WHEAT TEMPEH[1]

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ABSTRACT

Among the cultures tested, *Rhizopus oligosporus* NRRL 2710 gave the most satisfactory wheat tempeh, whereas such cultures as *R. oryzae* NRRL A-9847 and *R. arrhizus* NRRL 1526, known to make good soybean tempeh, proved unsuitable because of undesirable odor or poor growth. The yield of wheat tempeh by the procedures described was approximately 84.5% (on dry solid basis) after 20 hr. of fermentation and 64.5% after 43 hr. Unlike soybean tempeh, wheat tempeh still possesses a very pleasant odor and acceptable taste even after 43 hr. of incubation. As the fermentation progressed, the pH of wheat fell from 6.8 to 5.7 and then gradually rose to 6.7, presumably because of protein breakdown. Soluble nitrogen and reducing substances increased steadily whereas total nitrogen remained fairly constant. Proteolytic enzyme having optimal pH 5.5 was responsible for the breakdown of protein. Of the vitamins analyzed, niacin and riboflavin of wheat tempeh greatly exceeded that of wheat; thiamine appeared to be less. Thus, this new fermented wheat product may provide vitamins, as well as calories and proteins, at low cost.

Species of the genus *Rhizopus* have long been used to ferment soybeans in Indonesia. The fermented product is known as tempeh. Tempeh is not only more acceptable in flavor than the original soybean, it is also said to be more easily digested (1). Although conflicting results (2–4) have been reported for the nutritional value of tempeh as observed in rat growth, the process of fermentation retains a large part of the nutritive value of soybeans, increases the hydrolysis of soybean protein (5) and soybean oil (6), and enriches the content of some B-vitamins (7). Wheat has also been reported as a suitable substrate for preparing a tempeh-type product by *Rhizopus oligosporus* NRRL 2710 (8). Our purpose was to investigate further the capability of other *Rhizopus* strains for wheat fermentation, the processing losses in preparation of tempeh from wheat, and the effect of *Rhizopus* on wheat.

Materials and Methods

Preparation of Fermented Product. Pure-culture fermentation was carried out in Petri dishes. Portions of 35 g. of cracked Conley hard red spring wheat were placed in a wire colander and washed thoroughly with running tap water. The washed wheat was then transferred to

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a beaker, covered with excess water, and boiled for 12 min. (8). Any water remaining was drained off. The well-drained grains were cooled to room temperature before inoculation with a spore suspension of *Rhizopus*. The spore suspension was prepared by adding 2 ml. of sterilized distilled water to each slant. To inoculate each sample, 0.5 ml. of spore suspension was then used. The inoculated grain was packed lightly in a sterilized Petri dish and incubated at 31°C. for various lengths of time as shown under "Results and Discussion."

Chemical Analyses. Moisture contents of samples before and after inoculation were determined by drying a portion of each in an oven to constant weight at 90°C. The pH determination was made directly on a sample homogenized with water. The samples were then freeze-dried and ground into powder with a 6-in. Raymond hammer mill for other analyses.

The semi-micro Kjeldahl method was used to determine both total and soluble nitrogen. For soluble nitrogen, the sample was first homogenized with 7 volumes of water and then centrifuged for 30 min. at 32,000 $\times$ *g*. The supernatant was used for determination of soluble nitrogen.

Vitamins were assayed by microbiological methods. Thiamine was determined by turbidimetry according to Sarett and Cheldelin (9) except that Mylase P was used for enzymatic hydrolysis. The niacin and riboflavin assays were done by titration as described in AOAC Official Methods (10).

Proteolytic activity was measured according to the casein digestion method of Kuntz (11). After 25 mg. of fermented wheat was homogenized with 1 ml. of citrate buffer at pH 3.0 or 5.5 (12), the homogenized sample was mixed with 1 ml. of 1% casein in the same buffer. The digestion was carried out at 40°C. for 10 min. and then stopped by the addition of trichloroacetic acid. The amount of solubilized protein was estimated by measuring the ultraviolet light absorption at 280 mμ.

The activity of lipase was assayed by the method of Fiore and Nord (13). After 250 mg. of the sample to be tested was homogenized with 5 ml. of buffer at pH 5.6, 5 ml. of 4% olive oil in 1% polyvinyl-alcohol was added to the homogenate. The mixture was incubated at 40°C. for 2 hr. with constant shaking. At the end of incubation time, 15 ml. of 1:1 alcohol-acetone solution was added to stop the reaction and break the emulsion. The free fatty acids formed were titrated with 0.05*N* sodium hydroxide and with phenolphthalein as the indicator.

