Reducing weight loss of retorted soy protein tofu by using glucose- and microwave-pre-heating treatment

Yee K. Kaye, Azhar Mat Easa* & Noryati Ismail

School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia

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Summary Soy protein isolate tofu with or without glucose was subjected to a pre-heating treatment in a microwave oven before being thermally processed at 121 °C for a range of time between 5 and 25 min. The occurrence of the Maillard reaction during the processing of tofu containing glucose was shown by a decrease in pH and a change in colour of the tofu from pale white to brownish yellow. Retorted tofu prepared with glucose had a reduced weight loss and lower solubility in 1% sodium dodecyl sulphate solvent. This effect was most pronounced in the tofu containing glucose which had been preheated. Similarly, a lower percentage of glucose loss during retorting was seen in tofu containing glucose which had been preheated, which indicated that some level of the Maillard reaction may have occurred during the microwave pre-heating treatment. The decrease in solubility in sodium dodecyl sulphate solvent was thought to be due to the formation of the 'Maillard network'. It was hypothesized that this had provided a protection to the hydrophobic regions of the protein against attack from the solvent. The glycation effects and formation of the 'Maillard network' within tofu's internal structure were suggested as the major reasons for the reduced weight loss of tofu containing glucose.

Keywords Maillard reaction, 'Maillard network', protein solubility, retorting.

Introduction

The texture of thermally processed high protein foods can be influenced by a loss of moisture from the internal structure of the foods and formation of intermolecular cross-links within the food systems. The presence of small compounds such as reducing sugar in the food system is not regarded as an important element that can contribute to texture. The sugars have most often been associated with the occurrence of the Maillard browning reactions that affect the sensory and nutritional qualities of foods. The reaction between carbonyl compounds of reducing sugars and amino groups of protein could be characterized in a number of ways. These include a formation of coloured compounds (Hill *et al.*, 1992), a decrease in pH (Mat Easa *et al.*, 1996) and increase in net charge on the protein molecules as a result of glycation (Yaylayan *et al.*, 1992). During thermal processing the Maillard reaction is also known to produce a number of aromatic compounds, thus enabling unpleasant-tasting foods to be transformed into desirable flavour-rich products.

However, another aspect of the Maillard reaction that is often overlooked is its potential to induce a formation of strong non-disulphide cross-links (Hill & Mat Easa, 1998). These cross-links, which could be termed the 'Maillard cross-links', have been shown to enhance the gelation properties of an aqueous system containing a protein and a reducing sugar (Cabodevila *et al.*, 1994; Mat Easa, 1996) and affect the extrusion behaviour of soy extrudates (Hill & Mat Easa, 1998). Even though very little information is available on the influence of the

*Correspondent: Fax: 604 6573678;
e-mail : Azhar@usm.my

Maillard reaction on the texture of foods, Hill & Mat Easa (1998) postulated that such a reaction can contribute to the texture of foods during thermal processing. Therefore, the effect of incorporating a low molecular weight reducing sugar such as glucose into a high protein food such as tofu merits study. Tofu was chosen as the model for the study due to its ease of preparation and to the fact that retorted tofu has been reported to undergo a loss in weight due, possibly, to the breakdown in the protein microstructure (Nonaka *et al*., 1996). The weight loss of retorted tofu is known to cause shrinkage in size and change in texture (Kah Hui, 1998). It was envisaged that if strong cross-links occurred within the tofu structure upon retorting, a denser network could result and these could affect the final texture and the weight loss of tofu. An increase in net charge on the protein molecules as a result of glycation (Hill *et al*., 1992) could be expected to influence moisture retention.

It was our intention to investigate the effect of microwave pre-heating treatment and retorting on the occurrence of the Maillard reaction and its effect on percentage weight loss of a tofu system containing glucose.

