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Effect of Solvents and Extraction Methods on Total Anthocyanins, Phenolic Compounds and Antioxidant Capacity of *Renealmia alpinia* (Rottb.) Maas Peel

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Abstract

Vega Arroy J.D., Ruiz-Espinosa H., Luna-Guevara J.J., Luna-Guevara M.L., Hernández-Carranza P., Ávila-Sosa R., Ochoa-Velasco C.E. (2017): Effect of solvents and extraction methods on total anthocyanins, phenolic compounds and antioxidant capacity of *Renealmia alpinia* (Rottb.) Maas peel. Czech J. Food Sci., 35: 456–465.

The effect of different solvents and extraction methods on total anthocyanins, phenolic compounds, and antioxidant capacity from x´kijit (*Renealmia alpinia* Rottb. Maas) peels was evaluated. In order to evaluate the effect of solvents on the bioactive compounds extraction efficiency and antioxidant capacity, a special cubic mixture design model was implemented with ethanol, methanol, and acetone as solvents and conventional (agitation – 1 and 6 h; Soxhlet – 2 and 4 h), novel (power ultrasound – 2.5 and 5 min) methods, and combination of extraction methods. Acceptable correlations between predicted and experimental data were obtained for total anthocyanins (R^2 = 0.95), phenolic compounds (R^2 = 0.78), and antioxidant capacity (R^2 = 0.97), with methanol exhibiting the highest extraction yield of bioactive compounds and resultant antioxidant capacity. Although the extraction of total anthocyanins (82.2–85.8 mg cyanidine/kg) and phenolic compounds (183.6–207.0 mg GAE/kg) was best carried out through Soxhlet, the ultrasonic treatment showed similar antioxidant capacity values (27.4–34.3 mg Trolox/kg) to those of 2-h Soxhlet. Moreover, a 5-min ultrasound pretreatment significantly increased (P < 0.05) phenolic compounds by 11, 21, and 12% when combined with agitation 1, 6, and 2-h Soxhlet treatments, respectively; while the antioxidant capacity increased by 26, 48, and 22% for the same treatments. Ultrasound might be used as a valuable, green alternative procedure for improving the solvent extraction of bioactive compounds.

Keywords: ultrasound; extraction process; x´kijit peel; bioactive compounds; antioxidants

The human organism naturally produces reactive oxygen species (ROS) which have long been linked to cancer, diabetes, cardiovascular and other chronic diseases (Ma *et al.* 2009). The World Health Organization (WHO 2003) indicates that prevention, rather than treatment, is the most effective strategy against illnesses. In this regard, adequate dietary habits can play an important preventive role. According to recent studies, regular consumption of phytonutrient-rich fruits and vegetables is associated with a reduced risk of those diseases (Erkan *et al.* 2008). Thus, studies on the characterisation of new sources of dietary antioxidants are of the utmost importance.

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X´kijit (*Renealmia alpinia* Rottb. Maas) is an aromatic, rhizomatous plant cultivated in some regions of Mexico (Macía 2003). This plant produces an ovoid, seeded, yellow-pulp fruit of about 1.5 cm long and 3.5 cm in diameter. Pulp roughly represents 20% of its total weight, with the rest being peel (80%) (Miguel-Sánchez *et al.* 2015). As with other fruits, colour serves as a maturity index; x´kijit fruit shifts from brown to red to blue to purple and finally to black. However, when the fruit turns purple, it is considered to be edible (Macía 2003). Some ethnomedicinal properties have been attributed to pulp extract, peel and leaves, including antiemetic, antinausea, and febrifuge effects and as snake venom neutralizer (Gómez-Betancour & Benjumea 2014). Moreover, recent studies indicate that x´kijit fruit contains different bioactive compounds such as vitamin C, phenolics, carotenoids and anthocyanins (peel), which may exhibit high antioxidant capacity (Miguel-Sánchez *et al.* 2015). Anthocyanins are especially found in peel (Luna-Guevara *et al.* 2015), where their concentration (6.7 mg cyanidine/kg of fresh weight) resembles that of other blue-purple fruits such as certain berries (Koca *et al.* 2008). Anthocyanins are a group of phenolic compounds that not only provide colour to many fruits and vegetables, but also have been proven to possess important biological activities as antioxidant, antimutagenic, anticancer and antiobesity agents (Moldovan *et al.* 2012).

