International Food Research Journal 29(5): 1110 - 1119 (October 2022)
Journal homepage: *http://www.ifrj.upm.edu.my*

Application of Folin-Ciocalteau colorimetric method in the determination of total tannin in maize and soybean food products

[1,2]*Adegbusi, H. S., [2]Ismail, A., [2]Mohd Esa, N. and [3]Azuan Mat Daud, Z.

[1]Department of Biological Sciences, Nigeria Police Academy Wudil, P.M.B 3474, Kano, Nigeria
[2]Department of Nutrition, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Department of Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Article history

Received:
12 April 2021
Received in revised form:
1 October 2021
Accepted:
23 February 2022

Keywords

Folin-Ciocalteu colorimetry, plant-based food, total phenolics, total tannin, validation parameters

Abstract

Yellow maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.) are local raw materials used in the formulation of complementary foods in Nigeria. The presence of antinutritional factors such as tannins in them could disrupt the nutritional status in infants and young children, thus leading to malnutrition. However, data on the total tannin contents in maize, soybean, and their products remain limited. This could be due to the lack of a fast, accurate, and inexpensive analytical method for tannin determination. The present work thus evaluated the Folin-Ciocalteu (FC) assay for the accurate quantification of total tannin from yellow maize, soybean, and their products. Techniques including soaking, dehulling, oven-drying, boiling, and frying were used to process the raw materials, prior to grinding and subsequent formulation of their products. The FC method was validated to quantify the total tannin contents from extracts of tested samples by ultraviolet-visible (UV-Vis) spectrophotometry. The original extracts from the tested samples, and external standards from tannic acid and total phenolics (µg/µL) were used for method validation. The method validation showed that the instrumental response to standard tannic acid and the investigated analytes were specific, linear (R^2 = 0.998), precise (% CV < 20%), and accurate (recovery = 91%). The limits of detection (LOD) and limits of quantification (LOQ) were 0.03 and 0.09 µg/µL, respectively. The validation complied with the requirements to ensure the reliability of the results. The combined processing techniques were also effective in reducing the total tannin content of maize (0.213 to 0.041% TAE) and soybean (0.257 to 0.064% TAE) by 81 and 75%, respectively. The present work demonstrated the suitability of the FC method as an analytical tool for the quantification of total tannin from plant-based food products.

DOI
https://doi.org/10.47836/ifrj.29.5.13

Introduction

Tannins are polyphenolic secondary plant metabolites that occur as compounds of relatively high molecular weight, ranging between 500 - 20,000 Dalton (Da), and capable of binding and precipitating alkaloids, saccharides, proteins, and other macromolecules from aqueous solutions (Krzyzowska *et al.*, 2017). Tannins' structural complexity and diversity result in their broad classification into hydrolysable tannins (HTs) and condensed tannins (CTs), or proanthocyanins (PAs) (Engstrom, 2016; Delimont *et al*., 2017).

In plants, tannins are essential as a defence mechanism against pathogens and herbivores, and growth control of nearby plants (Furlan *et al.*, 2010). Although tannin-rich diets may have medicinal effects in relation to cardiac diseases and cancers due to the antioxidant activity of tannins, their antinutritional property in the nutritional context is of concern in the human population, especially among those less than five years old in developing countries (Delimont *et al.*, 2017). Tannins exert antinutritional effects through protein precipitation, inhibition of digestive enzymes, and micronutrient chelation (Khasnabis *et al.*, 2015), thus causing a reduction in

*Corresponding author.
Email: khalidadegbusi@yahoo.com

protein digestibility, micronutrient bioavailability, and net metabolisable energy (Khasnabis *et al*., 2015). The reduction in bioavailability of minerals in the human system by tannins is well documented. For instance, the intake of large amounts of cereal, legume, tannic acid, tea, and other tannin-rich foods has been found to reduce iron availability before absorption due to the formation of an insoluble tannin-iron complex, and similarly, cases of iron deficiency anaemia (IDA) aggravation were reported in human populations whose diet staples were high in tannin (Delimont *et al.*, 2017).

