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Optimization of a Pilot-Scale Process for Producing Lupin Protein Isolates with Valuable Technological Properties and Minimum Thermal Damage

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This paper describes a pilot process for obtaining protein isolates from white lupin seed with improved water solubility and technofunctional properties as well as reduced thermal damage. After a careful optimization of the process parameters, two valuable food ingredients were prepared: lupin protein isolate type E, with a useful emulsifying capacity, and lupin protein isolate type F, with a high capability of foam formation and stabilization. The spray-drying process was particularly critical for inducing some thermal damage, but a careful selection of the conditions permitted ingredients having only marginally impaired lysine bioavailability to be obtained. The reproducibility of the protein extraction process was tested on two different lupin varieties.

KEYWORDS: Protein extraction; lupin protein isolate; nonenzymatic browning; furosine; HMF; thermal damage; technofunctional properties

INTRODUCTION

Seeds of *Lupinus albus* (white lupin) have been used as food by the Mediterranean populations for over 3000 years (*1*). Before consumption, traditionally they were soaked in water to remove the alkaloids responsible for their bitter taste (*2*), whereas, starting from the second half of the 20th century, sweet varieties have been selected and domesticated, the seeds of which may be consumed directly without any previous treatment, because the alkaloid concentration is much lower (*3*). The introduction of these cultivars has enormously broadened the possibility of using white lupin in both human and livestock nutrition. The high protein content (∼35−40%) indicates that white lupin has the potential to become a useful source of protein concentrates and isolates, to be exploited, for example, in replacements for milk, egg, or meat proteins.

White lupin has some characteristics that may be positively considered by consumers and the food industry: the content of antinutritional components is very low (*4, 5*), the level of phytoestrogens is negligible (*6*, *7*), the beanlike flavor is rather faint, and no genetically modified varieties are commercially available. Other favorable features of lupin protein concentrates and isolates are related to some key technofunctional properties, that is, emulsifying and foaming properties and the optimization of cost in comparison to most animal proteins (*8*).

In addition, the recent literature has pointed out some possible health benefits: a moderate daily intake of lupin protein extract led to a reduction of total and low-density lipoprotein (LDL) cholesterol (*7*), when administered to an established animal model of hyperlipidemia, and a specific protein fraction was able to control hyperglycemia in a rat model (*9*). Moreover, recent in vitro studies showed positive effects of lupin protein isolates on bile-acid binding, angiotensin converting enzyme inhibition, and DPPH radical scavenging activity (*10*).

The main objective of this research was to develop an efficient pilot process for obtaining lupin protein isolates from white lupin kernels (*11*). A recent report has indicated that protein isolates from white lupin may have very good water solubility as well as emulsifying and foaming properties if they are not denatured (*12*). As these features are very critical in determining the application potential of any ingredient in food manufacture, the investigation of the influence of different processing conditions on the technofunctional properties of the isolated protein fractions was another main objective of this work.

The nutritional value of protein (*13*) may be impaired by the industrial processing, mostly as a result of nonenzymatic browning (NEB), which involves reducing sugars and the free amino acids or the side chains of protein-bound lysine and arginine (*14*). Taking this into consideration, the process was optimized also with the objective of reducing the thermal damage of the final products in order to compete with other

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Table 1. Modification of Processing Conditions in Trials T3 and T4 Compared to the Initial Process, T1

	T1	T3 + T4
UF-input	acid extract + precipitation supernatant	acid extract
UF-temperature	40 °C	cold, ∼15 °C
diafiltration	supernatant of precipitation + deionized water	osmosis water
pasteurization LPI-F	before spray-drying, longer storage time 60 °C, 45 s	plate heat exchanger integrated in UF 60 °C, 30 min
spray-drying LPI-F	after ∼10 h of storage	immediately after UF

commercial food ingredients. This was done by measuring two classical molecular markers of NEB (*15*): *N*ϵ-furoylmethyl-L-lysine (furosine) and 5-(hydroxymethyl)-2-furancarboxaldehyde (HMF). Furosine is produced by acid hydrolysis of the Amadori compounds formed in the early stage of NEB (*16*) and is useful to measure the early stage of this reaction, whereas HMF, being an intermediate of the 1,2-enolization route of the decomposition of the Amadori rearrangement product, is a marker of the middle stage of NEB (*14*).

MATERIALS AND METHODS

Materials. Two varieties of white lupin seeds were used as raw material for the protein extraction: *L. albus* Typ Top obtained from a Chilean grower (var. Baer) and *L. albus* Ares obtained from Terrena, FR.

