Research Article

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Raw and roasted pistachio nuts (*Pistacia vera* L.) are 'good' sources of protein based on their digestible indispensable amino acid score as determined in pigs

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Abstract

BACKGROUND: Pistachio nuts may be consumed as raw nuts or as roasted nuts. However, there is limited information about the protein quality of the nuts, and amino acid (AA) digestibility and protein quality have not been reported. Therefore, the objective of this research was to test the hypothesis that raw and roasted pistachio nuts have a digestible indispensable AA score (DIAAS) and a protein digestibility corrected AA score (PDCAAS) greater than 75, thereby qualifying them as a good source of protein.

RESULTS: The standardized ileal digestibility (SID) of all indispensable AAs, except arginine and phenylalanine, was less in roasted pistachio nuts than in raw pistachio nuts ($P < 0.05$). Raw pistachio nuts had a PDCAAS of 73, and roasted pistachio nuts had a PDCAAS of 81, calculated for children 2–5 years, and the limiting AA in the PDCAAS calculation was threonine. The DIAAS values calculated for children older than 3 years, adolescents, and adults was 86 and 83 for raw and roasted pistachio nuts respectively. The limiting AA in both raw and roasted pistachio nuts that determined the DIAAS for this age group was lysine.

CONCLUSION: The results of this research illustrate that raw and roasted pistachio nuts can be considered a good quality protein source with DIAAS greater than 75; however, processing conditions associated with roasting may decrease the digestibility of AAs in pistachio nuts.
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Keywords: protein digestibility corrected amino acid score; digestible indispensable amino acid score; pistachio nuts; pig model

INTRODUCTION

Pistachio nuts (*Pistacia vera* L.) have been identified in archeological excavations indicating that they have been associated with human activity for centuries.[1] Pistachio nuts are thought to be originally native to Iran, Afghanistan, and central Asia,[2] but they are currently being cultivated and grown in a variety of climates.[1] Iran, the USA (only California), and Turkey are the top three producers of pistachio nuts, and in 2018 these countries produced approximately 551 000 t, 448 000 t, and 240 000 t of pistachio nuts respectively.[3]

The majority of edible nuts (e.g. pistachios, cashews, chestnuts, pecans, brazil nuts, peanuts, macadamia nuts) are rich sources of lipids, protein, and minerals.[2,4] Lipids make up a significant portion of the composition of edible nuts, ranging from approximately 450 to 700 g kg^{-1},[2,4,5] but pistachio nuts have among the least concentration of lipids, at approximately 450 g kg^{-1}.[6] The concentration of protein in edible nuts ranges from approximately 75 to 220 g kg^{-1} if a nitrogen-to-protein conversion factor of 5.3 is used,[7] and pistachio nuts have a greater protein concentration (approximately 210 g kg^{-1}) than most other nuts.[6] In addition, mineral concentrations in edible nuts range from approximately 10 to 35 g kg^{-1} [4] (approximately 32 g kg^{-1} in pistachio nuts[6]), and pistachio nuts are rich in vitamin A, vitamin E, vitamin B_1, vitamin B_6, vitamin K, and potassium, magnesium, calcium, and copper.[5,6]

Pistachio nuts, at the time of harvest, contain 400 to 500 g kg^{-1} moisture on a fresh weight basis and must be dried to about 50 g kg^{-1} moisture to inhibit fungal growth, prevent rancidity, improve sensory characteristics, and extend shelf life.[8,9] Pistachio nuts can be consumed in raw or roasted forms. Several health benefits in relation to metabolic conditions and cardiovascular health have been associated with the bioactive compounds and lipid composition of pistachio nuts.[6,10] However, to our knowledge the quality of protein in pistachio nuts and the effect of roasting on protein quality have not been studied. Therefore, the objective of this work was to determine the ileal digestibility of amino acids (AAs) in raw and roasted pistachio nuts and to test the hypothesis that the protein digestibility corrected AA score (PDCAAS) and the digestible indispensable AA score (DIAAS) in both raw and roasted pistachio nuts are greater than 75.

