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Influence of moderate electric field pretreatment on protein extraction from lupin flour

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ABSTRACT

Lupin protein quality is notably affected by the presence of anti-nutritional factors (ANF) such as polyphenols, alkaloids and saponins, along with anti-technological factors (ATF) like polyphenols and fat. This research addresses the impact of moderate electric field-assisted (MEF) pretreatment on lupin flour (LF) prior to protein extraction by isoelectric precipitation, focusing on its effects on ANF and ATF content, protein yield and techno-functional properties. Pretreatments were carried out for 3 min using water and ethanol–water (1:4 *v*/v), under two conditions: (i) conventional mechanical stirring (952 rpm) and (ii) MEF (from 150 to 300 V) at 60 °C. Pretreatment decreased noticeably ANF and ATF content of both LF and lupin protein isolate (LPI). Furthermore, it yielded a lupin protein concentrate (LPC, avg. 62 g protein/100 g) with reduced levels of ANF and ATF. LF pretreatment increased the recovery of protein (avg. 12 %). Compared to conventional pretreatment, MEF application reduced fat (avg. 21 %), saponins (avg. 37 %), polyphenols (avg. 38 %) and antioxidant activity (avg. 8 %) and improved LPI's techno-functional properties, including water and fat absorption index (avg. 17 and 68 %, respectively), foaming (avg. 122 %) and emulsifying properties (avg. 10 %). The LPC fraction also exhibited promising characteristics, indicating its potential as a valuable ingredient in food formulations. These findings highlight the effectiveness of MEF pretreatments as a strategy for reducing ANF and ATF, enhancing protein extraction and overall quality.

1. Introduction

The growing awareness of climate change and the urgent need for sustainable crops have accelerated the exploration of alternative protein sources that can meet future dietary requirements while minimizing environmental impact. Among plant-based proteins, legume crops have emerged as promising candidates due to their capacity to enrich soils through nitrogen fixation and their relatively low resource requirements. Specifically, lupin stands out due to its high protein content, typically ranging from 30 to 40 %, and its well-balanced amino acid profile. Lupin proteins provide essential amino acids, including lysine, which is often limited in other plant-based proteins. Furthermore, lupin is naturally low in fat and free of gluten. However, despite these advantages, the broader use of lupin as a protein source is hindered by the presence of anti-nutritional factors (ANF), such as alkaloids, polyphenols and saponins, and anti-technological factors (ATF), such as fat. These compounds can reduce protein digestibility, hinder extraction processes and alter the taste and texture of final protein products (Adrar et al., 2019; Bou et al., 2022; Navarro del Hierro et al., 2018). In order to address these challenges, new pretreatment technologies are being explored to enhance protein extraction while reducing the retention of ANF and ATF. Techniques such as high-power ultrasound (Navarro-Vozmediano, Dalmau, Benedito and Garcia-Perez, 2025b), thermal processing (Piccini et al., 2019; Sapna et al., 2019), enzymatic treatments (Perović et al., 2020) and fermentation (James et al., 2020; Ruan et al., 2020) have shown potential in mitigating these limitations, while also having the potential to modify the techno-functional properties of proteins.

One emerging approach that has gained attention for its effectiveness and energy efficiency is the use of moderate electric field (MEF). MEF processing operates at low field strengths (< 1 kV/cm) and is applied continuously during the extraction process, leading to a significant energy release into the medium. This continuous application induces simultaneous cell stress, which may result in membrane electroporation,

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and volumetric ohmic heating (Gavahian et al., 2018). Thus, MEF has the potential to enhance extraction efficiency by disrupting cell membranes and improving mass transfer (Astráin-Redín et al., 2024; Donsì et al., 2010; Fronza et al., 2021). Previous studies had demonstrated MEF effectiveness in extracting lipids from rice bran (Lakkakula et al., 2004), betanin from beet (Kulshrestha & Sastry, 2003) pectin from pomegranate peel (Sharifi et al., 2022), anthocyanins from grape skin (Pereira et al., 2020) or polyphenols from blueberry (Pataro & Ferrari, 2021) and rambutan peels (Torgbo et al., 2022). However, this technology has not been previously employed for ANF and ATF extraction prior to protein isolation, making this a significant area for further investigation. Furthermore, the use of MEF may induce structural modifications in proteins, which may alter their techno-functional properties, such as emulsifying and foaming capacities, as well as water and fat absorption, key factors for the development of food products (Cheng et al., 2023; Joeres et al., 2023; Subaşı et al., 2021; Wang et al., 2022). MEF is also advantageous as it operates at low energy levels and may reduce the use of organic solvents, making it an environmentally friendly alternative to traditional extraction methods (Bou et al., 2022).

In this context, the objective of this study was to investigate the application of moderate electric field-MEF as a pretreatment for lupin flour, assessing its impact on protein extraction, the removal of ANF and ATF, and the techno-functional properties of the recovered protein fraction.

2. Materials and methods

2.1. Plant material

Seeds of yellow lupin (*Lupinus luteus* L. var. *tremosilla*) were acquired from Semillas Batlle S.A (Barcelona, Spain). Whole seeds were milled into flour, achieving a final particle size between 200 and 1000 μm, using an industrial vertical hammer mill (Sitem-gran Ibérica S.L., 22 kW).

2.2. Conventional and moderate electric field pretreatment

The experimental setup for flour pretreatments is illustrated in Fig. 1. Conventional pretreatment (CV) was performed at 952 rpm using a mechanical stirrer (RZR 2021, Heidolph, Germany) with two solvents: water and ethanol-water (1:4 *v*/v) and a flour-to-solvent extraction ratio of 1:6 *w/v*. The procedure was carried out at 60 °C for 3 min in a 200 mL jacketed vessel equipped with a chiller-heater unit (Model 89,202–974, VWR, Pennsylvania, United States) to ensure accurate temperature control. On the other hand, the moderate electric field (MEF) system consisted of an AC power supply (BK Precision 9833) and a Teflon treatment chamber with internal dimensions of 9 × 8 × 8 cm, equipped with two stainless steel electrodes (9 × 8 cm) positioned 8 cm apart. To ensure optimal sample homogenization, the treatment chamber was placed on a magnetic stirrer. Due to variations in the conductivity of the solvents, different voltages were applied to reach the target temperature of 60 °C (150, 200 and 300 V for water and 250, 275 and 300 V for ethanol-water, at 1200 Hz). These voltage settings resulted in an electric field strength ranging from 18.75 to 37.5 V/cm. In this case, the initial temperature was set at 25 °C and was gradually increased to 60 °C using MEF technology. The time required to reach 60 °C was influenced by both the applied voltage and the solvent used. Once the temperature reached 60 °C, it was maintained constant until the 3 min of pretreatment were completed applying an ON-OFF control loop to the MEF voltage using a temperature probe (Pt100) connected to a basic process controller (E5CC, Omron Electronics Iberia S.A.U., Madrid) and supervised with a PC via the RS422 interface from a Visual Studio application. The custom software also allowed to record power consumption and temperature data in real time. Both CV and MEF pretreatments were conducted in triplicate.

After the pretreatments, the treated flour mixture was centrifuged at 5000 rpm for 10 min. Then, the resulting solid fraction (pellet), designated as "Separated flour" in Fig. 1, was freeze-dried for 48 h to reach a final moisture content of approximately 7 % w.b. and stored at −26 °C. This fraction, referred to as pretreated lupin flour (PLF) was used for further processing and analysis. The yield of PLF was calculated with Eq. (1).

$$PLF\,Extraction\ yield\left(\frac{g\,PLF}{100\,g\,LF}\right) = \frac{m_{PLF}}{m_{LF}} \times 100 \quad (1)$$

where, m_{PLF} is the mass of the resulting pretreated lupin flour (PLF) and m_{LF} is the initial mass of lupin flour (LF).

