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EFFECT OF SUNFLOWER SEEDS ADDITION ON THE NUTRITIONAL VALUE OF GRASS PEA TEMPEH

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Nutritional parameters of grass pea tempeh in comparison with products obtained with the addition of sunflower seeds were evaluated. Mixed tempeh was characterised by significantly increased amount of lipids with higher polyunsaturated fatty acids (PUFA) (60%) content and improved n-6 to n-3 fatty acids ratio (16:1). The addition of sunflower seeds resulted in the rise in *in vitro* protein availability of the products. The maximum protein availability was obtained for sunflower tempeh (72%) and the product made from grass pea and sunflower seeds in the proportion of 3:2 (62%). The sum of sulfur amino acids present in the latter object amounted to 171% of the FAO reference pattern. Levels of other essential amino acids were also higher than the amounts recommended by the FAO. Enriching grass pea seeds in sunflower seeds resulted, however, in lowering both the level and the *in vitro* availability of carbohydrates. Organoleptic assessment showed that the mixed tempeh was estimated better than the product prepared with grass pea seeds only.

INTRODUCTION

Grass pea (*Lathyrus sativus* L.*)* is a legume plant only locally known in Poland but worth of popularization due to valuable proteins, relatively high content of mineral compounds and little cultivation requirements. The applications of grass pea as well as other legumes are, however, limited by the presence of antinutrients which are usually removed by the culinary process, that is soaking and subsequent cooking. Other treatment which results in high nutritional quality of the product together with antinutrients elimination is fungal fermentation tempeh type, very popular in Indonesia [Astuti *et al*., 2000].

The purpose of the study was to estimate the nutritional profile of tempeh obtained from grass pea seeds and to assess whether the application of sunflower seeds as an additional fermentation substrate may enhance the value of the product. Sunflower seeds were chosen mainly as a source of lipids in order to enrich the flavor of grass pea tempeh since grass pea seeds are very low-fat substrate [Grela, 1994]. In this way the final products would resemble typical soy tempeh which should increase their general acceptability by consumers. Moreover, sunflower seeds are very popular in Poland and commonly used as a food ingredient.

MATERIALS AND METHODS

Materials

Research was conducted on grass pea (*Lathyrus sativus* L.) seeds cultivar 'Krab' obtained from the company 'Spójnia Hodowla i Nasiennictwo Ogrodnicze' in Nochowo, Poland, and dehulled sunflower seeds (China origin), purchased in a local healthy food store.

Preparation of seeds

Grass pea seeds thoroughly cleaned with water and dried with filter paper were cooked in tap water (1:4 v/v seed to water) for 30 min. Then, they were soaked in tap water for 18 h at room temperature. Next, the seeds were dehulled by hand and boiled for 15 min in tap water acidified to pH 4.5-5.0 with vinegar.

Dehulled sunflower seeds were cooked in acidified tap water (1:4 v/v seed to water) for 15 min.

Preparation of fermented products

After cooking, the seeds were cooled (temperature <35°C), drained and mixed thoroughly with vinegar (1.4 mL/100 g raw seeds) and tempeh starter culture (0.39 g/100 g raw seeds, starter type B containing *R. oligosporus* DSMZ 1964 culture suitable for the fermentation of legume-grains mixtures; purchased in the company 'Top Cultures', Zoersel, Belgium). Inoculated material was placed in a perforated plastic bag and formed in a shape of compact small packets 3 cm in height. Fermentation was conducted for 31 h at 32°C (until the seeds were covered with white mould). Tempeh was sliced into approximately 10-mm thick slices and then steamed for 10 min. Next, the product was crumbled and dried at 60°C for 20 h. Dried tempeh was ground in a seed mill (1 mm of mesh diameter) and stored at 4°C until analysed.

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Five types of tempeh were prepared: from grass pea seeds (G), from sunflower seeds (S) and from mixed seeds of grass pea and sunflower (G:S) at ratios of 4:1, 3:2 and 2:3 (w:w).

Analytical methods

Dry matter content was obtained with a moisture analyser (type WPS 110 S, RADWAG, Radom, Poland).

Total protein (g/kg d.m.) was evaluated on the basis of nitrogen level according to the Nessler method [Marczenko & Balcerzak, 1998] in samples previously mineralized in a Hach Digesdah® Digestion Apparatus at 280°C (Hach Company, Loveland, Colo. USA). The nitrogen level was multiplied by 6.25.