Materials and methods

A commercial soy protein isolate powder (PP_{TM}500E, Protein Technologies International, St Louis, USA) and an industrial grade glucono-δ-lactone were used for the preparation of the tofu. Other chemicals and reagents used were of analytical grade and were either obtained from Fluka Chemical Corporation, Busch, Switzerland or Sigma Chemical Company, St Louis, USA.

Preparation of tofu

Soy protein isolate tofu with or without 5% glucose was prepared by dispersing 24.5 g of soy protein isolate powder in 300 mL of distilled water. The dispersion was stirred manually at a constant speed for around 5 min. After heating in a water bath at 50 ± 2 °C, the slurry was poured into a beaker filled with a freshly prepared 50 mL solution of 0.02 M glucono-δ-lactone (62 ± 3 °C), without stirring. The mixture was left for 15 min at around 50 °C for curd formation before transferring to a perforated round-shaped plastic container lined with a cheese cloth to drain off the water. A pressure of 1.6 mPa was applied for 2 h after which the tofu gel was firm enough for the pre-heating treatment in the microwave oven.

The effect of microwave pre-heating was studied by heating the tofu in a Sharp R-8320E microwave oven (output power of 650 W) at medium heat for 3 min. A round-shaped tofu sample with a diameter of 8 cm and a height of 2.3 cm was then cut into two halves and put into a can (300 × 407) containing distilled water which made up a 4 mm headspace. Before retorting, tofu was exhausted at 85 ± 3.0 °C for 15 min in a steam bath and seamed. The can was then retorted in a laboratory size autoclave (Hirayama HA –240M) at 121 °C for a range of time between 5 and 25 min. After thermal processing, the can was cooled down immediately under running water and stored at ambient temperature for 2 h before opening for analysis. At least three cans were taken out from each retorting treatment to perform the analysis.

pH measurement

The pH of the tofu was measured using a 691 Digital pH Meter (Metrohm Herisau, Switzerland).

Solubility estimation

Tofu was homogenized by using a Waring blender running at high speed for 30–40 s. Then 0.5 g of the homogenized tofu particulates was extracted in 10 mL of 1% sodium dodecyl sulphate (SDS) solvent for 14 h while shaking. The mixture was centrifuged using a Kubota 5100 centrifuge (Kubota Corporation, Tokyo, Japan) at 3500 r.p.m./2330 g for 20 min to remove undissolved materials. The supernatant was filtered through a Whatman number 4 filter paper. A volume of the supernatant was removed for protein determination using the Lowry method (Lowry *et al*., 1951).

Weight loss

The weight loss of tofu during retorting was estimated according to the methods of Nonaka *et al*. (1996);

$$\%\text{weight loss} = (a - b)/a \times 100\% \qquad (1)$$

where: a = initial weight before retorting, b = final weight after retorting.

Percentage loss of glucose

The glucose content of tofu and the can's liquor was estimated according to the methods of Nelson (1944) and Somogyi (1952) with some modifications. A 2-mL aliquot sample was mixed with 2 mL of copper Somogyi reagent and boiled for 10 min. After cooling under running water, 1.0 mL of Nelson reagent was added followed by 10 mL addition of distilled water. The absorbance at 660 nm was measured using a Shimadzu UV–160A spectrophotometer (Fischer Scientific, Pittsburgh, USA). Glucose concentration was determined after plotting a standard curve for glucose in the concentration range of 0–100 μg mL^{-1}.

Percentage loss of glucose was estimated according to the following equation:

$$\%\text{loss of glucose} = a/b \times 100\% \qquad (2)$$

where: a = amount of glucose in the can's liquor after exhausting and/or retorting, b = actual amount of glucose in tofu before exhausting and/or retorting.

The actual amount of glucose, b, in the tofu had to be determined as some glucose was lost during tofu preparation. The initial amount of glucose in tofu before exhausting was between 4.2 and 4.6%.

Data analysis

A minitab software version 10 for Windows was used to perform a one-way ANOVA. Least significant difference (LSD) was used to determine a statistical significant difference at $P \leq 0.05$.