Protein Extraction in Pilot Scale. *Pilot Plant Equipment*. The following equipment was used for protein processing: a de-huller from Streckel & Schrader, capacity = 200 kg/h; a zig-zag sifter from Alpine Hosakawa, capacity = 60 kg/h; a flaking mill from Streckel & Schrader, capacity = 250 kg/h; a deoiling plant including flash desolventizer from E&E Verfahrenstechnik GmbH, maximum volume = 1500 L; stirring tanks, 3 × 2 m^3, stainless steel; a decanter centrifuge from Westfalia, CB 300, 2.200 m^2 equivalent surface; a disk-type separator from Westfalia, SC-20, 20.000 m^2 equivalent surface; an ultra-/diafiltration unit, Pall, type cross-flow, cutoff = 10 kDa, surface area = 10 m^2; and a spray-dryer, APV, cocurrent, evaporation rate for protein solutions = up to 40 kg/h.

Process Description. In total four different trials were performed: trial 1 (T1) was the initial procedure, whereas trials 2, 3, and 4 (T2, T3, and T4) were used to optimize the processing conditions and to verify the reproducibility (see **Table 1** for a summary of the main characteristics of T1, T3, and T4).

Trial 1 (T1): Initial Procedure. The main features of this procedure are shown in **Figure 1**. After dehulling of the lupin seeds in an underrunner disk sheller, the kernels and hulls were separated in an air sifter and the kernels were flaked using a roller mill. The lupin flakes were deoiled using either hexane or supercritical carbon dioxide and then used as input material for the protein extraction plant. Extraction from deoiled flakes was performed under mild conditions using a two-stage process (*11*). In the first stage, the deoiled flakes (180 kg) were mashed in cold water (1800 L) under acid conditions (pH 4.5) to separate the low molecular weight compounds, such as the oligosaccharides and the off-flavors (e.g., alkaloids) from the basic proteins. The clarified acid extract was concentrated by ultra-/diafiltration to give the lupin protein isolate F (LPI-F). In the second stage, the main storage protein fraction was extracted at neutral pH and separated from the insoluble fibers, then lupin protein isolate E (LPI-E) was enriched from the resulting protein extract by using acid precipitation at the isoelectric point. In the initial procedure also the supernatant of the precipitation was ultrafiltered to obtain lupin protein isolate F. Finally, the protein isolates were pasteurized and spray-dried.

Trial 2 (T2): Optimization of the Spray-Drying Conditions (T2). The precipitated protein fraction was produced using the same steps

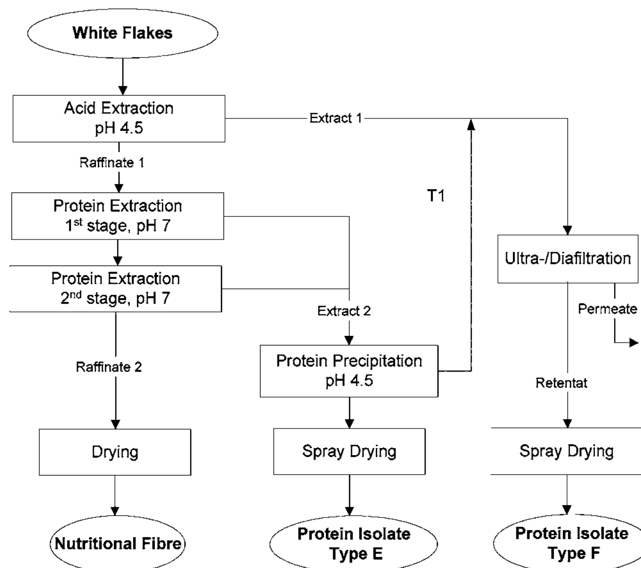

Figure 1. General procedure for the extraction and separation of lupin protein isolates from white flakes.

and conditions as in T1. The precipitate was used as input material for the spray-dryer, and the settings were changed in order to evaluate the thermal damage by measuring HMF and furosine. The three different series of conditions investigated are the following: *trial 2a*, constant temperature of hot air (T_{in} = 160 °C) and variation of the temperature of exhausted air (T_{out} = 65−80 °C) in five steps; *trial 2b*, variation of the temperature of hot-air (T_{in} = 160−220 °C) in five steps and constant temperature of exhaust air (T_{out} = 75 °C); and *trial 2c*, same settings as for trial T2b, but a two-stage drying instead of a single stage one.