Determination of tannins can be achieved by allowing a suitable aqueous solution of methanol, ethanol, acetone, ethyl acetate, or their mixture to pass through powdered plant tissue, and dissolve soluble phenolics present therein (Sieniawska and Baj, 2017). While methanol is often preferred to extract tannins of low molecular weight, acetone is suitable to extract tannins of high molecular weight due to less reactivity with acetone (Sieniawska and Baj, 2017). The powdered sample is recommended for pre-treatment before proper extraction with non-polar organic solvents such as *n*-hexane and petroleum ether, or polar dichloromethane to remove lipids and chlorophylls, and to prevent enzymatic reaction that may interfere with tannin recovery (Arapitsas, 2012). Furthermore, the yield of solvent extraction of tannins can be enhanced by subjecting the solution to sonication (Bouaoudia-Madi *et al.*, 2019). Sonication is responsible for the disruption of the cell walls and reduction of the particle size, thus accelerating the dissolution of the analytes into the extraction solvent (Annegowda *et al.,* 2011). With sonication-assisted extraction, Lu *et al.* (2012) and Sousa *et al*. (2016) found 27.2 - 630 mg/g of tannin yield. Other extraction methods and their tannin content yields (mg/g) are as follows: solid-liquid or traditional extraction, 0.609 - 850 (Chowdhury *et al.*, 2010; Aires *et al*., 2016); supercritical fluid extraction, 26.4 - 770 (Ashraf-Khorassani and Taylor, 2004; Talmaciu *et al*., 2016); pressurised water extraction, 52.9 - 382 (Vergara-Salinas *et al*., 2013; Ravber *et al*., 2015); and microwave-assisted extraction, 4.1 - 529 (Huma *et al.*, 2018; Maškovic *et al*., 2018). Following extraction, tannins are quantified using an appropriate analytical technique. In terms of the Folin-Ciocalteu (FC) method, ultraviolet-visible (UV-Vis) spectrophotometry is the preferred method for tannin quantification due to its speed, simplicity, low-cost, and practicality (Galvão *et al*., 2018). Also, UV-Vis spectrophotometry allows for the quantification of the total tannin content, both monomeric and polymeric tannins, thus avoiding the underestimation of the analytical response (Galvão *et al.*, 2018). Although other specific techniques such as high-performance liquid chromatography (HPLC) may be used for tannin quantification, only monomers of tannins can be quantified (Galvão *et al*., 2018).

Maize and soybean are locally available foods in Nigeria, used for making and formulating complementary foods. Some researchers have worked extensively on their nutrient composition but not specifically on their phenolic components, especially tannins, which are known to hinder essential mineral and protein bioavailability. The immobilisation of nutrients by tannins is a contributing factor to malnutrition among 6 - 23 months old children in developing countries including Nigeria, where tannin-rich maize, soybean, and their products are consumed as staples. In view of tannin's antinutritional effect on human nutrition, it is essential that a fast, simple, and efficient technique be adopted as a screening strategy for monitoring its safe level, and managing its content in food products commonly consumed by the human population, perhaps to forestall its antinutritional effects in humans, especially during complementary feeding when malnutrition is prevalent in Nigeria and other low-income countries. In the present work, crude extracts from maize, soybean, and their products were quantified for total tannin content (TTC) using the FC method. The analytical method was also validated using concentrations of standard tannic acid and the crude extract from tested samples, as well as total phenolics (TP, µg/µL). The present work will provide the first line data on TTCs of flours from yellow maize, soybean, and their products, based on the FC method.

Materials and methods

Yellow maize and soybean were purchased from the Nigerian market for tannin analysis. Samples were identified at Bayero University, Kano and given the following voucher numbers: yellow maize (BUKHAN0277), soybean (BUKHAN0088).

Analytical grade reagents were used including FC reagent (2*N*), polyvinyl pyrrolidone (polyvinyl polypyrrolidone, PVPP), and standard tannic acid purchased from Sigma Aldrich, USA. Acetone and

anhydrous sodium carbonate were purchased from R&M Chemical, Malaysia. Sulphuric acid was purchased from Fischer Scientific, Malaysia. Distilled water was obtained from the Nutrition Laboratory, Universiti Putra Malaysia (UPM), Malaysia.

Raw material processing

Maize and soybean flours were produced following the procedures described by Ibironke *et al*. (2014) and Achidi *et al*. (2016). Soybean seeds were cleaned for debris and stones. The cleaned soybeans were soaked for 3 h, dehulled, boiled for 0.25 h, oven-dried at 80°C for 24 h, and roasted under an open flame for 0.5 h until golden brown. Maize was cleaned, washed with tap water, and oven-dried at 80°C for 24 h. Dried samples were dry-milled and sieved using a laboratory sieve of 710 µm aperture to obtain large particle-free and smooth maize flour (MF) and soybean flour (SBF). These flours were further used to formulate maize-soybean flour (MSF), in a ratio of 72:28 (g/100 g), that met the 16% protein requirement in complementary food, using the material balance equation as described by Chiba (2009). Additional two products, unprocessed maize flour (MFunp) and unprocessed soybean flour (SBFunp) were also prepared, which did not undergo soaking, dehulling, boiling, oven-drying, or roasting. These flours were separately filled into airtight polyethylene bags, and stored in tightly covered aluminium tins. These flours were kept in a cool, dried cupboard prior to the analytical procedure.

TTC analysis

FC colorimetric method

TTC quantification was carried out following the procedures described by Zaklouta *et al*. (2011), with minor modification to the usage of 14.8 g of anhydrous sodium carbonate dissolved in 200 mL of distilled water.

