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Antioxidant Properties of Sacha Inchi (*Plukenetia volubilis*) Shell Extracts as Affected by Solvents used for Prior Decolorization

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Abstract: Sacha inchi (*Plukenetia volubilis*) shell is a potential source of phenolics with antioxidative activity and its extract can be used to prevent lipid oxidation in some food matrices. However, the sacha inchi extract has been fully exploited due to the dark brown colour properties associated with pigments. Thus, decolourization of sacha inchi shells before extraction using solvents could be a means to bring about the extract with a lighter colour, which could be applied in foods without constraints. The effects of different solvents used for decolourization in sacha inchi (*Plukenetia volubilis*) shell powder on antioxidant properties were investigated. The solvents used were methanol, acetone, chloroform and propanol. The ethanolic extracts' total phenolic content (TPC) and total flavonoid content (TFC) decreased when solvents were employed for prior decolourization. Among all solvents, the ethanolic extracts from sacha inchi shell powder decolourized using chloroform (CHE) showed the highest TPC (9.94 mg GAE/g dry extract) and TFC (7.20 mg CE/g dry extract). Also, extracts from chloroform decolourized shell powder had the highest antioxidant activities (2,410.01, 111.60 and 4.58 μmol TE/g dry extract for 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities, ferric reducing antioxidant power (FRAP), respectively, and 0.52 mmol EDTA /g dry extract for metal chelating assay) compared to other extracts. Therefore, chloroform was the appropriate solvent for decolourization, and the resulting extract had higher antioxidant properties than others.

Keywords: Sacha Inchi; Decolorization; Antioxidant Properties; Phenolic Compound; Extraction

1. Introduction

Most herbs contain various phenolic compounds, including flavonoids, phenolic acids, tannins, stilbens and lignans [1]. Phenolic compounds, the secondary metabolites of plants, have the aromatic ring bearing one or more hydroxyl substituents [2]. The differences in antioxidant activities of the phenolic compounds are contributed by structures and the number of hydroxyl groups [3]. Phenolic compounds have different functions, including biomembrane interaction or transition metal chelation, free radical scavenging, inhibition of various enzymes, and quenching of reactive oxygen species (ROS), by which their antioxidant activities exhibit differently [3]. Phenolic compounds have different physiological and biomedical characteristics such as anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective, and vasodilatory effects [4]. Nowadays, herb extracts are used as natural preservatives for fresh and processed foods due to their antioxidant activities [5].

Pigments are widely distributed in almost all plants, including chlorophyll, carotenoids, flavonoids and phytochrome. [6]. Among the pigments, chlorophyll and carotenoids belong to the most abundant organic pigments in nature [7]. They are largely biosynthesized in various plants and can be extracted and used as food colourants [8]. There are partially soluble in a polar solvent [9]. Consequently, both pigments are inevitably co-extracted into plant extracts. Thus, the co-existed pigments can impact unwanted colour on the resulting plant extract, limiting food colouring applications [10]. In this context, it is helpful to carry out the decolourization of plant materials to maximize the application of plant extracts rich in phenolic compounds. Many organic solvents have been used to decolour pigments from plant materials. Those solvents include polar solvents (methanol and propanol), polar aprotic solvent (acetone), and nonpolar solvent (chloroform) [11]. Olantunde et al. [11] reported that polarity and type of solvent showed a profound impact on pigment removal from guava leaf. Benjakul et al. [3] found that extraction solvent, decolourization, and drying methods directly influenced lead seed extract's yield and antioxidant activity.

