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Effect of NaCl on textural changes and protein and lipid degradation during the ripening stage of sufu, a Chinese fermented soybean food

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Abstract: Sufu is made by solid state fungal fermentation (using *Actinomucor elegans*) of tofu, followed by salting and maturation in dressing mixtures containing salt, alcohol and various other ingredients. NaCl in dressing mixtures strongly affected the changes in textural properties and the hydrolysis of protein and lipid of sufu. Higher salt contents (14% w/w) resulted in increased hardness (+100%) and elasticity (+18%) and reduced adhesiveness (−30%). Hardness and elasticity could be used to judge the extent of sufu ripening. SDS-PAGE showed the disappearance of all protein subunits at 80 and 110 g kg^{-1} salt content; however, some protein subunits were still detectable at 140 g kg^{-1} salt content after 60 days of ripening. Higher ratios of free amino nitrogen to total nitrogen (FAN/TN = 0.4–0.45) and free amino acids to crude protein (FAA/CP = 0.24–0.26) were observed in sufu with lower (80 g kg^{-1}) salt content. FAN/TN and FAA/CP in white sufu (obtained with dressing mixtures containing only salt and alcohol) were higher than those in red sufu (obtained with dressing mixtures containing angkak or kojic red rice) owing to different dressing mixture compositions. Increases in free fatty acids (FFA) were also observed during ripening. FFA levels in sufu with lower salt content increased rapidly during the first 30–40 days and then increased slowly, probably resulting from the formation of fatty acid esters. Lowering the salt content (80 g kg^{-1}) can shorten the ripening time to 40 days, which is of benefit to manufacturers. However, sufu will spoil, ie undergo souring, during the ripening stage at salt contents of 50 g kg^{-1} or lower.

Keywords: sufu; NaCl; texture; protein and lipid degradation; ripening

INTRODUCTION

Sufu (*fu-ru* written in hieroglyphics) is a traditional Chinese fermented soybean curd and a highly flavoured, soft creamy cheese-type product which can be used in the same way as cheese.[1,2] This fermented product with a relatively high protein and lipid content has been widely consumed by Chinese people as an appetiser for many centuries.

There are many different types of sufu produced by various local processes in China,[3] with mould-fermented sufu being the most popular type of product.[4] Four steps are normally involved in making this type of sufu: (1) preparing tofu (soybean curd), (2) preparing pehtze (*pizi*) by solid state fungal fermentation of tofu using eg *Actinomucor elegans*, (3) salting of pehtze and (4) ripening in dressing mixture.[5] Hydrolysis of protein and lipid occurs mainly during the ripening stage, which usually takes 3–6 months. Traditionally, the ripening stage took over 6 months, since the salt content in some sufu exceeded 140 g kg^{-1}. Presently, the salt content in most products, especially red sufu, is still >100 g kg^{-1},[6] which results in ripening periods of 3 months or longer. A reduction in salt content would have the combined advantages of shortening the ripening period and reducing dietary sodium intake. Hardness and smoothness were identified as important factors influencing consumer acceptability of tofu.[7] These textural properties are also important factors influencing consumer acceptability of sufu and could also be used as parameters for judging the extent of ripening of sufu during its production.

Like in other fermented foods such as cheese[8] and miso,[9] salt has a multiple role in sufu. During the salting period the pehtze absorbs the salt and free water until the salt content of pehtze reaches an equilibrium level. The absorbed salt will later impart a salty taste to the sufu, but it will control the microbial growth and enzyme activity in sufu as well. In addition,

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salt influences physical and biochemical changes in the product. Not surprisingly, the salt concentration in sufu is considered one of the most important factors affecting its quality. However, little quantitative information on its effect on the hydrolysis of protein (the major component of tofu) and lipid is available.

Chou and Hwan[10] investigated the effect of ethanol in dressing mixtures on some biochemical changes in white sufu during ripening and showed that the added alcohol delayed the degradation of soybean proteins. In the present study the effect of salt on such biochemical changes as hydrolysis of protein and lipid during the ripening of red and white sufu prepared with *A elegans* was investigated.

