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Antioxidant activity and sensory analysis of murtilla (*Ugni molinae* Turcz.) fruit extracts in an oil model system

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SUMMARY: An oil model system was used to analyze the antioxidant activity of Chilean fruit extracts and to determine their odor sensory effect. Hydroalcoholic extracts from wild and 14-4 genotype murtilla (*Ugni molinae* Turcz.) fruit were assessed by the Response Surface Methodology. The optimal conditions for producing high total phenolic-content extracts were 49.5% (v/v) ethanol at 30 ºC, which yielded 18.39 and 26.14 mg GAE·g^{-1} dry matter, respectively. The optimized extracts were added to a lipid model system and evaluated via the Schaal Oven Test. After 96 hours, 150 and 200 mg·kg^{-1} oil of the wild and 14-4 genotype extracts, respectively, showed an antioxidant capacity similar to TBHQ (200 mg·kg^{-1} oil) in terms of peroxide values and odor. Thus, murtilla fruit extracts are a natural source of antioxidants for protecting lipidic foods, such as soybean oil.

KEYWORDS: *Antioxidant activity; Murtilla; Oil model system; Oil organoleptic characteristic; Response Surface Methodology; Total polyphenol content*

RESUMEN: ***Actividad antioxidante y evaluación sensorial de extractos de frutos de murtilla (*****Ugni molinae** ***Turcz.) en un sistema modelo aceitoso.*** Se analizó la actividad antioxidante de extractos de una fruta chilena en un sistema modelo aceitoso y se determinó el efecto sobre las características organolépticas. Se utilizaron extractos hidroalcohólicos de frutos de murtilla (*Ugni molinae* Turcz.) silvestre y del genotipo 14-4, y se aplicó la Metodología de Superficie de Respuesta. Las condiciones óptimas para obtener extractos con altos contenidos de fenoles totales fueron 49,5% (v/v) de etanol a 30 °C, lo que produjo 18,39 y 26,14 mg AGE g^{-1} materia seca, respectivamente. Los extractos optimizados se añadieron a un sistema modelo aceitoso y se evaluaron a través de una prueba de estabilidad en horno Schaal. Después de 96 horas, los extractos de frutos silvestre (150 mg·kg^{-1} aceite) y genotipo 14-4 (200 mg·kg^{-1} aceite) mostraron una capacidad antioxidante similar al TBHQ (200 mg·kg^{-1} aceite) en términos de índice de peróxido y respecto a las características organolépticas. Se concluye que los extractos de frutos de murtilla son una fuente natural de antioxidantes para la protección de los alimentos lipídicos, tales como el aceite de soja.

PALABRAS CLAVE: *Actividad antioxidante; Características organolépticas del aceite; Contenido de polifenoles totales; Metodología de Superficie de Respuesta; Murtilla; Sistema modelo aceitoso*

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1. INTRODUCTION

Lipid oxidation is the most important cause of food deterioration and leads to changes in flavor and nutritional compounds that reduce palatability. For the food industry, these changes cause enormous commercial damages, which justifies the continuous search for new technologies to help improve food product shelf life, such as changing package constituents, reducing oxygen contact with food, increasing food production care and adding antioxidant compounds (Zhang *et al.*, 2013; Alves *et al.*, 2015; Cichello, 2015). Antioxidants from natural or synthetic sources are capable of retarding or reducing the oxidation reaction in lipid compounds (Shahidi, 1997). However, the use of synthetic antioxidants in foods may be restricted or prohibited because high quantities of lipid compounds may be detrimental to human health. Therefore, many researchers have focused on obtaining antioxidants from natural sources and assessed the activity of these antioxidants against free radicals and evaluated their potential uses in foods (Karpińska *et al.*, 2001; Scheuermann *et al.*, 2002; Yanishlieva *et al.*, 2006; Brewer, 2011; Shah *et al.*, 2014; Shi *et al.*, 2014).

Among the most studied natural sources of antioxidants are fruits, herbs and spices, which possess phenolic compounds capable of acting as antimicrobials, anti-allergens, anti-teratogens, antithrombotics, cardio-protectors and vasodilators (Zhang *et al.*, 2011; Bektas *et al.*, 2012; Simin *et al.*, 2013; Del Monte *et al.*, 2015; Claro *et al.*, 2015).

