Phytase influence on soymilk protein colloid stability studied with thermographic method

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Abstract. Coagulation of soymilk is well studied from a technological point of view. However, physicochemical features of the process of soymilk protein colloidal stability destruction may be of interest both from a purely scientific point of view and for improving production processes. In this study, an attempt was made to analyze the role of phytinates in formation of the colloidal stability of soymilk proteins. A possible role of the dissociation of phosphate groups of phytic acid in the rise of an electric charge stabilizing soy protein micelles in water solution has been suggested. The ability of phytase to cut off the phosphate groups of phytic acid was used to substantiate our assumption. To study the kinetics of coagulation of soymilk proteins, a thermographic method was used. The results of the experiments show that the addition of phytase to soymilk can significantly accelerate its acid coagulation, which is an indirect confirmation of the above assumption.

1 Introduction

Milk substitutes based on plant proteins are becoming more and more popular. Most often, protein products from legumes, nuts, seeds and cereals are currently used [1-4]. Their popularity is due to various circumstances. For example, such products are consumed by vegetarians, believers during fasting, or those who are forced to give up cow's milk for medical reasons. In addition, milk replacer products based on plant proteins are consumed by those who are not satisfied with the ethical production of milk, the care and nutrition of animals, as well as gourmets who want to diversify their diet.

Soymilk has been one of the most popular milk substitutes since ancient times. From soymilk, other products can be obtained, for example, soy cheese “tofu” [5]. The coagulation step is the most important step in tofu production. Its purpose is to coagulate proteins and fats in soymilk. For the production of different types of tofu, various coagulants are used: salts, acids, and enzymes [6–8]. They can be used singly or in combination.

Despite the fact that the coagulation of soy milk is quite well studied from a technological point of view, at present there is not a sufficient general theory describing the loss of colloidal stability of soy milk proteins under the influence of various coagulation factors.

It is known that most of the phosphates in plant proteins are represented by phytic acid or phytinates associated with plant proteins [9]. Phytases are a group of enzymes that hydrolyze the phosphate ions of the phytic acid molecule. In principle, the dissociation of phytinates is

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one of the sources of electric charge of soy protein micelles, which can become one of the factors that ensure the coagulation stability of soymilk. In this work, an attempt was made to analyze the role of phytinates in the formation of the colloidal stability of soymilk proteins.

2 Materials and Methods

2.1 Experimental set

To prepare reconstituted soymilk, 40 g of dry soymilk (LLC "Uspekh", St. Petersburg, Russia) was thoroughly mixed in 160 ml of distilled water and left for several hours (6-12 hours) for complete dissolution and swelling at a temperature of 4-6 ºC.

A 20% solution of glucono-delta-lactone (GDL) (Roquette Italia S.p.A, Cassano Spinola Alessandria, Italy) in distilled water, prepared immediately before use, was used as a coagulant.

To study the role of phytinates in the colloidal stability of soy proteins, a 10% phytase solution (Huirui Chemical Technology Ltd., Shanghai, China) with an activity of 100,000 U/g was used.

To observe the coagulation of soymilk samples, the thermographic method [10] was used, based on measuring the temperature difference between two thermometers placed in the sample, one of which is heated. The temperature difference between two thermometers depends on the intensity of convection near the heated one. Therefore, an increase in the viscosity of the solution leads to an increase in the temperature difference of the thermometers.

Coagulation of two identical samples of soymilk was carried out simultaneously in two identical temperature-controlled cells with a volume of 100 ml at a temperature of 30 ºC. To measure the temperature difference, we used two pairs of a FOTEMP 4-channel optical thermometer (Weidmann Technologies Deutschland GMBH, Dresden, Germany). One of the optical sensors of each pair was located near the surface of the resistor, on which a thermal power of 1 W was dissipated. The second optical sensor was placed at a distance of about 4 cm from the resistor. Both sensors were immersed in the studied soymilk to a depth of about 5 cm. The thermometer data were recorded in a file.

2.2 Theoretical background

Soymilk is a very conditional product, and its composition is highly dependent on both the quality of the original beans and the method of preparation. From the point of view of constructing a model that describes the coagulation of soymilk, the most important factor is the ability of phytic acid to form complexes with metals, primarily with calcium and magnesium [11]. On the one hand, this property of phytinate-glycinine complexes reduces the nutritional value of soymilk, and on the other hand, in our opinion, it is the main reason for the destruction of colloidal stability in soymilk.