A considerable amount of flour with high protein content was solubilized in the supernatant. The protein from this fraction was recovered by readjusting the pH of the solubilized flour to the pI of lupin proteins (4.7) (Domínguez et al., 2023), using 4 N HCl to facilitate precipitation. Subsequently, the resulting precipitates were centrifuged at 5000 rpm

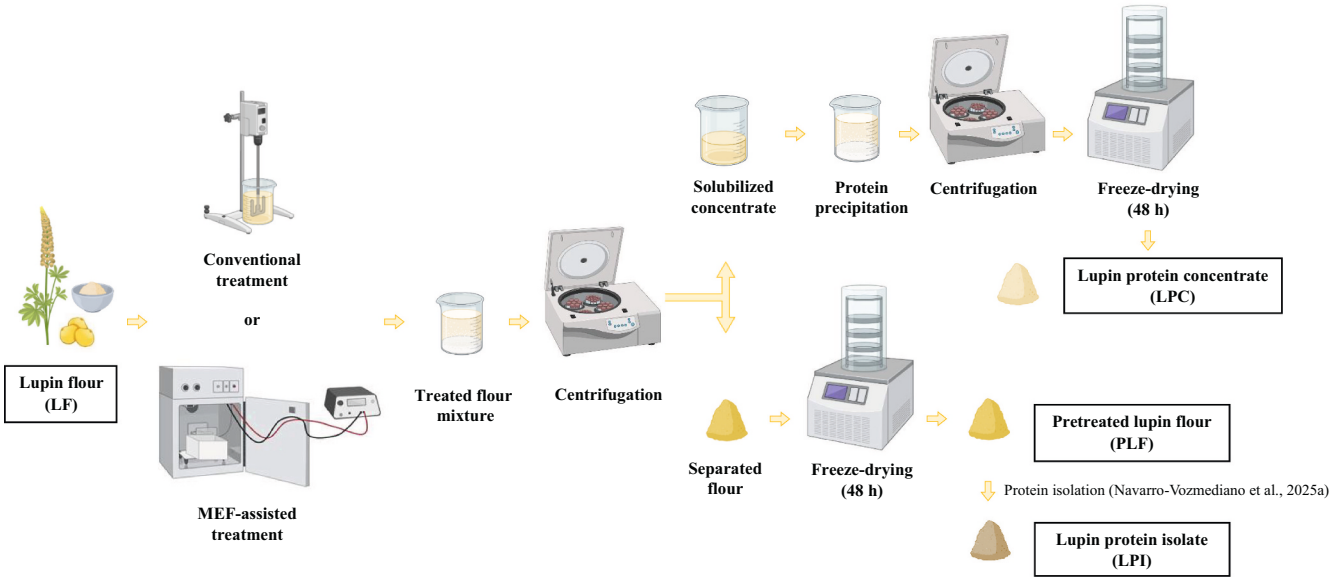

Fig. 1. Scheme of lupin flour pretreatments.

for 10 min, freeze-dried for 48 h to reach a final moisture content of approximately 7 % w.b. and stored at −26 °C. This fraction, referred to as lupin protein concentrate (LPC), was also subjected to chemical analysis and techno-functional characterization due to its high protein content. The extraction and protein yield of LPC were calculated with Eqs. (2) and (3), respectively.

$$LPC\,Extraction\ yield\left(\frac{g\,LPC}{100\,g\,LF}\right) = \frac{m_{LPC}}{m_{LF}} \times 100 \quad (2)$$

$$LPC\,Protein\ \ yield\left(\frac{g}{100\,g\,LF}\right) = PC_{LPC} \times \frac{m_{LPC}}{m_{LF}} \quad (3)$$

where, m_{LPC} is the mass of lupin protein concentrate (LPC), m_{LF} is the initial mass of lupin flour (LF) and PC_{LPC} is the protein content of the LPC determined as explained in Section 2.4. Yields in Eqs. (1) to (3) are referred to dry matter.

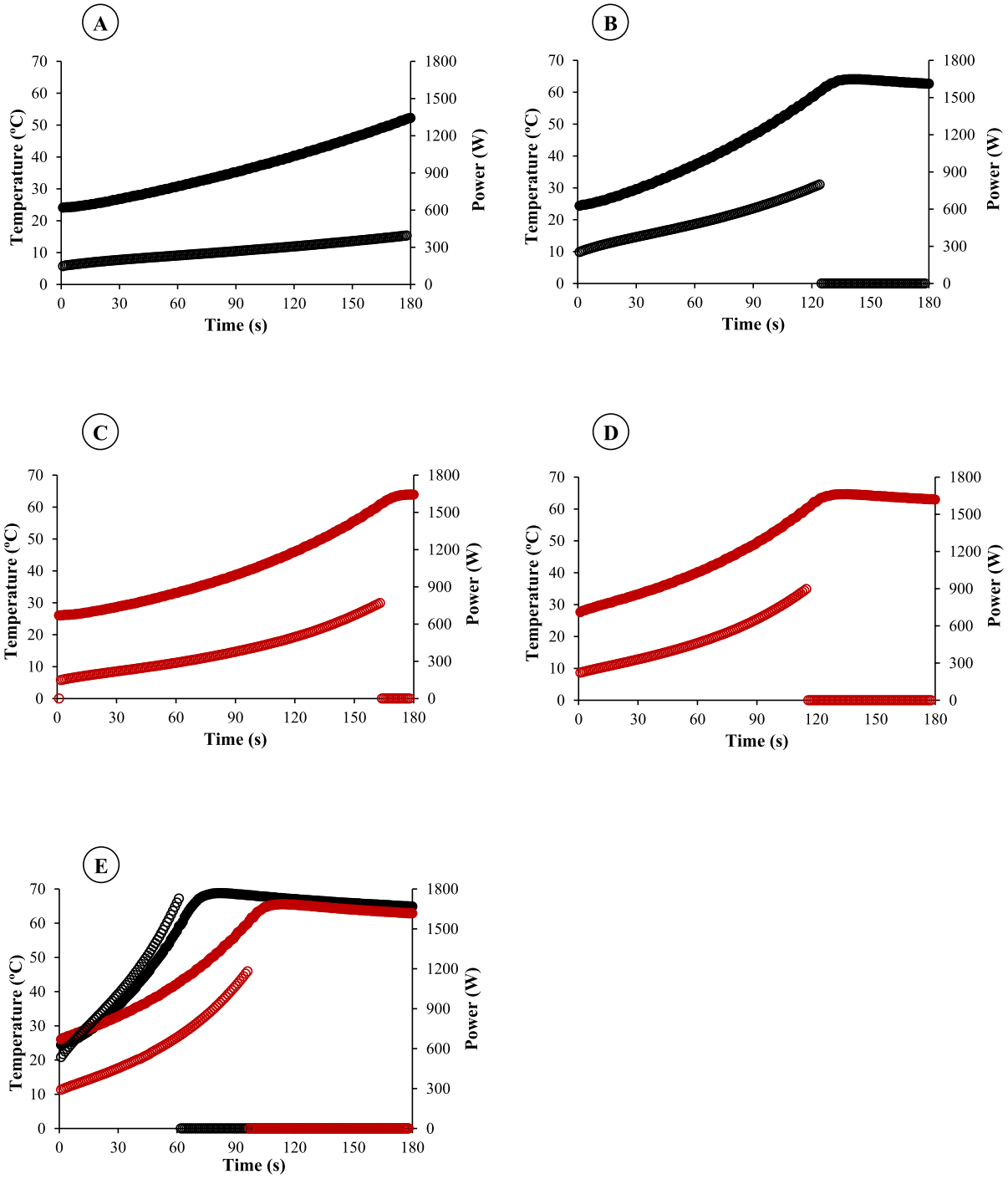

Fig. 2. A. Evolution of temperature (filled dots) and variation of electrical power (unfilled dots) during MEF pretreatment of lupin flour dispersions in water (black) and ethanol–water solvents (red) at 150 (A), 200 (B), 250 (C), 275 (D) and 300 V (E). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3. Protein isolation procedure

