# Physico-chemical properties of tempeh produced from chickpea seeds

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**Abstract:** Chickpea seeds are rich source of protein (24.63 %), fat (5.62 %), carbohydrates (64.60 %), ash (3.30 %) and fiber (1.85 %). The ant-nutritional factors of raw chickpeas were 7.98, 4.64, 10.96 and 269.38 mg /g dry matter, phytic acid, tannins, trypsin and total phenols, respectively. These seeds are a good source of K (771.77), Ca (156.13), Na (107.34), Mg (152.58), Cu (0.98), Fe (6.85) and Zn (3.83 mg /100 mg). Tempeh was produced from chickpea flour after soaking, blanching (whole seeds), blanching (dehulled) and inoculated with a suspension of *Rizophus oligospours.* The product was evaluated for nutritional quality. Protein in tempeh (28.85 %) was higher than that recorded in raw seeds. However, fat (2.84), ash (2.10) and fiber (1.68 %) were affected due to soaking, blanching and fermentation. Carbohydrates content (64.53 %) was not affected due to the previous treatments. Anti-nutritional factors of tempeh were reduced by 71.18, 73.22, 89.78 and 67.84 % with phytic acid, tannins, trypsin and phenols, respectively compared with this content in raw chickpeas. Protein solubility, water solubility index and water absorption index. In-vitro protein digestibility, in tempeh was higher compared with raw chickpeas. Determination of color showed that ΔE (color difference) of tempeh was high (18.79). Also, essential amino acids reached to their high values in tempeh..
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**Key words:** Tempeh – blanching – anti-nutritional factors - chickpeas.

## 1. Introduction:

A pulse, including chickpea (Cicer arietinum L) is one of the most important crops of the world due to their nutritional quality. They are rich sources of carbohydrates, protein, vitamins and minerals *(Costa, et al., 2006* and *Gowen , et al., (2007 )*. Chickpea seeds are usually consumed at the raw green tender stage (unripe stage), called Malana, or in the form of mature dry seeds after parching as a popular snack food. (*Alajaji* and *El-Adawy 2006).* Chickpeas contain also certained anti-nutritional constituents such as trypsin inhibitors, phytic acid and tannins *(Wang et al., 2010)*.

Some components of pulses or legumes can be fully or partially removed by different processing as soaking and cooking Mubarak*, 2005; Ramakrishna et al.2006* and El- *Maki et al. 2007)*.

Fermentation is the one of the processes that decrease the levels of anti-nutrients in food grains and increase minerals extractability *(Badau et al., 2005).* Tempeh is a solid-substrate fermentation patty consisting of cooked acidified beans and /or grains. This product has been a protein staple in Indonesia for centuries (*Bates, and Schmidt 2002)*. Tempeh in Indonesia is usually produced by fermenting soybeans (i.e., one of the most important grains of legume family) with *Rhizopus oligosporus*, but other pulses or solid substrates such as barley kernels *(Feng et al. 2005; Feng et al. 2007)* and chickpeas *(Reyes-Moreno et al. 2000)* can be used as well. Studies on organic characteristics of tempeh (such as protein, lipids, vitamin, phytate, isoflavone, and carbohydrates) have been published elsewhere *Eklund-Jonsson et al. 2006; Azeke et al. 2007; Angulo-Bejarano et al. 2008)*. It's easy to make tempeh at home at a very low cost. Dehulled soybeans are soaked overnight, cooked for about 30 min and mixed with tempeh starter. After 36 to 48 hours incubation you have delicious fresh tempeh. Tempeh starter contains spores of *Rhizopus oligosporus or Rgizopus oryzae*.(*Bates* and *Schmidt 2002).*

Furthermore, chickpea has several undesirable attributes, such as long cooking time, protease inhibitors, phytates and phenolic compounds, which must be decreased or eliminated for the effective utilization of this legume *(Mila´n-Carrillo, et al., 2000)*. Solid state fermentation (SSF) represents a technological alternative for processing a great variety of legumes and/or cereals to improve their nutritional quality and to obtain edible products with palatable sensory characteristics. Several other substrates have been used to prepare tempeh, e.g. common beans, chickpeas, rapeseed, lupine, home bean, ground nut, wheat, corn/soybean *(Cuevas-Rodrı´guez, et al., 2004).* The potential of using SSF to improve the nutritional value of cereals and/or legumes has been evaluated *(Egounlety, et al., 2002).* This procedure requires a relatively simple infrastructure and can produce chemical changes, e.g.

increases in soluble proteins and soluble carbohydrates; furthermore, it is possible to significantly decrease antinutritional factors, e.g. protease inhibitors, phytic acid, tannin content, and flatulence producing factors (*Hachmeister* and *Fung 1993).* The tempeh was obtained from fresh and hardened chickpea. The SSF process caused a significant increase ($p < 0.05$) in crude protein, true protein (19.6-19.9 to 23.2-23.4%), protein solubility, in vitro digestibility (68.6-73.1% to 79.9-80.5%), available lysine (2.19-3.04 to 3.19-4.07 g lysine/16 N) a significant decrease ($p < 0.05$) in lipids, minerals, and phytic acid (8.82-10.73 to 2.11 g phytic acid/g dry matter), and tannins (16.1-22.4 to 3 mg catechin / g dry matter (*Reyes-Moreno. et al., 2000).*

The objective of this investigation was to evaluate physico-chemical and nutritional properties of different processed chickpea seeds (soaking and blanching) and tempeh obtained from subsequent processed chickpea flours fermented by the solid state fermentation (SSF).

