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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).

2.9. Ferric reducing antioxidant power (FRAP)

The FRAP reagent was prepared minutes prior to initiation of the assay by mixing 10 mM TPTZ (1 mL, dissolved in 40 mM HCl), 20 mM $FeCl_3$ (1 mL) and 300 mM acetate buffer, pH 3.6 (10 mL) (Benzie & Strain, 1996). The sample (200 μL) was mixed with FRAP reagent (1.5 mL). The reaction was incubated at 37 °C for 5 min and measured at 593 nm. The results are expressed as mg of trolox equivalents per gram of extract (dw).

2.10. High-performance liquid chromatography coupled with electrospray ionization and mass detection (HPLC-ESI-TOF)

The seed and peel extracts were dissolved in methanol-water (3:1) at a concentration of 5 mg/mL; the polyphenol standards (epicatechin, catechin, caffeic acid, ferulic acid, quercetin, gallic acid, and rutin) were prepared in the same manner as the extracts. HPLC analyses were performed with the Ultimate 3000 Basic Automated (ThermoScientific-Dionex, CA, USA), and a Poroshell 120 EC-C18 column (2.7 μm, 4.6 × 50 mm; Agilent Technologies, Santa Clara, CA, USA). Mobile phase was carried out using a gradient of methanol and acetonitrile with a flow of 0.5 mL/min at 25 °C. The liquid chromatography was coupled to an electrospray mass spectrometer ESI-MS MicroTOF (Bruker Daltonik, Bremen, Germany), utilizing an electrospray interface operated in negative mode with a voltage of 4.5 kV. The drying gas temperature was 190 °C, the drying gas flow was 8 L/min, and the nebulizer gas pressure was 3 bar. The data obtained from the molecular ions were processed by means of Compass Data Analysis 4.1 (Bruker Daltonik) software and the SmartFormula Editor tool.

2.11. Antimicrobial activity

Bacterial strains: *Listeria innocua* (ATCC 33090), *Escherichia coli* (JMP101), *Lactobacillus sakei*, *Weissella viridescens*, and *Leuconostoc mesenteroides* were isolated from meat products and identified by rDNA16S in our laboratory. The antimicrobial activity of extracts alone or in combination with nisin was determined by the broth-based turbidimetric method (Othman et al., 2011) with some modifications. Forty microliters of inoculum (10^3 colony-forming units per mL) was mixed with one hundred and sixty microliters of extracts, nisin, or as a combination and then they were incubated at 35 °C. The optical density was monitored at 600 nm every 30 min with a 10 s agitation prior to each measurement for 24 h in a Synergy HT microplate reader. Lag time and maximum growth rate were calculated using Gen5™ software. Lag time was considered in the mixture design because it is the time during which a bacterium does not grow. The antimicrobial activity of nisin and its combination with the extracts was measured only with *L. innocua*.

2.12. Simplex lattice mixture design for optimization of antimicrobial and antioxidant response

A simplex lattice augment mixture design was utilized to determine the effect of interactions among nisin, seed extract, and peel extract on antimicrobial and antioxidant properties. Component proportions were expressed as a fraction of the mixture with a sum of one. Response variables comprised antioxidant (ORAC method) and antimicrobial (turbidimetric assay, measured as lag time) activities. Mixture design experiments were designed and analyzed using Statgraphics Centurion XV, version 15.2.6 (StatPoint Technologies, USA). A total of 10 combinations are presented in Table 1. The following polynomial equation of function X_i was fitted for each factor assessed at each experimental point, where Y is the predicted response and β_1, β_2, β_3, β_{12}, β_{13}, and β_{23} are constant coefficients for each linear and non-linear interaction terms:

$$Y = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \quad (1)$$

The data were analyzed by a general linear model. Optimum values of the independent variables were elucidated by conducting a three-dimensional response surface analysis of the independent and dependent variables.

