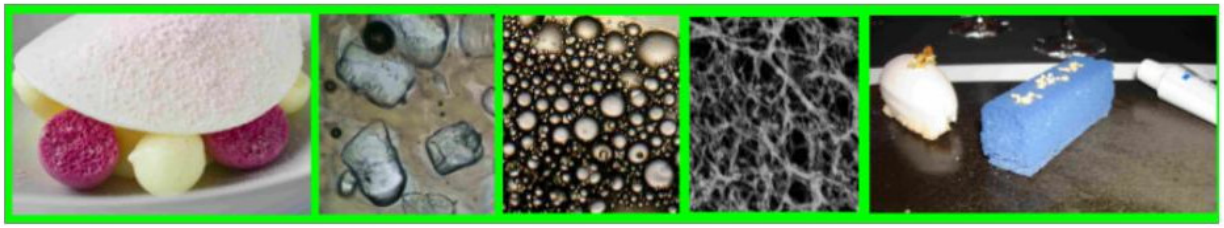


Inrae-AgroParisTech
International Centre for Molecular and Physical Gastronomy

International Journal of Molecular and Physical Gastronomy

Authors
Witcha Treesuwan, Hervé This

Title of the work
Monitoring the bioactive compounds in culinary transformation of soymilk: An in situ quantitative NMR study

Year 2015, Volume 1 (1), Number 1, pp. 1-9.

Published online:
5 March 2015

International Journal of Molecular and Physical Gastronomy

Monitoring the bioactive compounds in culinary transformation of soymilk: An in situ quantitative NMR study

Witcha Treesuwan[1*] and Hervé This[2,3]

[1] Institute of Food Research and Product Development, Kasetsart University, Bangkok, 10900, Thailand
[2] INRA UMR 1145, Laboratoire de chimie analytique, 16 rue Claude Bernard, 75005 Paris, France
[3] AgroParisTech, UFR de chimie analytique, 16, rue Claude Bernard, 75005 Paris, France

**** Corresponding author:***
W. Treesuwan, Institute of Food Research and Product Development, Kasetsart University, Bangkok, 10900, Thailand. Email: ifrwctw@ku.ac.th

ABSTRACT

Many products made from soybean are consumed in Asia. Soymilk, tofu and yuba are obtained by individuals at home. Changes of bioactive compounds during culinary process were rarely reported. In this study, the analytical method called "in situ quantitative nuclear magnetic resonance spectroscopy" (*is*q ^{1}H NMR) was applied to the quantitative determination of the variation in taste and functional molecules that are sucrose, lysine, arginine and valine during various culinary transformations of soymilk, involving fresh, thermal process, vacuum evaporation, tofu and yuba. Results suggest that thermal process at 100 ˚C increased the amount of free sucrose but significantly decreased the amount of lysine. Sweetness of soymilk could be simply enhanced. Evaporation of soymilk caused a higher reduction of lysine than thermal processing at 100 °C, indicating that complexation and Maillard reactions of lysine could be enhanced. The arginine content also decreased, albeit in less quantities. The changes of molecular conformation could also be monitored. In particular, resonances associated with the side-chain of valine were observed when most proteins were unfolded at high temperature. In mild acidic condition using glucono-delta-lactone as a coagulant, the amount of lysine was maintained higher than thermal process and evaporation while sucrose content was reduced. Tofu containing of lysine and sucrose could have a sweetness taste. However, there was no free sucrose in yuba. Understanding the change of taste molecules in soymilk during culinary transformation could lead to a better formulate soymilk products, thus this would be benefit to culinary application at home and industrial.

Key words: Molecular gastronomy, Soymilk, Sucrose, Amino, In situ quantitative ^{1}H NMR