Extraction of bioactive compounds from fruits, vegetables, and by-products is one of the most current areas of research (Radojković *et al.* 2012). Either traditional (maceration, agitation, Soxhlet, hydrodistillation) or novel (enzyme, microwave, ultrasound-assisted, using supercritical fluids) extraction processes have been reported (Corrales *et al.* 2008; Ghafoor *et al.* 2011). A number of factors including extraction time, temperature, pressure, type of solvent, solid-to-solvent ratio, among others might affect the process efficiency (Hernández-Carranza *et al.* 2016). Under selected conditions, some novel processes could allow reducing extraction time and temperature and/or increasing extract yield and quality, while decreasing solvent usage when compared to other conventional extraction methods such as Soxhlet (Wang & Weller 2006; Azmir *et al.* 2013). In this regard, green extraction alternatives, based on renewable plant resources, alternative solvent usage (different from petroleum) and emergent technologies that may allow reducing energy, cost and time investments have been vastly explored in recent times (Li *et al.* 2013). Ultrasound-assisted extraction (UAE) has been widely used for recovering bioactive compounds from several food materials including herbs, leaves, pulp, seeds, and peels, among others (Yang *et al.* 2011). Higher extraction yields of UAE processes have been linked to cavitation, the rapid formation and collapse of air bubbles in ultrasound-treated fluids that produces local rises in pressure and temperature and large amounts of energy that ultimately could increase the diffusion rates across the cell wall or its breakdown (Chandrapala *et al.* 2012), thus enhancing the liberation of cell contents (Khan *et al.* 2010). Therefore, the aim of this research was to evaluate the effect of different solvents and both conventional (agitation or Soxhlet) and potentially green (ultrasound-assisted) extraction methods on total anthocyanins, phenolic compounds and antioxidant capacity of x´kijit (*Renealmia alpinia* Rottb. Maas) peel.

MATERIAL AND METHODS

Raw material. X´kijit was acquired in edible stage from Cuetzalan del Progreso, Puebla, Mexico. X´kijit peel was manually separated from pulp and dried in an electric oven (model RE53; Redline, USA) at 60°C until constant weight (6–8 h). Dried peel was ground using a conventional grinder (model 80374; Hamilton Beach, USA). Powder was sieved (420 µm) and stored under dark conditions until used. The study was carried out in two stages; the first stage was the solvent selection (2014) while the second was the evaluation of extraction methods (2015).

Chemical reagent and solvent. Folin-Ciocalteu reagent, gallic acid, 6-hydroxy-2,5,7,8 tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were purchased from Sigma-Aldrich (USA). Solvents (ethanol, methanol, hydrochloric acid, and acetone) with ≥ 99.5% of purity were obtained from J.T. Baker (USA).

Moisture analysis. Moisture content of dried x´kijit peel was determined according to the oven-dry method (AOAC 18th edition, 2007). One g of x´kijit peel was placed in an oven (Digiheat 150L; JP Selecta S.A., Spain) at 110°C for 5 h; the weight loss was used to calculate the moisture content of the x´kijit peel.

Total anthocyanins. Total anthocyanins were determined following the pH differential method

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(Miguel-Sánchez *et al.* 2015) with some modifications. Ten ml of extract was mixed with hydrochloric acid (1 M) or sodium hydroxide (1 M) to reach pH of 1 or 4.5. Absorbance was evaluated at 520 and 700 nm using a UV-Vis spectrophotometer (model 6405; Jenway, UK). The concentration of total anthocyanins was calculated as cyanidin-3-glucoside equivalents (mg/kg) using Equations 1 and 2:

$$\text{Total anthocyanins (mg/kg)} = (A \times \text{MW} \times \text{DF} \times 1000)/\varepsilon \times 1 \quad (1)$$

$$A = (\text{Abs}_{520} - \text{Abs}_{700})_{\text{pH}=1} - (\text{Abs}_{520} - \text{Abs}_{700})_{\text{pH}=4.5} \quad (2)$$

where: A – difference in absorbance; MW – molecular weight of cyanidin-3-glucoside; DF – dilution factor; 1 – quartz cell pathway (1 cm), ε – molar extinction coefficient (26, 900 $M^{-1}cm^{-1}$)

Phenolic compounds. One ml of x´kijit peel extract was mixed with 1 ml of Folin-Ciocalteu reagent (0.1 N), after 3 min 1 ml of Na_2CO_3 (0.05%) was added. The mix was kept in the dark at room temperature for 30 min. Phenolic compounds were determined using a UV-Vis spectrophotometer at 765 nm. Total phenolic compounds were calculated as mg of gallic acid equivalents (GAE)/kg of dry weight using Equation 3:

$$\text{Phenolic compounds (mg GAE/kg)} = [(\text{Abs} - b)/m] \times 100 \quad (3)$$

where: Abs – absorbance; *b* – intercept; *m* – slope (l/kg GAE) of the linear regression; gallic acid standard curve was Abs = 0.0229 (mg GAE/kg) – 0.0234 (R^2 = 0.997)

