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Studies upon the *Actinomucor elegans* proteolytic activity during solid-state fermentation in obtaining "sufu"

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Abstract

In this paper we studied the role of solid-state fermentation due to Actinomucor elegans in obtaining sufu, a soybean food that is becoming popular in chinese dishes all over the world. Fungal proteases act upon tofu's proteins and protein hydrolysis provide the principal compounds of the mild, characteristic flavor of sufu. We studied the protein content evolution consecutive to fermentation and ripening processes and correlate our findings with a sensory analysis of resulted sufu.

Keywords: *Actinomucor elegans*, sufu, proteolytic activity

1. Introduction

Sufu is one of the most popular fermented soybean foods in China, and is becoming popular in Chinese shops all over the world. It is made by fungal solid-state fermentation of tofu (a vegetal cheese-like product) followed by salting and ripening in dressing mixture. Several types of sufu can be distinguished according to processing method or colour and flavour. [1, 2, 3]

Development of aromas through fermentation is a major characteristic of fermented soybean foods [4]. Also, raw soybeans contain significant levels of anti-nutritional factors, such as phytates, which are removed or destroyed (in majority) during soaking, cooking and fermentation of the soybeans [5]. Soybean fermentation has shown to improve the bioavailability of dietary zinc and iron and can result in increased levels of vitamins [6,7]. Therefore, soybean fermentation is an interesting process to study, bringing a lot of improvements in respect of sensory quality and nutritional value of the end-product, influencing product's flavor and texture and also its digestibility, bio-availability and detoxification function.

Fungal enzymes, produced during fermentation, act upon their respective substrates, and it is likely that hydrolysis of protein and lipid provide the principal compounds of the mild, characteristic flavor of sufu.

That is why we studied the protein content evolution consecutive to fermentation and ripening processes and correlate our findings with a sensory analysis of resulted sufu.

2. Materials and Method

We used two different tofu products, one of them obtained in our laboratory and the other was a commercial product (from Inedit S.R.L.).

To obtain tofu samples in the laboratory we washed soybeans and soaked overnight in water, and then ground into a slurry using a blender. The slurry was diluted and pressed to obtain soymilk. Coagulation was achieved by acid addition and the precipitate was pressed to remove excess water (soy whey) with cheesecloth bags using wooden planks. Finally, a soft but firm cake-like tofu resulted, which was then cut into cubes of desired sizes.

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A pure culture inoculum of *Actinomucor elegans* was prepared, starting from agar slant culture, by solid substrate culture in roux bottles. The medium used for solid substrate culture consists of bran and water (1:1.2~1.4). The spore suspension (~10^5 CFU/ml) was harvested and inoculated on the surfaces of both commercially and laboratory produced tofu, with manually operated sprayers.

The inoculated tofu was incubated at 25°C, with 96% relative humidity and a good aeration was provided. Thin yellow-white mycelia appeared on the surface of tofu samples after 20 hours and thicken after 48 hours. Moulded tofu was transferred to vessels containing a saturated alcoholic salt solution (consisting of 10% NaCl and 6% ethanol), and after 10 days at room temperature the salt content reaches over 10% and the moisture content decreased by 10-12%. Final levels of samples moisture content varied in the range 50-65% (w/w).

In order to evaluate the proteins level before and after fermentation and ripening we used Lowry's method, a widely-used protein assay. Under alkaline conditions, copper complexes with protein. When Folin phenol reagent (phospho-molybdic-phosphotungstic reagent) is added, the Folin-phenol reagent binds to the protein. Bound reagent is slowly reduced and changes color from yellow to blue. After 30 minutes absorbancy is read in a spectrophotometer at 660 nm. We used bovine serum albumin (BSA) as a standard [8].

We weighted 1 g from each sample (tofu and sufu) and diluted them in distilled water, obtaining 100 ml extract, homogenized for 30 minutes. From each extract we took 0.25 ml and filled with distilled water up to 0.5 ml. Then, we prepared the BSA standard solution (5 mg/5 ml) from which we took aliquots as shown in table 1. Both in extracts and in BSA solutions we added 5 ml copper alkaline reagent and 0.5 ml Folin reagent, and after incubating them at room temperature for 30 minutes we read absorbance at 660 nm.

The sensory analysis for obtained sufu was realized through a simple sensory analysis method, with 12 members' panel who evaluated the following features: form, color, consistency, flavor and taste, on a scale from 0 (not liked at all) to 5 (very good). We have calculated an average score for each sensorial feature and represented to obtain a graph. [9].

3. Results and Discussion

Absorbance values read for BSA standard curve obtaining and the standard curve resulted are shown in table 1 and figure 1, respectively.

