Functional properties of Australian sweet lupin protein isolated and tested at various pH levels

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Abstract: Australian sweet lupin has the potential to be a new source of vegetable protein due to its similar protein content to soy, the common source of vegetable protein used in the food industry. Investigation of its functional properties is essential to validate the potential application in the food industry. Lupin protein isolates were prepared by alkaline extraction at pH 9.0 followed by acidic precipitation at eight different pH levels i.e. 4.0, 4.2, 4.4, 4.5, 4.6, 4.8, 5.0 and 5.5. The range of pH employed here covered the isoelectric points of major legume proteins. The emulsifying and foaming properties of lupin protein isolate (LPI) samples were evaluated and compared with those of soy protein isolate (SPI). Lupin protein isolates prepared by precipitating at a pH range of 4.4 to 5.0 had no significant difference in their emulsifying and foaming properties. Emulsifying activity and emulsion stability of LPI samples were comparable to those of SPI. All LPI samples exhibited greater emulsifying activity and emulsion stability than SPI at pH 4.0. Foaming capacity and foam stability of LPI tested at a range of pH levels was also higher than that of SPI. The study provides a base for more flexible and economical process for making LPI at commercial level.

Keywords: Australian sweet lupin, *Lupinus angustifolius*, Protein isolates, Functional properties

INTRODUCTION

Australian Sweet Lupin (ASL), *L. angustifolius*, is a high protein grain legume. Utilization of ASL flour, that contains approximately 40% protein, has recently been studied in many foods to improve their nutritional value [1-4]. However, despite the fact that lupin protein has comparable quality to the commonly used soy protein, there is limited application of ASL protein in the food industry [5]. The main reason may be the lack of available information on the functional properties of ASL protein. Functional properties of food proteins that refer to the fundamental physicochemical properties which play a role in influencing the behaviour of proteins during processing and storage and the quality of the food formulations [6], are key determinants of its food applications. Of all the different types of functional properties, emulsifying properties and foaming properties are among the most important quality determinants in food formulations.

In a food system, molecules constantly bind to each other and promote aggregation of droplets that could cause emulsion instability [7] and is unacceptable in most foods. Separation of oil-in-water emulsion can be prevented by the presence of an emulsifier. Due to their hydrophilic ('water-loving') and hydrophobic (or lipophilic, known as 'oil-loving') structures, proteins are amongst the most effective emulsifiers commonly applied in the food industry. Their outer hydrophilic hydrocarbon terminals can interact with the surrounding aqueous phase while the lipophilic interfaces interact with the surrounding oil phase. This results in the reduction of the interfacial tension between molecules of one immiscible liquid and another immiscible liquid (for example, oil and water). As a strong and cohesive physical barrier is formed on the surface of the emulsion, protecting the oil droplets from being re-dispersed to the emulsion, the mixture is stabilized [7-9]. One of the examples of commonly used good emulsifier is the lecithin present in egg yolk. However, lecithin is a relatively expensive food ingredient and a low cost substitution is in demand.

In aerated food products such as marshmallow, whipped toppings, meringues and sponge cakes, it is important to incorporate a large volume of air ('foam') into the food system. Foam is defined as 'a colloidal dispersion of a gas phase in a liquid phase'[8]. The surface activity of proteins allows uniform distribution of fine air cells in the protein matrix. In addition, the unique structure of protein molecules allows them to rapidly adsorb at the air-water interface during bubbling. Egg white is one of the most common ingredients used in the food industry to create stable foam with high volume. The food industry is, however, looking at other alternative with effective foaming capacity and foam stability to replace such a high cost ingredient.

Hettiarachchy and Kalapathy [9] reported that emulsifying and foaming properties of proteins are dependent on the hydrophobicity and structural flexibility of proteins, which is largely affected by pH. Zhang et al. [10] highlighted the necessity of testing functional properties over a range of pH levels. The reason is that pH is influential in the flexibility of protein, which in turn, is essential in adsorbing to the oil/water and air/water surface and reducing the interfacial tension. In fact, these two capacities are crucial to shaping the emulsifying and foaming properties of protein.

The objective of this study was to investigate the emulsifying and foaming properties of different ASL protein isolates over a range of pH in comparison with soy protein isolate. The results provide an indication of the potential application of ASL protein isolate as a new source of vegetable protein in the food industry.

MATERIALS AND METHODS

Preparation of protein isolates

The ASL flour was defatted using hexane. The flour was mixed with deionized water at 1:10 (flour: water; w/v) for 30 min at room temperature ($20\pm2°C$) using a Warring blender.

