Food Sci. Technol. Res., **8** (1), 70–74, 2002

Method for Measuring the Degree of Maceration of Fermented Soybean

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Received September 3, 2001; Accepted November 30, 2001

The force-deformation curve of bulk soybean fermented with *Rhizopus* could be described by the equation, $F=C\cdot(\Delta\varepsilon)^n$. The degree of maceration of fermented soybean (a measure of softness) was estimated by the *n*-value of the power of the equation and depended on the *Rhizopus* strain used. Of the *Rhizopus* strains tested, *R. oligosporus* TISTR3001 (well known as a dominant tempeh processing species) and *Rhizopus* sp. LKN (isolated from a tempeh starter) gave high degrees of maceration corresponding to n=1.3 (initial value of 1.8) and 1.6 (initial value of 1.8) respectively, for 60 h of fermentation of raw soybeans at 30°C. On the other hand, the *R. oligosporus* TISTR3001 and *Rhizopus* sp. LKN for sterilized soybeans decreased to *n*-values of 1.5 from initial value of 1.7 and to 1.3 from initial value of 1.7, respectively, for 60 h of fermentation at 30°C. *n*-Values less than 1.5 were considered to indicate a considerably high degrees of maceration.

Keywords: degree of maceration, soybean, fermentation, *Rhizopus*

The relationships of mechanical (physical) properties of force-deformation (-compaction) and the biochemical properties of food materials have been reported on processed foods (Okadome *et al*., 1996; Sugiyama *et al*., 1998; Segnini *et al*., 1999: Horiuchi & Sugiyama, 1999; Agblor & Scanlon, 2000, vegetables (Lu *et al*., 2000; Komai *et al*., 1998), fruits (Duprat *et al*., 1997; Martinez-Navarrete & Chiralt, 1999), meat (Cierch & Majewska, 1997), and polysaccharide gels (Benzion & Nussinovitch, 1997; Yoshiyama *et al*., 1999). Some researchers (Peleg, 1997; Lu & Chen, 1998; Mcullecnct & Gross, 1999; Hubert & Poursartip, 2001) have developed measurement methods of rheological properties for biological materials and their mathematical analysis.

Legumes and grains fermented with microorganisms have improved their nutritional values and digestibility as human food (Steinkraus, 1986; Hachmeister & Fung, 1993; Fudiyansyah *et al*., 1995). Some typical examples are tempeh (Indonesian traditional fermented soybean (Hesseltine, 1965; Steinkraus, 1983)) and natto (oriental fermented soybean (Hesseltine & Wang, 1967; Yokotsuka, 1985)). They are popular soybean food products fermented with *Rhizopus oligosporus* and *Bacillus subtilis* (natto), respectively. Boiled soybean (dehulled) as raw material is processed by fermentation with a suitable fungi or bacillus. During fermentation, the initially hard tissue of the soybean converts to soft tissue by the maceration effects of microbial growth. As a result, the digestibility and nutritional values of the soybean are improved with fermentation (Wang & Hesseltine, 1981; Nout & Rombouts, 1990; Bisping *et al*., 1993; Rehms & Barz, 1995; Yamanishi *et al*., 1995).

Since hardness or softness of fermented soybean food is important in ascertaining their physical and chemical properties during and after fermentation, the degree of maceration (degree of softness) of the fermented soybean is an important factor in food quality, particularly in texture.

With regard to the degree of maceration of various solid foods, methods presented so far for determining this parameter have been based on the weight loss of fruit or vegetable pieces to be macerated (Zetelaki-Horváth, 1974), sieving (Sreenath *et al*., 1984) or centrifugal separation (Zetelaki-Horváth & Urbányi, 1978) of fragments, or the absorption of the liquefied fruit and vegetable samples into filter paper discs (Tantchev *et al*., 1990). Generally, the hardness or softness of the solid specimen is believed to be given in the equation, $F=C(D)^n$ in force F and deformation D relationship where C is constant and n is the deviation from linearity (Peleg & Campanella, 1989; Roy *et al*., 1989). However, most specimens tested were solid foods (a block of food) and to date there has been almost no research on a measurement method for bulk food (gathering of small granules) such as fermented soybeans reported.

This paper was sought to establish a novel method for measuring the degree of maceration of bulk soybeans fermented with *Rhizopus* strains.