Results and discussion

Changes in the pH of soy protein isolate tofu as a function of heating time are shown in Fig. 1. Tofu prepared without glucose did not show much change in pH as a result of retorting. The initial pH of tofu was 5.75 ± 0.05 and this was attributed mainly to the release of gluconic acid from the hydrolisis of glucono-δ-lactone. However, the pH values of tofu containing glucose decreased to lower values upon exhausting and continued to decrease with increasing retorting time. This decrease in pH with heating is known to occur due to formation of organic acids and loss of basic amino groups of protein as a result of the Maillard reaction (Hill *et al.*, 1992) between carbonyl group of glucose and amino groups of soy protein. The Maillard reaction also caused a change in colour of the tofu from pale white to brownish yellow (results not shown). For tofu prepared without glucose, the microwave pre-heating treatment did not seem to affect the pH values during retorting. However, the pH values of preheated tofu containing glucose was slightly lower than that retorted without the microwave treatment.

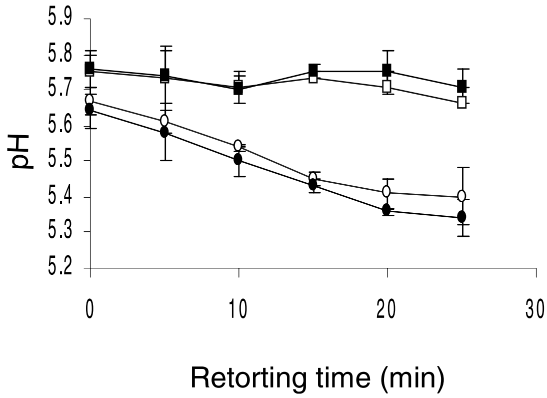

Figure 1 pH values of soy protein isolate tofu retorted without pre-heating treatment (□), with pre-heating treatment (■), prepared with glucose and retorted without pre-heating treatment (○) or with pre-heating treatment (●), as a function of retorting time . Error bars indicate standard deviation of three determinations.

Retorted tofu has been reported to undergo a loss in weight due possibly to the breakdown in the protein microstructure (Nonaka *et al.*, 1996), causing a shrinkage in size and change in texture (Kah Hui, 1998). In these experiments, the microwave pre-heating treatment caused a 16.5 ± 3.4% loss of moisture in tofu. Percentage weight loss due to retorting tofu is shown is Fig. 2 as a function of retorting time at 121 °C. For all tofu samples, weight loss during exhausting was between 3.5 and 10.0%. Preheated tofu containing glucose had the lowest percentage of weight loss upon exhausting. During the first 5 min of retorting, the percentage weight loss of unmicrowaved tofu retorted without glucose increased

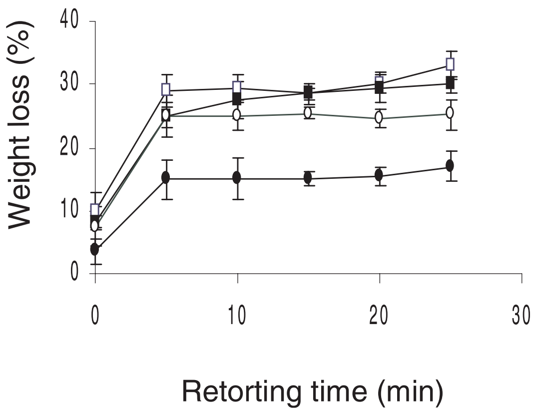

Figure 2 Weight loss of soy protein isolate tofu retorted without pre-heating treatment (□), with pre-heating treatment (■), prepared with glucose and retorted without pre-heating treatment (○) or with pre-heating treatment (●), as a function of retorting time. Error bars indicate standard deviation of three determinations.

significantly ($P < 0.05$) to 29.2%. The weight loss of preheated tofu prepared without glucose and unmicrowaved tofu prepared with glucose increased to about 25.1% after 5 min. However, tofu prepared without glucose showed a slight but continuous increase in weight loss during further retorting, whereas the weight loss of unmicrowaved tofu prepared with glucose remained at around 25.1% during further retorting.