Crude fat content was measured by gravimetric method [AOAC 920.39] after Soxhlet extraction with hexane. Ash content was determined by using a muffle furnace (type FCF-5 SH, CZYLOK, Jastrzębie Zdrój, Poland) at 550°C to a constant weight [ICC-Standard No.104/1]. Total carbohydrate content was obtained by difference between 1000 and sum of total ash, crude fat and total protein.

Amino acids were determined by ion-exchange chromatography with post-column derivatization with ninhydrin using an automatic amino acid analyzer (Ingos, Czech Republic) according to a standard protocol of the manufacturer after hydrolysis of powdered samples performed in 6 mol/L HCl containing 0.5 g/100 mL phenol. Sulphur-containing amino acids were analysed as oxidation products obtained by performic acid oxidation followed by standard hydrolysis. Tryptophan was not determined.

Fatty acid extraction was conducted as described by Folch *et al.* [1957]. Fatty acids esters were obtained by the AOAC Official Method 991.39. The fatty acid composition was analysed by gas chromatography using Thermo Trace GC Ultra (Thermo Electron Corporation, USA) and a flame ionizing detector. The separation was done in a Suplecowax 10 column (carrier gas helium at a flow rate of 7.5 mL/min, the initial column temperature was 190°C, final – 210°C).

Protein and carbohydrates availability was estimated in an *in vitro* test.

The *in vitro* method described by Żyła *et al.* [2000] was modified in order to obtain conditions of the human gastrointestinal tract. To this end, 0.5 g of material was incubated with the addition of 1.7 mg of pepsin (Sigma, Steinheim, Germany, the declared activity 4750 U/mg) dissolved in 0.1 mol/L HCl at 37°C, pH 2.0 for 2 h. Next, 2.5 mg of pancreatin (Sigma, from porcine pancreas, 8x United States Pharmacopeia) dissolved in 0.1 mol/L $NaHCO_3$ was added and the sample was incubated in dialysis tubes (Sigma – Aldrich, cellulose membrane 25 mm x 16 mm, retaining most proteins with molecular weight of 12,000 or greater) at 37°C, pH 7.0 for 4 h. The applied incubations simulated the stomach and small intestine medium conditions, respectively. In dialysate, the level of soluble protein was estimated by the method of Lowry *et al.* [1951] and the level of reducing sugars was obtained by the method of Miller [1959]. The availability of protein and carbohydrates was expressed as% total protein or carbohydrates, respectively, released from material during *in vitro* digestion.

Organoleptic assessment

Organoleptic assessment was conducted during Science Festival in Kraków (17 May 2007). Tempeh steamed slices were salted or not salted and served to 15 non-trained panelists on white paper plates with plastic forks at ambient temperature (23°C). The parameters evaluated included taste, odour and general acceptability using a 5-point hedonic scale where 5 represented the highest order of preference as in Mugula & Lyimo [2000].

Statistic analysis

Data were analysed using Statgraphics Plus for Windows. The results were evaluated statistically using the Student's t-test. To determine significant differences, the LSD test was used at $p<0.05$. The statistical analysis was not conducted for fatty acids and amino acids profiles and for total carbohydrates.

RESULTS AND DISCUSSION

Fat content and fatty acids profile

Grass pea tempeh contained a small amount of fat (Table 1) due to low lipids level in grass pea seeds (below 20 g/kg, non-published data). This is consistent with results reported by other authors on products obtained from different low-fat seeds: common bean (11 g/kg) [Paredes- Lopez & Harry, 1989] cowpea (13 g/kg) [Osundahunsi & Aworh, 2003], and chickpea (26 g/kg) [Reyes- Moreno *et al*., 2004]. Along with the rise in the dose of sunflower seeds in mixed tempeh, the increase in fat content was observed. The products prepared with the dominating share of sunflower seeds had fat level similar to that obtained in soy tempeh by Osundahunsi & Aworh [2003] – 250 g/kg, and Kiers *et al*. [2000] – 250-295 g/kg.

The introduction of sunflower seeds as an additional substrate in tempeh fermentation resulted not only in about 30-fold rise in crude fat but also in an increase in PUFA content (α-linolenic acid, γ-linolenic acid, linoleic acid) in total fatty acids from 52% (grass pea tempeh) to about 60% (Table 2). The observed results may be considered as advantageous from the nourishing point of view. However, the high level of sunflower fat rich in polyunsaturated fatty acids may also be connected with greater susceptibility of the product to lipid peroxidation processes and hence may decrease its durability.

TABLE 1. Proximate composition of grass pea and sunflower tempeh.