Antioxidant capacity. One ml of x´kijit peel extract was mixed with 1 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (0.004%). The mixture was kept in the dark at room temperature for 30 minutes. The antioxidant capacity was determined at 517 nm using a UV-Vis spectrophotometer and reported as mg of Trolox per kg DW as expressed in Equation 4:

$$\text{Antioxidant capacity (mg Trolox/kg)} = [(\text{Abs} - b)/m] \times 100 \quad (4)$$

where: Abs – absorbance; *b* – intercept; *m* – slope (l/kg Trolox) of the linear regression; Trolox standard curve was Abs = 4.809 (mg Trolox/kg) – 4.5113 (R^2= 0.997) (Luna-Guevara *et al.* 2015)

Solvent selection. In order to evaluate the solvent efficiency, ethanol, methanol and acetone were employed as extraction solvents. An agitation method (220–230 rpm, 1 h in dark and room temperature) was carried out for selecting the best solvent in terms of extracting capability of bioactive compounds (total anthocyanins and phenolic compounds) and the resulting antioxidant capacity of peel extracts. A special cubic mixture design model was developed using the Design Expert program 6.0.6 (Stat Ease Inc., USA), with ten different experimental mixing points and three replicates for the individual solvent. A mathematical model was obtained (Equation 5) and fitted to the experimental data using a linear regression to estimate model parameters for each response

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_1X_2 + \beta_5X_1X_3 + \beta_6X_2X_3 + \beta_7X_1X_2X_3 \quad (5)$$

where: Y – response; β – regression coefficients; X_1 – ethanol; X_2 – methanol; X_3 – acetone

In order to evaluate the factors that significantly affected the responses (Y), an analysis of variance was performed at a significance level of 0.05. Excel® Solver Add-in was used as optimisation tool for selecting the solvent or combination of solvents that maximise the extraction of bioactive compounds while exhibiting the greatest antioxidant capability. All extractions were done in triplicate.

Extraction methods

Magnetic stirring extraction. One g of peel powder was placed in a dark flask, adding 200 ml of extraction solvent. The mix was later stirred at 220–230 rpm for 1 or 6 h using a magnetic stirred hot plate (model SP131015Q; Thermo Scientific Cimarec, USA).

Soxhlet extraction. One g of peel powder was placed in a cellulose cartridge and 200 ml of extraction solvent was poured in a flask covered with aluminium foil to delay light degradation. The system was put in refluxing for 2 h (4 refluxing) or 4 h (8 refluxing) at 65°C. The resulting extract was transferred to a 200 ml volumetric flask and diluted to the mark with the extracting solvent. The extracts were processed as described above.

Ultrasound-assisted extraction. Ultrasound-assisted extraction (UAE) was performed using an ultrasonic device with a 22-mm sonotrode (UP 400 S; Hielscher Ultrasound Technology, Germany). This processor works at 24-KHz with an output power of 400 W, although the actual power delivered to the solvent was 30 W, determined calorimetrically

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as described by Chow *et al.* (2003). One g of peel powder and 200 ml of extraction solvent were placed in a 500-ml jacketed glass reactor covered with aluminium foil to delay light degradation. The sample was sonicated for 2.5 or 5 min, keeping the temperature below 25°C by constantly circulating cool water using a recirculated water bath (AD07R-20-AA1B; Polyscience, USA).

Combined methods. In order to evaluate the effect of combined methods on the efficiency of the extraction process, several combinations were implemented, all using a 5-min ultrasound pretreatment coupled with: (a) 1-h stirring; (b) 6-h stirring; (c) 2-h Soxhlet, and (d) 4-h Soxhlet. All extraction processes were done in triplicate and independently of the method used, all the extracts obtained were cotton-filtered and immediately used for evaluating total anthocyanins, phenolic compounds, and antioxidant capacity (Hernández-Carranza *et al.* 2016).

Statistical analysis. Statistical differences (α = 0.05) were assessed by one-way analysis of variance (ANOVA) using Tukey's test for pairwise comparisons in Minitab 15 software (Minitab Inc. State College, USA).

RESULTS AND DISCUSSION

Solvent selection. Solvent selection is one of the most important steps in bioactive compounds extraction from fruits, vegetables, and by-products (Gan & Latiff 2011). Contrary to fresh vegetal tissues, dry cells have lost their capacity to undergo diffusion and osmosis because of the cell wall and middle lamella desiccation (Toma *et al.* 2001). Therefore, a correct rehydration process is paramount to allow cell tissues to take hydroxyl groups. Table 1 shows the capability of pure and combined solvents (according to the mixture design) for the extraction of total anthocyanins and phenolic compounds from x´kijit peel, as well as the resulting antioxidant capacity. Bioactive compounds and antioxidant capacity of x´kijit peel were significantly affected (P < 0.05) by the type of solvent. This may be due to the effect of each solvent on solubility, diffusion kinetics and mass transfer of bioactive compounds (Cacace & Mazza 2003). Moreover, it is well known that the affinity between solvent polarity and the compound of interest plays an important role in extraction processes (Złotek *et al.* 2016). It is important to point out that although the methodologies used in this study are nonspecific, these are generally used as a first-step approach to study the bioactive compounds concentration of unexplored fruits. Figure 1 displays the response surface plot from the mixture design showing the effects of the three solvents used and their binary and ternary combinations on the total anthocyanins (A), phenolic compounds (B), and antioxidant capacity (C) from extracts of x´kijit peels. Pure methanol exhibited the greatest capability for extracting both total anthocyanins and phenolic compounds, although the antioxidant capacity was

