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NUTRITIONAL QUALITY, AMINO ACID PROFILES, PROTEIN DIGESTIBILITY CORRECTED AMINO ACID SCORES AND ANTIOXIDANT PROPERTIES OF FRIED TOFU AND SEITAN

Dina ANWAR[1] , *Ghadir EL-CHAGHABY [1]
[1] *Regional center for Food and Feed, Agricultural Research Center, Giza, Egypt*
ghadiraly@yahoo.com
*Corresponding author
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Abstract: *As the world population is increasing, tofu and seitan may play an important role in human nutrition as inexpensive protein-rich food sources. In order to increase the consumption of tofu and seitan, modified processes are needed to meet the needs of different consumers. In this study, the aim of the work is to set up fried tofu and seitan that couple high nutritional value and good sensory features and to evaluate their nutritional profile and protein quality as well as the consumer's acceptance. The average values of tested organoleptic parameters of tofu and seitan resulted in a positive influence after frying process. Cooking loss due to frying was lower in case of fried seitan over fried tofu. The protein content of both fried tofu and seitan samples was higher than 20%, fat and energy of the control were increased after frying process. Minerals and antioxidant capacity were higher in the modified products as compared to the traditional ones. Most of essential amino acid values were enhanced after frying seitans especially with wheat flour coated seitan, however, raw tofu was proven to be a rich source of essential amino acids over fried ones and different seitan samples. Tofus were found to be higher in essential amino acid index (EAAI) and biological value (BV) over seitan samples. Tofu samples with higher amino acids content had higher values of digestibility and PDCAAs. Overall, the new formulations of tofu and seitan could be used to enhance their nutritional quality and taste.*

Keywords: *Nutritional profile, protein quality, sensory evaluation, mineral content*

1. Introduction

Proteins are the main constituents of agricultural raw materials with two main functions: bio- and techno-function. Bio-functionality of proteins is related to their nutritional and physiological properties, while techno-functionality is related to their physico-chemical ones [1]. One of the earliest indications of the importance of the increment of food protein resources was to overcome the world malnutrition. It is expected that the world demand for proteins of animal origin would be doubled by 2050 [2]. The replacement of animal proteins by novel plant proteins has been driven by sustainability assurance in the protein food sources and considered the simplest way to solve the shortage in animal protein sources. Protein rich plant-based foods are legumes, grains, nuts and dairy replacers. The current ready-made meat and dairy replacers are based on wheat, soy and lupine, more than other plant based protein sources from the fact that they have an amino acid composition of quite high quality [3]. A growing awareness in the population about healthy and sustainable foods has lead to a rising interest in plant protein based meat alternatives in many European countries and worldwide [4].

Soybean foods are rich in protein and have been shown to offer specific health benefits. Tofu (soy bean curd) is one of the most important food products made from soy bean protein [5]. It is an important food for eastern Asians due to its good nutrition and digestibility [6]. Western countries have recently increased interest in eating tofu due to its benefits to human health and the United States has increased tofu consumption [7]. It has been increasingly used in numerous Asian dishes, replacing dairy products due to comparatively low cost and high protein bioavailability [8].

Tofu is usually considered as salt – or acid – coagulated water based gel, with soy lipids and proteins trapped in its gel networks [5]. It is a cheap, nutritious and versatile meat or cheese substitute with bland taste and porous texture and hence called "Tofu is meat without bone". The main health benefits of tofu will show up in significantly lower total cholesterol, triglycerides and low-density lipoprotein (bad cholesterol) if one eats tofu regularly instead of meat [9]. On the other hand, cereals (e.g wheat, oats, etc) can be considered the other side of plant protein sources. Wheat gluten is becoming a very large player in the industry, with new production facilities for gluten and textural wheat products expanding all over the world [10]. Gluten can be readily prepared by gently washing dough under a stream of running water. This removes the bulk of the soluble and particulate matter to leave a protein aceous mass that retains its cohesiveness on stretching [11]. Soy protein and wheat gluten have been the dominant raw materials for meat surrogates for a long time. These proteins have unique techno-functional taste and nutritional properties depending on their origin and how they are processed [4]. Historically, wheat protein has been used for thousands of years as a meat substitute called seitan in China, Japan, Korea, and Russia [10]. Gluten can be flavored in a variety of ways; when simmered in a traditional broth of soy sauce, ginger, garlic, it is called seitan. Seitan contains small amount of sodium and extremely low fat protein. In addition, gluten (setian) has a very similar texture to meat, making it ideal for vegetarian dishes meant to mimic meat-based ones. Seitan is a great option for vegans who cannot eat soy. Recently, western countries have increased the interest in eating tofu and seitan which are considered as high protein source especially for vegetarians. Despite their high functional properties, they are not typically consumed in the basic Arab countries diet because of their unpleasant flavor. The acceptability level of foods depends mostly on cultural habits, therefore, when a food is created or modified, it is extremely important to evaluate it with consumers or potential consumers. Thus, the objective of this study is to prepare tofu and seitan by two different methods and to evaluate the products in terms of sensory characteristics, nutritional quality, amino acid scores and antioxidant capacity.

