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The effects of lupin (*Lupinus angustifolius*) protein isolation on its dietary fibre and whey proteins

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Abstract

The study was conducted to evaluate the effects of protein isolation on dietary fibre and whey proteins of lupin flour. Commercially available flour was used in-order to understand the possible commercial applications of the results generated by the project. Protein isolation process from lupin flour that was first defatted, resulted in three products: (1) the dietary fibre fraction; (2) the protein isolate; and (3) the soluble lupin whey protein supernatant. The total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) contents in the flour were 28.9, 19.1, and 9.9%, respectively. The TDF, IDF and SDF of the dietary fibre fraction were 72.1, 65.8, and 6.3%, respectively. A salting out process for the isolation of the whey protein fraction with different salts including trichloroacetic acid was evaluated. Emulsification and foaming properties of the whey proteins were significantly affected by the type of salt. The functional properties of the control whey protein isolated by a simple additional centrifugation step were significantly better than those isolated with any of the salts. The results showed that lupin whey protein with good functional properties can be produced from the whey fraction.

Keywords: insoluble dietary fibre, lupin, protein extraction, soluble dietary fibre, total dietary fibre

1. Introduction

Lupins (*Lupinus angustifolius*) have been used in the Mediterranean region for about 4,000 years (Todorov *et al.*, 1996). Lupin seeds contain significantly higher levels of protein and dietary fibre compared to other similar grain legumes. Protein is one of the main ingredients utilised by the food industry for functional properties. Traditionally animal based proteins were used, however, due to cost and various other reasons the food industry is looking for alternative plant based proteins with good functional properties. Jayasena and Nasar-Abbas (2011) and Nasar-Abbas and Jayasean (2012) successfully incorporated lupin in to biscuits and muffins with good consumer acceptability indicating that lupin can be used in human food product development.

The demand for legume based proteins by the food industry has increased due to its lower production cost and potential health benefits (Martínez-Villaluenga *et al.*, 2006). Lupin flour contains 32 to 42% protein (Hall *et al.*, 2005a) making it one of the best sources of protein. One of the by-products of lupin protein extraction process is the dietary fibre fraction. The other by-product is the whey fraction, which still has functional proteins that may be utilised in food formulations.

Isoelectric precipitation has been the most popular method for commercial protein isolation process (Lusas and Riaz, 1995). However, the isoelectric precipitation is not capable of recovering all proteins, the soluble whey proteins are lost in the by-products (Millan *et al.*, 1994; Oomah and Bushuk, 1983). The incomplete recovery of proteins has been identified as one of the major concerns in the commercialisation of lupin protein isolation (Sgarbieri and Galeazzi, 1978). According to a study conducted by Jayasena *et al.* (2004) more than 20% of the lupin proteins were lost in the whey fraction. The soluble lupin whey

fraction proteins have good functional properties, which make it useful for the food industry.

Demand for plant protein rather than animal protein has been increasing because of the high input costs for animal protein extraction and animal diseases (Jayasena and Quail, 2004). Further Kyle (1994) and Jayasena and Quail (2004) report it is more cost effective to isolate protein from lupins. The co-product obtained during the lupin protein isolation process is the dietary fibre fraction. Due to its higher dietary fibre and protein contents lupins have the potential to provide unique health benefits. Studies have shown the consumption of lupin incorporated food products has the potential to decrease risk factors for obesity (Lee *et al*., 2009), cardiovascular disease (Belski *et al*., 2011) and gastrointestinal illnesses (Johnoson *et al*., 2006). The food industry is on the lookout for dietary fibre and proteins as separate ingredients for use in food formulations. The reason for such a need is that some products require only protein and other products require only dietary fibre as an ingredient in its formulation. Such variations in requirements are due to the distinct needs of each and every product.

Total dietary fibre (TDF) consists of two forms that are based on their water solubility. Insoluble dietary fibre (IDF) includes celluloses, hemicelluloses and lignins (Johnson *et al*., 2006). In western countries, the amount of dietary fibre intake in the daily diet is usually below the recommendations and has been shown to correlate with certain diseases (Betancur-Ancona *et al*. 2004). As a result of associated health benefits dietary fibre is becoming more popular in food product development. Some studies have shown that adding lupin kernel fibre in normal white bread can reduces the risk of cardiovascular disease, obesity, and serum lipids (Hall *et al*., 2005b; Lee *et al*., 2006, 2009). Other studies on fibre contained in lupin flour have shown reductions in blood insulinaemic response, reduction in the risk of colon cancer, elongate satiety and improving bowel function (Hall *et al.*, 2005a; Johnson *et al*., 2003, 2006).

