

Levels of Some Antinutritional Factors in Tempeh Produced From Some Legumes and Jojobas Seeds

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Abstract—Three legumes i.e. soybean, kidney bean and mung bean, and jojoba seed as an oil seed were processed into tempeh, a fermented food. Changes in phytic acid, total phenols and trypsin inhibitor were monitored during the pretreatments (soaking, soaking-dehulling, washing and cooking) and fermentation with *Rhizopus oligosporus*. Soaking was found to reduce total phenol and trypsin inhibitor levels in soybean, kidney bean and mung bean. However, phytic acid was reduced by soaking in kidney bean and mung bean. Cooking was the most effective in reducing the activity of trypsin inhibitor. During fermentation, a slight increase in the level of trypsin inhibitor was noticed in soybean. Phytic acid and total phenols were decreased during fermentation in soybean, kidney bean but mung bean failed to form tempeh because the antifungal activity of herein a protein in mung bean, which exerts both chitinase activity and antifungal activity against a variety of fungal species. On the other hand, solid-state fermentation of jojoba seeds was not effective in reducing their content from cyanogenic glycosides (simmondsin).

Keywords—Antinutritional factors, cyanogenic glycosides (Simmondsin), tempeh.

I. INTRODUCTION

LEGUMES play an important role in human nutrition since they are rich source of protein, calories, certain minerals and vitamins. In African diets, legumes are the major contributors of protein and calories for economic and cultural reasons [1]. However, their role appears to be limited because of several factors including low protein and starch digestibility [2], poor mineral bioavailability [3] and high antinutritional factors [4].

The most important antinutritional factors ANFs antiphenological substances in legumes include protease inhibitors, phenolic substances, non-protein amino acids, lecithin's, saponins, flatulence produces and non-starch polysaccharides [5]-[8].

Removals of undesirable components are very essential in improving the nutritional quality and organoleptic acceptability of legumes and in turn help to effectively utilize their potential as human food. Several food processing methods such as germination [9]-[11], soaking and dehulling [12], [13], cooking [14], [15], fermentation [16], [17] are known to reduce antinutritional factors effectively and

upgrade the nutritional quality of legumes. The most effective treatments are fermentation [18].

Rhizopus oligosporus is a food-grade fungus that has been widely used in solid-substrate bioconversion systems to produce value-added food products [19].

Tempeh is a traditional Indonesian fermented food in which fungi, particularly *Rhizopus* sp., play an essential role; this product is gaining acceptance elsewhere. 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 [20], [21].

Sharma and Sarbhoy [22] made tempeh in the laboratory, employing *Rhizopus stolonifer*, *R. arrhizus*, *R. oryzae*, *R. microsporus*, *R. oligosporus* and *R. chinensis* for fermentation. The best results were obtained by *R. oligosporus*, which did not give good flavor and color as well as formed a compact mass covered with a white moldy mass.

This study was undertaken as part of efforts to introduce tempeh technology in Egypt and to evaluate the combined effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on some antinutritional factors of soybean, kidney bean, mung bean and jojoba seeds.

II. MATERIALS AND METHODS

A. Materials

1. Seed Samples

Mung bean (*Vigna radiata* kawmy1), soybean (*Glycine max.* L.) Clark, kidney bean (*Phaseolus vulgaris* L.) Nebraska, jojoba (*Simmondsia chinensis* L.) varieties were obtained from Agriculture Research Center (A.R.C), Ministry of Agriculture, Giza, Egypt. The seeds were thoroughly cleaned from dust and other extraneous material prior to use.

2. Mould Strains

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

B. Chemicals

Trichloroacetic acid, ammonium molybdate sodium phytate, gallic acid, tris hydroxymethyl aminomethane, trypsin, benzoyl-DL-arginine-p-nitroaniline (BAPA), dimethyl sulfoxide were purchased from Sigma Chemical Company,

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C. Media

Mould strain was activated and maintained on PDA medium [23]. After sterilization at 121°C for 15min, the medium was acidified to pH 3.5 ± 0.2 by adding 1.0ml of sterile lactic acid solution (10%) to each 100ml of sterilized medium at 50°C.