Fresh and dried murtilla (*Ugni molinae* Turcz.) fruit, which is a Chilean berry, exhibit antioxidant activity via phenolic compounds (Alfaro *et al.*, 2013; Augusto *et al.*, 2014; Suwalsky and Avello, 2014; Rodríguez *et al.*, 2014; Junqueira-Gonçalves *et al.*, 2015; Jofré *et al.*, 2016). For being a seasonal fruit, the drying process is considered an adequate technique for its preservation which saves the antioxidant properties provided by its polyphenolic components (Alfaro *et al.*, 2014).

In this study, experiments using the Response Surface Methodology were performed to analyze the effects of different solvents and temperatures on the extraction of the phenolic content of dehydrated murtilla (*Ugni molinae* Turcz.) fruit. The antioxidant activity of the extracts obtained under the optimized conditions was then evaluated in refined soybean oil as a model system, and the effect of the extracts on the organoleptic characteristics of the oil were determined by a sensory evaluation.

2. MATERIALS AND METHODS

Folin–Ciocalteu phenol reagent was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents were purchased from Fisher Scientific (Nepean, Canada) and were of ACS grade or better. Refined soybean oil without synthetic antioxidants was donated by Cargill (Mairinque, São Paulo, Brazil).

2.1. Plant material and extract preparation

Murtilla fruits (*Ugni molinae* Turcz.) were obtained from wild and cultivated plants. The wild fruits were obtained from native vegetation in Puerto Saavedra (38°, 45’ S, 73° 21’ W), La Araucanía Region, Chile. The cultivated fruits (14-4 genotype) were grown in the germ plasm bank at the experimental station of Instituto de Investigaciones Agropecuarias (INIA) at Puerto Saavedra. The wild and cultivated fruits were harvested on April 18, 2011 and dried on April 19, 2011 using an industrial drying cabinet (1.8 m long, 2.2 m high, 3.5 m deep) at a constant temperature of 70 °C for 6 hours until a final moisture content of 5.4% was reached, according to Alfaro *et al.* (2014). The fruits were vacuum packed and protected from light and oxygen. The hydro-alcoholic extracts were prepared according to the method described by Ribeiro *et al.* (2008) with certain modifications. Different concentrations of ethanol:water and different temperatures were used throughout the experiments (see Table 1 for the conditions) in an attempt to improve the extraction of polyphenols. The dehydrated fruit was ground, and 1.0 g of the samples was placed in a 250 mL Erlenmeyer with 30 mL of solvent (v/v, ethanol/water) and heated to a specific temperature (see Table 1), which was maintained for 50 minutes under agitation in a water bath (Model NI 1322-220, Nova Instrumentos, Brazil). The extracts were then centrifuged at 2057 *g* for 15 minutes, filtered using Whatman N° 2 filter paper, and stored under refrigeration at 7 °C in amber flasks until analysis.

2.2. Experimental design, murtilla fruit extraction and total phenolic content

The Response Surface Methodology and a multiple regression analysis were performed to determine the effects of the solvent ratio (ethanol:water) and temperature on the total phenolic content (dependent variable) in the murtilla fruit extracts to identify the best conditions for obtaining high antioxidant activity. Adjustments were performed to generate a second order mathematical model that included linear and quadratic terms as well as their interactions. To estimate the experimental error, four assays with coded values related to zero levels, which represented the central point, were performed. To complete the design, trials referencing the axial points positioned at a distance α ($\alpha = 2^{n/4}$) from the central point were conducted. At the end of the design, twelve trials had been performed. The experimental design is shown in Table 1.

TABLE 1. Coded levels and actual values of the independent variables used to optimize the extraction conditions of wild and 14-4 genotype (14-4) murtilla fruits. Their effect on the total phenolic content (TPC) was used as a dependent variable

Assay	Coded levels		Actual values		TPC (mg GAE·g^{-1} dry matter)*	
	Ethanol	Temperature	Ethanol (%)	Temperature (°C)	Wild*	14-4*
1	-1	-1	14.4	34.4	14.2	20.4
2	1	-1	84.6	34.4	9.9	8.8
3	-1	1	14.4	55.6	14.6	22.6
4	1	1	84.6	55.6	11.6	17.6
5	-1.41	0	0	45	11.3	14.6
6	1.41	0	99	45	7.3	5.5
7	0	-1.41	49.5	30	18.3	26.1
8	0	1.41	49.5	60	18.8	27.9
9	0	0	49.5	45	17.5	25.2
10	0	0	49.5	45	17.7	23.6
11	0	0	49.5	45	18.0	27.2
12	0	0	49.5	45	19.4	27.7

*Values represent the mean of three replicates expressed as mg gallic acid equivalent (GAE) of dry matter (g).