Table 1. Mixture design of solvent selection for the extraction of phenolic compounds, total anthocyanins, and antioxidant capacity from x´kijit peel

Experiment	Decoded variables (%, v/v)			Responses[§]		
	X_1 ethanol	X_2 methanol	X_3 acetone	TA	PC	AC
1	1.00	0.00	0.00	3.3 ± 0.0^{fg}	40.6 ± 0.2^{g}	18.3 ± 0.5^{c}
2	0.50	0.50	0.00	3.1 ± 0.1^{fg}	39.0 ± 0.5^{g}	15.1 ± 0.2^{d}
3	0.50	0.00	0.50	14.6 ± 0.4^{b}	47.8 ± 0.3^{ef}	20.9 ± 0.1^{b}
4	0.00	0.50	0.50	7.8 ± 0.2^{d}	56.5 ± 0.9^{c}	7.3 ± 1.0^{e}
5	0.00	0.00	1.00	2.2 ± 0.0^{g}	51.4 ± 0.3^{de}	18.6 ± 0.7^{c}
6	0.67	0.17	0.17	5.0 ± 0.2^{e}	47.5 ± 0.4^{f}	21.4 ± 0.1^{b}
7	0.17	0.67	0.17	10.7 ± 0.1^{c}	70.3 ± 1.4^{a}	20.1 ± 0.4^{bc}
8	0.17	0.17	0.67	3.6 ± 0.1^{f}	61.3 ± 1.9^{b}	19.2 ± 0.5^{c}
9	0.33	0.33	0.33	5.4 ± 0.5^{e}	53.6 ± 0.7^{cd}	21.3 ± 0.4^{b}
10	0.00	1.00	0.00	15.7 ± 0.0^{a}	66.0 ± 2.3^{a}	23.5 ± 0.1^{a}

[§]mean ± standard deviation; different letters within the same column are statistically different (P > 0.05); TA – total anthocyanins (mg cyanidine/kg); PC – phenolic compounds (mg GAE/kg); AC – antioxidant capacity (mg Trolox/kg)

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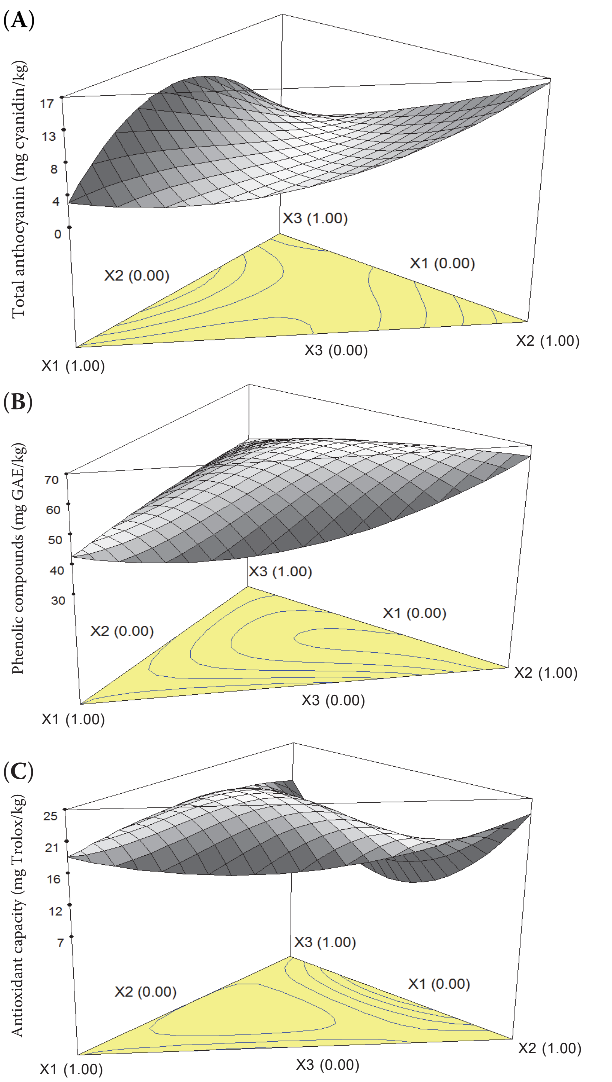

Figure 1. Mixture design plot of total anthocyanins (**A**), phenolic compounds (**B**), and antioxidant capacity (**C**) extraction with ethanol (X_1), methanol (X_2), and acetone (X_3) from x´kijit peel

slightly higher with a 45 : 20 : 35 mixture of ethanol, methanol and acetone, respectively. Therefore, it can be assumed that most of the bioactive compounds present in x´kijit peel are highly polar molecules.