INTRODUCTION

Food products prepared from soy (*Glycine max* (L.) Merr.) such as soymilk (obtained by hot water extraction from soybean), tofu (by protein aggregation of soymilk) and yuba (film on top of soymilk) are consumed all around the world, especially in Asia[1]. Because such products are reported to have nutritional advantages (soybean is rich in proteins and phytoestrogen[2]; soy products are free of lactose, cholesterol and gluten[3]), they have been extensively studied[4-5]. The protein composition, lipid, minerals, vitamins and organic acids in soymilk were initially reviewed by García and co-workers[6]. Volatile compounds that presented the characteristic of soymilk were analyzed using solid-phase microextraction-gas chromatography. Commercial soymilks could contain a total of 30 volatile compounds, hexanal, benzaldehyde, and pentanal being major volatile[7]. In 2003, when fermentation by lactic acid bacteria (LAB) and bifidobacteria was applied to produce the fermented soymilk, reduction of stachyose and raffinose together with the increment of sucrose, fructose, glucose and galactose were observed[8]. Proteins in soymilk played an important role in texture of tofu because of their cross link functionality. Soy proteins mainly contain glycinin and β-conglycinin in which glycinin had bigger molecular weight than β-conglycinin. Selective denaturation of these proteins by two-step heating that was treating at 75 °C for 5 min followed by 95 °C for 5 min therefore resulted in a more stable tofu than by one-step heating process. According to the two-step processing temperature of soymilk, the resultant tofu gained higher Young's modulus and decreased the syneresis rate[9]. The mixture of water, oil and proteins in soymilk represented the oil-in-water emulsion that stabilized by proteins. However, formation of a thin film on the soymilk surface could be observed during heat treatment. These yuba generally contained 57 % protein and 24 % oil but the study of yuba formation mechanism revealed that protein particles especially β-conglycinin were main functional molecule[10]. Yuba could be formed without oil but not β-conglycinin. Understanding the change of bioactive compounds during culinary transformation could lead to a precise recipe or innovation in food industry.

Chemistry techniques based on extraction, fractionation, chromatography and spectroscopy such as high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE)[11] have been regularly used to determine the content in proteins, saccharides, isoflavones and vitamins in soymilk and soybean related products. Nuclear magnetic resonance (NMR) spectroscopy has been introduced in food analysis from 1990s[12], not only for structure identification but also for quantification (q NMR) because spectra of all components with concentrations higher than the detection threshold (around 0.1 mg in 1 g of fresh product) can be detected at high resolution[13]. Recently, a new non-destructive and fast analytical method called "*in situ* quantitative nuclear magnetic resonance spectroscopy" (isq ^{1}H NMR) was applied to the quantification of bioactive compounds in plant and animal tissues with no preliminary preparation of samples[14].

Both q NMR and isq NMR were applied to a lot of food systems, such as solutions, pigments extracts (for example, solutions containing chlorophylls, their derivatives, and carotenoids, extracted from immature pods of *Phaseolus vulgaris* L.). The q ^{1}H NMR gives higher precision for chlorophyll a, a', b, b', their derivatives and carotenoids than other methods[15]. The content of saccharides in aqueous extraction from onion (*Allium cepa* L.) bulbs was also studied, revealing rapidly the content in 3 saccharides, 17 amino acids and 5 organic acids[14]. During methodological comparisons of isq ^{1}H NMR and other methods based on extraction, it was shown that a higher concentration of glucose and sucrose in carrot root was observed by isq ^{1}H NMR method compared with other methods[16-17]. Therefore, isq NMR is useful for the quantitative determination of the bioactive compounds in food matrix. Although soymilk

and other related products were good nutrition resource, their bitter taste prevented them from consumer acceptance. Therefore, the aim of this work was to follow the changes of bioactive compounds which were responsible for taste and nutrient like saccharides and amino acids in soymilk after the culinary transformations such as thermal process, evaporation, coagulation (tofu) and film formation (yuba) by using the isq ^{1}H NMR method. Sucrose was the main saccharide in soymilk and it also represented the importance of sweet taste and energy providing molecule. Three amino acids that are arginine (Arg), lysine (Lys) and valine (Val) were monitored as Arg and Val contributed to a bitter taste while Lys contributed to a sweet taste of soymilk. Moreover, Lys and Val are essential amino acid while Arg and Lys were important for building muscle as they involved in the protein synthesis.

MATERIALS AND METHODS

All samples, hardware, and solutions used at each step were precisely weighed using a balance with precision 0.00001 g (Mettler Toledo AG 153).

Chemicals
Deuterated water (D_2O, 99.9 %), 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid, sodium salt (TSP; 98 %) were from Sigma-Aldrich. Ethylacetate (99.8 %) was from *Chromanorm*. Methanol (99.9 %) and chloroform (99.0 %) were from *Carlo Erba*. Sucrose, for biochemistry and microbiology was from *Merck*. L-Arginine (98 %), L-;ysine (98 %) and L-valine (98 %) were from *Sigma*.