The mathematical model was evaluated based on the coefficient of determination (R^2) and F test, which were determined by a variance analysis using Statistica 11 software (Stat Soft, 2013). The general equation is $Y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_2^2x_2^2 + b_{12}x_1x_2$, where Y is the dependent variable, x_1 and x_2 are the coded levels of the explanatory variables, b_0 is the central point and b'_s is the estimated coefficient from the minimum square method. The total phenolic content of the extracts was the dependent variable in the experimental design. The measurements were determined in triplicate according to the method described by Singleton *et al.* (1999) using the Folin-Ciocalteu solution as a reagent and gallic acid as a standard. The hydroalcoholic extracts were diluted in ethanol at concentration of 1:10 (extract: ethanol, v/v). An aliquot of 0.5 mL of the diluted sample was transferred to a test tube, and 2.5 mL of the Folin-Ciocalteu reagent:water solution (1:10, v/v) were added. The mixture was vortexed at room temperature in the dark, and after a 5 minute resting period, 2.0 mL of a sodium carbonate 4% (m/v) solution was added, and the mixture was agitated again and kept at rest for two hours at room temperature and protected from light. The absorbance was read at 740 nm using a UV-1203 spectrophotometer (Shimadzu Corporation, Japan). The results were calculated using the standard curve of gallic acid with known concentrations (2.5 to 50 µg·mL^{-1}), and the results were expressed in mg of gallic acid equivalent (GAE) of dry matter (g).

2.3. Accelerated stability assessed via the Schaal Oven Test to measure the antioxidant activity against lipid oxidation

Ten treatments were established to evaluate the antioxidant ability to prevent lipid oxidation via accelerated stability tests. These treatments included the addition of 50, 100, 150 and 200 mg GAE kg·oil^{-1} of the wild and 14-4 genotype extracts of murtilla to soybean oil and 200 mg of TBHQ kg·oil^{-1} to soybean oil. The control treatment consisted of refined soybean oil alone. The total phenolic contents (determined by the Folin-Ciocalteu method) were used to calculate the concentrations of the added extracts (µg GAE·mL^{-1}).

For each trial, the samples were prepared in triplicate, binned in 50 mL beakers and randomly distributed in the oven. The established temperature was 63 ± 2 ºC and after 36, 72 and 96 hours, the samples were removed for peroxide and ultraviolet absorption analyses.

2.4. Antioxidant activity measure by the peroxide value (PV) and ultraviolet absorption (UV)

The peroxide value was determined according to the Cd 8b-90 method (American Oil Chemists' Society, 2009), and the results are expressed in meq O_2·kg^{-1} oil. The specific absorbance was determined according the Ch 5–91 method (American Oil Chemists' Society, 2009), and the results were expressed in $E^{1\%}_{1\ cm}$. The absorbance was read at 232 nm using a UV-1203 spectrophotometer (Shimadzu Corporation, Japan).

2.5. Sensory analysis

The discriminative test was conducted using an unstructured scale with 6 trained tasters to evaluate the odor (rancidity). For this test, 20 mL from each treatment were kept in black plastic cups labeled with three-digit numbers and randomly distributed in the oven at 43 ºC (Institute of Food Technologists, 1981). The complete block design

was applied, and the results were analyzed using the statistics discussed in section 2.6.

2.6. Statistical analysis

All evaluations were conducted in triplicate, and the results were expressed as the mean ± standard deviation. The data were statistically analyzed via an analysis of variance (ANOVA) and Tukey's test (α = 95%) using SAS (Statistical Analysis System, USA) software.

3. RESULTS AND DISCUSSION

3.1. Effect of solvent and temperature on the total phenolic content

The total phenolic contents of the murtilla fruit extracts using different extraction conditions are shown in Table 1. The estimated effects on the total phenolic content for each murtilla fruit extract (wild and 14-4 genotype) are shown in Table 2.

According to Table 2, the extraction process for both samples of murtilla were affected by the solvent because the linear and quadratic effects were statistically significant. However, the effects of increases in temperature, which promoted an increase in the total phenolic content of the extract were not statistically significant ($p < 0.05$).