Sacha inchi (*Plukenetia volubilis* L.) is an oleaginous plant belonging to the Euphorbiaceae family. Sacha inchi seeds consist of the shell (33-35%) and kernel (65-67%), and the kernel has 54% of oil and 33% of proteins [12]. Sacha inchi seeds are mainly industrialized into oil by taking advantage of the unique fatty acids, composed of approximately 34% linoleic acid and 51% linolenic acid [13]. The industry of sacha inchi oil has grown significantly worldwide in the last years because of the demand for high-quality oils from the functional foods and nutraceutical industries. The main by-products of the sacha inchi oil extraction process are the shell and the press cake. These by-products are generally used as animal feed or discarded without further use. Consequently, finding feasible ways to add value to these by-products is essential for the sacha inchi industry. Several investigations stated that plant-derived by-products generated from processing and commercialization are valuable sources of antioxidant bioactivity rich in phenolic compounds [14-15].

The extracts from sacha inchi shells have been studied to prevent lipid oxidation in some foods potentially, but they have not been fully employed due to the dark brown colour associated with pigments. Decolourization of sacha inchi shells before extraction using a few selected solvents could be useful to lighten the extract, which would allow the extract to be used in various food products. Thus, the present investigation aimed to understand the influence of multiple solvents used to decolourise sacha inchi shell powder and study the antioxidant properties of extracts.

2. Materials and Methods

2.1 Chemicals

All chemicals used were of analytical grade and were procured from Merck (Darmstadt, Germany)

2.2 Preparation of sacha inchi shell powder

Sacha inchi (*Plukenetia volubilis* L.) shell (exocarp and mesocarp part) were obtained from Ban Obboon OTOP (One Tumbon One Product), Phatthalung province, Thailand. Sacha inchi shell powder was prepared

following Chotphruethipong et al. [16]. Sacha inchi shells were blended using a blender (Panasonic, Model MX-898N, Berkshire, UK) and sieved through stainless steel (sieve 80 mesh).

2.2.1 Decolorization

Sacha inchi shell powder (50 g) was mixed with various solvents (500 mL), including methanol, acetone, propanol, and chloroform. The mixtures were stirred continuously for 30 min to remove pigments. Afterwards, the mixtures were filtered using a Whatman filter paper No. 1 (Whatman International Ltd., Maidstone, UK). The residues were subjected to another-round decolourization. The decolourized powder was dried in an oven (Memmert, Schwabach, Germany) at 70°C for 3 hr. The powder was named "decolourized sacha inchi shell powder" and stored in a polyethene bag in darkness at ambient temperature (28°C–30°C) before further ethanolic extraction.

2.3 Preparation of sacha inchi shell extracts

The preparation of sacha inchi shell extract was performed following Chotphruethipong et al. [16]. Ethanol was applied as the extraction medium by mixing with decolourized shell powder at a solid/solvent ratio of 1:15 (w/v) with continuous stirring for 60 min, followed by centrifugation at 5,000 x g for 30 min at 25°C using a Sorvall Model RC-B plus centrifuge (Newtown, CT, USA). The supernatants were collected via filtration through Whatman filter paper No. 1**.** They were subsequently evaporated at 40°C using a rotary evaporator (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan). Finally, They were lyophilized using a Dura-TopTM lp freeze dryer (FTS systems Inc., Stone Ridge, NY, USA). The dried extracts samples were stored in a desiccator before analysis. The dried extract samples without the decolourization step (subsection 2.2.1) were applied as a control.

2.4 Analyses

2.4.1 Extraction yield

The extraction yield was calculated according to the method of Benjakul et al. [3], and it was expressed as a percentage (%) of the weight of crude extracts relative to the weight of dry raw materials.

2.4.2 Color values

Colour values of the sacha inchi shell extract powders with and without decolourization was determined on a colourimeter (ColorFlex, Hunter Lab Reston, VA, USA) according to the method of Siomos et al. [17] and Olatunde et al. [11]. Calibration was performed using the Manufacturer's standard white plate. Lightness (L*), redness/greenness (a*) and yellowness/blueness (b*) were measured. Hue angle, chroma value, and ΔE were calculated as follows:

$$\text{Hue angle } (h^{\circ}) = 180+\tan^{-1}(b^{*}/a^{*})$$

$$\text{Chroma value } (C^{*}) = (a^{*2}+b^{*2})^{1/2}$$

$$\Delta E = (\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{1/2}$$

Where ΔL*, Δa*, and Δb* are the differences between the corresponding colour parameter of the sample and that of white standard (L* = 90.77, a* = –1.27, and b* = 0.50).