The pH of the suspension was adjusted to 9.0 using 1M NaOH followed by stirring for 1h. The suspension was centrifuged at 3000 rpm for 15 min. To the supernatant, 1M HCl was added to attain required pH points i.e. 4.0, 4.2, 4.4, 4.5, 4.6, 4.8, 5.0 and 5.5. The solution was stirred for 1 h. The precipitated protein was separated by centrifugation at 3000 rpm for 15 min. The resulted lupin protein isolates were freeze dried and named as LPI-4.0, LPI-4.2, LPI-4.4, LPI-4.5, LPI-4.6, LPI-4.8, LPI-5.0 and LPI-5.5, respectively. The procedure was carried out in triplicate for each type of isolate. The LPI-4.5 served as control-A as 4.5 is the most commonly used pH for legume protein precipitation. For a comparison, soy protein isolate was prepared by treating defatted soy flour in a similar way with protein precipitation at its most commonly used isoelectric point of pH 4.5 and was referred to as SPI-4.5 (control-B).

The freeze dried protein isolates were ground to a particle size of 60μm prior to the assessment of functional properties.

Functional Properties

Emulsifying activity

The method employed by Naczk, Rubin and Shahidi [11] was used to evaluate emulsifying activity[12]. A 1% protein isolate suspension was made in distilled water. The pH of the suspension was adjusted to 2, 4, 6 and 8 with 0.1M NaOH or 0.1M HCl in order to study the effect of pH level on the EA of the protein isolate. The protein isolate suspension (100 mL) with a specific pH level was homogenised using Polytron PCU-2 homogeniser (Switzerland) for a period of 10 min. At the 5th min, 100mL of canola oil (Coles Farmland®) was added gradually to the suspension with continuous stirring. The emulsion was later centrifuged at 3000rpm for 10 min in a Hettich Universal 16 centrifuge

(Germany). Volume of the emulsified layers was recorded to calculate EA based on the following formula:

EA (%) = [volume of emulsified layer (mL)/total volume of suspension (mL)] x 100

Emulsion stability

Sample preparation for the determination of emulsion stability (ES) was similar to that of EA, with an additional step of heating before centrifuge [11]. The emulsion was heated in a Ratek WB 20 water bath (Boronia, Victoria) at 85°C for 30min, then cooled under running tap water for 5 min to room temperature (20±2°C). Samples were centrifuged at 3000 rpm for 10 min in a Hettich Universal 16 centrifuge (Germany) before the volume of the emulsified layer recorded ES was calculated using the following formula:

ES (%) = [volume of emulsified layer (mL)/total volume of suspension (mL)] x 100

Foaming capacity

To determine if foaming capacity (FC) of the protein isolates be affected by pH of the medium, protein suspensions (1% w/v) were made with the pH adjusted to 4, 6 and 8 by addition of 0.1M NaOH or 0.1M HCl [13]. Samples were individually beaten in a domestic Sunbeam cake mixer at 'Hi' speed for 5 min. The mix was immediately transferred to 1000 mL graduated cylinder. The volume of the foam was recorded and FC values were calculated based on the following formula:

FC (%) = [foam volume (mL)/total volume of suspension (mL)] x 100

Foam stability

Foam stability (FS) of the protein suspensions was recorded over a period of two hours. The data were recorded for foam volume remaining at 30, 60, 90 and 120 min. The FS at

each time interval was calculated as per cent of the initial foaming volume using the following formula:

FS (%) = [foam volume (mL) at a time interval/initial foam volume (mL)] x 100

Statistical analysis

Data were statistically analyzed using one-way analysis of variance (ANOVA). The means were compared using Tukey's post-hoc test at *P≤0.05*. All data were analyzed by the SPSS version 17.0 software.