2.13. Statistical analysis

Radical scavenging activity and the characteristics of byproducts and extracts were analyzed at least six times and data were expressed as mean ± standard deviation. One-way ANOVA and the Tukey's multiple comparisons test were conducted utilizing the software Prism ver. 5.0 (GraphPad, San Diego, CA, USA). A p-value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Physical characteristics of avocado samples and the resulting extracts

The average weight of the avocado fruit was 153.89 ± 16.06 g, where the 15.22 ± 4.7% corresponds to the seed and 16.12 ± 0.3% to the peel of the fruit's total weight (Table 2). The morphometric results of the fruits, such as seed and peel weight as well as moisture composition, are similar to those previously reported by Rodriguez-Carpena et al. (2011). These data show that the Hass avocado variety possesses a considerable byproducts percentage (approximately 30%). Yields of the extraction method were 8 ± 2.3% in the peel and 13.2 ± 3.4% in the seed. The color of extracts is different from the color of the material from which they were extracted (Table 3). Additionally, the peel extract presents a slightly tamarind-like fruity odor.

Table 1
Experimental conditions of mixture design.

Mixture	Proportions and final concentrations in assay (mg/mL)			Variable responses	
	Nisin	Seed	Peel	ORAC (μg trolox equivalents/mL)	Lag time (h)
1	1	0	0	31.99	24
2	0.5	0.5	0	62.63	23.61
3	0.5	0	0.5	156.45	24
4	0	1	0	106.56	7.18
5	0	0.5	0.5	241.92	7.01
6	0	0	1	285.18	7.10
7	0.33	0.33	0.33	185.51	21.48
8	0.667	0.167	0.167	100.34	24
9	0.167	0.667	0.167	142.27	18.62
10	0.167	0.167	0.667	245.56	18.81

Table 2
Physical characteristics of avocado samples.

		Whole fruit	Seed	Pulp	Peel
Weight (g)		153.89 ± 16.06	28.66 ± 14.46	100.44 ± 23.89	24.78 ± 2.22
Moisture (%)		–	55.40 ± 8.3	76.45 ± 12.5	74.99 ± 15.3
Color	L*	23.85 ± 0.65	31.00 ± 1.44	61.72 ± 0.93	23.85 ± 0.65
	a*	1.25 ± 0.51	6.15 ± 2.09	−1.48 ± 0.39	1.25 ± 0.51
	b*	9.1 ± 0.36	5.97 ± 1.23	31.02 ± 1.76	9.1 ± 0.36

– Not evaluated.

Table 3
Antioxidant and radical scavenging capacity, total phenols and flavonoids content.

	Seed extract	Peel extract	Reference compound
Color	L* = 79.96 ± 0.18 a* = 10.35 ± 0.07 b* = 27.26 ± 0.07	L* = 41.2 ± 0.27 a* = 10.70 ± 0.06 b* = 21.16 ± 0.2	NE
Total phenols (mg GAE/g extract)	5.7 ± 0.2[a]	19.7 ± 1[b]	NE
Total flavonoids (mg QER/g extract)	2.8 ± 0.8[a]	10.9 ± 1.03[b]	NE
Tannin content (mg GAE/g extract)	0.09 ± 0.001	0.04 ± 0.001	NE
ORAC (μg Trolox equivalents/g extract)	1.6 ± 0.09[a]	216.8 ± 2.5[b]	NE
FRAP (mg Trolox equivalents/g extract)	9.5 ± 1.6[a]	23.1 ± 5.9[b]	NE
$O_2^{\bullet -}$	0.87 ± 0.03[a]	0.83 ± 0.08[a]	0.25 ± 0.02[b]
OH•	430 ± 86[a]	26.5 ± 1.0[b]	0.49 ± 0.04[c]
$^1O_2$	2854 ± 72[a]	1741 ± 3[b]	2.9 ± 0.6[c]
$ONOO^-$	612 ± 184[a]	198 ± 44[b]	3.0 ± 1.4[c]
H_2O_2	0.8 ± 0.03[a]	0.6 ± 0.09[a]	11.0 ± 0.2[b]

Data are expressed as the mean ± standard deviation. N = 6–8 independent experiments. Values with different letters (a–c) within a row are significantly different ($p < 0.05$). GAE = Gallic acid equivalents, QER = Quercetin equivalents. NE = not evaluated. The data of scavenging capacities are expressed in IC50 (μg/mL).

