



Almond (*Prunus dulcis* L.) Protein Quality*

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Abstract. Three marketing varieties of almonds; Carmel, Mission, and Nonpareil; were analyzed for proximate composition and protein nutritive quality. Moisture, lipids, protein, ash, sugars, and tannins ranges were 3.05–4.33%, 43.37–47.50%, 20.68–23.30%, 3.74–4.56%, 5.35–7.45%, and 0.12–0.18%, respectively. No detectable hemagglutinating and trypsin inhibitory activities were present in Carmel, Mission, and Nonpareil almonds. Amino acid analyses indicated the sulfur amino acids (methionine + cysteine), lysine, and threonine to be the first, second, and third limiting amino acids in almonds when compared to the recommended amino acid pattern for children 2–5-year old. However, compared to the recommended amino acid pattern for adults, sulfur amino acids were the only limiting amino acids in almonds tested. True Protein Digestibility (% TPD) values for Carmel, Mission, and Nonpareil were 88.55 ± 1.26 , 92.25 ± 1.05 , and 82.62 ± 1.47 , respectively. Protein Digestibility Corrected Amino Acid Scoring (PDCAAS) values suggested almond proteins to be of poor nutritional quality.

Key words: Almond, Amino acid, Digestibility, Hemagglutinating activity, PDCAAS, Trypsin inhibitory activity

Introduction

Almonds are readily accepted worldwide and are therefore used in a variety of food products. Incorporating almonds in food products adds value to that product in a variety of ways. Some of the important contributions made by almonds to a food product include nutritive value (protein, lipids, minerals, vitamin E, and fiber), desirable texture (e.g., crunchy texture of toasted almonds), and flavor (nutty and sweet) characteristics.

Globally, the US is the largest almond producer and almond exporter [1]. In 2003, the US accounted for 44.15% (757,500 metric tons) of global almond production of 1,705,578 metric tons. In 2002, US exported shelled almonds valued at \$ 681,424,000 (~67% of the total global exports of shelled almonds valued at \$ 1,017,238,000). Five marketing varieties—Carmel, Mission, NePlus, Nonpareil, and Peerless; account for ~90% of the US almond production. Despite being high in lipids, almonds have a nutritionally desirable fatty acid profile with oleic (65%) and linoleic (25%) acids accounting for approximately 90% of total almond lipids [2]. Almond protein composition and

protein properties [2–4] including biochemical purification of the major storage protein, AMP or amandin, have been reported earlier [5].

With the exception of a few sensitive individuals allergic to almonds, almonds are globally enjoyed and used either as a snack food or as an ingredient in other foods. AMP contains key reactive polypeptides recognized by IgE from almond allergic patient sera [6], is present in globally important almond genotypes and hybrids [7], and is antigenically stable toward various food processing methods [8]. Consequently, AMP is an excellent marker protein for detecting trace (ppm) quantities of almonds (raw or processed) using rabbit polyclonal antibody-based Enzyme-Linked Immunosorbent Assays (ELISAs) [6, 8, 9].

Despite the long history of global cultivation and ready consumer acceptance of almonds, data on almond protein quality are limited. Protein polypeptide composition analyses of almond genotypes and hybrids indicated no significant differences between globally important almond genotypes and hybrids [7], suggesting protein compositional similarity among the tested genotypes and hybrids. We therefore investigated protein nutritional quality of three major marketing varieties of shelled, raw, whole almonds—Carmel, Mission, and Nonpareil.

Materials and Methods

Materials

Shelled, raw, Carmel, Mission, and Nonpareil almond seeds were obtained from Almond Board of California, Modesto, CA. Sources of laboratory chemicals, reagent or better grade, have been reported earlier [5].

Methods

When needed, samples were powdered using either a mortar and pestle or Osterizer blender (Galaxie Model Number 869-18R) for homogeneity. Samples were stored in an airtight container at -20°C until further use.

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Moisture. An accurately weighed sample (~1 g) was placed in an aluminum pan and dried in a previously heated vacuum oven (95–100 °C, 25 in. Hg) to a constant weight ([10], Official Method 925.40).

