Journal of the Science of Food and Agriculture
J Sci Food Agric **83**:7–12 (online: 2002)
DOI: 10.1002/jsfa.1273

Study of the effect of using different levels of tahinah (sesame butter) on the protein digestibility-corrected amino acid score (PDCAAS) of chickpea dip†

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Abstract: This study was conducted to investigate the effect of using different proportions of tahinah (0–25%) on the protein digestibility-corrected amino acid score (PDCAAS) of chickpea dip and to evaluate this parameter when chickpea dip is consumed with wheat bread. Protein quality was evaluated using the methods of amino acid score and true protein digestibility in weanling Sprague–Dawley rats. The levels of tahinah that provided the best true protein digestibility and protein digestibility-corrected amino acid score were 20 and 25%. Values of true protein digestibility were significantly higher ($P \leqslant 0.05$) than for the other types of chickpea dip (0.87 and 0.88 respectively). The consumption of wheat bread with chickpea dip led to a marked improvement in the true protein digestibility of the protein mixture (0.90); however, the protein digestibility-corrected amino acid score did not change in the same manner owing to the relatively low amino acid score. It can be concluded that the addition of tahinah to chickpea led to an increase in the protein digestibility-corrected amino acid score of chickpea dip (based on laboratory rat requirements for essential amino acids) and that the consumption of chickpea dip with bread led to an improvement in the protein digestibility of chickpea dip but not in the protein digestibility-corrected amino acid score.

Keywords: chickpea dip; tahinah (sesame butter); true protein digestibility (TD); protein digestibility-corrected amino acid score (PDCAAS)

INTRODUCTION

Chickpea (*Cicer arietinum* L) is a member of the Leguminosae and is the fifth most important legume in the world.[1] It is classified among the high-protein seed crops, with a mean value of 22.2%.[2,3] Chickpea dip, which is prepared from chickpea, tahinah (sesame butter) and lemon juice or citric acid,[4] is a very popular dish in South Asian as well as Mediterranean countries, including Jordan. However, the deficiency of legumes, including chickpea, in sulphur-containing amino acids and the deficiency of sesame protein in lysine are well known.[3,5–8]

There are many methods to evaluate the protein quality of food mixtures, including protein digestibility, chemical score and protein digestibility-corrected amino acid score (PDCAAS).[9] Digestibility of a protein is defined as the fraction of ingested nitrogen which is absorbed.[10] It can be used as a biological indicator of protein quality and can be measured by rat bioassay as 'true digestibility (TD)', in which allowance is made for faecal nitrogen loss on a protein-free diet.[9,11]

Chickpea and sesame seeds have been reported to contain different types of antinutritional factors, the most important of which are trypsin inhibitors.[12,13] Such substances have the ability to inhibit the proteolytic activity of the digestive enzymes trypsin and chymotrypsin, thus hindering protein digestibility. The protease inhibitors that have been isolated from soybeans and other legumes are the so-called Kunitz and Bowman–Birk inhibitors.[14]

Chemical score is the most theoretically sound and commonly used chemical method.[9,11] However, this method does not take into account the bioavailability of amino acids or protein digestibility. PDCAAS is adopted as a current concept in protein quality evaluation methodology since it is more relevant to human requirements.[15] Therefore, and since varying proportions of tahinah are used in the preparation of chickpea dip, the objective of this work was to study the effect of using different proportions of tahinah in improving the protein digestibility and PDCAAS of chickpea dip.

Because chickpea dip is usually consumed in East

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† This paper was presented at the First Jordanian Conference for Food and Nutrition, Amman, Jordan, 11–12 July 2001
(*Received 19 February 2001; revised version received 19 June 2002; accepted 20 September 2002*)

Table 1. Components of 6 kg chickpea dips

Percentage of tahinah (cooked chickpea basis)	*Cooked chickpea (g)*	*Tahinah (g)*
0	6000	0
10	5400	600
15	5100	900
20	4800	1200
25	4500	1500

Mediterranean countries with white wheat bread in a ratio of 1:2 (w/w), this study was also aimed at identifying the extent to which the consumption of chickpea dip with wheat bread would improve the protein digestibility and PDCAAS of the food mixture.

