Kinetics of degradation of saponins in soybean flour (*Glycine max.*) during food processing

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Abstract

Saponin glycosides present in a wide variety of plants have the ability to haemolyse red blood cells. They are known to be relatively heat stable. The present study aims at the development of a kinetic model for degradation of saponins in soybean flour (*Glycine max.*) subjected to a defined set of processing conditions. This study was carried out at isothermal conditions over a temperature range of 80–130 °C, and also under nonisothermal conditions in three different cooking methods viz., open pan, pressure cooking and cooking in a recently developed and patented fuel efficient 'EcoCooker'. The degradation of saponins was adequately modeled by the Arrhenius equation. Using the time–temperature data of the nonisothermal heat process and isothermal kinetic rate parameters, a mathematical model has been developed to predict the degradation of saponins in any nonisothermal heating process of known time–temperature profiles.

Keywords: Saponins; Degradation; Kinetics; Soybean; Cookers

1. Introduction

Saponins are a diverse group of biologically active glycosides that occur in a wide variety of plants. Each saponin consists of one or more sugar moieties bonded to a 'sapogenin' aglycon. The sugar moieties may be glucose, galactose, or a pentose or methylpentose, while the aglycones can be divided into triterpenoid (C_{30}) and steroid (C_{27}) sapogenins (Schwarz, 1993). Soybean is known to contain triterpenoid saponins (Bondi, Birk, & Gestetner, 1973; Wolf & Betty, 1970). The aglycones are divided into soyasapogenols A, B, C, D and E; their glycosides are correspondingly called as group A saponins, group B saponins and so on, respectively. Triterpenoid saponins are synthesized in plants via the isoprenoid pathway by cyclization of 2,3-oxidosqualene (Haralampidis, Trojanowska, & Osbourn, 2002).

They generally have a characteristic bitter or astringent taste (Rackis, Sessa, & Honig, 1966) and are highly toxic to cold-blooded animals. Group A soyasaponins are strongly responsible for this taste (Okubo et al., 1992). Saponins have detergent properties, forming oil-in-water emulsions and producing copious quantities of foam when dissolved in water. They are known for their ability to haemolyse red blood cells. Ethanol extract of soyabean has higher haemolytic effect than that extracted with saline and 0.01 M phosphate buffer (pH 7.2) (Birk, Bondi, Gestetner, & Ishaaya, 1963; Oboh, Olaleye, & Akinyosoye, 1999).

The major dietary source of saponin is legumes. The presence of saponins in soya is of particular importance because of its increasing use as a source of proteins and

nutraceutical(s). Soybean saponins possess membranolytic activity, as indicated by their interaction with human colon carcinoma cells. This explains their role as an anticarcinogen (Rao & Sung, 1995; Sung, Kendall, & Rao, 1995). The blood cholesterol-lowering properties of soybean saponins are of particular interest in human nutrition. Saponins cause a depletion of body cholesterol by preventing its reabsorption, thus increasing its excretion. Soluble fibers in legumes are known to increase the viscosity of gastric and intestinal contents, and may be one of the factors responsible for the lowering of cholesterol levels (Amarowicz, Shimoyamada, & Okubo, 1994; Rao & Gurfinkel, 2000). Studies on health benefits of saponins suggest their hepatoprotective and hypocholesterolemic activity, but these studies are limited to cell culture and few animal studies. Studies on rats have shown soybean saponin to have an anabolic effect on bone components, suggesting its role as a nutritional factor in the prevention of osteoporosis (Ono & Yamaguchi, 1999).

Total saponin content in the seed hypocotyls fraction of soybean ranges from 0.62% to 6.16%. Saponin content in soybean is dependant on the variety rather than on the cultivation year. Soybean harvested at different degrees of maturity has varied saponin content, although the nature of this variation is insufficient to exert an influence upon the varietal distribution. Distribution of saponins in soybean plants was investigated and found that acetyl-soyasaponins A1 and A4 occur only in seed hypocotyls of soybean plants (Shimoyamada, Kudo, Okubo, Yamauchi, & Harada, 1990).

Kinetic studies help food process engineers and scientists to optimize processing systems and design processes, to improve and optimize existing processes and develop control systems for processing operations in terms of reaction rates. These studies help to predict how quick the reaction mixture will attain equilibrium condition, and also in predicting the reaction mechanism (Atkins, 1990). The rate can vary with temperature, heating protocol, moisture content or water activity, pH, pressure, amount of reactants and many other experimental conditions (Fennema & Tannenbaum, 1985). Knowledge of kinetic modeling aids predicting the intentional or unintentional food quality losses as a function of processing conditions.