RESULTS AND DISCUSSION

Emulsifying Activity

Emulsifying activity [12] of the protein samples measured in pH 2, 4, 6 and 8 suspensions is summarized in Table 1. The LPI samples had comparable EA values to that of SPI-4.5 at pH 2, 6 and 8. The EA value of SPI sample at pH 2, 6 and 8 corresponds to the findings by Morales-de Leon et al. [14] who recorded an EA value of 57.8% (pH not mentioned) for soy protein sample. At pH 4 SPI-4.5 showed a very poor EA of 4.0% whereas LPI samples maintained their EA within the range of 50-54%. The lower EA of SPI-4.5 at pH 4 could be due to the well-established fact that isoelectric point of soy protein lies at pH 4.5 and its solubility is minimum at adjacent pH points. With major protein fractions of soy protein being insoluble at pH approximate to 4, its ability to orient and act as an effective emulsifier is inferior [15]. Another reason could be the difference in type of proteins in the lupin and soy. Glycinin constitute to a major fraction of soy protein [15] whereas, globulin is the main type of protein in lupin [16]. Different types of protein exhibit different behaviour; one type of protein may be more unfolded than the other. When unfolded, more of its 'inner-bound' lipophilic (hydrophobic) groups are exposed. The exposed lipophilic

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groups associate with fat globules while the outer hydrophilic groups allow the protein to orient freely in the oil-in-water emulsion, thus resulting in an increase in EA.

The EA of LPI samples were also comparable to other legume proteins. EA of pigeon pea, black bean and cowpea protein isolates were about 50%, 55% and 60%, respectively [14,17]. Lqari et al. [18] reported a higher EA value of 74% for LPI. However, they used a 1.75% LPI suspension whereas 1% suspension has been used in this experiment. Values of EA are affected by the amount of soluble protein in the solution [19]. Soluble protein promotes oil droplet entrapment, and subsequently, improves its emulsion properties. Adequate amount of soluble protein in the solution is required to promote entrapment of oil droplets for a better EA [20].

Sample LPI-4.5 had higher EA than those of other LPI samples (except LPI-4.6) at pH 2. However, at pH 4 and 6, which is the pH range of most of the foods, LPI-4.5 had similar EA to those of LPI-4.6, LPI-4.8 and LPI-5.0 (Table 1). It can be concluded that the precipitation pH is not critical in relation to the emulsifying properties of LPI. Previous studies also revealed that lupin protein isolates prepared at a pH range of 4.4 to 5.0 had similar protein contents, protein recovery and yield [21]. The high EA of lupin protein isolates which is quite stable at a range of pH highlights its suitability as a vegetable based emulsifier to replace animal based emulsifiers in different food products particularly those with pH around 4.

Emulsion Stability

Emulsion stability (ES) of the protein samples at pH 2, 4, 6 and 8 is presented in Fig. 1. The ES of the LPI sample showed a variation at different pH levels. All of the LPI samples showed similar or better ES compared to SPI-4.5. At pH 4, SPI-4.5 had the lowest ES (4%) whereas all LPI samples showed ES >50%. The ES of LPI samples was higher than those

reported for some other legume proteins. ES of pigeon pea and cowpea protein isolates were 45% and 50%, respectively [17] and those of black bean protein isolate were 42% [14]. Lqari et al. [18], however, reported a higher ES values of 70% for LPI. They applied a higher concentration (1.75%) of LPI suspension than that used in the present experiment (1%).

Foaming Capacity

Foaming capacity (FC) of the protein isolates depended both on the isolation pH and pH of the testing solutions (Table 2). At pH 4 and 6, LPI-4.8 and LPI-5.0 samples had lower FC than the LPI-4.5 sample. At pH 4, all LPI samples had significantly higher FC values than SPI-4.5 sample.

In general FC increased with the increase in pH of the solution from pH 4 to pH 8 (Fig. 2). The finding is in agreement with previous studies [13,22-24]. Such phenomenon is due to the solubility of the protein. Snyder and Kwon [15] stated that solubility and the ability of the protein to unfold are the two crucial factors to promote foaming. As pH approaches the isoelectric point of a protein, the net charge on the protein structure is minimal. The protein is less soluble and has less flexibility to unfold, leading to an increase in the surface tension. Consequently, less protein is adsorbed to the air-water interface, leading to a reduced FC [25-27]. On the other hand, as the pH increases towards the alkaline region, the net charge of the protein is increased [23], the molecular interaction is enhanced and the FC is improved.

The FC of LPI samples were higher compared to values reported for other types of proteins. Fresh egg white (8% suspension) and mung bean protein isolate (8% suspension) produced 625% and 650% FC [28]. A 10% milk protein suspension produced 420% FC [29]. The FC of LPI is also greater than that of pea (320%), broad bean (250%) and black bean

(18.8%) isolates [14,29,30]. It is interesting to note that Lqari et al. [18] reported a low FC of 119% when 3% Spanish lupin protein isolated at pH4.3 was used. The result suggested that Australian lupin protein might have superior FC than Spanish lupin protein.