Hartono (2003) showed that the use of defatted lupin flour resulted in higher yield and better purity of the protein isolate. The effects of the high pH environment on the dietary fibre component have not been studied. The TDF, IDF, and soluble dietary fibre (SDF) should be measured to evaluate the impact of the protein isolation process. The objectives of the present study were to (1) evaluate the impact of the protein isolation process on the fibre component of lupin flour; (2) isolate the whey protein from the by-products; and (3) determine the functionality of the isolated whey proteins.

2. Materials and methods

Materials

Commercial lupin flour was obtained from Coorow Seeds (Perth, Western Australia). Megazyme Total Dietary Fibre Kit 200 Assays was obtained from Deltagen (Perth, Western Australia). All other chemicals and reagents were obtained from Sigma Aldrich (New South Wales, Australia, Australia). All analysis was performed in triplicate.

Defatted flour preparation

Hartono (2003) demonstrated that the yield of protein isolates can be increased by first defatting the lupin flour, which could be the industry standard. The lupin flour was defatted by solvent extraction in a 1 kg Soxhlet setup (Chemistry Centre of Western Australia, Perth, WA, Australia). Approximately 1 kg of lupin flour was extracted each time with six litres of hexane for 48 hours. The defatted flour was air dried for 12 hours before analysis and protein isolation.

Lupin flour fractionation

Following the method developed by Jayasena *et al*. (2005) lupin protein was isolated from the lupin flour and the defatted lupin flour (DFLF) and the fibre fraction was obtained. The protein isolation method included the homogenisation (IKA RW20DZm homogeniser; IKA Labortechnick, Staufen, Germany) flour in de-ionised water at a ratio of 1:10 (w/w) at 24 °C for 30 minutes. Once homogenised the pH was increased to 10 with 1N NaOH and further mixed for 30 minutes while maintaining the pH to solubilise the proteins. The mixture was then centrifuged (Eppendorf 5810R centrifuge; Eppendorf, Hamburg, Germany) at 3,000 rpm for 20 minutes to separate the insoluble fibre fraction. The insoluble wet fibre was freeze dried at 100 Pa and -50 °C for 48 hours in preparation for analysis. By adjusting the pH to 4.5 with 1N HCl of the solubilised protein was precipitated and collected after centrifuging at 3,000 rpm for 20 minutes. The process is shown in Figure 1.

Whey protein isolation

The effect of salts on the isolation of lupin whey proteins were determined by treating the whey fraction with calcium chloride, potassium phosphate, potassium citrate, sodium citrate, sodium sulphate, sodium acetate, sodium phosphate, sodium chloride, calcium citrate and trichloroacetic acid (TCA).

Solutions of the different salts were made up to a concentration of 80 g/l in deionised water. For each 800 ml treatment of the whey fraction, 250 ml of the salt solution