## 2. Materials and Methods:

### Materials:

### Samples:

Chickpea (*Cicer aritinum*, L.) variety Giza was obtained from the Field Crops Research Institute, Agricultural Research Centre (A.R.C.) Ministry of Agriculture, Giza, Egypt. Chickpea cleaned and stored at 4ºC in tightly sealed containers until used.

### Chemicals:

The chemicals used in this study (trichloroacetic acid, ammoniom molybdate and sodium phytate, gallic acid, tris (hydroxymethyl) aminomethane, benzoyl-DL-arginine-*p*-nitroaniline (BAPA) dimethyl sulfoxide were purchased from Sigma Chemical Company St. Louis, MO., USA.). Trypsin enzyme which used for in-vitro protein digestibility (IVPD) from bovine pancreas type IIII, 16.500 BAEF Umg $^{-1}$, pepsin (P-7000) and Vanillin reagent were purchased from Sigma Chemical Company ( St. Louis, MO, USA). Potato dextrose agar (PDA) media was obtained from Oxoid.

### Mineral standards:

Standard solution (1000 ppm) of macro elements; potassium (K), calcium (Ca), sodium (Na), and magnesium (Mg) as well as micro elements; copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) were provided by Merck (Darmstadt, Germany). The standards were prepared from the individual 1000 mg/L. Working standards were prepared from the previous stock solutions.

### Mould strain:

The strain *Rizophus oligospours* (NRRL 2710) used for production of tempeh was provided from Microbiological Resource Center (Cairo, MIRCEN), Faculty of Agriculture, Ain Shams University, Egypt.

### Methods:

### Activation of *Rizophus oligospours:*

*Rizophus oligospours* was activated and maintained on PDA medium *(APHA, 1992)* broth at 25ºC for 24 h, then was propagated in PDA at 25ºC**.** The culture was conserved at 4ºC. Using separate sterile pipettes, a series of decimal dilutions were prepared ($10^5$ to $10^{10}$) from an overnight culture grown in PDA broth. All dilutions were shacked in a vortex and the volum of suspension contained about$10^9$ spores was collected.

### Processing methods:

The effect of some physical treatments (i.e. soaking, blanching with whole seed, blanching with dehuled seed and fermentation strains of *R. oligosporous*), individually and in combination for reducing anti-nutritional factors (ANFs) of the tested seeds were studied.

After each step for every particular processing method , the samples were dried at 50 °C for 20 h. in a hot air oven and ground in an electric mill to pass a 60 mesh sieve screen .The powdered samples were stored in plastic containers under cooling until analysis for their anti-nutritional factors.

### Physical methods:

### Soaking:

Chickpea seeds were soaked in distilled water (1:10, w/v) for 12 hrs, at room temperature (~ 25 ºC). The soaked seeds were drained and rinsed three times with 600 ml distilled water.

### Dehulling:

Hulls were removed by hand after soaking *(El-Beltagy, 1996).*

### Blanching treatments:

Seeds were blanching in distilled water (100°C) in the ratio of 1:10 (w/v) on a hot plate for 30 min.

### Production of chickpea tempeh flour:

Tempeh flour was prepared using the procedure described by *Reyes-Moreno et al. (2004).* As observed in Fig. (1) Chickpea seeds were soaked at 25 ºC for 16 h in four volumes of a 0.9 M acetic acid solution (pH 3.1). Seeds were then drained and their seed coats removed hand. The cotyledons were then cooked at 90 ºC for 30 min, cooled at 25 ºC, inoculated with a suspension of R. oligosporus (1 X $10^9$ spores/ l), and packed in perforated polyethylene

bags (15 X 15 cm). Solid state fermentation (SSF) was carried at 30-37 ºC for 43-77 h. The resulting chickpea tempeh was dried at 52 ºC for 12 h, cooled at room temperature (25ºC) and mille. Chickpea tempeh flour was kept at 4ºC in tightly sealed containers until used.

**Analytical Methods:**

**Proximate composition:**

Protein, fat, crude fiber and ash were determined according to the methods described in the *A.O.A.C. (2000)*. A total carbohydrate was calculated by difference. All the measurement of analyzed samples were made in triplicate.

**Determination of phytic acid:**

The phytic acid content in both raw and treated seeds samples was determined according to the method of *Mohamed et al., (1986)* using chromogenic (solution methanol, concentrated $H_2SO_4$, concentrated HCL, elemental mercury and ammonium molybdate). The amount of phytic acid content was expressed as mg/g sample (dry weight basis).