Sample preparation
Commercial soymilks ("*Bio boisson au soja, nature*") were purchased daily from the local grocery store. Experiments were carried out within 3 days after the box was opened and stored at 4 ˚C. In order to analysis, sample was mixed with D_2O at 7:1 ratio (w/w).

Commercial soymilk was mixed with D_2O. For thermal processing, the commercial soymilk was heated at 75 °C or 100 °C for 60 min using electrical hot plate (IKA RCT Classic) controlled with a thermocouple and stirred magnetically (spinning rate 1000 rpm). After cooling, liquid samples were mixed with D_2O.

A variation of water content in different samples (fresh and thermal process) may lead to the difficulty in comparison of the obtained result. Therefore, dehydration of soymilk samples involving fresh, thermal process, vacuum evaporation, tofu, and yuba was considered. For evaporated soymilk, the commercial soymilk was transferred into a 250 mL round-bottom flask and vacuumed for 6 hrs. A water bath temperature was set at 60 ˚C. Evaporated soymilk was cooled down to room temperature before being frozen at -20 °C. For making soft tofu, 60 mg of glucono-δ-lactone (GDL) were added in 20 g of commercial soymilk. The solution was heated in a water bath at 95 ˚C for 60 min, before storage at room temperature; soft tofu was formed during this final storage, then cleaned under running distilled water until water was clear. For yuba, the same process as producing a heated soymilk was applied but temperature was kept at 85-90 ˚C without stirring for 3 hours. After this thermal processing step, a soft film on the top surface was carefully removed and rinsed by distilled water until washing water was clear. Samples were frozen (-20 ˚C) overnight and freeze-dried in lyophilizer machine for 2 days.

The lyophilisate resultant was dissolved in D_2O at a ratio of 1:35 (w/w). Solution samples of about precisely 700 mg were loaded into a NMR tube (quartz, 5 mm diameter). A capillary tube containing a known concentration of TSP was put in a tube before ^1H NMR acquisition. All analyses were done in triplicate.

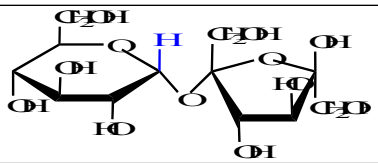
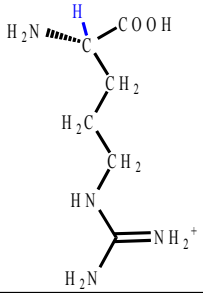
q ^1H NMR

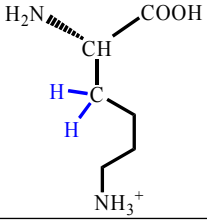
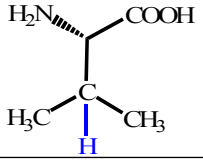
A superconducting Ultrashield 300 MHz (7.05 T) 54 mm magnet NMR spectrometer BZH 30/300/70 E *Bruker Biospin* (Germany) was used. All ^1H NMR spectra were recorded at a temperature of 21 ˚C in order to avoid different mutarotation equilibrium for the saccharides present in the samples[17] . For each spectrum, 64 scans of 32 k data points were recorded with a spectral width of 6172.8 Hz, an acquisition time of 5.3 s, and a recycle delay of 25 s per scan to allow complete relaxation and absolute quantification. The analysis of each sample was performed using D_2O as an internal lock. The acquisition procedure (shimming, gain signal and Fourier transformation using XWIN NMR 3.5 software) were automated.

Identification and quantification

The major components in soymilk samples were identified and quantified by q ^1H NMR. The identification was carried out by comparison with reference data from standard compounds and literature. The main saccharide was sucrose (Suc) and selected amino was arginine (Arg), lysine (Lys) and valine (Val). When TSP was used as reference for identification, the best results for quantitative determination were obtained using: the doublet at 5.42 ppm (H_1) for Suc, the triplet at 3.22 ppm ($H_{1\alpha}$) for Arg, the multiplet at 1.77-1.85 ppm ($2H_\beta$) for Lys, and the multiplet at 2.27 ppm (H_β) for Val as indicated in table 1. The identified compounds were quantified by reference to the peak area of the known concentration of TSP in capillary tube. The area of the interesting resonances in all spectra was integrated and autocorrected three times using *NMR Notebook 2.50* software. The area of the TSP resonance was used as a fixed reference of area equal to 1.