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MATERIALS AND METHODS

The protocol for the experiments in this study was reviewed and approved by the Institutional Animal Care and Use Committee at the University of Illinois. Two protein sources, raw pistachio nuts and roasted pistachio nuts, were used in this study (Table 1). Pistachio nuts were roasted in a commercial rotary batch roaster. Roasters were preheated to 176 °C. Once nuts were added, nut temperature was gradually increased to 115 °C. They were then removed from the roaster and allowed to cool to room temperature. The entire roasting process took approximately 30 min. Raw and roasted pistachio nuts were ground using a roller mill prior to addition to the diets.

Diets, animals, and feeding

Three diets were prepared (Tables 2 and 3) with the two pistachio proteins included in one diet each as the only AA-containing ingredient. A nitrogen-free diet was also formulated and used to measure basal endogenous losses of AAs and crude protein (CP). Vitamins and minerals were included in all diets to meet or exceed current nutrient requirement estimates for growing pigs.[11] Diets also contained 4 g kg^{-1} titanium dioxide as an indigestible marker, and all diets were provided in meal form. Samples of each pistachio protein and of all diets were collected at the time of diet mixing and used for chemical analysis.

Twelve growing barrows (initial body weight 60.9 ± 3.2 kg) were equipped with a T-cannula in the distal ileum.[12] Following surgery, pigs were randomly allotted to a two-period switch-back design with three diets and four replicate pigs in each period; therefore, there were eight replicate pigs per treatment. Each period was 9 days; therefore, the entire experiment lasted 18 days. Pigs were housed in individual pens (1.2 m × 1.5 m) in an environmentally controlled room. Each pen had smooth sides and fully slatted tribar floors. A feeder and a nipple drinker were installed in each pen.

All pigs were fed their assigned diets in a daily amount equivalent to 40 g kg^{-1} of the body weight for each pig. Two equal meals were provided daily at 08:00 and 16:00, and water was available at all times. Pig weights were recorded at the beginning of each period and at the conclusion of the experiment. The amount of feed supplied each day was also recorded.

Sample collection

Experimental periods were 9 days, with the initial 5 days being the adaptation period to the diet. Fecal samples were collected on days 6 and 7 and stored at −20 °C, and ileal digesta were collected for 9 h (from 08:00 to 17:00) on days 8 and 9 following standard procedures. In short, a plastic bag was attached to the cannula barrel and digesta flowing into the bag were collected. Bags were removed when filled with ileal digesta, or at least once every 30 min, and immediately frozen at −20 °C to prevent bacterial degradation of AAs in the digesta. On the completion of the first experimental period, animals were deprived of feed overnight, and the following morning the new experimental diet was offered.

Chemical analysis

At the end of the experiment, fecal samples were dried and ground through a 1 mm screen in a Wiley Mill (model 4; Thomas Scientific, Swedesboro, NJ, USA) prior to chemical analysis. Ileal digesta samples were thawed, mixed within animal and diet, and a subsample was collected for chemical analysis. Ileal digesta samples were lyophilized and finely ground prior to chemical analysis. Samples of all ingredients, diets, fecal samples, and ileal digesta samples were analyzed for dry matter (Method 927.05)[7] and for nitrogen by combustion (Method 990.03)[7] using a Leco FP628 analyzer (Leco Corp., Saint Joseph, MI, USA). A nitrogen-to-protein conversion factor of 6.25 was used to calculate CP as recommended by the Food and Agriculture Organization of the United Nations (FAO) for the calculation of PDCAAS and DIAAS.[13,14] All diets, fecal samples, and ileal digesta samples were also analyzed for titanium (Method 990.08),[15] and all diets, ingredients, and ileal digesta samples were analyzed for AAs (Method 982.30 E (a, b, c)).[7]

Calculations

Values for apparent ileal digestibility (AID), basal endogenous losses, and standardized ileal digestibility (SID) of CP and AAs in each diet were calculated based on equations from Stein *et al.*[16] The standardized total tract digestibility (STTD) of CP was also calculated.[17]

Values for the STTD of CP were used to calculate PDCAAS using the following equation:[13]

$$\text{PDCAAS}\,(\%) = \frac{\text{Mass}\,(\text{mg})\ \text{of limiting AA in 1 g of test protein}}{\text{Mass}\,(\text{mg})\ \text{of the same AA in 1 g of reference protein} \times \text{Standardized total tract digestibility}\,(\%)} \times 100 \quad (1)$$