A different response of weight loss during retorting was seen in the tofu containing glucose that was microwave preheated. Upon exhausting, the percentage weight loss of this tofu was 3.5% and this increased to around 15.0% during the first 5 min of retorting. The weight loss, however, remained at around 15–17% during further retorting. Since the tofu retorted without glucose was preheated in the microwave oven under similar conditions, the difference in percentage weight loss of the preheated tofu could well be due to the presence of glucose. It is possible that some stage of the Maillard reaction had occurred between glucose and tofu protein during the microwave treatment. This could be indicated by the difference in pH values of preheated tofu prepared with glucose and that retorted without pre-heating treatment (Fig. 1). Thus, the decreased weight loss of tofu containing glucose could be attributed partly to the increased net charge on the protein molecules as a result of glycation due to the Maillard reaction (Yaylayan *et al*., 1992; Hill *et al*., 1992). A similar assumption was made for soy protein gels containing reducing sugars, which had a reduced level of synerisis upon thermal processing (Cabodevila *et al*., 1994). Increasing the net charge on the protein molecules enabled entrapment of more water molecules, even though the pH was decreasing to lower values. However, it was also likely that the formation of covalent cross-links during heating altered the protein network (Hill & Mat Easa, 1998) and this helped in reducing weight loss of the tofu. These changes in the network of a protein gel system can be assessed by solubilizing the protein gel particles in a disrupting solvent such as a 1% sodium dodecyl sulphate solvent, which is known to be capable of disrupting hydrophobic interactions of protein and rendering the gel particles soluble. Figure 3 shows the relationship between protein solubility in the solvent as a function of retorting time. Before retorting, the solubility values of exhausted tofu samples prepared without glucose were between 99.1 and 105.3%. This could indicate that the protein network of this tofu was formed almost entirely by hydrophobic interactions (Kohyama *et al*., 1995). The percentage solubility of the tofu decreased with retorting time to about 60% after 25 min of retorting. This occurred because of the increasing importance of disulphide bonds as

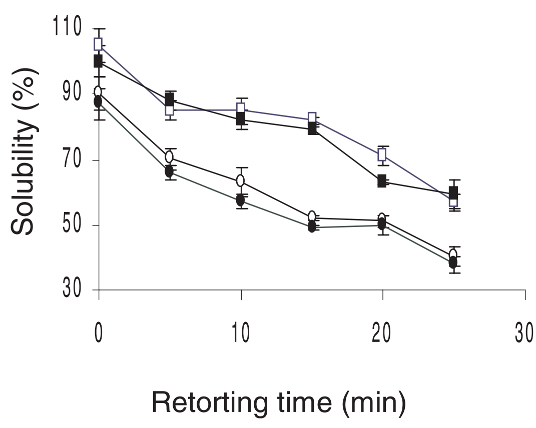

Figure 3 Solubility values in 1% SDS of soy protein isolate tofu retorted without pre-heating treatment (□), with pre-heating treatment (■), prepared with glucose and retorted without pre-heating treatment (○) or with pre-heating treatment (●), as a function of retorting time. Error bars indicate standard deviation of three determinations.