Lipids. Lipids were quantified using AOAC Official Method 948.22 [10]. A known weight of the sample (~10 g/thimble) was defatted in a Soxhlet apparatus using petroleum ether (boiling point range 38.2–54.3 °C) as the solvent (flour to solvent ratio of 1:10 w/v) for 8 h. Defatted samples were dried overnight (~10–12 h) in a fume hood to remove petroleum ether and weighed.

Lipid content(%)

$$= \frac{[\text{Initial wt. of full fat flour(g)} - \text{final wt. of defatted flour(g)}]}{\text{Initial wt. of full fat flour (g)}} \times 100$$

Defatted samples were homogenized using either a pestle and mortar or Osterizer blender and stored in plastic screw-capped bottles at –20 °C until further analysis.

Protein. Micro-Kjeldahl method was used to determine total proteins. Briefly, 0.1 g sample was placed in a micro-Kjeldahl flask. A catalyst [mixture of 0.42 g CuSO₄ + 9.0 g K₂SO₄], few glass beads (to prevent sample bumping), and 15 ml conc. H₂SO₄ (36 N) were added to each sample. Sample digestion was done at 410 °C for 45–75 min [until clear green solution was obtained which ensured complete oxidation of all organic matter]. The digest was diluted with 50 ml of distilled water and the micro-Kjeldahl flask was attached to the distillation unit. After adding 45 ml of 15 N NaOH, sample distillation was done to collect released ammonia into a boric acid solution containing the indicators methylene blue and methyl red. Borate anion (proportional to the amount of nitrogen) was titrated with standardized 0.1 N H₂SO₄. A reagent blank was run simultaneously. Sample nitrogen content was calculated using the following formula.

%N

$$= \frac{(\text{ml H}_2\text{SO}_4 \text{ for sample} - \text{ml H}_2\text{SO}_4 \text{ for blank}) \times \text{Normality of H}_2\text{SO}_4 \times 1.4007}{\text{Weight of sample(g)}}$$

Protein (%) = N (%) × 5.18 ([10], Official Method 950.48).

Ash. Accurately weighed sample (~0.1 g) was placed in a ceramic crucible (previously heated and cooled till constant weight obtained) and subjected to ashing at 550 °C in a muffle furnace until constant weight ([10], Official Method 923.03).

Total Soluble Sugars. Soluble sugars were determined as described by Dubois et al. [11]. A known weight of defat-

ted sample (~0.1 g) was extracted with 1 ml distilled deionized (DI) water containing 1 mM NaN₃ for 1 h, centrifuged (16, 100 × g, 10 min) and the supernatant collected. To 100 μl of the suitably diluted supernatant, 100 μl of DI water followed by 200 μl of lead acetate (20% w/v) were added and vortexed to thoroughly mix the contents. To this mixture were added 200 μl Na₂SO₄ (20% w/v), sample vortexed, and centrifuged (16100 × g, 10 min). The supernatant was suitably diluted. A known aliquot (μl) of the diluted supernatant was made to 400 μl with distilled water and 10 μl of 80% (w/v) phenol and 1 ml concentrated H₂SO₄ were added with vigorous vortexing after each addition. Absorbance was read at 490 nm. Glucose standard curve (0–100 μg glucose) was prepared simultaneously. Total soluble sugars were expressed as glucose equivalents.

Amino Acid Composition. Total amino acid composition of the sample was determined using a Pico-Tag Column Amino Acid Analyzer (Waters Chromatograph Division, Milford, MA). Accurately weighed sample was hydrolyzed in 600 μl of 6 M HCl in the presence of nitrogen (18 h, 110 °C). The sample was treated with a 2:2:1 v/v/v ethanol: triethylamine: water solution and dried. The dried sample was then treated with a 7:1:1:1 v/v/v/v ethanol: triethylamine: water: PITS solution (99.9%) and held for 20 min at 25 °C in a nitrogen atmosphere and dried. Fifty μl of 5 mM sodium acetate buffer (pH 7.6, 40 °C) containing 6% v/v acetonitrile was added to the sample for analysis by HPLC. Norleucine was used as an internal standard to calculate percentage recovery for each amino acid. Tryptophan content was determined by the colorimetric method (No. 3) of Spies and Chambers [12]. Amino acid composition was reported as g of amino acid per 100 g of protein.

