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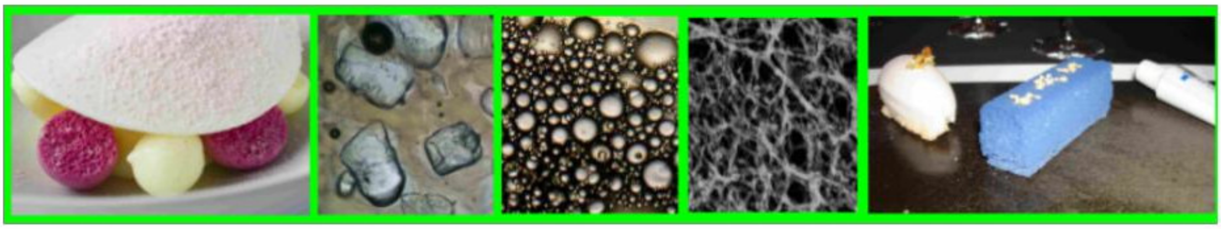

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Monitoring the bioactive compounds in culinary transformation of soymilk: An in situ quantitative NMR study. Part 2: Discussion

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Changes of Suc content during culinary transformation
Culinary transformations trigger changes in physical and chemical properties. Various chemical changes could occur during process but saccharides and proteins appear the main ones. Therefore, Suc, Arg, Lys and Val were selected for monitoring the chemical changes from the culinary transformation of soymilk. The result clearly revealed that Suc concentration increased after heating. This corresponds to the report that the amount of saccharides significantly increased after heating in yuba production[19]. The mechanisms for sucrose increase could relate to the interaction of Suc and other components. Soybean contained a sucrose binding protein (Suc transporter protein) as well as glycinin and lecithin that were able to bind sugar[20-21a, 18b]. In fresh soymilk, the doublet at 5.42 ppm (H_1) of Suc might be hindered due to Suc-protein binding. When proteins were denatured by thermal processing, free Suc molecules could be enhanced. Suc content was much reduced in tofu whereas coagulation was occurred. The protein network in this acidic coagulation tofu may squeeze out Suc. However some Suc could be trapped in tofu but not in yuba. Yuba on top of soymilk surface could be formed at the temperature of 65-75 ˚C when proteins connect into a network together with oil droplet. Reasons that no Suc was observed in yuba could be related to the structure of protein-lipid network where Suc (a hydrophilic molecule) could not bind. Therefore it could be removed by rinsing process during sample preparation before lyophilized. These suggested that a sweetness of soymilk could be simply enhanced by boiling of soymilk. Sweetness from sugar was lost when soymilk was processed into yuba.

Changes of amino content during culinary transformation
Two major proteins (glycinin and β-conglycinin) which represented 80 % of the total protein are present in soymilk. Glycinin is a plant globulin containing Arg (6.41 %), Lys (4.98 %) and Val (6.05 %), among other amino residues. The β-Conglycinin is composed of three protein subunits containing Arg (6.97 %), Lys (5.05 %) and Val (5.53 %), and other amino residues. Combination of these two proteins leaded to the total amount of Arg, Val and Lys at

6.65 %, 5.83 % and 5.01 %, respectively. In this study, the isq ^{1}H NMR detected only the content of free amino acid (AA). The observation in fresh soymilk revealed that ratio of Arg was higher than Lys which coincidentally corresponds to the level of amino residue content in total proteins. For free Val, the content of 10.96 % is much greater than Val residue in protein (expectation of 5.83 %). High Val content suggests the uncorrelation between the amount of free Val and Val residue in protein.

In the thermal treatment, the amount of Arg and Lys in soymilk was reduced as compared to their content in the fresh soymilk. Extensive reduction of Lys was observed when temperature was increased from 75 ˚C to 100 ˚C. The opposite was found for the Val concentration, which increased significantly especially at 100 ˚C. The changes of these amino concentrations in the thermal treatment might be difficultly affected by hydrolysis because soymilk was heated in a non-acidic condition. Both Arg and Lys are basic amino residue which might be prone to oxidation and Maillard reaction in a presence of sugar especially xylose[22], causing a decrease in their concentration. The dramatic decrease of Lys indicates the high reactive property of Lys. Our finding based on the isq ^{1}H NMR method corresponded to the previous report on heat treatment and Lys stability[23-25]. Significant reduction of Lys in soybean meal after heat treatment by autoclave for a long time was reported[23]. Heat treatment of amino acid especially Lys in a presence of reducing sugar potentially converted reactive Lys to unreactive Lys. If excessive heat treatment was further applied, the amino content would be reduced by Maillard reaction[24]. Mottram D.S. reviewed that thermal treatment generated pathways for compound conversion especially for amino acid via Maillard reaction[25]. Maillard reaction products from soy produced the volatile molecules like furanmethanol and 4-hydroxy-2,5-dimethyl-3(2H)-furanone, while some generated compounds reacts with other compounds in food matrix yielding more complex molecules[25]. Extensive reduction of Lys over Arg in soymilk at 100 ˚C indicates that Lys is reactive to the reaction rather easier than Arg which corresponds to other reports[26]. Evaporation even produced lower amount of Arg and Lys. These indicate that a drastic Maillard reaction could also occur in a process using vacuum at low temperature. Therefore these results suggest that both Lys and Arg in soymilk were very sensitive to the culinary involving thermal process. Decreasing of Arg in soymilk from thermal process especially by evaporation could consequently reduce a bitterness of soymilk.

Val signal was significantly increased especially after thermal process at 100 ˚C. This might not correlate with Val concentration since some experiment reported that Val concentration was unaffected by heat in the heated soybean products[24]. This might due to the high sensitivity of the isq ^{1}H NMR method over chromatography technique which compounds were analyzed regardless of structural conformation. Since soymilk contained amino acids in both free and peptide form, they could be affected differently by heat. Dynamics of free Val could be monitored efficiently by NMR technique[27]. The chemical shift of Val side chain especially at H_{β} is known for the torsional dependence as described by Karplus parameter. The isopropyl group of Val side-chain generally preferred *trans* conformation but non staggered rotamers may presence from some free Val in fresh soymilk. Hence, increasing of Val signal at 100 ˚C in this study suggests that some restraint Val side-chain might rotate freely. The conformational change of Val side-chain might consequently lead to a bigger area at 2.27 ppm in the obtained spectrum. Taste was perceived by a mechanism that the taste molecule bound to receptor hence the binding was a molecular conformational dependence process[28]. While boiling soymilk usually provided less bitterness, conformational change of Val side-chain might therefore contribute in the process.

Physical properties of tofu and yuba were dramatically changed from soymilk when liquid becomes soft gel (tofu) and thin film (yuba). These physical change affected the concentration of Suc and amino. In tofu, a 60 % reduction of Suc was observed as related to a fresh soymilk while yuba contained none of Suc. Only few Suc was trapped during formation of protein network in tofu but it could not be trapped in yuba which generated from a formation between protein and lipid. Unfortunately, higher level of Lys was found in tofu and yuba as compare with thermal treatment at 100 ˚C. This corresponds to the report that unreactive Lys could be hydrolyzed to Lys in a presence of glucono-delta-lactone[29]. While yuba seems to composed of nonpolar compounds like protein and fat, there was less amount of *trans* Val. These suggest that natural sweetness of tofu could be contributed by Suc and Lys. Yuba tastes differently from tofu as it expected for a less sweetness because it contained no Suc and low *trans* Val. In this study, interactions and behavior of Suc and some amino in various treatment of soymilk were demonstrated, however many questions have been arisen including changing of these bioactive compounds in other food matrix.