FC reagent is a mixture of phosphotungstic and phosphomolybdic acid. In alkaline medium, phosphomolybditungstic acid oxidises total phenols in a tannin-containing sample and becomes reduced to a mixture of blue oxides of tungsten and molybdenum. The absorption at 725 nm was proportional to the total quantity of phenolic compounds originally present in the sample. This method was used to determine the total free phenolic groups, comprising of either HT or PA (Sieniawska and Baj, 2017). To determine the amount of TTC present in the sample, PVPP was added to precipitate tannins, and thereafter, TTC content was established by the difference between the reaction product of total polyphenols and the reaction product of residual non-adsorbed or non-tannin polyphenols (Galvão *et al*., 2018).

Sample extraction and ultrasound treatment

Solvent extraction of tested samples was conducted in an ultrasonic bath (Powersonic 405, Korea) with the working frequency fixed at 20 kHz, as described by Rusydi and Azrina (2012). About 500 mg dried ground sample of a food product, and 5 mL of 50% aqueous acetone were added into 20 mL centrifuge tube, and subjected to extraction in ultrasonic water bath at 25°C for 0.5 h. The temperature was set and maintained constant by circulating external cold water. After extraction, the tube was centrifuged for 10 min at 3,000 rpm and 4°C. The supernatant, original extract (oE) was collected into a new 20 mL tube, and kept in a 4°C refrigerator prior to tannin analysis. Triplicate samples of each product were extracted under dim light.

TTC determination

Determination of total phenolic content (TPC)

Into a 20 mL tube, 450 µL of distilled water and 50 µL of a sample's original extract (oE) were added. Subsequently, 250 µL of FC reagent followed by 1,250 µL of sodium carbonate solution were added. The mixture was vortexed at each step of addition. After 40 min of incubation in the dark, the absorbance was measured at 725 nm using a UV-Vis spectrophotometer (UV-180, Japan). The concentration of the total phenol was derived through extrapolation from the standard curve shown in Figure 1. Distilled water in a 1 cm path length quartz cell was used to zero the spectrophotometer, and triplicate extracts of each product were measured. The TPC was calculated using Eq. 1:

$$\text{TPC (\% tannin acid equivalent)} = [(A / 0.05) / 1000] \times 1 \quad \text{(Eq. 1)}$$

where, A = concentration (µg/µL) of the aliquot obtained from the standard curve, and 0.05, 1000, and 1 = conversion factors to percentage.

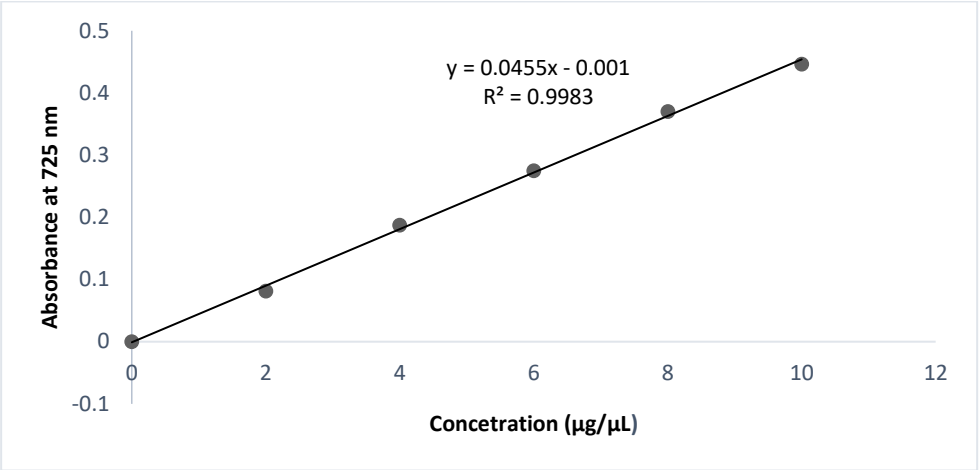

Figure 1. Calibration curve of standard tannic acid.

Determination of residual non-adsorbed phenolic content (RNAPC)

A total of 100 mg of PVPP and 1 mL of distilled water were added into a 20 mL tube. Subsequently, 1 mL of oE of a sample was added. The mixture was vortexed at each step of addition. The mixture was incubated at 40°C for 10 min. Then, the tube was centrifuged at 3,000 rpm and 4°C for 10 min. After centrifugation, 100 µL of the supernatant was placed in a 20 mL tube containing 400 µL distilled water, and vortexed. Subsequently, 250 µL FC reagent was added, followed by 1,250 µL sodium carbonate solution, and vortexed. The preparation was kept in the dark for incubation. After 40 min of incubation, the absorbance was measured at 725 nm using a UV-Vis spectrophotometer. The concentration of RNAPC in the supernatant was derived through extrapolation from the standard curve as shown in Figure 1. Distilled water in a 1 cm path length quartz cell was used to zero the spectrophotometer and triplicate extracts of each product were measured. The RNAPC was calculated using Eq. 2:

RNAPC (% tannic acid equivalent) =
$[(B / 0.1) / 1000] \times 2$ (Eq. 2)

where, B = concentration (µg/µL) of the aliquot obtained from the standard curve, 0.1 and 1000 = conversion factors to percentage, and 2 = two-fold dilution.

