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Comparison of Extraction Methods for the Quantification of Selected Phytochemicals in Peanuts (*Arachis hypogaea*)

YVONNE C. CHUKWUMAH,[†] LLOYD T. WALKER,*[,†] MARTHA VERGHESE,[†] MPOKO BOKANGA,[‡] SIMON OGUTU,[†] AND KYSHA ALPHONSE[†]

Department of Food and Animal Sciences, Alabama A&M University, Post Office Box 1628, Normal, Alabama 35762, and United Nations Development Project, UNIDO Regional Industrial Development Center, 1181 Aguiyi Ironsi Street, Maitama, Abuja, Nigeria

Peanuts have been reported to contain bioactive phytochemicals, particularly isoflavones (genistein, daidzein, and biochanin A) and *trans*-resveratrol. Currently, limited data are available regarding the levels of these bioactive compounds in peanuts with variations in reported levels. The purpose of this study was to compare four methods of extraction [stirring, sonication, Soxtec, and microwave-assisted sonication (MAS)] for runner peanuts. Quantification of the selected compounds was conducted by reverse-phase high-performance liquid chromatography (RP-HPLC). The results showed that the MAS and Soxtec methods extracted significantly higher amounts of the phytochemicals. Also, the defatted peanuts gave significantly higher amounts of the phytochemicals compared to the nondefatted peanuts. The high levels of the isoflavones may be attributed to heat-induced conversion of conjugate glycosides to aglycons. The MAS and Soxtec methods may be used for total isoflavone content quantitation, while sonication or stirring may be the method of choice for quantitation of isoflavone composition (aglycons and glycoside conjugates) in peanuts.

KEYWORDS: Peanut (*Arachis hypogaea* L.); groundnut; extraction methods; phytochemical; high-performance liquid chromatography; microwave-assisted sonication

INTRODUCTION

Peanuts (*Arachis hypogaea* L.) have been shown to have a favorable nutrient profile as well as bioactive components that elicit cardioprotective effects (*1*−*5*). An oil seed belonging to the plant family Leguminosae, it has been reported to contain nonnutritive bioactive phytochemicals, such as *trans*-resveratrol, stigmasterol, β-sitosterol, *p*-coumaric acid, and particularly the isoflavones daidzein, genistein, and biochanin A (*6*). Isoflavones are an important group of phytochemicals that have been reported not only to have anticarcinogenic properties but also to play a role in the mitigation of osteoporosis in postmenopausal women (*7*−*9*). This is partly due to their antioxidative (*10*, *11*) and estrogenic properties (*12*−*14*).

Although several reviews have summarized qualitative and quantitative data on these bioactive compounds (*15*−*18*), there are limited data regarding the levels of these compounds in peanuts and peanut products. Also, there is a wide variation in the levels reported for these compounds. This may be due not only to the diversity in the extraction methods used with their different extraction efficiencies but also to the use of different peanut cultivars (*19*).

The extraction method and solvents used are very critical for estimation of the concentration of any compound in foods. This creates a need for standardization of extraction protocols and analytical techniques for the quantification of these compounds in peanuts. Isoflavones have been extensively studied in soy and soy products and to some extent in peanuts and peanut products (*15*−*18*, *20*, *21*). In soy, a total of 12 chemical forms have been identified (*20*, *21*). These forms consist of the β-glycosides, the acetyl and malonyl derivatives (mainly present in raw, unprocessed soy), and the aglycons (**Figure 1**). Concentrations of the different forms of isoflavones in soy have been shown to be affected by processing and storage conditions (*22*). Previous studies showed that moist heat treatment of isoflavones promotes the conversion of the predominant 6″-*O*-malonyl form to β-glycosides (*23*) while dry heat processing promotes the conversion of the 6″-*O*- malonyl form to 6″-*O*-acetyl form through decarboxylation (*24*). Other considerations would include photochemical isomerization of the compound of interest such as *trans*-resveratrol (**Figure 1**), which is abundant in the skin of grapes and has been reported to be present in peanuts. Thus, a method for the extraction of *trans*-resveratrol from peanut would have to include protective measures such as absence of light, use of inert atmosphere, or addition of an antioxidant with higher antioxidative activity than *trans*-resveratrol (*25*).

