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Effect of Thermal Processing on Available Lysine, Thiamine and Riboflavin Content in Soymilk

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Abstract: Soymilk was heated over a range of temperatures (90–140°C) and times (0–6 h). The available lysine, thiamine and riboflavin content of the soymilk samples were determined. There was no significant change in available lysine during a 3 h heating period at 95°C. At elevated temperatures of 120 and 140°C, optimum heat processed soymilk gave higher measured values of available lysine than did soymilk processed at 95°C. Prolonged heating at 120 and 140°C caused a decline in available lysine. Kinetic data on the thermal degradation of thiamine and riboflavin in soymilk were fitted with first-order kinetics and the kinetic parameters were determined. © 1998 SCI.

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Key words: soymilk; available lysine; thiamine; riboflavin; thermal processing

INTRODUCTION

A major source of nutritive loss during processing is the deterioration in protein quality caused by non-enzymatic Maillard browning. Maillard browning primarily involves the reaction of free amino groups and reducing sugars. The majority of the free amino groups participating in the reaction are the ε-amino groups of the essential amino acid lysine. If the ε amino acid group is blocked through chemical bonding, the affected lysine residue in the protein becomes unavailable for use in the body for protein synthesis. Although lysine is present in fairly high concentrations and may not be the growth-limiting amino acid in the proteins of soymilk, the extent of its loss may provide an index of general heat damage to the nutritional quality of the soymilk protein. Kwok and Niranjan (1995) have reviewed the effect of thermal processing on the protein quality and vitamins in soymilk. It has been shown that there is a high correlation between available lysine content in soymilk protein and protein efficiency ratio (PER) once the antinutritional factors in soymilks have been inactivated by heating and drying under various conditions (Van Buren *et al* 1964; Hackler *et al* 1965). Hackler and Stillings (1967) noted that the highest PER value for soymilk processed at 121°C is greater than the best PER value for soymilk processed at 93°C. It has also been reported that soymilk produced by direct steam processing, using rapid hydration hydrothermal cooking (RHHTC) at temperatures of 121–154°C, had a higher reactive lysine value and nutritional properties than that produced by traditional methods (Hung 1984; Kim 1989). All these results suggest that heat treatment at high temperatures under optimum conditions may result in better protein quality.

The amounts of thiamine and riboflavin in soymilk are comparable to those in cow's milk. Thiamine is water soluble and is one of the most unstable vitamins to moist heat treatment. Different processes involved in soymilk production such as soaking and blanching of the soybeans, and the subsequent heat treatments may lead to the loss of thiamine. Miskovsky and Stone (1987) compared the thiamine retention in soymilk processed by RHHTC, a high temperature (121–154°C)

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short time direct steam-infusion cooking method, with soymilk processed by the traditional extraction method which involved cooking the soy flour slurry at 99°C for 60 min. RHHTC soymilk was found to contain a higher concentration of thiamine. The greater retention was attributed to a shorter period of exposure to heat. No significant differences were found in riboflavin between the two soymilks indicating that it is less heat sensitive.

The purpose of this study was to investigate the kinetics of the thermal degradation of available lysine, thiamine and riboflavin in soymilk processed over a range of temperature (90, 120 and 140°C) and time (0–180 min.)

MATERIALS AND METHODS

Preparation of soymilk

Canadian No 1 soybeans (*Glycine max*) were soaked in water (bean-to-water, 1 : 10) for 14 h at 5°C. The soaked beans, along with the soaked water, were blended in a Waring blender at low speed for 5 min. The slurries were filtered through a nylon filter bag. The insoluble residue was discarded and the filtrate, containing about 6·5% total solids, was used in the experiments.

Heat treatment of soymilk

Heat treatments of soymilk were carried out in stainless-steel capillary tubes of 2 mm internal diameter, 0·56 mm wall thickness, and 6 m length, corresponding to a holding capacity of about 22 ml. The tubes were spiral-coiled with a valve at each end. The capillary tubes, which had a thin wall and large surface area, were highly efficient in heat transfer. The tubes were filled with soymilk using the suction created by a water aspirator. The filled tubes, with the valves closed, were immersed in a water bath for temperatures below 100°C, or in polyethylene glycol for higher temperatures. The heating temperatures and times cover a range from 90 to 140°C and 0 to 180 min, respectively. At the end of the heating time, the tubes were immediately transferred to a cold water bath. The time required to heat up the soymilk in the tubes to temperatures between 90 and 140°C varied between 6 and 9 s, while, for cooling, it took 3–7 s. Thus, the heating-up and cooling-down times were negligible in comparison with the holding time.