Washe, Muller & Schafer [31] reported an FC value of 1025% for LPI samples however they used a 5% LPI suspensions compared to 1% solutions used in this study. Protein concentration is indeed an influential determinant in FC of proteins [15].

Foam Stability

Results of the foam stability (FS) at 30th, 60th, 90th and 120th min of all protein samples at pH4, 6 and 8 suspensions are reported in Fig. 3. All LPI samples demonstrated higher FS than the SPI-4.5 at pH 4. At 120th min the FS of LPI samples were around 60% of the initial value whereas that of SPI-4.5 was 7% (Table 3). The FS values of SPI-4.5 improved at pH 6 and pH 8 (48% and 61% respectively) however still lower than LPI samples with FS of around 70% (Table 3). The data also exhibited that FS of all samples decreased over the time period (Figs 2, 3 and 4). The results confirm those of Lqari et al. [18] that revealed a high FS over 120 min when 1.75% LPI suspension was whipped.

The FS of LPI sample in this study was much higher than those of other proteins. Morales-de Leon et al. [14] reported that 2% fresh black bean protein isolate suspension had a FS of 50% of the initial after 30 min. An 8% suspension of mung bean protein isolates showed an FS value of 20% and 8% fresh egg white suspension showed an FS value of 46% after 120 min [28].

The pH of the suspension had a significant effect on the FS of most of the protein isolates. Foaming stability was, generally, lower at pH 4 than at pH 6 and pH 8 (Table 3). However, FS was not affected by the type of LPI. The LPI samples isolated at different pH points had similar FS at 120th min at a given pH of the suspension. In fact the LPI samples exhibited

superior FS to SPI-4.5 at pH 4 and pH 6. Such findings indicates that LPI samples have a great potential in food formulations requiring foaming characteristic, such as cakes, marshmallows, whipping cream and frozen desserts.

CONCLUSIONS

Lupin protein isolates prepared by precipitating at a pH range of 4.4 to 5.0 had no significant difference in their emulsifying and foaming properties. This provides more flexibility in the isolation process. Maintaining a predetermined pH during the protein isolation is an expensive process. The emulsifying and foaming properties of LPI samples were better than the SPI sample, particularly under acidic pH condition. It may be concluded that LPI could be more applicable than SPI in a wider range of food preparations due to its superior functional properties at low pH. LPI can be applied in food products particularly those with low pH such as yoghurts, salad dressings, fermented products and supplemented sports drinks. The information would be helpful in exploration the future LPI applications in the food industry.

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Table 1: Emulsifying activity of protein isolates at various pH levels

Samples	Emulsifying activity			
	pH2	pH4	pH6	pH8
LPI-4.0	54.7 ± 1.0^{b}	50.7 ± 0.7^{b}	52.3 ± 0.9^{ab}	53.4 ± 0.8^{b}
LPI-4.2	55.8 ± 0.2^{b}	50.5 ± 0.3^{b}	52.5 ± 1.3^{ab}	53.6 ± 1.0^{b}
LPI-4.4	55.1 ± 0.8^{b}	50.9 ± 0.4^{b}	52.3 ± 0.3^{ab}	53.1 ± 1.1^{b}
LPI-4.6	56.2 ± 0.7^{ab}	51.4 ± 0.7^{ab}	52.1 ± 0.6^{ab}	52.9 ± 0.4^{b}
LPI-4.8	55.5 ± 1.7^{b}	51.3 ± 0.8^{ab}	53.0 ± 0.4^{ab}	53.0 ± 0.1^{b}
LPI-5.0	55.6 ± 0.2^{b}	51.5 ± 0.6^{ab}	51.8 ± 0.8^{ab}	52.8 ± 0.7^{b}
LPI-5.5	55.9 ± 0.9^{b}	51.3 ± 1.2^{ab}	51.2 ± 0.6^{b}	52.6 ± 0.3^{b}
Control-A (LPI-4.5)	58.8 ± 0.8^{a}	53.5 ± 1.2^{a}	53.5 ± 1.4^{a}	53.2 ± 0.3^{b}
Control-B (SPI-4.5)	57.1 ± 1.1^{ab}	4.0 ± 0.2^{c}	53.2 ± 0.2^{ab}	57.0 ± 0.9^{a}

Values (means ± s.d., n=3) with the same letter within a column are not significantly different (p≤ 0.05)