Tempeh	Crude lipid (g/kg d.m.)	Total protein (g/kg d.m.)	Total carbohydrates (g/kg d.m.)
Grass pea	12.2[a][1]	334.9[d]	639.5
Grass pea: sunflower 4:1[2]	177.1[b]	283.2[c]	524.4
Grass pea: sunflower 3:2	342.2[c]	251.2[b]	388.1
Grass pea: sunflower 2:3	415.8[d]	253.8[b]	305.8
Sunflower	625.3[e]	215.1[a]	135.2

[1]values with different letters differ significantly ($p<0.05$); [2]weight ratio of seeds.

TABLE 2. Fatty acid composition of grass pea and sunflower tempeh (percent share in total fatty acids).

Fatty acid	Tempeh				
	grass pea	grass pea: sunflower 4:1[1]	grass pea: sunflower 3:2	grass pea: sunflower 2:3	sunflower
4:0	0.36	0.28	0.25	0.07	0.06
14:1	0.10	0.078	0.06	0.01	0.01
15:0	0.28	0.226	0.18	0.02	0.02
16:0	15.20	14.508	14.37	5.84	5.49
16:1 n-7	0.35	0.296	0.27	0.07	0.06
17:0	0.38	0.267	0.21	0.05	0.05
17:1	0.10	0.095	0.09	0.03	0.03
18:0	6.48	4.6	3.60	5.34	5.41
18:1 n-9	20.24	17.8	18.13	25.50	26.48
18:1 n-7	0.74	0.876	0.87	0.47	0.36
18:2 n-6	47.17	52.247	54.27	60.47	60.00
18:3 n-6	2.28	2.348	1.57	0.10	0.04
18:3 n-3	2.80	3.449	3.60	0.31	0.07
20:0	0.47	0.354	0.31	0.13	0.32
20:1	0.26	0.361	0.41	0.06	0.12
22:0	0.30	0.223	0.18	0.79	0.79
24:0	0.71	0.44	0.30	0.21	0.18
24:1	0.15	0.104	0.10	0.02	0.02
Total	98.18	98.55	98.89	99.51	99.48

[1]weight ratio of seeds.

As shown in Table 2, the percent share of individual fatty acids in their sum was dependent on the seeds ratio in the fermentation substrate. Generally, tempeh obtained from grass pea and sunflower seeds in proportion 3:2 was characterised by fatty acids profile similar to that obtained for the product made with sunflower seeds only, whereas the product obtained with the prevailing amount of grass pea seeds showed the percent share of fatty acids concurrent with grass pea tempeh. This dependence was true for a considerable number of saturated and unsaturated fatty acids (Table 2).

The addition of sunflower seeds resulted in lowering the ratio of n-6 to n-3 fatty acids in tempeh, as compared to the product obtained from grass pea seeds alone (16:1 and 18:1, respectively). Hence, tempeh containing sunflower seeds was characterised by improved PUFA profile from the nourishing point of view. However, it should be mentioned that it still significantly differed from dietary recommendation, *i.e.* 4:1 for adults [Ackman, 2001].

Protein content and availability. Amino acids profile

Grass pea tempeh contained about 330 g/kg total protein (Table 1) of which 47% were available *in vitro* (Table 3). The obtained total protein level was lower than the one measured in soy and lupine tempeh (450 g/kg) [Zamora & Veum, 1988; Agosin *et al*., 1989] but higher than that of cowpea and chickpea tempeh (260 g/kg) [Osundahunsi & Aworh, 2003; Reyes-Moreno *et al*., 2004]. Introducing the increasing doses of sunflower seeds resulted in significant gradual lowering of the total protein content in tempeh. However, it did not influence the amount of soluble proteins released from tempeh to dialysate under conditions of the *in vitro* test, which was similar for products containing only sunflower seeds or grass pea seeds (the exception was tempeh G:S 2:3), (Table 3). As a consequence, tempeh with sunflower seeds had higher (even above 50%) *in vitro* protein availability, calculated on the basis of the soluble proteins released during the *in vitro* test, as compared to grass pea tempeh. The highest value was obtained for tempeh made from sunflower seeds alone. Legume seeds contain compounds which have a negative effect on protein digestibility, such as phytic acid or trypsin inhibitors. Short fungal fermentation may cause temporary increase

TABLE 3. Availability of tempeh proteins and carbohydrates estimated by *in vitro* test.