2. Matherials and methods

2.1. Raw material

Dried soy beans (*Glycine max*), Giza 111 cultivar were obtained from the Field Crops Research Institute, Agricultural Research Center. Wheat flour was purchased from the local market. The chemicals were obtained from El-Gomhoria Company for chemicals (El-Mataria, Egypt).

2.2. Preparation of tofu

Tofu was prepared by the following method **[12]**: one kilogram of soy bean seeds were washed, soaked in 4L of water for 16 hrs at room temperature, drained and rinsed with water. Soaked seeds were then processed by hand dehulling and the cotyledons were ground in low speed blender with addition of water (8 L) for 2 minutes followed by high speed for 5 minutes. The resultant bean puree was heated to 80-85^{o} C with constant stirring.

The slurry was filtered through cheese cloth to separate soy milk from residue (okara) and the liquid was heated to boiling. The soy milk was allowed to cool to 70° C and while stirring, a saturated solution of calcium sulphate (2% of dry soy bean weight) was added to soy milk until it began to coagulate. The suspension was allowed to form a curd without further stirring. After one hour, the curd was transferred into cheese cloth lined on plastic strainer. The curd was pressed to separate the whey. At the end of pressing, the curd had attained the required firmness and consistency of tofu. The cloth was removed and the tofu was stored refrigerated until used.

2.3. Fried tofu preparation

Fried tofu was prepared by mixing optimum quantities of onion paste (12%), corn starch (4.2%), bicarbonate (0.7%), salt (1.5%), spices (0.6%) and chili sauce together with fresh tofu (74%). The tofu mix was then formed into discs of 14g. The produced tofu patties were coated with wheat flour (7.0%) and fried using sunflower oil at 170°C for 20 sec. the fried tofus were cooled till examination.

2.4. Seitan preparation

To produce seitan, wheat flour was mixed with tap water as needed to make dough. The dough was washed repeatedly under running water to remove starch and some bran until a gluten- protein was observed. Seitan was stored in a refrigerator until used.

2.5. Fried seitan preparation

Seitan (wheat gluten) was soaked in mixed ingredients of onion juice, yoghurt, lemon juice, soy sauce, salt and spices and allowed to rest for one hour. Seitan was crosswise cut into four pieces then simmered in water for 15 min.

Cooked seitan was cut into small pieces then coated with wheat flour (1) or chickpea flour (2). Coated seitans were fried using sunflower oil at 170° C for 20 sec and the fried seitan was cooled till examination.

2.6. Investigations

For sensory evaluation: samples from each of tofu and seitan patties were fried in a preheated pan for 5 min (2.5 min. on each side) before coded and evaluated for test, color, odor, texture, bite and overall acceptability using 10-point descriptive scales, where a score of 1 denotes for extremely poor and a score of 10 denotes for excellent (as described before by **[13].** The weight of tofu and seitan patties were measured before and after frying to determine cooking loss and calculated according to the equation below:

$$\text{Cooking loss\%} = \frac{\text{Cooked weight} - \text{raw weight}}{\text{Raw weight}} \times 100 \quad (1)$$

2.7. Chemical determinations

2.7.1. Proximate analysis

Moisture, protein, fat (ether extractable), fiber and ash contents were determined according to methods described in **[14]**. While, total carbohydrates were estimated by difference according to **[15]**.

2.7.2. Minerals content

Potassium, magnesium, phosphorous and iron were analyzed by atomic absorption spectrophotometry 3300 Perken Elmer, while, calcium was analyzed by ICP optima 2000 DV Perken Elmer according to the method described in the **[14]**.

2.7.3. Soluble, in soluble and total dietary fiber

Soluble dietary fiber (SDF), in soluble dietary fiber (IDF) and total dietary fiber (TDF) were determined according to the methods in **[16]**.

2.7.4. Antioxidant assay

Total antioxidant capacity of the samples was determined using the phosphomolebdenum method **[17]** using ascorbic acid as standard. The results were expressed as milligram ascorbic acid equivalent per 100 milliliters (mg AAE/100 ml).