Determination of available lysine

Available lysine was determined by the method of Hall *et al* (1973). The soymilk sample was diluted three times with deionised water. The 0·5 ml of the diluted soymilk, 0·5 ml of 1 M $NaHCO_3$ and 1 ml of 1% trinitrobenzenesulphonic acid (TNBS) (Sigma Chemical Co, St Louis, MO, USA) were added and the mixture incubated for 75 min at 40°C. At the end of the incubation period, 3·0 ml of 11 M HCl was added. The reaction mixture was hydrolysed in a boiling water bath for 2 h. After filtering through Whatman no 42 filter paper and diluting to 10 ml, a 4-ml aliquot of the hydrolysate was extracted with two 5-ml volumes of diethyl ether. After removing the ether layer, the aqueous layer was diluted to 10 ml and the absorbance was measured at 415 nm. Pure L-lysine monohydrochloride (Aldrich Chemical Co, Gillingham, UK) was used as a standard.

Determination of thiamine and riboflavin

The high-performance liquid chromatographic (HPLC) method to determine thiamine and riboflavin in soy products, developed by Fernando and Murphy (1990), was modified. The soymilk samples were lyophilized in a laboratory freeze-drier for 48 h and the dried samples were stored in screw-cap test tubes at 5°C until use. About 0·6–0·75 g of freeze-dried soymilk solids were reconstituted in 10 ml deionized water. The mixture was stored at 5°C for 15 h for complete hydration. The solution was adjusted to pH 2 with 5 M HCl and autoclaved at 121°C for 15 min to hydrolyse the protein-bound vitamins without causing heat damage. The pH of the cooled sample was increased to 4·5 by 1 M NaOH to isoelectrically precipitate most of the proteins. The sample was centrifuged at 5000 × g for 5 min, and filtered through Whatman no 42 filter paper. The filtrate was made up to 25 ml with deionised water, refiltered through 0·45-μm membrane filter, and used in the quantitation of riboflavin via HPLC or subjected to oxidation for thiamine analysis followed by HPLC analysis. The oxidised thiamine solution was neutralised by concentrated H_3PO_4 to ensure a pH level acceptable to the C_{18} column. The vitamins were separated and quantified by HPLC on a reversed-phase C_{18} system (Rainin 5 μm RP-18 Microsorb-MV 100A° column) with fluorescence detection (Dynamax FL-1 detector). The vitamins were monitored fluorimetrically at 436 nm excitation and 536 nm emission for riboflavin; 360 nm excitation and 426 nm emission for thiochrome. The mobile phase was 15 mM sodium acetate in 40% methanol, pH 5·5, instead of acetonitrile, to increase the polarity in order to give a better separation.

The standard curves were prepared from thiamine hydrochloride and riboflavin standards (Sigma). Thiamine and riboflavin standard solutions were injected into the HPLC system and the peak areas in the chromatogram were recorded. Linearity was observed in the chromatographic response to the concentration of the vitamin. The linear regression equation of the calibration curve obtained for thiamin was

$y = 35{\cdot}74x + 0{\cdot}88$ ($r = 0{\cdot}997$), and that for riboflavin was $y = 37{\cdot}66x - 0{\cdot}05$ ($r = 0{\cdot}995$), where y is the peak area and x is the concentration of the vitamin in mg kg^{-1}.

Recovery efficiency was analyzed by spiking samples with 50 µl of the standard stock solutions of thiamine (100 mg kg^{-1}) and riboflavin (50 mg kg^{-1}).