Tannins. Tannins were determined as described earlier [13]. Briefly, a known weight of the sample (~0.1 g) was extracted in an acidified methanol (1% HCl, v/v) with continuous vortexing and centrifuged (16, 100 × g, 10 min). Aliquots of the supernatant were immediately analyzed for tannin using 2% vanillin assay. Catechin standard curve (0–1 mg/ml) was prepared simultaneously. Tannin content was expressed as catechin equivalents.

Hemagglutinating Activity (HA). Microtiter plate assay was used to determine hemagglutinating activity (Sigma Chemical Co., St. Louis, MO; [14]) of the sample and compared to a soybean lectin (Sigma Chemical Co., St. Louis, MO, Product No. L1395). HA activity of the sample extracted in 0.01 M phosphate buffered saline, pH 6.8 was determined under standard agglutination conditions defined as: Agglutination of a 2% (v/v) suspension of fresh human blood group A erythrocytes after 1 h incubation at 25 °C. HA was determined from serial dilutions in 0.01 M phosphate

buffered saline, pH 6.8. One hemagglutinating unit (HU) was defined as the least amount of hemagglutinin (highest sample dilution), producing positive evidence of agglutination.

$$\text{Hemagglutinating activity (HU/g sample)} = \frac{\text{HD} \times \text{dilution factor} \times \text{volume for extraction (ml)}}{\text{wt. of sample (g)}}$$

Trypsin Inhibitor Activity (TIA). TIA was determined according to the method approved for soy products [15] with suitable modifications. Defatted soybean flour (Williams 82) was used as a reference standard. Briefly, a known weight of sample and standard (~25 mg) was extracted with 1.5 ml of 0.01 N NaOH in a microcentrifuge tube for 3 h with constant and vigorous shaking provided. When necessary, pH of the suspension was adjusted between 8.4 and 10.0 using 0.1 N NaOH. Sample was then centrifuged (16, 100 × g, 10 min) and supernatant used for analysis. Final assay volume was 1.5 ml. Suitable blank and “0” sample (no TIA) were simultaneously included for analysis. One trypsin unit was defined as an increase of 0.01 absorbance units (AU) at 410 nm per 10 ml reaction mixture (therefore a factor of 10/1.5 = 6.67 was used in calculations). Samples were suitably diluted to obtain 40–60% trypsin inhibition (typically 40–60% of “0” sample AU) under the assay conditions. TIA was expressed as trypsin inhibitor unit per mg sample (TIU/mg sample).

Trypsin Inhibitor Units (TIU)/mg sample/standard

$$= \frac{(\text{“0” sample AU} - \text{sample AU} - \text{blank AU}) \times \text{dilution factor} \times \text{volume for extraction (ml)}}{0.01 \times 6.67 \times \text{volume of diluted sample used for assay (}\mu\text{l)} \times \text{sample wt. (g)} \times 1000}$$

True Protein Digestibility (%TPD) in Vivo. Weanling male Sprague-Dawley rats were housed in individual cages and kept under controlled conditions of temperature and light with free access to water. Rats were fed a standard rodent chow diet based on the American Institute of Nutrition (AIN) 93 g diet with 14% protein as casein [16] for acclimatization for 5 days. Following this test period, seven rats per treatment group were fed either a protein free diet (control) or a test diet (containing raw whole almond flour) for a 5 day preliminary period and a 5 day balance period. During this 10 day study period, rats were weighed daily to monitor growth. Prew weighed food was provided and food intake measured daily after accounting for spillage. Feces were collected, dried, ground, and analyzed for daily spillage and were analyzed for nitrogen content. Nitrogen content of feed and feces was determined by micro-Kjeldahl method no. 950.48 [10].

Protein Digestibility Corrected Amino Acid Score (PDCAAS). PDCAAS value was calculated following the recommendations of FAO/WHO Joint Expert Consultation on

Protein Quality Evaluation (1989) as described by Henley and Kester [17]. $\text{TPD}(\%) = \frac{I - (F - F_k)}{I} \times 100$ where, I = intake nitrogen, F = fecal nitrogen and F_k = metabolic/endogenous fecal nitrogen. $\text{PDCAAS} = \text{TPD}(\%) \times \text{amino acid score of the limiting essential amino acid}$.

