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A New Evaluation of Browning Reactions of Korean Traditional Soy Sauce Mash During Fermentation

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Abstract

To re-evaluate the browning reactions of fermented soybean products, soy sauce mash with added glucose and/or tyrosine was fermented for 152 days in the presence or absence of oxygen. Glucose negatively affected brown pigmentation either singly or with tyrosine. Tyrosine-added soy sauce mash initially browned at the same rate as the control mash until 127th day and then the former continued to brown at the same steady rate while the control mash stopped further browning. Aerobically incubated mash browned much more than anaerobically incubated one when the browning was compared on the 152nd day of fermentation. More than half of the mash browning was found to be due to the oxygen-related browning during the limited 152 days of fermentation time. Both oxygen-related and oxygen-unrelated browning reactions were found to contribute to the browning of soy sauce mash. Oxygen-related browning, however, was found to be more important than the Maillard browning reaction.

Introduction

Korean fermented soybean products, especially soy sauce and soybean paste, are believed to be browned during koji preparation, fermentation in salt brine, and storage. There have been many works[1-11] to investigate browning reactions of fermented soybean products.

The conventional and most prevailing hypothesis for the browning of fermented soybean products has been the Maillard reaction, while some workers maintain that Maillard reaction may not be the primary browning reaction when they studied with simulated soy sauce systems.[7,10,11] Okuhara et al.[10] and Okuhara et al.[11] reported that pentoses contributed only 10-20% of total browning of soy sauce and that solutions containing sugars and amino acids browned at much slower rates than actual soy sauce did. They concluded that Maillard reaction may not be the most important browning reaction and that peptides and other unknown factors were more important. Hashiba[7] who also worked with simulated soy sauce systems found that the browning rate of the mixture was approximately 10% of that of actual soy sauce.

Lee[12,13] who analyzed amino acid composition of soy sauce and soybean paste reported that tyrosine was in low concentration in soy sauce compared to that in soybean paste. Chang[5] studied the changes in amino acid composition of soy sauce during aging and reported that the content of tyrosine decreased rapidly while that of other amino acids did not change appreciably. His explanation for the phenomenon was that tyrosine and pentoses reacted to generate pigments by Maillard reaction. This, however, was not probable because those amino acids which are known to react with sugars at a similar or even faster rate[14] did not decrease as tyrosine did. Therefore tyrosine is thought to disappear during aging in salt brine through specific reaction(s).

Kyung[15] wrote a review advocating a good possibility of enzymatic browning of fermented soybean products in addition to the conventional Maillard reaction. Park and Kyung[16] isolated twenty six strains of brown-pigmenting bacteria from home-made soybean paste. They extracted crude tyrosinase enzyme from the culture extract of a pigmenting bacterium and found that the enzyme preparation produced brown pigment with tyrosine and dopa as substrates.

The objective of this communication is to further prove the enzymatic browning hypothesis proposed in this laboratory by following the pigmentation of fermenting soy sauce mash over an extended period of time. The effects of glucose, tyrosine, and oxygen on mash browning were studied.

Materials and Methods

Koji preparation

Washed soybean was soaked in cold water overnight and boiled for three hours before it was cooled, mashed, and molded into blocks with a dimension of $10 \times 15 \times 15cm$. The surfaces of the soybean blocks were heavily inoculated with spores of a strain of *Aspergillus oryzae* obtained from the Department of Food Science and Technology, Dongguk University. The blocks were incubated at 20°C for five days before they were halved and dried.

Soy Sauce Fermentation

Dried koji (100g) was mixed with other ingredients in 1000*ml* beakers as shown in Table 1. The beakers were tightly covered with plastic wrap. Uncovered water-filled Petri dishes were placed at the bottom of the incubator (25°C) to minimize water loss due to evaporation from the fermenting mash. Air was bubbled into the mash for two minutes immediately before sampling to mix the mash thoroughly and to provide oxygen. Approximately 5*ml* of liquid part of mash was taken, centrifuged to remove soybean debries, and filtered through membrane filter (0.2μm, Whatman, Maidstone, England) before optical densities (OD) were measured at 490*nm*. Anaerobic condition was obtained by BBL GasPak Anaerobic Systems (BBL Microbiology Systems, Cockeysville, MD).

Indigenous pigment concentration of koji

Dried koji (100*g*) was mixed with 180g of NaCl and 820*ml* of water, stored at 0°C overnight, ground to paste, and stored again at 0°C for 24 hours before it was squeezed through linen. The liquid was filtered through membrane filter (0.2μm, Whatman) before OD was measured at 490*nm*.

Effects of NaCl on Pigment Formation

A pigment-forming bacterium was inoculated into yeast extract (0.3%)-malt extract (0.3%)-peptone (0.5)-glucose (1.0%) liquid medium and browning was followed in the presence of different concentrations (0, 2, 4, 6, 8, 20%) of NaCl. NaCl was added before the bacterium was incoulated and after pigmentation was once started. The measurement of brown pigmentation was the same as mentioned earlier.

