

## ANALYSIS OF PROTEIN CONCENTRATE FROM KAHAI (*CARYODENDRON ORINOCENSE* KARST) SEEDS CULTIVATED IN AMAZONIA OF ECUADOR

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### ABSTRACT

**Objective:** The aim of this study was to obtain Kahai protein concentrate from *Caryodendron orinocense* Karst cultivated in the Amazonian region of Ecuador, to characterize its proteins using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and native electrophoresis methods, and to determine its content of total polyphenols.

**Methods:** Kahai seeds (*C. orinocense* Karst) were utilized to obtain Kahai protein concentrate at pH 3.0, pH 4.0, pH 5.0, and pH 6.0 using the isoelectric precipitation method. The proteins were characterized using the SDS-PAGE and native-PAGE electrophoresis methods. Total polyphenols were determined using the colorimetric assay method.

**Results:** The best treatment to obtain Kahai protein concentrate was at pH 5.0 with a 21.91% yield using the cold extraction method. The best treatment was at pH 6.0 with a 11.07% yield using the heat extraction method. Kahai concentrates presented a complex profile of proteins with molecular weights between 14 and 97 kDa. It was possible to obtain at the same time polyphenols at pH 5.0 with a value of 1028.58 mg gallic acid equivalents/100 g protein of sample.

**Conclusion:** This study suggests that Kahai protein concentrates possess a high content of proteins and polyphenols that can be used to elaborate functional foods.

**Keywords:** Kahai, Proteins, Kahai protein isolate, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, Polyphenol.

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### INTRODUCTION

Proteins, starch, and oil are important components of vegetal foods sources. World population is increasing with a high demand of vegetal foods. New crops are necessary so that these components can be obtained from new food sources. *Caryodendron orinocense* Karst, named Kahai, is an Amazonian fruit with a high content of proteins (17.78%), lipids (28.29%), and carbohydrates (34.60%) [1]. Lipids from Kahai seeds are used to elaborate different pharmaceutical and cosmetic products, and Kahai proteins are rarely used. The indigenous of Amazonia of Ecuador and Colombia have used Kahai oil in the preparation of ointments and different foods [1,2]. The production of Kahai protein isolate/concentrate using the isoelectric solubilization-precipitation method can be an effective process approach to expand the applications of this low-value flour sub-product in processed vegetal products in countries with this type of crops such as Ecuador and Colombia. The isoelectric solubilization-precipitation method is a selective, pH-induced solubilization method. In this process, globulin and albumin proteins are first solubilized at either acidic or alkaline pH values and then separated from fat and insoluble materials (i.e., fibre, carbohydrates). Solubilized proteins are finally precipitated at their isoelectric point, (pI), and isoelectric solubilization-precipitation processing recovers functional and nutritional proteins at high yields [3-5]. Proteins play a major role in the functional and sensory characteristics of several food products [6], and Kahai protein could be one of these products. The objective of this study was to obtain Kahai protein concentrates of Kahai seeds and their characterization using the electrophoresis method. The polyphenols content was also evaluated.

### METHODS

#### Kahai protein flour and proximate analysis

Kahai was obtained in the Coca region in Ecuador. The Kahai oil sample was obtained from Kahai seeds using the cold and heat pressing

methods. Kahai flour was defatted through extraction with hexane (1:10 w/v) at room temperature for 24 h, under continuous stirring during the first 5 h. After drying at room temperature, the flour was stored at 4°C until used. Analytical methods such as moisture, fat, total fiber, and soluble solids content were determined, according to the methods of the Association of Official Analytical Chemists (AOAC) [7], numbers 9250.10, 930.09, 985.29, and 923.03, respectively. The protein content of the samples was determined using the micro-Kjeldahl method AOAC number 920.152, % (N×6.25). Carbohydrate percentage was calculated with the formula: % Carbohydrates = 100 - (% moisture + % proteins + % fat + % soluble solids + % total fiber). Contents were expressed on a dry weight basis.