D. Production of Bean Tempeh

Tempeh was prepared in laboratory by the fermentation of soy bean and kidney bean with *R. oligosporus*. Mold spores were prepared according to the method of [24]. In this method potato dextrose agar slant was inoculated with mold strain, incubated at 28°C for a week and the produced spores were harvested with 3ml of 0.1% peptone water. Spores suspension contained approximately 3.22 X10⁵ spores/ ml.

Tempeh was produced using by traditional Indonesian technology as modified by [25] as can be seen in Fig. 1. Beans were sorted, washed, soaked overnight in tap water (1:3 w/v) for 12–14h and hand-dehulled. After washing, the beans were boiled in water (1:3 w/v) for 30 min, drained, cooled to room temperature, and packed in perforated polyethylene bags (15x15cm). A suspension of *R. oligosporus* (1x10⁶ spores/ml) was used to inoculate the bags which contained 100g of cooked beans. Solid state fermentation was carried out at 27–30°C for 48h. The resulting tempeh was dried at 50°C for 12h, cooled at 25°C and stored at 4°C.

E. Analytical Methods

1. Proximate composition

Moisture, fat, ash, crude fiber, protein contents (N X 6.25) of different seeds was determined according to [26].

2. Determination of Trypsin Inhibitor

Trypsin inhibitor activity (TIA) was measured by the method described by [27].

3. Determination of Phytic Acid

The phytic acid content in both raw and treated seeds samples was determined according to the method of [28] using chromogenic solution.

4. Determination of Total Phenolics

Total phenolics were determined from a modified assay described by [29], which was modified by [30].

5. Determination of Simmondsin

Simmondsin was determined in jojoba seeds as follows:

1. Extraction of SMT from Vegetal Material

In order to perform the extraction of simmondsin's (SMs) and simmondsin's ferulates (Fs), the samples (50g) were processed according to [31], in brief, ground, sifted (mesh no. 40), defatted (through Soxhlet with n-hexane during 12h) and extracted with methanol (3× 50ml) through shaking at room temperature during 30min. The resulting extracts were concentrated at reduced pressure (40-50°C), dissolved with methanol HPLC grade and taken to a final volume of 1ml.

2. Spectrophotometric Measurement UV–vis

The stock solution (0.022mg/ml) was prepared from the pattern sample of SMs and Fs in methanol. Different aliquots of stock solution were used to obtain six calibration standards in concentration range from 0.008 to 0.020mg/ml. The calibration curves were performed at two wavelengths, 220 and 323nm.

F. Sensory Evaluation and Acceptability of Soy and Kidney Bean Based Tempeh

Tempeh which fermented by *Rhizopus oligosporus* and characterized by the best chemical and microbiological attributes were used for sensory evaluation. Fresh tempeh was prepared using the following method. A 250g of tempeh cake were cut into slices of 0.5cm thickness, spicing in mixture contains (100ml onion juice, 1g NaCl salt, 0.5g black pepper, 1g cloves, 1g ginger, 5ml lemon juice) for 12h at 5°C. The spiced tempeh slices were coated with egg and crumb, and then fried in hot sunflower oil for 5min for each side.

Samples of tempeh were served to 10 panelists and the parameters assessed included the color, odor, taste appearance and overall palatability. Judges scored all parameters on 7 points scale, with higher values denoting better quality as the procedures previously described [32].

G. Statistical Analysis

Results are expressed as the mean value ± standard deviation (SD) of three replicates; Data were statistically analyzed using Analysis of Variance and Least Significant Differences [33]. Significant differences were determined at the (P <0.05) level.

III. RESULTS AND DISCUSSION

A. Characteristics Features and Acceptability of Legumes Tempeh

During the present study tempeh was produced by solid state fermentation of soybean, mung bean, kidney bean and jojoba seeds using *Rhizopus oligosporus*. Many attempts were done to produce tempeh from the tested seeds, but *Rhizopus oligosporus* failed to grow on mung bean and this may be attributed to the presence of some antifungal component (chitinase) in this legume seed as mentioned [34].

According to the characteristics of tempeh mentioned elsewhere [35], [36], the obtained tempeh was classified into three degrees of quality (good, acceptable and bad). Generally soy and kidney bean tempeh cakes have firm texture and earthy odor resembling a cross between mushroom and fresh yeast. Mycelia on the upper surface (Figs. 1, 2) were denser than on lower surface.



