Functionality of Purified Yellow Pea Protein Isolates for Food Application

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ABSTRACT

Protein isolates from yellow pea have been extracted and isolated at different pH conditions varying from 4 to 8. Rheological and functional properties such as surface colour, gel strength, gel point, emulsion and foam properties were investigated and compared to a commercial pea protein isolate (PPI).

INTRODUCTION

The increasing demand for protein due to increasing population has shifted focus from animal protein towards plant proteins. Plant proteins remain, with the exceptions of soy and gluten, underutilized in the food industry largely due to insufficient structural performance[1].

Legume proteins have received increased attention due to a well-balanced amino acid profile for human consumption including yellow pea protein with especially high content in lysine, but unfortunately the presence of antinutrients such as phytic acid, tannis and protease inhibitors have limited its utility to human consumption[2].

To overcome this challenge highly soluble yellow pea protein isolates have been extracted and purified using Expanded Bed Adsorption (EBA) technology by the company Upfront Chromatography A/S. The EBA-technique utilises the fact the protein can be bound to special designed ultra-high density adsorbent beads, which in this case furthermore have been optimized to remove the major anti-nutritional factors in a pea proteins. The high-density beads are positioned in a chromatography column, where water, and no organic solvent, is used to purify the protein. The design of the columns allows high purity proteins to cost effectively be purified from large crude streams.

Yellow pea soluble protein consists mainly of two larger storage proteins vicilin (7S) and legumin (11S). Previous studies show that the isolate production method has large influence on the ratio of storage proteins and the physiochemical properties of the protein isolates. Legume protein isolates are often isolates using an alkaline extraction method followed by and isoelectric precipitation salt extraction processes[1,3].

Changing the isolation method and varying pH conditions during extraction is expected to alter and improve the functional and rheological properties of yellow pea protein isolates, which will enhance pea protein isolates potential in food applications.

In the following study, important rheological and functional properties covering emulsion, foam and gelling properties have been investigated for four pea protein isolates extracted and isolated at different conditions and compared to a

commercially produced pea protein isolate (PPI).

MATERIAL AND METHODS

Extraction and drying of protein fractions

Proteins from yellow peas were isolated at different pH conditions with a 30 cm in diameter expanded bed adsorption chromatography column with 35 L adsorbent. A load ratio of 1:2 was used and elution was performed with 30 mM NaOH. The yellow pea flour was mixed with water in ratio 1:7. Extraction time was 1 hour. Extraction pH: Sample 1 and 4: pH 6.5, sample 2 and 3: pH 8.0. After extraction the raw material was left to settle for 20 min and only the supernatant was loaded on the column. 0.5 mg/ml sulphite was added to the extraction for sample 3. Loading pH was 6.5 for sample 1, pH 8.0 for sample 2 and 3 and pH 4.5 for sample 4.

The four protein isolates from the chromatographic process were ultrafiltrated and diafiltrated (10 kD cut-off membrane) and freeze dried. The results are compared to a commercial pea protein isolate (PPI) provided from MEGA (Natur Drogeriet).

pH adjustments

Protein isolate samples were adjusted to pH 4 and 7 using 0.1M and 1M HCl and NaOH.

Emulsion capacity and stability

Emulsions at pH 4 and 7 were prepared by mixing 0.1 g of protein isolate with 10 mL demineralised water and adjusting pH. The solutions were left at room temperature overnight to fully stabilise and dissolve. 10 mL (8.96 g) oil was added to the protein solution followed by 5 min homogenisation using an Ultra Turrax at 20,000 rmp. The emulsion capacity is measured after centrifugation at 1,500xg for 5 min by measuring the resulting height of the emulsion. The emulsion stability is measured by heating the emulsion at 80°C for 30 min and subsequently cooling the emulsions for 15 min followed by 5 min centrifugation at 1,500xg.

Foam capacity and stability

The foam capacity and stability were determined by mixing 0.3 g protein isolate with 10 mL demineralised water in a 50 mL beaker and adjusting pH to 4 and 7. The mixture was homogenized for 5 min. using an Ultra Turrax at 20,000 rpm. Immediately after, the initial foam volume was noted and again after 5, 10, 20, 45, 60, 90 and 120 min. The percentage volume increase is used to express the foam capacity and stability.

Gelling ability

The gelling ability was measured by mixing 2.5 g protein isolate with 20 mL demineralised water and adjusting pH to 4 and 7. The mixtures were measured in a cup and bob system on a rheometer (Physica MCR 301, Anton Paar). The samples were heated and cooled over a temperature range from 25°C to 95°C at 2°C/min. with a 5 min. hold time at the final temperature for both the heating and cooling phase. Both storage (G′) and loss (G′) modulus were recorded throughout the temperature sweeps. After cooling, a frequency sweep from 0.1-20 Hz was performed and the gel strength was calculated at 1 Hz. The sol-gel thermal transition point was defined by G′=G′.

Colour measurement

The colour of the protein isolate powders were determined by pressing the powder in metal crucibles coved with non-reflective glass. It is ensured that no air pockets are present. The samples surface colour are measured according to the L*a*b* system (CIElab Minolta Chroma Meter CR-200). Duplicates containing five repeats were conducted.