Table 2: Foaming capacity of protein isolates at various pH levels

Samples	Foaming capacity (%)		
	pH4	pH6	pH8
LPI-4.0	390 ± 86^{ab}	616 ± 49^{a}	776 ± 25^{a}
LPI-4.2	380 ± 190^{ab}	595 ± 37^{a}	696 ± 80^{ab}
LPI-4.4	383 ± 87^{ab}	575 ± 66^{a}	798 ± 73^{a}
LPI-4.6	397 ± 109^{ab}	418 ± 54^{b}	761 ± 38^{ab}
LPI-4.8	356 ± 35^{b}	408 ± 38^{b}	591 ± 23^{b}
LPI-5.0	323 ± 45^{b}	403 ± 110^{b}	680 ± 32^{ab}
LPI-5.5	396 ± 25^{ab}	548 ± 31^{a}	643 ± 20^{ab}
Control-A (LPI-4.5)	541 ± 52^{a}	590 ± 36^{a}	658 ± 52^{ab}
Control-B (SPI-4.5)	187 ± 38^{c}	435 ± 37^{b}	583 ± 72^{b}

Values (means ± s.d., n=3) with the same letter within a column are not significantly different (p≤ 0.05)

Table 3: Foam stability at 120th min at various pH levels

Samples	Foam stability (% of the initial value)		
	pH4	pH6	pH8
LPI-4.0	57 ± 24^{a}	84 ± 9^{a}	84 ± 10^{a}
LPI-4.2	59 ± 11^{a}	75 ± 8^{a}	72 ± 6^{a}
LPI-4.4	67 ± 30^{a}	90 ± 2^{a}	70 ± 10^{a}
LPI-4.6	56 ± 28^{a}	69 ± 4^{ab}	71 ± 9^{a}
LPI-4.8	65 ± 15^{a}	79 ± 10^{a}	79 ± 4^{a}
LPI-5.0	57 ± 15^{a}	68 ± 6^{ab}	66 ± 5^{a}
LPI-5.5	76 ± 5^{a}	73 ± 7^{a}	68 ± 12^{a}
Control-A (LPI-4.5)	80 ± 6^{a}	73 ± 6^{a}	72 ± 4^{a}
Control-B (SPI-4.5)	7 ± 2^{b}	48 ± 13^{b}	61 ± 12^{a}

Values (means ± s.d., n=3) with the same letter within a column are not significantly different (p≤ 0.05)
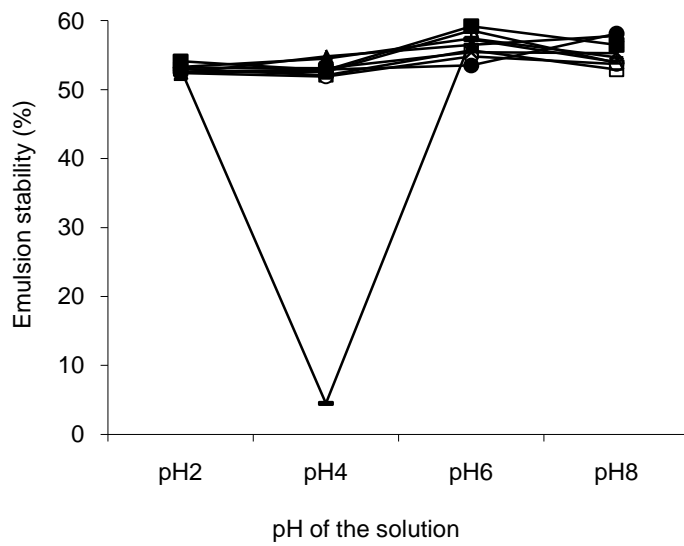
Fig.1. Emulsion stability of protein isolates at various pH levels: LPI-4.0 (□), LPI-4.2 (○), LPI-4.4 (Δ), LPI-4.6 (×), LPI-4.8 (■), LPI-5.0 (●), LPI-5.5 (▲), LPI-4.5 (+), SPI-4.5 (−)
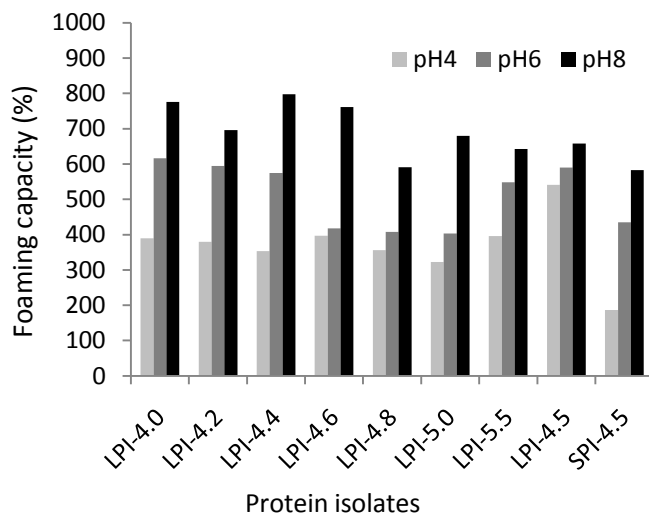