In their study of the efficiency of extraction methods for soy isoflavones, Achoun et al. (*26*) reported that the use of sonication

* Corresponding author: telephone (256) 372-4166; fax (256) 372-5432; e-mail lloyd.walker@email.aamu.edu.
[†] Alabama A&M University.
[‡] United Nations Development Project.

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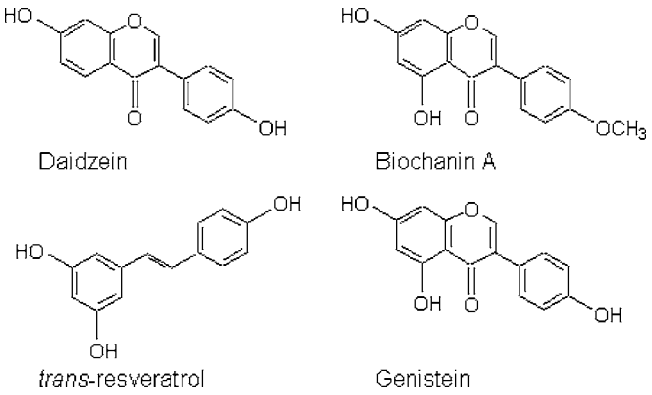

Figure 1. Structures of the isoflavones daidzein, genistein, and biochanin A and the stilbene *trans*-resveratrol.

for 15 min was as effective as five sequential extractions by vigorous mixing at room temperature for 2 h. The efficiency of ultrasonication can be explained by the finding that sonication simultaneously enhances the hydration and fragmentation process while facilitating mass transfer of solutes to the extraction solvent without significant decomposition of the solvent (*27*). Also, the microwave-assisted solvent extraction technology (MAE) has been shown to be more advantageous over conventional extraction methods, particularly the time-consuming Soxhlet (reflux) method and has been applied in area of extraction of phytochemicals (*28*−*30*). The Soxhlet extraction method has been applied to isoflavone extraction from soy; however, it has been shown that extraction at high temperatures causes changes in isoflavone composition due to conversion of the malonyl forms to acetyl forms or β-glycosides (*23*, *24*).

Due to differences in the compounds' solubilities in different solvents, extraction solvents also play an important role in extraction efficiency. A previous study comparing the effect of three solvents (acetonitrile, ethanol, and methanol) on isoflavone extraction showed that the addition of either water or hydrochloric acid greatly improved the extraction efficiency as opposed to using the solvents alone, since this increased the polarity index of the solvent (*31*, *32*). However, it was also shown that acidification of the extraction solvent favored chemical transformations of the isoflavones (*32*). In a study comparing the extraction efficiencies of polyphenols from peanut skin by use of water, 80% ethanol, or 80% methanol, the authors found 80% aqueous ethanol to be the most efficient of the three solvents (*33*).

The objective of this study was to compare four common methods of extracting phytochemicals from nondefatted and defatted peanut samples, with the purpose of evaluating the efficiencies of these methods in the extraction of selected free isoflavones (daidzein, genistein, and biochanin A) and *trans*-resveratrol from peanuts. This will provide researchers with information on appropriate extraction method for studies on the phytochemical composition of runner peanuts, thus facilitating the provision of information necessary for studies regarding the establishment of peanut consumption levels.

