The fruits were transported in plastic containers to the Agriculture Academic Unit of the Autonomous University of Nayarit for processing. Previously, they were sanitized with chlorinated water and their seeds were removed by hand. The pulp and peels were ground in a mobile hammer and blade fodder mill, without a sieve, with an added gasoline engine to obtain a paste without seeds (PWS). Likewise, an entire lot of whole avocados with seeds, was also ground (PS). By the end, both pastes were packed in polyethylene bags and stored in refrigeration (4 °C) for 60 d to then be analysed, with sampling every 15 d.

Determination of lipid oxidation by TBA

The value of Thiobarbituric acid (TBA) was determined following the method by Biwas *et al.* (2017). The result was expressed as the concentration of malondialdehyde (MDA) in mg kg⁻¹ and to perform calculations, a standard tetraethoxy propane (TEP) curve was used.

Evaluation of the antioxidant capacity using 1,1-diphenyl-2-picrylhydrazyl (DPPH●)

The ability to trap the free radical DPPH was measured based on Guija-Poma *et al.* (2015). The results were expressed in µmol equivalent to Trolox per g (µmol TE g⁻¹) and a standard Trolox curve (6-hydroxy-2, 5, 7, 8-tetramethylchromo-carboxylic acid) was used, from 0 to 500 µmol L⁻¹.

Determination of total phenolic compounds

The concentration of phenolic compounds was determined with the Folin-Ciocalteu reagent, following the method described by Shi *et al.* (2018). A standard gallic acid curve (0 to 400 mg L⁻¹) was used, and the result was expressed in mg gallic acid equivalents (GAE) per g of sample.

Statistical analysis

The data were analysed as a complete randomized design with a one-way analysis of variance (ANOVA). The treatments consisted of avocado paste with and without seeds, analysed jointly by storage time. In case a significant difference was found, the Tukey's test was performed ($p \leq 0.05$). The analyses were done using SPSS Statistics v. 22.0 (IBM, 2013).

RESULTS AND DISCUSSION

Regarding results of lipid oxidation for the avocado by-product paste with seed (PS) and without seed (PWS), in general terms, a significant reduction can be observed in the concentration of MDA during storage after 60 d ($p \leq 0.05$), although the PWS displayed the lower final values, making it the best treatment (Figure 1). The degree of oxidation (concentration of MDA) found for both pastes in this study was lower

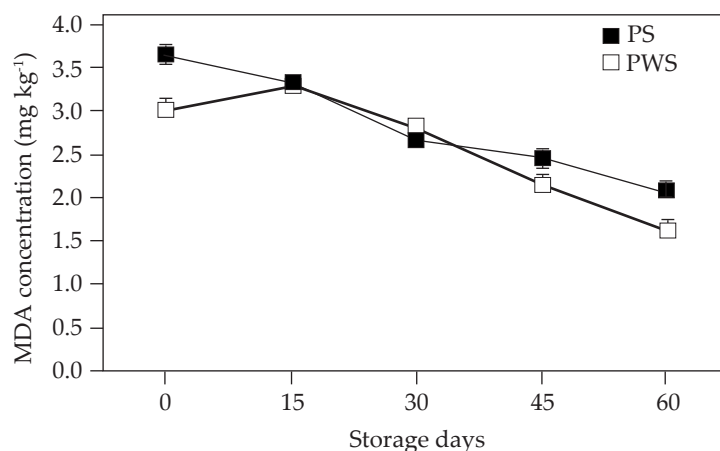


Figure 1. Concentration of malondialdehyde (MDA) of avocado by-product paste with seed (PS) and without seed (PWS) during storage in refrigeration. The data show the mean of $n = 3$. Bars represent the standard deviation.

in comparison to the one found by Plaitho *et al.* (2017) for all sampling points, who determined the effects of sterilization on the stability of the storage (6 months at 37 °C) of white and black sesame by-product paste. Those authors concluded that such a stability depends on the types of seeds and that it relates to the amount of free fatty acids, fibre, vitamins B, minerals and lignans (for example, sesamin, sesamol and sesamol), which are phenolic compounds with a high antioxidant capacity.

Avocado not only contains phenolic compounds, but also high concentrations of tocopherols and, in lower amounts, tocotrienols, which together represent the tocopherols with antioxidant and vitamin E activities. One of the most interesting chemical properties of the tocopherols (tocopherols and tocotrienols) is their ability to protect polyunsaturated lipids from oxidation, able to reduce peroxide radicals by donating hydrogen atoms (Delgado *et al.*, 2020). It has even been determined that the extracts obtained from “Hass” avocado residues present antioxidant activity against lipid oxidation in pork for hamburgers (Rodríguez-Carpena *et al.*, 2012).