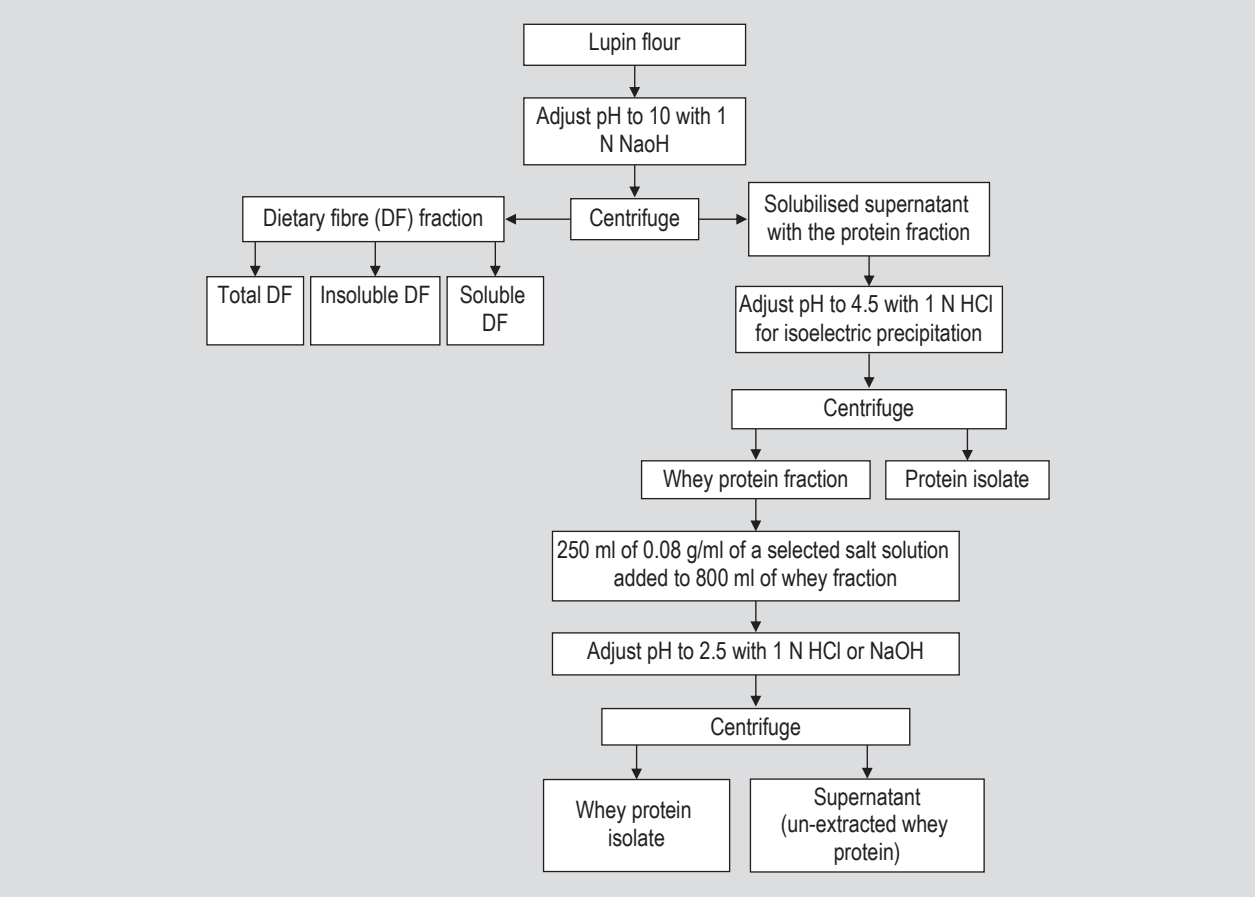

Figure 1. Lupin protein fractionation process.

was added. The pH of the solution was then adjusted to 2.5 with 1N HCl or 1N NaOH while stirring and maintained for 30 minutes as recommended by Batista (1999). The solution was then centrifuged for 15 minutes at 3,000 rpm. The supernatant was decanted and the whey protein isolate was separated. Two control samples were performed, the first one was with no salt treatment and the second was TCA, which was used as an internal control as a previous study has shown it can isolate almost all of the whey proteins fraction (Hartono, 2003).

Moisture content

Moisture was determined by the oven drying method at 105 °C until constant weight was achieved as per AOAC method 925.09 (AOAC, 2008a).

Protein content

Protein content was determined by the Kjeldahl method as per AOAC 960.52 and the N factor used was 6.25 (AOAC, 2008b).

Fat content

Fat content was determined by the Soxhlet fat extraction method as per AOAC method 963.15 (AOAC, 2006) in a BUCHI Soxhlet Fat Extraction E-816 apparatus (Soxhlet Buchi Fat Extraction Unit Model E-816; Buchi Labortechnik AG, Flawil, Switzerland).

Dietary fibre analysis

Total, soluble, and IDF levels were determined as per the AOAC method 991.43 (AOAC, 2005) using the MES-TRIS buffer (Sigma Aldrich, New South Wales Australia).

Functional properties of the whey protein isolates

Functional properties of the lupin whey protein isolates, the emulsifying activity (EA), emulsifying stability (ES), foaming capacity (FC) and foam stability (FS) were evaluated to determine their applicability in food formulation.

Emulsifying activity

EA of the protein isolates were determined as per the method employed by Naczk *et al.* (1986). A 1% protein isolate suspension was prepared with deionised water and the pH adjusted to 6.0 with 0.1 N NaOH or 0.1 N HCl while stirring and maintained for 10 minutes.