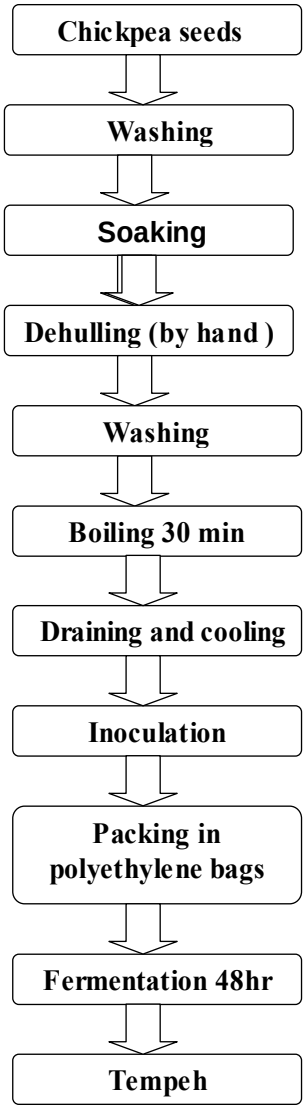


**Fig. 1. Flow-sheet of Tempeh production.**

**Determination of tannins:**

Tannins were assayed in accordance with the modified vanillin-HCL method of *Price et al., (1978)* and catechin was used as the reference standard.

**Determination of trypsin inhibitor:**

Trypsin inhibitor activity (TIA) was measured by the method described by *Hamerstrand et al. (1981).*

**Determination of total phenolics:**

Total phenolics compounds were estimated according to the method described by *Meda et al. (2005).*

**In vitro protein digestibility procedure (IVPD)**:

In vitro digestibility of protein was determined by successive pepsin trypsin enzyme system according to method of *Chavan et al., (2001)* with minor modification.

**pH values:**

The pH of flour samples was recorded using a pH meter. Each flour sample (10 g) was suspended in 100 ml of boiling distilled water. After cooling, the slurry was shaken (1500 rpm, 25 ºC, 20 min) using an orbital shake (HANNA instruments Hi 9021 Microprocessor pH Meter according to *AACC (1995).*

**Bulk density:**

Bulk density determined according to the method of *Berrios,( 2007 )*. The flour samples were gently filled into 10 ml graduated cylinders, previously tarred. The bottom of the cylinder was gently tapped on a laboratory bench several times until there was no further diminution of the sample level after filling to the 10 ml mark. Bulk density was calculated as weight of sample per unit volume of sample (g/ml). Measurements were made in triplicate.

$$\text{Bulk density (g/ml)} = \frac{\text{Weight of sample (g)}}{\text{Volume of sample (ml)}}$$

**Color:**

Color was measured by using a spectro-colorimeter (tristimulus color machine with CIE lab color scale) (Hunter, Lab Scan X E, Reston VA) calibrated with a white standard tile of Hunter Lab color standard (LX No 16379); X = 77.26, Y = 81.94 and Z = 88.14 (L*= 92.43, a*= - 0.86, b*= - 0.16). Color difference (ΔE) was calculated from a, b, and L parameters, using Hunter-Scot field's equation *(Hunter. 1975).*

$$\Delta E = (\Delta a^2 + \Delta b^2 + \Delta L^2)^{1/2}$$

Where: $a = a - a_o$, $b = b - b_o$ and $L = L - L_o$. Subscript *o* indicates color of control Hue angle ($t_g^{-1}$ b/a) and saturation index ($\sqrt{a^2 + b^2}$) were also calculated.

**Minerals determination:**

Mineral contents, ie. Copper (Cu), magnesium (Mg), manganese (Mn), iron (Fe) and zinc (Zn) were determined according to the method of *A.O.A.C. (2000)* using Atomic Absorption Spectrophotometer, Perkin-Elmer 2380. The flame photometer was applied for calcium (Ca), potassium (K) and sodium (Na) determination according to the method described by *Pearson (1976).*

**Amino acids determination:**

After hydrolysis of chickpea flour with 6 N HCL at 110 ºC for 24 hrs, the HPLC apparatus (Waters Assoc, USA) was used for identifying the amino acid of the tested samples according to *Millipore Cooperative (1987)* modified PICO-TAG method. Amino acid score was calculated according to *FAO /WHO (1985)* as follows:

$$\text{Amino acid score (\%)} = \frac{\text{mg of *EAA in 1 g of test protein}}{\text{mg of EAA in 1 g of **reference protein}} \times 100$$

* Essential amino acids

** EAA (1985) FAO/WHO

**Water absorption index (WAI) and water solubility index (WSI):**

Water absorption index (WAI) and water solubility Index (WSI) of chickpea flours were determined by slightly modifying the method of *Anderson, et al., (1969)*. WAI and WSI were calculated by the equations:

$$\text{WAI} = \frac{\text{Weight of sediment}}{\text{Weight of dry solids}}$$

$$\text{WSI \%} = \frac{\text{Weight of dissolved solids in supernatant X 100}}{\text{Weight of dry solids}}$$

**Statistical analysis:**

The results were statistically analyzed by analysis of variance and least significant difference (L.S.D.) at 0.05 levels according to the method described by *Snedecor and Cochran (1980).*

**3. Results and Discussion**

**Chemical composition of chickpea seeds and their tempeh:**

Chemical composition of raw chickpeas indicate that these seeds are a good source of protein, fat, ash, fiber and total carbohydrates (Table1).These results coincide with those reported by *costa et al., (2006)*.On the other hand, chemical composition of

raw chickpea seeds were slightly or highly affected by different processing, i.e. soaking in water and blanching as well as the final tempeh product producing from the seeds.