The calculation of PDCAAS was accomplished using the reference protein pattern for preschool children from 2 to 5 years of age.[13] Values were calculated using a nitrogen-to-protein conversion factor of 6.25,[13] as well as a conversion factor of 5.3.[7,18]

Values for the SID of AAs were used to calculate DIAAS reference ratios for each AA using the following equation:[19]

$$\text{Digestible IAA reference ratio} = \frac{\text{Digestible IAA content in 1 g protein of food}\,(\text{mg})}{\text{Mass}\,(\text{mg})\ \text{of the same dietary IAA in 1 g of the reference protein}} \quad (2)$$

where IAA is the indispensable AA.

The DIAAS was calculated for children older than 3 years, adolescents, and adults as recommended by the FAO[14] using the following equation:

$$\text{DIAAS}\,(\%) = 100 \times \text{Lowest value of the digestible IAA reference ratio} \quad (3)$$

DIAAS was calculated using a nitrogen-to-protein conversion factor of 6.25.[14]

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Table 1 Analyzed nutrient composition (g kg^{-1}) of ingredients, as-fed basis

Item	Raw pistachio nuts	Roasted pistachio nuts
Dry matter	961	973
Ash	28.7	28.8
Crude protein		
Nitrogen × 6.25	271	251
Nitrogen × 5.3	229	212
Indispensable amino acids		
Arginine	23.1	23.1
Histidine	5.3	5.4
Isoleucine	11.0	11.3
Leucine	17.0	17.4
Lysine	12.9	12.9
Methionine	3.8	3.9
Phenylalanine	13.6	13.8
Threonine	7.3	7.5
Tryptophan	3.4	2.7
Valine	14.4	14.9
Total	111.8	112.9
Dispensable amino acids		
Alanine	10.1	10.5
Asparagine	20.4	20.7
Cysteine	4.5	4.6
Glutamic acid	44.6	44.8
Glycine	10.6	10.8
Proline	9.0	9.3
Serine	12.0	12.2
Tyrosine	7.0	7.1
Total	118.2	120.0
Total amino acids	230.0	232.9

Table 2 Ingredient composition (g kg^{-1}) of experimental diets, as-fed basis

Item	Raw pistachio nuts	Roasted pistachio nuts	Nitrogen free
Pistachio nuts	500.0	510.0	—
Cornstarch	286.5	276.5	677.5
Cellulose (Solka-Floc)	40.0	40.0	40.0
Soybean oil	40.0	40.0	40.0
Limestone	7.0	7.0	5.5
Dicalcium phosphate	15.5	15.5	21.0
Sodium chloride	4.0	4.0	4.0
Magnesium oxide	—	—	1.0
Potassium carbonate	—	—	4.0
Sucrose	100.0	100.0	200.0
Titanium dioxide	4.0	4.0	4.0
Vitamin mineral premix[a]	3.0	3.0	3.0

[a] The vitamin–micromineral premix provided the following quantities of vitamins and microminerals per kilogram of complete diet: vitamin A as retinyl acetate, 11 136 IU; vitamin D_3 as cholecalciferol, 2208 IU; vitamin E as DL-α-tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B_{12}, 0.03 mg; D-pantothenic acid as calcium D-pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; copper, 20 mg as copper sulfate and copper chloride; iron, 126 mg as ferrous sulfate; iodine, 1.26 mg as ethylenediamine dihydriodide; manganese, 60.2 mg as manganese sulfate; selenium, 0.3 mg as sodium selenite and selenium yeast; and zinc, 125.1 mg as zinc sulfate.