3.2. Composition and antioxidant capacity

The content of phenols, tannins and flavonoids, as well as the antioxidant capacities of each extract are illustrated in Table 3. In all cases, the peel extract possesses more compounds that confer greater antioxidant capacities than those of the seed extract, except in tannin content. These results are similar to other reports in which the peel extract has more total polyphenol content than the seed extract using other solvent and extraction process (Rodriguez-Carpena et al., 2011; Widsten, Cristina, Fletcher, Pajak, & McGhie, 2014). We extracted polyphenols with water because it is a food grade solvent and has lower ecological impact if compared to other organic solvents such as methanol or acetone. Additionally, we used an aqueous extract for compatibility with nisin in the mixture solution.

The differences in the composition of both by-product extracts were observed among the scavenging antioxidant activities (Table 3). Seed and peel extracts were able to scavenge in a concentration-dependent manner (data not shown) in all of the reactive species described in this study. IC_{50} values for $^1O_2$, OH• and $ONOO^-$ were different for the extracts and the reference compounds. IC_{50} values for H_2O_2 and $O_2^{\bullet -}$ did not exhibit a significant variation, but they were different with respect to the reference compound. In all cases, the peel extract IC_{50} values were lower than those of the seed extract. Also the peel extract was significantly different in OH•, $^1O_2$ and $ONOO^-$ inactivation when compared to the seed extract. This suggested that the peel extract is more effective than the seed extract in scavenging ROS. Similar results were observed in another study which demonstrates that peel extracts of Hass avocados and Shepard avocados have more total phenolic content and antioxidant activity than seed extracts (Kosińska et al., 2012).

When peel and seed extracts were analyzed by HPLC-ESI-TOF (Table 4), it was found that the peel extract had almost the same number of compounds as the seed extract (Fig. 1S). However, the seed presents common compounds reported previously in databases compared to the peel, and this was easy to identify. Epicatechin was the only polyphenol standard presented in both extracts. Some of the identified compounds (Table 4) have been reported previously in other studies such as epicatechin, procyanidins and chlorogenic acid in both extracts (Rodriguez-Carpena et al., 2011; Widsten, et al., 2014). Other identified compounds, such as phenolic acids, have been reported in the pulp fruit (Hurtado-Fernández, Carrasco-Pancorbo, & Fernández-Gutiérrez, 2011). It is well known that members of the same group of polyphenols (phenolic acids, flavonols, flavone, etc.) do not have similar antioxidant activity despite having certain molecular similarity as this activity is a result of the environment and the presence of other compounds (Parker, Miller, Myers, Miguez, & Engeseth, 2010; Tabart, Kevers, Pincemail, Defraigne, & Dommes, 2009). It is likely that even though both extracts have similarities in composition, they do not have the same antioxidant capacity potentially due to the presence of unidentified compounds that could increase or have no effect on the antioxidant or antimicrobial activity observed in the extracts.

3.3. Antimicrobial activity

The peel and seed extracts themselves do not present antimicrobial activity against any bacteria analyzed in this study. The extracts show the same values of lag time and maximum growth rate as those of the control. In the case of *L. innocua*, the lag time was 1.2 ± 0.03 h and the maximum growth rate was 6.66 ± 0.14 h^{-1} for the control and for the peel and seed extracts. There are studies

Table 4
Phenolic profile and tentative identification of compounds found in water extract of avocado peel and seed.