Trial 3 (T3): Optimization of the Separation Conditions. A third trial (T3) was planned to take into account the results of T2 and other modifications in order to decrease the deterioration of the proteins while maintaining their technofunctional properties. The main focus was on the optimization of the ultrafiltration (UF) process to produce LPI-F with improved microbial quality and functional properties. LPI-E was produced by aqueous extraction and isoelectric precipitation following the initial process protocol (T1), whereas some main modifications were introduced in the production of LPI-F: (i) only the acid extract was used as input of the ultrafiltration process; (ii) filtration at lower temperature (15 °C) to inhibit microbial growth; (iii) longer pasteurization time (30 min at 60 °C instead of 45 s at 60 °C); and (iv) in-line pasteurization immediately after the UF with integrated plate-heat exchanger.

Trial 4 (T4): Reproducibility. A fourth trial (T4) was performed to prove the reproducibility of the process conditions and the resulting quality of the LPI as well as the transferability to other varieties of lupin. The process conditions in T4 were similar to those of T3, but the raw lupin seeds were from *L. albus* var. Ares instead of *L. albus* var. Typ Top.

Proximate Analysis of Protein Isolates. The chemical composition (dry matter, nitrogen, ash, and oil contents) of the recovered lupin protein isolates were analyzed in accordance with the official method (*17*).

Microbiological Analysis. Ten grams of each sample was diluted with 90 mL of physiological saline solution (0.9% NaCl) and 0.1% Tween 80 and thereafter homogenized. This solution was diluted further to produce a dilution series ranging from 10^{-1} to 10^{-6}. Diluted samples were plated in Petri dishes containing standard plate count agar (PCA; Merck no. 1.05463) and incubated at 30 °C. The aerobic mesophilic count [colony-forming units (CFU/g)] was determined after 3 and 6 days of incubation time as an average of three plates. Spore-forming bacteria were cultivated on Caso agar (Merck no. 1.05458) for 6 days at 30 °C. Detection of bacteria of the Coli-Group was done with VRB-agar (Merck no. 1.04030) after 24 h of incubation at 37 °C. The existence of *Salmonella* spp. was checked in 25 g samples by enrichment in RVS-bouillon and subsequent cultivation/isolation using different selective media (Rambach-agar, BPLS-agar).

Materials for the Evaluation of NEB. HPLC-grade methanol and acetic acid were purchased from Baker (Deventer, The Netherlands); the water used as eluent in the HPLC analysis of furosine was from Fluka (Milan, Italy); the water used in all other procedures as well as as eluent in the HPLC analysis of HMF was produced with a Milli-Q water purification system (Millipore, Billerica, MA). Furosine (purity = 99.5%) was from Alltech (Milan, Italy) and HMF (purity = 99%) from Sigma-Aldrich (St. Louis, MO). SPE cartridges High Capacity C_{18} Extract-Clean (bed-weight = 500 mg, tube size = 4 mL, particle size = 50 μm, pore size = 60 Å, carbon loading = 17%) and 0.45 μm disposable nylon filters for HPLC eluents and samples were from Alltech.

Analysis of Furosine. The determination of furosine was done by RP-HPLC following a procedure previously reported for milk (*18*). In a screw-cap Pyrex vial, an aliquot of sample corresponding to ~500 mg of protein (as determined by C,H,N analyzer, N × 5.4) was added with 8 mL of 8 N HCl; the closed vials were sealed under vacuum and kept at 110 °C for 23 h. After filtration on a paper filter, the filtrate (0.5 mL) was applied on an Extra-Clean C18 cartridge, prewetted with 5 mL of methanol and 10 mL of water. The displaced liquid was discarded, and then furosine was eluted with 3 mL of 3 N HCl. The HPLC analyses were conducted with a Hewlett-Packard HP-1050 quaternary pump fitted with a Rheodyne injector (20 μL, loop) and equipped with a HP-1050 variable wavelength detector (HPLC-VWD). The analyses were carried out on a C_8 furosine-dedicated column (5 μm, 250 mm × 4.6 mm, Alltech). Conditions: eluent A, 0.4% acetic acid in water; eluent B, 0.3% KCl in eluent A (w/v); gradient, 0% B for 13.5 min, 0−50% B in 7 min, then 50% B for 2 min; flow rate = 1.2 mL/min; UV detection at 280 nm. Furosine was eluted at t_R 22−25 min. Quantification was performed by the external standard method: the results were expressed as milligrams per 100 g of protein.

