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# Investigations of Amino Acids Profile, Fatty Acids Composition, Isoflavones Content and Antioxidative Properties in Soy Okara

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Soy okara powder was evaluated for protein content, oil content, *in vitro* digestibility, amino acids profile, fatty acid composition, isoflavones content and antioxidative properties. Besides, okara powder was also assessed in terms of meeting the daily requirement of essential amino acids. Dry okara contained 31.06 % protein and 8.62 % oil content and exhibited 48.5 % *in vitro* protein digestibility. Branched chain amino acid *e.g.*, leucine was the most concentrated essential amino acid (3.37 g/100 g), followed by phenyalanine (3.03 g/100 g). Methionine was the least concentrated (0.52 g/100 g) essential amino acid. Concentration of genistein (57.9 mg per100 g) was found to be higher than daidzein (33.0 mg per 100 g). Single serving of okara (6 g protein) was the most efficient in meeting the daily demand of aromatic amino acids (phenylalanine + tyrosine). Total phenolic content, ferric reducing antioxidative power and free radical scavenging activity was found to the magnitude of 0.65 mg GAE/g, 0.248 mmol/100 g and 5.21 % DPPH reduction, respectively. The study exhibited the nutritional and the nutraceutical value of okara.

**Keywords: Okara, Amino acids, Fatty acids, Isoflavones, Antioxidative properties.**

## INTRODUCTION

During tofu-making, okara is the fibrous residue left after the hot water extraction of soy milk from the ground soybean. Approximately, 1 kg of soybean processed for tofu-making yields about 1.1 kg of soy product [1]. In general, it is considered as a waste product and is either disposed off, thereby posing problem for the environment, or used as animal feed. However, okara has been shown to offer numerous health benefits such as antidiabetic [2], antihyperlipidemia [3], anti-obesity [4] and antihypertension, attributed to angiotensin converting enzyme inhibition property [5]. With the rising incidences of diabetes, cardiovascular disease and obesity in the country, this by-product from soy milk and tofu preparation may be used as an ingradient in Indian foods. Replacement of wheat flour with okara powder, to the magnitude of 10-25 %, without affecting the taste has been reported in the bread and noodle making [6]. Besides, fresh and dried okara may be used as ingradient in making Indian snacks like upma, bhajjia, sev, *etc*. Though, studies pertaining to biochemical components like protein, fat, fibre in okara have been reported sufficiently [7-9]. However, limited studies related to amino acid profile [10], *in vitro* digestibility, fatty acid profile [9], antioxidative value [8] are available. In the present investigation, soy okara samples were collected the manufacturing of tofu in Takai tofu plant was evaluated for protein content, oil content, *in vitro* digestibility, amino acids profile, fatty acid composition, isoflavones content and antioxidative properties. More importantly, the efficiency of okara protein in terms of meeting the daily requirement of essential amino acids was also computed.

## EXPERIMENTAL

Okara was sampled in triplicate from the Takai tofu plant installed at ICAR-Directorate of Soybean Research, Indore.

**Procedure:** A fixed quantity (25 g) of fresh okara was oven-dried in triplicate at 65 °C until all the replicates became moisture free. This moisture free okara was used for estimation of protein, oil, *in vitro* digestibility, amino acids profile, fatty acids composition and isoflavones content. For assessment of antioxidative value, the dried okara powder (1 g) was extracted with 15 mL of 70 % aqueous acetone at room temperature in the dark over-night. The mixture was centrifuged at 3000 rpm for 10 min and supernatant was decanted in a blank clean vial and stored at 4 °C in the dark for further analysis of total phenolic content (TPC), free radical scavenging activity (FRSA), ferric reducing antioxidative power (FRAP) assay.

**Protein content and *in vitro* digestibility:** For estimation of protein content, dried okara was subjected to the estimation of nitrogen content through Microkjeldhl method and the value

obtained was multiplied by 6.25. *In vitro* protein digestibility (IVPD) of okara samples was determined by pepsin digestion method [11]. Moisture free okara (350 mg) was taken and 5 mL of 0.075 N HCl and 0.5 mL of pepsin solution (2 mg dissolved in 1mL of 0.075 N HCl) was added to it. The tubes were incubated at 37 °C in water-bath shaker and enzyme action was stopped after 24 h by adding 5 mL of 10 % (w/v) trichloroacetic acid (TCA). The digest was passed through Whatman No. 2 filter paper and the residue was washed with warm water on the filter. Nitrogen in the residue was estimated by Kjeldahl method. *In vitro* protein digestibility was obtained by calculating the difference between the amounts of nitrogen in the sample before and after *in vitro* digestion with pepsin. Nitrogen was multiplied by the factor 6.25 to obtain crude protein.