The TTC was calculated using Eq. 3:

TTC (% tannic acid equivalent) =
% (TPC − RNAPC) (Eq. 3)

Tannic acid standard curve preparation

A stock of standard tannic acid solution (mg/mL) was prepared by dissolving 15 mg of standard tannic acid in 15 mL of distilled water. Subsequently, 1 mL of stock solution was dissolved in 10 mL of distilled water for working solution (0.1 mg/mL = 0.1 µg/µL). To draw the standard curve, different concentrations of standard solution, ranging from 2 - 10 µg/µL, were prepared from the working solution following the procedures described by Zaklouta *et al*. (2011). Accurately, 20, 40, 60, 80, and 100 µL aliquots of tannic acid working solution were diluted to the required concentrations of 2, 4, 6, 8, and 10 µg by 480, 460, 440, 420, and 400 µL of distilled water, respectively, prior to adding 250 µL of FC reagent and 1,250 µL of sodium carbonate solutions, and vortexed. The blank standard solution consisted of a mixture of 500 µL of water, 250 µL of FC reagent, and 1,250 µL of sodium carbonate without a standard sample. The whole mixture was allowed to incubate in the dark for 40 min, and the absorbance for tannic acid level was measured using a UV-Vis spectrophotometer at 725 nm. Distilled water in 1 cm path length quartz cell was used to zero the spectrophotometer, and triplicate absorbance for each level of tannic acid standard solution was taken under similar conditions as oE.

Validation of analytical method

To assure the reliability of the results, the FC method used was validated by UV-visible spectrophotometry using analytical performance parameters of specificity, linearity precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ), as recommended by the

International Conference of Harmonization (ICH). All extraction and dilution operations were done under dim light. oE from a tested sample and concentrations of standard tannic acid and TP (µg/µL) were used for the validation, as described in Blainski *et al*. (2013) with modification, for easier quantification and to avoid dilution errors that could produce inaccurate results.

Specificity

Specificity of an analytical procedure is the ability to exactly and specifically measure the substance of interest when other components were present. In the present work, the specificity was determined by adding 20 µL of oE from a tested sample to each level of standard tannic acid concentration (2, 4, 6, 8, and 10 µg/µL), and the subsequent steps as earlier explained, which was used in constructing the standard curve, were followed to plot the specificity curve for tested samples. If the curve on superimposition is parallel to that of linearity, it can be concluded that the method is specific (Blainski *et al.*, 2013).

Linearity

Linearity of an analytical procedure is its ability, within a given range, to provide directly proportional results between target parameter concentration and measurement of instrument response. In the present work, a standard curve was obtained for standard tannic acid over a concentration range of 2 - 10 µg/µL of triplicate measurements at each level, and linear regression was used to calculate the curve.

Accuracy

Accuracy evaluates the degree of closeness between analytical results found in a given sample and the true reference value of that sample, as determined by perfect measurement. Accuracy can be evaluated by certified reference materials (CRM), method comparison, and recovery trials. In the present work, a recovery trial was used to evaluate the accuracy in which 100 µg/µL of oE from MFpr and MSF and 20 µg/µL of oE from unprocessed maize flour (MFunp), unprocessed soybean flour (SBFunp) and processed soybean flour (SBFpr) were spiked with standard tannic acid at different concentration levels of 0.0125, 0.025, and 0.050 µg/µL respectively. The percentage recovery was calculated using Eq. 4:

$$\text{Recovery (\%)} = 100[(AR - A)/R] \quad \text{(Eq. 4)}$$

where, AR = concentration of a sample spiked with a standard of known amount, A = concentration of a spiked-free sample, and R = amount of standard added to the sample. Triplicate samples were tested at each level.

Precision

Precision of an analytical procedure evaluates the proximity of results obtained from a series of measurements of the same homogeneous sample under prescribed conditions. Precision may be calculated through three different levels: repeatability, intermediate precision, and reproducibility. The present work evaluated precision through repeatability of standard tannic acid solutions at 4, 6, and 8 µg/µL, six times respectively, using Eq. 5:

$$P\ (\%) = 100(SD/N) \quad \text{(Eq. 5)}$$

where, P (%) = precision in percentage, SD = standard deviation, and N = mean of the concentrations.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD of an analytical procedure is the lowest amount of target parameter in a sample that can be detected by the apparatus without being quantitated as an exact value. The LOQ of an analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with precision and accuracy under operational conditions. The limits reflect the capacity of the measurement instrument but not the limitation of applied methodology or extraction technique. The limits were calculated from the extrapolation of linearity using Eqs. 6 and 7:

$$LOD = 3.3(STEYX/SL) \quad \text{(Eq. 6)}$$

$$LOQ = 10(STEYX/SL) \quad \text{(Eq. 7)}$$

where, STEYX = standard error of a set of absorbance and its corresponding concentrations, and SL = slope of the standard curve.