#### Protein concentrate obtained from Kahai flour

Kahai protein concentrate was prepared according to Acosta *et al.* and Añón [8] with modifications. The defatted flour was suspended in water in a 1:10 w/v, and the suspension was adjusted to pH 8.0 by adding 2M NaOH. The suspension was stirred for 1 h and then centrifuged at 4500 g for 30 min at 25°C. The supernatant was adjusted to pH 2.0, pH 3.0, pH 4.0, pH 5.0, and pH 6.0 with 2 N HCl and centrifuged for 20 min at 4500 g. The pellet was suspended in a small volume of water, neutralized with 0.1 M NaOH, lyophilized, and then frozen at -20°C. The content of protein isolate was determined using the Dumas method [9].

#### Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of Kahai protein concentrate was carried out according to the method proposed by Laemmli [10], in the SDS-PAGE system, using 12% polyacrylamide gel/100 mL of resolving gel; 4 g acrylamide/100 mL of stacking gel was used in a Mini-PROTEAN Tetra Cell electrophoresis system (Bio-Rad, Hercules, CA, USA). Polypeptide bands were stained in Coomassie Brilliant Blue G-250 for 12 h. Relative molecular masses

of proteins present in kahui protein concentrate were determined by a comparison to molecular weight markers 6.5–200 kDa kaleidoscope pre-stained standards (Bio-Rad) which have mix of proteins: myosin (198 kDa),  $\beta$ -galactosidase (125 kDa), bovine serum albumin (BSA) (88 kDa), carbonic anhydrase (37 kDa), soybean trypsin inhibitor (31 kDa), LYS (17 kDa), and aprotinin (6.5 kDa). NATIVE-PAGE analysis of the Kahai protein was carried out by loading samples with 2.5 mg per well and run at 220 V constant voltage per gel [11]. The stacking gel, separatory gel, and the running buffer were prepared in the same way as in the SDS-PAGE method, except that no SDS was used. Molecular weight calibration kit for SDS electrophoresis (GE Healthcare, Uppsala, Sweden) containing phosphorylase B (97 kDa), BSA (66 kDa), OVA (45 kDa), carbonic anhydrase (37 kDa), trypsin inhibitor (20.1 kDa), and LA (14.4 kDa), was used. Polypeptide bands were stained in Coomassie Brilliant Blue G-250 for 12 h.

#### Analysis of Kahai protein concentrate using reversed-phase ultra-high-performance liquid chromatography (RP-UHPLC)

Kahui protein concentrates at pH 2.0, pH 3.0, pH 4.0, pH 5.0, and pH 6.0 were analyzed using RP-UHPLC on Agilent 1200 infinity series UHPLC System (Agilent Technologies, Waldbronn, Germany). The variable wavelength detector was 214 nm. The column used was EC C18 (Agilent Poroshell 120, 4.6×50 mm×2.7  $\mu$ m of particle size). Samples were eluted at 1.0 mL/min with a linear gradient from 0% to 70% of Solvent B (acetonitrile and trifluoroacetic acid [TFA], 1000:0.270 v/v) in solvent A (water and TFA, 1000:0.370 v/v) during 10 min. The injection volume was 100  $\mu$ L for each duplicated sample [12].

#### Extraction of polyphenols

After the precipitation of Kahai proteins using water at different pHs, the supernatants were separated and then lyophilized for 48 h. Then, the dry samples were stored at  $-20^{\circ}\text{C}$ .

#### Determination of total polyphenols

Total phenolics in the obtained extracts were estimated by a colorimetric assay based on the procedures described by Singleton and Rossi [13] with some modifications. Briefly, 1 mL of sample was mixed with 1 mL of Folin-Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min. Then, the absorbance was read at 725 nm using a spectrophotometer (Thermo Scientific Evolution 200). Gallic acid was used for constructing the standard curve (0–0.075 mg/mL). The results were expressed as mg of gallic acid equivalents (GAEs)/100 g of dry sample.

#### Statistical analysis

Results are presented as means $\pm$ standard deviation from three replicates of each experiment. Differences between mean values were determined by the analysis of variance. The *post hoc* analysis was performed using the Tukey test. All tests were considered statistically significant at  $p < 0.05$ . The statistical analysis was performed using the software package Prism 4 for Windows, version 4.3 (GraphPad Software Inc., www.graphpad.com).

