称号及び氏名	博士（農学）TRI　HANDOYO
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論　文　名	「Characterization of Fermented Soybean and Buckwheat by *Rhizopus oligosporus*　and Its Application for Food Ingredients」（*Rhizopus oligosporus* を用いた発酵大豆及び蕎麦の特性と食品への応用）
論文審査委員	主査　森田　尚文 副査　川崎　東彦 副査　原　　正之

論文要旨

Rhizopus oligosporus is one of the popular microorganisms in East Asia region, since it was used for fermented soybean (tempeh) production. Tempeh has been a favorite food and staple source of protein, and is now rapidly becoming more popular all over the world as people look for ways to increase their intake of soybeans. However, the effect of *R. oligosporus* on nutritional and rheological properties of soybean and buckwheat is not yet well understood. The author focused on characterization and application of fermented soybean (FeS) and buckwheat (FeB) as new functional ingredients for food processing.

Characteristics and Functional Properties of Fermented Soybean and Its Application for Bread-making

Fermented soybean (tempeh) was prepared from boiled soybean by inoculation with powdered *R. oligosporus*. The total amount of free amino acids increased to the level of 3-10 folds, and it showed positive correlation with protease activity during fermentation in tempeh. The essential amino acids of FeS were the highest at 72 hr of incubation, except for threonine which was variable. Lysine fluctuated, which had lower amount at 48 hr. Semi-essential amino acid, histidine was variable. Non-essential

amino acid, such as alanine, cysteine, proline and serine gradually increased more than 10 times at 72 hr of fermentation. Other amino acids, such as asparagine, glutamine, ornithine, and γ-aminobutyric acid (GABA) fluctuated, but they showed higher amount at 72 hr of fermentation, except that GABA was higher amount at 24 hr.

Phytic acid decreased by approximately 76% during fermentation, and indicated that mold produced extra cellular hydrolytic enzymes (phytase). Phytase degraded phytic acid to inositol phosphate and free phosphate. *R. oligosporus* decreased the level of lipid and increased free fatty acids, showing that the lipase activity had negative correlation with lipid contents in FeS. Cytoplasm of FeS at 24 hr became longer and thinner than that of the control because of the degradation of cell. It became irregular after 48 and 72 hr of fermentation. Perhaps, *R. oligosporus* also degraded some components of cell, including lipid, proteins, and carbohydrates.

Texture property showed that weakness, modulus of elasticity, and surrender value of 48 hr of FeS were significantly higher than those of other fermentation times. The 24 hr of FeS showed more soft texture than that of 48 hr of fermentation. Mycelium was overgrown and bound strongly among soybean grains into a solid after 48 hr of fermentation. Incubation at 30°C for 24 hr was enough to allow spore germination and abundant growth of mycelium; more than 24 hr, the color of spore resulted in the grey or black and affected the color properties of tempeh. The color values (lightness and hue) of FeS developed from light to dark during fermentation, indicating that the growth of the mold had a distinct effect on L^* values and chromaticity coordinates (a^* and b^*). When the ratio of matured mold became higher than immatured one, the development of flour color gradually glimmered.

The loaves baked with the addition of FeS became smaller than the control, along with the increase in the amount of substitution and loaf volume clearly decreased. The staleness of bread with FeS substitution for wheat flour was clearly retarded compared with that without FeS substitution during storage.

General Characteristics and Hypoallergenic Properties of Fermented Buckwheat and the Application for Noodle-making

Boiled buckwheat for 30 min was inoculated with *R. oligosporus* at 30°C and 85% relative humidity. The bioavailability of mineral of FeB increased during fermentation, showing that the amounts of K, P, Mg and Mn in the homogenates distinctly increased

during the time course of fermentation. The Na, Cu, Zn, and Fe increased at 24 hr of fermentation and then decreased after fermentation for 48 hr or more. In contrast, Ca and Cu decreased during the time course of fermentation.

Buckwheat fermented by *R. oligosporus* increased total and free amino acids during the course of fermentation to the level of 50-fold or more than those of the control without fermentation. Non-essential amino acids, especially alanine and asparagin, rapidly increased with the course of fermentation, suggesting that glutamic acid was catalyzed to form alanine and asparagin by amino transferase. Also, GABA increased during the growth of mold, showing that GABA synthesis caused to decrease the amount of glutamic acid.