The first and the second limiting essential amino acids in almonds were methionine + cysteine and lysine, respectively.

Statistical Analysis. Appropriate data were analyzed for statistical significance using SPSS (Version 10, Chicago, Illinois) and Least Significant Difference (LSD, $p = 0.05$).

Results and Discussion

Chemical Composition

Almond proximate composition (Table 1) is consistent with an earlier report [2]. Amino acid composition (Table 2) data suggest sulfur amino acids (methionine + cysteine) to be the first limiting amino acid, followed by lysine and threonine, in total seed proteins. Ezeokonkwo and Dodson [18] reported tyrosine, lysine, and methionine to be limiting amino acids in tropical almonds (*Terminalia catappa*). When FAO/WHO recommendations are followed, recalculation of data reported by Ezeokonkwo and Dodson indicates lysine to be the first limiting amino acid [$\text{Lys} (3.39/5/80) = 0.584$] in tropical almonds as chemical scores for Tyr + Phe ($2.12 + 3.04 = 5.16$)/6.30 = 0.819 and for Met + Cys ($1.48 + 1.92 = 3.4$)/3.5 = 0.971 no longer represent chemical scores consistent with first and second limiting amino acids. In view of such differences in different amino acids being limiting in almonds, it is important to learn whether such variation is due to almond cultivar

Table 1. Almond chemical composition

Chemical	Carmel	Mission	Nonpareil
Moisture	3.05 ± 0.01	4.33 ± 1.14	4.01 ± 0.07
Lipid	47.5 ± 0.36	44.1 ± 0.55	43.3 ± 0.62
Protein	20.6 ± 0.24	23.3 ± 0.38	21.0 ± 0.19
Ash	3.74 ± 0.11	4.56 ± 0.12	4.53 ± 0.02
Carbohydrate ^a	25.0 ± 0.72	23.6 ± 0.84	27.0 ± 0.62
Sugars ^b	5.35 ± 0.00	6.23 ± 0.10	7.45 ± 0.36
Tannins ^c	0.16 ± 0.02	0.12 ± 0.00	0.18 ± 0.00
TIA ^d	ND	ND	ND
HA ^e	ND	ND	ND

Note. Values are expressed on g per 100 g edible portion (Mean ± SEM).

^aCarbohydrate is calculated by difference. ^bSugars: Total soluble sugars. ^cTannins are expressed as catechin equivalents. ^dTIA: Trypsin Inhibitory activity (units) – not detected (ND). ^eHA: Hemagglutinating activity (units) – not detected (ND).

Table 2. Almond amino acid composition

Amino acid	Carmel	Mission	Nonpareil	FAO/WHO ^a	FAO/WHO ^b	<i>T. catappa</i> ^c
Asx	11.93	13.17	11.87			6.38
Glx	34.55	34.86	36.13			15.52
Ser	3.41	3.45	3.38			2.64
Gly	5.83	5.64	5.79			6.04
His	1.93	1.93	2.14	1.9	1.6	2.96
Arg	9.4	9.68	9.33			6.22
Thr	1.95	1.91	1.93	3.4	0.9	2.94
Ala	4.25	4.12	4.11			3.24
Pro	4.5	4.33	4.27			1.82
Val	3.39	3.3	3.17	3.5	1.3	2.74
Met	0.4	0.37	0.42	2.5	1.7	1.48
Cys	0.26	0.28	0.24			1.92
Ile	2.88	2.83	2.71	2.8	1.3	3.58
Leu	6.19	5.77	5.81	6.6	1.9	7.31
Phe	4.38	4.1	4.25	6.3	1.9	3.04
Tyr	1.51	1.39	1.19			2.12
Lys	2.14	1.99	2.36	5.8	1.6	3.39
Trp	1.08	0.89	0.89	1.1	0.5	0.9
LEAA ^a	Carmel	Mission	Nonpareil			<i>T. catappa</i> ^c
First	Met/Cys	Met/Cys	Met/Cys			Lys
Second	Lys	Lys	Lys			Val
Third	Thr	Thr	Thr			Phe/Tyr
LEAA ^b	Carmel	Mission	Nonpareil			<i>T. catappa</i> ^c
First	Met/Cys	Met/Cys	Met/Cys			—
Second	—	—	—			—
Third	—	—	—			—
E/T (%)	23.94	22.71	23.26			43.62

Note. All values are expressed as g/100 g protein. Data are corrected for 100% recovery. LEAA: Limiting Essential Amino Acid. E/T represents essential to total amino acid ratio.

