Influence of Sodium Chloride on the Levels of Flavor Compounds Produced by Shoyu Yeast

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This paper reveals the amount of flavor compounds produced by yeast depends greatly on concentrations of sodium chloride in the culture media and shoyu. Flavor compounds such as isobutyl alcohol, *n*-butyl alcohol, isoamyl alcohol, 3-(methylthio)-1-propanol, and 2-phenylethanol, which are derived from amino acids by the actions of shoyu yeast, were decreased with an increase of sodium chloride concentration in the fermented broth. However, the production of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF) biosynthesized through the pentose−phosphate cycle reached a maximum at a sodium chloride concentration of 16%. In shoyu brewed in the laboratory scale in various concentrations of sodium chloride, however, not only HEMF but also the alcohols drived from amino acids were produced in the highest quantities at the sodium chloride concentration of 17−18%.

Keywords: *Shoyu; shoyu flavor; sodium chloride; 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone*

INTRODUCTION

Shoyu is the Japanese name for soy sauce. It is made mostly by fermentative methods and mainly used as an all-purpose seasoning in Japanese cuisine. Five different types of syoyu are available in Japan: (1) Koikuchi, (2) Usukuchi, (3) Tamari, (4) Shiro, and (5) Saishikomi. Of all shoyu consumed in Japan, 85% is of the Koikuchi type. Koikuchi is made from a mixture of soybeans and wheat kernels in almost equal amounts and is characterized by deep reddish brown color and strong and pleasant aroma. The first attempt to identify the flavor components of shoyu was made by Tahara (1987). Since then, many studies on shoyu flavor have been reported. To date, over 280 volatiles have been detected from shoyu (Nunomura and Sasaki, 1986). But, to date, no reports have appeared concerning the effects of the content of sodium chloride on the production of flavor compounds. This is the first report on the effects of the content of sodium chloride on the production of flavor compounds by shoyu yeast.

MATERIALS AND METHODS

Microorganisms. The yeast strain used in this investigation was of the stock cultures of our laboratory.

Basal Medium and Enzymes-Added Medium. These were described in a preceding paper (Sasaki, 1996).

Cultivation. Two milliliters of the preceding enzymes-added medium and 0.75 g of the fine powder of steam-sterilized defatted soybean were dispensed into a l2 mL screw-cap vial. The mixture was incubated at 37 °C for 24 h to hydrolyze the defatted soybeans. Soon after the incubation, sodium chloride was added to the hydrolysates. The concentrations of sodium chloride were varied from 6 to 26% with an interval of 2%. The vials were inoculated with a 10 μL cell suspension of the shoyu yeast strain, *Zygosaccharomyces rouxii* No. 210, ATCC 13356, as a starter. The vials were loosely capped and incubated stationarily at 30 °C for 20 days. The vial was shaken once every 2 or 3 days to accelerate the growth of the yeast.

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Preparation of Shoyu in Laboratory Scale. One hundred and sixty grams of shoyu koji was added to 240 mL of brine to a 500 mL wide-mouthed plastic bottle. The brine was prepared at 11 different concentrations of sodium chloride ranging from 6 to 24% at regular intervals of 2%. Three samples (moromi or mash) were prepared for each concentration, for a total of 33 samples (3 × 11). These mashes were shaken vigorously, and then they were placed in a room at 30 °C. They were agitated gently every 2 or 3 days. After 10 days, they were inoculated with a starter culture broth of the strain *Z. rouxii* No. 210, ATCC13356. They were stirred vigorously with a spatula every day during the first week and once every 10 days thereafter. After 8 months of incubation at 30 °C, the matured mashes were filtered at high pressure (100 kg/cm^2) through cloth to obtain the liquid part of the mash.

Determination of Flavor Compounds, Ethanol, and Sodium Chloride. Quantitative determinations of flavor compounds and ethanol were accomplished according to the GC method previously described in detail (Sasaki et al., 1991). Sodium chloride was determined according to the Fr Mohr method (Ayers, 1960).

RESULTS AND DISCUSSION

Effects of Sodium Chloride Content in the Medium on Formation of Flavor Compounds. The concentrations of flavor compounds and ethanol in the fermented broth are shown in Table 1. The flavor compounds, such as isobutyl alcohol, *n*-butyl alcohol, isoamyl alcohol, 3-(methylthio)-1-propanol, and 2-phenylethanol, which are derived from amino acids by the actions of shoyu yeast, were decreased with an increase of sodium chloride concentration in the fermented broth. On the other hand, the formation of 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone (HEMF) biosynthesized from D-xylulose 5-phosphate in soybeans or wheat through the pentose−phosphate cycle (Sasaki et al., 1991; Sasaki, 1996) showed a peculiar behavior. That is, its concentration reached the maximum at a sodium chloride concentration of 16%. This result may reveal that the pentose−phosphate cycle against the glycolytic pathway becomes most active at a sodium chloride concentration of 16%. It supports the fact that