Processing methods such as soaking and discarding the soaked water reduced saponins content by 3–65% in legumes (Curl, Price, & Fenwick, 1985; Duhan, Khetarpual, & Bishnoi, 2001; Kataria, Chauhan, & Gandhi, 1988; Kataria, Chauhan, & Punia, 1989; Khokhar & Chauhan, 1986). Pressure cooking and ordinary cooking of soaked and dehulled legume seeds reduce the same by 28–38% (Duhan et al., 2001). The maximum reduction (81–84%) in saponins was observed for soaked, dehulled and autoclaved sample of faba beans (Sharma & Sehgal, 1992). Sprouting reduced saponin content of moth beans by 56–66%. Enzymatic degradation may be a possible explanation for this observation (Khokhar & Chauhan, 1986). Saponin content was significantly decreased (46–53%) in foods prepared from oven-heated seeds (Gahlawat & Sehgal, 1992).

Different processing methods are being used in usual household cooking. Some of them are normal open pan cooking, pressure cooking and slow cooking using slow cookers. A fuel-efficient cooker has been recently developed in our institute (Joshi & Patel, 2003). The principle of this cooker is based on multiple effect evaporation; slow heating proportional to pick up rate of heat by the cooking vessel, and insulation; and on the logic of combining these principles in one unit. A survey of literature over the past 50 years did not give any information on the degradation kinetics of saponins in foods cooked differently.

The main objectives of the present study were to (i) determine the kinetic parameters for saponins degradation in soybean flour (*Glycine max.*) over a temperature range of 80–130 °C (isothermal conditions), (ii) study the degradation kinetics of saponins under different cooking methods (nonisothermal conditions), (iii) develop a mathematical model relating the calculated kinetic rate data from the isothermal conditions and extend the same by taking into account the time–temperature profiles under different cooking methods (nonisothermal conditions), and (iv) apply this model to predict the degradation of saponins for nonisothermal process from the time–temperature data, and comparing it with the observed degradation. This could then be used to asses the nutritional values of the cooked item as a function of method of cooking.

2. Materials and methods

Defatted soybean flour was received as a gift sample from Marico Industries Ltd., Mumbai. Saponin standard was obtained from E. Merck, Germany. TLC plates of silica gel GF_{254} (0.25 mm) were purchased from E. Merck, Germany. All the reagents used were of analytical grade.

2.1. Heat treatment

Heat treatments were carried out at different temperatures (80, 90, 100, 110, 120, and 130 °C) over a time period of 0–60 min. A water bath was used as a heating device for temperatures up to 100 °C, while for the studies at 110, 120 and 130 °C an autoclave (Parr) was used. Ten grams of defatted soybean flour was transferred into a 100 ml beaker containing 100 ml distilled water pre-heated to the desired temperature conditions and the saponin content of the entire mass was analyzed at specified time intervals.

2.2. Cooking methods

Normal open pan cooking (30 min at a gas flow rate of 15 ml/s), pressure-cooking (20 min at a gas flow rate of 15 ml/s) and the newly developed slow cooker named 'EcoCooker' (30 min at 'sim', i.e. at a gas flow rate of 6 ml/s and 30 min holding period) were selected as different cooking methods for the defatted soybean flour. The time of heating to desired temperature and gas flow rates for open pan and pressure-cooking were selected as per the protocol used in household practices. The heating time and gas flow rates for 'EcoCooker' were selected as per the instructions given for its usage (Joshi & Patel, 2003). The samples were withdrawn periodically and analyzed for saponin content.

2.3. Time–temperature data

Time–temperature data for each cooking method was monitored using a thermocouple of ±1 °C accuracy.

2.4. Determination of saponins

2.4.1. Standard curve for saponins

Standard solutions were prepared by dissolving the saponins in 90% ethanol. Five microlitres of aliquots (containing 2–20 μg) were applied by Camag Linomat IV applicator on silica gel GF_{254} TLC plate, and run up to 9 cm using *n*-butanol–ethanol–ammonia (7:2:5) as the solvent system. The plates were visualized by saturating them with 15% sulphuric acid and heating for 15 min at 120 °C. The plates so developed showed four spots at R_f 0.39, 0.41, 0.44 and 0.48, which were densitometrically determined at 540 nm on a Camag III densitometer. The peak areas corresponding to each of these spots were summed (y) and plotted against the concentration of saponin, μg (x). The standard curve so obtained gave a regression equation, $y = 175.44x + 172.34$ ($R^2 = 0.99$).