2.7.5. Amino acids content

Amino acids determination was performed according to **[14]**. The system used for the analysis was Eppemdorf LC 3000 EZ chrom.

a- Essential amino acid index (EAAI)

The EAAI was calculated using Eq. (1) **[18]**.

$$EAAI = \sqrt[n]{\frac{100a \ x \ 100b \ldots 100h}{av \ x \ bv \ldots hv}} \quad (2)$$

Where n is the number of the essential amino acids; a, b,….h are the concentrations of the essential amino acids (lysine, isoleucine, valine, theronine, leucine, phenylalanine, histidine and methionine) in test sample, and a*v*, b*v*, …..h*v* are the concentrations of the essential amino acids in standard protein (%) (Casein)

b- Nutritional index (NI)

The NI was calculated using Eq. (2).

$$NI (\%) = \frac{EAAI \ x \ protein \ (\%)}{100} \quad (3)$$

c- *Biological value (BV)*

The BV was calculated using Eq. (3) **[18-19]**:

$$BV = 1.09 \ x \ EAAI – 11.7 \quad (4)$$

d- Predicted protein efficiency ratio (P-PER)

The *P*-PER was calculated using the regression equation, Eq. (4) **[20-21]**:

$$P\text{-}PER = - 0.468 + 0.454 (LEU) – 0.105 (TYR) \quad (5)$$

2.7.6. Determination of amino acid scores

Determination of the amino acid scores was first based on casein. In this method, essential amino acids were scored. Secondly, amino acid score was calculating using the following formula by **[22]**:

$$\text{Uncorrected amino acid score\%} = \frac{\text{mg of essential amino acids 1g of test protein}}{\text{mg of amino acid 1g of reference protein}} \ x100 \quad (6)$$

2.7.7. *In-vitro* protein digestibility determinations

The *in vitro* protein digestibility (IVPD) was measured using a multienzyme system (pepsin, chloramphenicol and pancreatin) according to the method of **[23]**. Crude protein (CP) was determined by the macro Kjeldahi technique (%N x 6.25) **[14]**. Protein digestability was calculated with the formula:

% protein digestibilty = (CP sample – CP undigested) / (CP sample) x 100 (7)

2.7.8. Protein digestibility corrected amino acid score (PDCAAs)

The indicator of protein quality was calculated based on the total amino acid contents of the sample, considering for its calculation t he limiting AA (g/16gN) of the sample in relation to the same AA of a reference protein and multiplied by the samples % digestibility according to **[24]**. The PDCAAs was calculated according to the following equation:

PDCAAs (protein digestibility corrected amino acid score) = Lowest uncorrected amino acid score x protein digestibility (%) (8)

3. Results and discussion

3.1. Sensory quality of fried tofu and seitan

The sensory evaluation was done on a ten-point scale by a ten panelists and the results are shown in **Table (1)**. Fried tofu was scored higher than 8.0 for the parameters considered (taste, odor, color, texture, bite and overall acceptability). In addition, 95% of consumers expressed the will of buying it. Fried tofu's taste had a level of acceptance near 'extremely like', therefore, incorporation of the ingredients in fried patties formulation increased the pleasant taste of tofu. Tofu has a mild flavor and a porous texture and, due to its neutral sensory characteristics, the tofu texture has an important role in the quality of the product, as well as in the consumer's acceptance **[25]**.

Table 1

Sensory evaluation of fried tofu and seitan

Products / Parameters	Tofu	Seitans	
	Fried tofu	**Fried seitan [(1)]**	**Fried seitan [(2)]**
Taste	9.55	8.70	8.80
Color	9.45	8.60	8.45
Odor	8.70	8.15	9.00
Texture	8.25	7.80	7.80
Bite	9.05	6.80	7.15
Over all acceptability	9.00	8.01	8.24
Cooking loss%	26.76%	2.15%	1.56%

(1) Fried seitan coated with wheat flour (2) Fried seitan coated with chickpea flour

The frying process increased the pleasant taste of the products; it also maintained the high average of texture. Tofu had high cooking loss (26.76%) which is probably due to its lower ability to hold the moisture during the frying process. **Table (1)** also shows the mean scores of the consumer's acceptance for fried seitan coated with wheat or chickpea flour. Both of them were scored higher than 8.0 for taste, color, odor and overall acceptability. Although, the tested seitans were characterized by similar ingredient contents, the use of chickpea flour instead of wheat flour involved an increase of the taste, odor and overall acceptability, probably due to a greater acceptance of chickpea by consumers. Taste and aroma are mostly the important attributes that influence the sensory properties of products. Cooking seitan (deep fat frying/70° C) resulted in low cooking loss percentage.

3.2. Proximate composition

The proximate composition of tofus and seitans are presented in **Table (2)**. The results showed that moisture percentage was higher in raw tofu compared to fried tofu. While, changes in moisture content of different seitan samples were not noticeable. The protein content of raw tofu was 11.29% of fresh matter which was lower than that of fried tofu (21.21%), probably due to the high moisture content of raw tofu.