MATERIALS AND METHODS

Pure standards of *trans*-resveratrol, genistein, daidzein, and biochanin A were purchased from Sigma Chemical Co., St. Louis, MO. Ethanol, acetonitrile, glacial acetic acid, water (HPLC-grade), and *n*-hexane were purchased from Fisher Scientific Inc., Pittsburgh, PA. Runner peanuts were provided by Golden Peanut Co. Equipment used included a Robot Coupe Blixer RSI BX3 processor (Robot Coupe USA, Inc.), a Carver Mechanical Press (Carver Inc., Wabash, IN), a rotary evaporator (Büchi Labortechnik, AG), a Sharp Carousel 700 W microwave oven (Sharp Electronics Corp.), a high-performance infrared thermometer (Cole-Palmer Instrument Co., Vernon Hills, IL), an ultrasonicator (model 4001H, Zenith Ultrasonic Inc.), and a Soxtec system HT6 (model 1043, Tecator Inc.). A Varian Prostar gradient liquid chromatography system (Varian Inc, Walnut Creek, CA) with a UV/visible detector coupled to a Star Workstation 5.52 was used for HPLC analysis with a C_{18} reverse-phase column, 250 mm length × 4.6 mm i.d., 5 μm particle size (Zorbax SB C18, Agilent Technologies Inc., Newcastle, DE).

Sample Preparation. Five hundred grams of peanut kernels with the skin intact was frozen with liquid nitrogen prior to milling via a Robot Coupe processor. Half of the milled sample was then defatted by use of the Soxtec system based on the AOAC (1990) official method of analysis for fat determination (*34*). Another 250 g of whole peanuts with the skin was mechanically defatted by use of the Carver press, after which the crushed peanuts were milled. All milled samples were stored in airtight amber jars at −20 °C prior to extraction. Aqueous ethanol (80%) was used for the extraction of phytochemicals from the samples by the four methods of extraction described below.

Sample Extraction: *(A) Stirring Method.* Ten grams each of the milled peanut samples [nondefatted peanuts (A), mechanically defatted peanuts (B), and hexane-defatted peanuts (C)] were suspended in 100 mL of 80% aqueous ethanol (*23*, *24*) in amber jars. The suspensions were stirred for 2 h at room temperature and then centrifuged at 1200*g* for 20 min at 20 °C. The supernatants were evaporated to dryness in a rotary evaporator at 38 °C, and each residue was resuspended in 5 mL of extraction solvent and stored at 4 °C prior to HPLC analysis.

(B) Sonication Method. Ten grams each of the milled peanut samples (A, B, and C) were suspended in 100 mL of 80% aqueous ethanol in mason jars. The jars were placed in a sonication tank $^3/_4$ filled with water. The suspensions were sonicated for 2 h in a 450 W ultrasonicator at combined frequencies of 25, 40, and 80 kHz. Each suspension was then centrifuged at 1200*g* for 20 min at 20 °C and the supernatants were evaporated to dryness in a rotary evaporator at 38 °C. The residues were resuspended in 5 mL of extraction solvent and stored at 4 °C prior to HPLC analysis. Since the temperature of the sonication tank could not be controlled, a separate experiment was conducted in which the change in temperature of the peanut suspension during sonication was determined. The temperature was monitored every 30 min for 3 h.

(C) Soxtec Method. The Soxtec system HT6 (Tecator HT Inc.) was used for extraction of phytochemicals from the peanut samples. Five grams each of the milled peanut samples (A, B, and C) were weighed into extraction thimbles and covered with cotton wool. Into each Soxtec extraction cup, 50 mL of 80% aqueous ethanol was added. Polyphenols were extracted from the samples via the recommended procedure for sample extraction with ethanol as the solvent by setting the unit at the boiling position for 30 min and then at the rinsing position for 1 h. The extracts were then allowed to cool to room temperature and evaporated to dryness in a rotary evaporator at 38 °C. The residues were each resuspended in 5 mL of 80% aqueous ethanol and stored at 4 °C prior to HPLC analysis.