MATERIALS AND METHODS

Micro-organism

Actinomucor elegans (Academia Sinica AS 3.227) is commonly used as a starter in commercial sufu production in China. Starting from an agar slant culture, a pure culture inoculum of *A elegans* AS 3.227 was prepared by liquid substrate culture in Roux bottles, as is common practice in Chinese sufu factories. The medium consisted of soy whey (by-product from tofu manufacture) to which maltose (20–30 g kg^{-1}) and peptone (15–20 g kg^{-1}) were added prior to sterilisation by autoclaving. After incubation at 28 °C for 72 h, medium and biomass were harvested and homogenised to obtain a spore suspension containing $\sim 10^5$ CFU ml^{-1}.

Sufu preparation

The tofu used as raw material for sufu was provided by Beijing WangZhiHe sufu manufacturer (China) and was cut into pieces (3.2 × 3.2 × 1.6 cm). The pieces were inoculated with *A elegans* by spraying the spore suspension onto their surfaces. The inoculated tofu pieces were placed, evenly spaced, in plastic trays. The loaded trays were piled up in an incubation room with controlled temperature (around 25 °C), relative humidity (around 90%) and air circulation to ensure adequate aeration. Fresh pehtze, ie tofu overgrown with *A elegans* mycelium, was obtained after incubation for 48 h.

The pehtze was transferred to a container (20 l), and salt was spread between the layers of pehtze as they piled up in the container. During a period of 5 days the pehtze absorbed the salt until the salt content of pehtze reached about 140–150 g kg^{-1}.

For the ripening of sufu, 12 pieces of salted pehtze (about 200 g fresh weight) were placed in individual wide-mouthed glass bottles with a capacity of 340 ml, after which dressing mixture (about 140 ml) was added to the pehtze. In order to reach the required final salt level, salt was also added to some dressing mixtures to obtain sufu with 110 and 140 g kg^{-1} salt content. For red sufu the dressing mixture consisted of angkak or kojic red rice[4], alcoholic beverage (rice wine)[11] to a final alcohol content of 50 g kg^{-1}, sugar, chiang (wheat-based miso[12]) and spices. For white sufu the dressing mixture consisted only of alcoholic beverage (final alcohol content 50 g kg^{-1}). The filled bottles were closed and incubated at 25–28 °C for 80 days.

Sampling for analysis

Two bottles were drawn randomly from each batch on each sampling day during the ripening period. The dressing mixture was decanted and the sufu pieces were tested.

Determination of textural properties

Pieces of tofu, pehtze and sufu were tested in triplicate. Texture profile analysis (TPA) of sufu was performed with a rheometer (NRM 2002J, Fudo Kabushiki Kaishia, Tokyo, Japan). A cylindrical plunger of 8 mm diameter with a weight beam of 2 kg was used. The plunger travelled to 75% depth of the sufu sample. The speeds of the crosshead and the recording chart were set at 60 and 100 mm min^{-1} respectively. Textural parameters, ie hardness (firmness), elasticity (springiness) and adhesiveness, of sufu were calculated from the curve according to Bourne.[13]

Hardness was defined as the force required to compress sufu, measured as the maximum height of the curve during the first compression. Elasticity was defined as the extent to which sufu returned to its original shape after decompression and was expressed as the horizontal distance between the point where the second curve started and the point where the second curve reached its peak. Adhesiveness was defined as the negative force area following the first compression, representing the work necessary to pull the compressing plunger away from the sufu sample.

Biochemical analyses

Sufu pieces were homogenised using a mortar and pestle prior to analysis in duplicate. Contents of total nitrogen, free amino nitrogen and crude lipid in each sample were analysed according to the Kjeldahl method, the formol titration method and the Soxhlet extraction method respectively as described by Nielsen.[14]

Free fatty acid (FFA) content was determined according to Pike.[15] To the extracted sufu lipid sample, neutralised 95% ethanol and phenolphthalein indicator were added. The sample then was titrated with NaOH and the per cent FFA was calculated as oleic acid.

Determination of total free amino acid content was performed with a modified method according to Niven *et al*.[16] The lyophilised sample homogenates were dissolved in sulphosalicylic acid and the supernatants were applied to the amino acid analyser (Hitachi 835-50, Tokyo, Japan) for determination of total free amino acids.