Fig. 1 Soybean tempeh



Fig. 2 Kidney bean tempeh

Lateral side of tempeh piece (Figs. 3, 4) and a selection through the tempeh cakes revealed that, beans are tightly bound together and the spaces between them completely filled with mycelia. Good tempeh cake could easily cut into workable pieces, which are ready for consumption. Really good soy and kidney bean tempeh are different in some external features. Upper surface of good soy tempeh is covered with white mycelia and the lower surface with cotyledons white in color. In addition piece of soy tempeh keep its regular configuration by time.



Fig. 3 Lateral side of soybean tempeh piece

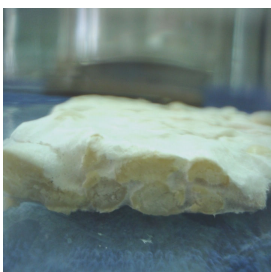


Fig. 4 Lateral side of kidney bean tempeh piece

On the other hand, good kidney tempeh was characterized by a white color. Upper surface is covered with cottony white

mycelia, while lower surface with cotyledons white in color. According to the characteristics of tempeh mentioned before the obtained soy and kidney bean tempeh was classified to a good tempeh.

Acceptable and good tempeh externally resemble to each other. However, acceptable jojoba tempeh was characterized by darker color, yeast odor, and moderately firm textures. Spaces between seeds were moderately bound together and not completely filled with mycelia. On the other hand, jojoba tempeh cake is soft, viscous, tends to break up and the spaces between seeds partly filled by mycelia. Upper surface is covered with slightly dense mycelia, but the seeds themselves still visible (Fig. 5).

In conclusion fermentation of some legumes and jojoba seeds with 3.22×10^5 spores suspension /100g seeds and incubation at 25-27°C for 18-24h resulting in good tempeh in the case of soy and kidney bean and acceptable tempeh for jojoba seeds.



Fig. 5 Jojoba seeds tempeh

Soy and kidney tempeh which characterized by the best of features attributed were used for sensory evaluation after frying in oils for 15min. The quality attributes including, color, taste, appearance, and overall acceptability were used to evaluate the cooked samples (Table I). Judges scored all parameters on 7- pointes scale, with higher value denoting better quality as described earlier [32].

Fried kidney and soy tempeh are shown in Fig. 6.



Fig. 6 Fried soy bean and kidney bean tempeh

Fried soy tempeh (Table I) was good in most of the tested sensory attributes, and has the following scores 5.3, 4.7, 5.3, 5.2 and 4.9 for color, odor, taste, appearance and overall acceptability, respectively. However, fried kidney tempeh was acceptable in all sensory attributed and has the following scores, 4.8, 4.5, 4.3 and 4.3 for color, odor, taste, appearance, and overall acceptability, respectively.

TABLE I
SENSORY EVALUATION OF FRIED SOY BEAN AND FRIED KIDNEY BEAN
TEMPEH SAMPLES FERMENTED WITH *RHIZOPUS OLIGOSPORUS*

Parameters	Fried soybean tempeh	Fried kidney bean tempeh
Color	5.33 ^a ± 0.65	4.83 ^a ± 1.27
Odor	4.67 ^a ± 1.27	4.50 ^a ± 1.27
Taste	5.33 ^a ± 0.89	4.25 ^b ± 1.48
Appearance	5.17 ^a ± 0.94	4.33 ^a ± 1.56
Overall palatability	4.92 ^a ± 1.38	4.33 ^a ± 1.37

Similar results were obtained by [37] who suggested that good tempeh cakes can be produced by inoculation of soy and lupine seeds with 3.22×10^5 *R. oligosporus* or *R. stolonifera* spores suspensions / 100g seeds and incubation at 30°C for 20 and 28h. Sensory evaluation of the cooked soy and lupine tempeh, showed a positive response for all sensory attributes.