RESULTS AND DISCUSSION

Effect of thermal processing on available lysine content in soymilk

The TNBS method of Kakade and Liener (1969), revised by Hall *et al* (1973), for determining available lysine was chosen after reviewing the literature for procedures available and after preliminary trials. Concon (1975) developed a spectrophotometric method for the determination of lysine in cereal grains without hydrolysis of the sample. This procedure is based on the reaction of dinitrobenzenesulphonate (DNBS) which, under prescribed conditions, is quite specific for the ε-amino groups of proteins or free lysine. The method of Concon required less time but suffered from poor repeatability when applied to the determination of available lysine in soymilk. The TNBS method of Hall yielded values of available lysine in soymilk protein similar to reported literature values with reasonably good reproducibility so this method was used throughout this study.

The available lysine content in soymilk, subjected to various degrees of heat treatment, is given in Table 1. When heating was prolonged at 95°C, there was no significant change in available lysine. This result is in agreement with the work of Van Buren *et al* (1964) and Hackler *et al* (1965) who reported that little change took place in the available lysine content of soymilk during a 6 h heating period at 93°C. When the heating temperatures were elevated to 120°C and 140°C, there was a marked initial increase in available lysine followed by a gradual decline on prolonged heating. The initial rise in available lysine is somewhat surprising since protein damage caused by heat is usually accompanied by a decline in available lysine. These results, however, can be explained by the complicated quaternary structure of the 7S and 11S globular proteins in soymilk. The chemical structure and heat-induced phenomena of soybean proteins have been reviewed by Yamauchi *et al* (1991). It is known that the 7S (β-conglycinin) and 11S (glycinin) fractions are made up of subunits which undergo association and dissociation reversibly. These changes are affected by factors such as temperature, pH, and ionic strength. Heating causes dissociation of both 7S and 11S into subunits. Further heating causes interaction between the dissociated subunits of 7S and 11S to form a soluble polymerised aggregate and insoluble precipitate. The available lysine content measured in soymilk protein is affected not only by the amount of free ε-amino groups of lysine but also by the extent of dissociation and association of the 7S and 11S globulins induced by heat. Chemical reactions such as the Maillard reaction and cross linking between polypeptide chains by acylation of free amino groups will result in a decrease in available lysine. On the other hand, heat-induced unfolding and dissociation of the globulins may have higher measured available lysine values than those of the native, unheated proteins because of the greater accessibility of the test reagent. Khaleque and Wallace (1975) found that the nutritive value (available lysine, available essential amino acids and pepsin digestibility) of soymilk samples prepared by the carbonate presoaking procedure was higher than that of the standard sample prepared from beans presoaked in water and processed at 115°C for 18 min. The higher availability of lysine and the higher pepsin digestibility of the proteins from carbonate presoaked preparations are possibly due to the unfolding of the protein molecule as a result of the combined action of heat and alkali, thus enabling more amino groups to become available to react with the test reagent and also favouring enzymic attack. Since soy protein is compactly folded and a high intensity of heat treatment is necessary to unfold the protein molecule completely, it is possible that some ε-amino groups may be buried within the protein molecule and thus may be unable to react with the test reagent (TNBS). It appeared that heating at 95°C and moderate heating at 120 and 140°C

TABLE 1
Effect of heat treatment on the available lysine content in soymilk (mean ± SD, $n = 3$)

Temperature (°C)	*Heating time* (*min*)	*Available lysine* (*g per 16 g N*)
95	0	5·18 ± 0·13
	30	5·19 ± 0·15
	60	5·28 ± 0·15
	90	5·12 ± 0·16
	120	5·02 ± 0·16
	150	5·19 ± 0·12
	180	5·16 ± 0·05
120	0	5·06 ± 0·12
	10	5·95 ± 0·17
	20	5·93 ± 0·14
	30	5·85 ± 0·07
	40	5·70 ± 0·15
	50	5·42 ± 0·19
140	0	5·17 ± 0·10
	1	5·06 ± 0·21
	3	5·63 ± 0·06
	5	5·58 ± 0·26
	7	5·22 ± 0·10
	10	4·78 ± 0·16

