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Study of Selenium Distribution in the Protein Fractions of the Brazil Nut, *Bertholletia excelsa*

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The high selenium content of the Brazil nut, *Bertholletia excelsa*, makes this seed a healthy food qualified as an antiradical protector. The studied nut contained 126 ppm of selenium. Selenium was found to be distributed in the nut protein fractions. The water-extracted fraction, which represented 17.7% of the cake protein, was the richest in selenium with 153 ppm. Analysis by HPLC-MS showed that selenium was linked by a covalent bond to two amino acids to form selenomethionine and selenocystine. The selenomethionine represented a little less than 1% of the total amount of methionine.

KEYWORDS: Brazil nut; *Bertholletia excelsa*; protein; selenium; covalent bonds

INTRODUCTION

Brazil nut is the fruit of a big tropical tree of the Lecythidacea family (*Bertholletia excelsa* and *nobilis*) widespread in the Latin American basin of the high and middle Amazon, until Bolivia. The trees can exceed 30 m in height, which explains why the local populations wait for the natural fall of the nuts, which occurs in January. Fruits are then dried to make them favorable to preservation or transformation. A tree produces >150 kg of fruits. Fruits may weigh up to 1 kg and contain 12−20 nuts. The Brazil nuts have a thick woody brown-gray hull. Their length is from 3 to 5 cm. The hull contains a very white almond with dark brown tegument.

This gathered fruit is consumed traditionally by local populations, but it is also used by local industry, and 90% of the production is exported to the United States, England, and Germany. It is consumed in these countries as dry nibbling fruits or as raw material for confectionery, like almonds or groundnuts.

The hull represents half to the three-fifths of the nut total weight. The fat content of peeled almonds is 50−75% (*1*, *2*); although known for their protein content, 15−17% by fresh weight and ∼50 wt % of its defatted flour, they are also reputed for an interesting content in oil microcomponents such as tocopherols, sterols, and phospholipids (*3*).

However, the nut's exceptional nutritional qualities, and in particular its high content in selenium and magnesium, sulfur amino acids (*4*, *5*), and the balance in its essential fatty acids, could make it an essential ingredient entering in the composition of food with strong nutritional potential.

A proposal of an average British diet was calculated to provide ∼60 μg of Se/day, of which 50% was derived from cereals and cereal products and another 40% from meat and fish. Milk, fats, fruits, and vegetables provided little or no Se (*6*). The U.S. National Research Council has interpreted information from animals as suggesting a human requirement of ∼60−120 μg of Se/day with toxicity occurring after prolonged ingestion of 2400−3000 μg of Se/day (*7*). Selenium can be toxic at very high amounts. Toxicity for man has seldom been observed. However, toxicity was reported in the areas of the world where the levels of selenium ingestion are extremely high. Symptoms of this toxicity observed in man include increased brittleness of the nails or hair, cutaneous eruptions, irritability, nausea, or vomiting. These side effects were observed in roughly 10% of the Chinese people consuming >1000 μg of selenium/day.

Reported selenium contents of 16−30 μg/g (*8*) and 35 μg/g (*9*) make seed a food described as beneficial as an antiradical-izing guard or in cancer prevention (*8*). The daily consumptions observed are 30−220 μg/day. The recommended daily intakes are calculated from these data and go from 1 to 5 μg of selenium/kg of body weight. Few cases of toxicity were related to selenium in food, and some studies mention that 500 μg/day could be a maximum daily intake in the long term.

The principal functions of selenium in the body that have been discovered to date are summarized by Dr. Clarke from the University of Arizona(*10*). Selenium is found in the active site of many enzymes such as thioredoxin reductase, which catalyzes reactions of oxidation/reduction. An enzyme that helps to prevent the process of oxidation, glutathione peroxidase, needs

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selenium for its formation. Selenium seems to improve the immune system and its response to infections. There are indications that it supports the natural death of cells, which destroys infectious bacteria. The enzymes P450, which help to detoxify some procancerous substances, can be induced by selenium. Selenium inhibits the action of prostaglandins, which are responsible of inflammatory reactions in the body. Male fertility can be increased by selenium, which improves the mobility of spermatozoids. At high amounts, selenium can decrease the growth rate of the tumoral cells.