Table 2

Proximate composition (g/100g) and the energy value of tofus and seitans

Products / Macronutrients	Tofus		Seitans		
	Raw tofu	**Fried tofu**	**Raw seitan**	**Fried seitan [(1)]**	**Fried seitan [(2)]**
Moisture	74.92	44.61	49.30	48.30	51.59
Protein	11.29	21.21	34.27	21.71	24.52
Fiber	0.36	0.37	0.16	0.02	0.36
Carbohydrate	4.15	14.20	15.24	22.17	16.82
Lipid	7.84	15.08	0.78	6.40	5.09
Ash	1.44	4.53	0.25	1.40	1.62
Energy (Kcal/100g)	132.3	277.36	205.06	233.12	211.17

(1) Fried seitan coated with wheat flour (2) Fried seitan coated with chickpea flour

Tofu manufacture is in many respects similar to cheese-making, the essential difference being that a lactic acid-producing bacterial starter culture is not employed in its preparation **[26]**. Solids of tofu are composed chiefly of protein of high quality- the Net protein utilization (NPU) of tofu is reported to be 65% making it equivalent to chicken meat in terms of assimilability and digestibility. Soybean protein showed hypochloesterolemic and soy isoflavones have antiatherogenic effect **[27]**. It was also reported that the soy protein decreased effectively serum concentration of total cholesterol and triglycerides as compared to animal proteins **[28]**. On the other hand, seitan contained 34.27% of protein while it reached 21.71% and 24.52% of fresh matter for fried seitan coated with wheat or chickpea flour, respectively. It was clear that use of chickpea flour caused an increase in protein content of fried seitan over wheat flour. The fiber content of tofus and seitans was comprised between 0.02% and 0.37% of fresh matter. Frying tofu result an increase in carbohydrates (10%), lipid (7.24%) and ash (3.1%). Deep-fat frying of tofu with subsequent fat absorption increase the energy value (from 132.3 to 277.36 kcal/100g for tofus before and after frying, respectively). Similar trends were observed for seitan samples, the fried seitan either coated with wheat of chickpea flour contained high carbohydrates, lipids and ash in compare with raw seitan. The highest energy value was recorded for fried seitan with wheat flour (233.12 kca/100g) over other seitans. The high energy value in cooked burger patties was attributed to the reduction in moisture content during cooking **[29]**. The predicted dietary protein intake of 100g of raw and fried tofu and seitan for males and females was shown in **Fig (1)**. Raw tofu can supply about 21.2 and 24.5% of the protein requirement for males and females, respectively and this percentage would increase with fried tofu.

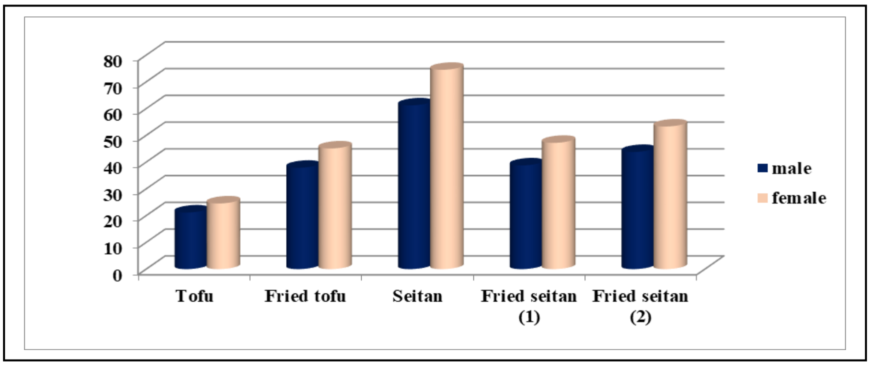

Fig. (1): % Dietary protein intake of raw and fried tofu and seitan

On the other hand, raw seitan can supply about 61.2 and 74.5% of recommended daily protein for males and females, respectively while these percentages were decrease with fried seitans but it still provide more than 38% of the protein requirement for both males and females.

3.4. Mineral content

Mineral content of raw and fried tofu is shown in the **Table (3)**. The results showed that K and Mg were the most abundant minerals in raw tofu; however, Ca and P recorded higher mean values over other minerals in fried tofu. The ratio of Ca/P of raw and fried tofu was 0.707 and 1.383, respectively. According to many authors a good food has a Ca/P ratio higher than one, while a poor food has a ratio lower than 0.5**[30]**. The ratio of Ca/P in the food is related to teeth and bone formation and therefore children and youth need higher intakes of calcium and phosphorus. Since the ratio of Ca/P in tofus either raw or fried is above 0.5, tofu can be considered a good food. As regards the seitan samples, the lowest mean value of Fe, K and P was recorded by raw seitan. Fried seitan coated with wheat flour contained big amounts of K and P which recorded the highest value among other seitan samples. On the other hand, fried seitan with chickpea flour yielded the highest Mg level followed by raw seitan and fried seitan with wheat flour. Calcium was not detected in raw seitan but it was recorded as 204.0 and 177.3 mg/kg for fried seitan with wheat and chickpea flour, respectively. These results may be related to the yoghurt addition in the seitan's broth. A slight increase was also shown in Ca/P ratio of fried seitan with chickpea flour over wheat flour.

Finally, the different ingredients added to tofu and seitan recipes before the frying process enhanced their mineral content and thus, fried tofu and seitan can provide high amount of minerals which are associated with good human health.