An additional electric charge of the soymilk micelles arises mainly during the dissociation of the phytinate-glycinine complex (GllyPhy), which we represent with a simplified scheme:

$$\begin{aligned} \mathrm{GlyPhy} &\leftrightarrow 2\mathrm{H}^{+} + \mathrm{GlyPhy}^{2-} \\ \mathrm{CaGlyPhy} &\leftrightarrow \mathrm{Ca}^{2+} + \mathrm{GlyPhy}^{2-} \end{aligned} . \qquad (1)$$

This scheme makes it easy to explain the main features of soymilk coagulation. Indeed, an increase in the acidity of the medium leads to a shift of the equilibrium of the first of reactions (1) to the left and the corresponding decrease of the micelle negative electric charge, which determines repulsion of micelles. An increase in the concentration of calcium ions in

soymilk whey leads to a similar effect, shifting the second reaction in scheme (1) to the left. In any case, the repulsion of micelles decreases, which leads to their sticking together, that is, coagulation.

3 Results and discussion

First, the possibility of coagulation of soymilk using phytase was tested. However, no significant effect was observed for small doses of phytase added to reconstituted soymilk. Most likely, this fact is due to the strong dependence of phytase activity on medium acidity. The active acidity of the reconstituted soymilk was pH = 6.8 ± 0.2, while in [12] it is reported that phytase activity drops to almost zero when the acidity decreases to pH > 6. Only the addition of more than 1 g of phytase per 100 ml of reconstituted soymilk led to the observation of the effect of increasing the viscosity but not to the coagulation of soymilk (Fig. 1).

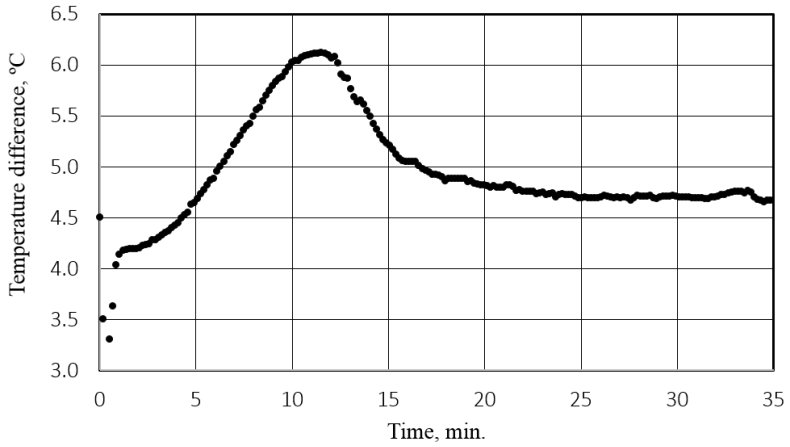

Fig. 1. Thermogram of 100 ml soymilk sample after adding of 2 g phytase.

An increase in the temperature difference of the thermometers by about 1.5 ºC corresponds to a noticeable increase in the viscosity of soymilk. In addition, the time to reach the maximum viscosity of soy milk corresponds quite well to the kinetics of phytic acid hydrolysis by phytase [13].

The absence of milk coagulation because of the hydrolysis of phytinates can, in our opinion, be explained by several factors. It is possible that phytase activity at neutral acidity is insufficient for complete hydrolysis of phytinates, or some phytinates are sterically inaccessible to phytinase. In addition, phosphate residues of phytic acid can participate in the formation of bonds between micelles of soy proteins.

According to [12], the maximum activity of phytase is achieved at pH ≈ 5, however, at such acidity values, soymilk is subjected to acid coagulation. Fig. 2 demonstrates a thermogram of acid coagulation of reconstituted soymilk using GDL as the acid agent. The same figure shows experimental data on the kinetics of acidity increase in soymilk. As can be seen from the figure, an increase in the viscosity of milk or the beginning of its coagulation corresponds to an increase in acidity to the values of pH < 5.

Since it is not possible to study the effect of phytase on the colloidal stability of soy proteins at high acidity due to the acid coagulation of soymilk, experiments were carried out on the effect of phytase on the acid coagulation of soymilk.