(D) Microwave-Assisted Sonication Method. Ten grams each of the milled peanut samples (A, B, and C) were suspended in 100 mL of 80% aqueous ethanol in mason jars and sonicated for 2 h in a 450 W ultrasonicator at combined frequencies of 25, 40, and 80 kHz. The suspensions were irradiated in a 700 W microwave oven until a temperature of 70 °C was attained and then irradiated intermittently for a total of 30 s with power off for 10 s in between 6 s of irradiation. The temperature was monitored with a calibrated infrared thermometer. After irradiation, the suspensions were centrifuged at 1200*g* for 20 min at 20 °C and each supernatant was evaporated to dryness in a rotary evaporator at 38 °C. The residues were each resuspended in 5 mL of 80% aqueous ethanol and stored at 4 °C prior to HPLC analysis.

HPLC Analysis. Aliquots of sample extracts were filtered through a nylon filter (45 μm) prior to HPLC analysis. The method of Lamuela-Raventos et al. (*35*) was modified by adjusting the gradient elution profile to correct for the coelution of genistein and biochanin A (**Figure 2**). Method validation (linearity, precision, accuracy, and limits of detection and quantitation) was performed with pure standards. The sample injection volume was 50 μL. A constant flow rate of 1.5 mL/

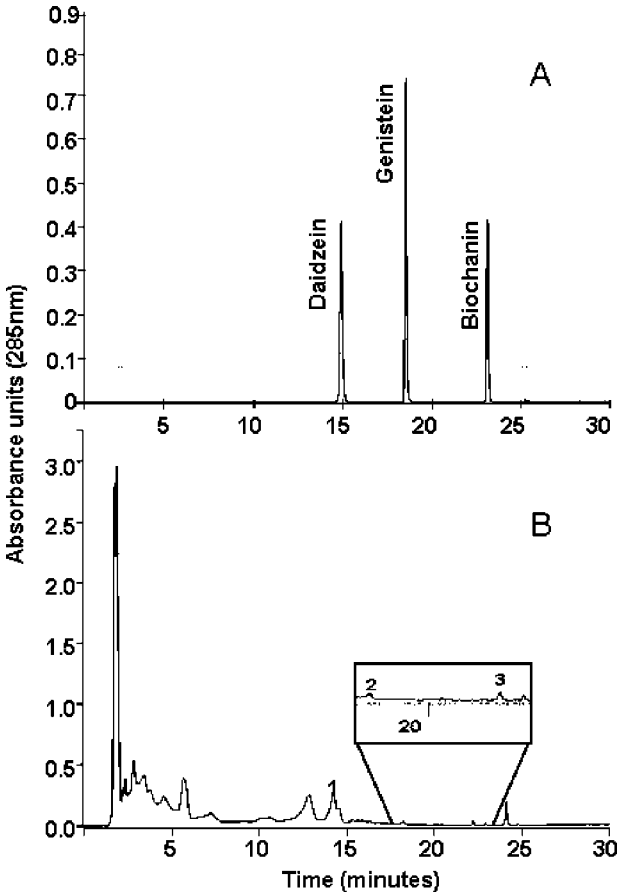

Figure 2. HPLC chromatograms of isoflavones in the standard mixture (**A**) and in peanut sample extract (**B**), identified by their retention times at 285 nm: daidzein (peak 1), genistein (peak 2), and biochanin A (peak 3).