Table 3

Mineral composition (mg/kg) of tofus and seitans

Products / Minerals	Tofus		Seitans		
	Raw tofu	**Fried tofu**	**Raw seitan**	**Fried seitan (1)**	**Fried seitan (2)**
Fe	24.00	13.24	22.46	76.88	71.02
K	239.6	411.1	225.8	2688	506.4
Mg	3010	137.3	184.4	69.23	309.0
Ca	12.19	4597	Nd	204.0	177.3
P	17.23	3323	3.51	1603	918.3
Ca/P	0.707	1.383	-	0.127	0.193

(1) Fried seitan coated with wheat flour (2) Fried seitan coated with chickpea flour

3.5. Dietary fiber content

The results of soluble, insoluble and total dietary fiber shown in **Table (4)** indicate that total dietary fiber (TDF) levels in the tofu and seitan ranged from 2.12 to 5.95%. On the other hand, the content of soluble diatery fiber (SDF) and insoluble dietary fiber (IDF) in raw tofu was lower than that in fried tofu which may be due to the lost soluble carbohydrates in the whey during tofu making. These results were in agreement with previous results **[31]** .In the case of seitans, fried seitan with chickpea flour had highest content of IDF followed by raw seitan and seitan with wheat flour.

Table (4):

Dietary fiber (%) of tofus and seitans

Products / Fiber	Tofus		Seitans		
	Raw tofu	**Fried tofu**	**Raw seitan**	**Fried seitan (1)**	**Fried seitan (2)**
IDF	1.88	2.82	1.57	1.09	4.45
SDF	1.61	1.66	1.77	1.03	1.50
TDF	3.49	4.48	3.34	2.12	5.95

(1) Fried seitan coated with wheat flour (2) Fried seitan coated with chickpea flour

Dietary fibre is regarded as one of the most important ingredients in human diet **[32]**. The characteristics of fibre such as particle size, bulk volume, surface area and adsorption as well as organic molecules are more effective in human digestive system **[32-33]**. It was observed that the addition of dietary fibre in foods can improve the overall qualities such as cooking prosperities and textural characteristics as well as taste **[34]**.

3.6. Antioxidant Capacity

The antioxidant capacity of raw and fried tofu and seitan is shown in the **Fig (2)**. Fried tofu showed high antioxidant level (861.6 mgAAE/100g), up to 1.8 fold of antioxidant capacity of raw tofu (487.9 mgAAE/100g). In this context, it was reported that isoflavones, aglycones and proteins present in tofu have antioxidant properties protecting from lipid oxidation **[35]**. Fried seitan coated with wheat flour contained higher levels, up to 2.4 fold of antioxidant than raw seitan. Although it is believed that that deep-fat frying changes the quality of fried foods and nutritional quality of foods **[36]**, but in the present study, the use of onion paste in fried tofu recipe and lemon juice for seitan may be the reason for improvement of their antioxidant capacity which can reduce the harmful effects of frying process.

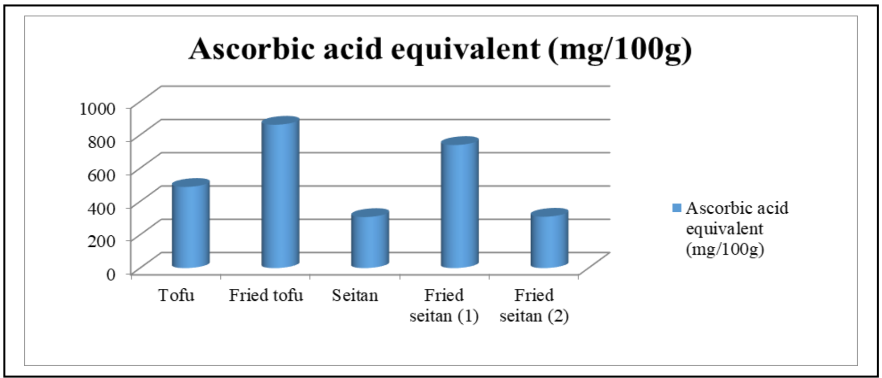

Fig. (2): Antioxidant activity of raw and fried tofu and seitan

3.7. Amino acids profile and protein quality of tofus and seitans

Amino acid composition is critical to evaluate the quality of a dietary protein source through quality also relates to other food properties (e.g presence of metabolic interfering substances digestibility and chemical integrity) with potential to manipulate the proportion of utilizable amino acids. Particularly, the essential amino acids content in protein source primarily influences the quality of that protein **[37]**. **Table (5)** shows the amino acids composition of the amino acids composition of tofu and seitan before and after frying in g/16gN. The sum of essential amino acids (TEAA) ranged from 25.77g in fried seitan with chickpea flour to 37.72g in raw tofu. Raw tofu was higher than 36% considered for an ideal protein **[38]** but it is lower than that in casein standard of 39.18%. Leucine and lysine content were higher in tofus and these values were higher than the recommended dietary requirements of FAO for adult foods while, the methionine content was the lowest amino acid in tofus. Seitan samples contained low amounts of lysine. However, fried seitan coated with chickpea flour caused an increase in lysine content, up to 1.18 fold than wheat flour and 1.23 fold than raw seitan.Nevertheless, seitans contained appreciable amount of leucine and phenylalanine, but it is still lower than that in tofu samples. Fried seitan coated with wheat flour contained 1.76g/100g of methionine which recorded the highest amount in seitan samples followed by raw seitan (1.66g/100g) and fried seitan with chickpea flour (1.65g/100g). These relative

