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Effect of Enzymes on the Degree of Maceration of Soybean Fermented by *Rhizopus* Strains

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Relationship between the degree of maceration (given in n-value) of fermented soybean and macerating enzyme formation was observed on raw and sterilized soybeans fermented for 12, 24, 36 and 48 h at 30°C with 7 *Rhizopus* strains. Among 7 *Rhizopus* strains tested, *Rhizopus oligosporus* (TISTR3001, known as a dominant tempeh processing strain) gave the most powerful maceration degree (n-value in the equation, $F=C(\Delta\varepsilon)^n$) of n= 1.4 (initial value of 1.8) and n = 1.1 (initial value of 1.7) for raw and sterilized soybeans respectively, for 48 h of fermentation Enzyme activities such as cellulase, pectinase, amylase and protease, markedly related to the degree of maceration. Among the enzyme formation, particularly both pectinase and cellulase gave the powerful maceration effect on the soybean fermented with *Rhizopus* since the formation of both enzymes coincided with the decrease of n-value.

INTRODUCTION

Several *Rhizopus* species are of considerable interest in the food and enzyme industry. They have been used in solid-state fermentation for several centuries, particularly in Asia for preparing many fermented foods such as tempeh from soybean (Cook, 1982; Hesseltine, 1965; Nout and Rombouts, 1990; Steinkraus, 1986). During fermentation, the initially hard tissue of the soybean converts to soft tissue by the maceration effect of fungal growth. The mycelium of *Rhixopus* can invade the intercellular lamella material and can solubilize it by extracellular enzymes (Nout and Rombouts, 1990). In spite of importance of the degree of maceration (softness or hardness) as an important factor for the quality of fermented soybean such as digestivity, texture and flavor, a few investigations (Jurus and Sundberg, 1976; Tsen et al., 1985) have reported regarding the degree of maceration of soybean during the fermentation in the past.

This paper deals with the relationship between the degree'of maceration and the change in enzyme formation during soybean fermentation by various *Rhixopus* strains.

MATERIALS AND METHODS

Microorganisms

Seven *Rhixopus* strains, i.e., four authentic *Rhizopus* strains (*javanicus* (IFO5442), *oligosporus* (TISTR3001), *arrhizus* (TISTR3247), sp. (UQM186F)) and three *Rhixopus* isolates (sp. All and sp. F98 from fermented foods, sp. LKN from tempeh starter) were used in this study.

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Soybean

Dehulled soybean (product of USA) was purchased from Kyuto Bussan Co. Ltd. (Fukuoka, Japan).

Preparation of fermented soybean

Raw and sterilized soybeans used as substrate were prepared and inoculated with desired *Rhixopus* strains by the method of Manurukchinakorn and Fujio (1997). Fermentation was carried out at 30 ˚C for 12, 24, 36 and 48 h.

Measurement of the degree of maceration

In accordance with Manurukchinakorn and Fujio (1997), the degree of maceration was determined by force-deformation test on bulk soybean specimen with a rheometer (R-UDJ-DM, Sun Kagaku Co. Ltd., Tokyo, Japan).

Preparation of enzyme solution

Enzyme formed was extracted by adding 100 ml of distilled water to 50g of the fermented soybean. The mixture was crushed and mixed thoroughly before placing in the cold room at 4 ˚C for 12 h. The enzyme solution was obtained after squeezing of the crushed mixture mentioned above by using cheese-cloth and centrifugation at 12500xg for 20 min.

Determination of enzyme activities

Enzyme activity was determined toward protease, amylase, cellulase and pectinase activities. For protease, reaction mixture was composed of 0.5 ml crude enzyme solution and 2.5 ml of haemoglobin solution (2% haemoglobin in acetate buffer 0.05 M, pH 4.5). The reaction was done by incubation at 40°C for 20min and the reaction was stopped by the addition of 4 ml of 5% trichloroacetic acid. After removal of the undigested haemoglobin by filtration, the acid soluble products were determined with a spectrophotometer at 280nm. Protease activity was defined as the amount of enzyme solution which changed the reading at 280 nm, equivalent to 1 μmole tyrosine per mm at 40 "C. For amylase, reaction mixture was composed of 0.1 ml of enzyme solution, 1 .O ml of 2% soluble starch solution and l.Oml acetate buffer (0.05 M, pH 5.0). The reaction was done by incubation at 40 ˚C for 10 min. One unit of amylase activity was defined as 1 μmole reducing sugar (as glucose) released per min per ml of enzyme solution. For cellulase, reaction mixture was composed of 0.5 ml enzyme solution, l.Oml of 0.5% carboxymethylcellulose solution and 1.0 ml of citrate buffer (0.05 M, pH 5.5). The reaction was done by incubation at 40 ˚C for 20 min. One unit of cellulase activity was defined as 1 μmole reducing sugar (as glucose) released per min per ml of enzyme solution. For pectinase (as polygalacturonase), reaction mixture was composed of 0.2 ml enzyme solution, 1 .O ml of 1% pectic acid solution and 1 .O ml of phosphate buffer (0.05 M, pH 7.0). The reaction was done by incubation at 40°C for 15min. The reducing sugar was determined by DNS method (Miller, 1959). One unit of pectinase activity was defined as 1 μmole reducing sugar (as α-galacturonic acid) released per min per ml of enzyme solution.

