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Study on the pigmentation by the Maillard reaction: Browning of cheese during storage, and formation of a yellowish pigment in a model system containing thiamine.

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As the Maillard reaction brings formation of pigments and aroma compounds, this reaction has a large effect on the palatability of foods. From the standpoint of used materials, two different methods have been used for the study on the pigmentation by the Maillard reaction; one is foods itself and the other is a model system. In this study, I selected cheese as the former, and a model system containing thiamine as the latter from the reason as described below.

The browning of cheese is often observed during ripening and storage. Although the browning seems to be caused by the Maillard reaction, there is little chemically clear evidence on the relationship between the browning and the Maillard reaction. Further we do not know the reason why some cheese samples are more susceptible to browning than others. Therefore, one aim of this study is to examine the browning of cheese and to clarify the relationship between the browning and the Maillard reaction.

Thiamine is a water-soluble vitamin and has an amino group in the structure. A lot of studies have shown that thiamine is involved in the Maillard reaction of foods and body. Although several aroma compounds are known to be formed from thiamine during food processing or heating, there is no report on a colored compound derived from thiamine except for thiochrome, which is an oxidative derivative of thiamine. Therefore, the second aim of this study is to find a pigment derived from thiamine by the Maillard reaction.

At first, I examined the chemical mechanism of the browning of cheese. A Cheddar cheese sample (Cheddar cheese A), which turned intensively brown during storage, was used for the study. The cheese was extracted with hexane, ethyl acetate, ethanol, and methanol, successively, before the browning of each extract and residue was evaluated by being stored at 70˚C and for 3 or 7 d. As a result, the methanol extract turned intensively brown and the residue extracted with methanol turned little brown. Further a spot in the methanol extract detected by TLC disappeared during storage of the methanol extract. Thus, I tried to isolate the compound using chromatographic procedures. As a result, the spot was identified as galactose. This result suggests that galactose is an important factor of the cheese browning. From another Cheddar cheese sample (Cheddar cheese B), which turned brown during storage, lactose was identified. In Cheddar cheese C, which turned little brown during storage, neither galactose nor lactose was detected. Next, cheese samples to which such sugars as galactose, glucose, or lactose was added were stored, before being evaluated to the susceptibility to browning. As a result, galactose-added cheese turned the most intensively brown. Further, the sugar contents and the browning susceptibilities of nine kinds of retail cheese were examined. The

decrease in sugars of nine kinds of retail cheese during storage correlated with the ΔL*-, Δa*-, and ΔE-values of these cheese samples. In general, high amount of amino acids, peptides, and proteins exist in ripe or mature cheese. Consequently, sugars, especially galactose, were considered to be the limiting factor for the Maillard reaction causing the browning of ripe or mature cheese during storage.

In a study of a model system, a solution containing glucose, lysine, and thiamine was prepared and heated at 100˚C for 6 h, before being analyzed with DAD-HPLC. As a result, two colored peaks showing absorption maxima at 380 nm and 390 nm, respectively, were detected. Although the latter was identified as thiochrome, the former was not identified. Thus, I tried to isolate and identify this compound (TGL3). After a 5 L of solution containing glucose (30 mM), lysine (30 mM), thiamine (30 mM), and 0.5 M phosphate buffer (pH 7) was heated at 120˚C for 4 h, its pH was adjusted to 8 and applied to a column of synthetic resin, DIAION, from which TGL3 was eluted with 0.1% HCl. The pH of the fractions containing TGL3 was adjusted to 10, before TGL3 was extracted with ethyl acetate. After the extract was concentrated *in vacuo*, the obtained yellow paste was applied to a column of silica gel, which was eluted with a mixture of ethyl acetate and methanol (32:1; v/v). The fractions containing TGL3 were collected and cooled at refrigerator. As a result, ca. 30 mg of yellowish needles were obtained. This crystal was applied to TLC, elemental analysis, LC-MS, and NMR. Although the molecular formula was determined $C_{10}H_{12}N_4O$, the chemical structure was not identified. The compound was then reduced with $NaBH_4$, and analyzed. Further, this compound was dissolved in DMSO, before its NMR analysis was done to detect the coupling of protons in NH group. Finally, the structure of TGL3 was determined 1-(2-methyl-6,9-dihydro-5*H*-pyrimido[4,5-e][1,4]diazepin-7-yl)ethan-1-one. This compound was a novel yellowish pigment containing pyrimidine and diazepine. This structure suggests that this compound was formed by condensation of a degradation product of thiamine and a tetrosone derivative formed by the Maillard reaction between glucose and lysine.

The present study showed that at first galactose was a limiting factor for the browning of cheese. The result will lead novel methods to repress the browning of cheese during processing and storage. In the second study, a novel pigment derived from thiamine was identified. This result gives a fundamental knowledge for the formation of pigments by the Maillard reaction.