Analysis of HMF. The determination of HMF was performed by RP-HPLC using a procedure previously proposed for milk (*19*). Each sample (100 mg) was hydrolyzed with 1 mL of 0.3 N oxalic acid at 100 °C for 3 h. After rapid cooling in ice, 0.5 mL of trichloroacetic acid solution (40% w/v) was added; then the sample was centrifuged at 12063*g* for 12 min and filtered through a 0.45 μm filter before injection into HPLC. The chromatographic analyses were performed on a C_{18} Spherisorb ODS-2 column (250 × 4.6 mm, 5 μm, Merck). Conditions: mobile phase, 100% sodium acetate buffer 0.08 mM, adjusted at pH 3.6 with acetic acid; flow rate, 1 mL/min; injection volume, 20 μL; wavelength, 284 nm. HMF was eluted at t_R 7−9 min. The quantification was performed by the external standard method, using a calibration curve in the range of 0.25−5 mg/L: the results were expressed as milligrams per 100 g of protein.

Analysis of Technofunctional Properties. *Water-Binding Capacity.* The analyses were conducted according to the AACC official method (*20*).

Oil-Binding Capacity. This was determined by dispersing the sample in oil and subsequent centrifugation following a method described by Ludwig et al. (*21*).

Protein Solubility. This was determined according to the method of Morr et al. (*22*), whereas the NSI value was determined in accordance with the official AOCS method (*23*) or AACC official method (*24*).

Emulsifying Capacity (EC). The protein solution (1% w/w) was stirred at constant temperature (20 °C) in an 1 L laboratory reactor (IKA) with a stirrer and an emulsifying system (Ultra-Turrax, IKA-Werke GmbH & Co. KG, Staufen, Germany). The oil was automatically added by a titration system (Metrohm GmbH & Co. KG, Herisau, Switzerland). The conductivity was continuously measured and used as parameter for the determination of the inversion point of the emulsion. The amount of oil added until the inversion point of the emulsion was used to calculate the emulsifying capacity (milliliters of oil per gram of protein).

Emulsion Stability (ES). Emulsions [1:10:10 (w/v/v)] were prepared in a 1 L laboratory reactor (IKA) with a stirrer and an emulsifying system (Ultra-Turrax, IKA-Werke GmbH & Co. KG). The homogenization was done at 11000 rpm for 5 min. After homogenization, the emulsion was poured into four centrifuge tubes. The tubes were heated for 30 min at 80 °C and then stored at 5 °C for 12 h. After storing, the samples were centrifuged for 10 min at 4500*g* and 20 °C. The stability of the emulsion after centrifugation was calculated as follows (eq 1):

$$\text{ES}\ (\%) = \frac{\text{vol of emulsified layer}}{\text{total vol}} \times 100 \qquad (1)$$

Foam Capacity (FA) and Foam Stability (FS). Foams were generated using a whipping machine (Hobart N 50, Hobart GmbH, Offenburg, Germany). The foaming activities of 5% protein solutions were obtained by comparing the foam volume after 8 min of whipping with the volume of the starting protein solution. The foaming capacity was calculated according to eq 2:

$$\%\ \text{FC} = \frac{\text{foam vol after whipping}}{\text{vol of protein solution}} \times 100 \qquad (2)$$

For measuring the foam stability, foams were immediately poured into a 250 mL graduated glass cylinder. Foam volume after 60 min was recorded for calculating foam stability (eq 3).

$$\%\ \text{FS} = \frac{\text{foam vol (60 min)}}{\text{initial foam vol}} \times 100 \qquad (3)$$

Table 2. Mass and Protein Balance of the Protein Extraction: Comparison of Trials T1, T3, and T4[a]

	yield (%)					
	T1		T3		T4	
lupin protein isolate	DM[b]	protein	DM	protein	DM	protein
LPI-E	22	39	29	51	28	52
LPI-F	6.8	10.9	2.4	3.6	2.6	4.9
total (E + F)	28.8	49.9	31.4	54.6	30.6	56.9

[a] Yields relate to either the input dry matter or protein of white flakes, considering the input = 100%. [b] Dry matter.

RESULTS AND DISCUSSION

Separation of Lupin Protein Isolates. A sequence of experiments was planned to select the best conditions for the separation of the lupin protein isolates. The first trial (T1) was used to establish the full process including the production of both LPI-E and LPI-F. For the spray-drying a two-stage procedure was applied with the aim of reducing the share of fine particles. The resulting products were analyzed for the NEB products HMF and furosine as markers of the thermal damage and for the microbial quality and the technofunctional properties, as explained in the following paragraphs.

Mass and Protein Balance. The protein extraction in T1 delivered two main lupin protein fractions: LPI-E, recovered by isoelectric precipitation, and LPI-F, recovered by ultrafiltration. About 29% of the input dry mass and around 50% of the initial protein could be recovered as protein isolates (**Table 2**); 22% of the input dry mass corresponding to 39% of protein was recovered as LPI-E, whereas the yield of LPI-F was 6.8% of the dry mass and 10.9% of protein, respectively.