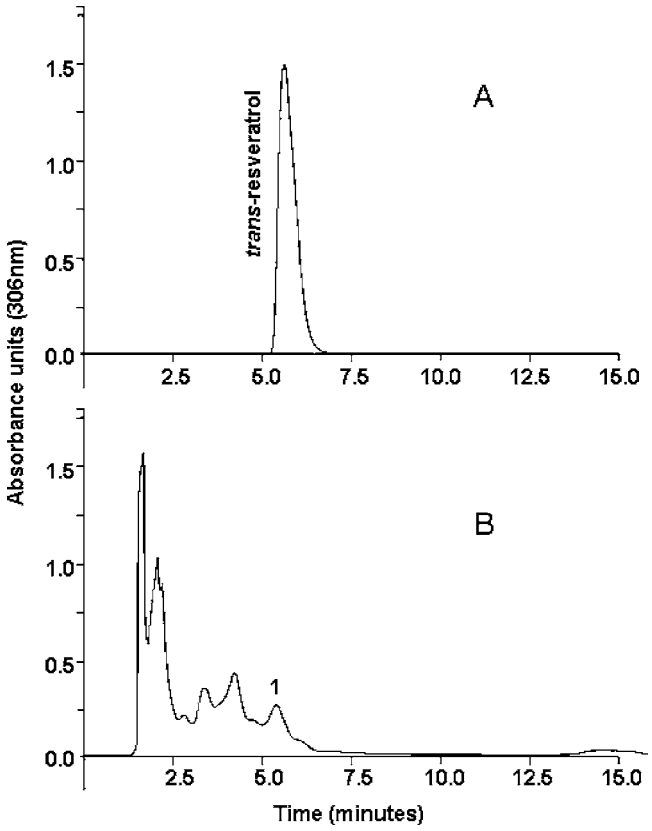

Figure 3. HPLC chromatograms of pure *trans*-resveratrol standard (**A**) and peanut sample extract (**B**) showing *trans*-resveratrol (peak 1) identified by its retention time at 306 nm.

min was used with two solvents (A and B). Solvent A was 80% acetonitrile and 20% solvent B. Solvent B consisted of 52.6 mL of glacial acetic acid in 900 mL of water (HPLC-grade). The elution profile was as follows: 0 min, 22% A, 78% B; 10 min, 22% A, 78% B; 25 min, 100% A, 0% B.

The eluates were monitored at 285 nm for isoflavones (**Figure 2A**) and at 306 nm for *trans*-resveratrol (**Figure 3A**). After each analysis, 100% acetonitrile was passed through the column for 5 min prior to re-equilibration with 22% A and 78% B for 10 min. Quantification of analytes was done by reference to pure standards (area of standard versus concentration in micrograms per milliliter).

HPLC Method Validation: *(A) Determination of Accuracy.* Accuracy of the modified HPLC method was established by spiking the peanut samples with stock solutions (1000 μg/mL) of biochanin A and *trans*-resveratrol at three different levels (0.5, 1.0, and 1.5 μg/mL) in triplicate. The analytes were estimated by HPLC analysis and the accuracy was expressed as percent analyte recovered at the three different levels with their respective standard deviations.

(B) Determination of Precision. Precision was evaluated by injecting 50 μL each of pure standard solutions (5 μg/mL) of biochanin A, daidzein, genistein, and *trans*-resveratrol 10 times. The method precision was expressed as relative standard deviation (RSD) or percent coefficient of variation (% CV) of data.

(C) Determination of Linearity. Linearity of the isoflavones and *trans*-resveratrol standard curves was determined at concentrations of 2, 4, 6, 8, and 10 μg/mL (biochanin A and genistein) or 20, 40, 60, 80, and 100 μg/mL (daidzein and *trans*-resveratrol). Linear regression analysis was performed on the data, and linearity of the standard curves is reported as correlation coefficients.

(D) Determination of Limits of Detection and Quantitation. The limit of detection (LOD) and limit of quantitation (LOQ) were determined by analyzing pure standards (stock concentrations of 2 μg/mL) that were diluted in series to obtain the lowest concentration of analyte that gave a measurable response with a signal-to-noise ratio of 3.

Statistical Analysis. Statistical analysis (ANOVA) of data was done with SAS version 9.1, and where significant, mean separations were done using Tukey's Studentized test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Method Validation: *(A) Linearity.* A linear relationship was observed for all the analytes investigated. The correlation coefficients (r) were 0.9989 for *trans*-resveratrol, 0.9957 for daidzein, 0.9960 for biochanin A, and 0.9973 for genistein.