After the removal of ANF and ATF from flours, protein extraction was carried out by alkaline extraction, followed by acid precipitation at the isoelectric point of lupin protein pH 4.7 (Domínguez et al., 2023). A detailed explanation of this method is described in Navarro-Vozmediano, Bou, García-Pérez, Dalmau, & Benedito (2025a). Extraction and protein yields of LPI were calculated using equivalent expressions to those presented in Eq. (2) and (3) for the LPC.

2.4. Chemical analysis

The analysis of protein, fat, polyphenol, saponin and alkaloid content as well as antioxidant activity was conducted following the procedures described by Navarro-Vozmediano, Bou, García-Pérez, Dalmau, & Benedito (2025a).

2.5. Characterization of techno-functional properties of the LPC and LPI

The evaluation of water and fat absorption indexes, foaming and emulsifying properties and instrumental color was carried out in accordance with the methods outlined by Navarro-Vozmediano, Bou, García-Pérez, Dalmau, & Benedito (2025a).

2.6. Statistical analysis

Experimental results were reported as mean value±SD and were analyzed by one-way variance analysis (ANOVA, $p < 0.05$) to determine significant differences between samples. Additionally, the effects of moderate electric field and type of solvent on chemical composition of PLF, LPC and LPI and techno-functional properties of LPC and LPI were examined by multifactorial analysis of variance (ANOVA, $p < 0.05$) considering interactions of level 2 between the factors. LSD test (Least Significant Difference) intervals were used to determine significant differences between averages. Statistical tests were carried out using the Statgraphics Centurion XVIII software (Statpoint Technologies, Virginia, United States).

3. Results and discussion

3.1. MEF pretreatment characterization

As illustrated in Fig. 2, at 300 V, the MEF pretreatment of flour using water as solvent showed a fast temperature increase, reaching 60 °C in 62 s. In contrast, when the ethanol-water was used as solvent, the target temperature was reached after 95 s (Fig. 2E). This difference is mainly due to the significantly ($p < 0.05$) lower electrical conductivity of the LF-ethanol-water mixture compared to the LF-water mixture (873 and 1623 μS/cm, respectively), which affects the heat generation due to Joule effect. However, a longer MEF treatment may be advantageous, as it allows for prolonged cell stress, which may lead to electroporation. In terms of power consumption, the water solvent consumed 1.18 kWh, while the ethanol-water solvent consumed 1.59 kWh (Table 1). This higher energy requirement for the ethanol-water system can be attributed to its lower conductivity (higher electrical resistance), which diminishes energy conversion efficiency and consequently increases overall power consumption. A similar trend was observed for the rest of the voltage settings (Fig. 2 and Table 1). These measurements could contribute to elucidate if the effects observed on protein extraction and techno-functional properties are modulated by the thermal effect or the electric field.

Table 1
Average ± SD of the time required to reach temperature set point (60 °C) and the power consumption at different voltages applied during MEF pretreatments.

	Voltage (V)	Time (s)	Consumption (kWh)
Water	150	180 ± 0	2.39 ± 0.03
	200	130 ± 11	2.49 ± 0.54
	300	62 ± 2	1.18 ± 0.06
Ethanol-water	250	156 ± 10	2.67 ± 0.33
	275	112 ± 5	1.78 ± 0.10
	300	95 ± 3	1.59 ± 0.08

3.2. Effect of flour pretreatment on the extraction yields and protein content of PLF, LPC and LPI

Extraction yields and protein content of PLF, LPC and LPI after LF pretreatments are summarized in Table 2. Notably, the experimental results revealed a significant ($p < 0.05$) influence of pretreatment variables. Thus, for water solvent, MEF application significantly ($p < 0.05$) increased PLF extraction yield (avg. 15 % compared to CV) while significantly ($p < 0.05$) reduced LPC's (avg. 35 % compared to CV). No clear effect was observed for LPI. Overall, MEF application significantly ($p < 0.05$) reduced protein content in all three fractions compared to CV. Additionally, increasing the voltage had no significant effect for both extraction yield and protein content of the three fractions ($p > 0.05$). A similar trend was observed for ethanol-water solvent (Table 2). During CV pretreatments, LF was exposed to 60 °C for 3 min. However, as seen in Section 3.1, under MEF-assisted conditions, the time required to reach 60 °C ranged from 62 to 180 s. Consequently, as the LF remained below the set-point temperature for at least 1 min, reduced solubilization may have occurred, leading to higher PLF yields and, correspondingly, lower LPC yields. Furthermore, protein content reductions may also be attributed to the co-precipitation of other compounds alongside the proteins during the extraction process (León-López et al., 2013; Navarro-Vozmediano, Bou, García-Pérez, Dalmau, & Benedito, 2025a; Navarro-Vozmediano, Dalmau, Benedito and Garcia-Perez, 2025b).

All LPI from PLF showed lower protein yields than LPI from LF (19.8 g/100 g LF). However, when combining the LPC and LPI fractions, the total protein yield (avg. 22.1 g/100 g LF, Fig. 3) was, on average, 12 % higher compared to LPI from LF, corresponding to a 52 % extraction of the total protein presented in LF (42.4 g/ 100 g LF). Total extraction yield, LPC + LPI, significantly ($p < 0.05$, Fig. 3) increased when pretreating with water compared to ethanol-water solvent.

3.3. Effect of flour pretreatment on the ANF and ATF content of PLF, LPC and LPI

3.3.1. Fat content

Although most of the pretreatments did not reduce the fat content (FC) of PLF compared to LF (3.79 g/100 g LF), reductions of up to 11 % could be achieved under specific conditions (Table 3). Additionally, the average FC of LPI from PLF was 57 % lower than LPI from LF (10.33 g/ 100 g LPI).

Compared to CV experiments, MEF-assisted pretreatment, using water as a solvent, led to a significant ($p < 0.05$) decrease in FC of PLF (avg. 19 %) and LPI (avg. 55 %). In contrast, the application of MEF led to a significant ($p < 0.05$) increase in FC of LPC (avg. 76 %) compared to CV. Nevertheless, no significant ($p > 0.05$) differences in FC were observed across the different voltages applied for the three fractions. The same pattern was observed with the ethanol-water solvent (Table 3). Thus, the cell stress induced by MEF promoted the release of the fat from the solid matrix (Gavahian et al., 2018). Based on this mechanism, it is reasonable to expect that conditions promoting fat reduction in flour have an inverse effect on LPC, as the extracted fat would either dissolve in the solvent or be retained in the LPC. Although higher voltages should theoretically induce a greater cell stress, this additional stress may not significantly enhance the extraction of specific compounds (Llavata et al., 2025). In this case, further electroporation was ineffective in enhancing fat release, likely because the highest extraction was already achieved at the lowest applied stress level with MEF at 150 or 250 V (for water and ethanol-water, respectively).