Table 3 Analyzed nutrient composition (g kg^{-1}) of experimental diets (as-fed basis)

Item	Raw pistachio nuts	Roasted pistachio nuts	Nitrogen free
Dry matter	952.9	961.6	936.7
Crude protein			
Nitrogen × 6.25	127.7	125.2	5.1
Nitrogen × 5.3	108.3	106.2	4.3
Indispensable AA			
Arginine	11.3	11.8	0.1
Histidine	2.7	2.8	0.1
Isoleucine	5.4	5.7	0.2
Leucine	8.6	9.0	0.3
Lysine	6.6	6.8	0.3
Methionine	1.9	2.0	0.1
Phenylalanine	6.4	6.6	0.3
Threonine	3.8	3.9	0.1
Tryptophan	1.5	1.5	0.2
Valine	7.2	7.5	0.2
Total	55.4	57.6	1.9
Dispensable AA			
Alanine	5.2	5.5	0.2
Asparagine	10.8	11.2	0.3
Cysteine	2.3	2.4	0.1
Glutamic acid	24.9	25.7	0.5
Glycine	5.4	5.7	0.2
Proline	4.9	4.9	0.5
Serine	6.0	6.2	0.1
Tyrosine	3.1	3.2	0.2
Total	62.6	64.8	2.1
Total AA	118.0	122.4	4.0

Statistical analysis

At the conclusion of the experiments, the normality of data was verified and outliers were identified using the UNIVARIATE and BOXPLOT procedures respectively (SAS Institute Inc., Cary, NC, USA). Once outliers were removed, data were analyzed by analysis of variance using the MIXED procedure in SAS (SAS Institute Inc.). The pig was the experimental unit for all analyses. Diet was the fixed effect, and pig and period were random effects. Treatment means were calculated using the LS MEANS statement in SAS; and if significant, means were separated using the PDIFF option in the MIXED procedure. An α value of 0.05 was used to assess significance among means.

RESULTS

All pigs remained healthy throughout the study and readily consumed their daily feed allowance. The concentration of CP (nitrogen × 6.25) was numerically greater for raw pistachio nuts (271 g kg^{-1}) than with roasted pistachio nuts (251 g kg^{-1}; Table 1). The AA in greatest concentration for both pistachio nuts was arginine (Arg), with leucine being the AA in second greatest concentration. Tryptophan and methionine were the AAs in least and second least concentration respectively, for both raw and roasted pistachio nuts.

The AID of CP was greater ($P < 0.05$) for raw pistachio nuts than for roasted pistachio nuts (Table 4), and the AID of all IAAs except Arg, isoleucine, and phenylalanine (Phe) was greater ($P < 0.05$) for

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Table 4 Apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (%) and amino acids (AAs, %) in pistachio nuts[a,b]

	AID				SID			
Item	Raw pistachio nuts	Roasted pistachio nuts	Pooled SEM	*P*-value	Raw pistachio nuts	Roasted pistachio nuts	Pooled SEM	*P*-value
Crude protein (nitrogen × 6.25)	77.22	67.46	2.70	0.005	94.45	85.19	2.70	0.007
Indispensable AAs								
Arginine	88.99	83.33	2.07	0.089	97.83	91.79	2.07	0.075
Histidine	80.88	71.56	2.09	0.008	88.83	79.22	2.09	0.006
Isoleucine	80.51	71.86	2.61	0.051	87.22	78.23	2.61	0.045
Leucine	81.29	72.85	2.51	0.048	87.99	79.25	2.51	0.043
Lysine	79.97	70.53	1.92	0.004	86.60	76.97	1.92	0.004
Methionine	82.72	76.08	1.96	0.037	86.94	80.08	1.96	0.033
Phenylalanine	81.31	71.90	2.81	0.057	86.90	77.31	2.81	0.054
Threonine	71.32	60.94	2.30	0.007	88.03	77.22	2.30	0.006
Tryptophan	83.57	76.24	2.12	0.044	91.81	84.48	2.12	0.044
Valine	79.50	70.06	2.69	0.044	87.77	78.00	2.69	0.039
Mean	81.85	73.45	2.39	0.045	89.76	81.05	2.39	0.040
Dispensable AAs								
Alanine	75.80	66.62	2.06	0.008	91.38	81.35	2.06	0.004
Asparagine	80.61	71.81	2.45	0.056	88.79	79.70	2.45	0.050
Cysteine	79.11	70.99	1.45	0.002	88.03	79.53	1.45	0.001
Glutamic acid	87.68	81.03	1.94	0.059	91.88	85.09	1.94	0.055
Glycine	67.05	51.07	6.48	0.007	107.73	89.61	6.48	0.003
Proline	46.71	35.08	10.27	0.120	208.48	196.86	10.27	0.120
Serine	81.83	73.81	2.43	0.038	91.69	83.36	2.43	0.033
Tyrosine	77.44	68.52	2.43	0.036	87.59	78.36	2.43	0.032
Mean	78.54	68.28	3.85	0.014	100.87	89.84	3.85	0.010
Total AAs	80.08	70.71	2.80	0.008	95.63	85.70	2.80	0.006

