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# PROTEOLYSIS IN TEMPEH-TYPE PRODUCTS OBTAINED WITH *RHIZOPUS* AND *ASPERGILLUS* STRAINS FROM GRASS PEA (*LATHYRUS SATIVUS*) SEEDS

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**ABSTRACT**

**Background.** Tempeh is a food product obtained from legumes by means of solid-state fermentation with *Rhizopus* sp. Our previous research proved that mixed-culture inoculum may also be successfully applied. The objective of present research was to study the proteolytic activity of *R. microsporus* var. *chinensis* and *A. oryzae* during tempeh-type fermentation of grass pea seeds, and the effect of inoculum composition on the protein level and *in vitro* protein bioavailability in products.
**Material and methods.** Fermentation substrate were soaked and cooked grass pea seeds. Material was mixed with single- or mixed-culture inoculum, and incubated in perforated plastic bags at 30°C for 32 hrs. In the products, the proteolytic activity (pH 3, 5 and 7), glucosamine, total protein and free amino acids levels, as well as protein *in vitro* bioavailability and degree of protein hydrolysis were obtained.
**Results.** The significant correlation was found between glucosamine content and proteolytic activity in grass pea seeds fermented with *Rhizopus* or *Aspergillus*. The activities of *Rhizopus* proteases were higher than *Aspergillus* ones, which corresponded with the degree of seed protein hydrolysis. Both strains showed the highest activity of protease at pH 3. Tempeh made with pure culture of *Rhizopus* had 37% protein of 69% *in-vitro* bioavailability. Mixed-culture fermentation improved nutritional parameters of products only when the dose of *Aspergillus* spores in the inoculum was equal and lower than that of *Rhizopus*. This process resulted in higher *in-vitro* bioavailability of protein, slightly more efficient protein hydrolysis and higher level of free amino acids, as compared to standard tempeh.
**Conclusions.** The activity of *A. oryzae* in tempeh-type fermentation is beneficial as long as it does not dominate the activity and/or growth of *Rhizopus* strain.

**Key words:** *Aspergillus oryzae*, *Rhizopus microsporus* var. *chinensis*, tempeh, protease activity, proteolysis

## INTRODUCTION

Tempeh-type fermentation is traditional Indonesian process of solid-state fermentation of legume seeds. A typical substrate is dehulled, soaked and cooked soybeans, but other legumes or grains may be also used. Tempeh fungus *Rhizopus* sp. produces thick white mycelium that overgrows the seeds and bounds them together in a compact 'cake'. As a result of mold hydrolytic enzymes action, tempeh products are characterised by higher nutritional quality and better digestibility of proteins and sugars, as well as lower antinutrients level, as compared to cooked seeds (Astuti et al., 2000). When *R. microsporus* var. *chinensis*

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strains are used, the flatulence-producing oligosaccharides of raffinose family are also decomposed due to α-galactosidase activity (Schwertz et al., 1997).

During traditional tempeh production the composition of tempeh microflora is complex, whereas in controlled laboratory conditions one specific strain is usually used for the fermentation. However, it is also possible to apply mixed inoculum consisting of *Rhizopus* tempeh strain together with yeasts or lactic acid bacteria (Feng et al., 2007; Starzyńska-Janiszewska et al., 2014). Our previous research proved that *Aspergillus oryzae* koji strain may be also used for mixed-culture tempeh-type fermentation. The results showed that grass pea tempeh obtained with inoculum consisting of specified doses of *Rhizopus* and *Aspergillus* spores had beneficial nutritional and bioactive parameters (Starzyńska-Janiszewska et al., 2012). *A. oryzae* and *R. oligosporus* were previously used by other authors for sequential solid-state fermentation of grass pea seeds (Kuo et al., 1995; Yigzaw et al., 2004).

The first objective of present research was to study proteolytic activity of *R. microsporus* var. *chinensis* and *A. oryzae* during single strain tempeh-type fermentation of grass pea seeds (experiment I). The other purpose was to determine the influence of *Aspergillus* addition to *Rhizopus* in the inoculum on the level and *in vitro* bioavailability of protein and content of total free amino acids in mixed-culture tempeh (experiment II).

## MATERIAL AND METHODS

### Materials

**Fermentation substrate.** Grass pea seeds (*Lathyrus sativus*) cultivar Krab were obtained from Spójnia Hodowla i Nasiennictwo Ogrodnicze (Nochowo, Poland).