Despite the differences in the results for each trial, both murtilla fruit extracts (wild and 14-4 genotype) showed the same relationship between the total phenolic content and the solvent. After the effects analysis, terms that were not significant were eliminated, and second-order regression coefficients were obtained.

The second-order polynomial equation for the total phenolic content (TPC) for wild murtilla is:

$$\text{TPC (mg GAE}\cdot\text{g}^{-1}\text{ dry matter)} = 18.09542 - 1.64877 * \%\text{Ethanol} - 4.76455 * \%\text{Ethanol}^2$$

and the mathematical model for the 14-4 genotype murtilla is:

$$\text{TPC (mg GAE}\cdot\text{g}^{-1}\text{ dry matter)} = 26.165239 - 3.66933 * \%\text{Ethanol} - 8.32753 * \%\text{Ethanol}^2$$

The analysis of variance (Tables 3 and 4) demonstrated that the proposed models were valid because the regressions were statistically significant. The F test showed that the lack of fit was not statistically significant ($p < 0.05$). The coefficients of determination (R^2) were higher than 0.90. Thus, the ANOVA results indicated that the F values calculated from the regressions of both models were three times higher than the F values that were tabulated. Therefore, the mathematical models were valid, and they can be used for predictive purposes.

The adjusted response surfaces of the mathematical models for murtilla fruit extracts from wild and 14-4 genotypes are shown in Figure 1. An ethanol concentration of 49.5% (v/v, ethanol:water) under all temperatures provided a higher yield of total phenolic content (18.39 and 26.14 mg GAE·g^{-1} dry matter for the wild and 14-4 genotype murtilla). Thus, 49.5% ethanol (v/v, ethanol:water) was the optimal solvent concentration for the extraction of this substrate. Values over 50% ethanol (v/v, ethanol:water) showed a reduced extraction efficiency.

Our results show that under the same solvent concentration, the total phenolic content of the 14-4 genotype murtilla fruit extract was significantly

TABLE 2. Estimated effects of the explanatory variables on the total phenolic content of the wild and 14-4 genotype murtilla fruit extracts

	Effect	Standard deviation	*p*-value
Wild			
Mean	**18.1932**	**0.4326**	**0.0000**
Ethanol (L)	**−3.2975**	**0.6127**	**0.0126**
Ethanol (Q)	**−9.5775**	**0.6868**	**0.0008**
Temperature (L)	0.6942	0.6127	0.3396
Temperature (Q)	−0.2458	0.6868	0.7441
Ethanol (L) x Temperature (L)	0.6862	0.8652	0.4856
14-4 genotype			
Mean	**25.9713**	**0.9553**	**0.0001**
Ethanol (L)	**−7.3387**	**1.3530**	**0.0123**
Ethanol (Q)	**−16.5590**	**1.5167**	**0.0016**
Temperature (L)	3.3676	1.3530	0.0886
Temperature (Q)	0.4874	1.5167	0.7690
Ethanol (L) x Temperature (L)	3.2805	1.9105	0.1845

Bold terms: statistically significant at 95% confidence ($p < 0.05$).
L = linear; Q = quadratic.

TABLE 3. Analysis of variance (ANOVA) for the total phenolic content of the wild murtilla fruit extracts

Source	Sum of squares	df	Mean square	*F* value
Model	171.5866	2	85.79331	96.96129
Residual	7.9634	9	0.88482	
Lack of fit	5.7179	6	0.952977	1.273172
Pure error	2.2455	3	0.748506	
Total	179.5500	11		

$F_{0.95;2;9} = 4.26$; $F_{0.95;6;3} = 8.94$; $R^2 = 0.95565$.

TABLE 4. Analysis of variance (ANOVA) of the total phenolic content of the 14-4 genotype murtilla fruit extracts

Source	Sum of squares	df	Mean square	*F* value
Model	565.3251	2	282.6625	43.98831
Residual	57.8327	9	6.425855	
Lack of fit	46.8821	6	7.813691	2.14063
Pure error	10.9505	3	3.650183	
Total	623.1578	11		

$F_{0.95;2;9} = 4.26$; $F_{0.95;6;3} = 8.94$; $R^2 = 0.90719$.