Results and Discussion

Strain Variation in Fermenting Wheat Tempeh by Rhizopus. In

the ARS Culture Collection there are 40 strains of *Rhizopus* known to make satisfactory soybean tempeh. The ability of these strains to produce enzyme systems varies greatly between different strains and between different species (12,14). Since soybeans and wheat are also quite different in their constituents, it is not surprising to find that some cultures which make good soybean tempeh are not suitable for making tempeh from wheat. Table I describes tempeh made from soybeans or wheat by some of the most common tempeh molds. All five

TABLE I

COMPARISON OF WHEAT AND SOYBEAN TEMPEH MADE WITH VARIOUS STRAINS OF *Rhizopus*

(All samples were incubated at 31°C. for 20 hr.)

STRAIN	SUBSTRATE					
	Wheat			Soybean		
	Growth	Odor	Taste[a]	Growth	Odor	Taste[a]
R. oligosporus						
NRRL 2710	Good	Yeastlike	Excellent	Very good	Pleasant	Good
NRRL A-9868	Good	Mild	Good	Good	None	Good
NRRL 2549	Good	Mild	Good	Good	Faint ammoniacal	Good
R. oryzae						
NRRL A-9847	Very poor	.....	Not tested	Very good	None	Good
R. arrhizus						
NRRL 1526	Good	Unpleasant	Not acceptable	Very good	None	Good

[a] The fermented products were sliced, dipped in salt water, and fried in vegetable oil for consumption. The taste of the products was judged by laboratory personnel immediately after cooking.

strains tested grew well on soybeans and produced good tempeh, although the fermentation with NRRL 2549 produced a faint ammoniacal odor in the tempeh. The odor is probably due to the highly proteolytic activity of this strain (12). Only three of the five strains, NRRL 2710, NRRL A-9868, and NRRL 2549, grew very well on wheat and gave good tempeh. Although wheat tempeh made by NRRL A-9868 and 2549 had a mild odor, none was noted in the cooked product.

Strain NRRL A-9847 grew very poorly on wheat. Since the grains must be bound into a compact cake by the growth of mold mycelium in the tempeh fermentation, this strain is unsuitable for making tempeh from wheat.

Strain NRRL 1526 grew very well on wheat, but the fermented product had a very unpleasant odor and taste. Some strains of *Rhizopus* are known to produce lactic, fumaric, and other organic acids. When wheat or other grain having a high starch content is used as substrate, the amylolytic enzymes produced by these strains hydrolyze starch to sugars which in turn are fermented into organic acids. These organic

acids are probably the cause of the undesirable taste. Since NRRL 1526 has been used to produce fumaric acid (15), it is a reasonable assumption that the unsatisfactory wheat tempeh made with NRRL 1526 is a result of the high starch content of the substrate and the fumaric acid production by the organism. Because of this, NRRL 1526 will probably be unsuitable to make a tempeh-type product from any other starchy grains.

Rhizopus oligosporus NRRL 2710 was used for the remaining portion of this study, because this strain gave the most satisfactory tempeh-type products regardless of substrates.

Fermentation Time and Acceptability of Wheat Tempeh Made with NRRL 2710. Hesseltine *et al.* (14) reported that when soybean tempeh is fermented in Petri dishes, the fermentation is completed after 20 hr. at 31°C. as judged by the development of mycelium and the acceptability of the product. Beyond this stage, sporulation and NH_3 production would be considered too far advanced to be accepted by consumers. In our study, wheat tempeh was fermented in Petri dishes from 8 to 43 hr. Although no growth was visible up to 8 hr. of fermentation, some growth was noted after 16 hr. The taste of the fermented products was not tested, because not enough mycelium developed to bind the grains together to make the fermentation complete. However, the grains were bound tightly together as a cake after 20 hr., and the taste of the product after being cooked was excellent. Even though some sporulation around the edge occurred after 24 hr., it was not noticeable in the product fermented 43 hr. The sporangia were probably obscured by the heavy development of white mycelium. All the products tasted like popcorn. Unlike soybean tempeh, the wheat tempeh still possessed a very pleasant odor and acceptable taste even after 43 hr. of incubation – distinct advantages, because harvest time does not have to be so rigidly controlled.