(B) Precision. The CV values for precision by repeatability ($n = 10$ injections) for *trans*-resveratrol, daidzein, genistein, and biochanin A were 0.64%, 1.56%, 2.75%, and 1.86%, respectively.

(C) Accuracy. The accuracy of the method at 285 and 306 nm was investigated by means of a recovery experiment and expressed as percent analyte recovered at the three different levels with their respective standard deviations. The percentage recoveries were 85.52% $\pm$ 5.84%, 93.16% $\pm$ 2.10%, and 98.34% $\pm$ 2.20% for biochanin A and 87.91% $\pm$ 6.09%, 78.76% $\pm$ 4.19%, and 93.89% $\pm$ 3.24% for *trans*-resveratrol.

(D) Limits of Detection. Detection limits of 0.1, 0.05, 0.025, and 0.005 μg/mL were obtained for daidzein, biochanin A, genistein, and *trans*-resveratrol, respectively.

Results: The results obtained showed that significantly (p value) higher amounts of isoflavones and *trans*-resveratrol were extracted from the hexane-defatted and mechanically defatted peanuts compared to the nondefatted peanuts by the MAS method (**Table 1**). The amounts of daidzein extracted by the four extraction methods were significantly (p value) lower in the nondefatted peanuts (**A**) than those extracted from the

Table 1. Biochanin A, Daidzein, Genistein, and *trans*-Resveratrol Levels in Extracts from Nondefatted, Mechanically Defatted, and Hexane-Defatted Peanuts by Four Methods of Extraction[a]

extraction method	amount, mg/100 g of peanut (dwb)			
	biochanin A	daidzein	genistein	*trans*-resveratrol
		(A) Nondefatted Peanuts		
Soxtec	0.043 ± 0.006 a	0.130 ± 0.016 a	0.024 ± 0.005 a	0.239 ± 0.058 b
MAS	0.045 ± 0.005 a	0.103 ± 0.028 a,b	0.020 ± 0.017 a	0.589 ± 0.152 a
sonication	0.038 ± 0.003 a	0.052 ± 0.015 c	0.013 ± 0.003 a	0.510 ± 0.166 a,b
stir	0.042 ± 0.003 a	0.072 ± 0.010 b	0.030 ± 0.009 a	0.784 ± 0.054 a
		(B) Mechanically Defatted Peanuts		
Soxtec	0.058 ± 0.007 b,c	1.063 ± 0.173 a	0.140 ± 0.054 a	0.087 ± 0.023 c
MAS	0.137 ± 0.013 a	0.327 ± 0.028 b,c	0.172 ± 0.038 a	1.953 ± 0.228 a
sonication	0.037 ± 0.012 c	0.125 ± 0.020 c	0.013 ± 0.003 b	0.331 ± 0.196 c
stir	0.067 ± 0.003 b	0.480 ± 0.083 b	0.030 ± 0.013 b	1.120 ± 0.307 b
		(C) Hexane-Defatted Peanuts		
Soxtec	0.133 ± 0.034 a	1.753 ± 0.756 a	0.227 ± 0.109 a	0.173 ± 0.063 c
MAS	0.088 ± 0.008 a,b	0.183 ± 0.076 b	0.082 ± 0.024 b	1.998 ± 0.226 a
sonication	0.037 ± 0.003 c	0.242 ± 0.024 b	0.057 ± 0.018 b	0.741 ± 0.140 b
stir	0.060 ± 0.000 b,c	0.195 ± 0.028 b	0.028 ± 0.008 b	1.128 ± 0.271 b

[a] Values are means ± SD for three replications and duplicate analysis on dry weight basis (dwb). Means with the same letter in each column are not significantly different ($p < 0.05$).