RESULTS

Time course of the degree of maceration of fermented soybean

The degree of maceration (Manurukchinakorn and Fujio, 1997) has been defined as the n-value in the equation, $F=C(\Delta\varepsilon)^n$, where F is force; C is constant and $\Delta\varepsilon$ is porosity change, by arrangement of measured force-deformation data of bulk specimen of fermented soybean, The smaller n-value in the equation corresponds to softer tissue.

Table 1 summarizes the n-values of raw and sterilized soybeans fermented by 7 strains of *Rhixopus*. The degree of maceration for raw and sterilized soybeans gave in initial n-value of 1.8 and 1.7, respectively. During fermentation, the tissue of fermented soybean became softer as shown by a decrease of n-value. The various n-values in Table 1 might be caused by different maceration abilities or growth rates of *Rhizopus* strains used. A tempeh strain, *Rhixopus oligosporus* (TISTR3001), gave the smallest n-value of 1.4 and 1.1 for raw and sterilized soybeans fermented at 30 ˚C for 48 h, respectively. The *Rhizopus* sp. LKN also gave the smallest n-value of 1.1 for sterilized soybean fermented at 30 ˚C for 48 h.

Table 1. *Summary* of n-values from the logarithmic plots of force-porosity change.

n-value	raw soybean/sterilized soybean				
Fermentation time (h)	0	12	24	36	48
Rhizopus sp.LKN	1.8/1.7	1.8/1.7	1.8/1.5	1.7/1.5	1.6/1.1
Rhizopus sp.A11	1.8/1.7	1.8/1.7	1.8/1.7	1.8/1.7	1.7/1.6
Rhizopus sp.F98	1.8/1.7	1.8/1.7	1.8/1.5	1.7/1.4	1.7/1.4
Rhizopus sp.UQM186F	1.8/1.7	1.8/1.7	1.8/1.7	1.7/1.5	1.6/1.4
R. javanicusIF05442	1.8/1.7	1.8/1.7	1.8/1.6	1.7/1.6	1.7/1.5
*R. arrhizus*TISTR3247	1.8/1.7	1.8J1.7	1.8J1.7	1.7/1.7	1.7/1.6
*R. oligosporus*TISTR3001	1.8/1.7	1.8J1.7	1.8J1.6	1.7/1.6	1.4/1.1

Enzymatic changes in raw soybean

The enzymes secreted with *Rhixopus* growth during fermentation will act on soybean maceration process. Fig. 1 (a, b, c, d) shows changes of enzyme activities with fermentation time on raw soybean. The protease activity (Fig. l-a) varied markedly among 7 *Rhixopus* strains used. Fig. l-b shows change of amylase activity with fermentation time. The high amylase activity may be caused by the inherent amylase in the raw soybean. Fig. l-c and d show changes of cellulase and pectinase activities with fermentation time, respectively. At 48 h fermentation, *Rhixopus oligosporus* (TISTR3001) seemed to be the best producer of cellulase (0.38U) and pectinase (3.38U) on raw soybean among 7 *Rhixopus* strains tested.

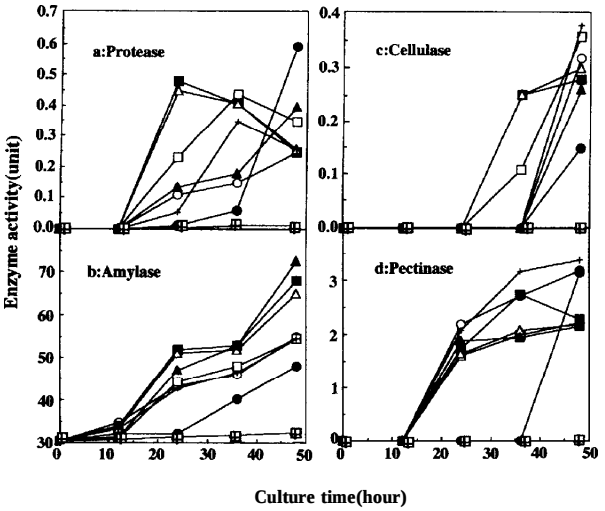

Fig. 1. Time courses of enzyme formation in the crude extract from raw soybean cultures of *Rhizopus* strains.
symbols: ❑ , control ○, *Rhizopus* sp. LKN
●, *Rhizopus* sp. Al 1 ■ , *Rhizopus* sp.F98
□, *Rhizopus* sp. UQM186F △, *Rhizopusjavanicus IF05442*
A, Rhizopus arrhizus TISTR3247 +, *Rhizopus oligosporus* TISTR3001