2.4.3 Total phenolic content

Total phenolic content (TPC) of the sacha inchi shell extracts with and without decolourization was determined spectrophotometrically with Folin-Ciocalteu's reagent (FCR) as described by Benjakul et al. [3]. The TPC was calculated as mg gallic acid equivalent (GE)/g dry extract.

2.4.4 Total flavonoid content

The total flavonoids (TFC) of the sacha inchi shell extracts without and with prior decolourization was determined by the aluminium chloride colourimetric method of Yang et al. [18]. TFC was expressed as mg catechin equivalent (CE)/g dry extract.

2.4.5 Antioxidant activities

DPPH radical scavenging activity was measured using the method of Wu et al. [19]. ABTS radical-scavenging activity was determined following Binsan et al. [20]. Ferric reducing antioxidant power (FRAP) was measured

as per the method of Benzie and Strain [21]. Activities were expressed as μmol Trolox equivalent (TE)/g dry extract. Chelating activity toward ferrous ion (Fe^{2+}) was determined by Boyer and Mccleary [22] and was expressed as mmol EDTA equivalent /g dry extract.

2.4.6 Statistical analysis

Experiments were run in triplicate using three different lots of samples. All data were subjected to analysis of variance (ANOVA) and differences between means were evaluated by Duncan's multiple range test [23]. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 24.0 for Windows, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Color values of ethanolic sacha inchi shell extract

Lightness (L*), Hue angle, Chroma values and ΔE* of the ethanolic sacha inchi shell extracts were in the range of 41.13-44.33, 246.04-247.12°, 15.46-17.40 and 49.54-52.42, respectively (Table 1). Those parameters were governed by solvents applied before decolourization. Decolourization increased the L* of sacha inchi shell extract. Ethanolic extract from acetone decolourization shell power had the highest L*- value, whilst the control exhibited the lowest value ($p < 0.05$). Hue angle, Chroma and ΔE* decreased when prior decolourization was carried out ($p < 0.05$). Hence, polarity and type of solvent exhibited a profound effect on pigment removal from sacha inchi shell powder. Olatunde et al. [11] found a similar trend, who reported that the Hue angle and Chroma decreased when prior dechlorophyllization was made. Therefore, decolourization using various solvents affected the colour of the resulting ethanolic extracts differently.

Table 1 Color of ethanolic sacha inchi shell extract with prior decolorization using different solvents*

Samples	L*	Hue angle	Chroma	ΔE*
CON	$41.13 \pm 0.12^{a**}$	247.12 ± 0.24^{c}	17.40 ± 0.14^{e}	52.42 ± 0.09^{d}
PRO	44.06 ± 0.12^{cd}	246.77 ± 0.07^{bc}	17.16 ± 0.16^{cd}	50.66 ± 0.24^{c}
MET	42.57 ± 0.29^{b}	246.44 ± 0.35^{ab}	17.02 ± 0.24^{bc}	50.12 ± 0.41^{b}
CHL	43.66 ± 0.50^{c}	246.09 ± 0.30^{a}	16.74 ± 0.09^{b}	49.87 ± 0.06^{ab}
ACE	44.33 ± 0.31^{d}	246.04 ± 0.14^{a}	15.46 ± 0.10^{a}	49.54 ± 0.23^{a}

*Values represent mean and standard deviation ($n = 3$).

**Different superscripts within the same column indicate significant differences ($p < 0.05$). CON: Extract without decolourization, PRO: Propanol decolourized extract, MET: Methanol decolourized extract, CHL: Chloroform decolourized extract and ACE: Acetone decolourized extract

3.2 TPC, TFC and extraction yield of ethanolic sacha inchi shell extract

The extraction yield of the sacha inchi shell extract was significantly influenced by the prior decolourization process. The extraction yield values with solvents treatment showed in the descending order: chloroform > propanol > methanol > acetone (Table 2). Decolourization using acetone had the lowest yield ($p < 0.05$). The data agreed with the findings from Olatunde et al. [11], which reported a lower yield of 6.73% for guava leaf extracted with prior chlorophyll removal using chloroform compared to the yield of 23.78% when extracted without chlorophyll removal.