defatted peanut samples (**B**, **C**), suggesting that defatting the peanut may enhance the extraction efficiency; however, the amount of genistein extracted by the sonication and stir methods from the mechanically defatted peanuts (0.130 and 0.030 mg/100 g) were not significantly different from the amounts extracted by these same methods from the nondefatted peanut. This implies that factors other than the absence of lipids may be responsible for the higher amounts of analytes observed in the defatted samples, especially since a similar observation was made about the amount of biochanin A extracted by sonication in both the hexane-defatted and mechanically defatted peanuts (0.037 mg/100 g) compared to the nondefatted peanut (0.038 mg/100 g). Also, lower amounts of *trans*-resveratrol were extracted by the Soxtec method from the defatted peanuts (0.087 and 0.173 mg/100 g) compared to the nondefatted peanuts (0.239 mg/100 g). Factors such as interference by other compounds (proteins and sugars) present in peanut and stability of analytes may also be responsible for the variations observed.

The amounts of isoflavones extracted by the Soxtec and MAS methods from the hexane-defatted and mechanically defatted peanuts were significantly (p value) higher compared to the nondefatted peanuts (**Table 1**). Although the amount of genistein extracted by the Soxtec method compared to the other methods was significantly higher in the defatted peanuts, there were no significant differences in the amounts of genistein extracted from the nondefatted peanut by any of the methods investigated. This may be due not only to the amount of sample used (w/w), taking the lipid content into consideration, but also to partitioning of the compounds into the extraction solvent in the presence of lipids. This was also the case for the methoxy derivative, biochanin A (**Table 1**). The amounts of genistein and biochanin A extracted by the sonication and stirring methods in all peanut samples were not significantly ($p < 0.05$) different. This may be because both methods are nonthermal compared to the Soxtec and MAS methods. Results also showed that a significantly ($p < 0.05$) higher amount of daidzein was extracted by the Soxtec method, while the MAS method extracted significantly ($p < 0.05$) higher amounts of *trans*-resveratrol from both the defatted and nondefatted peanuts (**Table 1**).

Previous studies on the isoflavone content of legumes reported the presence of daidzein, genistein, and biochanin A in peanut and peanut products. Liggins et al. (*18*) determined the levels of daidzein and genistein in fruits and nuts. In their study, dried peanut samples were extracted by 15 min of sonication in 80% aqueous methanol with overnight soaking prior to enzyme hydrolysis for the determination of total daidzein and genistein content. Although the amount of total genistein extracted from their peanut sample (0.016 mg/100 g) was comparable to amounts obtained from our nondefatted peanuts by all four extraction methods (0.013−0.030 mg/100 g), the amount of daidzein extracted by Liggins et al. (*18*) was relatively lower (0.008 mg/100 g) than the amounts extracted from the nondefatted peanut by all four methods (0.052−0.130 mg/100 g). It would be expected that with the enzymatic hydrolysis of the glycosidic conjugates of daidzin and genistin in their peanut sample, the amount of aglycons extracted by Liggins and coworkers would be relatively higher compared to the amount extracted from the nonhydrolyzed peanut samples in this study. However, this was not the case. This may be a result of partitioning of the aglycons in ethyl acetate after hydrolysis (*18*). A previous study on the effect of solvent polarity on extraction efficiency of isoflavones reported that extraction solvents with a polarity index (PI) between 6.7 and 7.4 were suitable for extracting isoflavones (*32*). Ethyl acetate has a PI of 4.3 and is therefore not as polar as 80% aqueous methanol (PI 7.1) or 80% aqueous ethanol (PI 5.6).

Mazur et al. (*15*), in their determination of isoflavones (genistein, daidzein, and biochanin A) and lignans in legumes, extracted isoflavones from legume powders with diethyl ether and quantified them in purified extracts. The amounts of biochanin A (0.006 mg/100 g) and daidzein (0.050 mg/100 g) extracted were relatively lower than those obtained in this study (**Table 1**). Also, since hydrolyzed samples were used by Mazur et al. compared to the samples used in this study, it is expected that higher amounts of aglycons would be extracted. This may be due to the use of diethyl ether as solvent for the extraction of these compounds. However, this was not the case with genistein. The amount of genistein extracted by Mazur et al. (*15*) was relatively higher (0.083 mg/100 g) than those obtained from the nondefatted peanuts used in this study (**Table 1**). This was expected since isoflavones primarily occur in legumes as glycosidic conjugates and in this study nonhydrolyzed samples were used. However, the Soxtec and MAS extracts of our defatted peanuts (**Table 1**) gave amounts of genistein (0.082−0.227 mg/100 g) that were comparable to that reported by Mazur et al. (*15*). The higher amounts of isoflavones observed in the