Table 2
Extraction yield, protein content and protein yield of pretreated flours (PLF) and their concentrates (LPC) and isolates (LPI).

	Voltage (V)	Extraction yields (g /100 g LF)			Protein content (g/100 g dm)			Protein yields (g/100 g LF)	
		PLF	LPC	LPI	PLF	LPC	LPI	LPC	LPI
Water	0	60.9 ± 2.8^{bA}	20.7 ± 0.5^{aA}	14.2 ± 1.7^{bA}	38.1 ± 0.1^{abB}	64.8 ± 0.6^{abA}	82.9 ± 1.2^{aB}	13.4 ± 0.3^{aA}	11.8 ± 1.4^{bA}
	150	69.6 ± 0.2^{a}	13.6 ± 0.6^{b}	17.8 ± 1.3^{ab}	40.8 ± 1.5^{a}	63.7 ± 0.1^{b}	76.8 ± 1.6^{b}	8.9 ± 0.4^{b}	13.7 ± 0.9^{ab}
	200	69.4 ± 0.5^{a}	13.2 ± 0.2^{b}	17.3 ± 1.2^{ab}	36.3 ± 1.3^{b}	65.3 ± 0.4^{a}	79.6 ± 0.5^{b}	8.6 ± 0.2^{b}	13.8 ± 0.9^{ab}
	300	70.7 ± 0.1^{aB}	13.3 ± 0.2^{bA}	19.6 ± 1.9^{aA}	36.2 ± 0.4^{bA}	61.5 ± 0.7^{cA}	78.6 ± 0.5^{bA}	8.2 ± 0.1^{bA}	15.4 ± 1.5^{aA}
Ethanol-water	0	69.8 ± 2.5^{cA}	11.9 ± 0.9^{bB}	15.8 ± 0.6^{aA}	42.0 ± 0.4^{aA}	58.9 ± 0.5^{bB}	88.5 ± 1.0^{aA}	7.0 ± 0.6^{bB}	14.0 ± 0.5^{aA}
	250	75.4 ± 0.4^{ab}	11.4 ± 0.3^{b}	14.9 ± 0.6^{a}	42.1 ± 0.9^{a}	65.6 ± 0.6^{a}	77.7 ± 0.5^{b}	7.5 ± 0.2^{b}	11.6 ± 0.4^{b}
	275	78.1 ± 0.7^{a}	15.1 ± 0.1^{a}	16.3 ± 1.9^{a}	35.7 ± 0.6^{b}	58.9 ± 0.5^{b}	79.3 ± 0.8^{b}	8.9 ± 0.1^{a}	12.9 ± 1.6^{ab}
	300	72.2 ± 0.4^{bcA}	13.9 ± 0.3^{aA}	16.8 ± 0.1^{aA}	37.5 ± 0.8^{bA}	59.2 ± 0.1^{bB}	78.8 ± 0.7^{bA}	8.3 ± 0.2^{abA}	13.2 ± 0.1^{abA}

0, refers to pretreatments without MEF application (Conventional pretreatments, CV). Values are presented as average ± SD. Different lowercase letters in columns indicate significant differences between voltages for each solvent ($p < 0.05$). Different uppercase letters in rows indicate significant differences between solvents (water and ethanol-water) at 0 and 300 V ($p < 0.05$).

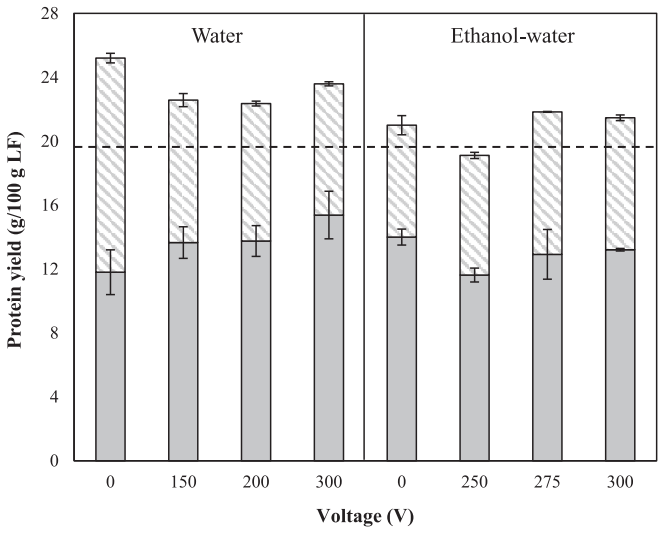

Fig. 3. Yields of protein concentrates (LPC) and isolates (LPI) from pretreated lupin flour (PLF) at the different voltages applied for each solvent. The black dashed horizontal line refers to the protein yield of LPI from non-pretreated lupin flour (LF). Striped segments represent LPC and grey segments represent LPI. 0, refers to pretreatments without MEF application (Conventional pretreatments, CV).

Overall, the use of ethanol-water as solvent exhibited slightly, but not significantly ($p > 0.05$), lower FC values compared to water. Differences in solvent polarity may affect the solubilization and extraction of fat from LF (Bader et al., 2011; Perrier et al., 2017).

3.3.2. *Total polyphenol content and antioxidant activity*

Regardless of pretreatment conditions used, average total phenolic content (TPC) in PLF (1.25 mg gallic acid/100 g PLF) was 54 % lower than in LF (2.72 mg gallic acid/100 g LF) and LPI from PLF showed an average of 27 % lower TPC values compared to LPI from LF (1.55 mg gallic acid/100 g LPI).

TPC in the three different fractions was significantly ($p < 0.05$) affected by pretreatment conditions (Table 3). Thereby, for the water solvent, a significant ($p < 0.05$) decrease in TPC was observed in PLF, LPC and LPI during the MEF experiments. Although increasing voltages caused a significant ($p < 0.05$) reduction in PLF's TPC, they had no significant ($p > 0.05$) influence on LPC and LPI. A similar trend was observed with the ethanol-water solvent. However, in this case, increasing voltages led to significantly ($p < 0.05$) higher reductions of TPC for the three fractions. Solvent choice also influenced TPC, with ethanol-water showing significantly ($p < 0.05$) higher extraction capacity than water, resulting in 16, 43 and 25 % lower TPC at 300 V for PLF, LPC and LPI, respectively. These results are in line with those obtained in previous works, which have demonstrated that the combination of cell stress-electroporation and ohmic heating improved the extraction of TPC from different vegetable matrices but also could led to the degradation and instability of polyphenols when exposed to the electric field (Al-Hilphy et al., 2020; Pereira et al., 2016). It is well known that higher voltages induce greater cell stress (Thamkaew & Gómez Galindo, 2020), which in this study resulted in enhanced polyphenol extraction from PLF for both solvents. However, for LPC and LPI obtained from PLF with water, the effect of voltage on extraction may have been masked by the removal of polyphenols during the LPC and LPI extraction processes. Moreover, ethanol's lower polarity enhances its affinity for polyphenols, leading to an improved extraction efficiency (Fu et al., 2016; Oreopoulou et al., 2019).

As for antioxidant activity (AA), PLF showed an average 10 % lower AA than LF (5.76 μM Trolox/100 g LF), while LPI from PLF showed an average of 36 % lower AA than LPI from LF (5.99 μM Trolox/100 g LPI).