B. Effect of Solid State Fermentation with *R. oligosporus* on Antinutritional Factors

1. Trypsin Inhibitor Activity

During tempeh procedure it was noticed that soaking for 12 h achieved loss in trypsin inhibitor activity ranged from 9.0, 16.7, and 11.5% in soybean, kidney bean and jojoba seeds, respectively. Dehulling of soaked seeds significantly reduced TIA by 13.7, 27.0, and 18.9% in soybean, kidney bean and jojoba seeds, respectively. This loss became greater when cooking was done after soaking and dehulling of the seeds. It has been found that cooking for 30min was very effective for inactivation of TIA by 90.0, 87.0 and 88.5% for soybean, kidney bean and jojoba seeds, respectively.

During fermentation with *R. oligosporus* a complete inactivation was observed in trypsin inhibitor activity in kidney bean and jojoba seeds. A slight increase in the level of trypsin inhibitor was noticed in soybean as can be seen in Table II.

TABLE II
EFFECT OF *R. OLIGOSPORUS* FERMENTATION FOR TEMPEH PRODUCTION ON TRYPSIN INHIBITOR CONTENT (MG/G) OF SOME LEGUMES AND JOJOBA SEEDS (DRY WEIGHT BASIS)

Treatment	Soybean	R %	Kidney bean	R %	Jojoba	R %
Raw seeds	58.13 ^a ± 0.38	-	13.70 ^a ± 0.04	-	5.30 ^a ± 0.20	-
Soaking 12 h.	52.89 ^b ± 0.16	9.0	11.41 ^b ± 0.13	16.7	4.60 ^b ± 0.04	11.5
Soaking (12 h.)+ dehulling	50.15 ^c ± 0.38	13.7	10.00 ^c ± 0.3	27.0	4.30 ^c ± 0.13	19.0
Cooking of dehulled soaked seeds	6.05 ^e ± 0.64	90.0	1.80 ^d ± 0.1	87.0	0.61 ^d ± 0.1	88.5
Tempeh	7.20 ^d ± 0.28	87.6	0.00 ^e	100	0.00 ^e	100

*Means in the same column with different letters are significantly (p < 0.05) different ** R= Reduction

These results are in agreement with earlier reports [38] who found that during fermentation with *R. oligosporus* a slight increase in the level of trypsin inhibitor was noticed in soybean whereas no activity was found in cowpea and ground bean.

2. Phytic Acid

During tempeh procedure it was observed that soaking soybean for 12h increased the phytic acid content from 35.01 to 37.04mg/g however, soaking caused loss in phytic acid content in both kidney bean and jojoba seed reached to 15 and 14%, respectively.

TABLE III
EFFECT OF *R. OLIGOSPORUS* FERMENTATION FOR TEMPEH PRODUCTION ON PHYTIC ACID CONTENT (MG / GM) OF SOME LEGUMES AND JOJOBA (DRY WEIGHT BASIS)

Treatment	Soy bean	R%	Kidney bean	R%	Jojoba	R%
Raw seeds	35.01 ^b ± 0.28	-	5.44 ^a ± 0.01	-	5.70 ^a ± 0.16	-
Soaking 12 h.	37.04 ^a ± 0.14	5.8	4.59 ^b ± 0.02	15.0	4.90 ^b ± 0.10	14.0
Soaking (12 h.)+ dehulling	34.33 ^b ± 0.59	2.0	4.20 ^c ± 0.01	22.0	3.90 ^c ± 0.03	31.5
Cooking of dehulled soaked seeds	32.30 ^c ± 0.60	7.7	3.90 ^d ± 0.06	27.8	2.04 ^d ± 0.03	65.0
Tempeh	28.13 ^d ± 0.13	19.6	3.53 ^e ± 0.04	34.6	1.70 ^e ± 0.01	70.0

*Means in the same column with different letters are significantly (p < 0.05) different ** R= Reduction

Dehulling after soaking 12h caused a loss in phytic acid reached to 2.0, 22.0, and 31.5% in soybean, kidney bean and jojoba seeds, respectively. A slight reduction in phytic acid (7.7%) occurred in soybean (Table III).

Cooking for 30 min significantly ($P \leq 0.05$) reduced phytic acid by 27.8 and 65.0% for kidney bean and jojoba seeds, respectively. Phytic acid is relatively heat-stable; hence, significant and prolonged inputs of energy are required for its destruction [39].