Similar results were reported by Ju & Howard (2003), where aqueous methanol (60%) showed a greater ability than aqueous ethanol (60%) and water for extraction of total anthocyanins and phenolic compounds from grape peel. Moreover, Khonkarn *et al.* (2010) pointed out that methanol was the solvent with the higher extraction yield and phenolic compound content from coconut, rambutan and mangosteen peels.

Table 2 presents the regression coefficients of the mixture design model used to evaluate the effect of pure or combined solvents in the extraction of bioactive compounds from x´kijit peel and the resulting antioxidant capacity of the extracts. All models were significant on bioactive compounds extraction and antioxidant capacity from x´kijit peel. Moreover, some mixtures significantly ($P < 0.05$) affected the extraction of phenolic compounds (ethanol-methanol) and total anthocyanins (ethanol-methanol and ethanol-acetone) from x´kijit peel, as well as its antioxidant capacity (ethanol-methanol, methanol-acetone and ternary mixture). Figure 2 shows the correlation between experimental and predicted data of x´kijit peel extracts, when a high correlation was observed

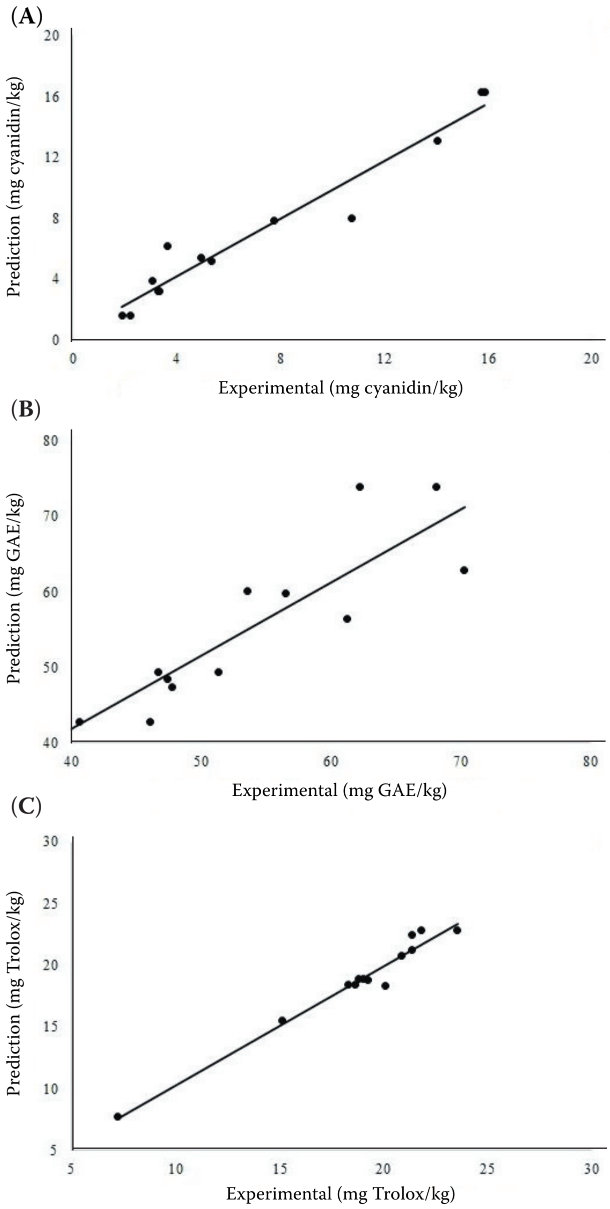

Figure 2. Experimental vs predicted data of the extraction of total anthocyanins (**A**), phenolic compounds (**B**), and antioxidant capacity (**C**) from x´kijit peel

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Table 2. Regression coefficients of special cubic models

Response	β_1	β_2	β_3	β_4	β_5	β_6	β_7	R^2
Total anthocyanins	3.239	16.267	1.637	–23.564*	42.573*	–4.420	–93.147	0.95
Phenolic compounds	42.834	66.443	49.400	–56.358*	5.150	7.415	327.692	0.78
Antioxidant capacity	18.412	22.873	18.929	–20.631*	8.521	–52.768*	259.582*	0.97

*significant (P < 0.05) coefficient in the mixture model

in total anthocyanins, phenolic compounds and antioxidant capacity; correlation values between data is necessary to apply models and evaluate the effect of different factors in bioactive compounds extraction and antioxidant capacity from any sample (Hernández-Carranza *et al.* 2016). According to the models, absolute methanol was the solvent used for the evaluation of traditional and novel extraction methods.