deficiencies in lysine in seitan samples and that of methionine in fried tofu can be largely attributed to their disparate composition of major storage proteins.
Raw and fried tofus were found to be rich in valine and phenylalanine. The histidine content in the studied samples followed a similar pattern, being the highest in tofus **(Table 5)**. Histidine has multiple roles including that in protein interactions, histamine synthesis, and repair and tissue growth **[39]**.

Table 5

Amino acid profile of tofus and seitans

Treatments	**T_1**	**T_2**	**T_3**	**T_4**	**T_5[1]**	**RDA%***
Amino acids	**Essential amino acids (g/100g cp.)**					
Theronine	3.86	3.78	2.44	2.54	2.51	1.50
Valine	5.06	4.90	3.44	4.00	3.30	2.60
Isoleucine	4.68	4.62	3.30	3.69	3.30	2.00
Leucine	7.91	7.38	6.51	7.02	6.52	3.90
Phenylalanine	5.17	5.03	4.66	4.64	4.28	2.50
Histidine	2.71	2.50	2.03	2.14	1.89	1.00
Lysine	6.40	5.22	1.89	1.97	2.32	3.00
Methionine	1.93	1.40	1.66	1.76	1.65	1.50
Conditionally essential amino acids (g/100g cp.)						
Tyrosine	3.71	4.09	3.20	3.11	2.84	-
Arginine	6.97	6.55	3.33	3.40	3.57	-
Cysteine	2.13	1.98	2.29	2.59	2.72	-
Proline	4.84	5.37	11.71	11.85	11.86	-
Glycine	4.48	4.15	3.45	3.95	3.44	-
Non-essential amino acids (g/100g cp.)						
Aspartic acid	10.77	10.41	3.08	3.66	4.38	-
Serine	4.88	4.64	4.20	4.45	4.01	-
Glutamic acid	18.20	18.45	39.30	33.80	28.94	-
Alanine	4.73	4.30	2.77	3.50	2.78	-
TEAA	37.72	34.83	25.93	27.76	25.77	
TCEA	22.13	22.14	23.98	24.90	24.43	
TNEAA	60.71	59.94	73.33	70.31	64.54	

(1)T_1= tofu, T_2 = fried tofu, T_3 = seitan, T_4 = fried seitan with wheat flour and T_5 = fried seitan with chickpea flour (2) TEAA=total essential amino acids, TCEA=conditionally amino acids, TNEAA=total non essential amino acids, TSAA=total sulphur amino acids, TArAA=total aromatic amino acids, TAAA=total acidic amino acids, TBAA= total basic amino acids. *RDA%=recommended dietary allowanced FAO/WHO, 2007 **[38]**.

Recent evidences have indicated the dietary essentiality of traditionally considered non essential (dispensable) amino acids as there is lack of substantive research on the assumption that nutritionally non-essential amino acids are adequately synthesized in human beings to meet the basal requirements **[40].** These amino acids, termed functional amino acids include aspartic acid, serine, glutamic acid, alanine, arginine, proline and glycine and also play important roles among others in intestinal integrity **[41]**, immune responses **[42]**, cell growth and differentiation **[43]** and antioxidant defense **[44]**. As shown in the **Table (5)**, glutamic acid was the predominant amino acid amongst the other amino acids ranging from 39.3g/100g in raw seitan to 18.20g/100g in raw tofu. Aspartic acid content was the highest in tofu samples while proline was found to be the highest in seitan samples. Both asparatate and serine play as precursors of other amino acids. Over all, the amino acids content of proteins in different samples can vary due to the kind of seed's protein and the procedures carried out to get the final products. Variation was found in the content of some of the amino acids including lysine, aspartic acid and glutamic acid. The nutritional quality of tofus and seitans is presented in the **Table (6)**. Tofu samples contained higher ratio of essential amino acids than total amino acids (TEAA/TAA %) as compared with seitans. However, the values of tofus and seitans were above 26% for ideal protein food for children and 11% for adult **[45]**. The sum of the acidic amino acids was higher in seitan samples than that in tofus. Conversely, tofu samples were found to have higher total basic amino acids than seitans. An observation on Asp/Glu showed that the level of Asp appeared to affect the glutamic value and vice versa as shown in the **Table (6)**.