TPC and TFC of the extracts showed values of 5.39-13.48 mg GE/g dry extract and 5.20-7.40 mg CE/g dry extract, respectively (Table 2). The TPC and TFC contents of the sacha inchi shell extracts decreased significantly ($p < 0.05$) when solvents were used before decolourization, and the descending trend for solvents used was: chloroform > propanol > methanol > acetone. The decreases in TPCs and TFCs with these solvents indicated were probably because the phenolic compounds were soluble in those solvents to some extent. Koddami et al. [24] found that extraction solvents such as water, acetone, ethyl acetate, alcohols (methanol,

ethanol and propanol) and their mixtures affected the phenolics yields. Therefore, some phenolic compounds were co-extracted along with the pigments during the decolourization process. Hence, less amount of phenolic content was contained in ethanolic extracts. Benjakul et al. [3] found that prior chlorophyll removal decreased the phenolic content of lead seed extracts. Olatunde et al. [11] also reported that the phenolic content of guava leaf extracts decreased when the solvent was used for prior decolourization.

Both polar solvents (methanol, water, propanol, and ethanol) and polar aprotic solvents (acetone, ethyl acetate, and dimethyl sulfoxide) were studied for extraction of phenolic compounds previously [25]. Using nonpolar solvents for decolourization, especially chlorophyll, could eliminate lipids from the leaf, thereby raising the efficacy of phenolic compound extraction. This was confirmed by the higher phenolic content observed in the extract pre-treated with chloroform compared to those found in the extracts with other solvents. Moreover, nonpolar solvents extracted phenolic compounds with low polarity [3]. Although with the benefits from using chloroform for decolourization, it showed lower phenolic content in chloroform-decolourized extract than that in control extract without decolourization.

Table 2 Extraction yield, total phenolic content and total flavonoid content of ethanolic sacha inchi shell extract with prior decolourization using various solvents*

Samples	Extraction Yield (%)	TPC (mg GAE/g dry extract)	TFC (mg CE/g dry extract)
CON	$15.53 \pm 0.01^{e**}$	13.48 ± 0.21^{d}	7.40 ± 0.02^{e}
PRO	5.62 ± 0.38^{c}	7.29 ± 0.47^{b}	6.49 ± 0.07^{c}
MET	5.14 ± 0.11^{b}	5.57 ± 0.38^{a}	5.53 ± 0.05^{b}
CHL	6.45 ± 0.01^{d}	9.94 ± 0.31^{c}	7.20 ± 0.15^{d}
ACE	3.15 ± 0.11^{a}	5.39 ± 0.07^{a}	5.20 ± 0.05^{a}

*Values represent mean and standard deviation ($n = 3$).

**Different superscripts within the same column indicate significant differences ($p < 0.05$). TPC: Total phenolic content, TFC: total flavonoid content, CON: Extract without decolorzation, PRO: Propanol decolorized extract, MET: Methanol decolorized extract, CHL: Chloroform decolorized extract and ACE: Acetone decolorized extract

3.3 Antioxidant activity of ethanolic sacha inchi shell extracts

3.3.1 DPPH and ABTS radical scavenging activities

The DPPH and ABTS radical scavenging activities generally increased when prior decolourization was carried out ($p < 0.05$) (Figure 1). The extract with chloroform decolourization showed the highest DPPH and ABTS scavenging activities ($p < 0.05$). This result agreed with the observation of Benjakul et al. [3] and Olatunde et al. [11], who found the increases in both DPPH and ABTS scavenging activities for chloroform decolourized lead seed and guava leaf extracts. In this study, the reductions in ABTS scavenging activities for extracts decolourized by acetone and methanol were contributed by the leaching out of some phenolic compounds with antioxidant activity during the decolourization process.