Western blot analysis showed that the 22 kDa protein was a major allergenic protein of Mancan buckwheat. It appeared in the control, but disappeared during the time course of fermentation. Allergenic proteins of buckwheat completely degraded to low molecular weight proteins after 24 hr of fermentation and protease activity showed positive correlation with the total amount of free amino acids.

SEM images of buckwheat grains after fermentation showed that the control buckwheat grain without treatment distinctly contained many raw starch granules with clearly round shape. After boiling, the round shape of raw starch granules was broken and the surface was somewhat viscous caused by gelatinized starch. However, after 48 hr of fermentation, the buckwheat images became irregular and typical image of gelatinized cereal products, where starch granules could not be distinguished. After 72 hr of fermentation, *R. oligosporus* caused buckwheat images to become big holes like vacuole in the grains. During fermentation, buckwheat structure was apparently more swollen and gelatinized than the cooked samples without fermentation.

The raw noodle made from 1CW alone (control) showed the presence of starch granules (large and small sizes), but the surface between gluten and starch were clearly observed and the raw starch granules showed smooth appearance. Noodle substituted with 30 %-FeB flour had somewhat different appearance as compared with the control, and contained swollen starch granules that were buried in viscous gluten sheet. The phenomenon was not observed for 1CW noodle but distinguished only in the FeB-substituted noodle sample.

The noodle made from 20 and 30 %-FeB substitution for wheat flour showed significantly higher elasticity than that of the control. The cutting property of noodle

substituted with 30 %-FeB flour was significantly softer than that of the control. Uncooked and cooked noodles made from 10, 20 and 30 %-FeB flour substitution showed significantly different color values from the control. The *L** value of the uncooked control noodle was significantly higher than those of uncooked noodles made from FeB substitution flour. In addition, the control had significantly lower values of *a** and *b** than the noodle substituted with FeB. The substituted noodles had higher hue (*a**, *b**) values than the control.

Conclusion

R. oligosporus affected physical and functional properties of the fermented soybean and buckwheat, resulting in degradation of protein to peptides and essential amino acids, and change of texture of soybean and buckwheat. Proteins and lipids in soybean were utilized to the metabolizing process for growth of mold. Generally, *R. oligosporus* increased the functional properties of soybean and buckwheat, including amino acids and minerals, and degraded the allergenic proteins.

The FeS contained a large amount of total proteins, minerals and amino acids. *R. oligosporus* decreased the phytic acid and increased the availability of minerals. The application of FeS resulted in different rheological properties for bread making, which the bread became smaller and more hardness.

The FeB contained larger amounts of amino acids, peptides and minerals, and lowered the amount of allergenic proteins, especially 22 kDa protein. Especially, fermented-buckwheat helps in the development of a new hypoallergenic foodstuff. *R. oligosporus* is one of the safety microbe to develop new food ingredients with higher functional property. Therefore, the application of fermented buckwheat also resulted in new noodles and available for human suffering from buckwheat allergenic protein.

審査結果の要旨

Rhizopus oligosporus（*R. oligosporus*）は発酵大豆、テンペの製造に使用されるため東アジア地帯では一般的な微生物の一つである。このテンペは主要な蛋白質の給源となるため、大豆のよさを知りその摂取を考えている人々には良く好まれている食品であり更にポピュラーになってきている。然しながら *R. oligosporus* の大豆と特に蕎麦の栄養学的、レ

オロジー学的な性質におよぼす効果については未だ十分に研究がなされていない。本研究はこのような見地から食品加工への新しい機能性素材としての発酵大豆(FeS)と発酵ソバ(FeB)の特性とその利用について検討した。