Table 6

Protein quality of tofu and seitan

Treatments	**T_1**	**T_2**	**T_3**	**T_4**	**$T_5^{(1)}$**
Parameters	**Nutritional quality$^{(2)}$**				
Total amino acids (TAA)	98.43	94.77	99.26	98.07	90.31
TEAA/TAA %	38.32	36.75	26.12	28.30	28.53
TNEAA/TAA%	61.67	63.24	73.87	71.69	71.46
TEAA/TNEAA	0.62	0.58	0.35	0.39	0.40
TSAA (Meth. + Cys)	4.06	3.38	3.95	4.35	4.37
Cys / TSAA %	52.46	58.57	57.97	59.54	62.24
TArAA (Pheny + Tyr)	8.88	9.12	7.86	7.75	7.12
Leu / Ileu ratio	1.69	1.60	1.97	1.90	1.98
Leu-Ileu (difference)	3.23	2.76	3.21	3.33	3.22
Leu / Ileu %	25.66	23.00	32.72	31.09	32.79
TAAA (Asp + Glu)	28.97	28.86	42.38	37.46	33.32
Asp/ Glu	0.59	0.56	0.08	0.10	0.15
Arg/lys	1.09	1.25	1.76	1.73	1.54
TBAA (Arg + Lys)	13.37	11.77	5.22	5.37	5.89
TEAA+Arg+His/TAA %	48.15	46.30	31.52	33.95	34.58
P-PER	2.734	2.453	2.152	2.393	1.705
EAAI %	95.90	87.12	64.67	69.07	64.92
BV %	92.83	83.26	58.79	63.58	59.06
Nutritional index %	43.15	33.26	43.72	29.00	32.88

(1)T_1= tofu, T_2 = fried tofu, T_3 = seitan, T_4 = fried seitan with wheat flour and T_5 = fried seitan with chickpea flour (2) TEAA=total essential amino acids, TCEA=conditionally amino acids, TAA=total amino acids, TNEAA=total non essential amino acids, TSAA=total sulphur amino acids, TArAA=total aromatic amino acids, TAAA=total acidic amino acids, TBAA= total basic amino acids, P-PER= Predicted Protein Efficiency Ratio, EAAI =essential amino acid index, BV= biological value.

Thus, the lowest Asp/Glu was recorded in raw seitan (0.08) while, it was of 0.59 in raw tofu and 0.56 in fried tofu. The Arg/lys ratios obtained in this work ranged from 1.09 to 1.76. It was previously reported that high ratio of Arg/Lys in the diet can produce beneficial hypocholesterolemic effects, improving the cardiovascular health, in addition to helps in hypertension regulation **[46-47]**. The values of predicted protein efficiency ratio (P-PER) of seitan samples were between 1.705 in fried seitan coated with chickpea flour to 2.393 in fried seitan with wheat flour whilst, it reached 2.453 for fried tofu and 2.734 for raw tofu **(Table 6)**. The P-PER in all seitan samples were lower than 2.88 in that was recorded in whole hen's egg **[48]** and 2.5 that found in reference casein **[49]**.

Nevertheless, the *P*-PER in raw tofu was higher than the value for reference casein and lower than whole hen's egg. The EAAI of tofu samples (raw and fried) were 95.90 and 87.12%, respectively, therefore, tofus are considered a good nutritional quality since the EAAI is ranged 80 – 90% as previously reported **[19]**. However, seitan samples were lower in the present study as compared with the values previously reported **[19]** showing that protein based food is in adequate when it's EAAI below 70%. The predicted BV exhibited the highest percentage in raw tofu followed by fried tofu and then fried seitan coated with wheat flour, while, raw seitan was the lowest. Scientifically, it is well known that a protein-based food nutritional is of good quality when its biological values (BV) are as high as 70 and up to 100% **[19]**.The increase in BV of the tofu samples either raw or fried is an indication of improved digestibility potential and effective utilization. **Table (6)** also showed the Nutritional index (NI) of tofu and seitan samples. The results showed that the raw tofu and seitan had higher NI followed by fried tofu and fried seitan with chickpea flour while, fried seitan with wheat flour was the lowest one.

3.7.1. Amino acid scores

Amino acid content in foods can be used to calculate the amino acid score, which provides a way to predict how efficiently protein will meet a person's amino acid needs **[38]**. **Table (7)** shows the amino acid scores for tofu and seitan samples analyzed in this study based on the essential amino acid content and the pattern for casein standard. As expected, lysine was the first limiting amino acid in cereal products. Seitans expressed the wheat protein (gluten) and therefore, lysine amino acid was the lowest score among other amino acids.