The objective of this work is to show that selenium is linked by a covalent bond to protein fractions of Brazil nut cake. To accomplish this study, experiments were carried out with Brazil nut samples very rich in selenium provided by JBA Agroconcept.

These nut samples, from a particular biotope, contain >120 ppm of selenium. Nut proteins were first separated on the basis of their affinity for different solvents (*4*, *5*, *11*−*13*). Then a liquid chromatography (LC) method was coupled to tandem mass spectrometry (LC-MS/MS) (*14*, *15*) to confirm the presence of selenocystine and selenomethionine in proteins. To protect the amino acids, enzymatic hydrolysis of proteins was carried out using proteases (*16*).

MATERIALS AND METHODS

Samples. Samples of Brazil nut used in this work were gathered in February 2000 in the Brazilian Amazonian region. The exact origin of this variety, which is very rich in selenium, is voluntarily held secret because of the future industrial applications that may arise from this study. From the beginning of the work, in April 2000, the nut samples were stored in a cabinet at ambient conditions with an average temperature of 25 °C. All of the analyses were done in 2 months.

Mineral Composition and Moisture Content. Total ash was obtained by calcination of 1 g of defatted cake containing 10% of lipid at 525 °C for 90 min (*17*). Mineral elements including selenium were dosed by plasma emission spectroscopy from Varian Vista ICP (*18*, *19*). Total nitrogen was analyzed by carbonization according to the Dumas method using an elementary analyzer Leco FP 528 (*20*).

Moisture content determination of *B. excelsa* nut was carried out in triplicate by drying at 105 ± 2 °C during 24 h.

Biochemical Analysis. Amino acids were determined in duplicate (two hydrolysates per sample, one chromatographic analysis per hydrolysate) by RP-HPLC of derived amino acid residues using a Pico Tag C 18 column (3.93 mm i.d. × 15 cm, Waters). Samples (50 mg) were hydrolyzed under vacuum in 6 N HCl for 24 h at 110 °C in sealed tubes. Amino acids were then reacted with phenylisothiocyanate (PITC) with 20 mL of an ethanol/triethanolamine/PITC mixture (7:1:1) to form phenylthiocarbamyl amino acids. Elution was performed with a gradient of acetonitrile in 0.14 M sodium acetate buffer (eluant A, 100% buffer; eluant B, acetonitrile 60% in buffer v/v). Tryptophan was not analyzed. Sulfur amino acids were analyzed after oxidation, in the form of cysteic methionine sulfone and cysteic acid. Oil was extracted from 20 g of Brazil nuts for 6 h by Soxhlet with hexane. Hexane was removed by evaporating and then flushing with nitrogen gas.

Fractionation of Brazil Nut Cake Proteins. The extraction process of Brazil nut proteins was based on the variation of pH. The temperature during the extraction and precipitation, besides the quantity of water, influenced the yield of extracted product. Total protein extraction was carried out on 10 g of defatted cake, which was dispersed in 150 mL of 1 N NaOH at pH 10 directly in centrifuge tubes that were kept under rotary agitation at 225 tr/min for 60 min and then centrifuged at 4700*g* at 20 °C for 20 min. The supernatant was then adjusted to pH 5 by the addition of 1 N HCl. Proteins precipitated, and separation was carried out by centrifugation at 4700*g* at 10 °C for 20 min. Supernatant was eliminated, and the protein pellet was dried at 60 °C for 16 h and then weighed.

Fractionation of proteins was carried out three times to obtain three different fractions. They were each analyzed independently. First of all, a water extraction at pH 7, followed by an extraction with 0.5 N NaCl at pH 8, and then an extraction with 1 N NaOH at pH 10 was done. The sum of these three extracts was compared with the quantity of proteins extracted directly with 1 N NaOH at pH 10. The name of the fractions was assigned per the paper of Rakipov (*21*).

Table 1. Mineral Composition of Brazil Nut *B. excelsa* (on Dry and Defatted Basis) Compared to Cambodia Nut *I. malayana* (*23*) and Walnut *J. regia* (*28*)

mineral	*J. regia* (μg/g)	*I. malayana* (μg/g)	*B. excelsa* (μg/g)
nitrogen	27200	23700	97470
phosphorus	5000	2700	23800
potassium	5000	5600	19690
calcium	900	1700	6060
magnesium	1280	2200	13380
sodium	25	123	20
iron	45	108	80
manganese	nd[a]	300	50
zinc	nd	35	115
selenium	nd	1.9	126

[a] Not determined.