Tempeh	Proteins released (g/kg d.m.)	Availability of proteins (%)	Carbohydrates released (g/kg d.m.)	Availability of carbohydrates (%)
Grass pea	158.7^{bc1}	47.40^{a}	212.1^{e}	29.29^{e}
Grass pea: sunflower 4:1[2]	163.4^{c}	57.67^{b}	148.9^{d}	24.14^{d}
Grass pea: sunflower 3:2	155.7^{bc}	62.05^{c}	90.3^{c}	20.73^{c}
Grass pea: sunflower 2:3	144.7^{a}	55.82^{b}	51.5^{b}	15.48^{b}
Sunflower	154.1^{b}	71.58^{d}	6.3^{a}	4.03^{a}

[1]values with different letters differ significantly ($p<0.05$); [2]weight ratio of seeds.

in trypsin inhibitors level due to their release from complexes with enzymes [Wang *et al*., 1972; Egounlety & Aworh, 2003]. The introduction of sunflower seeds to grass pea seeds resulted in lowering the amount of compounds mentioned above. Moreover, it might have contributed to enhanced metabolic activity of *Rhizopus oligosporus* strain by enriching fermentation substrate in energetically-advantageous lipids. As a consequence, the more dynamic proteolysis of the product could occur, thereby resulting in an increased level of partially-hydrolyzed proteins, more susceptible to the action of digestive enzymes during the *in vitro* test. Sunflower meal is proved to be a very good substrate for acid protease production in solid state fermentation [Ikram-ul-Haq *et al*., 2003].

Amino acids profile was estimated in selected products: grass pea tempeh and the product obtained from grass pea and sunflower seeds in the proportion of 3:2. Mixed tempeh was chosen on the basis of *in vitro* protein availability test. Tempeh G:S 3:2 was characterised by significantly lower crude protein content but protein availability higher by 30% than the product obtained from grass pea seeds alone.

The results obtained (Table 4) show that introducing sunflower seeds as a fermentation substrate resulted in an increase of amino acids level in the product, with the exception of lysine and tyrosine. Mixed tempeh contained less lysine (by 15%), as compared to grass pea tempeh, which may be explained by the difference in its level in fermentation substrates: 72 g/kg and 9.2 g/kg in grass pea and sunflower seeds, respectively [Pisulewska *et al*., 1997; Kunachowicz *et al*., 2005]. It should also be mentioned that both the analysed products contained lower levels of individual amino acids in comparison with soy tempeh [Zamora & Veum, 1988].

TABLE 4. Amino acids content of grass pea and grass pea: sunflower (3:2) tempeh (g/kg d.m.).

Amino acid	Grass pea tempeh	Grass pea: sunflower (3:2) tempeh
Ala	14.22	15.10
Arg	30.78	33.79
Asp	33.69	35.53
Glu	45.21	57.42
Gly	11.82	15.12
His	8.01	9.49
Ile	13.58	15.00
Lys	21.22	18.09
Leu	21.14	22.54
Phe	15.00	17.02
Pro	13.28	14.91
Ser	14.38	15.34
Thr	11.60	13.18
Tyr	11.46	10.96
Val	15.36	17.82
Met	3.12	4.03
Cys	6.49	10.25
Total amino acids	290.37	325.59
Essential amino acids	101.03	107.65

The levels of essential amino acids in grass pea tempeh were slightly lower or close to FAO recommendations [Chavan *et al*., 2001], except for phenylalanine with tyrosine and lysine whose content was higher than the presented recommended optimal values (Table 5). Whereas in the case of mixed tempeh, the obtained essential amino acids level was much higher than the amounts recommended by FAO. Thus, the results obtained show that the addition of sunflower seeds to grass pea seeds positively influenced the quality of tempeh as a source of these amino acids. It should be emphasized that the sum of sulfur amino acids present in the mixed tempeh was 171% of FAO reference pattern.

Carbohydrates content and availability

Grass pea tempeh was characterised by the highest level of total sugars of all the analyzed products, amounted to about 640 g/kg (Table 1). The obtained quantity was similar to findings concerning cowpea and chickpea tempeh – 690 g/kg [Osundahunsi & Aworh, 2003; Reyes-Moreno *et al*., 2004], and significantly higher than that measured in soy tempeh – 188 g/kg [Osundahunsi & Aworh, 2003]. Carbohydrates availability obtained in the *in vitro* test was slightly lower than 30% (Table 3). Adding sunflower seeds as a fermentation substrate resulted in a significant diminishing of both the level and the *in vitro* availability of sugars.