その内容は以下のように要約される。

北海道産のツルノコ大豆を水に浸漬、水切り、煮沸、水切り後、*R. oligosporus* 粉末を接種して30℃で培養を行い、FeSを調製した。FeSの遊離アミノ酸は培養期間中に3〜10倍に増加したが、この増加はテンペの発酵中のタンパク質分解酵素の活性と良い相関を示した。FeS中の必須アミノ酸はスレオニンを除き培養72時間で最も高い値を示した。なお、リジンは変動しており48時間では低い値を示した。また、準必須アミノ酸のヒスチジンは72時間で約10倍に増加した。その他のアスパラギン、グルタミン酸もほぼ同様な傾向を示したがγ-アミノ酪酸は 24 時間で最も高い値を示した。金属とキレート作用をするフィチン酸は約 76%が減少したことよりフィターゼの存在を明らかにし、イノシトール燐酸、遊離のリンが放出されることを明らかにした。また、リパーゼ等の酵素活性の増加と共に大豆細胞は24時間で細長くなり、48、72時間で不規則な形をとり細胞質の崩壊と共に脂質、蛋白質、糖質等の内容物が分解されたことを明らかにした。

FeSの弾性係数、弱化度などの粘弾性は48時間の培養で有意に高くなったが、胞子形成は24時間の培養で十分でテンペの外観の明度、彩度が変化し灰色に変わってきた。このFeSを小麦粉の 10%、30%代替することにより製パンをおこなうと代替量の増加に伴い比容積は減少したが、その保存性は明らかに改善することが分かった。

次に本菌を煮沸した玄蕎麦に接種して大豆の場合と同様に培養をおこない FeB を調製した。FeB中の全遊離アミノ酸は無発酵の対照に比べ約50倍に増加したが、中でもアラニン、アスパラギンが増加しており、この増加はGABAの生成とも関連することを示した。FeB中の蛋白質についてはウエスタンブロッテングの結果から蕎麦の主要なアレルゲンとして知られている22kDaの蛋白質が消失することを明らかにした。特に24時間の発酵後には蕎麦のアレルゲン蛋白質は完全に分解されていることが明らかになった。発酵をしていないコントロールの蕎麦穀粒の走査型電子顕微鏡写真では明らかに数多くの生澱粉粒の存在が認められたが、発酵後の蕎麦穀粒では澱粉粒が破壊され、その表面は澱粉の糊化により粘性を帯び丸くなり、大きな穴もみとめられた。

FeB の粉末を小麦粉に代替してソバ麺を調製して、対照のカナダ産の硬質粉の１CW を用いた場合の走査型電子顕微鏡写真と比較すると、１CW では大小サイズの澱粉粒の存在が認められ、グルテンと澱粉粒の表面が明確で、かつ澱粉の表面がはっきりと見られた。一方、30%のFeB粉末を代替して調製した麺では膨潤した澱粉粒が粘性をおびたグルテンシート中に埋もれていた。これはFeBで代替した粉でのみ認められ、１CWから調製した麺では認められなかった現象である。また、FeB を 20%、30%代替して調製した麺では明らかに高い粘

弾性を示し、30%の FeB を代替した麺の破断特性はコントロールのものよりも明らかに柔らかになった。また、FeB を 10%、 20%、30% 代替して、その茹で麺、生麺を比較すると明らかに異なった色調を示した。生麺の明度は茹で麺の明度に比べ明らかに高い値を示したが、彩度 a*、b*については生麺が茹で麺に比べ明らかに低い値を示した。

以上、本研究では *R. oligosporus* を用いて大豆発酵によるテンペと蕎麦に応用したソバテンペを調製しその特性を検討した。大豆、蕎麦に *R .oligosporus* を接種して培養することにより遊離アミノ酸、遊離脂肪酸の増加、GABA 等の機能性物質の増加、フィチン酸の減少などの効果が認められることを明らかにした。特に蕎麦においては主要なアレルゲン蛋白質である 22 kD a の蛋白質が消失することを明らかにした。発酵大豆を製パンに利用すると比容積は大きくはならないものの保存性が改良され、また発酵蕎麦テンペを麺に利用すると高い粘弾性を示すことより新しい食品素材として利用されることを明らかにした。このように発酵によりえられる蕎麦テンペのそば麺への利用は従来とは異なるそばの製造を示唆したものであり、同時にソバアレルギーを持つ人にも利用されるものであることを明らかにした。

以上の成果は、食品化学、食品製造学、食品物理学、食品化工学の分野に大きく貢献するものであり、本論文の審査並びに、最終試験の結果と併せて、博士（農学）の学位を授与することを適当と認める。