Table 1. Formulae for soy sauce mash

Mash code	Soybean koji (*g*)	Glucose (*g*)	Tyrosine (*g*)	NaCl (*g*)	Water (*ml*)
A	100	0	0	180	820
B	100	18	0	180	820
C	100	0	0.5	180	820
D	100	18	0.5	180	820
E*	100	0	0	180	820

* Anaerobically incubated.

Results and Discussion

Effects of Glucose and Tyrosine on Mash pH

pHs of soy sauce mashes containing added glucose decreased while those of the mashes without it increased. Tyrosine, on the other hand, did not influence pH at all. The difference in pH change could be mainly due to lactic acid produced by bacteria from the added glucose (B and D), and to ammonia released from amino acids by mash microorganisms with limited acid production from the limited amount of sugar (A and C).

The pHs of control and tyrosine-added mash simultaneously increased at a steady rate to about 7.6 in 152 days of aerobic fermentation. Under the anaerobic condition, the pH was 6.15 (Table 2) after the same period of fermentation time (152 days). In this case pH did not change from the starting point. The mashes without added glucose should have even higher pHs and the mashes with added glucose should have leveled-off pHs at around 5.0,

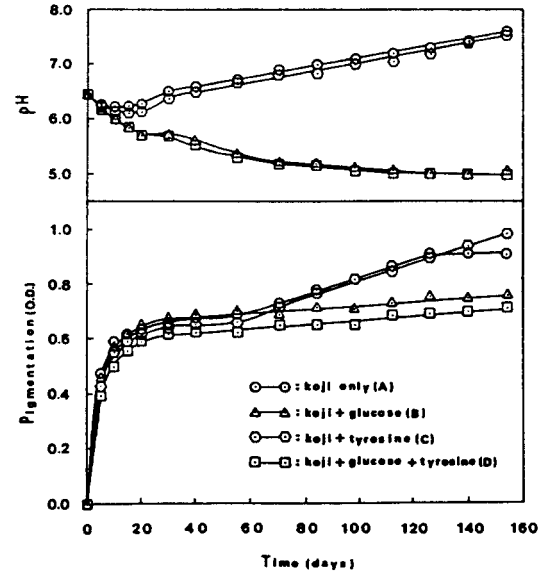

Fig. 1. pH and pigmentation changes during soy sauce fermentation

if the fermentation was allowed to proceed further.

Glucose which is one of the known reactants of Maillard reaction actually helped to lower pH to slow the Maillard reaction rate. Different pHs should have different influences on enzymatic browning reactions, too. Since both Maillard and enzymatic reaction rates are largely dependent on pH values, it seemed to be advisable to compare mash pigmentation between separate groups having a close pH range, i.e., B and D, and A and C.

Effects of glucose and tyrosine on mash browning

When glucose was added either singly or with tyrosine to soy sauce mash, the increase in brown pigmentation was only slight while pH decreased appreciably. Those groups without added glucose (A and C), however, showed increasing trends in pigmentation. These results can be explained that glucose-added groups experienced lower increase in pigmentation due to a decrease in pH and that glucose-unadded groups showed higher pigmentation due to a increase in pH. Glucose did not seem to be utilized as a Maillard reaction reactant, but metabolized to produce acids. If, however, the pigmentation was assumed to be caused by Maillard reaction, mash D which contained both sugar and amino acid should have shown more pigmentation than mash B because mash D contained additional amino acid Exactly the same phenomenon was observed with those mashes without added glucose. Tyrosine-added mash (C) browned at the same rate as control mash (A) until 127th day and after that period control mash did not increase in pigmentation while tyrosine-added mash kept increasing in pigmentation at the usual steady rate. If this difference was due to the difference in the concentration of Maillard reaction reactants, the browning rates had to be different from the beginning, i.e., mash C higher than mash A.

Okuhara et al.[11] heated soy sauce with added glucose to find that browing rate did not change. They concluded that glucose did not contribute to the browning of soy sauce. Yomo[6] reported that, when glucose was added to soy sauce, it actually inhibited browning of soy sauce. His finding, as it was also apparent in this research, seemed to be due to acid production from the added sugar and to the resultant low pH. The same interpretation can be applied to the fact that soy sauce made with more soybean had darker color and vice versa as reported by Noda,[17] Chung et al.,[4] and Fukushima.[18]

A possible explanation could be drawn that tyrosine, a substrate for enzymatic browning, was pretty much exhausted from the control mash (A) while mash C had additional tyrosine to be oxidized to cause further browning. Lowered pHs as shown in mashes B and D could have negatively affected enzymatic browning reaction.

Effect of Oxygen on Pigmentation

The mash incubated under anaerobic condition (E) reached OD of 0.71 after 152 days of fermentation (Table 2). This was only 32% increase from the original indigenous pigmentation of koji which was 0.54 OD units. The increase in OD under anaerobic condition (E) was 0.17 OD units while under aerobic condition (A) it was 0.39. The difference in OD increase (0.22) could be regarded as pigmentation due to the presence of oxygen. From the data obtained from the experiment of limited period of time, 56.4% of the pigmentation occurred during soy sauce fermentation was oxygen-related (enzymatic) browning and the rest due to oxygen-unrelated (Maillard) browning reaction.