**Table (1): Chemical composition of chickpea seeds (dry weight basis) and tempeh as affected by different processing methods.**

| Components % | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Raw seeds | Soaking seeds | Blanching (whole seeds) | Blanching (dehulled) seeds | Tempeh products | LSD at (5%) |
| **Protein** | 24.63[b] ± 2.0 | 24.86[b] ±1.0 | 24.12[b] ±1.0 | 24.37[b] ±2.0 | 28.85[a] ±2.0 | 3.04 |
| **Fat** | 5.62[a] ±0.02 | 5.71[a] ±0.02 | 4.93[a] ±0.02 | 5.17[a] ±0.02 | 2.84[b] ±0.02 | 1.15 |
| **Ash** | 3.30[a] ±0.02 | 2.98[d] ±0.02 | 3.22[b] ±0.02 | 3.11[c] ±0.02 | 2.10[e] ±0.02 | 0.02 |
| **Fiber** | 1.85[d] ±0.02 | 1.94[c] ±0.03 | 2.67[a] ±0.02 | 2.59[b] ±0.02 | 1.68[e] ±0.03 | 0.02 |
| ***Total Carbohydrates** | 64.60[b] ±2.0 | 64.51[b] ±3.0 | 65.06[a] ±3.0 | 64.76[b] ±3.0 | 64.53[b] ±2.0 | 4.62 |

*Calculated by difference.
-All values are means of triplicate determinations ± standard deviation (SD)
- Means within row with different letters are significantly difference (P < 0.05)

Protein content in raw chickpea seeds was slightly affected during soaking or blanching, but the reduction observed was not significant (P < o.o5). In contrast, protein value increased significantly (P < o.o5) in tempeh product by 17.13 % compared with the raw chickpea seeds. The reduction of protein content during the processing may be due to solid substrate fermentation (SSF) and due to both dehulling and leaching of solid material which may reflect an increase in mould biomass *(Paredes-Lopez et al., 1991; Sparringa* and *Owens, 1999)*. Our results are agreement with those reported by *Alajaji* and *El-Adawy (2006)* and *Daur et al., (2008)*.

Regarding to fat content, similar pattern as protein was observed with the soaking and blanching. However, significant decreases (P < 0.05) was detected in tempeh product; the reduction of fat was 49.47 % compared with the fat content in raw chickpeas seeds. The great reduction in fat content due to solid substrate fermentation (SSF) and the oxidation as well as utilization of fatty acids by the fungus as a source of energy *(Ruiz-Teran* and *Owens 1996)*.

Ash content in raw chickpea seeds was reduced significantly (P < o.o5) with the different treatments (soaking and blanching) and tempeh product (Table 1). These reductions were 9.70, 2.42, 5.76 and 36.36 % with soaking, whole seed blanching, dehulled blanching and tempeh, respectively. Data proved that, the highest reduction of ash was recorded with tempeh product. While the lowest reduction was detected with whole seed blanching. Data also proved that fiber content in soaking and blanching chickpea seeds increased significantly (P < 0.05) by 4.86, 44.32, and 40.00 % with soaking, whole seeds blanching and dehulled blanching, respectively. However, this value decreased significantly (P < 0.05) by 10.81 %. These results indicate the highest fiber content with the blanching. Increases of fiber content could be due to protein-fiber complexes formed after possible chemical modification induced by the soaking and cooking of dry seed *(Bressani, 1993)*. Similar results were noticed by *El-Adawy (2002)* and *Alajaji* and *El-Adawy (2006)*.

Soaking and dehulled blanching led to slightly decrease and increase, respectively on the content of total carbohydrates. However, this affect was not significant. Regarding to whole seed blanching, total carbohydrates were slightly increased significantly (P < 0.05) by 0.71 %. On the other hand, total carbohydrates in tempeh product not affected significantly (P < 0.05). These results are agreement with those reported by *Reyes-Moreno et al., (2000)* and *Egounlety* and *Aworh (2003)*.

These results proved that during tempeh production, soaking and blanching slightly decreased the chemical composition of raw seeds except carbohydrates. However, this reduction was the highest due to the fermentation process.

**Effect of different processing on anti-nutritional factors of chickpea and tempeh:**

Data in Table (2) indicate the content of anti-nutritional factors (phytic acid, tannins, trypsin inhibitor and total phenols) and their affected by different processing (soaking, blanching and tempeh production). Results proved that these factors decreased significantly (P < 0.05) due to the different

processing. The lowest reduction of the factors were 3.63 % (phytic acid), 16.85 % (tannins), 21.07 % (trypsin) and 19.21 % (total phenols) due to the soaking in water. These reduction increased by blanching, which recorded 32.10, 41.68, 74.45 & 27.71 %, (whole seeds) respectively and 41.10, 50.11, 78.92 & 36.34 % (dehulled), respectively. However, the highest reduction of these factors was detected in tempeh product, which recorded 71.18, 73.22, 89.78 and 67.84 % with phytic acid, tannins, trypsin and total phenols, respectively.