For the analysis of the EA, the protein isolate suspension was then transferred to a 250 ml measuring cylinder and homogenised in a high speed Polytron homogeniser for 5 minutes. At which time, 50 ml of canola oil was added gradually and continued for another 5 minutes. The emulsion was then centrifuged at 3,000 rpm for 10 minutes. Volume of the emulsified layer was recorded and the EA was calculated using the following formula:

$$\text{EA (\%)} = \frac{\text{volume of emulsified layer (ml)}}{\text{total volume suspension (ml)}} \times 100$$

Emulsion stability

Emulsion stability (ES) of the lupin whey protein isolates were determined as per the method used by Naczk *et al.* (1986). The samples were prepared as per the EA determination. The emulsion was then heated in a water bath at 85 °C for 30 minutes and cooled under running water to 20 °C. It was centrifuged at 3,000 rpm for 10 minutes. The volume of the emulsified layer was then measured and the ES calculated by the following formula:

$$\text{ES (\%)} = \frac{\text{volume of emulsified layer (ml)}}{\text{total volume of suspension (ml)}} \times 100$$

Foaming capacity

The method described by Sathe *et al.* (1982) was used to determine FC of the whey protein isolates. Into 1 g of the whey protein isolate 100 ml of deionised water was added and while stirring the pH was adjusted to 6.0 with 0.1 N NaOH or 0.1 N HCl and maintained for 10 minutes. The protein mix was transferred to a mixing bowl and beaten in a domestic cake mixer at 'Hi' for 5 minutes. The foam was transferred to a measuring cylinder and volume was measured. The FC was calculated using the formula:

$$\text{FC (\%)} = \frac{\text{foam volume (ml)}}{\text{total volume of suspension (ml)}} \times 100$$

Foaming stability

FS was expressed as the change in foam volume at 30-minutes intervals for a period of 2 hours (Sathe *et al.*, 1982). They were recorded as FS30 (foam stability at the 30th minute), FS60 (foam stability at the 60th minute), FS90 (foam stability at the 90th minute) and FS120 (foam stability at the 120th minute). The foam collected as per the foam capacity method was held in the measuring cylinder and the volume of foam was measured at the given time intervals.

Statistical analysis

The variability between the samples were analysed for statistical significance (P<0.05) via analysis of variance (ANOVA). Statistical analysis was conducted on SPSS software package (version 19; SPSS Inc., Armonk, NY, USA) running on a personal computer. Statistical significance was defined as P<0.05.

3. Results and discussion

The protein and crude fat contents (dry basis) of the DFLF samples are listed in Table 1. The results indicate significant differences (P<0.05) in the protein and fat contents between the two flour types.

The protein content in lupin flour and the DFLF was 42.0 and 55.6%, respectively. Hartono (2003) reported similar protein content for DFLF (48%). The increase in the protein content could be due to the percentage increase in other components within the flour as the fat is removed. The crude fat extraction process removed 90% of the fat from the DFLF. Hartono (2003) reported similar fat removal (86%) from *L. angustifolius* flour following the Soxhlet fat extraction technique.

The effect on dietary fibre due to the processing

One of the co-products of the lupin protein isolation process is the dietary fibre fraction (Figure 1). The TDF, IDF and SDF contents of the lupin flour and DFLF are shown in Table 2. The fat extraction process had no significant effect on the soluble fibre contents of the lupin flour.

The defatting has had a significant impact on the soluble and insoluble fibre contents of the dietary fibre fraction (Table 3). The insoluble fibre content was reduced by 12% and the soluble fibre content was increased by 46%. The change in the interaction between the dietary fibre and the

Table 1. Composition of lupin flour and defatted lupin flour (dry basis).

	Protein	Crude fat
Lupin flour (%)	42.0^{a}	6.0^{a}
Defatted lupin flour (%)	55.6^{b}	0.5^{b}

Mean values represented by different letters within the same column are significantly different at P<0.05.

Table 2. The percentage dietary fibre content of the flour.

	Soluble	Insoluble	Total
Lupin flour	8.3[a]	29.5[a]	37.8[a]
Defatted lupin flour	9.9[a]	19.1[b]	28.9[b]

Mean values represented by the same letters within the same column are not significantly different at $P<0.05$.

Table 3. The percentage dietary fibre content of the fibre fraction.

	Soluble	Insoluble	Total
Fibre fraction from lupin flour	4.3[c]	75.0[c]	79.3[c]
Fibre fraction from the defatted lupin flour	6.3[d]	65.8[d]	72.1[c]

Mean values represented by the same letters within the same column are not significantly different at $P<0.05$.

fat coupled with the effects of the changes in pH during the protein extraction could be a contributing factor in the change in the dietary fibre composition.

Hall *et al*. (2005a) report similar TDF, IDF and SDF in lupin flour (Table 2). However, the IDF content in the DFLF has been significantly reduced indicating that the defatting process has an impact on the fibre fraction. An example would be phosphate containing compounds such as innositol, which is soluble in organic solvents. Such compounds would show up in lupin flour but not in DFLF.