Extraction methods. Figure 3 shows the content of total anthocyanins (A), phenolic compounds (B) as well as the antioxidant capacity (C) of x`kijit peel extracted using different methods (agitation, Soxhlet and ultrasound). Bioactive compounds and antioxidant capacity were significantly affected by the extraction method. In general, power ultrasound showed the lower extraction of total anthocyanins (29.6–29.7 mg cyaniding/kg), compared to agitation (48.5–71.2 mg cyaniding/kg) and Soxhlet (82.2–85.8 mg cyaniding/kg) methods. It is important to note that the total anthocyanin concentration decreased with extraction times when using the Soxhlet method. This can be attributed to a degradation of anthocyanins or polymerisation reactions and the formation of new compounds with different response to analytical measurements (Spigno *et al.* 2007). It has been demonstrated that higher temperatures and longer extraction times could affect the yield of anthocyanin extraction (Moldovan *et al.* 2012). Corrales *et al.* (2009) reported similar total anthocyanin values (66.6–122.2 mg cyaniding/kg) in Dornfelder grape skin to those obtained in this study. Moreover, Corrales *et al.* (2008) evaluated the effect of conventional and novel technologies on anthocyanins extraction from grape skin. While ultrasound did not significantly increase anthocyanin extraction in comparison with conventional techniques, both high hydrostatic pressure and pulsed electric fields augmented anthocyanin yield by 44 and 77%, respectively. A similar trend to that observed with anthocyanins was seen in phenolic compounds extraction. Again, ultrasound extraction exhibited the lowest efficiency (93.8–100.8 mg GAE/kg) compared to agitation (117.0–141.5 mg GAE/kg) and Soxhlet (186.3–207.0 mg GAE/kg). It is well known that high temperatures may cause protein denaturation, leading to the collapse of

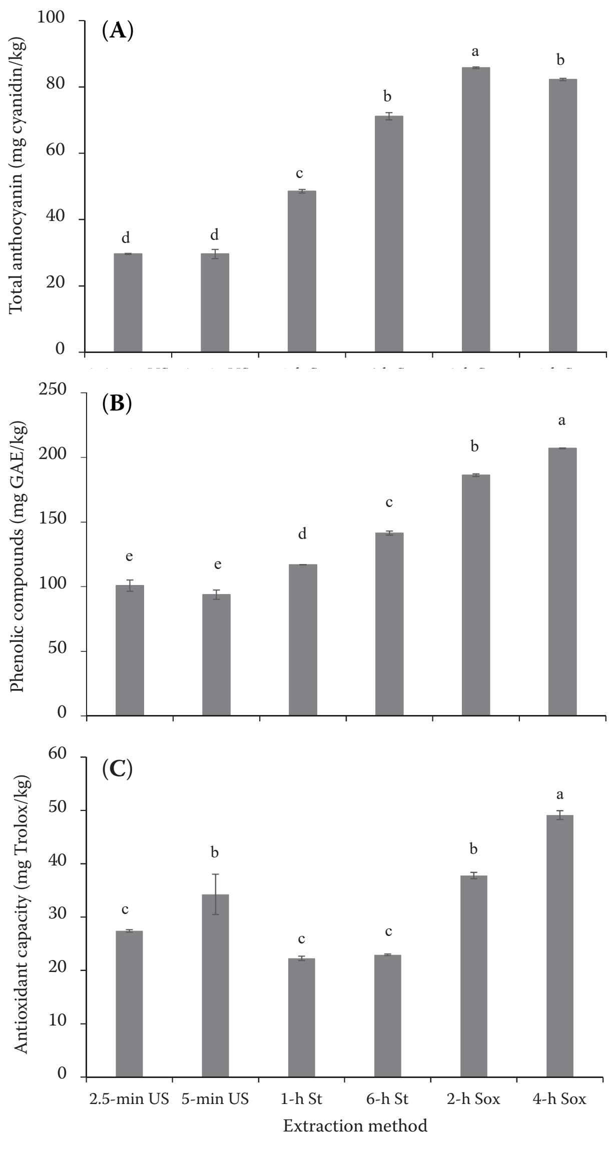

Figure 3. Evaluation of different extraction methods for total anthocyanins (**A**), phenolic compounds (**B**), and antioxidant capacity (**C**) from x´kijit peel (bars in the figure indicate the standard deviation)