Compound or possible compound	Formula or possibilities*	$[M-H]^-$ exp (*m/z*)	Theoretical mass	T_R (min)	Peel		Seed	
					Peak area	Area %	Peak area	Area %
Malic acid	$C_4H_6O_5$	133.0144	134.09	1.8			1679085.5	25.9
Quinic acid	$C_7H_{12}O_6$	191.0183	192.17	2.3	357672	3.4	668698.5	10.3
Succinic acid or methylmalonic acid	$C_4H_6O_4$	117.0198	118.09	2.6	626470	6.0	23783.2	0.4
7-Hydroxy-6,8-di-C-methylflavanone 7-O-arabinoside	$C_{22}H_{24}O_7$	399.1437	400.152	3.7			25899.8	0.4
Isorhamnetin	$C_{16}H_{12}O_7$	315.1054	316.26	5.6			42228.5	0.7
Kaempferide	$C_{16}H_{12}O_6$	299.1128	300.26	9.4			100845.5	1.6
3-*O*-*p*-coumaroylquinic acid	$C_{16}H_{18}O_8$	337.0924	338.30	10.1			51328.6	0.8
Chlorogenic acid	$C_{16}H_{18}O_9$	353.0835	354.31	10.96	376322	3.6	80251.6	1.2
Procyanidin (A or B)	$C_{30}H_{26}O_{12}$	577.1621	578.52	11.2	342976	3.3		
Epicatechin[a]	$C_{15}H_{14}O_6$	289.0855	290.27	11.7	315636	3.0	62105.8	0.9
Quercetin-3-*O*-arabinose	$C_{20}H_{18}O_{11}$	433.2306	434.37	12.6	35607	0.3		
Epicatechin Gallate	$C_{22}H_{18}O_{10}$	441.177	442.37	13.2			304539.3	4.7
Abscisic acid	$C_{15}H_{20}O_4$	263.1429	264.32	16.4	46252	0.4		
p-coumaroyl-D-glucose	$C_{13}H_{16}N_3O_7$	325.1979	326.09	28.4	161934	1.6		

TR = retention time; * = Possible formula in Smart Editor-Molecular formula (upward order of sigma of 30 and lower error than 5 ppm).
[a] Compound compared with standard.

in which the antimicrobial activity correlates with the total polyphenol and flavonoid content (Widsten et al., 2014). However, the antimicrobial activity also depends not only on the type, but also on the concentration of polyphenols in the sample and the target microorganism (Widsten et al., 2014). There are studies in which acetonic-water Hass avocado seed and peel extracts showed antimicrobial activity against pathogen strains (Rodriguez-Carpena et al., 2011; Widsten et al., 2014). In this study, the type of polyphenols that were extracted might not inhibit the growth of the bacteria studied, but they could inhibit other pathogenic bacteria. On the other hand, nisin itself, at a concentration of 0.78 mg/mL, demonstrates the minimum inhibitory concentration against *L. innocua*, calculated as the concentration that does not present changes in optical density after 24 h of incubation.

3.4. Effect of avocado extracts and nisin combinations in antioxidant and antimicrobial activities

Mix design optimization was conducted to evaluate the performance of antioxidant extracts (avocado seed and peel) with the antimicrobial nisin. The results for each mixture assay are shown on Table 1.

For the antioxidant and antimicrobial responses, full quadratic models were more acceptable due to the R^2 and R^2-adjusted values. Table 5 presents the coefficient values of the antimicrobial and antioxidant effects with their respective *p*-values. Coefficients with *p*-values >0.5 were not significant for either effect.

In the antioxidant response, the peel presents the higher coefficient value. Additionally, Fig. 1A and B indicate that antioxidant activity values increased by augmenting the peel content. The lowest antioxidant activity values were at the nisin edge. These results were expected because the highest antioxidant activity was found in the peel extract. Thus, the maximum effect was observed in a combination of 10% seed and 90% peel.