Water Extraction. Extraction solution was water at pH 7. Precipitation was carried out by adjustment to pH 5 by the addition of 1 N HCl. The fraction obtained was called "albumins".

Extraction of Proteins by NaCl. Extraction was carried out with the residue of the preceding operation, which was solubilized in a solution of 0.5 M NaCl at pH 8. Precipitation was carried out by adjustment to pH 5 by the addition of 1 N HCl. The fraction obtained was called "globulins".

Extraction of Proteins by 70% Ethanol. Extraction was carried out with the residue of the preceding operation, which was solubilized in 70% ethanol. Precipitation was carried out by adjustment to pH 5 by the addition of 1 N HCl. The fraction obtained was called "prolamins".

Extraction of Proteins by NaOH. Extraction was carried out with the residue of the preceding operation. Extraction solution was a solution of 1 N NaOH at pH 10. Precipitation was carried out by adjustment to pH 5 by the addition of 1 N HCl. The fraction obtained was called "glutelins".

Protein Enzymatic Hydrolysis. Protein hydrolysis was carried out enzymatically to retain the structure and composition of the amino acids released, in particular the selenoamino acids.

Ten grams of protein in 100 mL of demineralized water (p/p) was introduced into a reactor under agitation at 50 °C. pH was adjusted to 6 by 1 N NaOH because the enzymatically optimal pH is between pH 5.0 and 7.0.

Hydrolysis was carried out at 50 °C for 7 h by the addition of 0.1% (p/v) Flavourzyme (Novo Nordisk), which had both endoproteasic and exopeptidasic activities. At the end of the hydrolysis, reactional medium was heated at 90 °C for 5 min to denature the enzyme. Solution was then filtered in cascades up to 0.2 μm. The solution obtained was yellow and limpid.

Selenocystine and Selenomethionine Identification by LC-MS/MS. Determination of selenocystine and selenomethionine in Brazil nut hydrolysate was investigated by ion-pair reversed-phase liquid chromatography coupled with electrospray (ESI) tandem mass spectrometry under conditions previously described and optimized for the analysis of 20 underivatized proteinogenic amino acids (*22*). The amino acid separation was achieved on a Purospher STAR RP-18^{e} column with heptafluorobutyric acid as volatile ion-pairing reagent in an acetonitrile/water mobile phase.

This dosage was carried out on the enzymatic hydrolysates in order to avoid the chemical modifications due to usual acid hydrolysis. The step of sample preparation before analysis by LC was reduced to a dilution to $^{1}/_{10}$ of the enzymatic hydrolysates in demineralized water. No further derivatization step was necessary due to the detection of amino acid under their underivatized form.

Reagents. HPLC-grade acetonitrile was obtained from Baker. Heptafluorobutyric acid (HFBA), 99%, was from Aldrich (France).

Table 2. Amino Acid Composition (Percent) of Extracted Fractions of *B. excelsa* Protein Compared with Cambodia Nut Defatted Flour (*23*) and Bean Flour (*29*)

	B. excelsa					
amino acid	water (albumins)	NaCl (globulins)	NaOH (glutelins)	NaOH on cake (total proteins)	bean flour (%)	Cambodia nut (%)
aspartic acid	7.7	8.6	8.8	8.4	12.8	8.5
glutamic acid	18.0	16.0	16.1	17.7	15.9	19.4
serine	5.5	5.6	5.7	5.7	6.6	6.8
glycine	8.7	9.2	8.9	8.8	3.7	8.4
histidine	1.9	1.6	1.9	1.8	2.8	1.9
arginine	12.4	11.0	11.1	11.8	6.3	6.9
threonine	4.8	4.8	5.0	4.3	5.3	5.3
alanine	5.9	6.9	6.7	6.2	4.1	6.9
proline	5.0	5.0	5.1	5.0	4.5	4.9
tyrosine	2.4	2.6	2.5	2.4	3.2	2.8
valine	6.3	7.4	7.1	6.3	6.1	5.4
methionine	2.2	0.9	0.6	2.4	0.8	1.4
cysteine	1.1	0.6	0.6	1.0	0.6	1.7
isoleucine	3.9	4.5	4.1	4.0	5.0	4.4
leucine	8.0	8.3	8.5	7.9	8.8	7.2
phenylalanine	3.7	4.2	4.2	3.8	6.4	3.2
lysine	2.5	2.7	2.9	2.5	7.1	4.9

L-Selenocystine, 98%, and DL-selenomethionine, 99+%, were purchased from Acros; methionine and cystine were from Sigma. Deionized water (18 MΩ) obtained with an Elgastat UHQ II system (Elga) was used for the preparation of standard amino acid and ion-pairing reagent solutions. The hydrolysate sample was only diluted 1:10 with an aqueous solution of 0.1% HFBA before its injection in the LC-MS/MS system.