SDS-PAGE profile

SDS-PAGE (sodium dodecyl sulphate polyacrylamide gel electrophoresis) was performed using the PhastSystem from Amersham Pharmacia Biotech AB (Uppsala, Sweden).[17] The markers used were those of the LMW calibration kit that contained proteins with 14.4, 20.1, 30, 43, 67 and 94 KDa molecular weights. Samples were mixed with sample buffer at a concentration of 6 mg lyophilised sample powder ml^{-1}. The prepared samples and markers were then heated at 100 °C for 5 min. The SDS-PAGE was performed on PhastGel™ Gradient 8–25% using SDS buffer strips. The heated samples were applied using 8/1 (eight wells, each 1 µl) sample applicator/comb. The separation and visualisation were performed according to the manufacturer's manual.

RESULTS AND DISCUSSION

Properties of tofu, pehtze and salted pehtze

Table 1 presents the textural properties and chemical parameters of tofu, pehtze and salted pehtze, the intermediate products of sufu prior to the ripening procedure. Considerable differences can be observed between tofu, pehtze and especially salted pehtze, since the added salt has a great impact on the composition of the dry matter.

Chemical parameters of sufu during ripening

During the experiments, since the sufu was ripened in closed bottles, the moisture contents of sufu remained stable at 640, 610 and 590 g kg^{-1} for red sufu and 710, 670 and 640 g kg^{-1} for white sufu containing 80, 110 and 140 g kg^{-1} salt respectively. pH values decreased slightly from 6.6–6.8 after 10 days to 5.9–6.1 after 80 days of ripening.

Sufu with 50 g kg^{-1} salt content was also included in the experiments. However, this sufu was spoiled after 20 days, as evidenced by its low pH (<4.6) and off-flavour. This suggests that, for stability, sufu should have >50 g kg^{-1} salt content during ripening.

Changes in textural properties

Results of the TPA of red and white sufu with various salt contents are shown in Fig 1. All starting points were at 10 days instead of 0 days, because salted pehtze without dressing mixture cannot be compared with ripening sufu. Sufu at the ripening age of 10 days was considered to be a representative starting sample for this study. The hardness, elasticity and adhesiveness of red sufu were slightly higher than those of white sufu at the same salt content; this is probably due to the different dressing mixture compositions for red and white sufu. The salt levels in sufu greatly affected the textural changes. Higher salt contents resulted in higher values for hardness and elasticity, with lower adhesiveness. The hardness of sufu with 80 g kg^{-1}

