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Optimization of the antioxidant and antimicrobial response of the combined effect of nisin and avocado byproducts

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ABSTRACT

This study is focused on optimizing, by surface response methodology, the mixture of natural additives: nisin (an antimicrobial peptide) in combination with avocado seed or avocado peel extracts (both with antioxidant capacities) in order to maximize the antioxidant activity and antimicrobial response against some food-borne bacteria such as *Listeria*. The peel or seed extracts used in the mixture showed radical scavenging capacity and antioxidant activity due to their polyphenol composition, including kaempferide, epicatechin, chlorogenic acid, epicatechin gallate, among others. During optimization, the results showed that the major antioxidant response in the mixture was mainly provided by the peel extract compared with nisin, seed and their combinations in different proportions. In the antimicrobial response, a synergic effect was observed in the mixtures of each avocado byproduct extract with nisin. Maximum antioxidant and antimicrobial response were obtained with the mixture of 61% of peel extract with 39% of nisin ($p < 0.5$, desirability 0.76) at concentrations of 1 mg/mL for each.

1. Introduction

There is a current trend toward consuming more natural and healthy products at the expense of products with preservatives or synthetic additives. Thus, some studies are focused on finding new natural components that have a similar or better effect than synthetic additives that also maintain quality and lengthen shelf life without changing the sensory characteristics of the products.

The extraction process of agroindustrial byproducts generates phytochemicals that can be applied as functional food compounds, colorants, flavors, antioxidants, and stabilizers, among others (Roldán, Sánchez-Moreno, de Ancos, & Cano, 2008; Silva et al., 2014; Vijayalaxmi, Jayalakshmi, & Sreeramulu, 2014). In addition, the use of these waste materials would help reduce the generated environmental impact and contribute to a cost reduction in the industry.

Antioxidants play an important role in preventing the oxidation of food, which is a deterioration process that involves reactions among lipids, vitamins, proteins and sugars with reactive oxygen and nitrogen species (ROS). As a consequence of the oxidative deterioration there are changes in the quality, sensory, and nutritional characteristics of food products (Choe & Min, 2006). Vegetables and fruits are known for being good sources of antioxidants and the avocado fruit is a good example of this (Fu et al., 2011). Avocado (*Persea americana*) is an oleaginous fruit that matures on the tree and that ripens after its harvest (Kosińska et al., 2012). The Hass avocado variety is one of the main crops in Mexico; it is exported to USA, Asia, and Europe (FAO., 2014). The edible portion of the avocado contains essential nutrients and phytochemicals with health benefits (Dreher & Davenport, 2013). However, the peel and seed are underutilized and may be considered an alternative antioxidant source because they contain polyphenols, carotenoids,

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and chlorophylls which are responsible for the antioxidant activity (Rodriguez-Carpena, Morcuende, Andrade, Kylli, & Estevez, 2011). Even though avocado byproducts are known for their antioxidant capacity, their specific free-radical specific scavenging capacity is yet to be determined.

On the other hand, nisin, a peptide with antimicrobial activity produced by *Lactococcus lactis* sp *lactis*, is generally recognized as safe and has been used in several ways to control pathogens in foods (Juneja, Dwivedi, & Yan, 2012; Mills, Stanton, Hill, & Ross, 2011). The antimicrobial mechanism of nisin is due to the interaction with lipid II in the bacterial plasma membrane, with the subsequent formation of pores that induces leakage of the cytosolic contents (Bauer & Dicks, 2005). There are some studies about the interaction of nisin with certain antioxidant extracts or compounds that found a synergic effect when these are applied to food without sensorial changes (Abdollahzadeh, Rezaei, & Hosseini, 2014; Gao et al., 2014).

The aims of this study are: a) to know the composition, the antioxidant and antimicrobial capacities of avocado seed and peel extracts; b) to optimize a mixture of natural additives such as nisin (an antimicrobial) with avocado seed and peel (an antioxidant) through surface response and to maximize both responses; c) to come up with a new alternative for food additives.

2. Materials and methods

2.1. Chemicals

Nisin (2.5% w/w balanced with sodium chloride and denatured milk solids, 10^6 IU/g), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), Trolox, Folin-Ciocalteu's phenol reagent, gallic acid, quercetin, 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and other reagents were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA).