With respect to the antimicrobial effect, nisin-peel and nisin-seed interactions are significant and their coefficients are higher (Table 5), suggesting a synergistic effect. Seed and peel interaction was not significant for this response. Fig. 1C and D illustrate that the highest antimicrobial activity values were at the nisin edge. The maximum effect was observed at 70% of nisin with 25% of peel and 5% of seed. There are reports in which combinations of organic acids (lactic acid, acetic acid, or citric acid) with nisin increased antimicrobial activity against some pathogen strains such as *E. coli* O157:H7 (Fang & Tsai, 2003). Additionally, mixtures of nisin with the salts of organic acids or with ion chelators inhibited the growth of *Pseudomonas* sp, and *Listeria monocytogenes* (Wan Norhana, Poole, Deeth, & Dykes, 2012). The occurrence of some organic acids in the extracts could be responsible for the increased antimicrobial effect of nisin.

Another explanation for the antibacterial effect is the presence of phenolic compounds in the extracts. These compounds alter the function of bacterial cell membranes and, consequently, cause growth delay and inhibit bacterial multiplication. In addition, phenolic compounds can easily penetrate through biological membranes (Juneja et al., 2012). This, combined with the pore-forming ability of nisin, leads to the leakage of small molecules.

3.5. Optimization of the interaction of avocado byproduct extracts with nisin

To maximize effects (antimicrobial and antioxidant), a numerical and surface response optimization technique was employed.

Table 5
Estimated regression coefficients for variable responses.

Parameter	Antioxidant response			Antimicrobial response		
	Coefficient	Standard error	*p*-value	Coefficient	Standard error	*p*-value
Nisin	31.13	11.34		22.93	2.50	
Seed	102.59	11.34		7.71	2.50	
Peel	284.61	11.34		7.65	2.50	
Nisin-seed	10.71	52.28	0.8477	35.46	11.54	0.0372
Nisin-peel	35.56	52.28	0.5337	37.22	11.54	0.0321
Seed-peel	222.10	52.28	0.0132	6.12	11.54	0.6238
	R^2: 99.12, R^2adjusted: 98.02			R^2: 94.69, R^2adjusted: 88.05		