Table 1 The chemical shifts (ppm) and multiplicity of protons (highlight in blue) used for the quantitative determination of target compounds.

Compounds	Selected proton in structure	Chemical shift of selected proton (ppm)
Suc		5.42 (d)
Arg		3.22 (t)

Lys		1.77-1.85 (m)
Val		2.27 (m)

RESULTS

Changes in bioactive compounds during soymilk processing were analyzed by isq ^{1}H NMR. Samples from all kinds were used in the experiments including fresh soymilk immediately after opening of the box. A typical ^{1}H NMR spectrum of a soymilk sample was recorded by using the TSP as reference to a chemical shift at 0 ppm. Amino acids region appeared at 0.8 – 3 ppm, saccharide region appeared at 3 – 5.6 ppm and water molecules appeared around 4.8 ppm. Suc is the major saccharide in soymilk as well as in other soymilk products. Charged amino acids Arg and Lys were present in low amount (0.006 – 0.085 %) in fresh soymilk (Figure 1). We investigated these compounds in soymilk samples after culinary transformation using both liquid samples (Figure 1) and dried samples (Figure 2).

The amount of selected compounds in thermal processed soymilk is shown in Figure 1. Val concentration is higher than Suc, Arg and Lys in all samples. After thermal processing, Suc content increased from 0.23 to 0.54 %, Lys decreased at higher temperature, but Val increased from 0.82 to 1.94 % by heating at 100 ˚C.

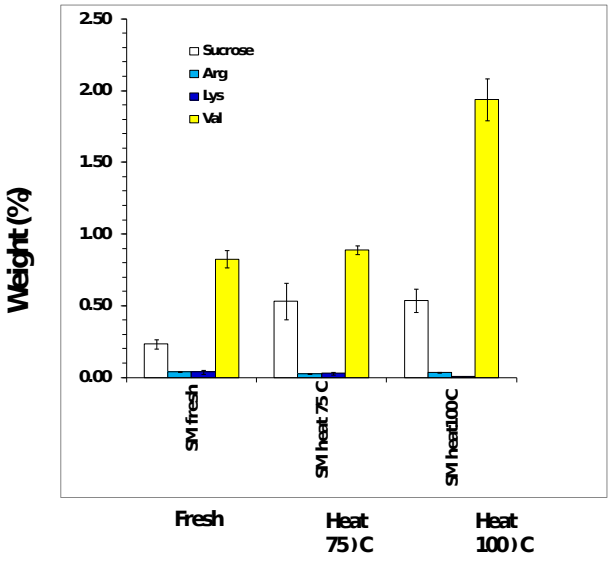


Figure 1 The amount of Suc, Arg, Lys and Val in fresh soymilk and after thermal processing. The percentages of bioactive compounds were calculated from the weight of the liquid samples.

Results from samples that were lyophilized are shown in Figure 2. Thermal processing increased Suc content from 5.57 to 7.06 %, Val content from 10.96 to 14.35 % but decreased the content in basic amino acid Arg and Lys. By evaporation, the amount of Suc and Val remained similar to the fresh soymilk, while basic amino acids Arg and Lys decreased to 0.27 % and 0.18 %, respectively, which was even lower than obtained from heated soymilk. In soft tofu, the content in Suc, Arg and Lys was much lower than the original soymilk by decreasing from 5.57 to 2.23 %, 1.50 to 0.32 %, and 0.79 to 0.34 %, respectively. Interestingly, in yuba, the Suc content was undetectable while the amount of Arg and Lys was similar to other treatments. Val concentration at 2.22 % was still the major content in soymilk product.