Among the few related studies is the one reported by Mepba *et al.* (2008), who evaluated the stability of avocado pastes with antioxidants (propyl gallate and ascorbyl palmitate) and the oxidative changes by performing an analysis of TBA-reactive substances. In their results, they reported that the oxidative rancidness increases until day 16, where the highest TBA values were found in the control sample, in comparison with the samples treated with antioxidants. Whereas in this study, the most important change in PS and PWS was the reduction in the values of TBA (expressed as the concentration of MDA) from day 30 to day 60 ($p \leq 0.05$). This may be due to the antioxidants, originally contained in the initial samples: phenolic compounds and tocopherols, as well as others that may be formed during the cold storage such as leucoanthocyanidins and catechins, which have a proven ability to counteract lipid oxidation (Ramírez-Martínez and Luh, 1973; Ramos-Aguilar *et al.*, 2019).

Antioxidant capacity

Regarding the determination of the antioxidant capacity by DPPH●, significant differences were observed between the pastes at the beginning of the experiment ($p \leq 0.05$), although their values are equalized toward the end of the storage period ($p > 0.05$) (Figure 2). It is well known that not only the pulp, but also the peel and seed of the avocado contain polyphenols, carotenoids, and chlorophylls, responsible for the antioxidant activity. They act as cell membrane stabilizers and neutralize the free radicals related to harmful events such as cell oxidative stress. Other active compounds are the procyanidins, which, in the case of the peel, are suggested to be the main phenolic compounds related to the antioxidant properties (Wang *et al.*, 2010). The antioxidant capacity of different avocado tissues by the stabilization of the radical DPPH● has previously been reported (Rodríguez-Carpena *et al.*, 2011; Kosinska *et al.*, 2012).

Since the beginning, lower values were observed for PWS and even a reduction in the antioxidant activity on day 15 of storage, yet by day 30, it increased again, with values near to those of PS ($p \leq 0.05$). Regarding that matter, despite the final values being similar, a higher antiradical activity was observed in most samples during storage in comparison with PWS. Therefore, should the paste be used in a fresh form, according to this technique, it would be recommended to keep the avocado seed ($p \leq 0.05$). Rodríguez-Carpena *et al.* (2011) reported a higher antioxidant activity in the seed in comparison with the peel, which was attributed to a high concentration of hydroxybenzoic acid, catechins and hydroxycinnamic acid, which may explain this behaviour.

On the contrary, Wang *et al.* (2010), Kosinska *et al.* (2012) and Calderón-Oliver *et al.* (2016) reported that an avocado peel extract presents a higher antioxidant capacity through the elimination of DPPH● radicals than the seed extract. The differences

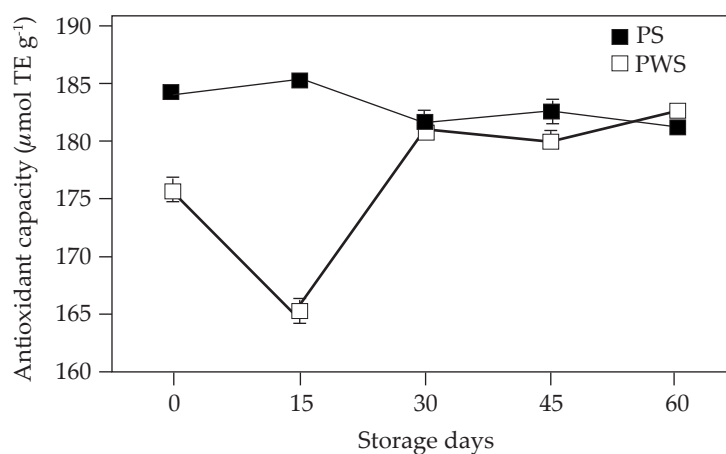


Figure 2. Antioxidant capacity (by DPPH● testing) of avocado by-product paste with seed (PS) and without seed (PWS) during storage in refrigeration. The data show the mean of $n = 3$. Bars represent the standard deviation.

between the studies may be due to the type of extract obtained before the analysis using the free radical. It is also worth mentioning that the antioxidant capacity of the avocado is affected by the season and time of postharvest maturation, since the fruit presents physiological and biochemical changes that include the biosynthesis and accumulation of pigments and lipid antioxidants in different tissues (Goulao and Oliveira, 2008). Differences may even be found in the behaviour regarding the antioxidant properties between the peel and seed of this variety studied (Rodríguez-Carpena *et al.*, 2011).