Fig.2. Effect of pH on the foaming capacity of the protein isolates
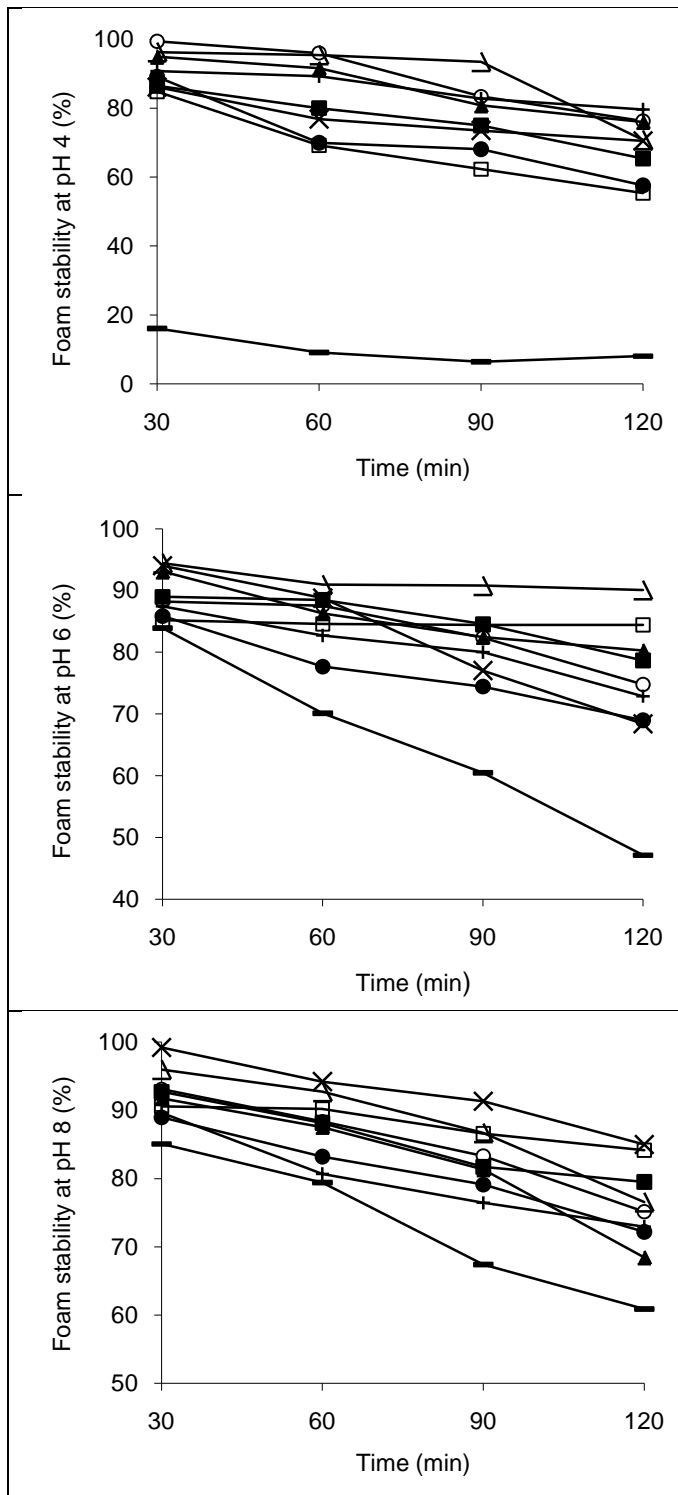
Fig. 3. Foam stability (% of the initial value) of different LPI samples at pH 4, 6 and 8: LPI-4.0 (□), LPI-4.2 (○), LPI-4.4 (Δ), LPI-4.6 (×), LPI-4.8 (■), LPI-5.0 (●), LPI-5.5 (▲), LPI-4.5 (+), SPI-4.5 (−).

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