Apparatus. LC-ISP-MS/MS was carried out using a Perkin-Elmer (Toronto, Canada) model LC-200 binary pump and a Perkin-Elmer Sciex (Forster City, CA) API 300 mass spectrometer triple-quadrupole with Turboionspray heated at 300 °C as ion source. The mass spectrometer was operated in positive ion mode. Nitrogen was used as curtain and collision gas. After optimization of MS parameters, state files were as follows; NEB = 9, CUR = 7, CAD = 1, IS = 5000, OR = 20, RNG = 200, Q0 = −5, IQ1 = −6, ST = −10, RO1 = −6, IQ2 = −15, RO2 = −20, IQ3 = −35, RO3 = −25, DF = −400, CEM = 2100; quad 1, 30 (0.010), 100 (0.050), 1000 (0.400), 2000 (0.742); quad 3, 10 (0.008), 100 (0.035), 1000 (0.285), 2000 (0.530). The NEB = 9 (nebulizer gas) corresponds to a flow rate of 1.08 L min^{-1}, and the CUR = 7 (curtain gas) corresponds to a flow rate of 1.02 L min^{-1}. The selective reaction monitoring (SRM) mode was used to monitor the parent and product ions of each amino acid. The dwell time was set at 100 ms, and the pause time was 5.0 ms. Injections were done by a Perkin-Elmer series 200 autosampler fitted with a 10-μL loop. Amino acid chosen ion transitions for MS-MS analysis were as follows: SeCys2 (*m*/*z* 337 → 247.5), Cys2 (*m*/*z* 241 → 152), SeMet (*m*/*z* 198 → 181), Met (*m*/*z* 150 → 104).

Separation was carried out on a Purospher STAR RP-18e 125 × 2 mm i.d. column, particle size = 5 μm, from Merck (Darmstadt, Germany); flow rate was 200 μL min^{-1}. For LC-ESI-MS/MS, a split of 1/20 was used to avoid too high flow rate of the ion source. For gradient elution, solvent A was 0.1% HFBA in water. Solvent B was acetonitrile; gradient starts at 100% A to 70% A in 15 min.

RESULTS

Mineral Composition. The moisture content of *B. excelsa* nut obtained in triplicate was found to be 2.75 ± 0.2%. This content appears to be normal for a nut and varies with the environmental conditions.

Ash content obtained by calcination in triplicate on a cake containing 10% lipids was found to be 10.7 ± 0.1%. Mineral content was higher than in other classical nuts such as walnut [*Juglans regia* (*25*)] or Cambodia nut [*Irvingia malayana* (*23*)].

Brazil nut was rich in phosphorus and magnesium (**Table 1**). Phosphorus, which can represent >2% of the cake, was present essentially in mineral form, because a small amount was found in oil after extraction with hexane (377 μg/g). This content was equivalent to that found in seeds such as sunflower, almond, and sesame. Magnesium content was high, ∼13.4 mg/g. Calcium content of ∼6 mg/g in the cake was low but higher than in the two other seeds.

Table 3. Calculation of Nitrogen/Protein Conversion Factor for Brazil Nut Proteins

amino acid	mol mass (g)	Brazil nut protein (%)	N/mol mass (g/g of amino acid)	N in protein (g/100 g)
aspartic acid	133.10	8.4	0.105	0.882
glutamic acid	147.10	17.7	0.095	1.681
serine	105.09	5.7	0.133	0.758
glycine	75.07	8.8	0.186	1.637
histidine	155.16	1.8	0.090	0.162
arginine	174.20	11.8	0.321	3.788
threonine	119.12	4.3	0.117	0.503
alanine	89.09	6.2	0.157	0.973
proline	115.13	5.0	0.122	0.610
tyrosine	181.19	2.4	0.077	0.185
valine	117.15	6.3	0.119	0.750
methionine	149.21	2.4	0.094	0.226
cystine	240.30	1.0	0.116	0.116
isoleucine	131.18	4.0	0.107	0.428
leucine	131.18	7.9	0.107	0.845
phenylalanine	165.19	3.8	0.085	0.323
lysine	146.19	2.5	0.191	0.477
total		100.0		14.344

Selenium content of this cultivar was particularly high; it reached 126 μg/g. No selenium trace could be detected in the oil extracted with hexane. This means that only 1 g of this cake or half of a nut contains the per-day quantity recommended for a person of 70 kg.