Statistical analysis

The TTC of five products was determined in triplicates. IBM SPSS version 25 was used to analyse the data, which was significantly differentiated by the Tukey's Honestly Significant Difference (HSD) test at the $p < 0.05$ level of significance.

Results and discussion

Specificity

Analysis of the specificity test's results, as shown in Figure 2, showed parallel curve lines when the linearity and specificity for MFpr, MSF, and SBFpr were superimposed. This thus indicated that the specificity of the analytical methodology was satisfactory and proved for the standard tannic acid, as the constant shifting of the new curves was proportional to the standard contribution that enabled parallel behaviour.

Linearity

The quantification range for linearity was 2 - 10 µg/µL, with an absorbance ranged from 0.081 - 0.446, thereby giving a regression equation for the relationship between them as $A = (0.046 \times C) - 0.001$. The coefficient of determination (R^2) value was 0.9983, which showed good linearity over the chosen range of standard concentrations as shown in Figure 1.

Precision

The precision of the method used at 4, 6, and 8 µg/µL of standard tannic acid was shown as a percentage coefficient of variation (% CV). The respective CV value of 0.3, 0.2, and 0.4% is shown in Table 1. It showed that the method was precise, and the precision of the instrument used was good and met the requirement for the quantification of TTC from the extracted samples. In addition, the spectrophotometric procedure was precise for standard tannic acid at the three levels of testing as their CVs were less than 5%.

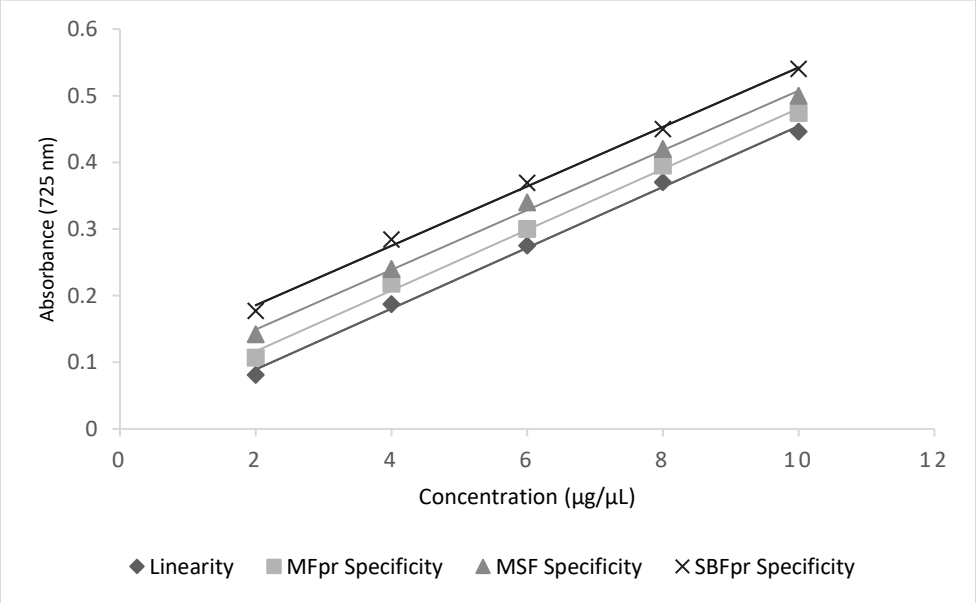

Figure 2. Curves of linearity and specificity for MFpr, MSF, and SBFpr.

Table 1. The repeatability of standard tannic acid (STA) at 4, 6, and 8 µg/µL.

STA (TP, µg/µL)	Absorbance (725 nm, *n* = 6)						M ± SD	CV (%)
4	0.190	0.188	0.184	0.187	0.182	0.185	1.116 ± 0.00	0.3
6	0.267	0.269	0.274	0.270	0.275	0.268	1.623 ± 0.00	0.2
8	0.360	0.370	0.389	0.373	0.369	0.365	2.226 ± 0.01	0.4

M: mean value of sex-duplicate determinations; SD: standard deviation of mean.