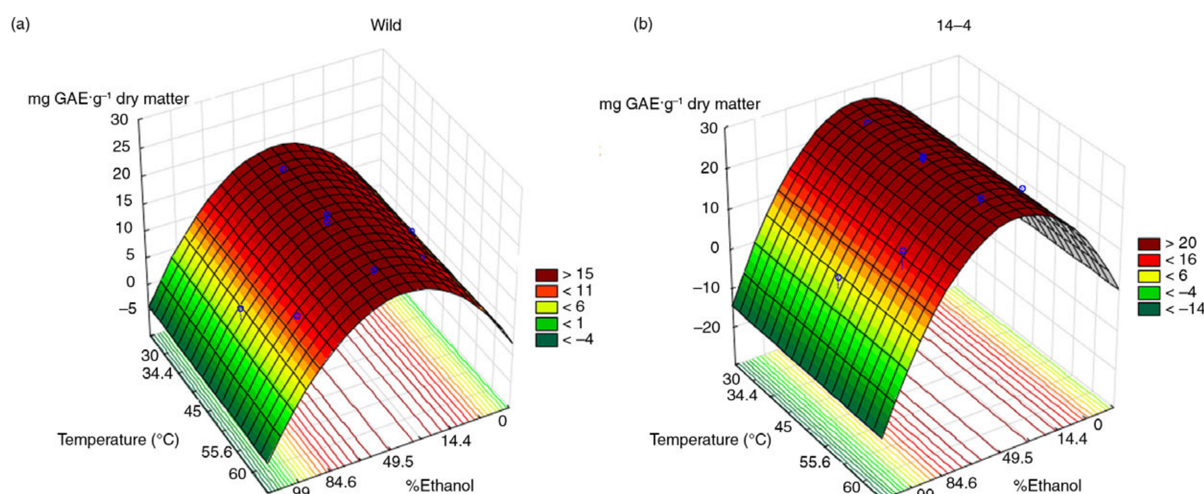

FIGURE 1. Effect of temperature and solvent concentration on the total phenolic content of the wild and 14-4 genotype murtilla fruit extracts.

higher than that of the wild murtilla fruit extract. This outcome is consistent with the concentration reported by Tsao *et al.* (2003), who found differences in the phenolic content of a cultivated variety of Canadian strawberries and the wild fresh fruit using the same extraction solvent.

Boeing *et al.* (2014) evaluated the effect of different solvents and their combinations on the extraction of antioxidant compounds from black mulberries (*Morus nigra*), blackberries (*Rubus ulmifolius*) and strawberries (*Fragaria* x *ananassa*), and they determined the total phenolic content, anthocyanin content and antioxidant capacity. For all of the berries, the acetone/water solvent mixture (70–30%, v/v) presented the optimal phenolic compound extraction capacity, whereas for black mulberries, the ethanol/water solvent mixture (50/50, v/v) presented the highest antioxidant capacity.

Radojković *et al.* (2013) evaluated the influence of solvent composition (ethanol/water, 40–80%, v/v), temperature (40–80 °C) and time (20–60 min) on the extraction yield of phenolic compounds, flavonoids, and saccharides and the antioxidant activity of black mulberries (*Morus nigra* L.). These authors observed that the optimal conditions within the experimental range of the studied variables were as follows: solvent composition of 58.7%, temperature of 58.1 °C and extraction time of 46.9 min.

According to the results of this study, the optimal conditions for obtaining extracts with a higher total phenolic content was the same for the wild and 14-4 genotype fruits under 49.5% ethanol

at 30 ºC. Because temperature did not have a significant influence on the amount of total phenolic compounds, a lower temperature was selected to save energy. Therefore, all of the subsequent analyses were performed using extracts produced under optimized conditions (49.5% and 30 ºC) for both samples. Certain components, such as quercetin, epicatechin, and gallic, benzoic and hydrocaffeic acids, were identified by a CG/MS analysis in a previous study (Augusto *et al.*, 2014).

3.2. Accelerated stability of soybean oil in the Schaal Oven Test

3.2.1. Peroxide value and ultraviolet absorption

The total phenol content determined by the Folin-Ciocalteu method was used to calculate the concentrations of the extracts, which were then added to soybean oil. The total phenolic content was 14.30 and 26.39 mg GAE·g^{-1} dry matter for the wild and 14-4 genotype murtilla fruit, respectively.

In Table 5 presents the results of the peroxide analysis, which showed that in all treatments, a gradual increase in peroxide value occurred over time. The TBHQ treatment showed the lowest peroxide values, which was expected because of the stability of TBHQ at higher temperatures.