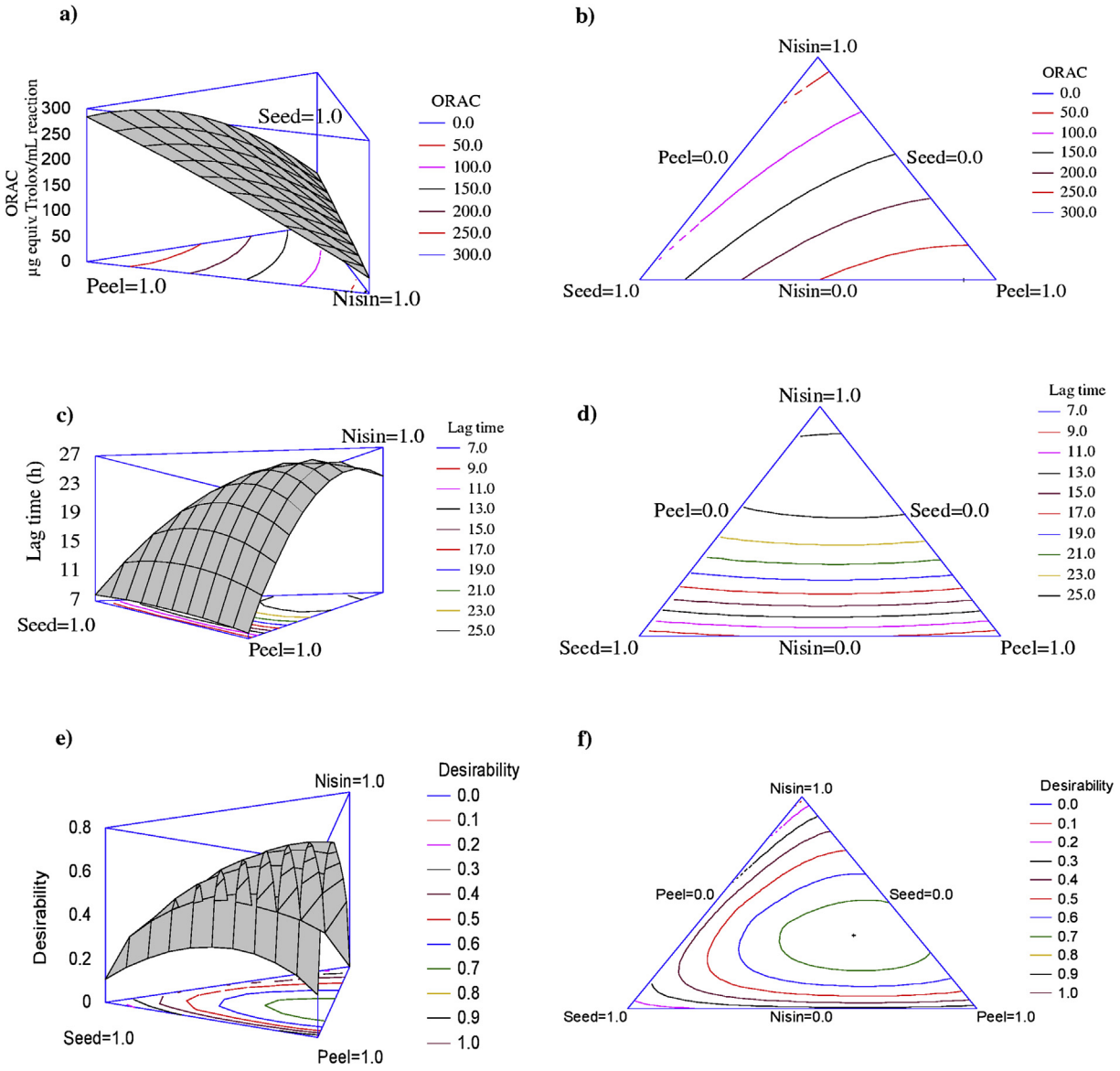

Fig. 1. Antioxidant and antimicrobial responses of the mixture of avocado seed, avocado peel and nisin. Antioxidant response: a) Surface response, b) Ternary contour plot. Antimicrobial response against *Listeria innocua*: c) Surface response, d) Ternary contour plot. Optimization maximizing antimicrobial and antioxidant responses: e) Surface response, f) Ternary contour plot.

This technique considers both responses as equal in weight and importance. Fig. 1E and F presents the estimated surface response and contour plot in which the maximum effect is observed in the interaction of nisin with peel ($p < 0.5$, desirability 0.76); with regard to numbers, a mixture of 39% of nisin and 61% peel maximized both effects (ORAC value of 194 μg of trolox equivalents/mL, and lag time of 22.4 h). The seed extract was discarded because it did not provide a good antioxidant or antimicrobial response compared with those of nisin or peel. It could be added that perhaps this mixture requires a final color, such as a natural color additive similar to curcumin (Dabas, Elias, Lambert, & Ziegler, 2011).

4. Conclusion

Peel extract presents more antioxidant and radical scavenging capacity than seed extract due to its polyphenols content, such as procyanidins, epicatechin and other non-identified compounds. These were mainly responsible for the antioxidant response in the mixture of the three natural additives utilized in this study. The peel or seed extract, in combination with nisin, presents a synergic effect in the antimicrobial response. The mixture of nisin and avocado peel comprises the optimal mixture that maximizes the antioxidant and antimicrobial response. The use of a natural antioxidant such as avocado peel, in combination with a natural conservative such as nisin, provides a novel combination that may reduce the amount of nisin employed at the industrial level, not only reducing costs, but also promoting the use of natural compounds that are derived from agro-industrial byproducts with good antioxidant and antimicrobial effects. This mixture also favors the trend towards the use of natural additives.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.lwt.2015.07.048.

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