As expected there was a significant increase in the TDF and the IDF content due to the protein isolation process. As the protein was solubilised TDF and IDF which are not water soluble would remain in the insoluble fraction and be removed as the dietary fibre fraction (Figure 1). The reduction in the SDF portion could be due to it being solubilised together with the protein. Part of the SDF consists of oligosaccharides, which are highly soluble in water and remains in the final whey protein fraction. Legumes such as lupin contain water soluble carbohydrates such as the raffinose family oligosaccharides (RFO), which humans cannot break down in the gut. These oligosaccharides ferment in the lower gut and is a major cause of flatulence (Muzquiz *et al*., 1999). The lowering of the RFO content due to the protein isolation process could be considered beneficial in food applications due to the removal of the flatulence causing oligosaccharides. A study by Smith *et al*. (2006) also indicates that oligosaccharides are a good source of nutrients for probiotic micro flora within the low gut. The concentrated IDF and TDF in the fibre fraction would be ideal for food applications as opposed to the use of the lupin flour as a dietary fibre source.

Whey fraction

The main by-product formed during the traditional protein isolation process is the whey fraction. The whey fraction consists of the soluble protein and other soluble matter. The whey protein fraction is thought to possess functional properties that are required in food applications such as whipperability which is ideal for use in the manufacture of products such as Pavlova and cakes. In the present study whey protein was isolated by adding different salts in order to salt-out the protein. Table 3 shows the percentage of protein that was isolated by the salting-out process. The highest recovery was from TCA which was similar to the results of Hartono (2003), who used only TCA to precipitate the whey proteins. However, due to potential harmful effects, TCA treated proteins cannot be used for food applications.

All salts, except sodium sulphate and TCA, had no significant effect on protein recovery (Table 4). Even though sodium sulphate recovered a significantly higher amount of protein, the protein content was lower than that of the control. A higher recovery but lower protein content is an indication that material other than protein has been removed from the liquid phase. The control and TCA treated sample had the highest protein content. It is the protein itself that possess the required functionality in food applications, higher protein content is required for good functionality in food products. The results indicated

Table 4. Protein recovery from the whey fraction.

Treatment	Protein recovered (%)	Protein content (%)
Control[1]	20.3[c]	80.8[ae]
Calcium chloride	18.0[c]	78.2[a]
Calcium citrate	22.4[b,c]	81.1[a]
Potassium phosphate	18.3[c]	53.9[b]
Potassium citrate	19.3[c]	53.2[b]
Sodium citrate	19.0[c]	65.1[f]
Sodium sulphate	27.8[b]	70.4[c]
Sodium acetate	18.4[c]	73.5[cd]
Sodium phosphate	17.5[c]	78.7[e]
Sodium chloride	22.6[bc]	74.6[d]
Trichloroacetic acid	49.9[a]	81.7[a]

[1] Control = lupin whey protein isolated by re-centrifuging without any salt treatment.
Values with the same letter within the same column are not significantly different ($P<0.05$).

it is possible to extract the proteins in the whey fraction by a simple second centrifugation. Thereby increasing the protein recovery and minimising the protein left behind in the whey fraction.

Emulsifying activity

The functional properties of proteins important in food applications include emulsifying and foaming properties. In the study, it was possible to determine the EA of only four protein isolates due to sample size limitations. The quantity of isolate was not sufficient to determine functional properties with samples treated with calcium chloride, calcium citrate, potassium phosphate, potassium citrate, sodium citrate and sodium phosphate. The EA of whey protein isolates were determined at pH 6 and the results are summarised in Table 5.

The highest EA was recorded for lupin whey protein isolated by sodium acetate followed by the control (Table 5). It is an indication that just by an additional centrifugation step the lupin whey protein isolate with good EA can be produced. The EA of lupin protein isolate obtained by Chih (2004) was at 53.5%, which is similar to the whey protein's EA. By incorporating the two protein fractions, a greater protein yield could be obtained.

Emulsion stability

ES was determined by heating the developed emulsion. As discussed by Pozani *et al.* (2002) by increasing the temperature the surface hydrophobicity of the protein molecules are changed and thereby its capacity to hold the emulsion is lost. The lupin whey protein isolated by sodium acetate was the most stable with a value of 46% and the next most stable emulsion was by the control at 44%. The whey protein isolated by the use of sodium acetate or by a simple centrifugation process delivers the best ES. However, any impact on flavour by the control sample would be minimal since there is no added salt. Even though the EA and ES of the control were statistically lower than that of sodium acetate, the control produced an isolate with reasonable emulsifying properties.