For the wild and 14-4 genotype murtilla fruit extracts, all of the treatments initially showed the same value (0.20 ± 0.00 meq O_2·kg^{-1} oil). When the wild murtilla fruit extract was applied to soybean oil, the 150 mg·kg^{-1} treatment showed the lowest peroxide values (2.01 ± 0.00 meq O_2·kg^{-1} oil) after 96 hours, and the value was statistically similar to the TBHQ treatment (2.01 ± 0.00 meq O_2·kg^{-1} oil). For the 200 mg·kg^{-1} treatment at 96 hours and the 150 mg·kg^{-1} treatment at 72 hours, the peroxide values (13.09 ± 1.01 meq O_2·kg^{-1} oil and 9.07 ± 1.01 meq O_2·kg^{-1} oil, respectively) were higher compared with the other treatments, which was likely caused by a pro-oxidant effect of the antioxidant compounds on the extract. According to Fukumoto and Mazza (2000), Carocho and Ferrera (2013) and Lee *et al.* (2016) the pro-oxidant effect can occurs when some antioxidant compounds are added in lipid systems. The protective effects of the wild murtilla fruit extracts were TBHQ = 150 mg·kg^{-1} > 100 mg·kg^{-1} > control > 50 mg·kg^{-1} > 200 mg·kg^{-1}.

For the 14-4 genotype murtilla fruit extract (Table 5), the 200 mg·kg^{-1} treatment after 96 hours and TBHQ (2.01 ± 0.00 meq O_2·kg^{-1} oil) showed the lowest peroxide values. The protective effects of the 14-4 genotype murtilla fruit were TBHQ = 200 mg·kg^{-1} > 100 mg·kg^{-1} = 150 mg·kg^{-1} > control > 50 mg·kg^{-1}.

Luzia and Jorge (2009) analyzed the antioxidant activity of lemon (*Citrus limon*) seed extract added to soybean oil, which was then subjected to thermal oxidation. The treatments were incubated at 80 ºC for 20 hours, and the samples were collected at various time intervals. After 5 hours, the authors observed peroxide values of approximately 2.75 meq O_2·kg^{-1} oil for the lemon extract and 3.92 meq O_2·kg^{-1} oil for TBHQ. The effect of the murtilla fruit extracts cannot be directly compared with that of the lemon extract because of differences in the temperature and time intervals applied for the Schaal Oven Test. However, the lemon seed extract peroxide value was similar to the value obtained for the murtilla extracts at 36 hours (Table 5), which shows the protective effect of the murtilla natural antioxidants. Asha *et al.* (2015) evaluated the antioxidant activities of butylated hydroxyanisole (BHA) and orange peel powder extract in ghee that was stored at three different temperatures for 21 days, and they observed that the ghee incorporated with orange peel extract showed reduced peroxide values compared with the ghee incorporated with BHA and the control ghee.

Table 6 shows that the control and 50 mg·kg^{-1} treatments had the highest values after 96 hours (13.19 ± 0.17 and 9.26 ± 0.73 $E^{1\%}_{1\,cm}$, respectively) for both murtilla extracts. For the wild murtilla fruit, the absorption values in all treatments increased,

TABLE 5. Peroxide values (meq O_2·kg^{-1} oil) of soybean oil added to the wild and 14-4 genotype murtilla fruit extracts obtained via the Schaal Oven Test

	Time (h)	Control	50 mg·kg^{-1}	100 mg·kg^{-1}	150 mg·kg^{-1}	200 mg·kg^{-1}	TBHQ
Wild murtilla extracts*	**0**	0.20 ± 0.00B	0.20 ± 0.00D	0.20 ± 0.00C	0.20 ± 0.00C	0.20 ± 0.00C	0.20 ± 0.00B
	36	0.60 ± 0.00Bc	6.03 ± 0.00Ba	2.01 ± 0.00Bb	2.01 ± 0.00Bb	1.51 ± 0.50Cb	0.20 ± 0.00Bc
	72	6.09 ± 1.05Ab	4.02 ± 0.00Cc	4.02 ± 0.00Ac	9.07 ± 1.01Aa	4.03 ± 0.00Bc	0.20 ± 0.00Bd
	96	6.21 ± 0.05Ac	9.32 ± 0.25Ab	5.04 ± 1.01Ac	2.01 ± 0.00Bd	13.09 ± 1.01Aa	2.01 ± 0.00Ad
14-4 Genotype murtilla extracts*	**0**	0.20 ± 0.00B	0.20 ± 0.00C	0.20 ± 0.00B	0.20 ± 0.00C	0.20 ± 0.00C	0.20 ± 0.00B
	36	0.60 ± 0.00Bcd	1.51 ± 0.50BCbc	3.02 ± 1.01Aa	2.01 ± 0.00BCab	2.01 ± 1.16Bab	0.20 ± 0.00Bd
	72	6.09 ± 1.05Aa	2.01 ± 0.00Bcd	1.34 ± 0.58Bcd	3.05 ± 0.99Bcb	5.03 ± 1.01Aab	0.20 ± 0.00Bd
	96	6.21 ± 0.05Ab	9.07 ± 1.01Aa	4.28 ± 0.25Ac	5.03 ± 1.01Abc	2.01 ± 0.00Bd	2.01 ± 0.00Ad