Like in TPC, AA was also affected by pretreatment variables (Table 3). Thus, for water solvent, MEF exhibited no significant ($p > 0.05$) effect on PLF. However, MEF pretreatments significantly ($p < 0.05$) reduced AA in LPC and LPI. While voltage had no significant ($p > 0.05$) impact on LPC, LPI's AA significantly ($p < 0.05$) decreased with increasing voltage (Table 3). On the other hand, for ethanol-water, MEF application significantly ($p < 0.05$) reduced AA of PLF and LPC but had no significant ($p > 0.05$) effect on LPI. Moreover, neither of the fractions showed significant differences ($p > 0.05$) due to the voltage applied. As mentioned in Section 3.3.1, although higher voltage induces greater cell stress, the extraction of antioxidant compounds may have reached its maximum at the lowest voltage, with no further changes observed as cell stress increased. While the choice of solvent had no significant ($p > 0.05$) effect on PLF's AA, significant ($p < 0.05$) differences were observed for LPC, with ethanol-water exhibiting higher AA levels for both CV (27 %) and 300 V (29 %) compared to water. On the other hand, LPI from PLF with ethanol-water solvent exhibited significantly ($p < 0.05$) lower AA than those from PLF with water (by 34 and 16 % for CV and 300 V, respectively), thereby reiterating the importance of solvent selection (Fu et al., 2016; Yusoff et al., 2022).

Remarkably, after MEF pretreatments, only 34 % of TPC was retained (avg. 29 % in PLF and avg. 5 % in LPC), while 74 % of the AA present in LF was preserved (avg. 64 % in PLF and avg. 10 % in LPC). Thus, although over 60 % of the polyphenols were removed, the remaining compounds maintained antioxidant properties. It has to be emphasized that no prior study has explored the influence of MEF pretreatment on the TPC and AA of PLF, LPC and LPI.

3.3.3. *Total saponin content*

On average, LF pretreatments diminished the total saponin content (TSC) of PLF by 17 % compared to LF (1.26 g oleanolic acid/100 g LF), while LPI from PLF showed a slightly higher TSC values (avg. 2.03 g oleanolic acid/100 g LPI, Table 3) compared to LPI from LF (1.99 g oleanolic acid/100 g LPI).

Table 3
Anti-nutritional and anti-technological factors of pretreated flours (PLF) and their concentrates (LPC) and isolates (LPI).

Voltage (V)		FC (g /100 g dm)			TPC (mg gallic acid/100 g dm)			AA (μM Trolox/g dm)			TSC (g oleanolic acid/100 g dm)			AC (g lupinine/g dm)		
		PLF	LPC	LPI	PLF	LPC	LPI	PLF	LPC	LPI	PLF	LPC	LPI	PLF	LPC	LPI
Water	0	4.77 ± 0.63^{aA}	4.24 ± 0.74^{bA}	7.38 ± 1.11^{aA}	1.93 ± 0.13^{aA}	1.18 ± 0.07^{aA}	1.43 ± 0.18^{aA}	5.04 ± 0.72^{aA}	5.03 ± 0.35^{aB}	5.32 ± 0.36^{aA}	1.58 ± 0.16^{aA}	2.14 ± 0.14^{aA}	2.03 ± 0.03^{aA}	0.23 ± 0.02^{aA}	0.016 ± 0.002^{aB}	nd
	150	3.84 ± 0.39^{b}	7.55 ± 0.10^{a}	3.26 ± 0.20^{b}	1.29 ± 0.14^{b}	1.17 ± 0.07^{a}	1.10 ± 0.07^{b}	5.37 ± 0.31^{a}	3.71 ± 0.08^{b}	4.37 ± 0.37^{b}	0.94 ± 0.10^{b}	1.98 ± 0.21^{a}	1.97 ± 0.18^{a}	0.23 ± 0.05^{a}	0.013 ± 0.000^{a}	nd
	200	3.95 ± 0.43^{ab}	7.49 ± 0.20^{a}	3.28 ± 0.09^{b}	1.19 ± 0.07^{bc}	0.97 ± 0.10^{b}	1.12 ± 0.10^{b}	5.10 ± 0.27^{a}	3.62 ± 0.07^{b}	3.64 ± 0.27^{c}	0.91 ± 0.19^{b}	2.03 ± 0.12^{a}	2.14 ± 0.39^{a}	0.21 ± 0.05^{ab}	0.014 ± 0.004^{a}	nd
	300	3.77 ± 0.28^{bA}	7.35 ± 0.22^{aA}	3.34 ± 0.21^{bA}	1.14 ± 0.04^{cA}	1.08 ± 0.14^{abA}	1.18 ± 0.06^{bA}	5.08 ± 0.30^{aA}	3.53 ± 0.09^{bB}	3.89 ± 0.15^{cA}	0.80 ± 0.09^{bA}	2.01 ± 0.14^{aA}	2.24 ± 0.13^{aA}	0.12 ± 0.01^{bA}	0.011 ± 0.003^{aA}	nd
Ethanol-water	0	4.24 ± 0.11^{aA}	4.33 ± 0.29^{cA}	9.78 ± 0.03^{aA}	1.45 ± 0.14^{aB}	1.36 ± 0.13^{aA}	1.27 ± 0.12^{aA}	5.71 ± 0.43^{aA}	6.39 ± 0.26^{aA}	3.49 ± 0.30^{aB}	1.07 ± 0.11^{aB}	1.49 ± 0.08^{bB}	1.91 ± 0.15^{aA}	0.24 ± 0.01^{aA}	0.023 ± 0.000^{aA}	nd
	250	3.58 ± 0.41^{ab}	7.64 ± 0.09^{a}	2.55 ± 0.36^{b}	1.11 ± 0.15^{b}	0.93 ± 0.20^{b}	1.18 ± 0.04^{b}	5.37 ± 0.44^{a}	4.56 ± 0.29^{b}	3.24 ± 0.17^{a}	1.13 ± 0.05^{a}	2.08 ± 0.10^{a}	1.97 ± 0.14^{a}	0.17 ± 0.02^{b}	0.013 ± 0.002^{b}	nd
	275	3.59 ± 0.43^{ab}	7.38 ± 0.11^{ab}	2.79 ± 0.48^{b}	0.90 ± 0.05^{c}	0.90 ± 0.11^{b}	0.94 ± 0.11^{c}	4.89 ± 0.44^{b}	4.60 ± 0.10^{b}	3.52 ± 0.25^{a}	1.04 ± 0.14^{a}	2.06 ± 0.10^{a}	1.94 ± 0.23^{a}	0.14 ± 0.01^{bc}	0.013 ± 0.001^{b}	nd
	300	3.36 ± 0.15^{bA}	7.18 ± 0.15^{bA}	2.84 ± 0.50^{bA}	0.96 ± 0.07^{cB}	0.62 ± 0.08^{cB}	0.89 ± 0.02^{cB}	4.82 ± 0.09^{bA}	4.56 ± 0.25^{bA}	3.28 ± 0.30^{aB}	0.88 ± 0.09^{bA}	2.06 ± 0.33^{aA}	2.04 ± 0.23^{aA}	0.11 ± 0.01^{cA}	0.012 ± 0.001^{bA}	nd

FC, fat content; TPC, total polyphenol content; AA, antioxidant activity; TSC, total saponin content; AC, alkaloid content. 0, refers to pretreatments without MEF application (Conventional pretreatments, CV). Values are presented as average ± SD; nd: non-detectable. Different lowercase letters in columns indicate significant differences between voltages within each solvent ($p < 0.05$). Different uppercase letters in rows indicate significant differences between solvents (water and ethanol-water) at 0 and 300 V ($p < 0.05$).