[a] Data are least-squares means of eight observations for roasted pistachio nuts and seven observations for raw pistachio nuts. SEM: standard error of the mean.
[b] SID values were calculated by correcting values for AID for the basal ileal endogenous losses. Endogenous losses of crude protein and AAs (g kg^{-1} dry matter intake) were as follows: crude protein, 23.09; arginine, 1.05; histidine, 0.23; isoleucine, 0.38; leucine, 0.60; lysine, 0.46; methionine, 0.08; phenylalanine, 0.38; threonine, 0.67; tryptophan, 0.13; valine, 0.62; alanine, 0.85; asparagine, 0.93; cysteine, 0.22; glutamic acid, 1.10; glycine, 2.31; proline, 8.32; serine, 0.62; tyrosine, 0.33.

Table 5 Standardized total tract digestibility (STTD) of crude protein (%) in pistachio nuts[a,b]

Item	Raw pistachio nuts	Roasted pistachio nuts	Pooled SEM	*P*-value
STTD of crude protein (nitrogen × 6.25)	92.11	91.64	0.84	0.698

[a] Data are least-squares means of eight observations for roasted pistachio nuts and seven observations for raw pistachio nuts. SEM: standard error of the mean.
[b] STTD of crude protein was calculated by correcting the value for apparent total tract digestibility of crude protein for the basal total tract endogenous loss of crude protein, which was determined as 12.05 g kg^{-1} dry matter intake.

raw pistachio nuts than for roasted nuts. The SID of CP and all IAAs except Arg and Phe was also greater ($P < 0.05$) for raw pistachio nuts than for roasted pistachio nuts. The STTD of CP for raw pistachio nuts did not differ from the STTD of CP in roasted pistachio nuts (Table 5).

The concentration of standardized ileal digestible CP in raw pistachio nuts was greater ($P < 0.05$) than for roasted pistachio nuts (Table 6). Concentrations of all standardized ileal digestible IAAs were greater ($P < 0.05$) in raw pistachio nuts than in roasted pistachio nuts, and the concentration of all dispensable AAs, except alanine and proline, was also greater ($P < 0.05$) in raw pistachio nuts than for roasted pistachio nuts.

The PDCAAS values calculated for the pistachio nuts were based on the requirement for children from 2 to 5 years (Table 7). The CP values used in the calculation of PDCAAS were calculated with a nitrogen-to-protein conversion factor of 6.25 and also using a conversion factor of 5.3. The PDCAAS calculated with the 6.25 conversion factor for roasted pistachio nuts (81) was greater

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Table 6 Concentrations of standardized ileal digestible crude protein (%) and amino acids (AAs, %) in pistachio nuts[a]

Item	Raw pistachio nuts	Roasted pistachio nuts	Pooled SEM	*P*-value
Crude protein (nitrogen × 6.25)	26.67	21.93	0.55	0.001
Indispensable AAs				
Arg	2.36	2.17	0.05	0.015
His	0.49	0.44	0.01	0.009
Ile	1.00	0.91	0.03	0.037
Leu	1.56	1.42	0.04	0.030
Lys	1.16	1.02	0.03	0.002
Met	0.34	0.32	0.01	0.049
Phe	1.23	1.10	0.04	0.019
Thr	0.67	0.60	0.02	0.011
Trp	0.33	0.23	0.01	0.001
Val	1.32	1.19	0.04	0.036
Total	10.45	9.40	0.25	0.011
Dispensable AAs				
Ala	0.96	0.88	0.02	0.017
Asp	1.89	1.69	0.81	0.868
Cys	0.41	0.38	0.01	0.002
Glu	4.27	3.91	0.08	0.007
Gly	1.20	0.99	0.04	0.007
Pro	1.92	1.92	0.07	0.976
Ser	1.15	1.04	0.02	0.012
Tyr	0.64	0.57	0.02	0.011
Total	12.46	11.08	0.34	0.013
Total AAs	22.95	20.51	0.53	0.006

[a] Data are least-squares means of eight observations for roasted pistachio nuts and seven observations for raw pistachio nuts. SEM: standard error of the mean.