Table 7

Essential amino acid score % of tofu and seitan relative to casein standard

Treatments	T_1	T_2	T_3	T_4	T_5	Casein standard
Essential amino acids						
Theronine	102.1	100.0	64.55	67.19	66.40	3.78
Valine	86.94	84.19	59.11	68.72	56.70	5.82
Isoleucine	103.0	101.7	72.69	81.27	72.69	4.54
Leucine	95.53	89.13	78.62	84.78	78.74	8.28
Phenylalanine	113.6	110.5	102.4	101.9	94.07	4.55
Histidine	106.2	98.03	79.61	83.92	74.12	2.55
Lysine	90.26	73.62	26.66	27.78	32.72	7.09
Methionine	75.09	54.47	64.59	68.48	64.20	2.57

T_1= tofu, T_2 = fried tofu, T_3 = seitan, T_4 = fried seitan with wheat flour and T_5 = fried seitan with chickpea flour

However, the highest score of seitan samples was recorded for phenylalanine. The results of the amino acid scores in tofu samples indicated that methionine was the limiting amino acid. It was also reported that legumes have low values of sulfur-containing amino acids such as methionine **[50]**. Generally, raw tofu had the highest score in all essential amino acid as compared with fried tofu and different seitan samples.

3.8. *In-Vitro* protein digestibility and protein digestibility corrected amino acid scores (PDCAAs)

The *in vitro* protein digestibilities of tofu and seitan samples are presented in **Table (8)**. The lowest values were observed for fried seitan with chickpea flour and fried seitan with wheat flour. Fried seitan with wheat flour had a relatively high amino acid score as compared to other seitans.

However, protein quality is not on the amino acid profile, but also on the protein digestibility. On the other hand, fried seitan with chickpea flour had low digestibility but had higher protein content than wheat flour and these differences in protein digestibility indicated that the preparation method may have an effect on the availability of amino acids. The main determinant of food protein quality is the content and availability of essential amino acids. These nutrients have been shown to play an important role in the growth, reproduction and maintenance of the human body **[38]**.

Table 8

% Protein digestibility and % PDCAAs of tofu and seitan samples

Foods	**% Protein digestibility**	**% PDCAAs**
Tofu	93.00	70
Fried tofu	89.64	49
Seitan	84.88	23
Fried seitan (1)	77.37	22
Fried seitan (2)	63.62	21

(1) Fried seitan coated with wheat flour (2) Fried seitan coated with chickpea flour

Bongnar [51] reported that frying without any additional ingredients, as it is normally the case, does not change the digestibility of protein. When reducing substances are added to the food that is fried, for instance, carbohydrates (flour), protein digestibility is lowered slightly, albeit significantly. On the other hand, the previous data showed that kind of flour can also affect the protein digestibility and use of wheat flour as a coating substance of seitan before frying is better than the use of chickpea flour to enhance the protein digestibility. As regards tofu samples, raw tofu sample was the highest in % digestibility (93%) as compared to other samples. These results are in accordance with previously reported results **[52]** showing that the digestibility for tempeh was 91.41 and this value was higher than that found in meat (90.79) or pure beef burger (90.04), even though temph is a form of soya and it was processed via fermentation without thermal processing. This study provided information on the amino acid content and protein digestibility of fried tofu and seitan and this is new information that was not available for consumers.

The protein digestibility- corrected amino acid score (PDCAAs) method has been considered to be a simple and scientifically sound approach for routine assessment of dietary protein quality of humans **[21]**. Higher PDCAAs for raw were recorded for tofu samples as compared with seitan samples **(Table8)**. The difference between the PDCAAs of raw and fried seitan was small but the PDCAAs of raw tofu were higher than that of fried tofu. It was earlier reported that a high protein quality of diets when PDCAAs values above 70 – 80%. The vegetable source foods with the lowest PDCAAS like wheat, maize and cassava, it is only when 50% of the protein is exchanged with an animal food source (i.e milk) that the increases are up to a level of 80% or above**[53]**. The addition of 25% milk powder brings PDCAAS values to a reasonable level, above 70 %, while the level, when adding meat, is only of 60 % or slightly above, for wheat and maize. The data on the composition of fried tofu and seitan are important to determine the nutritional value of these diets and can be useful for improving their protein quality to make a significant impact on growth.

4. Conclusions

Plant-based food preparations produced from pulses or grains have always been a major source of protein in human diet. Tofu and seitan have a very protein contents and can be considered as meat alternatives. This recent trend is being accompanied and promoted by a growing number of new food products from various raw materials. Frying method had a good effect on the pleasant and acceptability of tofu and seitan. Fried tofu and seitan contained a moderate amount of protein, which was above of 20% and high content of carbohydrates, lipids and energy in compare with traditional ones. However, the new recipes followed by frying process of tofu and seitan caused an increment in desired minerals and antioxidant activity. Fried tofu proved to be a good source of essential amino acids with respect to protein quality. A correspondence was found between essential amino acids of fried products and % digestibility and PDCAAs, since tofu samples with higher amino acids content had higher values of digestibility and PDCAAs.

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