caused little damage to the available lysine in soymilk. This is probably due to the fact that soluble sugars in soybeans are mainly sucrose, raffinose and stachyose, all of which are non-reducing sugars. The low reducing sugar content makes the loss of available lysine due to Maillard reactions insignificant when soymilk is moderately heated. On the other hand, heating at high temperatures (120 and 140°C) greatly facilitated the unfolding and dissociation of the globulins, exposing more free amino groups of lysine at the molecular surface, and hence resulted in higher measured values. With prolonged heating at these high temperatures, the unfolded protein molecules interact to form aggregates, blocking the free amino groups of lysine, and therefore decreased the measured available lysine values. These results conform with the work of Van Buren *et al* (1964) who found that the optimum heat processed soymilk at 121°C gave higher values of available lysine than did soymilk processed at 93°C. It is interesting to note that, in this present study, the maximum available lysine value reached is higher at 120°C than at 140°C. This is probably due to the greater extent of protein aggregation and/or chemical reactions taking place on prolonged heating at 140°C.

It has been shown that the available lysine content correlated significantly with the biological value (PER) (Van Buren *et al* 1964; Hackler *et al* 1965) and the pepsin digestibility (Khaleque and Wallace 1975) of heat-processed soymilk. The results in this study showed that the available lysine content in soymilk can be maximized, and the protein quality and digestibility improved, by proper control of processing temperature and time. Among the three different temperatures and the various heating times investigated, heating soymilk at 120°C for 10–20 min gave highest available lysine content.

Effect of thermal processing on thiamine and riboflavin in soymilk

The percentage recovery in the HPLC analysis, based on the mean and standard deviation of 12 determinations, were found to be 75 ± 6 and 71 ± 6 for thiamine and riboflavin, respectively. The data show that there are some losses of the vitamins. It should be noted that the recovery studies only test the efficiency of recovery of pure compounds, but do not measure the efficiency of extraction from the sample. High protein content of soymilk and occlusion of vitamins in the protein precipitate may account for the incomplete recovery. The thiamine and riboflavin contents in soymilk were calculated from the concentrations detected in the HPLC analysis divided by the % recovery.

The thiamine and riboflavin contents of raw soymilk were determined to have an average value of 14·06 and 2·44 mg kg^{-1} soymilk solids, respectively. The rate of destruction curves for thiamine and riboflavin in soymilk heated at different temperatures are shown in Figs 1 and 2. Each data point on these graphs represents the mean of two duplicate runs. The linear behaviour of the semi-log plots suggested that the thermal degradation of thiamine and riboflavin in soymilk is first order in nature. From the slopes of the straight lines (obtained by linear regression analysis), the interaction rate constants at each temperature were calculated. The reciprocal of the slope of

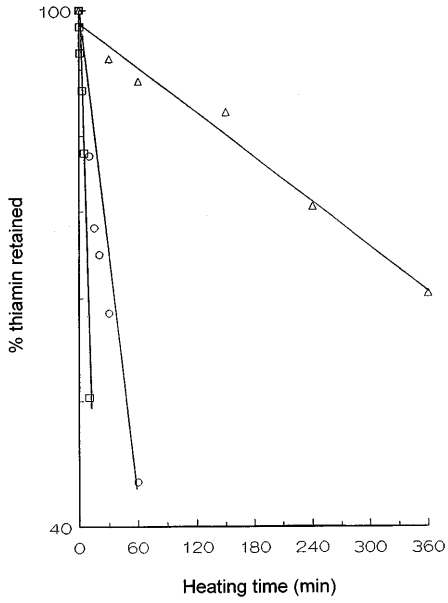

Fig 1. Rate of destruction curves for thiamine in soymilk. (△: 90°C, ○: 120°C, □: 140°C)

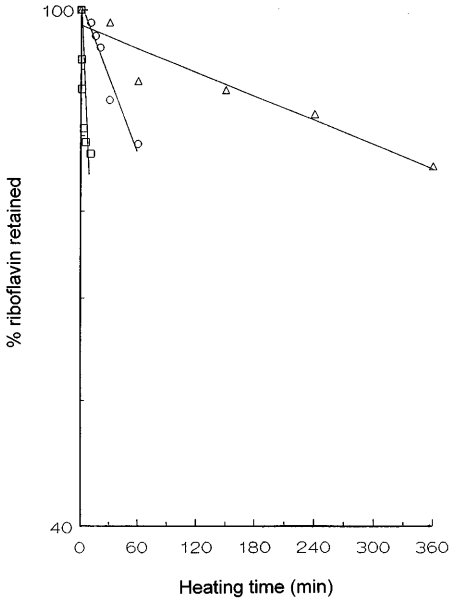

Fig 2. Rate of destruction curves for riboflavin in soymilk. (△: 90°C, ○: 120°C, □: 140°C)

the straight line is the decimal reduction time (D value), which is the time for 90% destruction of the vitamin in soymilk at the specified temperature. The D value can also be related to the first-order reaction rate constant: $D = 2{\cdot}303/k$. The calculated values for k and D, as shown in Table 2, represent the heat stability of these vitamins in soymilk.