**High performance liquid chromatography (HPLC) for estimation of amino acids content:** Dry okara (1 g) was hydrolyzed in 6 N HCl at 105 °C for 24 h and filtered. The supernatant so obtained was subjected to amino acids analysis through HPLC based amino acid analyzer procured from Waters India Private Limited following the method given by Seo [12]. ACCQ-Fluor reagent kit of Waters India Private Limited was used for derivatization of the amino acids in the hydrolyzed samples. First of all, ACCQ-Fluor reagent was reconstituted. For this purpose, the vial (2A) containing ACC-Q-Fluor reagent powder was tapped so that the compound is settled completely on the bottom followed by addition of 1 mL of ACCQ Fluor reagent (Vial 2B) and the mixture was incubated on heating block till the reagent powder completely dissolved. Borate buffer (70 µL) was added to 10 µL of the diluted hydrolyzed sample and vortexed. Subsequently, 20 µL of reconstituted ACCQ-Fluor reagent was added and heated for 10 min at 55 °C. A 5 µL of pre-derivatized amino acid mixture sample, standard and blank was loaded into HPLC, which was equipped with Waters 510 binary pump and Waters 2475 fluorescent detector. Separation of amino acids was carried out on ACCQ Tag column through mobile phase [ACCQ Tag Eluent as solvent A: 60 % Acetonitrile as solvent B] at a flow rate of 0.8 mL/min in a multi-step gradient. The gradient was: solvent A/solvent B: 100/0, 98/2, 93/7, 90/10, 70/30 at 0, 0.5,15,19, 35 min, respectively and was maintained for 3 min at 70/30 and brought to 100/0 at 40 min and run for another 10 min at this conc. before injecting next sample. The mobile phase was passed through Whatman (40 mm) filter paper prior to use. The column temperature was maintained at 37 °C. The fluorescence detector was set at excitation and emission wavelengths of 250 and 395 nm, respectively. Retention time observed for Asp (aspartic acid), Ser (serine), glu (glutamic acid), gly (glycine), his (histidinne), arg (arginine), thr (threonine), ala (alanine), pro (proline), tyr (tyrosine), val (valine), met (methionine), lys (lysine), ile (isoleucine), leu (leucine), phe (phenylalanine) was 13.07, 14.57,15.42, 16.61, 17.49, 21.6, 22.1, 23.72, 26.88, 31.69, 32.91, 33.59, 37.059, 37.61, 38.22, 39.50 min, respectively. Concentration of each amino acid was calculated by comparing the peak area of a particular amino acid in the sample chromatogram to the area of the corresponding peak in the standard amino acids mixture chromatogram. Table-1 presents the concentrations of all the essential and non-essential amino acids except tryptophan and cysteine, which are prone to acid-hydrolysis. Standard amino acid mixture solution was prepared by adding 40 µL of the standard into 960 µL of the distilled water. β-Mercaptoethanol (0.2 %) was added into 6 N HCl to give protection against the oxidation of methionine.

**Determination of isoflavones using HPLC:** Dry okara powder (125 mg) was extracted with 80 % methanol (5 mL) and concentrated HCl (1 mL) for 2 h in a boiling water bath as given by Vyn *et al*. [13]. HPLC conditions maintained has been described in our earlier study [14].

**Determination of fatty acid composition using gas chromatography:** Oil was extracted from the dried okara powder. A fraction of the oil was taken for preparation of fatty acid methyl esters (FAMEs) using 1 N sodium methoxide. FAMEs were injected into Shimadzu gas chromatograph (GC17A) for estimation of fatty acid composition as described in our earlier study [15].

**Determination of total phenolic content:** Total phenolic content of okara powder extract was determined by Folin-Ciocalteu's phenol reagent following the standard method [16].

**Free radical scavenging activity using DPPH (2,2-diphenyl-1-picryhydrazyl):** Free radical scavenging capacity of the dried okara extract was evaluated using an absolute ethanolic solution of DPPH following the standard procedure [17].

**Total antioxidant potential using ferric reducing antioxidative power assay:** Total antioxidant capacity of the dried okara extract was determined using the ferric reducing antioxidative power assay [18].