2.2. Avocado samples and extraction

Avocado fruits (*P. americana*, Hass variety) were purchased at the Central de Abastos in Mexico City and were maintained at room temperature until they reached ripeness ready-to-eat. Fully ripened fruits were manually separated into seed, pulp, and peel, and the weight for each component was obtained. Seed and peel were finely ground in a blender and dried at 40 °C for 24 h in an air flow oven. The moisture was measured by weight difference. Then, 50 g of dry avocado seed or peel was added to 500 mL of boiling distilled water. This mixture was boiled and stirred by a magnetic stirrer for 30 min (Xu et al., 2008). The extract was filtered with filter paper (Whatman No. 4). The filtrate was frozen and lyophilized at 5 mmHg at −50 °C (Freezone 2.5; Labconco Corp. Kansas, MO, USA). The lyophilized powder was stored at −20 °C. The extracts were dissolved in distilled water at 50 mg/mL before each characterization assay (color, radical scavenging capacity, and composition).

2.3. Color measurement

The color of the peel, seed, pulp, and the resulting extracts were measured utilizing a colorimeter (ColorFlex EZ spectrophotometer 45°/0°; HunterLab, Reston, VA, USA). The CIELAB color coordinates (L*, a* and b*) were set at a 10° angle observer and D65 light source.

2.4. Total phenolic content

Phenolics were measured using the Folin-Ciocalteu's phenol reagent (Singleton & Rossi, 1965) with modifications. Two hundred microliters of extract was mixed with 1 mL of Folin-Ciocalteu's reagent (1 N) and 0.8 mL of 7.5% Na_2CO_3. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 760 nm using a Synergy HT spectrofluorometer (Biotek Instruments Inc., Winooski, VT, USA). The results are expressed as mg of gallic acid equivalents (GAE) per gram of extract (dry weight, dw).

2.5. Total flavonoid content

Thirty-five microliters of extracts was mixed with 0.0105 mL of water and 0.0105 mL of 5% $NaNO_2$, 0.0105 mL of 10% $AlCl_3$ and 0.140 mL of 0.5 M NaOH, and incubated for 30 min at room temperature in the dark (Zhishen, Mengcheng, & Jianming, 1999). The absorbance at 510 nm was measured using a Synergy HT spectrofluorometer. The results are expressed as mg of quercetin equivalents per gram of extract (dw).

2.6. Tannin content

Tannin compounds were determined according to Makkar, Blummel, Borowy, and Becker (2006). Two hundred microliters of extract was mixed with 20 mg of insoluble polyvinylpolypyrrolidone. After 15 min of incubation at 4 °C, the tubes were centrifuged for 10 min at 15,000 × *g*. Then, 0.05 mL of supernatant was used to determinate tannin content by the same procedure used for total phenolic content. The tannin content was the difference between the total and non-absorbed phenolics. The results are expressed as milligrams of GAE per gram of extract (dw).

2.7. In vitro antioxidant capacity

Antioxidant scavenging assays, including superoxide anion ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), singlet oxygen ($^1O_2$), peroxynitrite ($ONOO^-$), and hydrogen peroxide (H_2O_2) were determined as previously described (Gaona–Gaona et al., 2011). $O_2^{\bullet -}$ was produced using the xanthine–xanthine oxidase reaction. The activity of xanthine oxidase was measured by uric acid production at 295 nm and scavenging capacity was measured by the nitroblue tetrazolium reduction at 560 nm. $OH^{\bullet}$ was produced by the Fenton reaction, and its generation was monitored by the increase in fluorescence (326 nm of excitation and 432 nm of emission) as a consequence of the reaction of $OH^{\bullet}$ with terephthalate. $^1O_2$ was produced by the reaction of H_2O_2 and sodium hypochlorite, and its production was determined by the increase in fluorescence (410 nm of excitation and 455 nm of emission) produced by its reaction with 1,3-diphenylisobenzofuran. $ONOO^-$ reacts with 2,7-dichlorodihydrofluorescein diacetate and produces a fluorescent product (485 nm of excitation and 520 nm of emission). H_2O_2 scavenging was determined using amplex red/horseradish peroxidase reagent. The fluorescence was measured at 550 nm of excitation and 590 nm of emission. The results of all scavenging assays are expressed as the extract concentration in μg/mL required to neutralize 50% of the reactive species present (IC_{50}).

2.8. Oxygen radical absorbance capacity (ORAC)

The ORAC assay was performed according to (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). The reaction mixture contained: 38.5 mM AAPH (25 μL), 30 nM fluorescein (150 μL) and sample (25 μL). The reaction was incubated and monitored for 1.5 h at 485 nm of excitation and 526 nm of emission. The areas under the curve and equivalents of trolox were calculated by Gen5™ software (Biotek Instruments), using a standard curve of trolox. The results are expressed as μg of trolox equivalents per gram of extract (dw).