MATERIALS AND METHODS

Kabuli chickpea (*C arietinum* L) and *Al-Kasih* tahinah were chosen for this study as they are widely used. They were processed into chickpea dip using the traditional methodology described by Pellett and Shadarevian.[16]

Cooked seeds were removed and preserved in a special stainless steel container. The cooking water was decanted and preserved for later use in the preparation of chickpea dip, as this water contains soluble proteins mobilised from the grains upon cooking.[17]

Sample preparation

Five chickpea dips (6 kg each) were prepared by combining specified quantities of chickpea and tahinah, except for the diet of chickpea alone (0% tahinah), as shown in Table 1. The ingredients were mixed thoroughly in a stainless steel blender (Valco, Milan, Italy). The chickpea dips were dried in an air-circulated drying oven (Blue M, Blue Islands, IL, USA) at 70 °C overnight. The dried chickpea dips were ground to pass through a 5 mm mesh (Retsch GmbH, Haan, Germany). They were then kept in polyethylene bags and stored in a refrigerator at 4 °C until used for the preparation of animal diets. The white bread used in one of the diets (mixture of bread and chickpea dip in 2:1 ratio) was dried at 70 °C for 5 h and ground to pass through a 5 mm mesh. Then 790 g of dried and ground bread was mixed with 500 g of dried and ground chickpea dip with 15% tahinah.

Protein quality evaluation

Six test diets were prepared containing 10% protein and 10% fat. These were compared with a casein diet (BDH Chemicals Ltd, Poole, UK) containing about 9% protein and 10% fat and a 'protein-free' diet based on starch, which contained a negligible amount of protein (the amount of fat added was similar to that of the other diets). In addition, equal amounts of vitamin and mineral mixtures were added to each of the eight diets. The ingredients of the diets used in the animal experiments are shown in Table 2 and the results of their proximate analysis are presented in Table 3. Proximate analysis was done according to AOAC methods.[18]

In vivo rat assay for true protein digestibility

The rats were of Sprague–Dawley strain, weaned at 23 days old and then fed a stock diet for 1 week. They were divided into eight groups of five rats each. The difference in mean weight between any two groups did not exceed 2 g. Each rat was individually caged in a stainless steel-bottomed cage with a polypropylene tray and box (North Kent Plastic Cages, Ltd, Dartford, UK) and maintained at a temperature of 25 (±2) °C. One group was fed the casein diet, another group was fed the protein-free diet (PFD) and the other six groups were fed the experimental diets containing different proportions of tahinah. Two similar experimental runs were conducted under the same conditions. The diets were fed for 12 days in each of the two runs.

Faeces from the preliminary period (the first 6 days of the experiment) were discarded, whereas faeces from the balance period (the last 6 days of the

Table 2. Composition of chickpea dip, casein and protein-free diets used to feed rats in true protein digestibility (TD) experiment (g kg^{-1})

	Chickpea dip diets							
Ingredient	*0% tahinah*	*10% tahinah*	*15% tahinah*	*20% tahinah*	*25% tahinah*	*15% tahinah + bread (1:2)*	*Casein diet*[a]	*Protein-free diet*
Test food	476.0	467.0	443.0	467.0	459.0	629.0	100.0	—
Corn oil	92.0	42.0	14.0	—	—	110.0	100.0	100.0
Corn starch	362.0	421.0	473.0	463.0	471.0	191.0	730.0	830.0
Fat-soluble vitamins[b]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Water-soluble vitamins[c]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Salt mixture[d]	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0

[a] Casein source: BDH Chemicals Ltd, Poole, UK.

[b] 1000 IU vitamin A, 100 IU vitamin D and 10 IU vitamin E g^{-1} fat.

[c] 0.5 g thiamin; 0.4 g riboflavin, 0.4 g pyridoxine, 45 g ascorbic acid, 4 g pantothenic acid, 4 g niacin, 75 g choline, 5 g inositol, 10 g *p*-aminobenzoic acid, 0.002 g cyanocobalamin, 0.02 g biotin and 0.2 g folic acid, made up to 1 kg with corn starch.