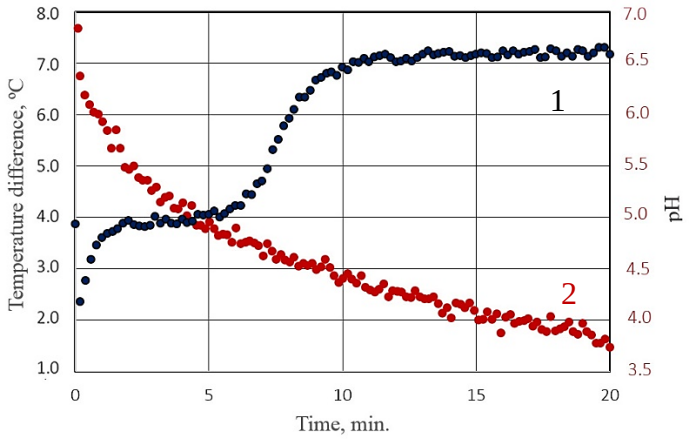

Fig. 2. Thermogram (1) and pH (2) of 100 ml soymilk sample after adding of 2 g GDL.

Fig. 3 shows a thermogram of the acid coagulation of soymilk, in which phytase was previously added. After 10 minutes of exposure at 30 ºC, the GDL solution was added to the measuring cells. Comparison of Fig. 3 and Fig. 2 shows a noticeable reduction in soymilk coagulation time after GDL addition. This can be explained from two points of view. On the one hand, a decrease in the negative electric charge because of the preliminary hydrolysis of phytinates by phytase makes it possible, in accordance with the first reaction of scheme (1), to achieve compensation of the remaining charge at a lower concentration of H^+ ions. On the other hand, an increase in phytase activity with a decrease in pH also leads to acceleration of compensation of micelle charge, which stabilizes the colloidal system of soymilk proteins. Both of these factors, of course, can act simultaneously.

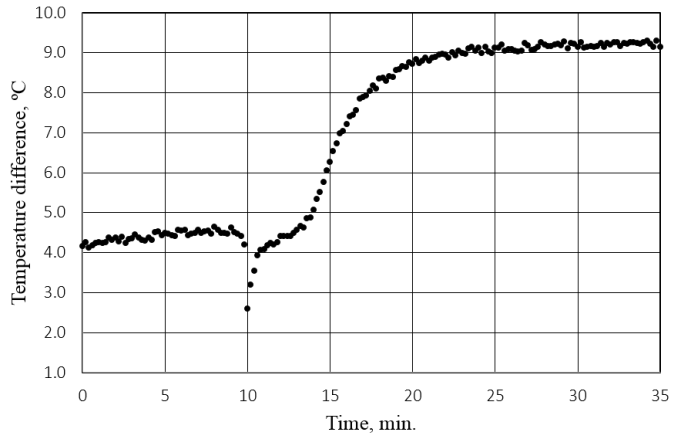

Fig. 3. Thermogram of 100 ml soymilk sample with 2 g of phytase to which after 10 minutes 2 g of GDL was added.

Experiments similar to those shown in fig. 3 with different amounts of GDL added definitely show the same trend towards a decrease in acid coagulation time after pre-fermentation of soymilk with phytase. The coagulation time was determined as the time interval between the addition of GDL and the achievement of the maximum value of the growth rate of the temperature difference on the thermogram.

The results of these experiments are shown in Table 1.

Table 1. Dependence of soymilk coagulation time on phytase addition.

Amount of GDL added, g/100 ml	Coagulation time after GDL addition, s	
	Without phytase	With 2 g/100 ml phytase
1.5	590 ± 10	430 ± 10
2.0	440 ± 10	270 ± 10
2.5	340 ± 10	210 ± 10

4 Conclusion

A possible role of the dissociation of phosphate groups of phytic acid or phytinates in creation of an electric charge stabilizing soy protein micelles in soymilk has been suggested. The ability of phytase to cut off the phosphate groups of phytic acid was used to substantiate our assumption. The results of our experiments show that the addition of phytase to soymilk can significantly accelerate its acid coagulation, which is an indirect confirmation of the above assumption.

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