Similar to the results already shown for TPC and AA, pretreatment conditions also influenced the final TSC achieved (Table 3). As for water solvent, MEF pretreatments aimed to significantly ($p < 0.05$) reduce TSC of PLF (avg. 44 % compared to CV experiments), while it had no significant ($p > 0.05$) effect on LPC. Moreover, increasing voltage had no significant ($p > 0.05$) effect for both fractions. As for ethanol-water solvent, MEF application at 300 V significantly ($p < 0.05$) reduced the TSC of PLF. In contrast, MEF treatment resulted in a significant ($p < 0.05$) increase of LPC's TSC, although no significant ($p > 0.05$) effect was observed due to the voltage (Table 3). Overall, MEF pretreatments at 300 V showed a significantly ($p < 0.05$) lower values of TSC for PLF by 37 % and a significantly ($p < 0.05$) higher values for LPC by 12 %, compared to CV. It is reasonable to expect that conditions favoring saponins extraction in LF would have an inverse effect on LPC, since the extracted saponins would likely remain in the solvent or be captured by the concentrate. On the other hand, the choice of ethanol-water as solvent exhibited significant ($p > 0.05$) lower values of TSC compared to water. These results are in line with those obtained by Navarro del Hierro et al. (2018) who reported that using ethanol-water as solvent, instead of water, supposed a 146 % increase saponins extraction from lupin. Thus, saponins might exhibit a stronger affinity for solvents with lower polarity than water, potentially improving their extraction from LF. As for the LPI, LF pretreatment conditions exhibited no significant ($p > 0.05$) effect on their TSC (Table 3).

Although no studies have specifically examined the use of MEF for saponin extraction, research on ginsenoside extraction from *Panax ginseng* using pulsed electric fields (Hou et al., 2010) reinforces our results and suggests its potential effectiveness for this purpose. As discussed in Section 3.3.1, the absence of significant differences between applied voltages may be because saponin extraction likely reached its maximum at the lowest voltage, with further increases in cell stress not leading to additional effects.

3.3.4. Alkaloid content

The alkaloid content (AC) in PLF (avg. 0.18 g lupinine/g dm) was on average 59 % lower than in LF (0.44 g lupinine/100 g dm), regardless of the pretreatment applied. AC in PLF ranged from 0.12 to 0.23 g lupinine/100 g PLF for water and from 0.11 to 0.24 g lupinine/100 g PLF for ethanol-water (Table 3). The application of MEF showed a significant ($p < 0.05$) reduction in the AC of PLF for both solvents. Notably, the higher the voltage, the higher the reduction observed (Table 3). As previously mentioned in Section 3.3.1, these findings suggested that the increased electroporation and permeabilization induced by higher cell stress, resulting from increased MEF voltage, may intensify alkaloid extraction.

LPC exhibited low AC, averaging 0.014 g lupinine/100 g dm (Table 3), which is below the limit for human consumption (0.02 g/100 g) (ACNFP, 1996), regardless of the pretreatment conditions. Moreover, all LPI samples showed no detectable presence of alkaloids. These results highlighted that the water-soluble nature of alkaloids minimized toxicity concerns, as they were only slightly retained in LPC and entirely removed during the protein extraction process (El-Adawy et al., 2001; Lqari et al., 2002). Therefore, LF pretreatment enabled the production of LPC with minimal AC and contributed to the reduction of alkaloid content in PLF.

3.4. Effect of flour pretreatment on the techno-functional properties of LPC and LPI

3.4.1. Water and fat absorption capacities

Overall, LPI from PLF exhibited higher water absorption index (WAI) values and similar fat absorption index (FAI) values compared to the LPI from LF (1.24 g water/g LPI and 2.63 g oil/g LPI for WAI and FAI, respectively) (Table 4). Moreover, the results revealed a significant ($p < 0.05$) influence of pretreatment variables.

MEF application during LF pretreatment with water solvent led to LPI with significantly ($p < 0.05$) higher WAI and FAI compared to CV

Table 4
Techno-functional properties of lupin protein concentrates (LPC) and lupin protein isolates (LPI) from pretreated lupin flours.

	Voltage (V)	WAI (g/g)		FAI (g/g)		FCA (%)		FS_{1h} (%)		FS_{2h} (%)		EAI (m^2/g)		ESI (min)	
		LPC	LPI	LPC	LPI	LPC	LPI	LPC	LPI	LPC	LPI	LPC	LPI	LPC	LPI
Water	0	2.11 ± 0.06^{bA}	1.51 ± 0.12^{bA}	2.26 ± 0.10^{bA}	1.62 ± 0.02^{bB}	67.9 ± 1.4^{bA}	71.8 ± 1.9^{dB}	26.1 ± 1.7^{dA}	52.6 ± 0.6^{aA}	6.8 ± 0.9^{dB}	34.3 ± 3.6^{aA}	14.6 ± 0.9^{bA}	16.3 ± 1.1^{bA}	24.7 ± 2.9^{aA}	19.8 ± 1.0^{cB}
	150	2.48 ± 0.07^{a}	1.54 ± 0.04^{ab}	2.43 ± 0.04^{ab}	2.71 ± 0.04^{a}	77.2 ± 1.5^{a}	163.1 ± 3.3^{c}	65.0 ± 4.5^{a}	46.6 ± 3.9^{b}	46.1 ± 2.5^{a}	25.9 ± 1.7^{b}	16.4 ± 0.8^{a}	16.6 ± 1.2^{b}	22.5 ± 2.0^{ab}	26.9 ± 1.8^{b}
	200	2.42 ± 0.08^{a}	1.66 ± 0.07^{ab}	2.79 ± 0.15^{a}	2.60 ± 0.14^{a}	57.9 ± 1.4^{c}	195.6 ± 4.4^{b}	35.6 ± 2.0^{c}	38.1 ± 2.4^{c}	13.4 ± 1.5^{c}	27.7 ± 2.8^{b}	15.9 ± 0.6^{a}	16.2 ± 0.4^{b}	24.1 ± 1.8^{ab}	19.0 ± 0.3^{c}
	300	2.46 ± 0.09^{aB}	1.85 ± 0.18^{aA}	2.82 ± 0.22^{aA}	2.71 ± 0.07^{aB}	68.8 ± 2.6^{bB}	202.3 ± 3.4^{aA}	51.2 ± 2.7^{bA}	28.9 ± 1.1^{dB}	21.4 ± 2.5^{bA}	25.0 ± 1.5^{bA}	16.8 ± 0.7^{aA}	17.9 ± 0.3^{aA}	21.7 ± 2.8^{bA}	32.7 ± 0.8^{aA}
Ethanol-water	0	2.42 ± 0.09^{cA}	1.59 ± 0.07^{cA}	2.51 ± 0.22^{bA}	1.89 ± 0.01^{bA}	69.9 ± 0.9^{bA}	97.4 ± 2.9^{dA}	21.2 ± 1.2^{cB}	37.9 ± 0.4^{bcB}	10.7 ± 0.7^{bA}	20.8 ± 1.4^{bB}	12.5 ± 0.7^{bB}	13.7 ± 0.2^{bB}	24.9 ± 3.1^{aA}	21.0 ± 0.5^{bA}
	250	3.19 ± 0.03^{b}	1.61 ± 0.04^{bc}	2.70 ± 0.31^{ab}	3.32 ± 0.19^{a}	51.1 ± 2.7^{d}	107.6 ± 1.6^{c}	35.6 ± 2.9^{ab}	35.3 ± 2.4^{c}	19.6 ± 0.6^{a}	21.2 ± 2.6^{b}	11.7 ± 0.8^{b}	13.6 ± 0.1^{b}	22.2 ± 2.0^{ab}	19.8 ± 2.0^{b}
	275	3.36 ± 0.00^{a}	1.74 ± 0.04^{ab}	2.78 ± 0.26^{ab}	3.01 ± 0.18^{a}	58.0 ± 1.2^{c}	155.8 ± 4.5^{b}	38.0 ± 1.6^{a}	53.0 ± 2.6^{a}	19.3 ± 0.8^{a}	36.1 ± 3.9^{a}	12.2 ± 0.7^{b}	13.5 ± 0.6^{b}	24.5 ± 2.3^{a}	17.1 ± 1.7^{c}
	300	3.35 ± 0.01^{aA}	1.79 ± 0.05^{aA}	3.15 ± 0.07^{aA}	3.17 ± 0.07^{aA}	97.0 ± 2.9^{aA}	173.2 ± 4.2^{aB}	32.6 ± 2.1^{bB}	40.2 ± 2.4^{bA}	21.1 ± 2.5^{aA}	25.5 ± 1.4^{bA}	15.5 ± 1.1^{aB}	15.0 ± 0.1^{aB}	21.0 ± 1.1^{bA}	24.0 ± 1.2^{aB}