Table 5. Mean values of emulsifying activity and stability at pH 6.

Treatment	Emulsifying activity (%)	Emulsion stability (%)
Control[1]	45.5^{b}	44.0^{b}
Trichloroacetic acid	42.6^{c}	37.6^{c}
Sodium acetate	49.3^{a}	46.0^{a}
Sodium sulphate	41.0^{d}	39.0^{c}
Sodium chloride	42.0^{cd}	38.6^{c}

[1] Control = lupin whey protein isolated without any salt treatment.
Values with the same letter within the same column are not significantly difference (P<0.05).

Foaming capacity

The foaming capacities at pH 6 of lupin whey protein isolates are presented in Table 6. The results show that the control and the sample isolated with TCA have the highest foaming capacities. Although sodium acetate treatment has the best emulsion properties, the treatment resulted in lower FC.

The FC of the whey protein in this study was lower than those reported by Wasche *et al.* (2001) and Chih (2004) for lupin protein isolates (non-whey protein). These authors found the foaming properties of lupin protein isolates to be 1,025% and 590%, respectively, at pH 6. The difference could be due to the difference in the type of protein under investigation in the two studies compared to the whey protein in the present study.

Foam stability

The FS at the different time intervals of the whey protein isolates are shown in Table 7. The foam stability at 30, 60, 90 and 120 minutes are represented as FS30, FS60, FS90 and FS120, respectively. The best stability was recorded for the control and the TCA treatment.

At pH 6, the foam stabilities of the control and the sample extracted with TCA were significantly better than the other samples at 30, 60, 90 and 120 minutes. Foams of both samples lost their stabilities in a similar manner over time, demonstrating that there was no difference in the performance of these two foams.

The superior performance in the FC and the FS of the lupin whey protein isolates obtained without any salt treatment

Table 6. Mean values of foaming capacity (FC) at pH 6.

Treatment	FC (%)
Control[1]	400^{a}
Trichloroacetic acid	400^{a}
Sodium acetate	360^{b}
Sodium sulphate	333^{bc}
Sodium chloride	300^{c}

[1] Control = lupin whey protein isolated without any salt treatment.
Values with the same letter within the same column are not significantly difference (P<0.05).

Table 7. Mean values of foam stability (FS) at pH 6.

Treatment	FS30 (%)	FS60 (%)	FS90 (%)	FS120 (%)
Control[1]	400[a]	400[a]	335[a]	270[a]
Trichloroacetic acid	405[a]	405[a]	333[a]	267[a]
Sodium acetate	340[b]	300[b]	250[b]	133[b]
Sodium sulphate	320[bc]	297[b]	250[b]	100[b]
Sodium chloride	290[c]	267[b]	233[b]	100[b]

[1] Control = lupin whey protein isolated without any salt treatment.
FS30, FS60, FS90, FS120 = foam stability at 30, 60, 90 and 120 minutes, respectively.
Values with the same letter within the same column are not significantly difference (P<0.05).

suggest that good quality foams from lupin whey fraction can be produced without any added salts. It would be simple and cost effective method of isolating the whey protein in a commercial setting. The good foaming and emulsifying properties of lupin whey protein isolates show the potential to be used in various food formulations. Examples of this food category include cakes, marshmallows, whipping cream and frozen desserts. The inclusion of lupin dietary fibre and whey protein in food applications can increase the health benefits of such products.

4. Conclusions

Defatting has no significant effect on the SDF content of lupin flour. Defatting resulted in the reduction of the IDF and TDF contents of the fibre fraction. The protein isolation process changes the ratio of the soluble and IDF in the fibre fraction but had no significant impact on the TDF content.

Emulsifying and foaming properties of the lupin whey protein isolates depend on the type of salt used. Lupin whey protein isolates with good functional properties can be produced by simple additional centrifugation step. Sodium acetate resulted in better emulsion properties but poor foaming properties. By extracting the whey protein by a simple re-centrifugation step the isolation of lupin protein can be maximised and still maintain a high level of functionality thereby increasing its commercial value. Lupin whey protein isolates has a great potential to be used in food formulations, such as in salad dressings, yogurt, gravies, whipping cream and cakes as a replacement for expensive egg proteins.

The co-products of lupin protein isolation process have the potential to be used as a cost effective sources of dietary fibre for food formulation. The next logical step is to conduct studies to evaluate the impact of the lupin fibre fraction and the whey protein in food formulations.

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