Accuracy

The result of the accuracy test, as shown in Table 2, showed a recovery range of 80 - 88, 88 - 92, and 94 - 100% for the lowest, intermediate, and highest levels of tannic acid concentration, respectively. The mean recovery of the samples was 92% with CV of 6.4%, which indicated that the method recovery rate was good, and the method accuracy for quantifying TTC through TP from the samples extracts was good and feasible. The recovery (%) of the analytical procedure showed performance within the limit of recommended values of 80 - 120% for the five tested samples, after fortification with a known amount of standard tannic acid solution. This could be attributed to the concentrations of TP and thus TTC present.

Table 2. Recoveries of TP (µg/µL) from 20 and 100 mg/mL of samples at 0.0125, 0.025, and 0.05 µg/µL standard tannic acid spiked.

Sample A	R (µg/µL)	AR (µg/µL)	Recovery (%, *n* = 3)
MFunp (20 mg/mL) [A] = 0.072 µg/µL	0.0125	0.082	80
	0.025	0.084	88
	0.05	0.109	94
MFpr (100 mg/mL) [A] = 0.137 µg/µL	0.0125	0.148	88
	0.025	0.160	92
	0.05	0.186	99
MSF (100 mg/mL) [A] = 0.207 µg/µL	0.0125	0.218	88
	0.025	0.230	92
	0.05	0.254	95
SBFunp (20 mg/mL) [A] = 0.138 µg/µL	0.0125	0.148	84
	0.025	0.160	88
	0.05	0.188	100
SBFpr (20 mg/mL) [A] = 0.106 µg/µL	0.0125	0.118	96
	0.025	0.129	92
	0.05	0.155	98
Mean value (%)			92
CV			6.2

n: triplicate determination at each concentration level; AR: concentration of sample spiked with standard of known amount; [A] = TP, is A concentration from standard curve; R: amount of tannic acid spiked to the sample A.

LOD and LOQ

For the standard tannic acid concentrations, LOD and LOQ were 0.02 and 0.07 µg/µL, respectively. This indicated that the sensitivity of the spectrophotometer used was capable of measuring TP as low as 0.07 µg/µL.

The coefficient of variation of TTC, as shown in Table 3, for tested diets ranged between 0.05 - 15%, which were within the acceptable 20% for complex analytes like tannin. This observation indicated that the method used for quantification was reliable.

Furthermore, the values of TTC (% TAE/dw) of tested samples ranged from 0.041 - 0.257, of which the content of unprocessed soybean flour (0.257% TAE/dw) was significantly higher at $p < 0.05$ than that of unprocessed maize flour (0.213% TAE/dw). The value of the TTC of processed maize flour (0.041% TAE/dw) was significantly lower at $p < 0.05$ than its unprocessed form (0.213% TAE/dw) by 81%. This is in conformity with the findings of Agume *et al.* (2016) and Chukwuma *et al*. (2016), in which TTC of maize flour was reduced by 22 and 50%, respectively, after processing. Similarly, the TTC of

processed soybean flour (0.064% TAE/dw) was significantly lower at $p < 0.05$ than its processed form (0.257% TAE/dw) by 75%. This is in conformity with the finding of Agume *et al.* (2017) who found the TTC of soybean flour to have been reduced by about 67% after processing. The TTCs among the flours of processed maize, soybean, and their product were not significantly different at $p > 0.05$. The combined methods of processing raw foodstuffs used in the present work might have contributed to the much greater reduction of TTCs of flours of maize, soybeans, and subsequently, in their product. Nevertheless, this is in agreement with previous reports that antioxidant polyphenols, such as tannin, were reduced during washing or soaking, and thermal processing (Suri and Tanumihardjo, 2016; Agume *et al*., 2016); and that soaking, dehulling, and thermal processing reduced tannin content in soybean flour (Joshi and Varma, 2016; Agume *et al*., 2017). Reduction in tannins may result from leaching into soaking water due to their water-solubility, dehulling of seed coats due to their concentration therein, and generally, thermal processing is known to reduce the phenolic content of foods (Suri and Tanumihardjo, 2016). Tannins are considered antinutritional factors due to their ability to interfere with and precipitate proteins, thus undermining their biological and nutritional utilisation. Regarding this, a reduction in TTCs can be considered to have a nutritional advantage.

Table 3. TTC obtained from the samples (dw^{-1})

Diet	TTC (% tannic acid equivalent, TAE)	TTC (% CV)
MFunp	0.213 ± 0.010^{f}	0.05
MFpr	0.041 ± 0.000^{g}	0.00
MSF	0.067 ± 0.010^{g}	15
SBFunp	0.257 ± 0.020^{h}	7.8
SBFpr	0.064 ± 0.008^{g}	13

TTC values of diets were expressed as mean ± SD of triplicate determinations. Means followed by different lowercase letter in a column are significantly different by the HSD-test at $p < 0.05$ level of significance.