*Results expressed as the mean ± standard deviation. Capital letters in the columns or lower case letters in the rows indicate that the values were not significantly different according to Tukey's test at 5% probability.

although the 150 and 200 mg·kg^{-1} treatments and the TBHQ treatment showed similar values after 96 hours (5.60 ± 0.37 $E^{1\%}_{1\ cm}$, 5.61 ± 0.28 $E^{1\%}_{1\ cm}$ and 4.32 ± 0.2 $E^{1\%}_{1\ cm}$, respectively). The protective effects of the wild murtilla fruit extracts were TBHQ > 150 mg·kg^{-1} = 200 mg·kg^{-1} > 100 mg·kg^{-1} > 50 mg·kg^{-1} > control. For the 14-4 genotype murtilla fruit, three treatments showed an increase in the absorption values after 96 hours. However, the 150 mg·kg^{-1} (5.18 ± 0.01 $E^{1\%}_{1\ cm}$) treatment did not exhibit this behavior, and its absorption value was not significantly different from the initial value (4.63 ± 0.13 $E^{1\%}_{1\ cm}$ at 0 hour) and the TBHQ value at 96 hours (4.32 ± 0.20 $E^{1\%}_{1\ cm}$). Therefore, the protective effects of the 14-4 genotype extracts were TBHQ = 150 mg·kg^{-1} > 100 mg·kg^{-1} > 50 mg·kg^{-1} > 200 mg·kg^{-1} = control.

Anwar *et al.* (2010) estimated the antioxidant activity of methanolic extracts from three varieties of barley in sunflower oil at 60 ºC, and after 10 days, the results varied from 8.38 ± 0.37 to 9.97 ± 0.83 $E^{1\%}_{1\ cm}$. These values were higher values than the values found in this study.

3.2.2. Sensory analysis

To complete the analysis, the total phenolic content determined via the Folin-Ciocalteu method was used to calculate the concentrations of extracts added to soybean oil. The total phenolic contents were 11.61 and 23.96 mg GAE·g^{-1} dry fruit for the wild and 14-4 genotype murtilla fruit extracts, respectively.

Table 7 shows that only the oxidized control treatment had a higher score for rancid odor at baseline, whereas significant differences were not observed for the other treatments. Therefore, the addition of murtilla fruit extracts (wild and 14-4 genotype at 150 or 200 mg·kg^{-1}) did not cause noticeable changes in the soybean oil odor compared with the pure condition (unoxidized control) or the addition of TBHQ, which is a common synthetic antioxidant used by the food industry. After 96 hours, all of treatments showed a higher rancid odor and all of the scores increased except for that of the unoxidized control. At 72 and 96 hours, the TBHQ treatment presented rancid odor scores that were lower than the samples treated with the murtilla fruit extracts. This outcome was expected because this synthetic antioxidant provides good protection at higher temperatures.

Ramsaha *et al.* (2015) analyzed the antioxidant potential of traditional plants of Mauritius, such as *P. betle* L. (Piperaceae), *M. koenigii* L. Sprengel (Rutaceae), *O. gratissimum* L. (Lamiaceae), *O. tenuiflorum* L. (Lamiaceae), and commercially available Mauritian green and black teas. The extracts of these plants were added to sunflower oil, and an odor evaluation was performed using a sensory panel. The results showed that the plant extracts and green tea effectively delayed the development of rancid odors in sunflower oil ($p < 0.05$).