Limited carbohydrates availability in the product containing sunflower seeds as compared to grass pea tempeh may be explained by different fungal metabolic activity during fermentation. In conditions of slight lipids supply, as grass pea seeds are low-fat ones, the main source of carbon and energy for *Rhizopus oligosporus* strain were polysaccharides. Hydrolysis of the latter in the fermentation substrate results in increased *in vitro* availability of carbohydrates present in the product [Prinyawiwatkul *et al*., 1996]. During tempeh fermentation, polysaccharides are hydrolysed as a result of the action of α-amylase, endoglucanase and β-glucosidase releasing glucose molecules, together with xylanase and cellulase decomposing xylans and cellulose, respectively [Miszkiewicz *et al*., 2004; Okadome *et al*., 2004]. On the contrary, in the presence of substrate rich in fat, the lipids were most probably main compounds metabolized by *R. oligosporus* during fermentation. Beuchat *et al*. [1974 in Grela, 1994] found that during fermentation of peanuts (*Arachis hypogaea*) containing

TABLE 5. Essential amino acid content of grass pea and grass pea: sunflower (3:2) tempeh (g/16 g N) in comparison with reference pattern.

Amino acid	Grass pea tempeh	Grass pea: sunflower (3:2) tempeh	Reference pattern FAO[1]
Phe+ Tyr	7.9	11.1	6.0
Ile	4.0	6.0	4.0
Leu	6.3	9.0	7.0
Lys	6.3	8.4	5.5
Met + Cys	2.9	6.0	3.5
Thr	3.5	5.2	4.0
Val	4.6	7.1	5.0

[1]Chavan *et al.* [2001]

TABLE 6. Mean score of organoleptic assessment of grass pea and grass pea: sunflower tempeh.

	Salted tempeh			Non-salted tempeh		
	taste	odour	acceptability	taste	odour	acceptability
Grass pea tempeh	3.6[2]	3.3	3.6	2.5	2.7	2.5
Grass pea: sunflower tempeh[1]	4.1	4.1	4.3	3.4	3.4	3.5

[1]each grass pea: sunflower tempeh (percent seeds ratio not distinguished); [2]scores: 1= dislike very much, 2= dislike moderately, 3= neither like nor dislike, 4= like moderately, 5= like very much.

500 g/kg of lipids and 130 g/kg of carbohydrates the former compounds were mainly used as a source of energy and carbon. The same mechanism could work during the fermentation of substrate enriched with a high dose of sunflower seeds in the case of our study. As a consequence, polysaccharides remained bound in the form of macro particles in the final product, being less susceptible to digestive enzymes during the *in vitro* test, which resulted in low availability of tempeh sugars.

Organoleptic assessment

The organoleptic assessment (Table 6) was conducted during presentation of tempeh products prepared by the Department of Biotechnology of University of Agriculture in Krakow for "Science Festival in Kraków 2007". Since it was conducted on rather small group of non-trained panelists chosen from visitors, the obtained information is not complete. However, the mean score of sensory analysis clearly shows that salted tempeh was more preferred (according to taste, odour, general acceptability) than the non-salted one. This is consistent with findings of Mugula & Lyimo [2000]. Most probably, further cooking treatment applied to the presented products, *e.g.* frying, could improve the acceptability of both salted and non-salted tempeh.

Tempeh made with the addition of sunflower seeds was estimated as more tasty than the product prepared with grass pea seeds alone. It should be mentioned that during the organoleptic assessment the percent grass pea: sunflower seeds ratio in tempeh was not distinguished and thus consumers compared only two kinds of product: grass pea tempeh and the mixed one.

CONCLUSIONS

The addition of sunflower seeds to grass pea seeds as a fermentation substrate caused a significant increase in fat content in products G:S 4:1 and 3:2, to the level similar to that observed in soy tempeh. Moreover, enriching the product in sunflower seeds resulted in more advantageous fatty acids composition. In spite of lowered crude protein content in the mixed tempeh, as compared to the product obtained with grass pea seeds alone, the protein availability increased. The highest *in vitro* availability was found for tempeh made from grass pea and sunflower seeds in the proportion of 3:2. This product was also characterised by advantageous amino acids content, which was consistent with FAO recommendations for essential amino acids. Thus, the addition of sunflower seeds resulted in the improvement of this parameter as compared to grass pea tempeh. Considering the results listed above together with observations from organoleptic assessment, we conclude that enrichment of grass pea seeds in sunflower seeds during tempeh fermentation resulted in higher nutritional and sensory quality of the products obtained.

In our opinion, the mixed product obtained in this study is an interesting example of practical application of grass pea seeds, poorly popular in Poland, in combination with seeds of sunflower – commonly used as food ingredients throughout our country.

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