Advantages and limitations of FC method

The FC method of tannin determination involves a colorimetric reaction that finds analytical measurement in UV-Vis spectrophotometry, which thus makes the method easier, more efficient, applicable in routine laboratory use, and low-cost. Tannin complexation, for instance, complexation with polyvinyl polypyrrolidone, allows tannin in selective precipitation from a solution of chemical substances. Consequently, the FC method can quantify the total tannin content of a test sample. However, despite FC's analytical advantages, it is prone to some limitations, such as lack of specificity in quantifying different types of tannin separately, either hydrolysable, condensed, or monomeric tannin. The colorimetric reaction product of the FC assay is the result of unspecific oxidation with polyphenols, *i.e.,* analytes that are either not phenols, or often thought of as phenols also oxidise. Thus, the intensity of colour observed after the reaction does not exclusively correspond to tannins.

Conclusion

The present work evaluated the performance of the FC method for the determination of total tannin content from yellow maize, soybean, and their blended products. The method was validated with validation parameters such as specificity, linearity, precision, recovery, LOD, and LOQ through UV-visible spectrophotometry. The standard tannic acid or total phenolics, and crude extract from tested samples were used for method validation. The FC method complied with the requirements for analytical use and for ensuring the reliability of the results. The combined processing techniques including soaking, dehulling, oven-drying, boiling, and frying applied on the raw materials were also effective in reducing TTC by 81% for maize, and 75% for soybean. The sonication-assisted extraction allowed for the use of simple and low-cost equipment, and provided an efficient and low energy procedure with reduced amount of solvent and/or time.

Acknowledgement

The authors sincerely thank all the staff of the Nutrition Science Laboratory, Faculty of Medicine and Health Sciences, UPM, particularly Syed Hasbullah, for the technical assistance during the laboratory work.

References

Achidi, A. U., Tiencheu, B., Tenyang, N., Womeni, H. M., Moyeh, M. N., Ebini, L. T. and Tatsinkou, F. 2016. Quality evaluation of nine instant weaning foods formulated from cereal, legume, tuber, vegetable and crayfish. International Journal of Food Science and Nutrition Engineering 6: 21-31.

Agume, A. S. N., Njintang, N. Y. and Mbofung, C. M. F. 2017. Effect of soaking and roasting on the physicochemical and pasting properties of soybean flour. Foods 6(12): 12.

Agume, A. S. N., Njintang, Y. N. and Mbofung, C. M. F. 2016. Physicochemical and pasting properties of maize flour as a function of the interactive effect of natural-fermentation and roasting. Journal of Food Measurement and Characterization 11(2): 451-459.

Aires, A., Carvalho, R. and Saavedra, M. J. 2016. Valorization of solid wastes from chestnut industry processing: Extraction and optimization of polyphenols, tannins and ellagitannins and its potential for adhesives, cosmetic and pharmaceutical industry. Waste Management 48: 457-464.

Annegowda, H. V., Bhat, R., Min-Tze, L., Karim, A. A. and Mansor, S. M. 2011. Influence of sonication treatments and extraction solvents on the phenolics and antioxidants in star fruits. Journal of Food Science and Technology 49(4): 510-514.

Arapitsas, P. 2012. Hydrolyzable tannin analysis in food. Food Chemistry 135(3): 1708-1717.

Ashraf-Khorassani, M. and Taylor, L. T. 2004. Sequential fractionation of grape seeds into oils, polyphenols, and procyanidins via a single system employing CO_2-based fluids. Journal of Agricultural and Food Chemistry 52(9): 2440-2444.

Blainski, A., Lopes, G. C. and de Mello, J. C. P. 2013. Application and analysis of the Folin Ciocalteu method for the determination of the total phenolic content from *Limonium brasiliense* L. Molecules 18(6): 6852-6865.

Bouaoudia-Madi, N., Boulekbache-Makhlouf, L., Madani, K., Silva, A. M. S., Dairi, S., Oukhmanou-Bensidhoum, S. and Cardoso, S. M. 2019. Optimiation of ultrasound-assisted extraction of polyphenols from *Myrtus communis* L. pericarp. Antioxidants 8(7): 205.

Chiba, L. I. 2009. Diet formulation and common feed ingredients. Retrieved from website: https://umkcarnivores3.files.wordpress.com/2012/02/animal-nutrition2.pdf

Chowdhury, S. A., Vijayaraghavan, R. and MacFarlane, D. R. 2010. Distillable ionic liquid extraction of tannins from plant materials. Green Chemistry 12(6): 1023-1028.

Chukwuma, O. E., Taiwo, O. O. and Boniface, U. V. 2016. Effect of the traditional cooking methods (boiling and roasting) on the nutritional profile of quality protein maize. Journal of Food and Nutrition Sciences 4: 34-40.

Delimont, N. M., Haub, M. D. and Lindshield, B. L. 2017. The impact of tannin consumption on iron bioavailability and status: A narrative review. Current Developments in Nutrition 1(2): e000042.