RESULTS AND DISCUSSION

The environment (acidic, alkaline and neutral) in which a protein is present affects the overall charge of the protein. The solubility of a protein is the most important functional property, which influences emulsification, foaming and gelation behavior[5]. Soluble pea proteins isoelectric point is typically found around pH ~5 at which point the proteins are least soluble. According to previous studies the hight solubility is found at pH~2 and 8[2,6].

Selected functional properties of the four different extracted pea protein isolates were evaluated at pH 4 and 7. The emulsion capacity (Fig. 1) of the protein isolates show that the isolates produced using the EBA technology regardless of pH during extraction and isolation have enhanced emulsion capacity at pH 4 compared to the commercial product (PPI). At neutral pH there is a less difference between all the isolates. The only difference between isolate 2 and 3 is addition of sulphite during isolation, which increase emulsion capacity. This could be due to more ions present to stabilise the emulsion.

Very few studies compare the emulsifying properties of pea protein isolate and in the studies available different units are used to calculate the emulsifying properties which makes it impossible to compare results.

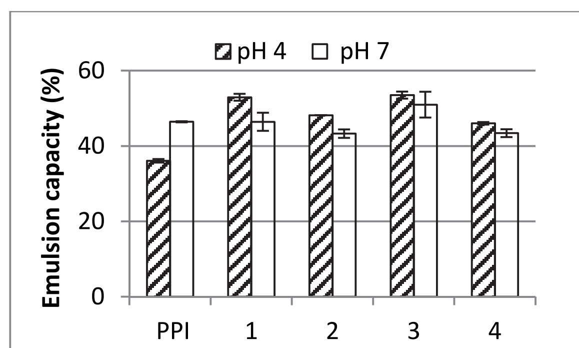

Figure 1. Emulsion capacity of commercial yellow pea protein isolate and samples 1-4 at pH 4 and 7.

For the commercial isolate the correlation between the emulsion capacity and stability (Figure 2) is that higher capacity yield equally higher stability. The opposite tendency is observed for isolate 1-4, where the capacity is high the stability is lower (pH 4) and opposite for pH 7. The difference could be because isolate 1-4 only contains the soluble proteins whereas the commercial product could contain less insoluble proteins as well. The stability of the proteins at pH 4 are higher for isolate 2, 3 and 4 compared to isolate 1 and PPI. At neutral pH there is a reduced difference between all the isolates and the addition of sulphite during extraction (isolate 3) does not increase the emulsion stability compared to the identical extraction without sulphite.

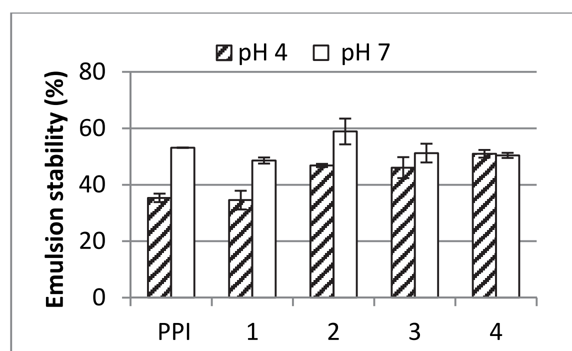

Figure 2. Emulsion stability of commercial yellow pea protein isolate and samples 1-4 at pH 4 and 7.

The foam capacity at pH 4 (Figure 3) of all the isolates is quite similar varying from 390-445%. The isolate extracted in acidic environment have reduced stability compared to the remaining isolates. The commercial pea isolate have reduced stability compared to the remaining isolates after approximately 1 hour.

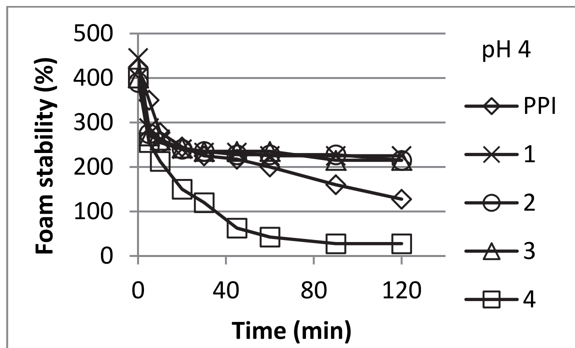

Figure 3. Foam capacity and stability of commercial yellow pea protein isolate and samples 1-4 at pH 4.

At neutral pH the foam capacity (Figure 4) is similar to the values at pH 4 varying from 360-450%. The reduced foaming stability of the isolate extracted in acidic environment is also pronounced at neutral pH. A similar behaviour is observed for isolate 1 extracted at neutral pH. The remaining samples have similar behaviour in the magnitude of the result observed at pH 4.