^aPreschool Child (2–5 years). FAO/WHO. ^bAdult (recommendations by the joint FAO/WHO Expert Consultation, 1959). ^cFrom Ezeokonkwo and Dodson [18].

or environmental and agronomic conditions involved in almond production. Critical evaluation of almond amino acid composition is therefore warranted. Legume proteins are typically limiting in sulfur amino acids while cereals are typically deficient in lysine. Cereals belong to *Graminae* (grass family) while almonds belong to *Rosaceae*, and legumes belong to *Leguminosae* that include many subfamilies including *Caesalpiniodae*, *Papilionoideae*, and *Mimosoidae*. Thus, almonds would appear to be quite distant from both cereals and legumes in terms of botanical relatedness. Almond proteins are soluble in aqueous media [2] and a single water soluble legumin (AMP or amandin) accounts for almost 70% of total soluble proteins in Nonpareil [5]. Legume proteins typically are also soluble in water or dilute aqueous salt solutions. Cereals on the other hand mainly contain prolamins (aqueous alcohol; soluble protein) and glutelins (proteins soluble in dilute aqueous acids/alkali). Recent reports [19, 20] have shown legumin and legumin-like proteins to be present in corn, however, legumin is only a minor protein in corn endosperm total proteins. Trypsin inhibitory activity as well as hemagglutinating activity was not detectable in almonds tested.

PDCAAS. TPD data suggest that almond proteins are quite digestible in vivo (Table 3) an observation consistent with our earlier reported high in vitro almond protein digestibility [2, 21]. The TPD values determined in the present investigation are consistent with the recently reported $TPD = 92.0 \pm 3\%$ (mean $\pm$ SD, $n = 6$) for tropical almond (*Terminalia catappa*) seeds [18]. It is important to note here that we used whole, natural almonds (powdered form) in our experimental diets while Ezeokonkwo and Dodson [18] used defatted seed flours. On the contrary, PDCAAS

Table 3. Almond TPD (%) and PDCAAS

Digestibility	Carmel	Mission	Nonpareil	LSD
TPD	88.55 $\pm$ 1.26	92.25 $\pm$ 1.05	82.62 $\pm$ 1.47	1.47
PDCAAS(Lys) ^a	0.33 $\pm$ 0.00	0.32 $\pm$ 0.00	0.34 $\pm$ 0.01	0.01
PDCAAS(Lys) ^b	1.19 $\pm$ 0.02	1.14 $\pm$ 0.01	1.22 $\pm$ 0.02	0.05
PDCAAS(Met/Cys) ^a	0.23 $\pm$ 0.00	0.24 $\pm$ 0.00	0.22 $\pm$ 0.00	0.01
PDCAAS(Met/Cys) ^b	0.35 $\pm$ 0.00	0.36 $\pm$ 0.00	0.32 $\pm$ 0.33	0.01

Note. Differences between two means within the same row exceeding the LSD value are significant ($p = 0.05$).

^aPreschool Child (2–5 years). ^bAdult.