**Table ( 2 ): Effect of different processing on anti-nutritional factors of raw chickpea seeds to produced tempeh.**

| Treatment | Phytic acid (mg/g dry matter) | *R% | Tannins (mg catechin/g dry matter) | *R% | Trypsin inhibitor (TI/mg/g sample) | *R% | Total phenols (mg/100g sample | *R% |
|---|---|---|---|---|---|---|---|---|
| **Raw seeds** | 7.98[a] ±1.0 | — | 4.63[a] ±0.03 | — | 10.96[a] ±1.0 | — | 269.38[a] ±3.0 | — |
| **Soaking seeds** | 7.69[a] ±1.0 | 3.63 | 3.85[b] ±0.03 | 16.85 | 8.65[b] ±0.02 | 21.07 | 217.64[b] ±4.0 | 19.21 |
| **Blanching (whole seeds)** | 5.42[b] ±0.02 | 32.10 | 2.70[c] ±0.03 | 41.68 | 2.80[c] ±0.02 | 74.45 | 194.73[c] ±3.0 | 27.71 |
| **Blanching (dehulled seeds)** | 4.70[b] ±0.02 | 41.10 | 2.31[d] ±0.03 | 50.11 | 2.31[c] ±0.02 | 78.92 | 171.50[d] ±3.0 | 36.34 |
| **Tempeh** | 2.30[c] ±0.02 | 71.18 | 1.24[e] ±0.03 | 73.22 | 1.12[d] ±0.03 | 89.78 | 86.64[e] ±3.0 | 67.84 |
| **LSD at (5%)** | 1.41 | — | 1.31 | — | 0.81 | — | 8.98 | — |

*Reduction

-All values are means of triplicate determinations ± standard deviation (SD)

- Means within column with different letters are significantly difference ($P < 0.05$)

The reduction of anti-nutritional factors may be due to leaching out of these compounds into soaking water *(Abd El-Hady* and *Habiba 2003* and *Ramakrishna et al., 2006)*. Also, phytase activity may be partially responsible for reducing the phytic acid level in fermented product *(Sharma* and *Khetarpaul, 1997)*. The relationship between decreases in phytic acid content and increases in fermentation time could be explained by phytase enzyme synthesis by Rhizopus, which hydrolysis phytic acid *(Reyes-Moreno et al., 2004)*. The decrease of poly phenols indicates the ability of micro flora to ferment phenolics *(Elyas et al., 2002)*. Similar findings were noticed by *Khandelwal et al., (2010)*.

**Nitrogen protein solubility and in-vitro protein digestibility (IVPD) values in raw, processed chickpea seeds and tempeh:**

Data presented in Table (3) demonstrate that raw chickpea seeds were rich in total nitrogen (3.94 %) and non-protein nitrogen (0.49 %). Beside, nitrogen protein solubility in both water and (0.5 N) NaCl was high which recorded 79.20 and 50.46 %, respectively. Also, results proved that in-vitro protein digestibility of raw chickpea seeds was high (66.19 %). The effect of different processing (soaking, blanching and tempeh production) on these values were slight or insignificant.

Regarding to protein and total nitrogen, data proved that their contents not affected significantly ($P < 0.05$) due to soaking or blanching. On the other hand, non-protein nitrogen content not affected significantly ($P < 0.05$) by soaking, but data showed that significantly decreased ($P < 0.05$) was observed in both whole seed and dehulled blanching, and the reduction was 20.41 and 24.49 %, respectively compared with raw chickpeas. In addition protein solubility in both water or (0.5 N) NaCl solution was affected significantly ($P < 0.05$) due to soaking and blanching. Protein solubility in water decreased by 5.11, 10.93 and 6.84 % with soaking, whole seed blanching and dehulled blanching, respectively. This reduction increased to 9.14, 20.02 and 15.48 %, in this order with (0.5 N) NaCl. These results indicate that protein solubility in (0.5 N) NaCl was lower than in water. Data also reveled that in-vitro protein digestibility increased significantly ($P < 0.05$) due to the different processing. The relative increase were 17.10, 11.77 and 13.57 % with the treatments of soaking, whole seed blanching and dehulled blanching, respectively. In tempeh flour, data showed that different processing caused significantly increased ($P < 0.05$) in protein (17.13 %), total nitrogen (17.26 %), non-protein nitrogen (14.29 %) and in-vitro protein digestibility (24.70 %). In contrast significantly decreased recorded in protein solubility in both water (29.68 %) and (0.5 N) NaCl (21.28 %).

**Table (3): Nitrogen protein solubility and In-vitro protein digestibility (IVPD) values in raw, processed chickpea seeds and tempeh.**