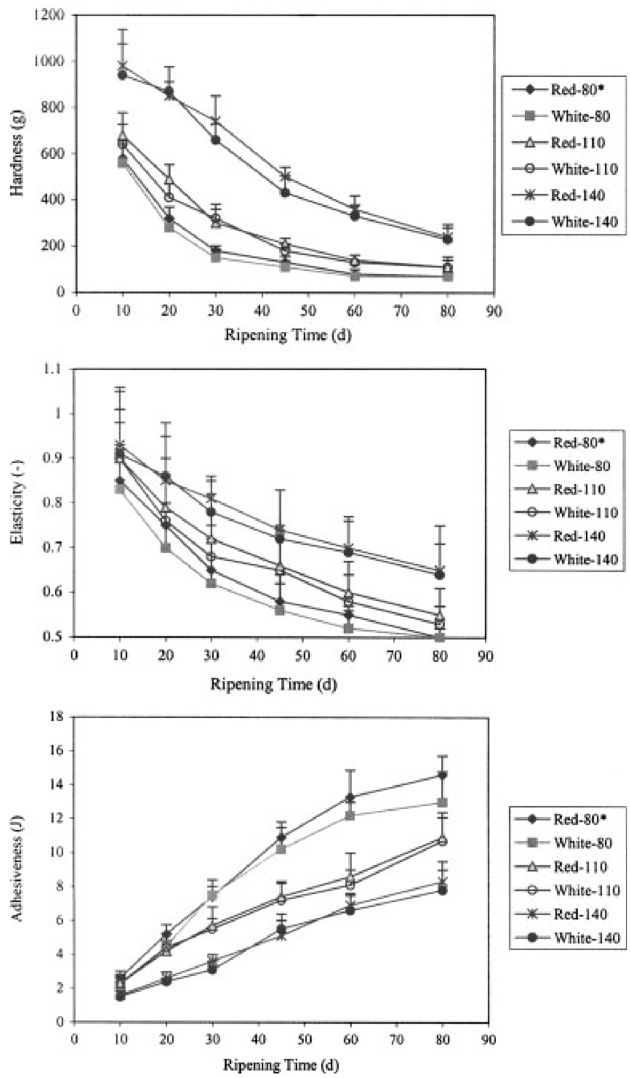

Figure 1. Texture profile analysis of sufu during ripening. Rheometer, probe diameter 8 mm. Triplicate analyses of duplicate samples. Error bars indicate standard deviation. *Red sufu with 80 g kg^{-1} total NaCl content, etc.

Table 1. Textural properties and chemical parameters of tofu, pehtze and salted pehtze

Sample	Hardness (g)	Elasticity	Adhesiveness (J)	pH	FAN ($mM\,g^{-1}$)[a]	Moisture ($g\,kg^{-1}$)[a]	Crude protein ($g\,kg^{-1}$)[b]	FAA ($g\,kg^{-1}$)[b]	Crude lipid ($g\,kg^{-1}$)[b]	FFA ($g\,kg^{-1}$)[c]
Tofu	560 ± 63[d]	1 ± 0	0.1 ± 0	6.9 ± 0.21	0.04 ± 0.002	743 ± 42	631 ± 45	1.3 ± 0.1	301 ± 18	37 ± 1.6
Pehtze	860 ± 55	1 ± 0	0.2 ± 0.03	7.0 ± 0.35	0.36 ± 0.02	703 ± 37	628 ± 38	18 ± 1.1	308 ± 21	64 ± 2.9
Salted pehtze	1380 ± 103	1 ± 0	3.8 ± 0.21	7.0 ± 0.27	0.26 ± 0.01	563 ± 41	375 ± 26	11 ± 0.9	184 ± 12	59 ± 3.3

[a] Fresh weight basis.
[b] Dry matter basis.
[c] Of crude lipid.
[d] Average ± standard deviation (triplicate analyses of duplicate samples).
FAN, free amino nitrogen; FAA, free amino acids; FFA, free fatty acids.

salt decreased rapidly from ~600 to ~100 g within 45 days, followed by a slow decrease. Even at the lowest ($80\,g\,kg^{-1}$) salt content, elasticity values did not decrease below 0.5.

TPA is widely used to evaluate the textural properties of tofu.[7,18] However, no published data refer to the textural properties of sufu. In the absence of comparative standards and based on limited subjective evaluations by sufu-manufacturing experts, we propose that sufu may be considered ready for packing and distribution when its hardness and elasticity have decreased to around 100 g and 0.55 respectively.

Degradation of protein during sufu ripening

Proteolysis is the principal and most complex biochemical event that occurs during the maturation of most cheese varieties.[19] Sufu is a cheese-like product, and proteolysis plays an equally important role during its ripening.

SDS-PAGE can easily separate major proteins and has been applied to monitor the hydrolysis of cheese[20] and fermented soybean.[17] In Fig 2 the SDS-PAGE profiles are shown for salted pehtze and subsequent red and white sufu with different salt contents at various ripening stages. The major protein subunits can be clearly identified in salted pehtze (lane 2) and in sufu containing $140\,g\,kg^{-1}$ salt after 10 days of ripening (lanes 5 and 8). Nevertheless, after 10 days, most protein subunits had been degraded to a large extent in sufu containing $80\,g\,kg^{-1}$ salt (lanes 3 and 6) and $110\,g\,kg^{-1}$ salt (lanes 4 and 7). All protein subunits had disappeared in sufu (lanes 3 and 6) containing $80\,g\,kg^{-1}$ salt at the age of 60 days. Some protein subunits were still visible in sufu (lanes 5 and 8) with $140\,g\,kg^{-1}$ salt content at the same age.

The increase in free amino nitrogen gives a further indication of the hydrolysis of protein. Fig 3 shows the change in the ratio of free amino nitrogen to total nitrogen (FAN/TN) during sufu ripening. FAN/TN in white sufu was higher than that in red sufu with the same salt content, probably owing to different dressing mixture compositions and microflora present.[6] FAN/TN in white sufu with $80\,g\,kg^{-1}$ salt content reached approximately 0.45 after 80 days, ie about two times higher than that in sufu containing $140\,g\,kg^{-1}$ salt.