[d] 0.21 g $Al_2(SO_4)_3 \cdot K_2SO_4 \cdot 24H_2O$, 309.85 g $CaCO_3$, 300 g $K_2HPO_4 \cdot 3H_2O$, 0.26 g $CoCl_2 \cdot 6H_2O$, 0.5 g $CuSO_4 \cdot 5H_2O$, 23.56 g $Fe_2(SO_4)_3 \cdot 7H_2O$, 51.13 g $MgSO_4$, 135.48 g $K_2HPO_4 \cdot 3H_2O$, 173 g NaCl, 0.26 g NaF, 0.26 g $Na_2B_4O_7 \cdot 10H_2O$ and 1.32 g Zn $SO_4 \cdot 7H_2O$.

Table 3. Proximate analysis of chickpea dip, casein and protein-free diets used in animal experiments (g kg^{-1} wet matter basis)[a]

	Chickpea dip diets							
Component	*0% tahinah*	*10% tahinah*	*15% tahinah*	*20% tahinah*	*25% tahinah*	*15% tahinah + bread (1:2)*	*Casein diet*	*Protein-free diet*
Moisture	50.1	57.4	65.4	61.6	58.6	54.7	81.5	86.0
Crude protein	105.0	93.8	100.0	119.4	120.0	94.7	94.1	2.5
Ether extract	144.5	148.6	148.5	140.5	144.5	112.6	107.0	125.6
Crude fibre	20.1	38.6	42.7	54.5	56.1	13.3	ND	ND
Ash	63.4	53.6	56.8	53.7	55.0	49.1	41.8	35.9
Nitrogen-free extract[b]	616.9	608.0	586.6	570.3	565.8	675.6	675.6	750.0
Calories (kcal (MJ))[c]	4188.1 (17.49)	4144.6 (17.33)	4082.9 (17.05)	4023.3 (16.80)	4043.7 (16.88)	4094.6 (17.03)	4041.8 (16.87)	4140.4 (17.39)

[a] Values are mean of triplicate determinations with CV < 5%.
[b] Calculated by difference.
[c] Calculated by multiplying grams of crude protein and nitrogen-free extract by 4 kcal and ether extract by 9 kcal.
ND, not detected.

experiment) were separated from any spilled food and collected for each group. The collected faeces were dried overnight at 100 °C (Memmert, Schwabach, Germany), then weighed and ground. Finally, the nitrogen content of the ground faeces was determined by the Kjeldahl method, and protein digestibility was calculated according to Pellett and Young[9] as follows:

$$\text{true digestibility (TD)} = \frac{I - (F - F_k)}{I}$$

where I is the nitrogen intake of the test group, F is the faecal nitrogen of the test group and F_k is the faecal nitrogen of the protein-free group.

Protein digestibility-corrected amino acid score

The protein digestibility-corrected amino acid score method was conducted in two steps. The first step involved the determination of the true protein digestibility of the casein and chickpea diets. In the second step the amino acid content, as documented in the literature,[16] was used to calculate the chemical score for the protein of the diets. PDCAAS was calculated according to the following equation:[15,19]

$$\text{protein digestibility-corrected amino acid score} = \text{true protein digestibility} \times \text{lowest amino acid score}$$

Statistical analysis

Statistical analysis of the data was performed using SAS. The data have been represented as mean ± SD. Analysis of variance (ANOVA) with Duncan's multiple-range test was used to find the differences among means for the parameters true protein digestibility and PDCAAS.[21] Significance was accepted at $P \leqslant 0.05$.

RESULTS

Amino acid score

Table 4 shows the essential amino acid requirements for laboratory rats and the essential amino acid

Table 4. Essential amino acid contents of casein and chickpea dip diets (mg g^{-1} protein) and suggested pattern of amino acid requirements for laboratory rats (mg g^{-1} protein)

			Chickpea dip diets[c]					
Amino acid	*Amino acid requirement for laboratory rats*[a]	*Casein diet*[b]	*0% tahinah*	*10% tahinah*	*15% tahinah*	*20% tahinah*	*25% tahinah*	*15% tahinah + bread (1:2)*
Isoleucine	42	54.3	47.88	46.10	45.00	45.00	44.00	40.81
Leucine	62	94.7	81.20	79.90	79.30	79.00	79.00	79.92
Lysine	58	85.0	74.82	64.96	59.74	55.68	52.20	43.50
Methionine + cysteine	50	35.4	30.00	35.00	37.50	40.00	43.00	39.40
Valine	50	63.1	52.93	53.50	48.00	54.00	54.00	52.10
Phenylalanine + tyrosine	66	111.5	112.82	109.60	108.00	107.00	106.00	107.00
Tryptophan	12.5	14.4	14.00	12.00	11.60	11.00	10.00	9.40
Threonine	42	42.0	40.00	40.00	40.00	40.00	41.15	35.15