values would suggest almond proteins to be of “poor quality” when compared with the recommended FAO/WHO (FAO/WHO Expert Consultation, 1989) pattern for children 2–5 years old. Gonzalez [22] compared protein quality of detoxified (cyanogenic glycosides removed) apricot kernel proteins with sweet almond seed proteins subjected to the same detoxification treatment and found detoxification treatment caused statistically ($p = 0.01$) significant reduction in digestibility coefficient (75.1 for treated versus 81.7 for untreated almond seed proteins). Calixto et al. [23] determined almond protein amino acid composition of five Spanish almond varieties, but did not determine protein quality, and opined “almond protein is considered to be of good nutritional quality.” Hall et al. [24] reported Protein Efficiency Ratio (PER) for Nonpareil blanched almond to be 1.61 ± 0.08 and 1.62 ± 0.15 in two experiments. When blanched almonds were further used for dry or oil roasting the PER values decreased ranging from 0.24 to 1.12. Beef, used as a control in the experiments by Hall and coworkers had a PER of 2.47 and 2.80. Cowan et al. [25], on the other hand, reported Lebanese grown sweet almonds contained 19.4% protein and corrected Protein Efficiency Ratio (PER) to be 0.32 when compared with that of the casein control (PER = 2.5). Ezeokonkwo and Dodson [18] reported the PER to be 1.95 (adjusted for casein PER = 2.5) for tropical almonds (*T. catappa*). Cowan et al. [25], based on amino acid supplementation of almond-based diets determined that methionine was the most limiting amino acid followed by lysine and threonine. Interestingly, Cowan and coworkers also found that although the order of limiting amino acids by biological and chemical methods did not agree, deletion of any one of the three limiting amino acids methionine, lysine, and threonine, resulted in very poor growth. Girard and coworkers (Ref. #19 in the paper by Cowan et al. [25]) found that addition of both lysine and methionine improved net protein ratio and net protein utilization when rats were fed (10% protein in the diet) using almonds as the protein source. However, when either of the two amino acids was added alone, there was no effect on protein quality. These data suggest that deficiency of lysine and methionine may be equally important for almond protein quality evaluation. Sathe et al. [5] also reported methionine, lysine, and threonine, respectively to be the first, second, and third limiting amino acids in AMP, the major storage protein in almonds, regardless of AMP preparation method. Based on the cDNA-derived sequence of prunin with 551 amino acid residues ([26], accession CAA55009, NCBI database) yield a calculated molecular weight of 72,870 g/mole with 2, 6, 6, and 12 moles of methionine, cysteine, lysine, and threonine, respectively. Using the appropriate amino acid MW, the g/100 g protein for cysteine + methionine (1.41 g), lysine (1.2 g), and threonine (1.96 g), yields chemical scores of 0.564 (methionine + cysteine), 0.207 (lysine), and 0.576 (threonine); indicating the order of limiting amino acids is

not the same noted earlier based on amino acid analysis of the isolated amandin [5]. Taken together, predicting protein quality based on amino acid composition alone may not always be sufficient. Based on available data it would therefore appear that in almonds, sulfur amino acids and lysine deficiencies may be equally important. Regardless of which amino acid is limiting, PDCAAS values suggest almond proteins to be of poor nutritional quality. Therefore, if almond proteins are to be used for development of high quality protein food products for 2–5-year olds, appropriate amounts of sulfur amino acids, lysine, and threonine should be incorporated in such products, preferably through judicious use of other food proteins such as milk, egg, and/or meat/fish proteins, known to be rich in those amino acids. Compared to the amino acid needs of adults, sulfur amino acids are limiting in almonds. Therefore, when consumed as a part of a balanced and varied diet rich in sulfur amino acids, almonds may serve as a valuable source of dietary proteins.

Based on literature data and our own results, it would appear that almond proteins are highly digestible. However, since published data and the data reported in the present investigation also suggest considerable variation in several essential amino acids, critical evaluation of almond amino acid composition followed by in vivo protein quality determination is warranted.

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References

1. FAO (2004) FAO STAT data <http://faostat.fao.org/Last> Accessed (SKS) May 15, 2004.
2. Sathe SK (1993) Solubilization, electrophoretic characterization, and in vitro digestibility of almond (*Prunus amygdalus*) proteins. *J Food Biochem* 16: 249–264.
3. Sathe SK, Sze-Tao KWC (1997) Thermal aggregation of almond protein isolate. *Food Chem* 59(1): 95–99.
4. Wolf WJ, Sathe SK (1998) Ultracentrifugal and polyacrylamide gel electrophoretic studies of extractability and stability of almond meal proteins. *J Sci Food Agric* 78(4): 511–521.
5. Sathe SK, Wolf WJ, Roux KH, Teuber SS, Venkatachalam M, Sze-Tao KWC (2002) Biochemical characterization of amandin, the major storage protein in almond (*Prunus dulcis* L.). *J Agric Food Chem* 50: 4333–4341.
6. Roux KH, Teuber SS, Robotham JM, Sathe SK (2001) Detection and stability of the major almond allergen in foods. *J Agric Food Chem* 49: 2131–2136.