Table 1. Absorbance values read for BSA standard curve obtaining

Dilution nr.	H_2O (ml)	BSA (mg)	Absorbance		A average	A average-A_{blank}
1	0,5	0	0,1006	0,1006	0,1006	0
2	0,475	0,025	0,1257	0,1533	0,1395	0,0389
3	0,45	0,05	0,1532	0,1739	0,16355	0,0629
4	0,425	0,075	0,1455	0,1378	0,14155	0,0409
5	0,4	0,1	0,1810	0,1824	0,1817	0,0811
6	0,35	0,15	0,2196	0,2196	0,2196	0,119
7	0,3	0,2	0,2484	0,2154	0,2319	0,1313
8	0,25	0,25	0,2526	0,2898	0,2712	0,1706
9	0	0,5	0,3271	0,4978	0,4125	0,3119

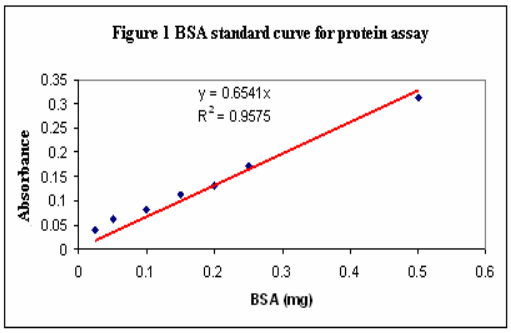


Figure 2. Laboratory obtained tofu

Table 2. Absorbance values read for tofu and sufu extracts

Sample	Extract (ml)	H_2O (ml)	Absorbance		$A_{average}$	$A_{average}$-A_{blank}
			A1	A2		
Blank	0	0,5	0,1006	0,1006	0,1006	-
Tofu (laboratory) 1	0,25	0,25	0,1347	0,1359	0,1353	0,0347
Tofu (laboratory) 2	0,25	0,25	0,1392	0,1358	0,1375	0,0369
Tofu (commercial) 3	0,25	0,25	0,1508	0,1488	0,1498	0,0492
Tofu (commercial) 4	0,25	0,25	0,1567	0,1467	0,1517	0,0511
Sufu from tofu 1	0,25	0,25	0,2443	0,2347	0,2395	0,1389
Sufu from tofu 2	0,25	0,25	0,2486	0,2358	0,2422	0,1416
Sufu from tofu 3	0,25	0,25	0,2548	0,2444	0,2496	0,1490
Sufu from tofu 4	0,25	0,25	0,2524	0,2412	0,2468	0,1462

Figure 3. Commercial tofu

Protein concentration calculation

From standard curve we have determinate protein concentration (mg) based on the absorbance values read at 660 nm.

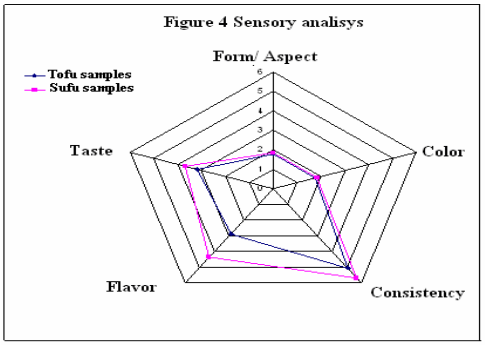
The obtained mathematical relation is:

$$y = 0.6541x$$; y = absorbance,
x = protein concentration (mg)

Results are gathered and shown in **Table 3**:

Sample extract	Protein concentration mg/100 ml extract	g/100g product
Tofu 1 laboratory	21,21	2,12
Tofu 2 laboratory	22,56	2,25
Tofu 3 commercial	30,08	3
Tofu 4 commercial	31,24	3,12
Sufu from tofu 1	84,94	8,49
Sufu from tofu 2	86,5	8,65
Sufu from tofu 3	91,11	9,11
Sufu from tofu 4	89,40	8,94

Apparently, the protein content for commercial tofu samples (3 and 4) is higher than laboratory obtained tofu protein content (samples 1 and 2), but that is only because of the higher humidity of the latter. All sufu samples were richer in protein than tofu samples they came from, even if we take into account the 10% loss of moisture. That fact, showed in Table 4, is explainable due to fungal protein accumulation during tofu samples solid state fermentation with *Actinomucor elegans,* and mycelium development. Some of those are structural proteins and some are functional proteins (enzymes). Although the mould grew only at the surface of tofu samples and the mycelium hardly penetrates into the tofu pieces, the brine enabled the enzymes to diffuse into the tofu pieces for substrate degradation. As a consequence of proteolytic and lipolytic activity of *Actinomucor elegans* are generated peptides, amino acids, amines, ammonia and other compounds responsible for the characteristic flavor of sufu.



Results obtained through sensory analysis show us that sufu has better sensorial properties than tofu. It is highly flavoured, compared to tofu which still has a touch of beanie flavour, has a creamy cheese-like consistency and a spicy taste. Tofu and sufu have different texture, due to important changes during fungal fermentation, which lead to a cake-like product (sufu) with a meat-like taste.

4. Conclusion

So, tofu and especially sufu are very good protein sources suitable for use as healthy, non-cholesterol, easy to prepare food from plant origin.

Sufu fermented from tofu is even easier to digest for children, elderly persons or ill persons because it represents also a good source of proteolytic enzymes. That is why it is recommended to be consumed as an appetizer and a side dish, especially with meat and rice.

Since tofu and sufu were well appreciated for their sensorial properties, are made from soybeans being nutritious protein food, we could recommend and consider them healthy food.

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