heating proceeded (Kah Hui, 1998). During retorting, the disulphide bonds were thought to stabilize the protein aggregates that were formed initially via hydrophobic interactions during the aggregation processes. For tofu containing glucose, the solubility values after exhausting were lower than tofu processed without sugar. A similar trend of decreasing solubility of tofu gel particles with retorting time was seen during further retorting. However, the solubility of tofu containing glucose was always lower than tofu processed without sugar. After 25 min of retorting, the solubility of tofu containing glucose was around 40%. This suggested that a different network existed in the tofu containing glucose, and since this was formed as a result of the Maillard reaction, it could well be termed the 'Maillard network' (Hill & Mat Easa, 1998). The nature of this network requires a separate study, but it is most probably comprised of additional covalent non-disulphide bonds (Hill & Mat Easa, 1998). The new network gave a form of protection to the hydrophobic regions of the protein against attack by the disrupting solvent. Thus the occurrence of the Maillard reaction followed by the formation of a 'Maillard network' within the tofu structure enabled more moisture to be entrapped. It can be imagined that such a dense network and a higher net charge on protein molecules would reduce the moisture loss of tofu during retorting. Even though a certain proportion of the Maillard reaction could occur in the unmicrowaved tofu containing glucose (Fig. 1), the reaction did not greatly influence the weight loss of tofu during retorting. If a pre-heating treatment was not performed, some glucose, together with moisture, could leach out to the can's liquor during retorting before an initial stage of the Maillard reaction could occur within the tofu internal structure. Thus, it can be expected that a reduced level of glycation occurred in the tofu containing glucose that was not preheated. More work will be done to test this hypothesis.

The percentage loss of glucose from retorted tofu as a function of retorting time is shown in Fig. 4. Before retorting, the percentage loss of glucose due to exhausting was relatively low, ranging between 3.8% and 4.1%. This result would suggest that, during exhausting, most glucose had been entrapped within the tofu, but a small proportion of glucose leached out from the tofu as a result of the mild heat treatment. During the first 5 min of retorting, however, the percentage loss of glucose increased significantly ($P < 0.05$) to higher values. This is almost similar to the trend observed for the weight loss of tofu seen in Fig. 2 and since glucose is soluble in water, this was expected to occur. Beyond the first 5 min of retorting, however, the level of glucose in the can's liquor decreased significantly ($P < 0.05$) with retorting time, even though the weight loss of most tofu had already reached a plateau (Fig. 2). This result would suggest that glucose in the can's liquor was involved in the Maillard reaction during retorting and it is probable that this occurred on the surface of tofu. It was also noted that the glucose content in the can's liquor of the preheated tofu was lower than that retorted without the pre-heating treatment. This may support the suggestion that the Maillard reaction had occurred during the microwave treatment. If some glucose took part in the Maillard reaction during microwave pre-heating and retorting, its level would be expected to decrease.

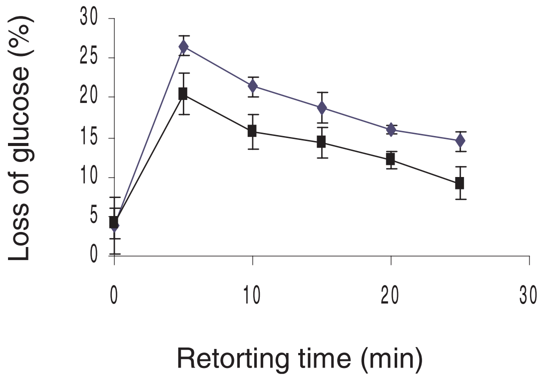

Figure 4 Percentage loss of glucose from soy protein isolate tofu prepared with glucose after retorting without pre-heating treatment (■) or with pre-heating treatment (♦), as a function of retorting time. Error bars indicate standard deviation of three determinations.

Conclusion

We suggest that the occurrence of the Maillard reaction within the tofu structure had caused a change in weight loss during retorting. However, induction of the reaction prior to retorting may result in a more pronounced effect. As sucrose could not induce a similar phenomenon and as browning occurred during processing, the Maillard reaction, probably through glycation effects and formation of new network, was thought to be the most probable mechanism. However, the specific roles of glucose, carbonyl compounds released during the reactions or other reducing sugars, during the thermal processing of high protein foods must await further studies.

Acknowledgments

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