## RESULTS AND DISCUSSION

Okara is the by-product generated during the manufacturing process of soy milk and tofu. In general, okara has been reported to contain 83.9-84.5 % moisture content. okara sample analyzed in this investigation was found to contain 75 % moisture. Table-1 presents the concentrations of protein, oil, amino acids, fatty acids, isoflavones and values of *in vitro* protein digestibility, total phenolic content, ferric reducing antioxidative power and free radical scavenging activity (FRSA) expressed in % DPPH reduction.

Okara sample in present study showed 31.06 % protein content, which is at par with the value reported in the previous studies [19,20]. Branched amino acid *i.e.*, leucine was the most concentrated essential amino acid (3.37 g/100 g) followed by phenyalanine (3.03 g/100 g). Isoleucine was the third most concentrated essential amino acid (2.17 g/100 g). Methionine, the sulphur containing essential amino acid was the least concentrated essential amino acid (0.52 g/100 g). Among non-essential amino acids, glutamic acid (4.19/100 g) followed by aspartic acid (3.13 g/100 g) was the most concentrated. Based upon the protein content of the okara sample, concentration of each amino acid (mg) per gram of okara protein was also computed (given in parenthesis in Table-1). These values were compared with the results of earlier study [10], pertaining to the contents of essential amino acids in okara and values were expressed in mg per gram of protein. The concentration of leucine (108.5 mg/g protein), the most abundant essential amino acid observed in the okara protein of the present study was higher compared to the level of this amino acid (81 mg/g

TABLE-1
BIOCHEMICAL PARAMETERS OF DRY OKARA

| Essential amino acids (g/100 g) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Phe | Leu | Isoleu | Lys | Val | Thr | His | Met | Total |
| 3.03±0.09 (97.5) | 3.37±0.09 (108.5) | 2.17±0.06 (69.8) | 1.68±0.05 (54.0) | 1.45±0.05 (46.6) | 1.15±0.05 (37.0) | 0.84±0.04 (27.0) | 0.52±0.02 (16.5) | 14.21 (440.7) |

| Non-essential amino acids (g/100 g) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Tyr | Pro | Ala | Arg | Gly | Glu | Ser | Asp | Total |
| 1.07±0.03 (34.44) | 1.26±0.04 (40.56) | 1.34±0.03 (43.14) | 1.86±0.05 (59.88) | 1.22±0.04 (39.27) | 4.97±0.18 (160.01) | 1.58±0.05 (50.86) | 3.13±0.15 (100.77) | 16.43 (528.9) |

| Per cent fatty acid composition of oil fraction | | | | |
|---|---|---|---|---|
| C16:0 | C18:0 | C18:1 | C18:2 | C18:3 |
| 15.34±0.84 | 2.59±0.49 | 27.12±0.35 | 49.76±1.2 | 5.14±0.37 |

| Isoflavones (mg/100 g) | | Antioxidative properties | | |
|---|---|---|---|---|
| Daidzein | Genistein | TPC (mg GAE/g) | FRAP (mmol/100 g) | % DPPH reduction |
| 33.0±2.71 | 57.9±3.67 | 0.65±0.04 | 0.248±0.01 | 5.21±0.23 |

TPC = Total phenolic content; FRAP = Ferric reducing antioxidative power
Crude Protein (%): 31.06±0.84; Oil Content (%): 8.62±0.23; *in vitro* Protein Digestibility (%): 48.5
Value given are mean ± standard deviation of triplicate samples. Values given in parenthesis are mg per gram of protein

protein) reported by Chan and Ma [10]. Phenylalanine (97.5 mg/g protein) was the second most concentrated essential amino acid in okara protein in the present study, while it was lysine (65 mg/g protein) in the study carried out by Chan and Ma [10]. Concentration of lysine content per gram of okara protein was slightly lower (54.0 mg/g protein) in present study. The same authors reported 51 and 28 mg per gram of protein for the contents of valine and histidine, respectively, in okara, which were in proximity to the values for the corresponding amino acids in our study. Total essential amino acids content, excluding tryptophan, in okara sample was 14.21 g/100 g. *In vitro* digestibility of okara was 48.5 %. Espinosa-Martos and Ruperez [21] reported indigestible fraction to the magnitude of 41.6 % in okara, which indirectly support the value of digestible fraction observed in this study.