Chemical Composition. As shown in **Table 3**, the protein content of LPI-E was 91.8% (N × 5.7) and that of LPI-F slightly lower, that is, 84%. The fat contents of both isolates were low, being 1.16 and 0.24%, respectively. These results clearly show that the protein isolation process was efficient in yielding high protein concentrations.

Microbial Data. The microbial status of LPI-E proved to be very satisfactory (see **Table 4A**), as the total germ number was ~10^4 CFU/g, which is considered to be the guide level of commercial plant protein ingredients (e.g., soy protein isolates). Spore-forming bacteria represented the biggest part thereof, whereas the content of coliform bacteria was not critical and salmonella could not be detected at all. On the contrary, the

Table 3. Chemical Composition of the Lupin Protein Isolates Produced in Trials T1, T3, and T4, in Accordance with the Official Method (*17*)

trial	product	dry matter (%)	protein[a] (%)	fat (%)	ash (%)
T1	LPI-E	96.17 ± 0.08	91.76 ± 0.13	1.16 ± 0.01	3.93 ± 0.1
T1	LPI–F	93.53 ± 0.04	83.39 ± 0.08	0.24 ± 0.01	5.50 ± 0.08
T3	LPI-E	94.33 ± 0.03	91.21 ± 0.01	1.24 ± 0.02	3.76 ± 0.05
T3	LPI–F	91.93 ± 0.03	77.09 ± 0.19	0.21 ± 0.03	5.80 ± 0.05
T4	LPI-E	94.29 ± 0.08	90.32 ± 0.22	1.06 ± 0.07	4.01 ± 0.04
T4	LPI–F	92.75 ± 0.01	73.80 ± 0.23	0.18 ± 0.01	9.62 ± 0.01

[a] N × 5.7

total germ number of LPI-F was ~10^9 CFU/g, an unacceptably high value, which was the main motivation for further optimization of the isolation process.

Thermal Damage. The results of the analyses of the samples produced with the initial procedure (T1) were the following: LPI-E, furosine = 25.7 ± 0.48 mg/100 g of protein, HMF = 4.8 ±.0.06 mg/100 g of protein; LPI-F, furosine = 53.6 ± 1.65 mg/100 g of protein, HMF = 7.6 ± 0.33 mg/100 g of protein. Considering the lysine content of these products, the values are in line with those reported by other authors in the case of dairy products (*18, 25,* 26) and soy and wheat flours (*27*).

The furosine values were used to calculate the amount of blocked lysine and consequently of available lysine (*28*). Because the lysine content of lupin storage proteins is 47.5 mg/g of protein, the content of available lysine in LPI-E is 47.2 mg/g (corresponding to 0.6% lysine loss). On the contrary, because LPI-F contains mainly γ-conglutin, the lysine content of which is 57.7 mg/g of protein, its available lysine value is 57.2 mg/g of protein (corresponding to 1.0% lysine loss).

Technofunctional Properties. The technofunctional properties of the protein isolates produced in T1 are presented in **Table 5**. Both isolates, LPI-E and LPI-F, showed very good solubility at pH 7, which indicates the native status of the recovered proteins. The water- and oil-binding capacities being ~1−2 mL/g protein were not very satisfactory. The LPI-E showed a good emulsifying capacity of 450 mg of oil/g of protein, comparable to those of commercial soy protein isolates and whole egg powders (*10*, *29*), whereas LPI-F showed a very good foaming capacity (2000%) and foam stability (95%), comparable to those of commercial egg white protein (*30*).

Optimization of the Spray-Drying Conditions. A second set of trials (T2a, T2b, and T2c) was planned with the aim of optimizing the spray-drying conditions. In general, higher temperatures are useful to optimize the operating efficiency, because higher water evaporation rates can be achieved; however, at higher temperatures more protein denaturation may occur, which is detrimental for the product quality. With this taken into consideration, temperature variations were applied by keeping constant either the hot air temperature or the exhausted air temperature. In addition, the influence of the operational mode (single-stage or two-stage drying) was investigated. Two-stage drying led to an increase of the mean particle size from ~25 μm in the one-stage process to 100 μm in the two-stage drying; however, the wettability did not improve correspondingly. The time to moisten a standardized powder layer of 0.02 g/cm^2 was in the same range (>600 s) for all of the samples. As the two-stage drying process has the disadvantage of leading to longer residence times in the dryer, which may adversely affect the protein quality, the thermal damage of these samples was investigated by analyzing HMF and furosine.