| Treatment | Protein % | Total nitrogen % | Non-protein nitrogen % | Protein solubility % | | In-vitro protein digestibility (IVPD) % |
|---|---|---|---|---|---|---|
| | | | | In water | In (0.5N) Nacl | |
| Raw seeds | 24.63 [b] ±1.53 | 3.94 [b] ±0.02 | 0.49 [b] ±0.02 | 79.20 [a] ±3.0 | 50.46 [a] ±3.0 | 66.19 [c] ±3.0 |
| Soaking Seeds | 24.86 [b] ±1.0 | 3.98 [b] ±0.02 | 0.43 [b] ±0.02 | 75.61 [a b] ±2.0 | 45.85 [b] ±2.0 | 77.51 [b] ±2.0 |
| Blanching (whole seeds) | 24.12 [b] ±1.0 | 3.86 [b] ±0.02 | 0.39 [c] ±0.02 | 70.54 [c] ±2.0 | 40.36 [c] ±2.0 | 73.98 [c d] ±2.0 |
| Blanching (dehulled seeds) | 24.37 [b] ±2.0 | 3.90 [b] ±0.02 | 0.37 [c] ±0.02 | 73.78 [b] ±3.0 | 42.65 [b c] ±2.0 | 75.17 [c] ±2.0 |
| Tempeh | 28.85 [a] ±2.0 | 4.62 [a] ±0.02 | 0.56 [a] ±0.02 | 55.69 [d] ±2.0 | 39.72 [c] ±3.0 | 82.54 [a] ±3.0 |
| LSD at ( 5%) | 3.04 | 0.6 | 0.06 | 3.80 | 4.46 | 4.23 |

-All values are means of triplicate determinations ± standard deviation (SD)
- Means within column with different letters are significantly difference (P < 0.05

From the results obtained, some traditional cooking methods decreased the values of protein and non-protein nitrogen. This decrease might be attributed to their diffusion into cooking water *(Alajaji* and *El-Adawy, 2006).* In protein solubility, all processing methods decreased the rat of solubility in both water and NaCl solution. Similar results obtained by *Helmy (2003)* who reported that the rate of decreasing ranged from 8.40 to 57.72 % in water and from 16.50 to 50 23 % in NaCl solution. Regarding to in-vitro protein digestibility, data showed increases with all the treatments. These increases could be explained by the elimination of anti-nutritional factors (e. g. hydrolysis of phytic acid during fermentation) and protein denaturation during the cooking step, which results in protein that are more vulnerable to enzyme action *(Angulo-Bejarano et al., 2008). Reyes-Moremo et al., (2000)* reported that solid state fermentation represents a technological alternative for a great variety of cereals and legumes or combination of them to improve the IVPD of tempeh from chickpea.

**Physico-chemical properties of different processed chickpea seeds and tempeh:**

Some physico-chemical properties of raw, processed chickpea seeds and tempeh were studied and data presented in Table (4). pH values, bulk density, water absorption and water solubility index in raw chickpea seeds were 6.0, 0.74, 1.90 and 27.94, respectively. Similar results was reported by *Kaur* and *Singh (2005).* pH, bulk density and water solubility index values were decreased significantly (P < 0.05) due to soaking, whole seed blanching and dehulled blanching. However, water absorption index increased significantly (P < 0.05) with these treatments. The relative increase of water absorption index were 9.47, 19.47 and 30.53 % and the reduction (%) of water solubility index were 11.92, 30.74 and 40.16 as affected by soaking, whole seeds blanching and dehulled blanching, respectively compared by raw chickpea seeds. Similar pattern was recorded with the tempeh, which pH, bulk density and water solubility index decreased significantly (P < 0.05), while water absorption index increased significantly (P < 0.05).

**Table (4 ): Physico-chemical properties of different processed chickpea seeds and tempeh.**

| Parameters | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Raw seeds | Soaking seeds | Blanching (whole seed) | Blanching (dehulled seeds) | Tempeh | LSD at (5%) |
| pH | 6.00 [a] ±0.02 | 5.92 [b] ±0.02 | 5.81 [c] ±0.02 | 5.75 [d] ±0.02 | 5.60 [e] ±0.02 | 0.06 |
| Bulk density | 0.74 [a] ±0.02 | 0.71 [b] ±0.03 | 0.69 [b] ±0.02 | 0.66 [c] ±0.02 | 0.59 [d] ±0.02 | 0.03 |
| Water absorption index (WAI) | 1.90 [d] ±0.02 | 2.08 [cd] ±0.02 | 2.27 [c] ±0.02 | 2.48 [b] ±0.02 | 3.96 [a] ±0.02 | 0.21 |
| Water solubility index (WSI) | 27.94 [a] ±3.0 | 24.61 [a] ±2.10 | 19.35 [b] ±2.08 | 16.72 [b] ±2.0 | 10.87 [c] ±1.0 | 3.88 |

-All values are means of triplicate determinations ± standard deviation (SD)
- Means within raw with different letters are significantly difference (P < 0.05)

The relative increase of water absorption index was 108.42 % and the reduction of water solubility index was 61.10 % as compared with raw chickpea seeds. The last results for tempeh seeds were due to partial protein denaturation and starch gelatinization occurring during the cooking step *(Angulo-Bejarano et al., 2008)*. Our results are agreement with those reported by *Milan-Corrillo et al., (2000)* and *Reyes-Moreno et al., (2004)*.

**Color values of raw, processed chickpea seeds and tempeh:**

Hunter color values of raw and different processed chickpea flour as well as tempeh from its were determined and data are presented in Table (5). Data proved that lightness values "L" of raw chickpea slightly increased due to the different treatments. The relative increase was 1.85, 4.45 and 5.25 % due to soaking, blanching (whole seeds) and blanched (dehulled), respectively. These results are agreement with those reported by *Reyes-Moreno et al., (2004)* and *Kaur* and *Singh (2005)*. Increasing of "L" value meaning a lighter color, but fermentation results in a slightly darker color due to the influence of mycelia color and the drying stamp *(Angulo-Bejarano et al., 2008)*.