[a] According to the FAO.[19]
[b] According to Steink *et al.*[20]
[c] According to Pellett and Shadarevian.[16]

Table 5. Amino acid scores for casein and chickpea dip diets based on amino acid requirements for laboratory rats[a]

Amino acid	Casein diet	Chickpea dip diets: 0% tahinah	10% tahinah	15% tahinah	20% tahinah	25% tahinah	15% tahinah + bread (1:2)
Isoleucine	1.29	1.14	1.10	1.07	1.07	1.05	0.97
Leucine	1.53	1.31	1.29	1.28	1.27	1.27	1.29
Lysine	1.47	1.29	1.12	1.03	0.96	0.90	**0.75**
Methionine + cysteine	**0.71**	**0.60**	**0.70**	**0.75**	**0.80**	0.86	0.79
Valine	1.26	1.06	1.07	0.96	1.08	1.08	1.04
Phenylalanine + tyrosine	1.69	1.17	1.66	1.64	1.62	1.61	1.62
Tryptophan	1.15	1.12	0.96	0.93	0.88	**0.80**	**0.75**
Threonine	1.00	0.95	0.95	0.95	0.95	0.98	0.84
Chemical score	**0.71**	**0.60**	**0.70**	**0.75**	**0.80**	**0.80**	**0.75**
Limiting amino acids(s)	Methionine + cysteine (sulphur amino acids)					Tryptophan	Lysine and tryptophan

[a] The figures in bold type denote the chemical scores.

contents of casein and the six types of chickpea dip. The amino acid scores for the protein of the diets were calculated from the suggested pattern of amino acid requirements for laboratory rats and are presented in Table 5.

True protein digestibility

Table 6 shows the protein digestibilities of casein and the six types of chickpea dip. It can be seen that the true protein digestibility of casein was, as expected, significantly higher than those of the chickpea diets ($P \leqslant 0.05$). Furthermore, the true digestibility of the chickpea dip with 15% tahinah plus bread (1:2) was significantly higher than those of the other chickpea dips ($P \leqslant 0.05$).

Protein digestibility-corrected amino acid score

This new method of protein quality evaluation is conducted by determining the true protein digestibility and the amino acid score. The PDCAAS values of casein and the six types of chickpea dip are shown in Table 6. It can be seen that the PDCAAS values of casein and chickpea/15% tahinah plus bread (1:2) were higher than that of chickpea/0% tahinah. This difference could be explained by the fact that the true protein digestibilities of casein and chickpea/15% tahinah plus bread (1:2) were high compared with that of chickpea/0% tahinah.

DISCUSSION

Amino acid score

Essential amino acids in the protein of casein and the six types of chickpea dip were compared with the suggested pattern of amino acid requirements for laboratory rats (Table 4). The calculated amino acid scores (Table 5) showed that all the chickpea dips were generally high in essential amino acids. This high level of essential amino acids in different chickpea dips is expected because of the relatively good quality of chickpea protein, compared with other plant proteins, and the ability of oilseed proteins (including sesame protein) to complement chickpea protein.

The decline in lysine content could be explained by the low content of lysine in sesame protein.[8] In other words, both sesame and wheat proteins, with their low lysine content, contributed to lowering chemical scores.