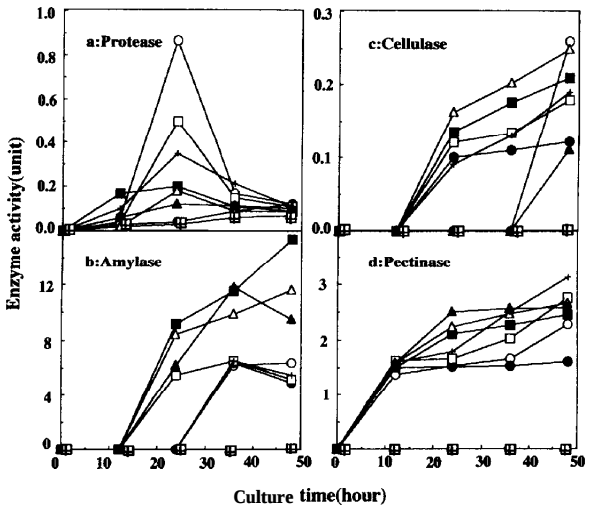

Fig. 2. Time courses of enzyme formation in the crude extract from sterilized soybean cultures of *Rhizopus* strains.
symbols: ❑ , control ○, *Rhizopus* sp. LKN
●, *Rhizopus* sp. All ■ , *Rhizopus* sp. F98
□, *Rhizopus* sp. UQM186F **A,** *Rhizopusjavanicus IF05442*
A, Rhizopus arrhizus TISTR3247 +, *Rhizopus oligosporus* TISTR3001

Enzymatic changes in sterilized soybean

Fig. 2 (a, b, c, d) shows the formation of enzymes on sterilized soybean with fermentation time. As shown in Fig. 2-a, LKN, UQM186F and TISTR3001 produced big amount of protease and the activities reached to the maximum at 24 h, then the steep decrease was observed. Fig. 2-b shows time courses of amylase formation which differ among strains tested. Fig. 2-c and d show time courses of cellulase and pectinase activities which increase with increase of fermentation time. At 48 h fermentation, *Rhixopus* **sp.** LKN seemed to be the best producer of cellulase (0.26U) while *Rhixopus oligosporus* (TISTR3001) was the best producer of pectinase (3.13U).

Comparing the enzyme formation on sterilized soybean with that on raw soybean, cellulase, pectinase and protease were formed at an earlier stage of fermentation time. The results suggest that *Rhixopus* strains tested can grow faster on sterilized soybean than raw soybean.

Relationship between the degree of maceration and enzymes formed

Fig. 3 shows the relationship between the degree of maceration (n-value) and cellulase and pectinase formation on soybean fermented by *Rhizopus oligosporus* (TISTR3001). During the fermentation, n-values of raw and sterilized soybeans decreased from 1.8 to 1.4 and 1.7 to 1.1, respectively. As shown in Fig. 3, the increase of cellulase and pectinase activities seemed to coincide with the decrease of n-values in both raw and sterilized soybeans. Despite sterilization or not, cellulase and pectinase are presumed to be the key enzymes in soybean maceration with *Rhixopus* growth.

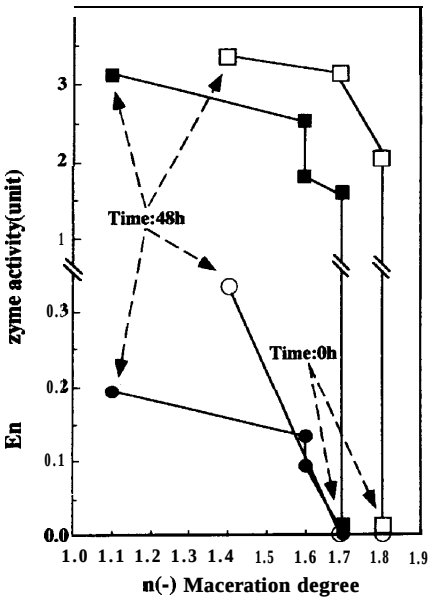

Fig. 3. Relationship between the degree of maceration and enzyme activity of soybean fermented by *Rhizopus oligosporus* TISTR3001.
Symbols: ●, cellulase ; ■ , pectinase: sterilized soybean
○, cellulase; □, pectinase: raw soybean