defatted peanuts may be attributed to the apparent increase in the amount of peanut meal used (w/w) for extraction in comparison to the nondefatted peanuts, which have about 40% lipid content. Also, there is thermal conversion of the conjugate glycosides to β-glycosides and aglycons (*21*) in the Soxtec and MAS extracts.

Trans-resveratrol was extracted from all peanut samples investigated, and results showed that significantly ($p < 0.05$) higher amounts of resveratrol were extracted by the MAS method. This suggested that the MAS method was more effective in the extraction of *trans*-resveratrol from peanuts compared to sonication alone or stirring. This may be due to the fact that while sonication causes a further disruption of the cells, thereby facilitating mass transfer of the cellular components, microwave heating may increase the solubility of *trans*-resveratrol in the solvent. Although the Soxtec extraction method also involved heating, this method extracted the least amount of *trans*-resveratrol. This may be attributed to the extended period of exposure (1.5 h) of the extract to heat, thereby resulting in loss of the compound by degradation and/or oxidation.

Previous studies on the *trans*-resveratrol content of peanuts reported amounts that were relatively lower than our finding. Sanders et al. (*16*) extracted *trans*-resveratrol from three market types (15 cultivars) of peanut by homogenization in 80% aqueous ethanol. The amount extracted ranged from 0.003 to 0.014 mg/100 g. Also, Ibern-Gomez et al. (*17*) extracted *trans*-resveratrol from different commercial peanut products using 80% aqueous ethanol. They reported amounts that were relatively lower (0.026−0.075 mg/100 g) than amounts extracted from peanuts used in this study (0.239−0.784 mg/100 g). Previous studies have shown that several factors affect the *trans*-resveratrol content of peanuts, such as infestation (*36*), maturity (*37*, *19*), and nut size (*38*). All these factors may have played a role in the observed variations in amounts of *trans*-resveratrol extracted.

The results obtained from this study have shown that the MAS and Soxtec methods extracted significantly (*p* value) higher amounts of the selected phytochemicals. However, the higher levels of the isoflavones observed by these extraction methods, which involved heat treatment, may be attributed to the conversion of conjugate glycosides to the aglycons as previously reported (*23*, *24*). However, Coward et al. (*39*) reported that although there was extensive conversion of the isoflavone malonyl conjugates to their corresponding β-glucosides with increased extraction temperature, the total amount of isoflavone extracted was constant. Among the other extraction methods, sonication and stirring methods, there were no significant differences in the levels of isoflavones extracted except for daidzein, genistein, and *trans*-resveratrol in the mechanically defatted peanuts, which gave significantly (*p* value) lower amounts with the sonication method. Although thermal degradation of genistein and daidzein has been shown to occur at 70 °C and above (*40*, *41*), the process of sonication resulted in a significant increase in temperature, with the final temperature after 2 h of sonication (34 °C) being well below 70 °C. It is also below the temperature for concentration of the extracts (38 °C) in the rotary evaporator.

Because no one method can adequately extract all target analytes from a sample matrix, the choice of an extraction method will greatly depend upon the analyte(s) of interest. The MAS and Soxtec methods may be used if the total isoflavone content is to be quantified, while sonication or stirring, which involve less thermal effects, may be the method of choice when quantifying the isoflavone conjugates and aglycons in peanuts. There is therefore a need to standardize the extraction protocol for quantitation of these bioactive compounds.

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