Thermal Damage. The results of the analyses of furosine and HMF in these experiments are presented in **Table 6**. The samples produced in the three sets of trials showed different extents of thermal damage. The samples of the T2a group (in which T_{in} was maintained constant at a low value and T_{out} was varied between 65 and 80 °C) suffered less thermal damage than the T2b group (in which T_{out} was maintained constant at 75 °C and T_{in} was varied between 160 and 220 °C). This indicates that, to reduce the thermal damage of the protein, it is very useful to keep the inlet air temperature at a low value.

The two-stage procedure, which may be useful to increase the particle size (agglomeration), proved to be detrimental from the point of view of the thermal damage that increased by roughly 60−70% compared to single-stage drying (T2c group of experiments).

Figure 2 permits comparison of the two NEB markers, to evaluate which one may be more useful for describing these experiments (to permit a direct comparison of the two markers, in this chart the units of measurement were converted to millimoles per kilogram). Although within each set of experiments the two parameters are not very well correlated, each set is very well separated from the others. HMF is less effective in discriminating between mild and drastic processes (T2a and T2b) than between one-stage or two-stage procedures (T2b and T2c sets), whereas furosine is able to separate all groups. However, the results suggest that both markers be measured in order to have a general picture of the consequences of the process parameter modifications, considering that these parameters are linked to different stages of the MR.

Optimization of the Processing Conditions and Reproducibility. In an additional trial (T3), the spray-drying process was adapted on the basis of the findings of the optimization program in trial T2, but processing conditions were modified to improve the separation and quality of the ultrafiltered protein fraction LPI-F. These process conditions were then repeated in trial 4 (T4), to prove the reproducibility of the process on a different variety of lupin seeds. As for the initial process, the quality of the resulting lupin protein isolates was evaluated by measuring the markers of NEB, the chemical composition, the functional properties, and the microbial status.

Thermal Damage. The results of T3 and T4 are reported in **Table 7**, together with the values referring to intermediate materials as lupin seeds, yellow flakes (undefatted), and white flakes (defatted). These last materials contain already some furosine (ranging from 9 to 12 mg/100 g of protein) and a large amount of HMF, which may be possibly explained with the high content of reducing sugars in lupin seeds (*5*). The purification steps applied to the seeds and flakes resulted only in an slight increase of furosine and, in particular, the defatting procedure affected only marginally the thermal damage of the flakes, measured by furosine. At the same time, however, a strong decrease of HMF was observed.

To compare the thermal damage of the lupin protein isolates, both HMF and furosine values of LPI-E were significantly lower in trial T3 than in trial T1, whereas the furosine value of T4 was very similar to that of T1. Because careful control of the pasteurization time and spray-drying conditions permitted reduction of the NEB, the thermal damage in the optimized conditions is only marginal (for example, the loss of available lysine in T4 is only 0.8%).

Both indices in LPI-F are much higher, a fact linked to the higher lysine content of γ-conglutin (57.7 mg/g of protein), the main component of this fraction. In addition, because the microbiological quality obtained in T1 was rather unsatisfactory, in the subsequent trials it was necessary to increase the pasteurization time to 30 min, which increased the values of

Table 4.

(A) Microbiological Status (CFU/Gram of Product) of Averaged Samples Taken from Trials T1, T3, and T4[a]

trial	product	total aerobic count[a]	spore-forming aerobic[a]	spore-forming anaerobic[a]	*Salmonella*	total coliforms[a]	molds[a]
T1	white flakes	3.4×10^2	na	na	negative	3.7×10^1	na
T1	LPI-E	1.2×10^4	6.9×10^3	1.4×10^3	negative	3.4×10^3	4.3×10^1
T1	LPI-F	3.7×10^9	1.0×10^4	1.3×10^4	negative	1.6×10^8	<10
T3	LPI-E	6.8×10^4	na	na	na	na	na
T3	LPI-F	3.2×10^3	na	na	na	na	na
T4	white flakes	1.7×10^5	na	na	na	na	na
T4	LPI-E	1.4×10^4	1.5×10^4	na	negative	<10	na
T4	LPI-F	9.2×10^3	2.6×10^3	na	negative	<10	na

(B) Assessment of Microbiological Status (CFU/Gram of Product) of Intermediate and End Products during Modified Processing of LPI-F (T3)

product	raw material	UF-retentate	UF-retentate (pasteurized)	LPI-F (spray-dried)
total aerobic count	3.4×10^2	1.6×10^4	1.6×10^2	3.2×10^3

[a] Ten gram samples. negative, not detectable in 2 × 25 g of sample; na, not analyzed.