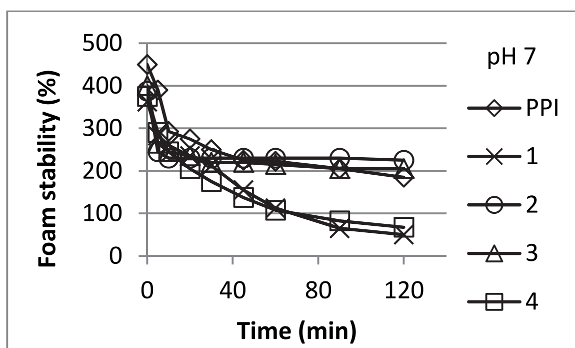

Figure 4. Foam capacity and stability of commercial yellow pea protein isolate and samples 1-4 at pH 7.

Pea protein isolates ability to form network and gels are an important property. Gelling occurs when the when the proteins form a three-dimensional network that can resist flow under pressure. All isolates could form gels at both pH 4 and 7 and the strength of the network is shown in Figure 5. The four isolates produced by EBA exhibited higher network strength compared to the commercial isolate at both pHs. The isolate extracted at acidic conditions create the strongest network at pH 7 compared to the other isolates. Previous studies suggest that the isolation method is of great importance to the gelling properties of the isolates[6], which is also observed in the present study.

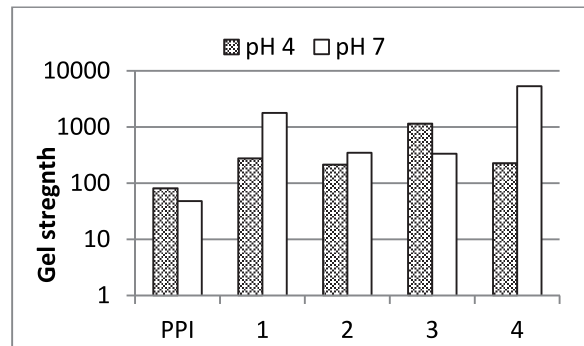

Figure 5. Gel strength at 1 Hz at 25°C of commercial yellow pea protein isolate and samples 1-4 at pH 4 and 7.

Tan δ is an indication for the elasticity of the network formed. A lower value indicated a more elastic network. The tan δ values (Table 1) for the gels shown in Figure 5 vary from 0.16 to 1.61 with most of the gels around 0.2-0.3, which indicates relative elastic gels, which is often desired in food applications[7].

Table 1. Tan δ of the gels produced by commercial yellow pea protein isolate and samples 1-4 at pH 4 and 7.

Samples	***tan δ*** *pH 4*	***tan δ*** *pH 7*
PPI	0.25	0.29
1	0.26	0.19
2	0.26	1.61
3	0.24	0.16
4	0.31	0.21

The gelling temperature (Table 2) varies from below 25°C to 82°C, which indicates that the proteins can be manipulated to achieve specific requirement for a specific purpose.

Table 2. Gel temperature of commercial yellow pea protein isolate and samples 1-4 at pH 4 and 7.

Samples	*Gel temp. pH 4 (°C)*	*Gel temp. pH 7 (°C)*
PPI	27.6	42.9
1	<25	<25
2	<25	75.3
3	25	82.2
4	39.8	51

The colour of the isolates was measured using the L*a*b* method (Figure 6). The values of the commercial isolate are in agreement with previous results[4]. PPI scores highest brightness value with a value over 80 and the highest yellowness value (~19). Whereas the four isolates 1-4 have brightness values around 73-79 and yellowness around 10. The green to redness value is small for all the samples and the standard deviation is relatively large. The reduced yellowness of the produced isolates could be described the EBA-technology in which some of the colour components could be removed during the isolation process.

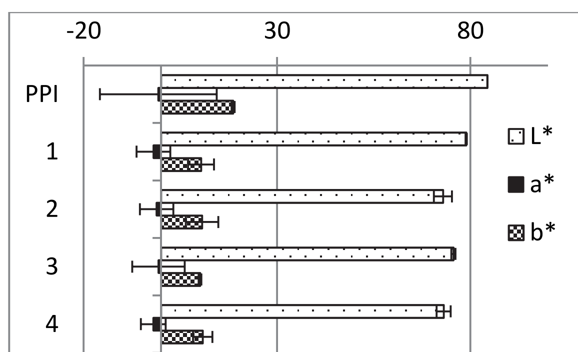

Figure 6. Colour (L*, a* and b*) of commercial yellow pea protein isolate and samples 1-4. L*=darkness(0) to brightness(100), a*= greenness (-) to redness (+) and b* blueness (-) to yellowness (+).

Overall, the colour of the isolates is reasonable compared to a desired white powder. A higher brightness value could be desired compared to commercial pea and soy protein[4].

CONCLUSION

Yellow pea protein isolates extracted and isolated by EBA technology at different pH conditions produced isolates with reduced brightness and yellowness compared to a commercial pea protein isolate (PPI). The isolates have enhanced rheological properties especially gelling properties with low tan δ values and wide gelling temperatures interval that can be used to design protein properties. The isolates have enhanced emulsion capacity and stability at pH 4 and enhanced foam stability above an hour at pH 4. The overall conclusion is that rheological and functional properties of yellow pea protein isolate can be enhanced by manipulating the extraction and isolation conditions using EBA-technology.

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