Materials and Methods

Soybean Dehulled soybean (product of the USA) purchased from Kyuto Bussan Co. Ltd. (Fukuoka) was used as raw material in the fermentation.

Microorganisms Seven *Rhizopus* strains, namely, four authentic strains (*R. javanicus* IFO5442, *R. oligosporus* TISTR-3001, *R. arrhizus* TISTR3247, *R.* sp. UQM186F) and three *Rhizopus* isolates (*R.* sp. A11 and *R.* sp. F98 from fermented foods, *R.* sp. LKN from tempeh starter) were used for fermentation.

Preparation of fermented soybean Fifty grams of soybeans was soaked in a 3% lactic acid solution for 3 h at 30°C in a 500 ml Erlenmeyer flask with a cotton plug. After draining completely, this was used as the raw soybean specimen (moisture

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content was 60%, wet basis). The sterilized soybean specimen was prepared by autoclaving the soaked raw soybeans at 121°C for 15 min. The *Rhizopus* inocula were precultured in test tubes (PDA medium) for 7 days at 30°C. Slants of the available *Rhizopus* strains were each added with 3 ml of sterile water and then mixed thoroughly. Inoculation of *Rhizopus* into both the raw and sterilized soybeans was done by transferring the suspensions including spores and mycelium fragments. Fermentation was carried out at 30°C for 24, 36 and 60 h. The control soybean specimen was prepared following the same procedures without inoculation.

Equipment A rheometer (R-UDJ-DM, Sun Kagaku Co. Ltd., Tokyo) was used to measure the relationship between force and displacement. The modified measuring-cell (Fig. 1), made of acrylic resin, consisted of a cylindrical container (3 cm in inner diameter 4 cm outer diameter, and 10 cm in height, cross-sectional area=7.07 cm^2) and a plunger (2.8 cm in diameter and 2 cm in height, cross-sectional area=6.16 cm^2). The bulk soybean (fermented or unfermented) specimen was first packed in the container to a height of 3 cm (13 g, wet weight), unless stated otherwise, and compressed by the plunger at a constant speed of 2 cm per min up to a maximum force of 1 kg against the plunger. The force exerted by the plunger and the resultant plunger displacement were recorded (Rika Denki Co., Ltd., Tokyo) as the force-displacement for the sample soybeans. Since the effect of the measuring device on measured values did not avoid to present measuring cell, measured values were described as intact values and then applied directly to compaction analysis.

Twenty replicated measurements were conducted on raw and sterilized specimens, which were not inoculated, to estimate error due to measuring techniques. Errors caused by differences in height of the packed specimen were estimated for packing heights of 3, 5, 6 and 7 cm. Data obtained were statistically analyzed for standard deviation at a 95% confidence limit.

The moisture content of the raw and sterilized specimens was determined by drying soybeans at 105°C for 24 h. Density of the soybean material was determined by putting 20 g of specimen in 60 ml of salad oil and measuring the resultant volume increase. The porosity of the packed specimen was calculated from the soybean volume and the bulk volume of the packed soybeans. Initial porosity of the packed soybeans was 0.45 for all specimens.

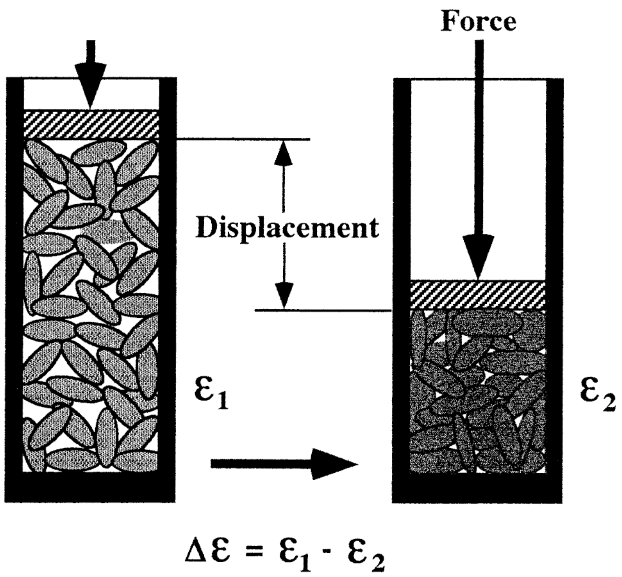

Fig. 1. Force-displacement measuring-cell. $\Delta\varepsilon$, the porosity change.