Ultimately, the degradation of protein in sufu leads to the liberation of free amino acids, which are considered to be important flavour-enhancing compounds in many fermented foods. In addition, volatile compounds formed from amino acids by decarboxylation, deamination, transamination and other transformations can make substantial contributions to sufu flavour. This is a major reason for the many different volatile compounds[21,22] encountered in sufu.

The ratio of free amino acids to crude protein (FAA/CP) is shown in Fig 4 as an indicator of protein degradation. FAA/CP in white sufu was larger than that in red sufu with the same salt content, which was similar to the corresponding FAN/TN. FAA/CP in white sufu with lower salt content ($80\,g\,kg^{-1}$) exceeded 0.25, and that in sufu with $140\,g\,kg^{-1}$ salt was less than 0.15 after 80 days.

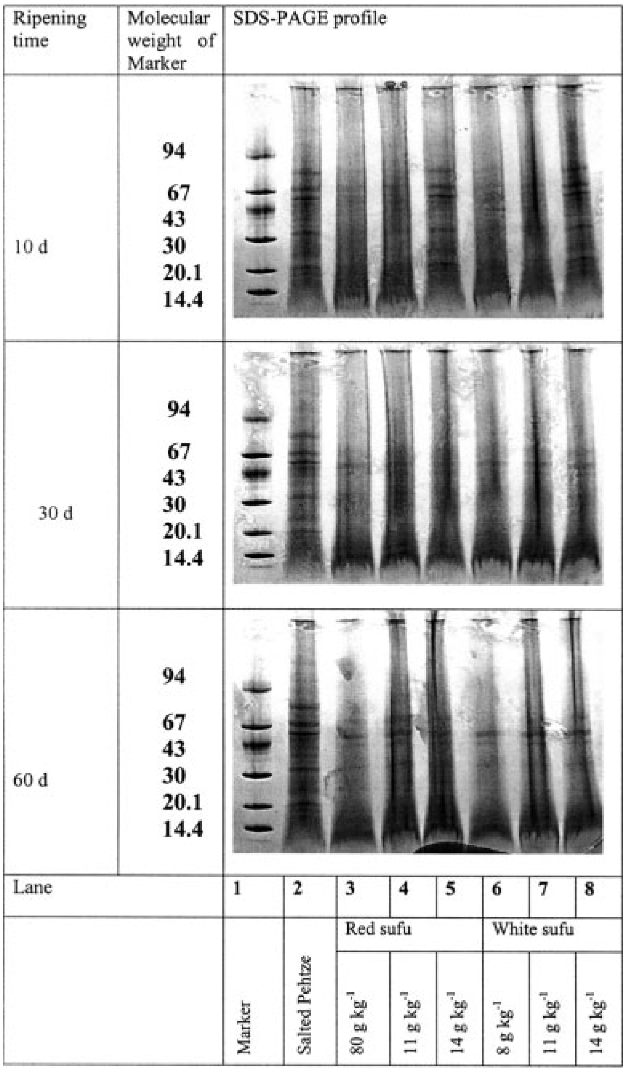

Figure 2. SDS-PAGE profiles of red and white sufu with different salt contents during ripening stage.

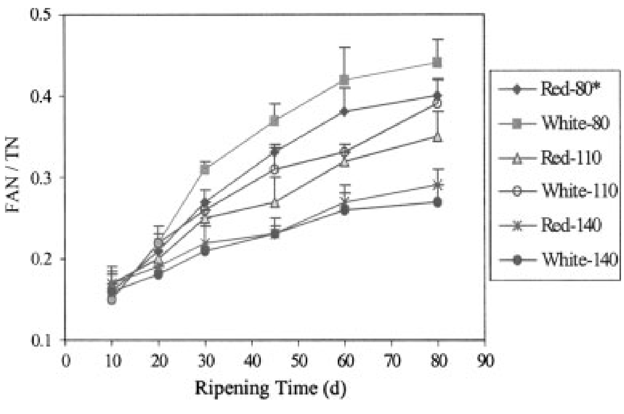

Figure 3. Ratio of free amino nitrogen to total nitrogen (FAN/TN) during sufu ripening as an indicator of protein degradation. Average and standard deviation (bars) of duplicate analyses of duplicate samples. *Red sufu with $80\,g\,kg^{-1}$ total NaCl content, etc.