Figure 3 gives the thermal destruction time curves for thiamine and riboflavin in soymilk as plots of the logarithm of the D values against the corresponding temperatures. The curves appeared to be straight lines, which were fitted with linear regression analysis. From the reciprocal of the slopes of these curves, the Z values (temperature difference in °C effecting a 10-fold change in D) were determined to be 30°C and 36°C for thiamine and riboflavin, respectively.

The effect of temperature on the destruction of thiamine and riboflavin can also be studied in terms of the Arrhenius equation:

$$\ln k = \ln k_0 - E_a/RT$$

in which k is the reaction rate constant, T the absolute temperature, R the gas constant, E_a the activation energy and k_0 the frequency factor. The Arrhenius plot, in which $\ln k$ is plotted against the reciprocal of the absolute temperature, is shown in Fig 4. The linearity of the plot indicates the conformity of the experimental data with the Arrhenius equation. From the slopes and intercepts of the Arrhenius plot, E_a and k_0 were calculated. Both E_a and Z characterize the temperature dependence of the reaction. The kinetic parameters k_0, E_a, Z were determined to be $1{\cdot}13 \times 10^{11}$ min^{-1}, 97·0 kJ mol^{-1} and 30°C for thiamine; and $6{\cdot}87 \times 10^{8}$ min^{-1}, 83·3 kJ mol^{-1} and 36°C for riboflavin, respectively.

Studies on the kinetics of the thermal degradation of thiamine in food products have been reported by earlier investigators. Mulley *et al* (1975) described the thermal degradation of thiamine in buffered solution (pH 6), and in pea puree, beef puree and peas-in-brine puree with first-order kinetics and E_a values were calculated to be in the range 113–123 kJ mol^{-1} (27–29·4 kcal mol^{-1}). However, some workers observed deviations from first order reactions. Mulley *et al* (1975) attributed the deviations to the heating equipment used by some workers which could not be operated under ideal conditions. The lag periods for come-up and cooling times could have introduced errors. In this study, the use of a capillary stainless steel tube for heating soymilk greatly facilitated the heat transfer. The more precise control of the heating temperature and time in this experiment resulted in more accurate kinetic data, and therefore

TABLE 2
Reaction rate constants (k) and decimal reduction time (D) for thermal degradation of thiamine and riboflavin in soymilk

Vitamin	*Temperature* (°C)	k (min^{-1})	D (min)
Thiamine	90	$1{\cdot}32 \times 10^{-3}$	1745
	120	$1{\cdot}29 \times 10^{-2}$	178
	140	$6{\cdot}66 \times 10^{-2}$	35
Riboflavin	90	$7{\cdot}05 \times 10^{-4}$	3268
	120	$4{\cdot}26 \times 10^{-3}$	540
	140	$2{\cdot}12 \times 10^{-2}$	109

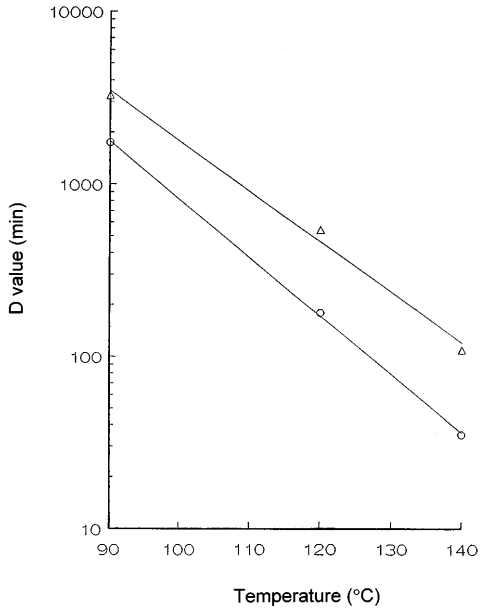

Fig 3. Relationship between decimal reduction time (D value) and heating temperature for thermal degradation of thiamine and riboflavin in soymilk. (○: thiamine, △: riboflavin)