This content was higher than those observed in cereal grains (0.11 μg/g), green vegetables (0.01 μg/g), roots (0.005 μg/g), or fruits (0.005 μg/g) (*24*, *25*) and similar to that observed in soybean (0.8−1.3 μg/g) in the United States (*26*). This cake can thus be considered as a rich raw material that could be used to formulate healthy food rich in selenium.

Protein Analysis. Composition in amino acids of the different protein fractions extracted with water, NaCl, and NaOH was analyzed (**Table 2**). The amino acid compositions of these three

Table 4. Selenium and Protein Contents Obtained from Protein Fractions Calculated with Conversion Factors

fraction (indicative name)	extraction mode	extracted proteins (g)/ 100 g of cake	extract N content (%)	proteins (g)/100 g of extract (factor 6.25/6.97)	Se (ppm)
albumins	water	17.7	14.76	92.2/100.0	153
globulins	NaCl	6.0	12.6	78.7/87.8	63
prolamins	EtOH 70%	0	0	0/0	0
glutelins	NaOH	3.0	10.93	68.3/76.2	56
total proteins	NaOH on cake	20.5	14.8	92.5/100.0	142

fractions did not differ significantly. The amino acid composition of *B. excelsa* proteins (**Table 2**) resembled that of bean and Cambodia nut (*23*). Lysine content was weak (3.3%), and sulfur amino acid content as methionine (6.3%) and cystine (2.2%) was high. This analysis, realized by the classical method, did not take into account the fact that selenium could replace sulfur in sulfur-containing amino acids.

To improve the calculation of protein contents in Brazil nut, we calculated the nitrogen/protein conversion factor (*27*). Nitrogen content obtained by total carbonization of the defatted cake was 9.74% (**Table 2**).

To determine this factor, the nitrogen content of each amino acid was calculated by taking into account its molecular weight. Then the percentage of nitrogen in proteins was established by multiplying the quantity of nitrogen by the percentage of nitrogen in protein. The factor of conversion thus obtained was 6.97 (**Table 3**). The defatted cake contained 97.47 mg/g of nitrogen (**Table 1**), which meant a protein content of $97.47 \times 6.97 = 679.36$ mg/g of protein and therefore 67.9% of proteins in the defatted cake.

Selenium Contents in Brazil Nut Cake Protein Fractions. Selenium was unequally distributed in the four protein fractions (**Table 4**). The water-extracted fraction, which was the most important in weight (with 17.7% of the cake), was the richest in selenium with 153 ppm. The NaCl-extracted fraction contained 63 ppm, and the one extracted with NaOH at pH 10 contained an equivalent level of 56 ppm. There was an absence of proteins from the prolamin family extracted with 70% ethanol. Nitrogen contents of the various fractions showed that proteins were very pure, especially if the new calculated factor of 6.97 was used. The purity of albumins was close to 100% and a little lower for globulins (87.8%) and glutelins (76.2%).

The total quantity of extracted proteins was generally close to 21%, with a maximum of 25%, which was obtained by mixing 10 g of oil cake with 170 mL of water instead of 150 mL and a precipitation by the addition of 1 N HCl until pH 5.5. Total protein extracted directly from cake with NaOH was extremely pure ($\cong$100%) and contained 142 ppm of selenium. The Brazil nut studied in this work showed a very high selenium content, much higher than the species quoted in the literature, which contained between 10 and 35 ppm of selenium (*8*, *9*), and that analyzed by our laboratory coming from other Amazonian areas such as Peru, where we found only 10 ppm of selenium (unpublished work).