2.4.2. Saponin content in soybean flour samples

Saponins were analyzed using the procedure described by Gurfinkel and Rao (2002) and Fenwick and Oakenfull (1983). For the estimation of saponins, 10 g soybean flour was transferred into a 500 ml conical flask containing 100 ml distilled water pre-heated to the desired temperature. After the specified time of heat treatment(s), the contents were extracted with ethanol (70 ml) for 4 h at 60 °C on a mechanical orbital shaker. The ethanol extract containing the saponin was then filtered, the residue washed with additional ethanol, and the total volume made to 100 ml. A cleanup procedure was then applied to separate the saponins from nonsaponin constituents of the extract. The extract was mixed with 0.4 M ammonium sulphate in 1:1 ratio and left to stand overnight at 20 °C. This resulted in the formation of nonsaponin precipitate. This precipitate was removed by centrifugation (8000 rpm, 30 min). The precipitate was washed three times with a 1:1 mixture of ethanol and 0.4 M ammonium sulfate to release any entrapped saponins. The final volume was made to 10 ml. Five microlitres was spotted as for standard saponins, and the total saponins estimated by using the regression equation as given above for the standard.

2.5. Kinetic calculations

A general reaction rate expression for the degradation kinetics can be written as follows (Labuza & Riboh, 1982; Ramaswami, Van De Voort, & Ghasal, 1989; Van Boekel, 1996).

$$-\mathrm{d}[C]/\mathrm{d}t = k[C]^m \quad (1)$$

where 'C' is the quantitative value of the concentration of the degraded molecule under consideration, 'k' is the reaction rate constant, and 'm' is the order of the reaction. The equation for first order rate kinetics can be obtained by the integration of Eq. (1) as

$$\ln([C_t]/[C]_0) = -kt \quad (2)$$

where '$[C]_0$' is the concentration of the reactants under consideration at time 0, and '$[C]_t$' is the value after reaction (heating) time 't'.

The relationship of the reaction rate to temperature can be quantified by the Arrhenius equation as indicated below

$$k = A_0 \exp(-E_a/RT) \quad (3)$$

where 'E_a' (cal M^{-1}) is the activation energy of the reaction, 'R' is the gas constant (1.987 cal M^{-1} K^{-1}), 'T' is absolute temperature (K), and 'A_0' is the pre-exponential factor.

Each experiment was done in triplicates and average values were taken for the analysis. Kinetic data were analyzed by regression analysis using MS Excel package.

3. Results and discussion

3.1. Amount of saponins in soybean flour at isothermal conditions

Table 1 shows the effect of heat treatment at different temperatures (80–130 °C) for different time periods on the resultant concentration of saponins. The initial concentration of saponins in soybean flour found in this study was 4.34 μg/g, whereas the saponin contents of defatted soy flour reported by Gurfinkel and Rao (2002) was 5.8 μg/g. The variation in the content may be due to varietal difference. During isothermal conditions, about 75% loss of saponins was observed.

Table 1
Effect of heating on saponin concentration[a] (μg/g) in soya flour[b] at various temperatures

Time (min)	80 °C	90 °C	100 °C	110 °C	120 °C	130 °C
10	3.08 ± 0.11	2.71 ± 0.27	2.65 ± 0.04	1.68 ± 0.15	1.77 ± 0.10	1.78 ± 0.15
20	2.96 ± 0.03	2.41 ± 0.12	2.47 ± 0.03	1.32 ± 0.15	1.31 ± 0.18	1.39 ± 0.06
30	2.74 ± 0.19	2.16 ± 0.24	2.04 ± 0.08	1.21 ± 0.12	1.08 ± 0.21	0.96 ± 0.06
40	2.61 ± 0.04	1.99 ± 0.12	1.76 ± 0.06	–	–	–
50	2.24 ± 0.09	1.83 ± 0.04	1.53 ± 0.13	–	–	–
60	2.03 ± 0.15	1.62 ± 0.03	1.48 ± 0.13	–	–	–

[a] Values are mean ± SD of three of more individual determinations.
[b] The saponin content of the soya flour chosen in the study was 4.34 ± 0.66 μg/g.