WAI, water absorption index; FAI, fat absorption index; FCA, foaming capacity; FS, foaming stability for 1 and 2 h; EAI, emulsifying activity index; ESI, emulsifying stability. 0, refers to pretreatments without MEF application (Conventional pretreatments, CV). Values are presented as average ± SD. Different lowercase letters in columns indicate significant differences between voltages within each solvent ($p < 0.05$). Different uppercase letters in rows indicate significant differences between solvents (water and ethanol-water) at 0 and 300 V ($p < 0.05$).

experiments. Although increasing the voltage produced slight increments, the differences were not statistically significant ($p > 0.05$). A similar trend was observed for the ethanol-water solvent (Table 4). Overall, LF pretreatments at 300 V resulted in LPI with significantly ($p < 0.05$) higher WAI and FAI values by 17 and 68 %, respectively, compared to CV experiments. As mentioned in Section 3.3.1, the lack of significant ($p < 0.05$) differences between the applied voltages may be due to the fact that the increase in cell stress did not result in substantial changes of WAI and FAI of LPI. Moreover, while the choice of solvent exhibited no significant ($p > 0.05$) effect on WAI, ethanol-water solvent resulted in significantly ($p < 0.05$) higher FAI by 17 % compared to water. This could be explained by the lower FC of the LPI obtained from PLF with ethanol-water solvent (Table 3). Generally, a lower FC in a protein isolate is associated with a higher FAI, as more hydrophobic sites are available for fat binding (Lawal, 2004).

As for LPC, both indexes exhibited remarkably higher WAI and FAI values (avg. 2.72 g water/g LPC and avg. 2.68 g oil/g LPC) than LPI from PLF (avg. 1.66 g water/g LPI and avg. 2.63 g oil/g LPI), which could be attributed to a larger carbohydrate fraction in LPC (Kaur et al., 2013; Tas et al., 2022). Pretreatment conditions also had a significant ($p < 0.05$) effect. For both solvents, MEF application resulted in LPC with significantly ($p < 0.05$) higher WAI and FAI. However, no significant ($p > 0.05$) differences between voltages were detected (Table 4). Similar to the LPI, the increase in cell stress due to higher voltage did not lead to significant changes in the WAI and FAI of the LPC. In particular, MEF pretreatments at 300 V resulted in LPC with significantly ($p < 0.05$) higher values of WAI and FAI by 28 and 25 %, respectively, compared to CV experiments. Moreover, while the choice of solvent exhibited no significant ($p > 0.05$) effect on FAI, ethanol-water solvent resulted in 26 % significantly ($p < 0.05$) higher WAI compared to water.

On one hand, the WAI is related to the content of hydrophilic amino acids, the presence of non-protein components and the type, quality and conformation of the proteins (Sathe & Salunkhe, 1981). On the other hand, the FAI is the ability of proteins to physically bind to fat through capillary attraction and is related to the presence of electrostatic interactions, hydrophobic forces and hydrogen bonds (Lawal, 2004). Thus, while the specific effects of MEF on WAI and FAI have yet to be explored, studies have demonstrated that PEF can alter protein structures by disrupting intermolecular interactions and exposing hydrophobic and hydrophilic regions (Liu et al., 2011; Liu & Zeng, 2010). As a result, these structural changes improve water and oil adhesion and increase the protein's ability to retain them (Malik et al., 2024). Given the similarities between both technologies, it is plausible that MEF may induce similar effects due the cell stress caused, which is consistent with our findings.

3.4.2. Foaming properties

The foaming capacity (FCA) of LPI from PLF was also influenced by pretreatment conditions (Table 4). Overall, while the FCA of LPI in CV pretreatments showed an average reduction of 18 %, the application of MEF resulted in LPI with an average 62 % higher FCA compared to LPI from LF (102.6 %). Particularly for water solvent, MEF application significantly ($p < 0.05$) increased LPI's FCA (avg. 120 %) and it was observed that the higher the voltage, the higher the FCA. In this case, the increased cell stress led to notable changes in LPI's FCA. The same pattern was observed for ethanol-water solvent (Table 4). The choice of solvent exhibited no significant ($p > 0.05$) effect on FCA and, overall, MEF pretreatment at 300 V resulted in the greatest increase in FCA for LPI (by 122 % compared to CV experiments). On the other hand, the foam stability (FS) of LPI from PLF exhibited, on average, 47 and 64 % lower values for FS_{1h} and FS_{2h}, respectively, compared to LPI from LF (79.1 % FS_{1h} and 75.5 % FS_{2h}). Additionally, the application of MEF resulted in higher FS values for the ethanol-water solvent and lower FS values for the water solvent, compared to CV (Table 4).

MEF pretreatment may have caused structural alterations in proteins, promoting their unfolding and disordering (Chen et al., 2022; Wang et al., 2022). Consistently, Subaşı et al. (2021) observed that MEF treatment in soy protein isolates led to modifications of protein conformation that increased their adsorption at the air–water interface, enhancing air bubble formation and improving FCA. Several studies have indicated that the foaming properties of protein isolates are influenced by their saponin content. Due to their amphiphilic nature, saponins reduce surface tension, facilitating greater protein adsorption at the air-water interface and promoting air bubble formation (Navarro

del Hierro et al., 2018; Navarro-Vozmediano, Bou, García-Pérez, Dalmau, & Benedito, 2025a). Although the differences were not statistically significant ($p > 0.05$), a consistent trend was observed suggesting that higher TSC may be associated with enhanced FCA (Tables 3 and 4). It is important to highlight that the foaming characteristics depend on the specific protein fractions solubilized at each processing step and may also be influenced by the levels of fat and carbohydrates present in protein concentrates and isolates (Omowaye-Taiwo et al., 2015; Wang & Kinsella, 1976).