Table 7 Protein digestibility corrected amino acid (PDCAA) reference ratios and protein digestibility corrected amino acid scores (PDCAAS) for raw and roasted pistachio nuts[a,b]

Item	Raw pistachio nuts	Roasted pistachio nuts	Pooled SEM	*P*-value
PDCAA reference ratio				
Histidine	0.95	1.04		
Isoleucine	1.34	1.48		
Leucine	0.88	0.96		
Lysine	0.76	0.81		
SAA[c]	1.13	1.24		
AAA[c]	1.11	1.21		
Threonine	0.73	0.81		
Tryptophan	1.05	0.90		
Valine	1.40	1.56		
PDCAAS				
FAO[d,e], (%)	73 (threonine)	81 (threonine)	0.76	<0.001
FDA[d,f] (%)	86 (threonine)	95 (threonine)	0.87	<0.001

[a] First-limiting amino acid (AA) is in parentheses.
[b] PDCAAS values were calculated from the standardized total tract digestiblity of crude protein in pigs: raw pistachio nuts, 92.11%; roasted pistachio nuts, 91.64%. SEM: standard error of the mean.
[c] SAA: sulfur AA (methionine and cysteine); AAA, aromatic AA (phenylalanine and tyrosine).
[d] PDCAAS values were calculated using the recommended AA scoring pattern for preschool children (2–5 years). The indispensable AA reference patterns are expressed as milligrams of AA per gram of protein: histidine, 19; isoleucine, 28; leucine, 66; lysine, 58; SAA, 25; AAA, 63; threonine, 34; tryptophan, 11; valine, 35.[13]
[e] Crude protein values used in the calculation of PDCAAS were calculated using a nitrogen-to-protein conversion factor of 6.25 as recommended by the Food and Agriculture Organization of the United Nations (FAO).[13,14]
[f] A nitrogen-to-protein conversion factor of 5.3 was used for crude protein in the calculation of PDCAAS as recommended by the US Food and Drug Administration for food labels (FDA).[18]

($P < 0.05$) than for raw pistachio nuts (73), and the PDCAAS calculated with the 5.3 conversion factor was also greater ($P < 0.05$) for roasted pistachio nuts (95) than for raw pistachio nuts (86). The first limiting AA in both raw and roasted pistachio nuts, when compared with the AA requirements of 2- to 5 year-old children, was threonine.

The DIAAS values were calculated based on the requirement for children older than 3 years, adolescents, and adults (Table 8). The DIAAS was numerically greater for raw pistachio nuts than for roasted pistachio nuts, with values of 86 and 83 respectively. Lysine (Lys) was the first limiting AA in both raw and roasted pistachio nuts when compared with the AA requirements for children older than 3 years, adolescents, and adults.

DISCUSSION

It is recommended that values for PDCAAS are determined using the rat model.[13] However, in this experiment, the pig was used to be able to determine PDCAAS and DIAAS in the same animal. All calculations for PDCAAS were done as recommended,[13] but because pigs were used, rather than rats, the values for PDCAAS obtained in this experiment may be called 'PDCAAS-like' values.[17] When using the pig as the model, values for AA digestibility determined at the end of the small intestine and corrected for the basal endogenous losses are typically called SID values.[16] In human nutrition, such values are often described as values for true ileal digestibility.[14]

In this study, PDCAAS was calculated using the recommended reference protein pattern for children from 2 to 5 years of age,[13] and DIAAS was only calculated using the recommended reference protein pattern for children older than 3 years, adolescents, and adults,[14] because younger children usually do not consume significant amounts of nuts. To our knowledge, PDCAAS and DIAAS have not been determined in pistachio nuts. However, peanuts, almonds, cashews, and brazil nuts have reported PDCAAS values of 69, 57, 90, and 63 respectively.[20] In addition, peanuts were assigned a DIAAS of 43.[21] With the exception of cashews, the PDCAAS and DIAAS obtained in this study for both raw and roasted pistachio nuts are greater than published values for other nuts. The DIAAS for pistachio nuts is also greater than for peas or kidney beans, but less than for soy proteins.[17,21] Thus, it appears that pistachio nuts, whether raw or roasted, have a protein quality that is better than that of most other nuts and also better than in pulse crops. Based on the DIAAS cut-off values describing protein quality,[12] raw pistachio nuts and roasted pistachio nuts can both be considered a 'good'-quality protein if consumed by children older than 3 years, adolescents, and adults. The implication of this