**Table ( 5 ): Hunter color values of different processed chickpea seeds and tempeh.**

| Treatment | L | a | b | a / b | Saturation | Hue | ΔE* |
|---|---|---|---|---|---|---|---|
| **Raw seeds** | 82.22 | 2.75 | 19.69 | 0.14 | 19.88 | 82.05 | __ |
| **Soaking seeds** | 83.74 | 2.68 | 18.63 | 0.14 | 18.82 | 81.81 | 1.85 |
| **Blanching (whole seeds)** | 85.88 | 3.16 | 27.72 | 0.11 | 27.90 | 83.50 | 8.83 |
| **Blanching (dehulled seeds)** | 86.54 | 2.76 | 28.09 | 0.10 | 28.23 | 84.39 | 9.45 |
| **Tempeh** | 68.91 | 7.12 | 30.47 | 0.23 | 31.29 | 76.85 | 18.79 |

*Color difference

Data in the same table indicate that redness "a" value was slightly decreased with soaking and slightly increased due to blanching. On the other hand, values of yellowness "b" were increased due to whole seed blanching and dehulled blanching by 40.78 and 42.66 %, respectively compared with the yellowness "b" value of raw chickpea. However, soaking caused slightly decrease (5.38 %). The same trend was observed in results of saturation and hue values. The color difference (ΔE) of chickpea was very little with soaking (1.85). However, these values were increased to 8.83 and 9.45 due to whole seeds and dehulled blanching, respectively.

Regarding to tempeh, results proved that this product had high values of redness "a" and yellowness "b", but had little lightness "L" compared with raw chickpea. In addition saturation and color difference (ΔE) were high values.

**Minerals content of raw, processed chickpea seeds and tempeh:**

Data presented in Table (6) shows macro and micro-elements content in raw chickpea seeds and their affected by different processing. Raw chickpeas are a good source of major elements (K, Ca, Na, & Mg) and lower or moderate source of micro-elements (Cu, Fe & Zn). These elements were reduced due to the different processing.

**Table ( 6 ): Minerals content of raw, processed chickpea seeds and tempeh.**

| Elements (mg / 100g) | Treatment | | | | |
|---|---|---|---|---|---|
| | **Raw seeds** | **Soaking seeds** | **Blanching (whole seeds)** | **Blanching (dehulled seeds)** | **Tempeh** |
| **Macro-elements:** **Potassium (K)** | 771.77 | 541.74 | 337.29 | 298.27 | 199.40 |
| **Calcium (Ca)** | 156.13 | 137.29 | 121.96 | 109.20 | 76.52 |
| **Sodium (Na)** | 107.34 | 104.83 | 102.35 | 100.40 | 69.85 |
| **Magnesium (Mg)** | 152.58 | 149.70 | 147.47 | 145.31 | 102.10 |
| **Micro-elements:** **Copper (Cu)** | 0.98 | 0.82 | 0.71 | 0.64 | 0.47 |
| **Iron (Fe)** | 6.85 | 6.73 | 6.34 | 5.96 | 4.13 |
| **Zinc (Zn)** | 3.83 | 3.51 | 3.39 | 2.97 | 2.09 |

The lowest reduction of minerals was detected due to soaking. These reduction were 29.81, 12.07, 2.34, 1.89, 16.33, 1.75 and 8.36 % with K, Ca, Na, Mg, Cu, Fe and Zn, respectively. On the other hand, the reduction of minerals increased with whole seed blanching by 56.30, 21.89, 4.65, 3.39, 27.55, 7.45 & 11.49 % and dehulled blanching by 61.35, 30.06, 6.47, 4.77, 34.69, 12.99 & 22.45 %, with K, Ca, Na, Mg, Cu, Fe & Zn, respectively. These reduction in minerals content might be attributed to the leaching of such minerals into soaking and blanching water *(Helmy, 2003)*. Similar results for beans and chickpeas were reported by *Wang et al., (2008* and *2010)*.

Minerals content was also reduced due to tempeh producing. The reduction (%) of K, Ca, Na, Mg, Cu, Fe and Zn were 74.16, 51.0, 34.93, 33.08, 52.04, 39.71 and 45,43, in this order. These reduction was higher than the reduction due to processing or soaking. Although, these reduction of minerals was high, the product of tempeh consider a suitable source of the minerals.

**Essential amino acids (EAA) profile of raw, processed chickpea seeds and tempeh:**

Essential amino acids (EAA) content of raw, processed chickpea flours (soaking and blanching) and tempeh are shown in Table (7). Data indicate that raw chickpea contained different types EAA, i.e. leucine, isolucine, lysine, methioine, phenyl alanine, therionine, valine, cystine and tyrosine at values ranged between 1.36 to 7.50 g/ 100g protein. The total of EAA was 39.89 g/ 100 g protein. These values proved that raw chickpea are a good source of these EAA.

Regarding to EAA in chickpea, data show that slightly decrease or increase and sometimes not affected due to the different processing (soaking and blanching) and tempeh production. Total EAA slightly increased in chickpea seeds (1.05 %) due to soaking and tempeh (5.54 %) production. However, whole seeds and dehulled blanching decreased EAA by 2.16 and 1.63 %, respectively. Similar results reported by *Alajaji* and *El-Adawy, (2006)* and *Bejarano et al., (2008)*.