Fermentation of soybean, kidney bean and jojoba seeds with *R. oligosporus* starter culture resulted in a decrease in phytic acid content (19.6% in soybean, 34.6% in kidney bean and 70% in jojoba seeds). These results are in agreement with earlier reports [38] who found that fermentation of soybean, cowpea, and ground bean with *R. oligosporus* for 36, 30 and 36h., respectively resulted in a decrease in phytic acid content (30.7% in soybean, 32.6% in cowpea and 29.1% in ground bean).

3. Total Phenolic Compounds

The result in Table IV showed that soaking for 12 h caused loss in total phenolic compounds reached to 19.7, 24.5 and 37.4% in soybean, kidney bean and jojoba seeds, respectively.

Dehulling after soaking 12h significantly reduced total phenolic compound by 28.1, 35 and 51.2% in soybean, kidney bean and jojoba seeds, respectively. Cooking dehulled beans

resulted in lowering the total phenolic content by 34, 38.3 and 63% in soybean, kidney bean and jojoba seeds, respectively.

Solid state fermentation with *R. oligosporus* slightly reduced total phenolic compounds from 26.82 and 139.10 mg/g for cooking-dehulled soybean and kidney bean to 22.31 and 128.86 mg/g, respectively for fermented samples.

TABLE IV

EFFECT OF *R. OLIGOSPORUS* FERMENTATION FOR TEMPEH PRODUCTION ON TOTAL PHENOLIC CONTENT (MG / GM) OF SOME LEGUMES AND JOJOBA SEEDS (DRY WEIGHT BASIS)

Treatment	Soy bean	R %	Kidney bean	R %	Jojoba	R%
Raw seeds	40.60 ^a ± 0.6	-	225.3 ^a ± 12	-	64.40 ^a ± 1.3	-
Soaking 12 h.	32.57 ^b ± 0.9	19.7	170.10 ^b ± 12	24.5	40.30 ^b ± 1.9	37.4
Soaking (12 h.)+ dehulling	29.15 ^c ± 1.6	28.1	146.70 ^c ± 3.1	35.0	33.00 ^c ± 0.10	51.2
Cooking of dehulled soaked seeds	26.82 ^c ± 2.6	34.0	139.10 ^{cd} ± 2.94	38.3	23.86 ^d ± 0.40	63.0
Tempeh	22.31 ^d ± 0.81	44.2	128.86 ^d ± 3.32	42.8	5.13 ^e ± 0.32	92.0

*Means in the same column with different letters are significantly different (p<0.05) different ** R= Reduction

On the other hand, fermentation of jojoba seeds with *R. oligosporus* significantly reduced total phenolic compounds from 23.86 mg/g of cooked samples to 5.13 mg/g of fermented ones (92% reduction over control samples).

4. Cyanogenic Glycosides

The results in Fig. 7 revealed that neither soaking nor dehulling had the ability to reduce simmondsin in jojoba seeds. However, cooking of dehulled seeds lower the simmondsin content by only 7.3 % below control value. Fermentation of jojoba seeds with *R. oligosporus* starter culture resulted in a decrease in the simmondsin content to 14.8 %.

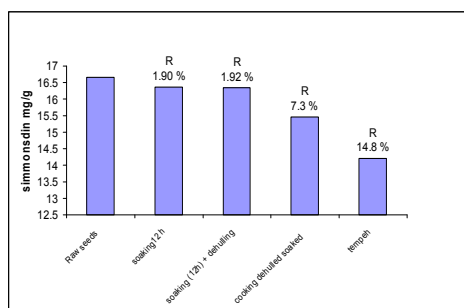


Fig. 7 Effect of tempeh production on simmondsin content in jojoba

This means that solid - state fermentation of jojoba seeds was not effective for reducing their content from cyanogenic glycosides (simmondsin).

IV. CONCLUSION

The current study revealed that soaking reduced total phenol and trypsin inhibitor levels in soybean, kidney bean and mung bean. Phytic acid reduced was by soaking in kidney bean and mung bean. Cooking was the most effective in reducing the activity in trypsin inhibitor. Phytic acid and total phenols decreased during fermentation of soybean, kidney bean. Solid-state fermentation of jojoba seeds was not

effective for reducing their content from cyanogenic glycosides (simmondsin).

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