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Table 8 Digestible indispensable amino acid (DIAA) reference ratios and digestible indispensable amino acid scores (DIAAS) for raw and roasted pistachio nuts[a]

	Reference pattern: older children, adolescents, and adults			
Item	Raw pistachio nuts	Roasted pistachio nuts	pooled SEM	*P*-value
DIAA reference ratio				
Histidine	1.09	1.07		
Isoleucine	1.18	1.18		
Leucine	0.91	0.90		
Lysine	0.86	0.83		
SAA[b]	1.17	1.18		
AAA[b]	1.62	1.58		
Threonine	0.95	0.92		
Tryptophan	1.75	1.38		
Valine	1.17	1.16		
DIAAS[c,d] (%)	86 (lysine)	83 (lysine)	2.02	0.260

[a] First-limiting amino acid (AA) is in parentheses. SEM: standard error of the mean.
[b] SAA: sulfur AA (methionine and cysteine); AAA: aromatic AA (phenylalanine and tyrosine).
[c] DIAAS was calculated using the recommended AA scoring pattern for older child, adolescent, and adult. The indispensable AA reference patterns are expressed as milligrams of AA per gram of protein: histidine, 16; isoleucine, 30; leucine, 61; lysine, 48; SAA, 23; AAA, 41; threonine, 25; tryptophan, 6.6; valine, 40.[14]
[d] Crude protein values used in the calculation of DIAAS were calculated using a nitrogen-to-protein conversion factor of 6.25 as recommended by the Food and Agriculture Organization of the United Nations.[14]

observation is that pistachio nuts may significantly contribute to the overall AA status of an individual provided they are consumed in combination with other high-quality proteins.

Compared with cereal grains, pistachio nuts, both raw and roasted, have a greater DIAAS than all reported cereal grains assigned a DIAAS.[17,19,21–23] The cereal grain with a DIAAS closest to pistachio nuts is raw, dehulled oats with a DIAAS of 77 for children older than 3 years, adolescents, and adults.[19] After cooking, the DIAAS of rolled oats decreased to 54 for children from 6 months to 3 years.[21] However, these DIAAS values were determined using different animal models (i.e. the growing rat and growing pig) and calculated with different reference protein patterns. To our knowledge, the effect on the DIAAS of cooking a food item has only been reported for beef topside steak, where it was concluded that cooking conditions affected the concentration of digestible AAs and the protein quality of meat.[24] In the current study, the concentration of all digestible IAAs in roasted pistachio nuts was significantly lower than in raw pistachio nuts, which indicates that roasting negatively affected the concentration of digestible AAs in pistachio nuts. Therefore, it is likely that roasting resulted in some heat damage of the pistachio nuts.

Pistachio nuts are commonly roasted prior to packaging, storage, and consumption to enhance flavor and texture, increase shelf life, and to reduce allergenicity.[25,26] The targeted moisture content of pistachio nuts post-roasting is approximately 30 g kg^{-1};[25] the roasted pistachio nuts used in this study had a moisture content of approximately 27 g kg^{-1}, indicating they were roasted following common industry practices. Food processing techniques involving heat often result in conformational changes to protein, such as denaturation, degradation, and aggregation, which may alter protein and AA digestibility.[26–28] Protein denaturation, or unfolding of the tertiary and secondary structure of the protein, is observed at temperatures of approximately 70–80 °C,[26] which may improve protein digestibility.[29] However, protein aggregation is observed at temperatures greater than 100 °C, and this may result in decreased protein and AA digestibility.[26,30] Protein aggregation may lead to modification and reduced digestibility of certain AAs, especially Lys, histidine (His), and Arg.[30] In the current study, the SID of Lys and His were greatly reduced in the roasted pistachio nuts compared with the raw nuts, indicating that the roasting process may have resulted in heat damage and protein aggregation.