## RESULTS

#### Kahui protein concentrates and its protein content

Kahui oil was extracted using either cold or hot press methods. Residual flour was used to obtain Kahai protein concentrates. This residual flour was defatted using hexane. The defatted Kahai flour was used to obtain Kahai concentrate protein (KCP) using the isoelectric precipitation method at different pHs (pH 3.0, pH 4.0, pH 5.0, and pH 6.0) with distilled water as solvent. The highest yield with the heat treatment was obtained at pH 5.0 with 11.07 $\pm$ 0.45%. The highest yield with the heat treatment was obtained at pH 6.0 with 21.91 $\pm$ 1.25% (Table 1). Yields of cold press at pH 3.0, pH 4.0, pH 5.0, and pH 6.0 presented statistical differences. The contents of protein in the KCP were analyzed using the Dumas method. The treatments at pH 3.0, pH 4.0, pH 5.0, and pH 6.0 presented a higher content of protein. The best treatment identified was at pH 5.0 with a value of 48.32%. At pH 4.0 and pH 6.0, there were no statistical differences with  $p < 0.05$  (Table 1). Furthermore, the content of proteins present in Kahai

protein concentrates was analyzed using the bicinchoninic acid (BCA) method. The results obtained were similar to the ones obtained using the Dumas method. The best treatment of the BCA method was at pH 5.0 with a value of 50.65% of protein (Table 1).

The profile of proteins from Kahai seeds was analyzed using the SDS-PAGE and native-PAGE electrophoresis methods.

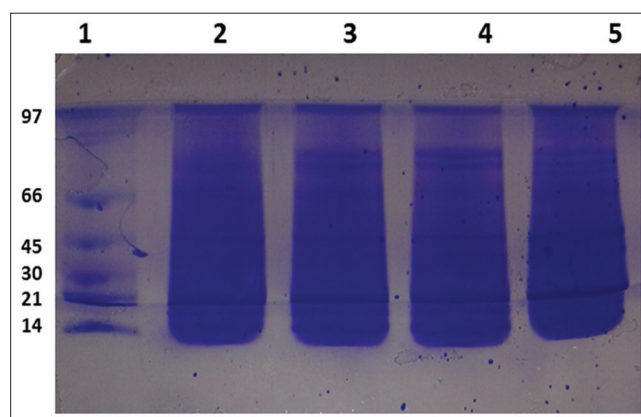
#### SDS-PAGE analysis

In the presence of the reductor agent 2-mercaptoethanol, the gel shows that at pHs 3.0, pH 4.0, pH 5.0, and at pH 6.0, there are higher contents of proteins as all bands were strongly stained with the solution of Blue Coomassie used in this study. It has been observed bands with 97, 45, 21, and 14 kDa strongly stained with the content of protein (Fig. 1).

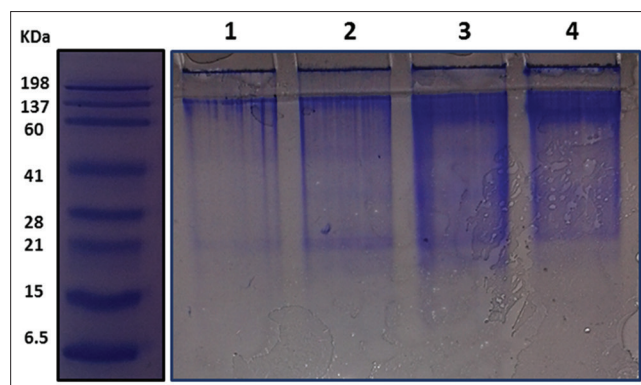
**Table 1: % Yield of Kahai protein concentrates obtained at different pHs and content of protein using BCA and Dumas method**