Table 5. Technofunctional Properties of LPI-E and LPI-F from Trials T1, T3, and T4

	LPI-E			LPI-F		
	T1	T3	T4	T1	T3	T4
protein solubility[a] (%)	64 ± 0.5	72 ± 0.3	69 ± 0.4	71 ± 0.3	67 ± 0.4	75 ± 0.4
water-binding capacity (mL/g of protein)	0.8 ± 0.2	0	0	1.3 ± 0.15	1.8 ± 0.1	1.0 ± 0.1
oil-binding capacity (mL/g of protein)	1.3 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
emulsifying capacity[a] (mL oil/g of protein)	450 ± 10	385 ± 5	410 ± 5	370 ± 7	570 ± 10	515 ± 5
emulsion stability[b] (%)	61 ± 2	63 ± 2	63 ± 1	74 ± 1.5	83 ± 1.5	93 ± 1
foam capacity (%)	1102 ± 10	1200 ± 16	1470 ± 14	2083 ± 24	1800 ± 10	1837 ± 15
foam stability (%)	73 ± 1	68 ± 1	87 ± 1	95 ± 1	90 ± 1	96 ± 1

[a] One percent solution at pH 7. [b] After heat treatment at 80 °C.

Table 6. Nonenzymatic Browning Measured as Furosine and HMF in Samples Produced in Trials 2a, 2b, and 2c Using Different Temperature Conditions during the Spray-Drying Procedure

trial_sample no.	temperature (°C) T_{in}	T_{out}	proteins (%)	furosine (mg/100 g of protein)	HMF (mg/100 g of protein)
first group					
T2a_1	160	65	81.39 ± 0.12	8.04 ± 0.48	1.42 ± 0.03
T2a_2	160	70	82.26 ± 0.04	8.39 ± 0.19	1.34 ± 0.03
T2a_3	160	75	82.47 ± 0.07	8.62 ± 0.25	1.31 ± 0.01
T2a_4	160	78	83.12 ± 0.31	8.10 ± 0.05	1.01 ± 0.07
T2a_5	160	80	83.19 ± 0.04	7.46 ± 0.81	1.31 ± 0.01
second group					
T2b_1	160	75	83.47 ±0.11	15.17 ± 0.53	1.46 ± 0.06
T2b_2	190	75	78.55 ± 0.04	17.77 ± 0.16	1.90 ± 0.35
T2b_3	200	75	78.65 ± 0.01	17.07 ± 0.09	1.75 ± 0.05
T2b_4	210	75	78.43 ± 0.04	17.61 ± 0.74	1.38 ± 0.08
T2b_5	220	75	78.56 ± 0.05	17.65 ± 0.39	1.22 ±0.05
third group					
T2c_1	160	75	83.46 ± 0.04	29.03 ± 0.79	4.23 ± 0.03
T2c_2	170	75	83.76 ± 0.02	24.40 ± 0.50	4.16 ± 0.13
T2c_3	180	75	83.31 ± 0.11	29.06 ± 0.43	4.06 ± 0.11
T2c_4	190	75	83.15 ± 0.23	29.07 ± 0.16	4.06 ± 0.03
T2c_5	200	75	83.19 ± 0.16	23.09 ± 0.49	3.51 ± 0.03

both furosine and HMF in T3 and T4. Despite this, however, the loss of available lysine in this food ingredient was only 1.8%.

Mass and Protein Balance. The mass and protein balances of the modified trials T3 and T4 are shown in **Table 2** in comparison to the initial process (T1). In the modified protein extraction in T3 ~31% of input dry mass corresponding to 55% of input protein could be recovered in protein isolates E and F: 29% of input dry mass (51% of protein) as LPI-E and 2.4% of dry mass (3.6% of protein) as LPI-F. The yields in T4 were equivalent, which proves the reproducibility of the optimized processing conditions. Compared to the first trial the yield of LPI-E in T3 and T4 increased by 7% on the dry mass and by 11% on the input protein, whereas the yield of LPI-F was lowered by around 4% (dry mass) and 7% (protein), respectively.

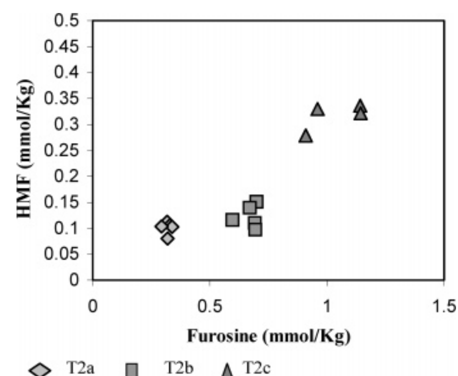

Figure 2. Comparison of the molecular markers of the thermal damage, as measured by furosine and HMF, of trials T2a, T2b, and T2c.