Taking into consideration the pattern of essential amino acid requirements for laboratory rats, sulphur-containing amino acids were the limiting amino acids in chickpea dips with 0, 10, 15 and 20% tahinah (Table 5). It can be further noticed that the amino acid scores of chickpea dips increased concomitantly with increasing tahinah proportion from 0 to 20% as a result of the ability of sesame protein to supplement chickpea protein with the two sulphur amino acids (methionine and cysteine) which are present in

Table 6. Protein digestibility-corrected amino acid scores (PDCAAS) for casein and chickpea dip diets (based on pattern of amino acid requirements for laboratory rats)

Diet	Amino acid score based on laboratory rats	True protein digestibility ($\overline{X}$)[a]	PDCAAS based on laboratory rats
Casein	0.71	0.95a	0.66
0% tahinah	0.60	0.77d	0.46
10% tahinah	0.70	0.85c	0.60
15% tahinah	0.75	0.86c	0.64
20% tahinah	0.80	0.87bc	0.70
25% tahinah	0.80	0.88bc	0.70
15% tahinah + bread (1:2)	0.75	0.90b	0.68

[a] Duncan's multiple-range test: means with the same letter are not significantly different ($P \leqslant 0.05$).

considerable quantities in many oilseed proteins, including sesame protein. In addition, it is clear from Table 5 that the amino acid scores of isoleucine, lysine and tryptophan decreased with increasing tahinah proportion, while methionine and cysteine increased concomitantly with increasing tahinah proportion.

For the casein diet it is obvious that the amino acid score is relatively low. This is because casein is a less than ideal reference protein and is deficient by 15–30% in meeting the sulphur amino acid requirements of the rat.[22] For that reason, it is recommended to supplement casein with 1% DL-methionine to allow casein to be of high nutritional value and to be used as a standard protein for comparative purposes in rat bioassay methods.[9]

True protein digestibility

The true protein digestibilities of the six types of chickpea dip were generally high, while the digestibilities for both 10 and 15% and both 20 and 25% tahinah were not significantly different ($P \leqslant 0.05$) (Table 6). For chickpea alone (0% tahinah) the true digestibility was low as compared with the other chickpea dip diets. The low protein digestibility of chickpea grains has been attributed to the presence of less digestible protein fraction(s), residual levels of antiphysiological factors (such as trypsin inhibitors, etc) and high concentrations of indigestible carbohydrates and tannins.[15] Also, the presence of significant amounts of fermentable carbohydrates that support maximum microbial growth in the large intestine would result in increased bacterial protein in the faeces. However, the digestibility of cooked chickpea alone is within the ranges reported by the FAO[3] and Chavan *et al*,[1] ie 0.77–0.88 and 0.76–0.93 respectively. It can be noticed from Table 6 that the true protein digestibility of chickpea dips increased concomitantly with increasing tahinah proportion, which could be explained by the limited types and amounts of trypsin inhibitors in sesame seeds in comparison with chickpea seeds.

Despite the fact that chickpea and sesame seeds contain antitryptic factors such as trypsin inhibitors, tannins and insoluble fibres,[3,23] it is possible that heat treatments of chickpea and sesame seeds are responsible for the destruction and inhibition of heat-labile trypsin inhibitors and the subsequent improvement in protein digestibility (TD).[12,24,25]

For chickpea dip with 15% tahinah plus bread (1:2) the true protein digestibility was significantly the highest ($P \leqslant 0.05$) among all types of chickpea dip. This elevation in digestibility of that food mixture is due to the high digestibility of wheat protein (90%),[26] as it lacks the antitryptic factors that are present naturally in legumes. The true protein digestibility of casein was, as expected, the highest, reaching about 0.95, which is consistent with the value of 0.95 reported by Dabbour and Takruri.[27] The high true digestibility of casein is attributed to the lack or absence of antinutritional factors (eg antitryptic factors) that accompany plant proteins.

Protein digestibility-corrected amino acid score

PDCAAS is the product of the limiting amino acid score and the true digestibility of protein.[15,19] From Table 6 it is clear that PDCAAS values were comparatively low owing to the low amino acid scores.[15,19]

The casein diet was used as a diet of reference protein; its protein digestibility was 95% and its chemical score was 0.71. Thus the PDCAAS obtained for casein was not the highest among the experimental diets because of its relatively low chemical score.

From the previous discussion it can be concluded that the addition of tahinah to chickpea led to an improvement in the amino acid score (based on amino acid requirements for laboratory rats), true digestibility and PDCAAS of chickpea dip mixtures. However, the ordinary consumption of wheat bread with chickpea dip led to an increase in the protein digestibility and PDCAAS of the protein mixture.

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