TABLE 6. Absorption values from the UV absorption analysis at 232 nm for the wild (a) and 14-4 genotype (b) murtilla fruit extracts added to soybean oil and then subjected to the Schaal Oven Test

	Time (h)	Control	50 mg·kg^{-1}	100 mg·kg^{-1}	150 mg·kg^{-1}	200 mg·kg^{-1}	TBHQ
Wild murtilla extracts*	**0**	4.63 ± 0.13C	4.63 ± 0.13C	4.63 ± 0.13C	4.63 ± 0.13B	4.63 ± 0.13B	4.63 ± 0.13A
	36	3.60 ± 0.25Dc	4.84 ± 0.13BCabc	5.90 ± 0.73Ba	5.09 ± 0.10ABab	5.25 ± 0.48ABab	4.29 ± 0.09Bbc
	72	10.70 ± 0.11Ba	6.18 ± 0.37Bb	5.61 ± 0.24BCb	4.83 ± 0.12Bc	4.82 ± 0.42ABc	4.31 ± 0.05AB
	96	13.19 ± 0.17Aa	9.21 ± 0.66Ab	7.86 ± 0.52Ac	5.60 ± 0.37Ad	5.61 ± 0.28Ad	4.32 ± 0.20ABe
14-4 Genotype murtilla extracts*	**0**	4.63 ± 0.13D	4.63 ± 0.13B	4.63 ± 0.13C	4.63 ± 0.13BC	4.63 ± 0.13C	4.63 ± 0.13A
	36	3.60 ± 0.25Cd	4.84 ± 0.26Bbc	5.68 ± 0.07Ba	4.49 ± 0.43Cc	5.18 ± 0.10Bab	4.29 ± 0.09Bc
	72	10.70 ± 0.11Ba	5.27 ± 0.36Bc	5.27 ± 0.05Bc	7.26 ± 0.23Ab	5.00 ± 0.15BCc	4.31 ± 0.05ABd
	96	13.19 ± 0.17Aa	9.26 ± 0.73Ab	6.81 ± 0.38Ac	5.18 ± 0.01Bd	12.72 ± 0.26Aa	4.32 ± 0.20ABd

*Results expressed as the mean ± standard deviation. Capital letters in the columns or lower case letters in the rows indicate that the values were not significantly different according to Tukey's test at 5% probability.

TABLE 7. Rancid odor score from the sensory analysis of the wild and 14-4 genotype murtilla fruit extracts added to soybean oil

	Treatments*						
Time (h)	**Unoxidized control**	**Oxidized control**	**Wild 150 mg·kg^{-1}**	**14-4 150 mg·kg^{-1}**	**Wild 200 mg·kg^{-1}**	**14-4 200 mg·kg^{-1}**	**TBHQ**
0	1.89 ± 0.29Ab	2.97 ± 0.35Ba	1.83 ± 0.53Bb	1.84 ± 0.49Cb	1.89 ± 0.64Cb	2.10 ± 0.37Cb	1.88 ± 0.21Bb
72	1.70 ± 0.28Ad	2.68 ± 0.07Ca	2.19 ± 0.29Bb	2.63 ± 0.29Ba	2.46 ± 0.16Bab	2.71 ± 0.34Ba	1.77 ± 0.30Bcd
96	1.67 ± 0.22Ac	3.80 ± 0.34Aa	3.60 ± 0.39Aa	3.67 ± 0.35Aa	3.61 ± 0.40Aa	3.81 ± 0.25Aa	2.53 ± 0.15Ab

*Results expressed as the mean ± standard deviation. Capital letters in the columns or lower case letters in the rows indicate that the values were not significantly different according to Tukey's test at 5% probability.

In our study, the TBHQ antioxidant had a better overall effect with respect to the release of odors from soybean oil compared with the murtilla fruit extract treatments.

4. CONCLUSIONS

The Response Surface Methodology was able to define the best extraction conditions for obtaining murtilla fruit extracts with a higher total phenolic content in an aqueous alcoholic solution with similar proportions (49.5% ethanol) and at a lower temperature (30 °C).

When added to soybean oil, the murtilla fruit extracts demonstrated good protective activity compared with other natural antioxidants reported in the literature. Although the protective level of the synthetic antioxidant TBHQ was not achieved, the hydro-alcoholic extracts of wild and 14-4 genotype murtilla fruit may be considered a natural source of antioxidants for addition to lipid foods. Additional studies are required to assess the most effective concentrations of these extracts against oxidation reactions.

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