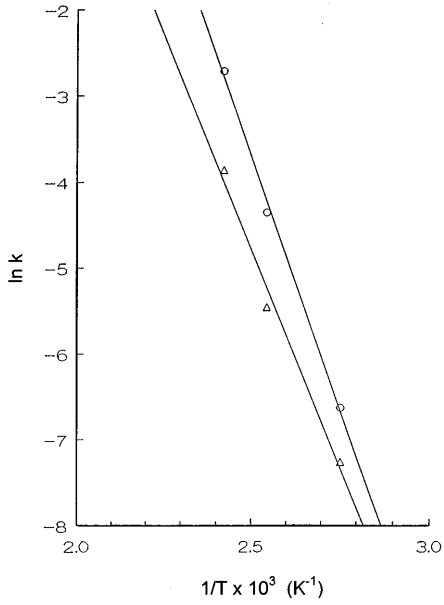

Fig 4. Arrhenius plot for thermal degradation of thiamine and riboflavin in soymilk. (○: thiamine, △: riboflavin)

first order kinetics were observed. Kessler and Fink (1986) fitted the kinetic data on the thermal decomposition of thiamine in cow's milk with a second order reaction kinetics and determined the kinetic parameters k_0 and E_a to be $5{\cdot}14 \times 10^{11}$ min^{-1} and 100·8 kJ mol^{-1}, respectively. These values are in close agreement with those for thiamine degradation in soymilk, as determined in this study, probably due to the fact that dairy milk and soymilk are similar in chemical composition.

As compared to thiamine, thermal degradation of riboflavin in soymilk was found to have greater D values (or smaller k) (Table 2) and smaller E_a (or larger Z), implying that riboflavin is more heat stable and less temperature sensitive than thiamine. Therefore, thiamine is a more critical nutrient factor to control during thermal processing. From the experimental results, about 12%, 32% and 3% of the thiamine was lost in soymilk processed at 90°C for 60 min, 120°C for 15 min and 140°C for 30 s, respectively, indicating the superiority of UHT heat treatment in maximizing vitamin retention. However, in the design of an optimum thermal process for soymilk, apart from maximising vitamin retention, the following conditions should also be obtained simultaneously: maximum destruction of bacterial spores, maximum destruction of antinutritional factors, maximum protein quality and minimum degradation of sensory quality. Under these constraints, some loss of vitamin is an inevitable consequence of thermal processing.

CONCLUSIONS

Heat treatment may cause an increase or decrease in the measured available lysine content of soymilk depending on the temperature and time used. Apparent increases may occur under optimum heating conditions due to the complete unfolding and dissociation of the soymilk protein, exposing more free ε-amino groups of lysine at the molecular surface. On the other hand, severe heating causes protein aggregation and chemical reactions such as Maillard reactions and cross-linking of the unfolded peptide chains, resulting in a decrease in the available lysine content. It was found that heating at 95°C for up to 3 h caused practically no change in available lysine in soymilk. Optimum heat processed soymilk at 120°C and 140°C gave higher measured values of available lysine than did soymilk processed at 95°C. The highest available lysine was found in soymilk processed at 120°C for 10–20 min. Extensive heating at 120 and 140°C caused a drop in available lysine. The available lysine content in heat-processed soymilk has a nutritional implication since it has been correlated with the biological value (PER) and the pepsin digestibility.

The kinetic data on the thermal destruction of thiamine and riboflavin in soymilk can be fitted with a first-order kinetics. Thiamine was found to be much more heat sensitive than riboflavin. The kinetic parameters k_0, E_a and Z were determined to be $1{\cdot}13 \times 10^{11}$ min^{-1}, 97·0 kJ mol^{-1} and 30°C for thiamine; and $6{\cdot}87 \times 10^{8}$ min^{-1}, 83·3 kJ mol^{-1} and 36°C for riboflavin, respectively.

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