Method	pH 3.0	pH 4.0	pH 5.0	pH 6.0
% Yield	6.98 $\pm$ 0.51 <sup>a</sup>	9.33 $\pm$ 0.40 <sup>b</sup>	11.07 $\pm$ 0.45 <sup>c</sup>	9.76 $\pm$ 0.63 <sup>b,c</sup>
% Yield	8.49 $\pm$ 0.27 <sup>a</sup>	14.08 $\pm$ 0.13 <sup>b</sup>	16.60 $\pm$ 0.35 <sup>c</sup>	21.91 $\pm$ 1.25 <sup>d</sup>
cold				
BCA	37.54 $\pm$ 4.52 <sup>b</sup>	36.33 $\pm$ 1.26 <sup>b</sup>	50.65 $\pm$ 2.82 <sup>a</sup>	49.65 $\pm$ 4.00 <sup>a</sup>
Dumas	35.05 $\pm$ 0.21 <sup>a</sup>	37.41 $\pm$ 0.11 <sup>a</sup>	48.32 $\pm$ 0.22 <sup>a</sup>	47.79 $\pm$ 0.01 <sup>a</sup>

Different letters show statistical difference between the groups ( $p < 0.05$ ) ANOVA and Tukey's test. BCA: Bicinchoninic acid, ANOVA: Analysis of variance



**Fig. 1: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of Kahai concentrates protein (KCP). Line 1: Molecular weight, Line 2: KCP at pH 3.0, Line 3: KCP at pH 4.0, Line 4: KCP at pH 5.0, and Line 5: KCP at pH 6.0**



**Fig. 2: Native-polyacrylamide gel electrophoresis analysis of Kahai concentrates protein (KCP). Line 1: KCP at pH 3.0, Line 2: KCP at pH 4.0, Line 3: KCP at pH 5.0, and Line 4: KCP at pH 6.0**

### Native-PAGE analysis

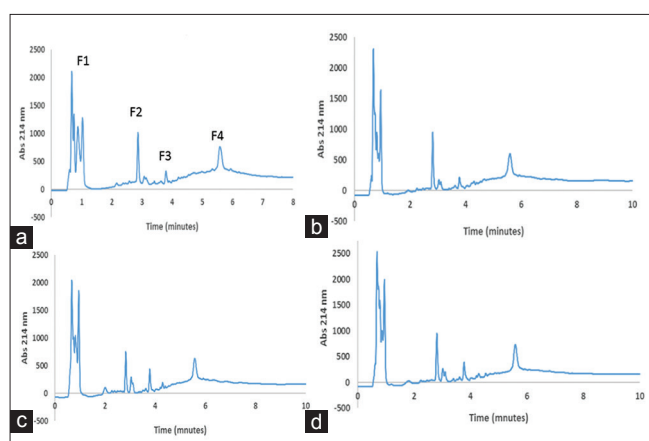
In the gel of polyacrylamide, it has been observed one band at all pHs assayed with molecular weight higher than 198 kDa. The gel shows bands between 21 and 137 kDa. These bands were strongly tinged with the solution Blue Coomassie (Fig. 2).

### RP-UHPLC analysis

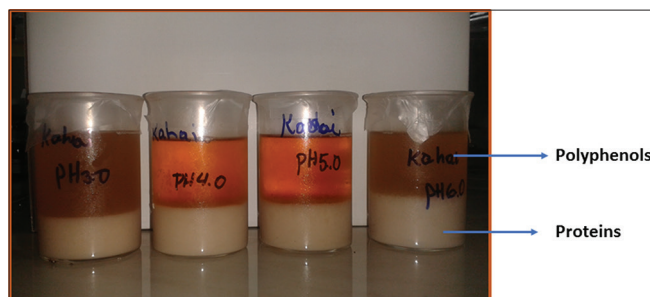
All Kahai protein concentrates were analyzed using the RP-UHPLC method for 10 min. The chromatograms show the proteins profile obtained from Kahai seeds. Fig. 3a and b shows four fractions with high capacity of absorbance at 214 nm. These fractions are present in all Kahai concentrates. These results are in accordance with the profile using the electrophoresis method. Fraction 1 (F1) presents low hydrophobicity being very polar [Fig. 3c]. The four fractions have the same time of retention. These proteins are acid proteins as proteins were obtained at low precipitation pHs. At acid pHs, the same proteins were obtained.