Of the two major isoflavones, genistein (57.9 mg per100 g) was found to be higher than daidzein (33.0 mg per 100 g) in the okara sample. The studies available in the literature for isoflavones values for okara are limited. Jackson *et al.* [22] reported daidzein and gensitein to the magnitude of 84 and 130 mg per 100 g of the dried okara, respectively. Lower values for these isoflavone forms in this study may be attributed to the varietal differences and processing methods for soy milk extraction [14,23,24]. Total phenolic content of dried okara was 0.65 mg GAE/g dry okara. This value was in the proximity of the total phenolic content (0.75 mg/g of dry okara) as reported for unfermented dry okara powder [8]. Ferric reducing antioxidative power of okara in this study was 0.25 mmol/100 g. Zhu *et al.* [25] assessed the reducing power of non-dried, oven-dried and freeze-dried aqueous extract of okara fermented by *Grifola frondosa* following the method of Shi *et al.* [26] and expressed their results in mg/mL, which was 1.19 in case of non-drying extract. The reducing power exhibited by the okara sample in their study supported our results. These authors reported free radical scavenging activity in terms of % DPPH reduction to the magnitude of 9.86 [$IC_{50}$ (mg/mL)] in fermented okara; the unfermented okara sample in the present study exhibited free radical scavenging activity to the magnitude of 5.21 % DPPH reduction.

Okara was found to contain 8.62 % oil content, which was lesser than reported by Rashad *et al.* [8]. Concentrations of palmitic acid, stearic acid, oleic acid, linoleic acid and α-linolenic acid were in the concentration of 15.34, 2.59, 27.12, 49.76 and 5.14 %, respectively. Compared to the soybean seed oil, oil fraction from dry okara was found to contain higher values of palmitic acid (C16:0) and oleic acid (C18:1) and lower value for α-linolenic acid (C18:3). Regular soybean seed oil contains 9-11 % palmitic acid, 19-23 % oleic acid and 7 % α-linolenic acid. A lower value for α-linolenic acid (5.14 %) in okara sample compared to the seed oil, may be ascribed to the oxidation of polyunsaturated fatty acids especially α-linolenic acid during oven-drying. Similar results were observed by Sengupta *et al.* [9] in microwave-dried okara.

Further, according to a health claim of Food and Drug Administration of United States, consumption of 25 g of soy protein per day reduces the risk of cardiovascular diseases and provides other health benefits, intake of 25 g protein can be distributed over 4 servings of 6 g protein each. Requirement of 6 g protein would come from the intake of 77.26 g of okara. Based upon the presence of essential amino acids (mg) per gram of the protein in okara (Table-1), the percentage of the daily requirement of essential amino acids for an adult of 70 kg, which can be provided through single serving of okara was computed. According to consultation of FAO/WHO/UNU [27], requirement for phenylalanine + tyrosine, leucine, isoleucine, lysine, valine, threonine, methionine and histidine were 25, 39, 20, 30, 26, 15, 10.4 and 10 mg per kg of the body weight of the adult, respectively. The daily requirement for a 70 kg adult for phenylalanine + tyrosine, leucine, isoleucine, lysine, valine, threonine, methionine and histidine is given in Table-2. Percentage of daily requirement for each of essential amino acids provided through single serving of okara was computed by dividing the daily requirement of the particular amino acid (for 70 kg body weight) by the quantity available of the same essential amino acid per serving of the product, multiplied by 100. In view of the recommendation of the daily requirement of phenyalanine in combination with tyrosine by FAO/WHO [27], the latter being the metabolic product of

TABLE-2
EFFICIENCY OF OKARA IN MEETING DAILY REQUIREMENT OF ESSENTIAL AMINO ACIDS FOR 70 kg ADULT

| | Phe+Tyr | Leu | Isoleu | Lys | Val | Thr | Met | His |
|---|---|---|---|---|---|---|---|---|
| Daily requirement (mg) | 1750 | 2730 | 1400 | 2100 | 1820 | 1050 | 728 | 700 |
| Availability per serving* (mg) | 792.01 | 650.9 | 419.18 | 324.5 | 280.1 | 222.1 | 98.7 | 162.2 |
| Efficiency for meeting essential amino acids per serving** | 45.2 | 23.8 | 29.9 | 15.4 | 15.3 | 21.1 | 13.6 | 23.1 |

*One serving was taken as 6 g protein, which was equivalent to 77.26 g of okara; **Per cent of daily requirement.

former catabolism, the availability of both the aromatic amino acids per serving of okara was computed together. Single serving of okara (6 g protein) was most efficient (45.2 %) in meeting the daily demand of aromatic amino acids (phenylalanine + tyrosine), followed by branched chain amino acids, namely, isoleucine (29.9 %) and leucine (23.8 %). Okara was the least efficient (13.6 %) in meeting methionine, sulphur containing amino acid.