Chemical Composition. The chemical analyses showed that the composition of LPI-E from trials T3 and T4 was very similar to the product obtained in the initial process (T1). The protein contents of the LPI-F were similar in T3 and T4, but slightly lower than in trial T1, whereas dry matter and fat contents were constant across the different trials (**Table 3**).

Microbial Data. Data of microbial analyses (**Table 4A**) showed that total aerobic count of LPI-E in T3 and T4 slightly increased compared to T1, but remained in an acceptable range

Table 7. Nonenzymatic Browining of the Protein Isolates LPI-E and LPI-F Produced in Trials T3 and T4 Measured as HMF and Furosine

sample	trial	HMF (mg/ 100 g of proteins)	furosine (mg/ 100 g of proteins)	loss of lysine[a] (%)
LPI-E	T3	2.15 ± 0.12	10.24 ± 0.66	0.21
LPI-F	T3	9.60 ± 0.01	110.5 ± 12.4	1.83
lupin seed	T4	70.90 ± 3.12	9.48 ± 0.13	0.06
yellow flakes	T4	61.70 ± 2.26	7.94 ± 0.08	0.07
white flakes	T4	44.15 ± 1.77	12.30 ± 0.85	0.14
LPI-E	T4	2.40 ± 0.11	31.88 ± 1.42	0.80
LPI-F	T4	5.75 ± 0.06	112.87 ± 1.03	1.81

[a] Loss of lysine was calculated from furosine by using the equation proposed by Erbesdobler et al. (*28*).

around the guide level of 10^4 CFU/g. The process optimization in T3 and T4 reduced the germ number of LPI-F by 6 orders of magnitude to $\sim 10^3$ CFU/g. This reduction was reached by a summary effect: cold ultrafiltration enabled the germ number to be kept at a low level (1.4×10^4 CFU/g of product) and subsequent pasteurization of the retentate lead to further reduction in germ number to $\sim 10^2$ CFU/g of product (**Table 4B**).

Technofunctional Properties. The changes of the technofunctional properties of the protein isolates produced by modified processing are presented in **Table 5**. The functional properties of LPI-E were only slightly influenced as these values are in a constant range within the different trials. Concerning LPI-F, the protein solubility, water- and oil-binding capacities, and foaming properties were only slightly influenced by the changes in the processing, but the emulsifying properties improved significantly in trials T3 and T4. The emulsifying capacity (EC) increased from 370 mL of oil/g of protein in T1 to 510−570 mL of oil/g of protein in T3 and T4 and the emulsion stability (ES) of the heated emulsion increased from 74 to 93% respectively.

Conclusion. The process optimization described in this paper permitted two added-value food ingredients to be produced from lupin seeds: LPI-E, endowed by a valuable emulsifying capacity, and LPI-F, which may be useful when foam production and stabilization are required. Good reproducibility of the protein extraction process and of the technofunctional properties of the food ingredients was assessed. The effort dedicated to the careful selection of the process parameters, especially during spray-drying, permitted thermal damage to be limited to levels comparable to other food ingredients (*18*, *27*, *31*).

Other relevant nutritional and biofunctional characteristics of these materials had been already presented in a preceding paper (*32*). A first observation was that the level of phytoestrogens (*32*) in these purified materials is negligible, as lupin seeds (*6*) have a much lower isoflavone content than soy seeds (*33*). This feature is important because some recent papers have demonstrated that isoflavones may have some toxicological implications (*34*). In addition, a proteomic investigation performed on LPI-E from T3 and based on two-dimensional electrophoresis coupled with HPLC-ESI-MS/MS (*32*) has demonstrated that the different protein fractions typical of lupin seed are still detectable in LPI-E, indicating that this optimized process modifies only marginally the quality of lupin protein. This latter result is very relevant because it has been demonstrated that some commercial soy protein isolates undergo extensive hydrolysis (*35*), which impairs greatly the structure of the 7S globulins, the hypocholesterolemic component of soy protein (*36*−*38*).

ABBREVIATIONS USED

CFU, colony-forming units; DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate; EC, emulsifying capacity; ES, emulsion stability; FC, foam capacity; FS, foam stability; furosine, *N*ε-(2-furoylmethyl)-L-lysine; HMF, 5-(hydroxymethyl)-2-furancarboxaldehyde; HPLC, high-performance liquid chromatography; LPI, lupin protein isolate; MR, Maillard reaction; NEB, nonenzymatic browning; PCA, plate count agar; RVS-bouillon, Rappaport−Vassiliades bouillon; UF, ultrafiltration; VRB-agar, violet-red-blue agar.

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