**Starter cultures.** *Rhizopus microsporus* var. *chinensis* tempeh strain (Institute for Microbial Resources. Taichung, Taiwan) and *Aspergillus oryzae* DSM 1861 koji strain were grown on malt-extract-agar (MEA) slants for 12 days at 20°C. Spores of each strain were harvested with sterile 8 $g \cdot l^{-1}$ saline solution supplemented with peptone (0.01 $g \cdot l^{-1}$) and Tween 80 (0.1 $ml \cdot l^{-1}$). Suspensions were filtered three times through nylon net filters (mesh diameter 11 μm, Millipore, Tullagreen Carrigtwohill, Ireland) to remove all fragments of mycelium. The spore density in the inoculum was obtained by spore-counting in a Thoma chamber and optical microscopy.

Experiment I – The seed portions were inoculated with $10^4$ spores of *R. microsporus* var. *chinensis* or *A. oryzae* per g of seeds and fermented up to 32 h. Samples were taken every 8 h.

Experiment II – A standard tempeh product was obtained with $10^4$ spores of *R. microsporus* var. *chinensis* per gram of seeds (A0). Mixed-culture tempeh-type products were made with inoculum containing *R. microsporus* var. *chinensis* ($10^4$ spores$\cdot g^{-1}$ seeds) and different doses of *A. oryzae*: $0.5 \cdot 10^4$ spores$\cdot g^{-1}$ seeds (A0.5), $10^4$ spores$\cdot g^{-1}$ seeds (A1), $1.5 \cdot 10^4$ spores$\cdot g^{-1}$ seeds (A1.5). Inoculated seed portions were fermented for 30 h (until the products were fully overgrown with mycelium).

**Fermentation procedure.** Grass pea seeds (60 g portions) were cleaned and cooked in tap water for 30 min. Next, they were soaked in distilled water for 18 h at room temperature. Then, the seeds were dehulled by hand and boiled for 15 min in sterile water acidified to pH 4.5–5.0 with lactic acid. After the water was discarded, the seed portions were drained, cooled (< 35°C) and mixed with appropriate amount of *R. microsporus* var. *chinensis* and/or *A. oryzae* spores (as specified above). Inoculated material was put into the perforated plastic bags (3 cm in height) and fermented at 30°C. Next, the products were steamed for 10 min (experiment II only), lyophilized and stored at 4°C for further analysis.

All experimental samples were prepared in duplicate and mixed within the treatment.

### Analytical methods

Dry matter (DM) was determined with a moisture analyzer (WPS 110S, Radwag, Radom, Poland).

To extract proteases, 1 g fermented material was extracted with 14 ml 0.05 M of citrate buffer (pH 3.0) or 0.05 M phosphate buffer (pH 5.0 and pH 7.0). Protease activity in crude supernatants was determined using the method described by Rick et al. (1974) with 0.25 $g \cdot l^{-1}$ haemoglobin as the substrate. One unit of protease activity (PA) was defined as the μmol Tyr $min^{-1}$ liberated by the enzyme at 37°C, calculated per gram of product dry mass.

Chitin hydrolysis was assayed as described by Desgranges et al. (1991). Glucosamine content in hydrolysed

samples was measured according to colorimetric method given by Tsuji et al. (1969) and calculated as mg glucosamine $g^{-1}$ product dry mass. To describe the growth of fungi, the background level of the seed glucosamine was subtracted from the glucosamine level.

Total protein content ($g \cdot kg^{-1}$ DM) was assayed on the basis of nitrogen level according to the Nessler method (Marczenko and Balcerzak, 1998) in samples previously mineralized in Hatch Digesdahl® Digestion Aparatus at 280°C (Hach Company, Laveland, Colo. USA). The nitrogen level was multiplied by 6.25. The content of free amino acids was measured according to Korenman (1973) colorimetric method, modified by replacing isobutanol with isopropanol.

Protein *in vitro* bioavailability was estimated according to the method described by Monsoor and Yusuf (2002) with slight modification, as described below. Adequate amount of sample (equivalent to 15 mg N) was suspended in 15 ml 0.1 M HCl containing 1.5 mg of pepsin (from porcine gastric mucosa, 4,220 $U \cdot mg^{-1}$ protein; Sigma-P6887), followed by gentle shaking for 3 h at 37°C. The suspension was than neutralized with 2 M NaOH to adjust the pH to 7.5 and mixed with 4 mg pancreatin (from porcine pancreas, activity equivalent to 8× US Pharmacopeia; Sigma-P7545) in 7.5 ml of phosphate buffer (0.2 M, pH 7.5, containing 1 mM NaCl and 0.02% $NaN_3$). The mixture was incubated for 24 h at 37°C. The undigested protein was precipitated with 30% trichloroacetic acid and separated by centrifugation (15,000 rpm, 20 min). The obtained supernatant was used to estimate digested protein. The protein susceptibility to enzymatic hydrolysis was expressed as % total protein digested in *in vitro* test.