Selenomethionine and Selenocystine Identification by LC-ESI-MS/MS. In the Brazil nut enzymatic hydrolysate, two compounds, **A** and **B**, with the same chromatographic retention times as the two standards of selenocystine and selenomethionine were identified in several different chromatographic systems. By LC-ESI-MS/MS, it was proven that both compounds **A** and **B** have different molecular masses. Compound **A** had the same molecular mass as the standard of selenocystine, and compound **B** had the same mass as the standard of selenomethionine. In addition, fragmentation by tandem mass spectrometry of compound **A** was rigorously the same as that observed for selenocystine under the same conditions and that of compound **B** was identical to that of selenomethionine. We thus showed the presence of two compounds, **A** and **B**, identical to the two standards. These results thus make it possible to affirm the presence of a covalent bond between selenium and protein for the two amino acids selenomethionine and selenocystine.

The content of selenomethionine obtained by LC by comparison with the standard was 111 μM/L of extract. The content of selenocystine was very weak and not easily quantifiable. The content of methionine (14054 μM/L) was 126 times higher than that of selenomethionine. Cystine content was 304 μmol L^{-1}. The corrected composition of Brazil nut protein, which took into account the content of selenomethionine and selenocystine, was thus not very different from the traditional composition.

Conclusion. Extraction of proteins in three steps, water, salt solution, and then NaOH, led to the obtaining of three protein families: albumin, globulin, and glutelin. The albumin family contained the most important part of selenium (153 ppm) and represented 17.7 g for 100 g of cake. The glutelins were also interesting, with 56 ppm of selenium and 3 g of proteins/100 g of cake, just like the globulins, with 63 ppm of selenium and 6 g of protein/100 g of cake.

The most recent study on this topic, which emanated from the American team of Vonderheide et al. (*9*), showed that the traditional Brazil nuts contain 35 ppm of selenium and that this selenium was linked by covalent bond to selenomethionine and in less quantity to selenocystine.

Tandem PLC-SM also made it possible to show that selenium was linked to proteins by covalent bonds and that selenium replaced sulfur in selenomethionine and selenocystine in part of these amino acids, almost 1% in the case of selenomethionine.

The studied nuts were thus extremely rich in selenium, much richer than in other published works. Extracted protein fractions were also very rich in selenium and could constitute a healthy food that could be interesting to consume with moderation. The daily consumption advised for a man (35 μg for 70 kg) is indeed very quickly reached because the nuts contain 126 μg of selenium/g. This product would have to be diluted in an adequate food mixture.

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LITERATURE CITED

(1) Antunes, A. J. Protein supplementation of navy beans with Brazil nuts. Ph.D. Thesis, Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI, 1975; 119 pp.

(2) Smith, N. J. H.; Williams, J. T.; Plucknett, D. L. Brazil nut. In *Tropical Forests and Their Crops*; Comstock Publishing Associates, Cornell University Press: Ithaca, NY, 1992; Chapter 9, pp 384−402.