Processing Losses in Making Wheat Tempeh. On the basis of 24 samples, it was found that 35 g. of cracked wheat (10.3% moisture) resulted in 84.8 g. of cooked and well-drained wheat (66.0% moisture). These data indicated a loss of 8.2% in total solid due to washing, cooking, and draining (Table II). There was a slight decrease in gross weight and an increase in moisture content of the product as incubation time increased. These changes resulted in additional loss of 3.3 to 27.3% in total solid due to fermentation. Therefore, on a dry-solid basis, 31.4 g. of wheat produced, after 8 to 43 hr. of incubation, 27.8 to 20.3 g. of fermented product, which represented a recovery of 88.5 to 64.5%. The loss of nitrogen accounted for a very small fraction of total

TABLE II

PROCESSING LOSSES DURING FERMENTATION OF WHEAT BY *R. oligosporus* NRRL 2710

PROCEDURE	LOSS OF SOLIDS [a]	LOSS OF GROSS WEIGHT	LOSS OF NITROGEN [a]	PROTEIN CONTENT [a] OF THE PRODUCT N × 5.83
	%	%	% *of total N*	%
Wheat (untreated)				16.3
Washing, cooking, draining	8.2		7.8	16.8
Incubation at 31°C., hr.				
8	3.3	0.9	1.8	16.6
16	5.6	1.1	4.2	16.8
20	7.2	1.6	3.6	17.4
24	10.7	1.9	3.6	18.1
30	18.1	4.0	3.6	19.2
43	27.3	6.0	2.0	22.6

[a] All values calculated on dry basis.

solid loss. In fact, the percentage of protein increased as the fermentation time increased. The increase of protein content reflected the great loss in carbohydrates or carbohydratelike substances.

Changes Occurring during Fermentation of Wheat. Some of the changes that occurred during the course of wheat fermentation by *R. oligosporus* are given in Fig. 1. For the first 8 hr. there was no visible

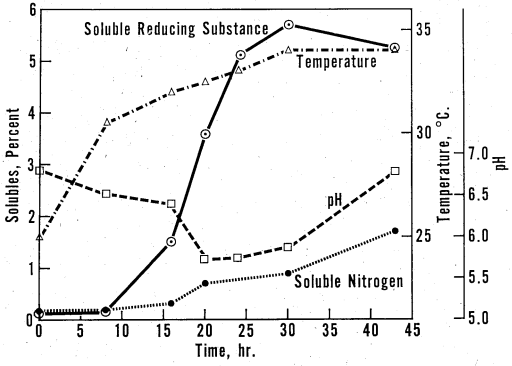

Fig. 1. Changes occurring during fermentation of wheat by *Rhizopus oligosporus* to make a tempeh-type product.

mold growth, the temperature of the inoculated wheat reached that of the incubator, and pH of the wheat tempeh fell slightly, from initial 6.8 to 6.5. These first few hours appeared to be the lag period during which germination of spores took place, and this was followed by several hours of slow growth. A fair amount of mold growth was noted after 16 hr. of incubation. The temperature of the wheat then rose above that of the incubator, and there was a slight increase in

soluble nitrogen and reducing substances, and a further decrease in pH. Thereafter, a marked drop in pH and a sharp rise in soluble nitrogen and reducing substances were observed, changes which reflected the rapid mold growth. At this stage, the grain was covered and bound with white mycelium. The pH fell to 5.7 and then gradually rose to 6.7, presumably because of a breakdown of protein. The temperature of the fermented wheat continued to rise and reached a maximum at 30 hr. of incubation; soluble nitrogen and reducing substances increased steadily. During the 43-hr. period of incubation, total soluble-reducing substances increased from 0.2 to 5.2% and total soluble nitrogen increased from 0.2 to 1.7%; total nitrogen changed slightly.