Total phenolic compounds

The total phenolic content increased from 33.78 ± 0.28 to 41.98 ± 1.75 for PS and from 34.56 ± 0.59 to 59.57 ± 0.66 mg GAE g⁻¹ for PWS ($p \leq 0.05$) during storage (Figure 3). It is interesting to notice the reduction and later increase for PWS on days 15 and 30, respectively ($p \leq 0.05$), which coincides with the antiradical activity values in the same sampling points. These increases, both in total phenolic compounds and in antiradical activity may be due to the formation of new compounds with a higher antioxidant capacity during storage in refrigeration. Regarding this, Ramírez-Martínez and Luh (1973) mentioned the formation of bioactive compounds in avocado pulp during its storage in freezing temperatures.

On the other hand, since day 30 and onwards, in all sampling points and until the end of the storage period, the PWS displayed higher values than the PS ($p \leq 0.05$), which may be due to the absence of seeds. Rodríguez-Carpena *et al.* (2011) and Kosinska *et al.* (2012) recorded a higher concentration of phenolic compounds in the avocado peel than in the seed of this same variety. For this reason, removing the seeds in the PWS promoted an increase in phenolic compounds values; this treatment was the best in terms of the concentration of such compounds. Wang *et al.* (2010) found a higher

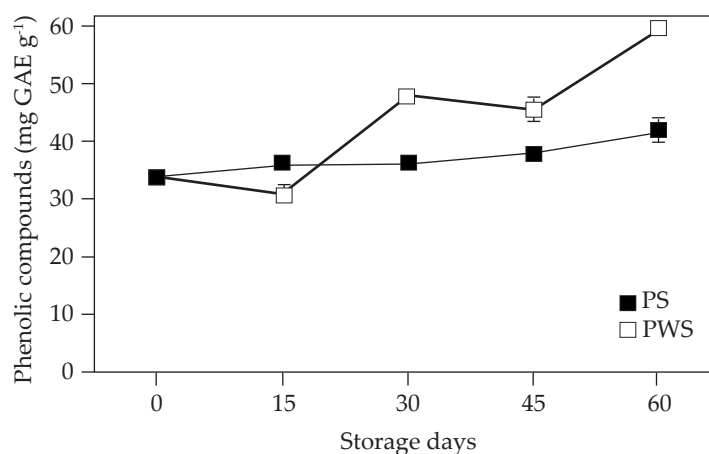


Figure 3. Total phenolic compounds in avocado by-product paste with seed (PS) and without seed (PWS) during storage in refrigeration. The data show the mean of $n = 3$. Bars represent standard deviation.

concentration of phenolic compounds in the seed than in the peel of this fruit, and they identified the procyanidins as the main representative of this group of oxidants. In the case of the peel, they identified chlorophyll and carotenoids as the main components. The differences between studies may be due to the factors discussed previously for the antioxidant activity. This study also used the pulp of fruits considered inadequate for sale or rejected for quality control reasons and which, if they were included as inputs in the food industry, would present other types of compounds related to beneficial effects in the organisms that consume them, such as fatty acids and essential amino acids (Gupta *et al.*, 2018).

In recent years, studies have been completed which formulate animal diets containing avocado by-products or paste with a low quality, or which do not comply with the characteristics with the required characteristics for marketing. Those studies were implemented to feed pigs and favourable results were found regarding the performance of consumption and food conversion, composition, and antioxidant protection in the muscle during cold storage (Hernández-López *et al.*, 2016b) and protection against lipid and protein oxidation in processed chops (Hernández-López *et al.*, 2016a). In aquaculture, there is a study reported in which avocado by-products were included in a diet for tilapia (*Oreochromis niloticus*) and their antioxidant properties improved (Jiménez-Ruiz *et al.*, 2019). As in previous studies, this study also used the pulp in the by-products studied, out of which the importance of including them in the diets of the fed organisms has already been mentioned (Gupta *et al.*, 2018).

This study becomes relevant by showing that the time of use of the stored avocado paste can be extended when there are, for example, high levels of waste of this fruit depending on the different harvest times among regions. On the other hand, when considering two of the analyses performed (lipid oxidation and concentration of phenolic compounds), the best option is to remove the avocado seed, as proven by the results for the treatment paste without seeds. It is important to mention that the paste with seeds also has attributes that can make it usable, although it is necessary to define its better final use. For example, when used as a food input, it would be recommendable to remove the seed, due mainly to the antinutrients it presents, which may affect the organisms being fed.

CONCLUSIONS

The pastes evaluated maintained their antioxidant properties and a low lipid oxidation in refrigeration, proving the feasibility of storing them in seasons in which the highest levels of avocado fruit are wasted until the moment in which they are used. Although removing the seed generally presented better results, according to the analyses, both pastes have the potential to be used in food production, particularly destined to animal feed, such as pigs, cattle, aquaculture or in the cosmetic industry, which would give them an added value. In the case of their use for the food industry, a key to consider is the antinutritional factors that may be found in the avocado seed included in one of the pastes, the removal of the seed is therefore recommended. In addition, this may contribute to reduce contamination when these fruits are discarded outdoors.

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