Solubilization of Soybean Tempeh Constituents During Fermentation[1]

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ABSTRACT

The solubility of tempeh solids in water reached 52%, and in 66% ethanol reached 28%, when followed during a 72-hr. fermentation period. The 66% ethanol-soluble components were mainly lipids, while the water-soluble components were rather evenly divided between crude protein, nitrogen-free extract (NFE), and lipids. At the end of the fermentation, two-thirds of the NFE and half the crude protein and fat had become water-soluble.

Tempeh, a popular food of Indonesia, consists of partially cooked soybeans overgrown with molds belonging to the genus *Rhizopus* (1,2). Steinkraus et al. (3) have reported an increase in soluble solids, soluble N, fiber, and pH during the tempeh process, as well as a decrease in reducing substances. Fatty acids were liberated (4) and lipids were metabolized by the mold. Further work is presented here dealing with the nature of the solubilized components.

MATERIALS AND METHODS

Tempeh was prepared by a pilot-plant procedure (5) from Clark-variety soybeans. Fermentation times of 24 to 36 hr. gave products typical of tempeh as used in Indonesia.

Analytical determinations were made on air-dried products ground in a Micro-Wiley mill to pass a 40-mesh screen. Previously described methods were used for pH, N, ammonia, and soluble solids (5), fiber (6), available lysine (7), and free fat and free fatty acids (4). Crude protein was derived by deducting the ammonia N from the total N, then multiplying by 5.7.

Bound fat remaining in ethyl ether-extracted material was obtained by an ethanol-chloroform (1:1, v./v.) treatment. Fatty materials in water and 66% ethanol were transferred to ethyl ether; the solvent was removed; and the residue was redissolved in ethyl ether, filtered, and the soluble fat measured in the filtrate as the weight of nonvolatile material remaining after evaporation of the ethyl ether.

RESULTS AND DISCUSSION

As fermentation progressed, there was an increase in fiber, nitrogen-free extract (NFE), ammonia, and free fatty acids (Table I). Bound fat had the same proportions of fatty acids as the free fat (4). Decreasing available lysine may be related to protein breakdowns, the rise in ammonia suggesting extensive deamination of amino acids. There may also have been nonenzymatic browning during fermentation and drying. These changes lead to a decreased nutritional value, as

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TABLE I. CHANGES IN TEMPEH COMPOSITION DURING FERMENTATION[a]

Constituents	Hours Fermented				
	0	12	24	36	72
Crude protein, %	43.2	42.4	41.5	40.6	41.6
Ammonia, %	0.05	0.22	0.40	0.47	0.59
Available lysine, g./16 g. N	4.06	3.70	3.74	3.64	3.4
Free fat[b], %	28.1	27.5	25.9	25.2	23.2
Bound fat[c], %	4.0	3.5	4.1	3.8	3.7
Fatty acid, % of total fat	2.2	18.5	19.3	19.1	29.4
Crude fiber, %	2.77	3.07	2.89	4.81	4.43
Nitrogen-free extract, %	21.1	23.1	24.3	24.1	24.3
pH	5.4	5.9	6.2	6.5	7.2
Moisture	0.8	0.2	0.9	1.0	2.2

[a]Analysis done on air-dried tempeh.

[b]Free fat was that extractable with ethyl ether.

[c]Bound fat was that not extractable with ethyl ether but extractable with ethanol-chloroform.

shown by Smith et al. (8) and Hackler et al. (9). Amines were detected in the 36- and 72-hr. samples by Feigl's method (10).

An examination of the soluble components of tempeh provides a more detailed picture of changes (Table II). Water dissolves many high-molecular-weight materials, as well as small molecules, but it is a poor solvent for lipids. Ethanol (66%) will not dissolve most high-molecular-weight plant materials, and it is a poor solvent for triglycerides, but it is a much better lipid solvent than water.

The proportion of water-soluble solids reached about half the total solids at the end of the fermentation. This material consisted of nearly equal parts of crude protein, fat, and NFE. A little less than a third of the solids—largely lipids—became soluble in 66% ethanol.

Water-soluble protein, quite low at zero time, rose rapidly during active mold growth, suggesting the production of proteolytic enzymes by the fungus (2). However, the disappearance of crude protein soluble in 66% ethanol shows that the

TABLE II. CHANGES IN SOLUBLE CONSTITUENTS OF TEMPEH DURING FERMENTATION[a]

Constituents	Extraction Liquid	Hours Fermented				
		0	12	24	36	72
Solids, %	Water	10.7	33	46	52	52
	66% Ethanol	9.5	19.2	22.3	23.3	28.6
Crude protein, %	Water	1.85	8.6	13.9	17.1	19.1
	66% Ethanol	0.44	0	0	0	0.7
Fat, %	Water	0.6	9.1	14.6	16.7	14.8
	66% Ethanol	1.4	16	21	20	23
Nitrogen-free extract	Water	8.2	15.1	17.1	17.7	17.5
	66% Ethanol	7.6	3.0	0.9	2.8	5.0

[a]Results expressed as % of total tempeh solids.

mold rapidly utilizes amino acids and low-molecular-weight peptides for its own growth. Thus the water-soluble nitrogen-containing materials at 24 hr. were probably intermediate-sized protein breakdown products. After growth ceased there was an opportunity for low-molecular-weight nitrogen compounds to accumulate.

The amount of water-soluble fat at zero hours equalled the quantity of free fatty acids. Since the pH of the tempeh started at 5.4 and rose as fermentation proceeded, one could assume that fatty acids were mainly in the carboxylate form and would appear in the water extract. Even so it was evident, especially at 24 and 36 hr., that a large part of the water-soluble fat consisted of additional components. Included would be mono- and diglycerides. The 66% ethanol-soluble lipid material constituted an even larger fraction of the total fat. The high hydrolysis rate of the first 24 hr. slows considerably later.

The rapid loss of 66% ethanol-soluble NFE represented the early utilization of the soybean sugars by the mold. Cooked soybeans contain about 3.5% sugar, largely sucrose and stachyose (11). In contrast, an increased water-soluble NFE was found after a brief incubation. The difference between the water-soluble NFE and the 66% ethanol-soluble NFE can be considered as water-soluble pectic and hemicellulosic-type material solubilized by mold enzymes (4). These NFE materials were converted to the soluble form early in the fermentation, leaving an NFE fraction resistant to digestion and remaining throughout the 72-hr. fermentation period. The solubilization of polysaccharide accounts for the softening effect of the mold on the partially cooked soybeans.

Others (8) have found that the loss of dry matter during fermentation was around 1 or 2%.

The tempeh-fermentation process brought about the solubilization of half or more of the soybean protein, fat, and NFE. Solubilization of proteins was accomplished without the development of bitter materials encountered in other cases of enzymatic proteolysis of soy protein (12). This is accompanied by the removal of stachyose and other complex sugars (7) thought to play a role in the flatulence phenomenon. These characteristics of the fermentation, together with softening of the soybean, may be important factors in explaining the popularity of this food in Indonesia.

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