Degree of protein hydrolysis was calculated as percentage of free carboxyl groups in fermented samples. In order to measure the total amount of carboxyl groups, the samples (1 g) were subjected to hydrolysis (6 M HCl, 24 h at 110°C). Then, the number of free carboxyl groups in hydrolysed and non-hydrolysed material was estimated in by the method of formol titration, as described in De Reu et al. (1995) paper.

### Statistical analysis

For each determination, four replications were made, with the exception of degree of protein hydrolysis (three). The results were statistically evaluated using one-way analysis of variance. To determine statistically significant differences, the least significant difference test was used at $P \leq 0.05$. Linear correlations between protease activity and glucosamine content were also evaluated. Data were processed using Statistica Version 8.0 (StatSoft, Inc., Tulsa, OK, USA).

## RESULTS AND DISCUSSION

### Experiment I. Proteolytic activity and protein hydrolysis during tempeh-type fermentation of grass pea seeds with *R. microsporus* var. *chinensis* or *A. oryzae* strain

The activity of proteases measured at pH 3.0, 5.0 and 7.0 in seeds fermented up to 32 h with *Rhizopus* or *Aspergillus* is shown in Table 1. Both strains showed similar dynamics of enzyme production during solid-state fermentation. Activities measured after 8 and

**Table 1.** Effect of incubation period on protease activity and glucosamine content of *Rhizopus microsporus* var. *chinensis* and *Aspergillus oryzae* during tempeh-type fermentation of grass pea seeds

| Species | Fermentation time, h | | | |
|---|---|---|---|---|
| | 8 | 16 | 24 | 32 |
| | Protease activity, $PA \cdot g^{-1}$ | | | |
| *Rhizopus* | | | | |
| pH 3.0 | 0.04 a | 0.07 ab | 1.37 f | 3.31 g |
| pH 5.0 | 0.15 bc | 0.18 c | 1.16 e | 1.23 e |
| pH 7.0 | 0.20 c | 0.16 bc | 0.48 d | 0.48 d |
| *Aspergillus* | | | | |
| pH 3.0 | 0.10 ab | 0.24 c | 0.80 e | 1.17 f |
| pH 5.0 | 0.14 ab | 0.16 b | 0.26 c | 0.55 d |
| pH 7.0 | 0.07 a | 0.09 a | 0.10 ab | 0.26 c |
| | Glucosamine, $mg \cdot g^{-1}$ DM | | | |
| *Rhizopus* | 7.36 a | 13.08 b | 29.02 c | 40.50 d |
| *Aspergillus* | 8.16 a | 9.80 b | 15.82 c | 26.01 d |

Means with different letters differ significantly ($P \leq 0.05$) for each parameter within the fungal strain.

16 h of the process were low and did not change significantly. The exception was *A. oryzae* protease activity at pH 3.0 which increased gradually and after 16 h exceeded 3-fold the value measured for *Rhizopus* enzyme. After that period, the activity of proteases in grass pea seeds in most cases gradually increased up to 32 h. The fermentation was stopped about this time, because the excessive prolongation of the process leads to ammonia production which decreases nutritional and sensory parameters of the product (Hedger, 1982). The activities of *R. microsporus* var. *chinensis* proteases measured after 24 and 32 h of fermentation were higher than that of *Aspergillus* at all pH values (3.0, 5.0 and 7.0). This was expected as high proteolytic activity is important feature of tempeh fungi (Baumann and Bisping, 1995).

*Aspergillus* strain is adapted for koji fermentation which is longer (~5 days) than tempeh process (Chou and Rwan, 1995). Aforementioned authors observed the maximum of proteolytic activity of *Aspergillus* strain after 94 h solid-state fermentation of rice seeds (Chou and Rwan, 1995). However, in case of the strain used in the present study, prolonged tempeh-type fermentation (up to 56 h) led to the decrease of enzyme activity at pH 3.0 (data not shown), starting from 32 hour of the process.