Engstrom, M. T. 2016. Understanding the bioactivity of plant tannins: Developments in analysis methods and structure-activity studies. Finland: University of Turku, PhD thesis.

Furlan, C. M., Motta, L. B. and dos Santos, D. Y. A. C. 2010. Tannins: What do they represent in plant life? In Petridis G. K. (ed). Tannins - Types, Foods Containing, and Nutrition, p. 4-5. Brazil: Nova Science Publishers, Inc.

Galvão, M. A. M., de Arruda, A. O., Bezerra, I. C. F., Ferreira, M. R. A. and Soares, L. A. L. 2018. Evaluation of the Folin-Ciocalteu method and quantification of total tannins in stem barks and pods from *Libidibia ferrea* (Mart. ex Tul) L.P. Queiroz. Brazilian Archives of Biology and Technology 61: e18170586.

Huma, Z. E., Jayasena, V., Nasar-Abbas, S. M., Imran, M. and Khan, M. K. 2018. Process optimization of polyphenol extraction from carob (*Ceratonia siliqua*) kibbles using microwave-assisted technique. Journal of Food Processing and Preservation 42: 1-10.

Ibironke, S. I., Fashakin, J. B. and Ige, M. M. 2014. Nutritional quality of animal polypeptide (crayfish) formulated into complementary foods. American Journal of Food and Nutrition 2: 39-42.

Joshi, P. and Varma, K. 2016. Effect of germination and dehulling on the nutritive value of soybean. Nutrition and Food Science 46(4): 595-603.

Khasnabis, J., Rai, C. and Roy, A. 2015. Determination of tannin content by titrimetric method from different types of tea. Journal of

Chemical and Pharmaceutical Research 7(6): 238-241.

Krzyzowska, M., Tomaszewska, E., Ranoszek-Soliwoda, K., Bien, K., Orlowski, P., Celichowski, G. and Grobelny, J. 2017. Tannic acid modification of metal nanoparticles: Possibility for new antiviral applications. In Andronescu, E. and Grumezescu, A. M. (eds). Nanostructures for Oral Medicine, p. 335-363. United States: Elsevier.

Lu, C., Wang, H., Lv, W., Ma, C., Lou, Z., Xie, J. and Liu, B. 2012. Ionic liquid- based ultrasonic/microwave-assisted extraction combined with UPLC-MS-MS for the determination of tannins in *Galla chinensis*. Natural Product Research 26(19): 1842-1847.

Maškovic, P. Z., Velicˇkovic, V., Đurovic, S., Zekovic, Z., Radojkovic, M., Cvetanovic, A., ... and Vujic, J. 2018. Biological activity and chemical profile of *Lavatera thuringiaca* L. extracts obtained by different extraction approaches. Phytomedicine 38: 118-124.

Ravber, M., Knez, Ž. and Škerget, M. 2015. Isolation of phenolic compounds from larch wood waste using pressurized hot water: Extraction, analysis and economic evaluation. Cellulose 22: 3359-3375.

Rusydi, M. and Azrina, A. 2012. Effect of germination on total phenolic, tannin and phytic acid contents in soy bean and peanut. International Food Research Journal 19: 673-677.

Sieniawska, E. and Baj, T. 2017. Tannin. In Badal, S. and Delgoda, R. (eds). Pharmacognosy - Fundamentals, Applications and Strategies, p. 199-232. United States: Academic Press.

Sousa, A. D., Maia, A. I. V., Rodrigues, T. H. S., Canuto, K. M., Ribeiro P. R. V., de Cassia, A. P. R., … and de Brito, E. S. 2016. Ultrasound-assisted and pressurized liquid extraction of phenolic compounds from *Phyllanthus amarus* and its composition evaluation by UPLC-QTOF. Industrial Crops and Products 79: 91-103.

Suri, D. J. and Tanumihardjo, S. A. 2016. Effects of different processing methods on the micronutrient and phytochemical contents of maize: From A to Z. Comprehensive Reviews in Food Science and Food Safety 15: 912-926.

Talmaciu, A. I., Ravber, M., Volf, I., Knez, Ž. and Popa, V. I. 2016. Isolation of bioactive compounds from spruce bark waste using sub- and supercritical fluids. Journal Supercritical Fluids 117: 243-251.

Vergara-Salinas, J. R., Bulnes, P., Zúñiga, M. C., Pérez-Jiménez, J., Torres, J. L., Mateos-Martín, M. L., ... and Pérez-Correa. J. R. 2013. Effect of pressurized hot water extraction on antioxidants from grape pomace before and after enological fermentation. Journal of Agricultural and Food Chemistry 61: 6929-6936.

Zaklouta, M., Hilali, M., Nefzaoui, A. and Haylani, M. 2011. Animal nutrition and product quality laboratory manual. Aleppo, Syria: ICARDA.