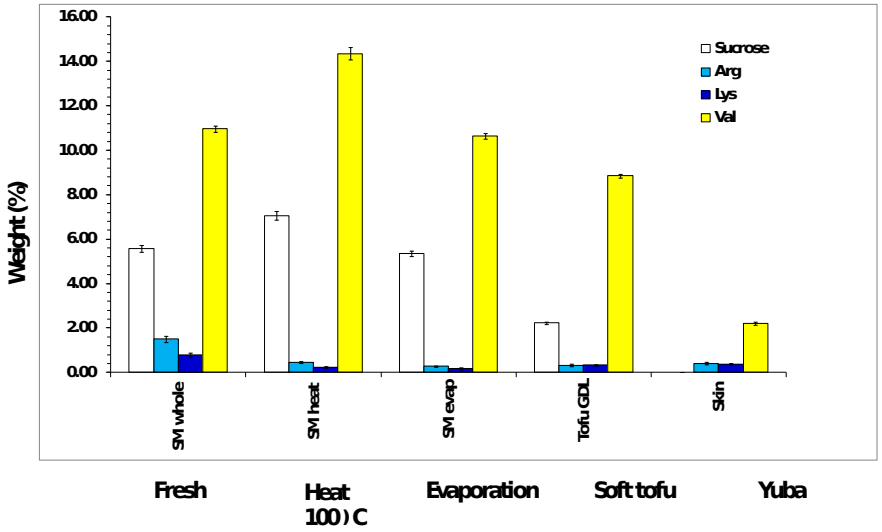


Figure 2 The amount of Suc, Arg, Lys and Val in soymilk, after culinary transformations. All samples were lyophilized for 2 days before dissolution in D_2O. The percentages of bioactive compounds were calculated from the weight of the dried samples.

DISCUSSION

See separate file, from the same authors.

DECLARATION OF INTEREST

The manuscript has not been submitted elsewhere. This study was funded by the Junior Research Fellowship Program 2011 from the French Ambassy, Bangkok, and partial supported from the Kasetsart University Research and Development Institute (KURDI).

REFERENCES

1. Ang, H. G.; Kwik, W. L.; Theng, C. Y. Development of soymilk-A review. Food Chem. 1985, 17(4), 235-250.

2. Nielsen, I. L. F.; Williamson, G. Review of the factors affecting bioavailability of soy isoflavones in humans. Nutr. Cancer. 2007, 57(1), 1-10.

3. Villares, A.; Rostagno, M. A.; García-Lafuente, A.; Guillamón, E.; Martínez, J. A. Content and Profile of Isoflavones in Soy-Based Foods as a Function of the Production Process. Food. Bioprocess Technol. 2011, 4(1), 27-38.

4. Messina, M.; Nagata, C.; Wu, A. H. Estimated Asian adult soy protein and isoflavone intakes. Nutr. Cancer. 2006, 55(1), 1-12.

5. Nishinari, K.; Fang, Y.; Guo, S.; Phillips, G. O. Soy proteins: A review on composition, aggregation and emulsification. Food Hydrocolloids. 2014, 39, 301-318.

6. García, M. C.; Torre, M.; Marina, M. L.; Laborda, F. Composition and Characterization of Soyabean and Related Products. Crit. Rev. Food Sci. Nutr. 1997, 37(4), 361-391.

7. Achouri, A.; Boye, J. I.; Zamani, Y. Identification of volatile compounds in soymilk using solid-phase microextraction-gas chromatography. Food Chem. 2006, 99(4), 759-766.

8. Wang, Y. C.; Yu, R. C.; Yang, H. Y.; Chou, C. C. Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacteria. Food Microbiol. 2003, 20(3), 333-338.

9. Liu, Z. S.; Chang, S. K. C.; Li, L. T.; Tatsumi, E. Effect of selective thermal denaturation of soybean proteins on soymilk viscosity and tofu's physical properties. Food Res. Int. 2004, 37(8), 815-822.

10. Chen, Y.; Ono, T. The mechanisms for yuba formation and its stable lipid. J.Agric. Food Chem. 2010, 58(10), 6485-6489.

11. Dentith, S.; Lockwood, B. Development of techniques for the analysis of isoflavones in soy foods and nutraceuticals. Curr. Opin. Clin. Nutr. Metab. Care. 2008, 11(3), 242-247.

12. Le Botlan, D.; Rugraff, Y.; Martin, C.; Colonna, P. Quantitative determination of bound water in wheat starch by time domain NMR spectroscopy. Carbohydr. Res. 1998, 308(1-2), 29-36.

13. Fan, T. W. M. Metabolite profiling by one- and two-dimensional NMR analysis of complex mixtures. Prog. Nucl. Magn. Reson. Spectrosc. 1996, 28(2), 161-219.