Using the STTD of CP in the calculation for PDCAAS generally overestimates the ileal digestibility of AAs for food items with lower AA digestibility, resulting in an inaccurate PDCAAS.[17,22] The current study also illustrates this point. The STTD of CP did not differ between the raw and roasted pistachio nuts. However, it is clear that the SID of CP and most AAs is greater for raw pistachio nuts than for roasted pistachio nuts. Thus, the greater PDCAAS for roasted pistachio nuts compared with raw pistachio nuts illustrates that PDCAAS values are unable to account for the decrease in digestibility that happens in heat damaged food items. In contrast, because DIAAS values are calculated based on the ileal digestibility of each individual AA, the reduction in protein quality that is a result of heat damage is reflected in the DIAAS, which is also clearly demonstrated in the present study. As a consequence, DIAAS more accurately represents the protein quality of heat damaged food proteins than PDCAAS does.

Annual consumption of pistachio nuts in the USA has doubled in the last decade, and is now around 200 g *per capita*, which is much greater than average global consumption of less than 10 g *per capita*. However, global production is increasing by 5–10% annually.

The CP and AA concentration for pistachio nuts used in this study are slightly greater than values reported in the literature and from the US Department of Agriculture Food Composition Database.[4,6,31,32] This is likely because the nitrogen-to-protein conversion factor of 6.25 was used to calculate CP. Considering the nitrogen concentration content in the pistachio nuts, the average nitrogen in the raw and roasted pistachio nuts was 43.3 g kg^{-1} and 40.1 g kg^{-1} respectively. If a nitrogen-to-protein conversion factor of 5.3 is used to determine the concentration of CP and AA in the two forms of pistachio nuts, those values are similar to values published in the literature.[4,6,31,32]

A conversion factor of 6.25 has been the standard for calculating CP from the total nitrogen content analyzed due to the early determination of the average nitrogen content of proteins being approximately 160 g kg^{-1}.[33] However, the nitrogen content of protein from different sources (i.e. plants and animals) has been reported to range from approximately 130 to 190 g kg^{-1},[34] equating to nitrogen-to-protein conversion factors of 7.69 to 5.26. The variable nitrogen concentration of protein is the result of differences in AA composition and differences among sources of proteins in concentrations of non-protein nitrogen, such as nucleotides, amines, amides, and so on.[33,35,36] To address these differences, nitrogen-to-protein conversion factors were determined for commonly consumed foods.[34] These conversion factors, known as 'Jones factors', are still used in the USA in food composition databases and on food labels.[7,17,32] When protein claims are made for a food item and reported on the food label,

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the PDCAAS has to be listed,[18] and the US Food and Drug Administration requires the food-specific nitrogen-to-protein conversion factor to be used in the calculation for PDCAAS.[16] For legumes, a conversion factor of 5.46 for peanuts and brazil nuts, 5.18 for almonds, and 5.30 for other nuts is required when calculating CP for food labels.[7,18]

However, when determining protein quality of food items using the PDCAAS or DIAAS methodology, the FAO[13,14] recommends a nitrogen-to-protein conversion factor of 6.25 for all test proteins. Consequently, when using specific conversion factors that are lower than 6.25, such as 5.3 for pistachio nuts, those food items will have a decreased CP content (quantity) but an increased calculated value for PDCAAS, which indicates the quality of the protein. This is illustrated in this study by a PDCAAS of 81 for roasted pistachio nuts if the 6.25 conversion factor is used, whereas the PDCAAS is 95 if the 5.3 conversion factor is used. The recommendation to always use a conversion factor of 6.25 standardizes the protein quality calculation and, therefore, reported PDCAAS and DIAAS values are representative of the nutritional value of nitrogen rather than of protein.[35]

CONCLUSION

This is the first study to determine protein quality of pistachio nuts by both the PDCAAS and DIAAS methods. Roasting pistachio nuts decreased ileal digestibility of AAs and the DIAAS value, whereas PDCAAS values were not reduced by roasting. However, pistachio nuts in both the raw and roasted forms are a 'good' source of protein, with a DIAAS greater than 75.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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