Results

Force-compaction curve Figure 2 (A, for raw soybean; B, for sterilized soybean) shows two typical force-displacement curves. Though the curves obtained from different specimens varied in shape, they generally exhibited common characteristics, some of which can be associated with descriptive textural parameters. There was an initial curved region as the soybean was packed into the measuring-cell, followed by a more linear region as the specimen was compressed further.

Reliability of data obtained Tables 1 and 2 summarize the effect of the packing height of the bulk soybean specimens on the measurement error. All values in Tables 1 and 2 were calculated from values from the starting position (at plunger position=0, displacement=0) to the final position (at a maximum force of 1

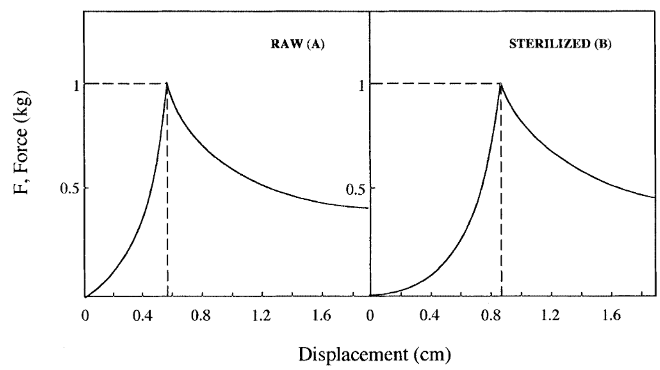

Fig. 2. Typical force-displacement curves obtained by a maximum force of 1 kg on the top surface of bulk soybean specimens. A, raw soybeans without fermentation; B, sterilized soybeans without fermentation.

Table 1. The error caused by difference in packing height of raw soybeans.

Sample amount (cm(g))	Maximum displacement[a] (cm)	Standard Deviation (cm)
3(13)	0.508±0.013	0.027
5(20)	1.195±0.029	0.065
6(25)	0.869±0.028	0.060
7(30)	0.881±0.019	0.060

[a]value at 95% confidence limit of the average of 20 replicates.

Table 2. The error caused by difference in packing height of sterilized soybeans.

Sample amount (cm(g))	Maximum displacement[a] (cm)	Standard Deviation (cm)
3(13)	0.625±0.016	0.034
5(20)	1.118±0.036	0.076
6(25)	1.087±0.021	0.060
7(30)	0.933±0.026	0.057

[a]value at 95% confidence limit of the average of 20 replicates.

kg applied by the plunger used). The standard deviation of 20 replicated measurements at 95% confidence limit ($p<0.05$) was lowest for a packing height of 3 cm and this height was used in all subsequent tests. The higher standard deviations for higher packing heights could be due to greater lateral-pressure dispersion on the vertical sides of the measuring cell. Reproducibility of the displacement values obtained for a packing height of 3 cm is shown in Tables 3 and 4 for raw and sterilized soybeans fermented for 48 h, respectively.

Degree of maceration of fermented soybean The relationship between the force (F) and the displacement (D) of solid specimen is commonly described by the equation, $F=C(D)^n$. Assuming that the displacement (D) for the bed of a solid specimen can be replaced by the change in porosity ($\Delta\varepsilon$) for a bulk specimen, the relationship between the exerted force and the change in porosity of the latter may conform to the equation:

$$F=C\cdot(\Delta\varepsilon)^n, \quad (1)$$

where F: loading force (kg), C: arbitrary coefficient (kg), $\Delta\varepsilon$: changes in porosity from the initial value (–), n: power coefficient (–).