DISCUSSION

Soybean (*Glycine max* (L.) Merr.) mainly composed of protein, lipid, carbohydrate, etc. is normally considered as oil seeds in western countries while in oriental countries it is considered as edible seeds. Particularly, soybean is still provided as popular food supplying protein source for human in Asian countries such as China and Japan. One of soybean processing methods is fermentation with selected microorganisms (Tempeh in Indonesia with *Rhizopus* strain; Natto in Thailand, Nepal, Japan, etc. with Bacillus *natto*). Cotyledon of soybean becomes softer by fermentation and the resultant texture is desirable simultaneously with the elimination of mal odor from soybean (Whitaker, 1978). The softness of soybean foods is one of the quality limiting factors of foods. Nakamura et al. (1995) have revealed that enzymatic maceration of vegetables might be initiated with protopectinases action on partial depolymerization of the middle lamella. Thereafter, the plant material transformed into suspension of loose cells with shear force. To know the degree of soybean maceration with *Rhizopus*, a measure for softness or hardness was introduced by force-deformation relationship of bulk soybean (Manurukchinakorn and Fujio 1997). This measure has been defined as a power value, n (a measure for softness), in an experimental equation, $F=C(\Delta\varepsilon)^n$ (where F is force; C is constant and $\Delta\varepsilon$ is porosity change). The maceration may follow the enzymes formed with fermentation and the enzyme activities may depend on the microorganisms and the potential of their enzyme formation. Within the limit of present experiment using *Rhixopus* strains, *Rhixopus oligosporus* TISTR3001 and sp. LKN showed the best capability for soybean maceration. This result might be caused by using two strains belonging to tempeh processing strain selected for soybean fermentation from of old. The enzymes related with maceration are presumed to be pectinase and cellulase. Pectinase and cellulase digest pectic substance and cellulose containing in soybean cotyledon as cementing material, respectively. The high amylase activity (Fig. l-b) in the control of raw soybean at the beginning of fermentation may be caused by the inherent enzymes in soybean. Raw soybean contains abundant α- and pamylase (Ofelt et al., 1955; Learmonth and Wood, 1960; Pomeranz and Mamaril, 1964). Both amylases in raw soybean might have been activated while soaking raw soybean in 3% lactic acid solution at 30 ˚C for 3 h before inoculation.

REFERENCES

Cook, J. H. 1982 Cassava: a basic energy source in the tropics. *Science,* 218: 755-762

Hesseltine, C. W. 1965 A millennium of fungi, food and fermentation. *Mycologia,* 57: 149-197

Jurus, A. M. and W. J. Sundberg 1976 Penetration of *Rhizopus oligosporus* into soybeans in tempeh. *Appl. and Envir. Microbiol., 32: 284-287*

Learmonth, E. M. and J. C. Woos 1960 The influence of soya flour in read doughs. IV. Alpha-amylase of soya. *Cereal Chemistry, 37:* 158-169

Manurukchinakorn, S. and Y. Fujio 1997 Development of method for measuring the degree of maceration of soybean fermented with *Rhizopus J. Fac. Agr., Kyushu Univ., 41:* 105-l 15

Miller, G. L. 1959 Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analyt. Chemistry, 39: 426-428*

Nakamura, T., A. R. Hours and T. Sakai 1995 Enzymatic maceration of vegetables with protopectinases. *J. Food Science, 60: 468-472*

Nout, M. J. R. and F. M. Rombouts 1990 Recent developments in tempeh research. *J. Appl. Bacteriol.*, *69: 609-633*

Ofelt, C. W., A. K. Smith and J. M. Mills 1955 Effect of soy flour on amylograms. *Cereal Chemistry, 32: 48-52*

Pomeranz, Y. and F. P. Mamaril 1964 Effect of *N*-ethylmaleimide on the starch liquefying enzymes from soy flour. *Nature, 203: 863-864*

Steinkraus, K. H. 1986 Fermented foods, feeds, and beverages. *Biotechnol. Advances, 4: 219-243*

Tsen, H. Y., T. S. Lai and C. F. Hwang 1985 The changes of enzymatic activities and biochemical constituents during the fermentation of defatted soybean meal with *Rhizopus thailandensis*. II. Different fermentation conditions and the comparision of biochemical and rheological properties. *J. Chin. Agr. Chem. Soc., 23: 300-307*

Whitaker, R. J. 1978 Biochemical changes occuring during the fermentation of high-protein foods. *Food Technol., 32: 175-180*