For both strains the highest proteolytic activity was determined at pH 3.0. Acidic proteases synthesis is crucial for producing high quality tempeh (Miszkiewicz et al., 2004). After 32 h of fermentation, protease activity at pH 3.0 was 2.7 fold and over 2 fold higher than one measured at pH 5.0 in case of *Rhizopus* and *Aspergillus*, respectively. This is consistent with the data given by Miszkiewicz et al. (2004) for proteolytic activity of *R. oligosporus* overgrowing pea seeds (pH 3 vs 5.5). The lowest proteolytic activity was measured at pH 7.0. Similarly, significantly higher activity of acidic (pH 3.0) than neutral (pH 6.0) *Aspergillus* protease was observed by Chou and Rwan (1995). It should be also mentioned, that according to Baumann and Bisping (1995), proteolytic capacity of the fungus depends on the strain and not on the species used. Heskamp and Barz (1998) reported that activities of proteases produced by *R. oryzae* isolate Fi and *R. microsporus* var. *chinensis* isolate Sur growing on soybeans were higher at pH 7.0 than 3.0.

The glucosamine assay is a good indicator of fungal biomass growth in condition of solid-state fermentation (Zheng and Shetty, 1998). Between 16 and 32 h of fermentation, the glucosamine level in grass pea seeds was on average 1.5 fold higher in case of *Rhizopus* than *Aspergillus* (Table 1). *Rhizopus* strain produces abundant aerial hyphae, whereas *Aspergillus* grows in the form of low, dense mycelium. The conditions of tempeh fermentation applied in our study were obviously favourable for *Rhizopus* growth. Lee et al. (2008) measured similar or higher level of glucosamine in black koji fermented for 3 days with *Aspergillus* vs. *Rhizopus* strains (solid-state fermentation of steamed seeds with double mixing).

For both *R. microsporus* var. *chinensis* and *A. oryzae*, the high significant linear correlation was found between the glucosamine content in seed dry mass and proteolytic activities during 32 h fermentation of grass pea seeds (Table 2). Correlation coefficients amounted to over 0.95 and 0.89 for acidic proteases and neutral protease, respectively. In case of both species, the highest glucosamine level corresponded to maximal activity of the enzymes (Table 1).

In order to compare the efficiency of proteolysis after 24 and 32 h of fermentation, the degree of protein hydrolysis in fermented grass pea seeds was measured (Table 3). The obtained values were lower in case of *Aspergillus*, amounting 10% and 30% of the data measured for *Rhizopus* growing on the substrate for 24 h and 32 h, respectively. This is consistent with levels of protease activity (Table 1). Ruiz-Terran and

**Table 2.** Correlations between glucosamine content and proteolytic activity in grass pea seeds fermented with *Rhizopus microsporus* var. *chinensis* or *Aspergillus oryzae*

| Species | Protease | Correlation coefficients |
|---|---|---|
| *Rhizopus* | pH 3.0 | 0.96 |
| | pH 5.0 | 0.95 |
| | pH 7.0 | 0.89 |
| *Aspergillus* | pH 3.0 | 0.96 |
| | pH 5.0 | 0.99 |
| | pH 7.0 | 0.89 |

**Table 3.** Degree of protein hydrolysis in grass pea seeds fermented with *Rhizopus microsporus* var. *chinensis* or *Aspergillus oryzae*

| Species | Fermentation time, h | |
|---|---|---|
| | 24 | 32 |
| *Rhizopus* | 15.6 c | 24.3 d |
| *Aspergillus* | 1.5 a | 7.6 b |

Means in a raw with different letters differ significantly ($P \leq 0.05$).

Owens (1996) showed that during fermentation of soybeans *R. oligosporus* utilizes plant protein as both energy substrate and the source of amino acids and peptides. Grass pea seeds are low-fat substrate (about 1% of fat) (Stodolak and Starzyńska-Janiszewska, 2009), as compared to soy seeds. The degree of protein hydrolysis measured in our study was higher than one reported by Sparringa and Owens (1999) for soybeans fermented with *R. oligosporus* (9.5% after 46 h of fermentation).