14. Tardieu, A.; De Man, W.; This, H. Using one-dimensional (1D) and two-dimensional (2D) quantitative proton (1H) nuclear magnetic resonance spectroscopy (q NMR) for the identification and quantification of taste compounds in raw onion (Allium cepa L.) bulbs and

in aqueous solutions where onion tissues are soaked. Anal. Bioanal. Chem. 2010, 398(7-8), 3139-3153.

15. Valverde, J.; This, H. 1H NMR quantitative determination of photosynthetic pigments from green beans (Phaseolus vulgaris L.). J. Agric. Food Chem. 2008, 56(2), 314-320.

16. Cazor, A.; Deborde, C.; Moing, A.; Rolin, D.; This, H. Sucrose, glucose, and fructose extraction in aqueous carrot root extracts prepared at different temperatures by means of direct NMR measurements. J. Agric. Food Chem. 2006, 54(13), 4681-4686.

17. Weberskirch, L.; Luna, A.; Skoglund, S.; This, H. Comparison of two liquid-state NMR methods for the determination of saccharides in carrot (Daucus carota L.) roots. Anal. Bioanal. Chem. 2011, 399(1), 483-487.

18. Balayssac, S.; Trefi, S.; Gilard, V.; Malet-Martino, M.; Martino, R.; Delsuc, M. A. 2D and 3D DOSY 1H NMR, a useful tool for analysis of complex mixtures: Application to herbal drugs or dietary supplements for erectile dysfunction. J. Pharm. Biomed. Anal. 2009, 50(4), 602-612.

19. Chen, Y.; Yamaguchi, S.; Ono, T. Mechanism of the chemical composition changes of yuba prepared by a laboratory processing method. J. Agric. Food Chem. 2009, 57(9), 3831-3836.

20. Pirovani, C. P.; Macêdo, J. N. A.; Contim, L. A. S.; Matrangolo, F. S. V.; Loureiro, M. E.; Fontes, E. P. B. A sucrose binding protein homologue from soybean exhibits GTP-binding activity that functions independently of sucrose transport activity. Eur. J. Biochem. 2002, 269(16), 3998-4008.

21. Contim, L. A. S.; Waclawovsky, A. J.; Delú-Filho, N.; Pirovani, C. P.; Clarindo, W. R.; Loureiro, M. E.; Carvalho, C. R.; Fontes, E. P. B. The soybean sucrose binding protein gene family: Genomic organization, gene copy number and tissue-specific expression of the SBP2 promoter. J. Exp. Bot. 2003, 54(393), 2643-2653.

22. Kwak, E. J.; Lim, S. I. The effect of sugar, amino acid, metal ion, and NaCl on model Maillard reaction under pH control. Amino Acids. 2004, 27(1), 85-90.

23. Kim, J. C.; Mullan, B. P.; Pluske, J. R. Prediction of apparent, standardized, and true ileal digestible total and reactive lysine contents in heat-damaged soybean meal samples. J. Anim. Sci. 2012, 90, 137-139.

24. González-Vega, J. C.; Kim, B. G.; Htoo, J. K.; Lemme, A.; Stein, H. H. Amino acid digestibility in heated soybean meal fed to growing pigs. J. Anim. Sci. 2011, 89(11), 3617-3625.

25. Mottram, D. S. Flavour formation in meat and meat products: A review. Food Chem. 1998, 62(4), 415-424.

26. Akagawa, M.; Sasaki, T.; Suyama, K. Oxidative deamination of lysine residue in plasma protein of diabetic rats: Novel mechanism via the Maillard reaction. Eur. J. Biochem. 2002, 269(22), 5451-5458.

27. Hansen, D. F.; Kay, L. E. Determining valine side-chain rotamer conformations in proteins from methyl 13C chemical shifts: Application to the 360 kDa half-proteasome. J. Am. Chem. Soc. 2011, 133(21), 8272-8281.

28. Shallenberger, R. S.; Symmetry, chirality and topology in taste; Taste chemistry; Springer: US; 1993.

29. Campbell, L. J.; Gu, X.; Dewar, S. J.; Euston, S. R. Effects of heat treatment and glucono-δ-lactone-induced acidification on characteristics of soy protein isolate. Food Hydrocolloids. 2009, 23(2), 344-351.