In fact Motai[12] reported that during the storage of fermented soybean products they were further browned and even blackened in the presence of oxygen. Kamada and Sakurai[9] reported that soybean paste extract and usukuchi soy sauce were browned in the absence of oxygen and that they were browned more in the presence of oxygen. These earlier findings well coincided with our present result.

Effect of NaCl on the Pigmentation by a Browning Bacterium

It was already reported that pigmenting bacteria existed in soy sauce koji and soybean paste.[4, 15] To examine the effect of NaCl on pigmentation, a pigmenting strain of *Bacillus subtilis* was cultivated in YMPGTB with different amounts of NaCl. When NaCl was added before the bacterial inoculation, the browning was decreased in

Table 2. pH and pigmentation of anaerobically incubated mash*

pH	Pigmentation (OD)
6.15	0.71

* Anaerobic condition was not interrupted until the final day.

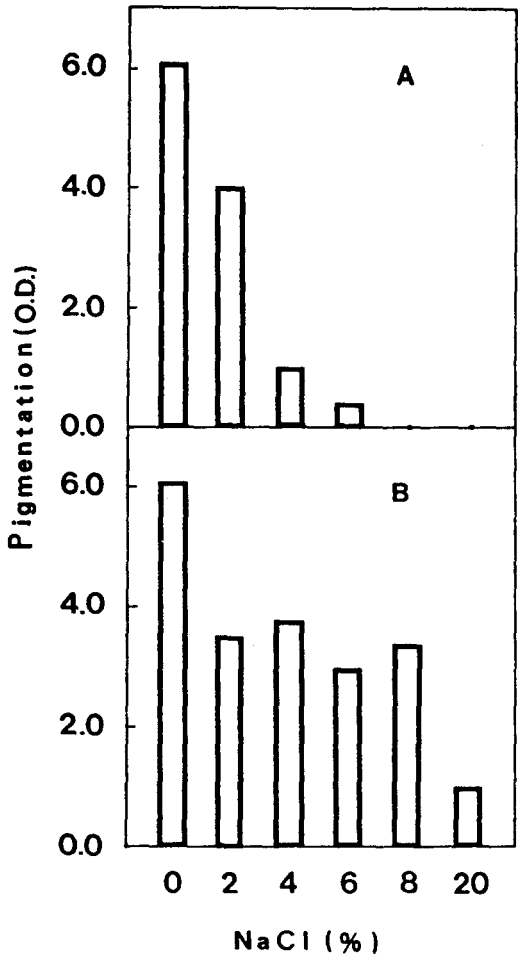

Fig. 2. The effect of NaCl on pigmentation by *B. subtilis* in the presence of tyrosine
NaCl was added before bacterial inoculation (A) and immediately after the initiation of pigmentation (B).

proportion to the amounts of NaCl (Fig. 2). With 8 and 20% NaCl, the bacterium did not grow and consequently no browning occurred. When, however, NaCl was added after browning was initiated, browning was less inhibited. Brown pigmentation at 20% NaCl was low but apparent. This suggests that NaCl negatively affects cell growth and pigmentation and that NaCl does not completely inhibit brown pigmentation once the enzyme is produced and released out of the cell.

Carol Shieh et al.[19] and Okada et al.[20] reported that the color of miso was darkened more when the concentration of NaCl decreased. They also mentioned that the content of free amino acids increased as NaCl content decreased. NaCl is known to interfere with the action of proteolytic enzymes.[21]

Conclusion

Browning increased when tyrosine was added into the fermenting soy sauce mash and also when oxygen was present. Sugar negatively affected the browning of the soy sauce mash. The positive effect of tyrosine and oxygen on browning suggested that enzymatic browning was one of the important browning reactions of fermented soybean products. It was concluded that oxygen-related (enzymatic) browning was more important than oxygen-unrelated (Maillard) browning.

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대두발효식품의 새로운 갈변기작에 관한 연구

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대두발효식품의 갈변현상을 확인하기 위하여 간장발효액에 포도당과 티로신을 따로 또는 함께 첨가하여 152일간 발효시키면서 이들이 갈변에 미치는 영향과 동시에 산소존재여부가 갈변에 주는 영향을 연구하였다. 포도당은 그 자체만으로나 또는 티로신과 함께 첨가되었을때 pH를 낮추었음은 물론 갈변을 저해하는 것으로 밝혀졌고 티로신만을 첨가하였을 때는 pH에는 영향을 주지 않았으나 발효후기에 갈변을 촉진시켰다. 산소의 존재하에 발효시킨 간장액은 산소가 없는 상태에서 발효시킨 간장액의 갈변과 비교했을때 산소가 있는 상태에서 발효시킨 것이 현저히 높은 갈변을 나타내었다. 대두발효식품의 갈변에는 산소가 없이도 이루어지는 마이야르반응과 티로신과 산소가 관련된 효소적갈변반응이 있는데 티로신과 산소가 첨가되었을 때의 갈변증가가 그렇지 않은 때보다 많은 것으로 보아 효소적갈변반응이 마이야르반응보다 더 중요함을 알 수 있다.