Saponins are reported to be relatively heat stable constituents (Onning, Jullerat, Fay, & Asp, 1994).

3.2. Kinetic data for degradation of saponins in soybean flour

In order to arrive at the reaction rate constants, a first order degradation was presumed. Accordingly, '$\ln([C]_t/[C]_0)$' was plotted vs. 't', from which rate constant, 'k' was calculated as the slope of the linear plot. Fig. 1 shows the representative plots for degradation of saponins at 90, 100 and 120 °C. A correlation coefficient >0.9 in all the cases confirmed the degradation to follow the first order kinetics as assumed. Similar data were obtained at all the other temperatures.

The time required for saponins to degrade to 50% of their original value, $t_{1/2}$, was calculated from the rate constant as '$0.693/k$'. Table 2 also shows the rate constants and '$t_{1/2}$' (min) for saponins in soybean flour. It is evident that the rate of saponins degradation increased with an increase in temperature. The rate constant increased from 0.0085 min^{-1} at 80 °C to 0.0309 min^{-1} at 130 °C. Activation energy E_a (Kcal M^{-1}) was calculated from the slope of the graph obtained by plotting '$\ln k$' vs. '$1/T$'. Fig. 2 shows the Arrhenius plot for saponins degradation in soybean flour. The activation energy obtained from the slope of linear plot was 7.567 Kcal M^{-1}.

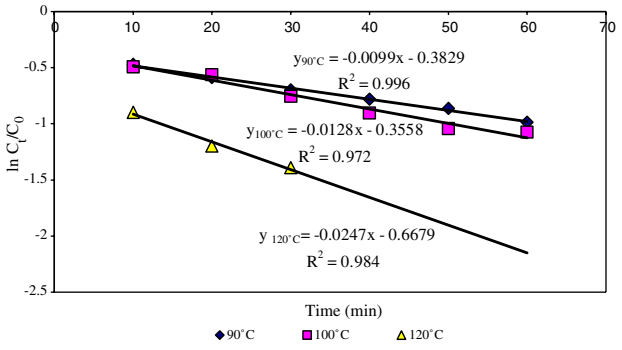

Fig. 1. First order plot of saponin degradation in soya flour at 90, 100 and 120 °C.

Table 2
Rate constant (k), correlation coefficient (R^2) and half-life ($t_{1/2}$) of saponin degradation in soya flour

Temperature (°C)	Rate constant k (min^{-1})	R^2	$t_{1\backslash 2}$ (min)
80	0.0085	0.96	81.53
90	0.0099	0.99	70.00
100	0.0127	0.97	54.57
110	0.0164	0.93	42.25
120	0.0247	0.96	28.06
130	0.0309	0.98	22.43

3.3. Time–temperature data of the three modes of cooking

To extend the results obtained from isothermal heat treatment experiments to the nonisothermal condition encountered in the three modes of cooking, viz. open pan cooking, pressure cooking and cooking in 'Eco-Cooker', time–temperature data during the processing of each was recorded (Fig. 3).

3.4. Degradation profile and half-life values of saponins in soybean flour under the three modes of cooking

Saponins degradation was followed in each of these modes of cooking similar to the experiment for soybean

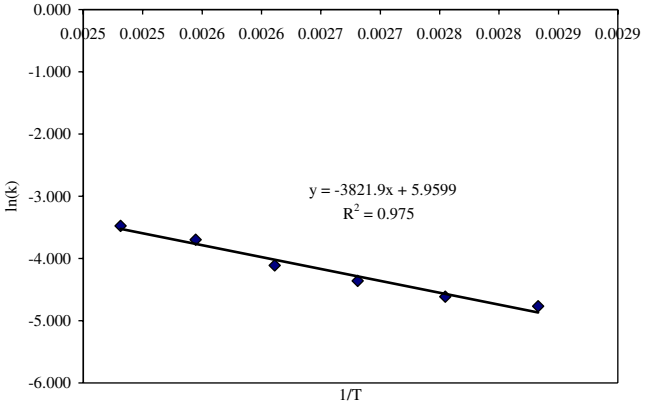

Fig. 2. Arrhenius plot for saponin degradation in soya flour.