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several cell structures including wall, membrane and certain organelles; this in turn improves mass transfer and increases the extraction of soluble constituents into the solvents (Thoo *et al.* 2010). Similar results were obtained by Jun *et al.* (2011) where 4-h Soxhlet extraction was more efficient in terms of green tea polyphenol extraction than a 40-min power ultrasound treatment. Figure 3B shows that the ultrasonic extraction time (2.5 or 5 min) did not have a significant effect ($P > 0.05$) on polyphenol yield, unlike both agitation and Soxhlet extraction procedures ($P < 0.05$), although, as previously reported, an increase in the extraction efficiency of bioactive compounds over time was expected. As mentioned before, one of the major advantages of novel technologies such as ultrasound when used as an extraction method is the possibility of being considered a green extraction alternative as both processing costs and time might be reduced. Consequently, this energy-wise effective, potentially eco-friendly procedure could also reduce the negative impact that overly long conventional extraction procedures might have on bioactive compounds stability. Although some reports indicate that long-time ultrasonic treatments, even with a cooling system to maintain the temperature, cause alteration on the bioactive compounds, probably due to competition between extraction and degradation of compounds (Saleh *et al.* 2016). Thus, short sonication times are preferred for minimising this possible effect while simultaneously maintaining the green character of the extraction (Achat *et al.* 2012; Jacotet-Navarro *et al.* 2016). As commented before, x´kijit is a mostly unknown fruit; to the best of our knowledge, no scientific data on it have been generated. However, phenolic compound values are higher than those reported by Molina-Quijada *et al.* (2010), where values in the range of 71.8–106.0 mg GAE/kg were reported for dry peel from different grape varieties.

Contrary to the behaviour observed in the extraction of total anthocyanins and phenolic compounds, the antioxidant capacity from ultrasonic x´kijit peel extracts was higher (27.4–34.3 mg Trolox/kg) than that obtained with extracts from the agitation method (22.3–22.9 mg Trolox/kg). Moreover, the antioxidant capacity of x´kijit peel obtained with a 5-min ultrasound treatment did not exhibit any significant differences ($P > 0.05$) from that attained with Soxhlet extraction for 2 h (37.8 ± 0.6 mg Trolox/kg). This can be a good indicator that x´kijit peel contains other bioactive compounds that increase the antioxidant capacity and ultrasound further promotes their extraction; moreover, there is evidence that processes carried out for longer times or at higher temperatures could negatively affect the antioxidant capacity of extracts. It is important to note that by applying ultrasound the extraction time could be reduced up to 24 times (compared to a 2-h Soxhlet extraction procedure), decreasing both the extraction costs and energy needed. The higher efficacy of ultrasound technology as an extraction method could be due to the cavitation phenomenon that promotes the hydration and fragmentation process improving the mass transfer of solutes to the solvent (Chemat *et al.* 2017). On the other hand, temperature and time effects on bioactive compounds extraction and antioxidant capacity of extracts are not clear yet. Some reports indicate that by increasing time and temperature the antioxidant capacity is enhanced (Thoo *et al.* 2010) while others indicate that at higher extraction times or temperature the antioxidant capacity is negatively affected (Xu *et al.* 2007).

Combined extraction methods. Findings from previous reports show that the application of ultrasound as a pretreatment increases the extraction yield of bioactive compounds (Balachandran *et al.* 2006; Khan *et al.* 2010). Thus, this approach was used with x´kijit peel to improve the recovery of bioactive compounds while maximising the antioxidant capacity. To evaluate the effect of ultrasound as a pretreatment, a new set of experiments was performed comparing the efficiency of individual conventional extraction methods with or without ultrasound assistance (Figure 4). Figure 4A shows the total anthocyanin content extracted from x´kijit peel using combined methods. The application of ultrasound as a pretreatment did not significantly change this parameter ($P > 0.05$) when compared to traditional processes. The only exception was the 4-h Soxhlet extraction where power ultrasound allowed a slight increase in anthocyanin content (3.6 ± 0.1%). Meanwhile, a significant ($P < 0.05$) increase in phenolic compounds was obtained using combined ultrasound and 1-h and 6-h agitation procedures (11 ± 0.5 and 21 ± 4.6%, respectively) (Figure 4B). Moreover, the ultrasound-assisted Soxhlet extraction showed an increase in yield (12 ± 1%) after the 2-h treatment, while longer Soxhlet extraction times did not significantly affect ($P > 0.05$) this parameter. It is important to note that the 2-h ultrasound-