### Experiment II. Proteolysis in mixed--culture tempeh-type products obtained with *R. microsporus* var. *chinensis* and *A. oryzae*

Tempeh made with the pure culture of *R. microsporus* var. *chinensis* was characterised by 37% protein of 69% *in-vitro* bioavailability (Table 4). The obtained protein level was about 12% higher than the one measured in tempeh made from seeds of the same grass pea variety with *R. oligosporus* DSM 1964 (Stodolak and Starzyńska-Janiszewska, 2008). However, Yigzaw et al. (2004) reported 20% higher amount of protein in Ethiopian variety of grass pea fermented with *R. oligosporus*. *In-vitro* protein bioavailability of grass pea tempeh made with *R. microsporus* var. *chinensis* was 30% higher than the one obtained previously with *R. oligosporus* DSM 1964 (Stodolak and Starzyńska--Janiszewska, 2008; Stodolak et al., 2009).

The addition of lower and equal dose of *A. oryzae* to *R. microsporus* var. *chinensis* spores in the inoculum positively influenced the protein level in grass pea tempeh. Zamora and Veum (1988) did not observe differences between the level of protein in soybeans fermented for about 20 h with *R. oligosporus* and *A. oryzae*. Mitchell et al. (1988) reported that *R. oligosporus* is better for protein enrichment of cassawa than *A. oryzae*.

The application of mixed inoculums with lower and equal dose of *A. oryzae* spores resulted in significantly higher *in-vitro* protein bioavailability of grass pea tempeh, as compared to the product made only with *Rhizopus* strain. This was accompanied by slightly more efficient protein hydrolysis and higher level of free amino acids, by over 12% (Table 4).

On the contrary, when the dose of *A. oryzae* spores in the inoculum exceeded the dose of *R. microspores* var. *chinensis* spores, the obtained product was characterised by the highest level of protein but the lowest *in-vitro* protein bioavailability of all analysed tempeh. It may be speculated that this product had high amount of fungal protein or less accessibility to hydrolytic enzymes applied in *in-vitro* model than grass pea protein. It is also worth noting that the higher dose of *A. oryzae* than *R. microspores* spores in the inoculum did not significantly change the free amino acids content in tempeh, but it slightly lowered its protein hydrolysis

**Table 4.** Parameters of tempeh-type products obtained as a result of 30 h fermentation of grass pea seeds with *Rhizopus microsporus* var. *chinensis* and *Aspergillus oryzae*

| Products | Protein $g \cdot kg^{-1}$ DM | Protein *in-vitro* bioavailability % | Protein hydrolysis % | Free amino acids $g \cdot kg^{-1}$ DM |
|---|---|---|---|---|
| A 0 | 363.0 a | 69.39 b | 17.6 | 2.22 a |
| A 0.5 | 365.0 ab | 73.07 c | 18.6 | 2.38 b |
| A 1 | 372.8 b | 77.15 d | 20.5 | 2.62 c |
| A 1.5 | 410.9 c | 65.82 a | 18.8 | 2.75 c |

Products: tempeh obtained with inoculum containing *R. microsporus* var. *chinensis* ($10^4$ spores$\cdot g^{-1}$ seeds) (A0) and different doses of *A. oryzae*: $0.5 \cdot 10^4$ spores$\cdot g^{-1}$ seeds (A0.5), $10^4$ spores$\cdot g^{-1}$ seeds (A1), $1.5 \cdot 10^4$ spores$\cdot g^{-1}$ seeds (A1.5).
Means in a column with different letters differ significantly ($P \leq 0.05$).

level. This may suggest that important part of *A. oryzae* proteolytic complex could be exopeptidases releasing amino acids and/or small peptides, rather than endoproteases. *A. oryzae* is proven to produce numerous proteolytic enzymes. The genomic sequence of this species contains 69 identified genes of exopeptidases, e.g. aminopeptidases (19), serine-type carboxypeptidases (12), dipeptidyl or tripeptidyl peptidases (9) (Kobayashi et al., 2007).

The results of our previous research showed that *A. oryzae* activity was beneficial for tempeh protein *in-vitro* bioavailability only when the dose of its spores in the inoculum was smaller than that of *Rhizopus* (Starzyńska-Janiszewska et al., 2012). It may therefore be concluded that, with regard to protein hydrolysis, the *A. oryzae* activity during tempeh fermentation is advisable, as long as it does not dominate the process. Too high activity of *A. oryzae* and/or too large amount of its mycelium did not influence positively the protein *in-vitro* bioavailability of grass pea tempeh. The negative competitive impact of *Aspergillus* on growth and/or activity of *Rhizopus* during the fermentation cannot be excluded. The results obtained by Feng et al. (2006) concerning co-cultivation of *R. oligosporus* with yeasts during tempeh fermentation of barley showed that yeasts at high inoculation level ($>10^4$ cfu per gram of substrate) affected negatively the growth of the mold.