Equation (1) can be transformed to

$$\log F=\log C+n\cdot\log \Delta\varepsilon. \quad (2)$$

A plot of $\log F$ versus $\log\Delta\varepsilon$ in equation (2) should yield a straight line if this equation is applicable. Figures 3 and 4 show typical logarithmic plots of force (F) and porosity change ($\Delta\varepsilon$) for *R. oligosporus* TISTR3001 and *Rhizopus* sp. LKN fermentation, respectively. The straight lines are recognized. Thus, equation (1) was applicable to the force-deformation relationship of the bulk specimen. Table 5 summarizes the n- and C-values obtained from the slope of each line and the intercept on the y-axis, respectively. The n-value may be characterized as the degree of maceration depending on the *Rhizopus* strain tested. Small n-values were interpreted as indicating high maceration effects by fermentation with the *Rhizopus* tested, and the variation in these values (Table 5) may be due to by differences in the maceration

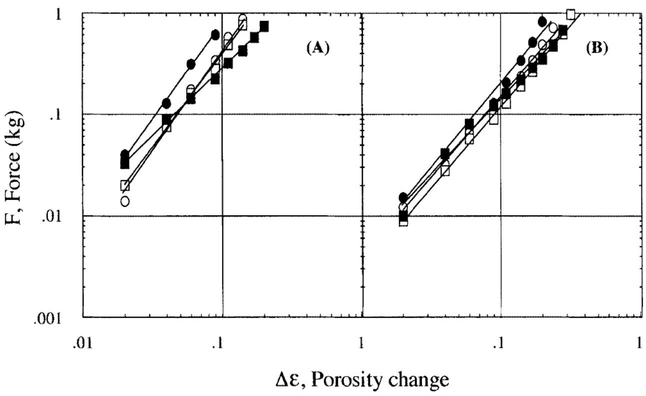

Fig. 3. Typical relationship between force and porosity change of raw (A) and sterilized (B) soybeans fermented with *Rhizopus oligosporus* TISTR-3001 for different fermentation durations.
●, control; ○, 24 h-fermentation; □, 36 h-fermentation; ■, 60 h-fermentation.

Table 3. The maximum displacement in force-displacement curves of raw soybeans fermented with 7 strains of *Rhizopus* for 48 h.

Strain	Displacement[a] (cm)	Standard Deviation (cm)
Control	0.559±0.012	0.026
Rhizopus javanicus IFO5442	0.653±0.010	0.022
Rhizopus arrhizus TISTR3247	0.583±0.013	0.029
Rhizopus sp. A11	0.653±0.013	0.027
Rhizopus sp. F98	0.712±0.025	0.054
Rhizopus sp. UQM186F	0.740±0.021	0.044
Rhizopus sp. LKN	0.779±0.019	0.040
Rhizopus oligosporus TISTR3001	0.779±0.022	0.048

[a]value at 95% confidence limit of the average of 20 replicates.

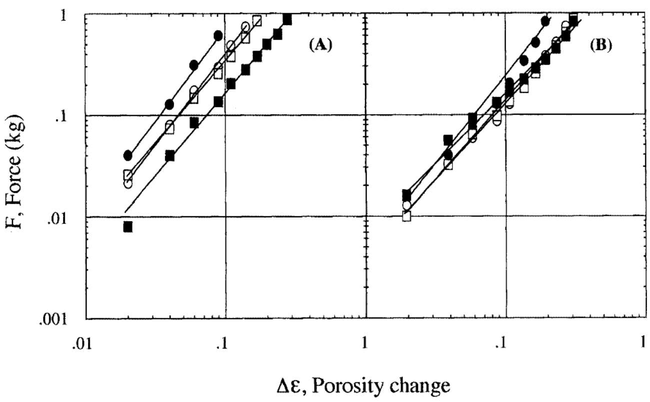

Fig. 4. Typical relationship between force and porosity change of raw (A) and sterilized (B) soybeans fermented with *Rhizopus* sp. LKN for different fermentation durations.
Symbols are the same as those in Fig. 3.

Table 4. The maximum displacement in force-displacement curves of sterilized soybeans fermented with 7 strains of *Rhizopus* for 48 h.

Strain	Displacement[a] (cm)	Standard Deviation (cm)
Control	0.553±0.013	0.028
Rhizopus javanicus IFO5442	0.766±0.025	0.054
Rhizopus arrhizus TISTR3247	0.663±0.015	0.032
Rhizopus sp. A11	0.741±0.019	0.042
Rhizopus sp. F98	0.875±0.014	0.029
Rhizopus sp. UQM186F	0.930±0.025	0.053
Rhizopus sp. LKN	0.962±0.022	0.048
Rhizopus oligosporus TISTR3001	0.963±0.024	0.051

[a]value at 95% confidence limit of the average of 20 replicates.