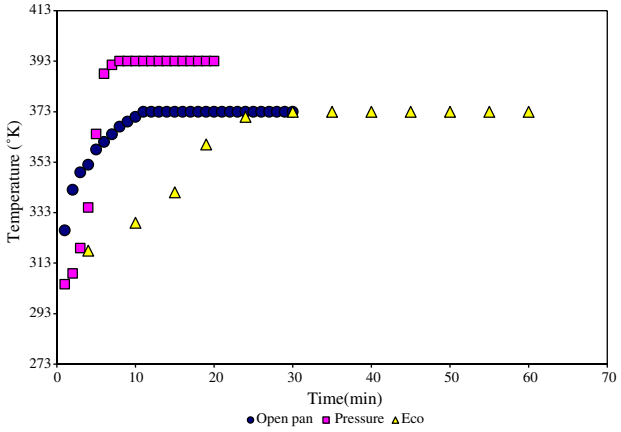

Fig. 3. Time–temperature profile of the different cooking methods used.

Table 3
Degradation profile and kinetics of saponin in soya flour at different cooking methods

Method of cooking	Time (min)	Saponin concentration[a] (μg/g)	Rate constant, k[b] (min^{-1}) (R^2)	$t_{1\backslash 2}$ (min)
Pressure cooking	10	1.75 ± 0.125	0.022(0.91)	
	20	1.65 ± 0.15		31.5
	30	1.31 ± 0.09		
Open pan cooking	10	2.32 ± 0.02	0.038(0.97)	
	20	1.82 ± 0.14		18.0
	30	1.55 ± 0.15		
EcoCooking[c]	10	2.68 ± 0.09	0.037(0.91)	
	20	1.85 ± 0.18		18.7
	40	1.50 ± 0.07		

[a] Values are mean ± SD of two or more individual determinations.
[b] The content of the saponin chosen in the study was 4.34 ± 0.66 μg/g.
[c] The readings were taken after 30 min holding period as per the protocol for EcoCooker.

flour heat treatment under nonisothermal conditions. The results are documented in Table 3.

3.5. Prediction of saponins loss during unsteady state heating/processing

To predict the amount of saponins degradation occurring in the soybean flour during a given nonisothermal state heating process, the following equation was derived from the integrated first order rate law:

$$k_i = A_0 \exp(-E_a/RT_i) \quad (4)$$

where 'k_i' is the rate constant at time 't_i' which depends on the temperature T (K) at that time. 'E_a' is the activation energy of the reaction, 'R' is the gas constant and A_0 is the pre-exponential factor, which have already been calculated from the isothermal experiments. The rate constant 'k_i' at each temperature was calculated using Eq. (4) substituting for "T" from the time temperature data under nonisothermal cooking operations. Knowing the rate constant k at that temperature, the rate dC/dt_i, the amount degraded during the time interval zero to t_i and the final concentration 'C' can be calculated as follows:

Rate = Rate constant $k_i \times$ initial concentration C.
Amount degraded during t_i (ΔC) = Rate $\times$ t_i.
Concentration after time $t_i = C - \Delta C$.

These calculations were extended for the entire period (heating and constant temperatures) for each cooking process. An Excel based computer program was used to compute the above parameters.

The total amount degraded after complete cooking $= \sum \Delta C$. The final concentration thus will be $= C_0 - \sum \Delta C$, where C_0 is the initial concentration of saponins present in the soybean flour. The computed values and the actual degradation obtained experimentally are given in Table 4. As seen, a good agreement between the actual and the calculated degradation/retention of saponins has been obtained. Using this method, the degradation of saponins can be predicted for any processing method, if the time–temperature profile of that processing operation is known.

Table 4
The actual and predicted retention of saponin in the cooking methods

Cooking method	Saponins (μg/g)[a]	
	Actual retention	Predicted retention
Open pan	1.55	1.52
Pressure	1.65	1.40
EcoCooking	1.50	1.56

[a] The saponin content of the soya flour chosen in the study was 4.34 ± 0.66 μg/g.

4. Conclusions

Slow cookers, as exemplified by 'EcoCooker', although fuel-efficient show no significant difference in the magnitude of degradation of saponins as compared to normal open pan and pressure-cooking studied in this work. This study merits investigations on the degradation of other antinutrient constituents subjected to different cooking methods. Based on the results of degradation of saponins and fuel savings, a favourable judgement of the slow 'EcoCooker' is suggested.

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