The recommendation of a precise dose of *Aspergillus* spores that could assure obtaining good-quality mixed-culture tempeh is difficult. According to our previous (Starzyńska-Janiszewska et al., 2012) and present observations, one of the important factors influencing results of different experiments might be the changeable viability of *Rhizopus* and *Aspergillus* strains' spores. We conducted tests concerning this parameter in years 2008–2013. Viability of spores harvested from 11-day old agar slants underwent serious fluctuations: 3–10% for *A. oryzae* and 13–20% for *R. microsporus* var. *chinensis* (data not published). In our opinion setting the dose of spores in the inoculum should be accompanied by simultaneous standardization of their viability. It would be advantageous to conduct such standardization in case of every fungal strain co-cultivated with *Rhizopus* in conditions of tempe-type fermentation.

## CONCLUSIONS

*R. microsporus* var *chinensis* and *A. oryzae* DSM 1861 showed similar pattern of proteases production during 32 h tempeh-type fermentation of grass pea seeds. However, the former strain was characterized by higher activity levels (experiment I). Changes in proteases activity corresponded to degree of grass pea protein hydrolysis and levels of fungal glucosamine. The protease activities measured at pH 3.0 were significantly higher than the ones obtained at pH 5.0 and 7.0.

On the basis of the results obtained in experiment II, as well as our previous research (Starzyńska-Janiszewska et al., 2012), it may be concluded that *A. oryzae* co-cultivation with *R. microsporus* var. *chinensis* may exert beneficial influence on protein *in vitro*-bioavailability, degree of protein hydrolysis and free amino acids content of tempeh. However, precise determination of the recommended dose of *A. oryzae* spores in mixed inoculum is a technical difficulty.

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## PROTEOLIZA W PRODUKTACH TYPU TEMPEH OTRZYMANYCH W WYNIKU FERMENTACJI NASION LĘDŹWIANU SIEWNEGO (*LATHYRUS SATIVUS*) SZCZEPAMI *RHIZOPUS* I *ASPERGILLUS*

### STRESZCZENIE

Tempeh to produkt spożywczy otrzymywany z nasion roślin strączkowych poddanych fermentacji szczepami z gatunku *Rhizopus*. Wcześniejsze badania autorów wykazały, że jest także możliwe zastosowanie inokulum mieszanego. Celem pracy było zbadanie aktywności proteolitycznej *R. microsporus* var. *chinensis* i *A. oryzae* w trakcie fermentacji nasion lędźwianu oraz określenie wpływu składu inokulum na poziom białka i jego biodostępność (szacowaną metodą *in vitro*). Substratem były namoczone i ugotowane nasiona lędźwianu siewnego. Materiał dokładnie mieszano z jednoszczepowym lub mieszanym inokulum, a następnie inkubowano w perforowanych woreczkach foliowych przez 32 h w 30°C. W otrzymanych produktach oznaczano aktywność proteaz (w pH 3 5 i 7), zawartość glukozaminy, białka i wolnych aminokwasów, biodostępność oraz stopień hydrolizy białka. Wykazano, że aktywność proteolityczna, oznaczona w nasionach lędźwianu fermentowanych szczepem *Rhizopus* lub *Aspergillus*, była silnie skorelowana z poziomem glukozaminy. Aktywność proteaz *Rhizopus* była większa niż *Aspergillus*, co było zgodne ze stopniem hydrolizy białka nasion. Największą aktywność proteolityczną pleśni oznaczono przy pH 3. Produkty typu tempeh otrzymane z udziałem czystej kultury *Rhizopus* zawierały 37% białka, z czego 69% było dostępne *in-vitro*. Zastosowanie mieszanego inokulum skutkowało poprawą parametrów odżywczych nasion, gdy dawka zarodników *Aspergillus* była mniejsza lub równa dawce zarodników *Rhizopus*. Takie produkty charakteryzowały się większą biodostępnością białek i nieco większym stopniem ich hydrolizy oraz większą zawartością wolnych aminokwasów od tempeh monoszczepowego. Wniosek: *A. oryzae* w procesie fermentacji typu tempeh ma pozytywny wpływ na jakość produktu pod warunkiem, że nie zdominuje aktywności i/lub wzrostu szczepu *Rhizopus*.

**Słowa kluczowe:** *Aspergillus oryzae*, *Rhizopus microsporus* var. *chinensis*, tempeh, aktywność proteaz, proteoliza

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