## Conclusion

In brief, dry okara powder, which is known for its fibre content was found to be rich in essential amino acids and very efficient in meeting the daily requirement of essential amino acids especially aromatic and branched amino acids. Besides, okara sample was found to contain good concentration of isoflavones content and antioxidative value.

## REFERENCES


1. D.K. O'Toole, *J. Agric. Food Chem.*, **47**, 363 (1999).
2. F. Lu, Y. Liu and B. Li, *Bioactive Carb. Dietary fibre*, **2**, 126 (2013).
3. M.J. Villanueva, W. Yokoyama, Y.J. Hong, G.E. Barttley and P. Rupérez, *Food Chem.*, **124**, 72 (2011).
4. K. Matsumoto, Y. Watanabe and S. Yokoyama, *Biosci. Biotechnol. Biochem.*, **71**, 720 (2007).
5. A. Jiménez-Escrig, M. Alaiz, J. Vioque and P. Rupérez, *Eur. Food Res. Technol.*, **230**, 655 (2010).
6. F. Lu, Z. Cui, Y. Liu and B. Li, *Adv. J. Food Sci. Technol.*, **5**, 960 (2013).
7. G.L. Wickramarathna and P.C. Arampath, *Ceylon J. Sci.*, **31**, 29 (2003).
8. M.M. Rashad, A.E. Mahmoud, H.M. Abdou and M.U. Nooman, *Afr. J. Biotechnol.*, **10**, 5504 (2011).
9. S. Sengupta, M. Chakrabort, J. Bhowal and D.K. Bhattacharya, *Int. J. Sci. Environ. Technol.*, **1**, 319 (2012).
10. W.M. Chan and C.Y. Ma, *Food Res. Int.*, **32**, 119 (1999).
11. C.L. Nanda, J.H. Ternouth and A.C. Kondos, *J. Food Sci. Agric.*, **28**, 20 (1977).
12. S.S. Seo, *J. Korean Chem. Soc.*, **49**, 278 (2005).
13. T.J. Vyn, X. Yin, T.W. Bruulsema, C.-J.C. Jackson, I. Rajcan and S.M. Brouder, *J. Agric. Food Chem.*, **50**, 3501 (2002).
14. V. Kumar, A. Rani, A.K. Dixit, D. Pratap and D. Bhatnagar, *Food Res. Int.*, **43**, 323 (2010).
15. V. Kumar, A. Rani and O.P. Joshi, *Indian J. Agric. Sci.*, **74**, 388 (2004).
16. V.L. Singleton and J.A. Rossi, *Am. J. Enol. Vitic.*,**16**, 144 (1965).
17. A. Mellors and A.L. Tappel, *J. Biol. Chem.*, **241**, 4353 (1996).
18. I.F. Benzie and J.J. Strain, *Anal. Biochem.*,**239**, 70 (1996).
19. K. Shuruga, A. Kato, K. Kadokura, W. Hiruma, Y. Sekino, C.A. Tony Buffington and Y. Komatsu, in ed.: H. El-Shemy, "Okara" A New Preparation of Food Material with Antioxidant Activity and Dietary Fiber from Soybean, In: Soybean and Nutrition, InTech, Ch. 16, pp. 311-326 (2007).
20. I. Mateos-Aparicio, A. Redondo-Cuenca and M.J. Villanueva-Suárez, *Food Chem.*, **122**, 339 (2010).
21. I. Espinosa-Martos and P. Ruperez, *Eur. Food Res. Technol.*, **228**, 685 (2009).
22. C.J.C. Jackson, J.P. Dini, C. Lavandier, H.P.V. Rupasinghe, H. Faulkner, V. Poysa, D. Buzzell and S. DeGrandis, *Process Biochem.*, **37**, 1117 (2002).
23. L. Coward, M. Smith, M. Kirk and S. Barnes, *Am. J. Clin. Nutr.*, **68**, 1486S (1998).
24. M. Kim and J. Lee, *J. Korean Soc. Appl. Biol. Chem.*, **54**, 833 (2011).
25. D. Zhu, H. Sun, S. Li, X. Hu, X. Yuan, C. Han and Z. Zhang, *Int. J. Biol.*, **7**, 82 (2014).
26. M. Shi, Z.Y. Zhang and Y.N. Yang, *Carbohydr. Polym.*, **95**, 200 (2013).
27. FAO/WHO/UNU, Report of a JointWHO/FAO/UNU Expert Consultation, United Nation University, p. 135 (2007).