**Amino acid scores of raw, processed chickpea seeds and tempeh:**

Leucine was increased in tempeh and it is clear from these results that tempeh content covered the daily recommended requirements of FAO/WHO for all essential-amino acids compared to raw, soaking, blanching (whole seed) and blanching (dehulled) where the later covered daily requirements of some essential-amino acids and not covered other some essential-amino acids especially Methionine + Cystine and threionine.

The EAA scores of proteins from raw, different processed chickpea seeds and tempeh were evaluated and data collected in Table (8). Comparing these scores with the suggested Ref. pattern of *FAO / WHO (1985*). From the results data proved that leucine, isolucine, lysine, (phenyl alanine + tyrosine) and valine were slightly or not affected due to the different treatment, so their limits covered the recommended requirements of FAO/WHO (1985). However, (methionine + cystine) and therionine were affected due to the different processing, but their limits were near to the requirements of FAO /WHO. (Methionine + cystine), therionine and leucine were found to be the first, second and third limiting amino acids in the raw and different processed chickpea seeds respectively. Tempeh flour had (methionine + cystine), lysine and therionine as the first, second and third limiting amino acids respectively. These results are agreement with those reported by *Alajaji* and *El-Adawy, (2006)* and *Angulo- Bejarano et al., (2008*).They reported that, (methionine + cystine) was the first limiting amino acid in raw and different processed chickpea flours (boiling, autoclaving and microwave cooking).

**Table (7 ): Essential-amino acids (EAA) profile of raw, processed chickpea Seeds and tempeh.**

| Essential-amino acids (EAA) (g / 100g protein) | Treatment | | | | |
|---|---|---|---|---|---|
| | Raw seeds | Soaking seeds | Blanching (whole seeds) | Blanching (dehulled seeds) | Tempeh |
| **Leucine** | 7.59 | 7.66 | 7.43 | 7.48 | 7.74 |
| **Isolucine** | 4.76 | 4.80 | 4.66 | 4.69 | 5.18 |
| **Lysine** | 6.00 | 6.07 | 5.87 | 5.94 | 5.63 |
| **Methionine** | 1.54 | 1.58 | 1.50 | 1.49 | 1.62 |
| **Phenyl alanine** | 5.57 | 5.62 | 5.45 | 5.51 | 6.20 |
| **Therionine** | 3.89 | 3.92 | 3.80 | 3.82 | 4.24 |
| **Valine** | 5.60 | 5.65 | 5.48 | 5.54 | 5.79 |
| **Cystine** | 1.36 | 1.39 | 1.33 | 1.30 | 1.55 |
| **Tyrosine** | 3.58 | 3.62 | 3.51 | 3.47 | 4.15 |
| **Total essential-amino acids** | 39.89 | 40.31 | 39.03 | 39.24 | 42.10 |

**Table ( 8 ): Amino acids scores of raw, processed chickpea seeds and tempeh.**

| Amino acids | Treatment | | | | | Ref. pattern (FAO/WHO 1985) | Amino acids scores | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Essential-amino acids (EAA) (g / 100g protein) | Raw seeds | Soaking seeds | Blanching (whole seeds) | Blanching (dehulled seeds) | Tempeh | | Raw seeds | Soaking seeds | Blanching (whole seeds) | Blanching (dehulled seeds) | Tempeh |
| Leucine | 7.59 | 7.66 | 7.43 | 7.48 | 7.74 | 7.00 | 108.43 | 109.43 | 106.14 | 106.86 | 110.57 |
| Isolucine | 4.76 | 4.80 | 4.66 | 4.69 | 5.18 | 4.00 | 119.00 | 120.00 | 116.50 | 117.25 | 129.50 |
| Lysine | 6.00 | 6.07 | 5.87 | 5.94 | 5.63 | 5.50 | 109.09 | 110.36 | 106.73 | 108.00 | 102.36 |
| Methionine +Cystine | 2.90 | 2.97 | 2.83 | 2.79 | 3.17 | 3.50 | 82.86 | 84.86 | 80.86 | 79.71 | 90.57 |
| Phenyl alanine +Tyrosine | 9.15 | 9.24 | 8.96 | 8.98 | 10.35 | 6.80 | 134.56 | 135.88 | 131.76 | 132.06 | 152.21 |
| Therionine | 3.89 | 3.92 | 3.80 | 3.82 | 4.24 | 4.00 | 97.25 | 98.00 | 95.00 | 95.50 | 106.00 |
| Valine | 5.60 | 5.65 | 5.48 | 5.54 | 5.79 | 5.00 | 112.00 | 113.00 | 109.60 | 110.80 | 115.80 |

## CONCLUSION:

It could be concluded that chickpeas are rich source of protein, fat, carbohydrates and minerals. Besides, they are contained anti-nutritional factors, i. e. phytic acid, tannins, trypsin inhibitor and phenols. These components partially affected by soaking, blanching and fermentation to produce tempeh product. However, the final products still a good source of the chemical composition, minerals and essential amino acids (EAA). In most cases, EAA limits covered the recommended requirements of FAO / WHO.

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