### Content of polyphenols from Kahai

The content of polyphenols present in the supernatant after separation of KCP was evaluated (Fig. 4). The content of



**Fig. 3: Reversed-phase ultra-high-performance liquid chromatography of Kahai protein concentrate obtained at different pHs. (a) Kahai protein concentrate at pH 3.0, (b) Kahai protein concentrate at pH 4.0, (c) Kahai protein concentrate at pH 5.0, (d) Kahai protein concentrate at pH 6.0. Column Zorbax EC C18 (Agilent Poroshell 120, 4.6×50 mm×2.7 μm of particle size)**



**Fig. 4: Obtention of polyphenols with water of Kahai at different pHs**

polyphenols was determined using a colorimetric assay with the Folin-Ciocalteu's phenol reagent. The highest content of polyphenols was obtained at pH 6.0 with a value of 1028.58 mg GAE/100 g protein of sample. At pH 5.0, the content was 962.49 mg GAE/100 g protein sample. pH 3.0 presents a low value with 644.34/100 g protein of sample (Table 2).

### DISCUSSION

Nowadays, the food industry has a great interest to get new protein sources, and this situation has grown dramatically in the past years [14]. The countries, high cost of proteins from animal sources, health problems such as allergies to animal proteins and many consumers choosing freely to refrain consuming animal proteins. This situation has led to a substantial search for alternative sources of proteins which can replace conventional sources more used [15,16]. Alternative sources have been thoroughly studied in recent years with proteins derived from plants (lupin, quinoa, amaranth, and sesame), bacteria, and yeasts being the most promising ones [17]. Among them, oil seeds are a good option as the protein-rich oil cake left after oil extraction is a by-product which can be valorized by the food industry [18]. Kahai seeds are used to extract the oil, and these oils are used for the pharmaceutical and cosmetic industry, but their proteins are less used. The protein content of an oilcake left after oil extraction accounts for 20–50% on a dry weight basis, similar to soybean which is extensively used in food industry [19]. However, at present, its utilization is limited to the production of animal feed. Kahai protein concentrate at pH 5.0 presents a high content of protein with a value of 50.65% of protein. Protein concentrates are considered economic if protein content ranges between 35 and 80% on a dry basis. Protein isolates are defined to have a protein content higher than 85%. For this reason, this vegetal protein is a good option for uses in the food industry.

The Folin-Ciocalteu's reagent assay is the common method used to determine the total phenolic content (TPC) of nuts. TPCs of nuts, expressed as mg of GAE/100 g of sample, were reported in Phenol-Explorer database [20,21] with a range between 47 and 3673. Chestnut contained the highest TPC (1580–3673 mg GAE/100 g), followed by pecan (1284–2016), walnut (1558–1625), pistachio (867–657), hazelnut (291–835), peanut (0.1–420), almond (47–418), Brazil nut (112–310), cashew (137–274), macadamia (46–156), and pine nut [22,23]. In the Phenol-Explorer Database, TPC is reported in fruits with values of orange (278.59 mg GAE/100 g), kiwi (179.71 mg GAE/100 g), mango (174.77 mg GAE/100 g), and papaya (57.60 mg GAE/100 g). In this study, we report TPC from Kahai with a value range of 644.34–1028.58 mg GAE/100 g in the samples analyzed. These values are high when compared to other foods with polyphenols. In the future, studies could identify the polyphenols present in Kahai seeds. Polyphenol and flavonoids have been reported with different biological activities such as antibacterial activity [24]. Polyphenols of Kahai could have biological activities such as antioxidant and antimicrobial activities.

### CONCLUSIONS

It was possible to obtain protein concentrate from Kahai seeds (*C. orinocense* Karst) and by-product flour with water as solvent obtaining high yields of protein content. It was possible to obtain polyphenols with high values. Kahai seeds are a good source of proteins to be used for animal and human nutrition. Kahai seeds can be an important source of bioactive compounds.

**Table 2: Content of polyphenols of Kahai**

Sample	pH 3.0	pH 4.0	pH 5.0	pH 6.0
% polyphenols	644.34±0.00 <sup>a</sup>	856.56±0.00 <sup>b</sup>	962.49±0.0003 <sup>c</sup>	1028.58±0.0009 <sup>d</sup>

Different letters show statistical difference between the groups ( $p < 0.05$ ) ANOVA and Tukey's test. ANOVA: Analysis of variance

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