S0021-8561(95)00650-9 CCC: $12.00

Table 1. Influence of Sodium Chloride on the Levels of Flavor Compounds Produced by Shoyu Yeast

	concentrations of sodium chloride (%), ethanol (%), and flavor compounds (ppm)									
compound	6	8	10	12	14	16	18	20	22	24
ethanol	3.15	2.06	2.48	2.65	2.01	1.33	1.67	0.81	0.26	0.27
isobutyl alcohol	30.30	30.40	27.85	24.31	20.34	13.57	10.89	5.73	2.60	2.35
n-butyl alcohol	6.65	4.30	4.50	5.33	6.12	7.95	8.51	2.84	0.29	0.34
isoamyl alcohol	55.52	48.10	37.62	32.11	26.31	15.12	13.76	5.16	2.23	1.72
3-(methylthio)propanol	7.02	5.48	3.96	3.23	3.02	2.52	2.20	1.68	0.98	0.73
2-phenylethanol	39.51	29.31	15.54	8.41	6.82	3.82	3.06	1.69	0.66	0.55
HDMF[a]	7.12	6.29	6.01	5.60	5.27	5.26	5.77	4.94	4.68	4.71
HEMF[b]	38.30	39.12	47.45	52.81	70.99	92.31	89.98	66.06	27.47	21.99
HMMF[c]	65.32	66.18	64.77	59.35	50.26	31.67	30.81	30.78	48.75	50.67

[a] 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone. [b] 4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone. [c] 4-Hydroxy-5-methyl-3(2*H*)-furanone.

Table 2. Influence of Sodium Chloride on the Levels of Flavor Compounds in Shoyu Mash Prepared in Laboratory Scale

	concentrations of sodium chloride (%) and flavor compounds (ppm)										
compound	9.47	11.28	13.40	14.90	17.08	18.68	19.52	21.40	23.35	23.92	25.32
isobutyl alcohol	15.15	20.28	43.95	21.05	43.80	37.16	31.01	23.01	17.62	13.22	10.92
n-butyl alcohol	2.43	5.33	9.48	6.63	11.99	13.10	11.69	7.01	2.10	1.00	0.23
isoamyl alcohol	22.80	31.11	51.67	26.04	49.87	40.54	30.44	21.14	16.59	12.17	10.39
3-(methylthio)propanol	3.75	5.13	7.54	5.33	9.08	9.65	9.03	9.93	9.07	7.82	7.17
2-phenylethanol	7.89	11.57	19.90	11.26	20.71	18.04	13.59	8.94	5.73	4.20	2.92
HDMF[a]	9.67	7.97	7.07	7.08	6.77	6.80	5.94	6.19	6.08	6.10	5.90
HEMF[b]	6.55	10.99	31.94	15.29	42.24	45.32	39.59	29.46	22.37	18.61	10.97
HMMF[c]	0.00	0.00	21.45	15.01	35.88	36.85	35.54	29.59	27.64	28.89	29.49

[a] 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone. [b] 4-Hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2*H*)-furanone. [c] 4-Hydroxy-5-methyl-3(2*H*)-furanone.

total flavor and taste of shoyu are decreased with a decrease of the content of sodium chloride.

On the other hand, the formation of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (HDMF) (Rodin et al., 1965; Re et al., 1973) and 4-hydroxy-5-methyl-3(2*H*)-furanone (HMMF) (Tonsbeek et al., 1968; Honkanen et al., 1980) caused by the nonenzymatic oxidative reaction was plentiful in the range of low sodium salt and then decreased with an increase of the amount of sodium chloride. However, at levels over 22%, the formation of these compounds increased again. The shoyu yeast becomes more active in higher concentration of sodium chloride, and, as result, the fermented broth becomes more reductive. Thus, the formation of HDMF and HMMF becomes lower. However, at a concentration of sodium chloride over 22%, the yeast becomes less active, and oxidation in the fermented broth occurs. Then, greater amounts of HDMF and HMMF are produced.

Effects of Sodium Chloride Content in the Shoyu Prepared at Laboratory Scale on Formation of Flavor Compounds. Shoyu was brewed at laboratory scale in various concentrations of sodium chloride. A total of 33 shoyu samples were prepared: 11 different levels of sodium chloride concentrations were prepared, and 3 samples of each were made. The concentrations of the flavor compounds in the shoyus were compared. Table 2 shows the results. The quantity of HEMF in each content of sodium chloride generally agreed with the data given in Tables 1 and 2, attaining a maximum at a sodium chloride concentration of 17.08−18.68%, though a distinctive point was observed at 13.40%. However, the levels of productions of the alcohol group, such as isobutyl alcohol, isoamyl alcohol, 3-(methylthio)-1-propanol, and 2-phenylethanol, which are formed from amino acids by the action of shoyu yeasts, showed different results from those obtained by using fermentation broth. That is, in the experiment using fermentation broth, the concentrations of these flavor compounds in media decreased as sodium chloride content increased, but in this test, the highest productions occurred at the sodium chloride concentration of 17−18% as was the case for HEMF. The reason for the difference between the results in the fermentation broth and in shoyu is not clear. It is not clear why productivity of flavor compounds by yeasts exhibited different trends between shoyu and the medium with defatted soybeans. Production of shoyu in laboratory scale might be a result of the slightly more anaerobic conditions for fermentation in the medium with defatted soybeans. It is interesting to note that the flavor compounds are most abundant in shoyu samples having a sodium chloride concentration of 17−18%, which is the most common concentration level for shoyu.

ACKNOWLEDGMENT

I thank Mrs. S. Yamamoto for skillful technical assistance. I greatly appreciate correction of the manuscript by Dr. N. Nunomura.

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Received for review October 2, 1995. Accepted May 15, 1996.[⊗]

JF9506500

[⊗] Abstract published in *Advance ACS Abstracts,* September 1, 1996.