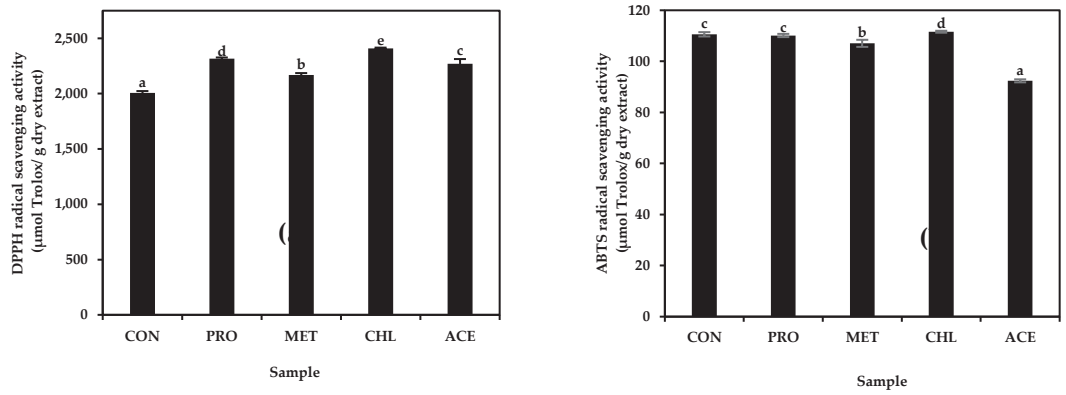

Figure 1. DPPH (a) and ABTS (b) radical scavenging activities of ethanolic sacha inchi shell extracts with prior decolourization using different solvents. Bars represent the standard deviation (n = 3). Different letters on the bars indicate significant differences ($p < 0.05$). CON: Extract without decolourization, PRO: Propanol decolourized extract, MET: Methanol decolourized extract, CHL: Chloroform decolourized extract and ACE: Acetone decolourized extract

Antioxidant activity in the hydrophobic system can be measured using DPPH scavenging activities, while ABTS scavenging activities are more appropriate to measure in hydrophilic and hydrophobic systems [26]. The results obtained from this investigation indicated the ability of all the extracts to donate a hydrogen atom to both types of free radicals, i.e., ABTS and DPPH radicals, and the scavenging abilities of extracts greatly depended on their phenolic contents [27], which also varied from the selection of solvents used for prior decolourization.

3.3.2 FRAP and metal chelating activity

The solvents used for prior decolourization of sacha inchi shell powder significantly influenced the ferric reducing antioxidant power (FRAP) and metal chelating activity of the final ethanolic extract ($p < 0.05$), with values of 2.03-4.58 µmol TE/g dry extract and 0.41-0.52 mmol EDTA equivalent/g dry extract, respectively (Figure 2). Ethanolic extract pre-decolourized with chloroform exhibited the highest FRAP and metal chelating activities ($p < 0.05$). Benjakul et al. [3] found increases in both FRAP and metal chelating activity for chloroform-decolourized lead seed extract compared to the extract with no chlorophyll removal. Olatunde et al. [11] also found that extract from chloroform-dechlorophyllized leaf powder showed the highest antioxidant activities compared to other solvents-treated extracts. The reduction in FRAP and metal chelating abilities on the acetone-, methanol-, and propanol-decolourized extracts could be because some antioxidant phenolic compounds were removed from the decolourization process.

Generally, antioxidant activities have positive correlations with TPC and TFC content. However, some antioxidant activities increased after decolourisation as TPC and TFC content decreased in this study. The results suggested that the phenolic compounds with higher antioxidant activity were more concentrated after pigment removal. Consequently, the higher antioxidative activity was obtained in the sacha inchi extract with prior pigment removal.