Activities of Proteolytic and Lipolytic Enzymes. Proteolytic enzyme systems are thought to play an important role in soybean tempeh fermentation. The enzymes break down the soybean protein, and perhaps this breakdown is one of the reasons that soybean tempeh is more easily digested than whole soybeans. When *R. oligosporus* was grown in a liquid medium of wheat or soybeans, the organism produced very active acid proteases having optimal pH 3.0 and 5.5, with the pH 3.0 type predominating (10). Both of the proteolytic enzyme systems were present during the course of wheat tempeh fermentation by *R. oligosporus* (Table III), but in this solid fermentation the amount of the pH 5.5 enzyme was greater than the amount of pH 3.0 enzyme. The difference might be due to the amount of aeration. It is likely that submerged cultivation has more aeration than moist solid cultivation. Aeration has long been known to affect the production of some extracellular enzymes produced by microorganisms (16). Considering the pH of the fermented wheat and the activities of the

TABLE III

ACTIVITIES OF PROTEASE AND LIPASE IN WHEAT FERMENTATION BY *R. oligosporus* NRRL 2710

FERMENTATION AT 31°C.	PROTEASE ACTIVITY [a]		LIPASE ACTIVITY [b] MEASURED AT pH 5.6
	Measured at pH 3.0	Measured at pH 5.5	
hr.			
0	...	...	0.4
8	...	0.7	1.3
16	1.3	7.9	4.1
20	6.5	12.2	5.0
24	8.7	12.0	5.8
30	9.7	18.7	5.9
43	14.4	32.3	5.5

[a] Protease activity expressed in terms of μmole of tyrosine formed/hr./g. of sample.
[b] Lipase activity expressed in terms of ml. 0.05*N* free acid formed/hr./g. of sample.

enzymes produced, one would speculate that the enzyme system having optimal pH 5.5 is more important in making wheat tempeh.

Lipase activity was also found (Table III) during all stages of wheat fermentation and it increased steadily as time of incubation increased. The very low activity of lipase in the control sample might be due to an intrinsic enzyme, which perhaps has not been completely inactivated by 12 min. of boiling.

Changes in Vitamins during Wheat Fermentation. A significant loss of niacin, riboflavin, and thiamine was noted during the preparation of wheat for fermentation, but the loss of niacin and riboflavin was reversed by the fermentation process. The effects of *R. oligosporus* on niacin, riboflavin, and thiamine in wheat are given in Table IV. There were no significant changes in the three vitamins during the lag period of growth. Both niacin and riboflavin increased throughout the rest of the growth period. On the other hand, the total amount of thiamine in the finished products appeared to be less than that of the control. The data strongly suggest that *R. oligosporus* has a great synthetic capacity for both niacin and riboflavin but not for thiamine. Practically no information is available regarding the ability of this mold to synthesize vitamins. Generally speaking, the requirement of thiamine for growth has been more commonly reported among filamentous fungi than any other type, and nicotinic acid has also been found to be essential for some filamentous fungi. However, none of the fungi isolated from nature have shown the need for riboflavin. Sorenson and Hesseltine (17) reported that *R. oligosporus* can grow on a defined medium free of vitamins. The present data seem to support their find-

TABLE IV

CHANGES IN VITAMINS DURING WHEAT FERMENTATION WITH *R. oligosporus* NRRL 2710

FERMENTATION AT 31°C.	NIACIN		RIBOFLAVIN		THIAMINE	
	Amount[a]	Total[b]	Amount[a]	Total[b]	Amount[a]	Total[b]
hr.	*μg./g.*	*mg.*	*μg./g.*	*μg.*	*μg./g.*	*μg.*
Whole wheat (untreated)[c]		4.3		119		570
Unenriched flour[c] (all-purpose)		0.9		46		62
0	46	3.8	0.4	35	3.2	260
8	51	3.9	0.4	35	3.7	288
16	65	5.0	1.0	79	2.8	211
20	112	8.5	2.2	170	2.9	223
24	135	9.7	3.2	233	3.0	213
30	240	14.5	4.4	304	3.5	241
43	422	25.5	9.0	539	3.7	226

[a] All values calculated on dry basis.
[b] Total amount of each vitamin in the fermented product obtained from 100 g. of wheat.
[c] Source: Composition of foods, U.S. Dept. Agr. Handbook 8, p. 120 (1963).

ings. As for thiamine, the organism might be able to synthesize the vitamin, but the rate of synthesis would be so slow that the organism would utilize readily available thiamine for maximum growth or other functions.

The increase of niacin and riboflavin content of wheat fermented by *R. oligosporus* could be significant to human nutrition, because the diet of large segments of the world's population is deficient in both these vitamins. This new fermented wheat product, wheat tempeh, may provide vitamins, as well as calories and protein, at low cost for large populations whose diets now are nutritionally inadequate.

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