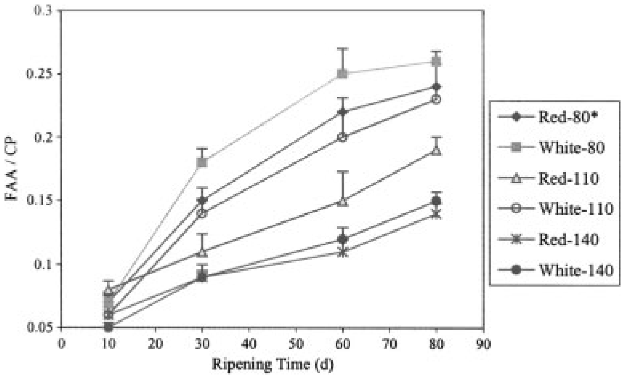

Figure 4. Ratio of free amino acids to crude protein (FAA/CP) during sufu ripening as an indicator of protein degradation Average and standard deviation (bars) of duplicate analyses of duplicate samples. *Red sufu with 80 g kg^{-1} total NaCl content, etc.

Comparing FAN/TN with FAA/CP in sufu, FAN/TN was much higher than FAA/CP. As an example, taking into account the absence of protein bands in SDS-PAGE, data for white sufu with 80 g kg^{-1} salt after 80 days indicate that approximately 27% of N occurs as monomeric amino acids and the remaining N occurs as peptides and amino acid degradation products.

Lipolysis during sufu ripening

Lipids represent the second largest class of compounds in sufu after proteins. Crude lipids decreased slightly (<20 of 184 g kg^{-1} in salted pehtze) during sufu ripening, regardless of sufu type (red or white) or salt content. Fungal lipases can decompose crude lipid into free fatty acids and di- and monoglycerides.

The changes in free fatty acid content expressed as fraction of crude lipid during sufu ripening are presented in Fig 5. Obviously, salt retarded the hydrolysis of lipids, resulting in a higher free fatty acid content in sufu containing less salt. The free fatty acid content in sufu containing 80 and 110 g kg^{-1} salt increased rapidly during the first 40 days of ripening, after which it continued to increase slowly. This trend probably results from the formation of fatty acid esters

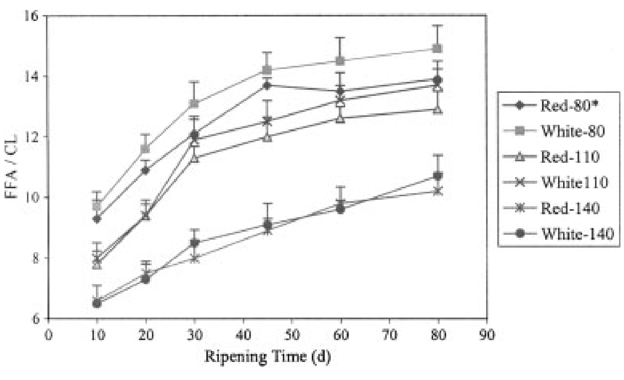

Figure 5. Ratio of free fatty acids to crude lipid (FFA/CL) during sufu ripening as an indicator of lipid degradation. Average and standard deviation (bars) of duplicate analyses of duplicate samples. *Red sufu with 80 g kg^{-1} total NaCl content, etc.

from free fatty acids and alcohol present in the dressing mixtures.[23]

Chou and Hwan[10] reported that the free fatty acid content in sufu ripened in brine containing ethanol increased during the first 30 days, then decreased as ripening progressed further. We still did not observe a decrease after 80 days even though 50 g kg^{-1} alcohol was present in the dressing mixtures we used.

CONCLUSIONS

Salt inhibits the enzymic ripening processes, resulting in the hydrolysis of protein and lipid. Lower salt content in sufu resulted in reduced hardness and elasticity and a greater extent of degradation of protein and lipid. The ripening time of sufu can be shortened by reducing the salt content, which is of benefit to manufacturers. However, sufu will spoil during the ripening stage at a salt content of 50 g kg^{-1} or below.

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