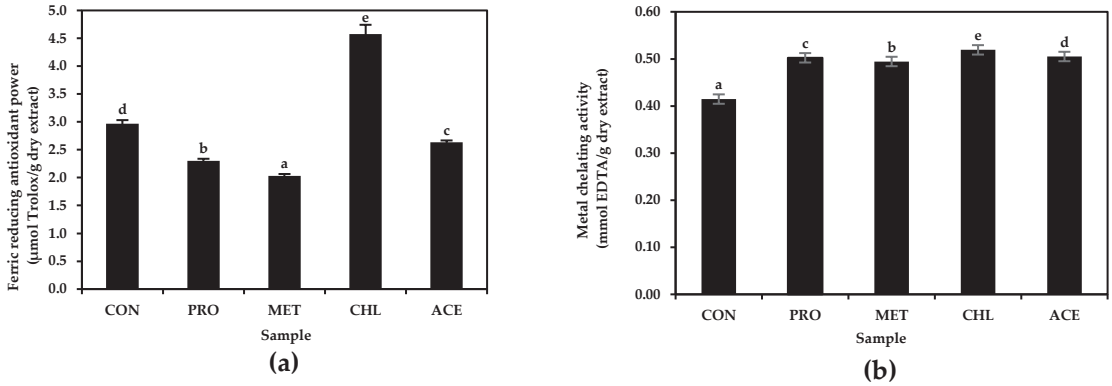

Figure 2. Ferric reducing antioxidant power (a) and metal chelating activity (b) of ethanolic sacha inchi shell extracts with prior decolourization using different solvents. Bars represent the standard deviation (n = 3). Different letters on the bars indicate significant differences ($p < 0.05$). CON: Extract without decolourization, PRO: Propanol decolourized extract, MET: Methanol decolourized extract, CHL: Chloroform decolourized extract and ACE: Acetone decolourized extract

4. Conclusions

Pigments removal before extraction of phenolic compounds from sacha inchi shell resulted in a decrease in extraction yield and a lower TPC and TFC. Among the selected solvents, chloroform was the best solvent for the decolourization process with a minimal negative effect on antioxidant properties and a positive impact on the better lightness and lower brownness; thus, it could be used in foods.

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Author Contributions:

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. D'Archivio, M.; Felesi, C.; Benedetto, R.D.; Gargiulo, R.; Giovannini, C.; Masella, R. Polyphenol, dietary sources and bioavailability. *Annali dell'Istituto Superiore di Sanità.* 2007, 43, 348–361.
2. Naczk, M.; Shahidi, F. Extraction and analysis of phenolics in food. *Journal of Chromatography A*. 2004, 1054, 95–111.
3. Benjakul, S.; Kittiphattanabawon, P.; Sumpavapol, P.; Maqsood, S. Antioxidant activities of lead (*Leucaena leucocephala*) seed as affected by extraction solvent, prior dechlorophyllisation and drying methods. *Journal of Food Science and Technology*. 2014, 51, 3026–3037.
4. Puupponen-Pimiä, R.; Nohynek, L.; Meier, C.; Ka€Hko€Nen, M.; Heinonen, M.; Hopia, A.; Oksman-Caldentey, K.M. Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology*. 2001, 90, 494–507.
5. Benkeblia, N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Journal of Food Science and Technology.* 2004, 37, 263–268.
6. Heldt, H.W. *Plant Biochemistry*, 3rd ed.; Academic Press: Burlington, MA, 2004; 16, 399–408.
7. Cai, J.Q.; Liu, X.M.; Gao, Z.J.; Li, L.L.; Wang, H. Chlorophylls derivatives: Photophysical properties, assemblies, nanostructures and biomedical applications. *Mater. Today.* 2021.
8. Roca, M.; Chen, K.; Pérez-Gálvez, A. *Chlorophylls. In Handbook on natural pigments in food and beverages*. Woodhead Publishing, 2016; 125–158.