7. Sathe SK, Teuber SS, Gradziel TM, Roux KH (2001) Electrophoretic and immunological analyses of almond (*Prunus dulcis* L.) genotypes and hybrids. *J Agric Food Chem* 49: 2043–2052.
8. Venkatachalam M, Teuber SS, Roux KH, Sathe SK (2002) Effects of roasting, blanching, autoclaving, and microwave heating on antigenicity of almond (*Prunus dulcis* L.) proteins. *J Agric Food Chem* 50(12): 3544–3548.
9. Acosta MR, Roux KH, Teuber SS, Sathe SK (1999) Production and characterization of rabbit polyclonal antibodies to almond (*Prunus dulcis* L.) major storage protein. *J Agric Food Chem* 47(10): 4053–4059.
10. AOAC (1995) Official Methods of Analysis, 16th edn. Arlington, VA: Association of Official Analytical Chemists.
11. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28: 350–356.
12. Spies JR, Chambers DC (1948) Chemical determination of tryptophan. *Anal Chem* 10: 30–39.
13. Sze-Tao KWC, Schrimpf JE, Teuber SS, Roux KH, Sathe SK (2001) Effects of processing and storage on walnut (*Juglans regia* L.) tannins. *J Sci Food Agric* 81: 1215–1222.
14. Lis H, Sharon N (1972) Soybean (*Glycine max*) agglutination. Minneapolis, St. Paul, Minnesota: Methods Enzymol 28: 360–368.
15. AACC (1983) Approved Methods of the AACC. Method 71–10. Determination of trypsin inhibitor activity of soy products. American Association of Cereal Chemists.
16. Reeves PG, Nielsen FH, Fahey GC Jr (1993) AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 123(11): 1939–1951.
17. Henley EC, Kester JM (1994) Protein quality evaluation by protein digestibility corrected amino acid scoring. *Food Technol* 48(4): 74–77.
18. Ezeokwonkwo CA, Dodson WL (2004) The potential of *Terminalia catappa* (tropical almond) seed as a source of dietary protein. *J Food Qual* 27: 207–219.
19. Woo YM, Hu DW, Larkins BA, Jung R (2001) Genomic analysis of genes expressed in maize endosperm identifies novel seed proteins and clarifies patterns of zein gene expression. *Plant Cell* 13: 2297–2317.
20. Yamagata T, Kato H, Kuroda S, Abe S, Davies E (2003) Uncleaved legumin in maize endosperm: Identification, accumulation, and putative localization. *J Exp Bot* 54(384): 913–922.
21. Sze-Tao KWC, Sathe SK (2000) Functional properties and in vitro digestibility of almond (*Prunus dulcis* L.) protein isolate. *Food Chem* 69(2): 153–160.
22. Gonzalez MA (1972) On the nutritive value of apricot (*Prunus armeniaca*) kernel. I. Comparative digestibility tests of detoxified apricot kernel and common sweet almond. *Rev Agroquim Tecnol Alimentos* 12(3): 436–443. Abstract Accessed through ISI Web of Science, Food Science, and Technology Abstracts (May 15, 2004).
23. Calixto FS, Canella J, de Toda FM (1982) A chemical study of the protein fraction of Mediterranean sweet almond varieties (*Prunus amygdalus*). *Z fuer Lebensmittel Untersuchung Forsch* 175(1): 34–37.
24. Hall AP, Moore JG, Gunning B, Cook BB (1958) The nutritive value of fresh and roasted, California-grown Nonpareil almonds. *J Agric Food Chem* 6(5): 377–382.
25. Cowan JW, Sabry ZI, Rinnu FJ, Campbell JA (1963) Evaluation of protein in middle eastern diets. *J Nutr* 81: 235–240.
26. Garcia-Mas J, Messeguer R, Arus P, Puidomenech P (1995) Molecular characterization of cDNAs corresponding to genes expressed during almond (*Prunus amygdalus* Batsch) seed development. *Plant Mol Biol* 27(1): 205–210.