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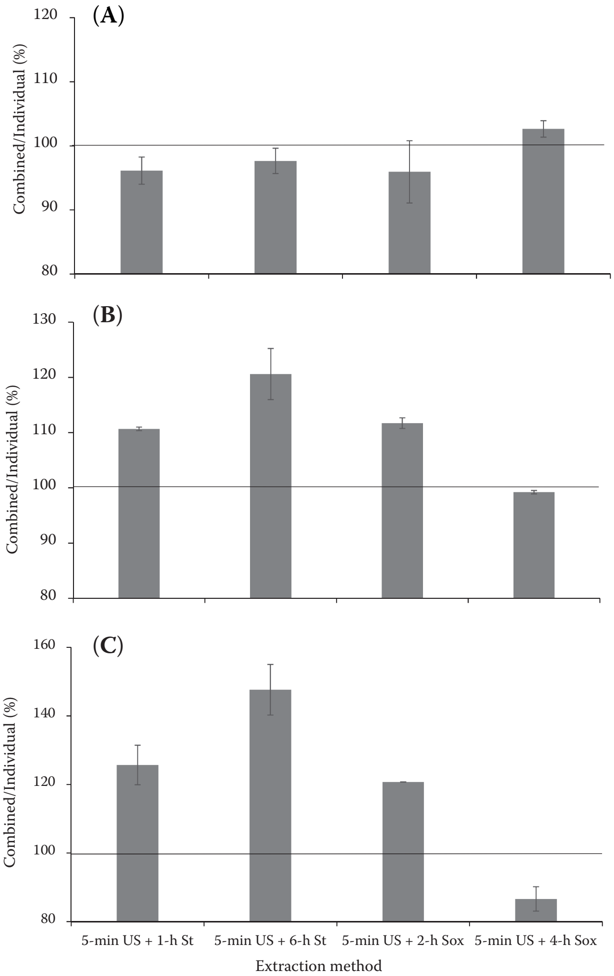

Figure 4. Evaluation of combined extraction methods for total anthocyanins (**A**), phenolic compounds (**B**), and antioxidant capacity (**C**) from x´kijit peel

assisted Soxhlet treatment extracted a similar amount of phenolic compounds (208.1 ± 0.7 mg GAE/kg) to that obtained with a 4-h Soxhlet process, thus reducing the extraction time by 50%. Figure 4C shows the effect of an ultrasound pretreatment coupled with agitation or Soxhlet extraction methods on antioxidant capacity of x´kijit peel extracts. It was observed that the effect of the treatments on antioxidant capacity showed similar trends ($R^2 > 0.98$) to those observed with phenolic compounds. An increase of 26 ± 5.8 and 48 ± 7.4% was observed after 1 and 6 h agitation, respectively. Moreover, the application of ultrasound as a pretreatment coupled with Soxhlet extraction only exhibited a significant increase in antioxidant capacity with the 2-h treatment (22 ± 0.1%). Additionally, antioxidant capacity values (46.2 ± 0.7 mg Trolox/kg) obtained with ultrasound plus 2-h Soxhlet extraction did not present any significant differences ($P > 0.05$) when compared with those obtained with 4-h Soxhlet extraction.

As commented before, the energy dissipated by the collapse of cavitation bubbles induces a greater penetration of the solvent into vegetal tissues, thus improving the mass transfer phenomenon. In addition, it has been shown that depending upon its intensity, ultrasonic pretreatment may enhance extraction due to the cell wall disruption that in turn releases cell materials thus facilitating the recovery of biologically active compounds compared to other methods (Patist & Batest 2008). In this sense, several researches have been conducted in order to evaluate the combined effect of ultrasound and other extraction methods (Corrales *et al.* 2008; Liu *et al.* 2011; Gomes *et al.* 2017). In general, results indicate that power ultrasound might be used as an effective tool for significantly ($P < 0.05$) increasing the extraction rate and yield of several bioactive compounds (Balachandran *et al.* 2006).

CONCLUSION

According to the planned mixture design, methanol was the solvent with the highest extraction yields of total anthocyanins and phenolic compounds from x´kijit peels. Besides, it also produced the extracts with the highest antioxidant capacity. On the other hand, the mathematical model obtained from the mixture design showed a good correlation between experimental and predicted data for total anthocyanins and antioxidant capacity. The 4-h Soxhlet procedure was a treatment that yielded the x´kijit peel extracts with both the higher concentration of bioactive compounds and the greatest antioxidant capacity; however, antioxidant capacity of the extracts obtained with ultrasound showed similar values to those obtained with 2-h Soxhlet extraction. The use of power ultrasound as a pretreatment for traditional extraction processes significantly increased both the separation of phenolics and the antioxidant capacity of x´kijit peel extracts while reducing extraction time and increasing energy efficiency, thus constituting the basis for developing a green extraction procedure. Values of bioactive compounds and antioxidant capacity obtained from x´kijit peel were similar to those reported with extracts from other food sources; therefore, x´kijit peel might be used as a potential source of natural antioxidants although more studies are necessary to evaluate its applicability in food systems.

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