9. Bohn, T.; Walczyk, T.; Leisibach, S.; Hurrell, R. Chlorophyll-bound magnesium in commonly consumed vegetables and fruits: Relevance to magnesium nutrition. *Journal of Food Science.* 2004, 69, 347–350.
10. Namal Senanayake, S. P. J. Green tea extract: Chemistry, antioxidant properties and food applications – A review. *Journal of Functional Foods.* 2013, 5, 1529–1541.
11. Olatunde, O.O.; Benjakul, S.; Vongkamjan, K. Antioxidant and antibacterial properties of guava leaf extracts as affected by solvents used for prior dechlorophyllization. *Journal of Food Biochemistry.* 2018, 42, 1–12.
12. Gutiérrez, R.M.P.; Mitchell, S.; Solis, R.V. *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*. 2008, 117, 1–27.
13. Chirinos, R.; Pedreschi, R.; Domínguez, G.; Campos, D. Comparison of the physico-chemical and phytochemical characteristics of the oil of two Plukenetia species. *Food Chemistry*. 2015, 173, 1203–1206.
14. Wanyo, P.; Meeso, N.; Siriamompun, S. Effects of different treatments on the antioxidant properties and phenolic compounds of rice bran and rice husk. *Food Chemistry*. 2014, 157, 457–463.
15. Do Prado, A.C.P.; da Silva, H.S.; da Silveira, S.M. Effect of the extraction process on the phenolic compounds profile and the antioxidant and antimicrobial activity of extracts of pecan nut [*Carya illinoinensis (Wangenh*) C. Koch] shell. *Industrial Crops and Products*. 2014, 52, 552–561.
16. Chotphruethipong, L.; Benjakul, S.; Kijroongrojana, K. Optimization of extraction of antioxidative phenolic compounds from cashew (*Anacardium occidentale* L.) leaves using response surface methodology. *Journal of Food Biochemistry*. 2017, 41, 1–10.
17. Siomos, A.S.; Gerasopoulos, D.; Tsouvaltzis, P.; Koukounaras, A. Effects of heat treatment on atmospheric composition and color of peeled white asparagus in modified atmosphere packaging. *Innovative Food Science and Emerging Technologies.* 2010, 11, 118–122.
18. Yang, J.; Martinson, T.E.; Liu, R.H. Phytochemical profiles and antioxidant activities of wine grapes. *Food Chemistry*. 2009, 116, 332–339.
19. Wu, H.C.; Chen, H.M.; Shiau C.Y. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International.* 2003, 36, 949–957.
20. Binsan, W.; Benjakul, S.; Visessanguan, W.; Roytrakul, S.; Tanaka, M.; Kishimura, H. Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). *Food Chemistry*. 2008, 106, 185-193.
21. Benzie, I.F.F.; Strain, J.J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" The FRAP assay," *Analytical Biochemistry.* 1996, 239, 70–76.
22. Boyer, R.F.; McCleary, C.J. Superoxide ion as a primary reductant in ascorbate-mediated ferretin iron release. *Free Radicals Biology & Medicine.* 1987, 3, 389–395.
23. Steel, R.G.D.; Torrie, J.H. *Principle and procedure of statistics; a biometrical approach,* 2n ed; McGraw-Hill Kogakusha, Ltd.: Tokyo, 1980, 1–633.
24. Khoddami, A.; Wilkes, M.A.; Roberts, T.H. Techniques for analysis of plant phenolic compounds. *Molecules*. 2013, 18, 2328–2375.
25. Złotek, U.; Mikulska, S.; Nagajek, M.; Świeca, M. The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (*Ocimum basilicum* L.) extracts. *Saudi Journal of Biological Sciences*. 2016, 23, 628–633.
26. Rubalya Valantina, S.; Neelamegam, P. Selective ABTS and DPPH-radical scavenging activity of peroxide from vegetable oils. *International Food Research Journal.* 2015, 22, 289–294.
27. Seo, J.; Lee, S.; Elam, M.L.; Johnson, S.A.; Kang, J.; Arjmandi, B.H. Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. *Food Science & Mitrition*. 2014, 2, 174–180.