Table 5. Summary of the n- and C-values obtained from the transformed curves of some *Rhizopus* strains used in this study.

strain	*Rhizopus* sp. LKN				*Rhizopus oligosporus* TISTR3001				*Rhizopus* sp. A11				*Rhizopus javanicus* IFO5442			
time(h)	0	24	36	60	0	24	36	60	0	24	36	60	0	24	36	60
raw																
n-value	1.8	1.8	1.7	1.6	1.8	1.8	1.7	1.3	1.8	1.8	1.8	1.8	1.8	1.8	1.7	1.7
C-value	50	25	8	13	50	25	50	6	50	50	40	40	50	40	25	13
sterilized																
n-value	1.7	1.5	1.5	1.3	1.7	1.6	1.6	1.5	1.7	1.7	1.7	1.7	1.7	1.6	1.6	1.6
C-value	10	4	4	3	10	6	5	4	10	10	8	7	10	10	5	4

capabilities of the different tested strains. *Rhizopus* strains with small *n*-values may secrete soybean macerating enzymes during fermentation, which may soften soybean tissue. Consequently, the *n*-value may be an effective measure of soybean maceration, samples with n>1.7 considered hard; n=1.5, medium soft; and n<1.5, soft. The *C*-value, at the intercept value on the y-axis, depends on the starting point which is an unstable measuring region, and this value is not significant in the relationship describing the degree of maceration in equation (1).

Discussion

Hardness or softness of a solid specimen has been defined as the force necessary to attain a given deformation (Szczesniak *et al*., 1963). Friedman *et al*. (1963) developed their texturometer (General Food Texturometer) and the hardness was defined as the ratio of height of a test specimen to a standard specimen. To compare the same texturometer with the Instron texturometer (Bourne, 1968), Brennan *et al*. (1970) also reported on the textural properties of rubbers. Using the same texturometer, Kapsalis *et al*. (1970) determined the textural properties of pre-cooked, freeze dried beef and of special bite-size space foods in relation to their moisture content. Their definition of the hardness for a solid specimen basically coincides with our definition of the degree of maceration, except for the bulk specimens tested. In a compressible solid-body, it is generally believed that the force-deformation relationship is characterized by the equation, $F=C(D)^n$ (Peleg & Campanella, 1989; Roy *et al*., 1989). This power-law equation is widely employed to measureing the softness of a solid specimen. A study by Moresi and Spinosi (1984) assessed of this textural property. They suggested that the rheological behavior of grape must follow a power-law relationship, which depends on the soluble-solid content.

Based on the power-law equation, we replaced the displacement term D with porosity change $\Delta\varepsilon$ in packing height for a bulk specimen and modified the equation to $F=C\bullet(\Delta\varepsilon)^n$. We determined this property by measuring the displacement of the bulk fermented soybean in the force-compaction curve at an arbitrarily selected maximum force. The maximum force of 1 kg selected in our study was such that the soybean specimen underwent significant displacement in specimen-height in the measuring-cell. The present modified equation is applicable to bulk specimen and the *n*-value can be interpreted as the degree of maceration of fermented bulk soybeans since this value depends on the maceration capability of *Rhizopus* strains tested. Peleg and Campanella (1989) reported on some experimental results concerning various soft solid-foods using a compression test (by Instron UTM model 1000) in the process of the theoretical derivation for mechanical sensitivity of soft solid-foods. They discussed the "*n*" value concerning only the concavity of the force-deformation pattern. In the case of our bulk specimen, a series of tests conducted on the soybean fermented by 7 varieties of *Rhizopus* strains revealed that the "*n*" value, which is the degree of maceration, might represent the softness of fermented soybean depending on the *Rhizopus* strain tested. A smaller "*n*" value means softer bulk of the soybean spacimen. The physical meaning of "*n*" in Eq. (1) corresponds to the overall order of compaction rate, depending on the softness (or hardness) of the soybean spacimen. Thus, the *n*-value is the gross compaction (or deformation) rate of a mass of food particles.

Acknowledgment We wish to thank to Thailand Institute of Scientific and Technological Research (TISTR) for donating authentic *Rhizopus* cultures.

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