(3) Chunhieng, T.; Hafidi, A.; Pioch, D.; Brochier, J.; Montet, D. Detailed study of Brazil nut (*Bertholletia excelsa*) oil biological active compounds. *Ol., Corps Gras, Lipides* **2004**, submitted for publication.
(4) Ampe, C.; Van Damme, J.; De Castro, L. A. B.; Sampaio, M. J. A. M.; Van Montagu, M.; Vandekerckhove, J. The amino acid sequence of the 2S sulphur-rich proteins from seeds of Brazil nut (*Bertholletia excelsa* H.B.K). *Eur. J. Biochem*. **1986**, *159*, 597−604.
(5) Sun, S. M.; Leung, F. W.; Tomic, J. C. Brazil nut (*Bertholletia excelsa* H.B.K.) proteins: fractionation, composition, and identification of a sulfur-rich protein. *J. Agric. Food Chem*. **1987**, *35*, 232−235.
(6) Thorn, J.; Robertson, J.; Buss, D. H.; Bunton, N. G. Trace nutrients. Selenium in British food. *Br. J. Nutr*. **1978**, *39*, 391−396.
(7) Food and Nutrition Board: no title. *Nutr. Rev*. **1976**, *34*, 37.
(8) Ip, C.; Lisk, D. J. Bioactivity of selenium from Brazil nut for cancer prevention and selenoenzyme maintenance. *Nutr. Cancer* **1994**, *21* (3), 203−212.
(9) Vonderheide, A. P.; Wrobel, K.; Kannamkumarath, S. S.; B'Hymer, B.; Montes-Bayon, M.; Ponce de Leon, C.; Caruso, J. A. Characterization of selenium species in Brazil nuts by HPLC-ICP-MS and ES-MS. *J. Agric. Food Chem.* **2002**, *50*, 5722−5728.
(10) http://www.selenium.arizona.edu/.
(11) Landry, J.; Moureaux, T. Hétérogénéité des glutélines de maïs: extraction sélective et composition en acides aminés des trois fractions isolées. *Bull. Soc. Chim. Biol.* **1970**, *52*, 1021−1037.
(12) Landry, J.; Moureaux, T.; Huet, J. C. Extractibilité des protéines du grain d'orge: dissolution sélective et composition en acides aminés des fractions isolées. *Bios* **1972**, *7*−*8*, 281−292.
(13) Landry, J. Extraction séquencée des protéines du grain d'orge. *C. R. Acad. Sci. Paris D* **1979**, *288*, 907.
(14) Ganther, H. E.; Kraus, R. J.; Foster, S. J. Identification of selenocysteine by high-performance liquid chromatography and mass spectrometry. *Methods Enzymol*. **1984**, *107*, 582−592.
(15) Léonil, J.; Gagnaire, V.; Mollé, D.; Pezennec, S.; Bouhallab, S. Application of chromatography and mass spectrometry to the characterization of food proteins and derivated peptides. *J. Chromatogr. A* **2000**, *881*, 1−21.
(16) Villanueva, A.; Vioque, J.; Sanchez-Vioque, R.; Clemente, A.; Bautista, J.; Millan, F. Production of an extensive protein hydrolysate by sequential hydrolysis with endo- and exoproteases. *Grasas Aceites* **1999**, *50*, 472−476.
(17) Mindak, W. R.; Dolan, S. P. Determination of arsenic and selenium in food using a microwave digestion-dry ash preparation and flow injection hydride generation atomic absorption spectrometry. *J. Food Compos. Anal.* **1999**, *12*, 111−122.
(18) Inter Institute Committee. Méthodes de référence pour la détermination des éléments minéraux dans les végétaux. *Oleagineux* **1973**, *28*, 87−92.
(19) Kingston, H. M.; Jassie, L. B. Introduction to microwave sample preparation: theory and practice. In *Professional Reference Book Series*; American Chemical Society: Washington, DC, 1988; 263 pp.
(20) Ebeling, M. E. The Dumas method for nitrogen in feeds. *J. Assoc. Off. Anal. Chem.* **1968**, *51*, 766−770.
(21) Rakipov, N. Cultures oléagineuses. In *Biochimies des Cultures Tropicales*; Mir: Moscow, Russia, 1987; p 255.
(22) Petritis, K.; Chaimbault, P.; Elfakir, C.; Dreux, M. Parameter optimisation for the analysis of underivatized protein amino acids by liquid chromatography and ionspray tandem mass spectrometry. *J. Chromatogr. A* **2000**, *896*, 253−263.
(23) Bandelier, J.; Chunhieng, T.; Ollé, M.; Montet, D. An original study of the biochemical and oil composition of Cambodian nut: *Irvingia malayana*. *J. Agric. Food Chem.* **2002**, *50*, 1478−1482.
(24) Mortensen, A.; Skibsted, L. H. Relative stability of carotenoid radical cations and homologue tocopheroxyl radicals. A real time kinetic study of antioxidant hierarchy. *FEBS Lett.* **1997**, *417*, 261−266.
(25) Kaiser, S.; Di Mascio, P.; Murphy, P.; Sies, H. M. E. Physical and chemical scavenging of singlet molecular oxygen by tocopherols. *Arch. Biochem. Biophys.* **1990**, *27*, 101−108.
(26) Wauchope, R. D. Selenium and arsenic levels in soybeans from different production regions of the United States. *J. Agric. Food Chem*. **1978**, *26*, 226−228.
(27) Morr, C. V. Recalculated nitrogen conversion factors for several soybean protein products. *J. Food Sci.* **1982**, *47*, 1751−1752.
(28) http://woodlandnut.com/walnut.html, 2002.
(29) Antunes, A. J.; Markakis, P. Protein supplementation of navy beans with Brazil nuts. *J. Agric. Food Chem.* **1977**, *25*, 1096−1098.

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