No clear trend of pretreatment conditions was identified for LPC's foaming properties. However, although LPC had a significantly ($p < 0.05$) lower FCA than LPI, most LPC samples still showed adequate FCA values. Similar results were found for FS, with significantly ($p < 0.05$) lower but acceptable values in LPC compared to LPI, suggesting that LPC could be also a valuable functional ingredient for food applications.

3.4.3. Emulsifying properties

Like FCA, the emulsifying activity index (EAI) and the emulsifying stability index (ESI) were also affected by pretreatment conditions (Table 4). In general terms, the EAI of LPI from PLF with water solvent showed no significant differences ($p > 0.05$) compared to LPI from LF (16.7 m^2/g and 22.6 min) but their ESI was significantly ($p < 0.05$) improved. Compared to CV experiments, MEF application showed a significant ($p < 0.05$) increase on the EAI, with the highest values observed at 300 V. Additionally, MEF pretreatment also influenced the ESI, with the highest values recorded after the 300 V application (Table 4). A similar trend was observed for ethanol-water solvent (Table 4). In summary, MEF pretreatments at 300 V led to LPI with significantly ($p < 0.05$) higher values of EAI and ESI by 10 and 28 %, respectively, compared to CV experiments. Moreover, the choice of ethanol-water solvent exhibited a significant ($p < 0.05$) decrease on both EAI and ESI (16 and 14 %, respectively) compared to water solvent. Notably, no similar research has been conducted on lupin or any other legume to date.

Slightly lower but comparable average EAI and ESI values were observed for LPC compared to LPI. Moreover, LPC exhibited the same trends as LPI (Table 4). Overall, the EAI of LPC and LPI from PLF could be compared to other protein isolates from different plant sources like sesame seed (15 m^2/g) (Fasuan et al., 2018) or soybean (14.6 m^2/g) (Singhal et al., 2016), showcasing their potential as functional ingredients in the development of new food products. As previously discussed in Sections 3.4.1 and 3.4.2, electric fields can influence protein conformation and enhance their solubility, promoting stronger interactions with lipids and improving surface activity, potentially improving emulsion formation and stability (Chen et al., 2022; Wang et al., 2022).

3.4.4. Color properties

LF pretreatments resulted in minimal variations in the LPI color parameters, with no clear trends observed (Table 5). However, the total color differences (ΔE) between LPI from PLF and LPI from LF were noticeable to the human eye, exceeding a 5 unit change in color (Fig. 4) (Mikulec et al., 2019). Despite these differences, the results indicated that both LPI from LF (L*: 66.6, a*: 8.6, b*: 64.2, Navarro-Vozmediano, Bou, García-Pérez, Dalmau, & Benedito, 2025a) and LPI from PLF (avg. L*: 59.5, a*: 7.6, b*: 51.4, Table 5) presented a brownish-yellow color, similar to cooked meat. This suggests its potential as an ingredient in plant-based meat products.

In the case of LPC, color parameters were significantly ($p < 0.05$) influenced by LF pretreatment as MEF application led to LPC with significantly ($p < 0.05$) lower L* and significantly ($p < 0.05$) higher a* and b* values, for both water and ethanol-water solvent. Nevertheless, increasing voltages showed no clear trend on color parameters (Table 5). LPC generally exhibited lighter and whiter tones (avg. L*: 73.1, a*: 3.3, b*: 44.5 for water solvent and avg. L*: 80.6, a*: 2.0, b*: 31.2 for ethanol-water solvent) compared to LPI (Table 5 and Fig. 4). This suggests its potential for applications in plant-based dairy alternatives, like yogurt or cheese, as well as nutritional shakes or ready-to-eat snacks. Interestingly, LPC and LPI from PLF obtained with ethanol-water solvent showed significantly ($p < 0.05$) lighter tones compared to those from PLF extracted with water solvent (Table 5 and Fig. 4). Color variations are typically influenced by the presence of carotenoids, which are associated with fat content due to their lipid-soluble nature (Bou et al., 2022; Siger et al., 2023). Therefore, an increase in redness and yellowness (indicated by higher a* and b* values) generally suggests a higher FC (Domínguez et al., 2023). However, as shown in Tables 3 and 5, this pattern is not always consistent. For example, LPC exhibited a higher FC but showed lower a* and b* values compared to LPI. Additionally, color can also be affected by the overall composition and protein structure (Llave et al., 2018; Xu et al., 2007). As discussed in earlier sections, MEF pretreatment alters both the composition of LPC and LPI, and seems to affect their protein conformation, resulting in significant color changes that may overshadow the influence of fat content or carotenoid presence. Moreover, the observed color changes in the LPI could also be partially attributed to protein aggregation, which can lead to darker or browner tones (Gomes et al., 2024; Kim et al., 2021).

Table 5
Color parameters of lupin protein concentrates (LPC) and lupin protein isolates (LPI) from pretreated lupin flours.

	Voltage (V)	L*		a*		b*		C*		h*	
		LPC	LPI	LPC	LPI	LPC	LPI	LPC	LPI	LPC	LPI
Water	0	77.6 ± 0.5^{aB}	48.9 ± 0.4^{dB}	2.2 ± 0.2^{cA}	9.5 ± 0.1^{aA}	37.1 ± 1.1^{cA}	62.8 ± 0.4^{aA}	37.2 ± 1.1^{cA}	63.5 ± 0.4^{aA}	86.6 ± 0.2^{aB}	81.4 ± 0.1^{aB}
	150	70.8 ± 0.3^{c}	56.7 ± 0.2^{c}	4.0 ± 0.3^{b}	8.1 ± 0.1^{b}	49.5 ± 1.3^{a}	50.1 ± 0.2^{b}	49.7 ± 1.3^{a}	50.8 ± 0.2^{b}	85.4 ± 0.3^{b}	80.8 ± 0.0^{d}
	200	69.7 ± 0.4^{d}	58.4 ± 0.3^{b}	4.5 ± 0.2^{a}	7.8 ± 0.0^{c}	50.7 ± 1.5^{a}	49.5 ± 0.1^{b}	50.9 ± 1.5^{a}	50.1 ± 0.1^{b}	85.0 ± 0.2^{c}	81.0 ± 0.0^{c}
	300	74.0 ± 0.4^{bB}	60.6 ± 0.1^{aA}	2.5 ± 0.3^{cA}	6.9 ± 0.0^{dB}	40.7 ± 1.4^{bA}	44.4 ± 0.1^{cB}	40.8 ± 1.4^{bA}	44.9 ± 0.1^{cB}	86.6 ± 0.3^{aA}	81.2 ± 0.1^{bB}
Ethanol-water	0	83.5 ± 0.3^{aA}	66.3 ± 0.3^{aA}	1.0 ± 0.1^{cB}	6.5 ± 0.1^{cB}	26.1 ± 0.5^{cB}	49.1 ± 0.6^{cB}	26.1 ± 0.5^{cB}	49.5 ± 0.6^{cB}	87.7 ± 0.2^{aA}	82.4 ± 0.0^{aA}
	250	79.0 ± 0.4^{d}	62.7 ± 0.5^{b}	2.6 ± 0.2^{a}	7.0 ± 0.2^{b}	33.4 ± 0.4^{a}	52.2 ± 0.6^{b}	33.5 ± 0.4^{a}	52.7 ± 0.7^{b}	85.5 ± 0.2^{c}	82.3 ± 0.1^{a}
	275	80.2 ± 0.6^{b}	61.8 ± 0.3^{b}	2.1 ± 0.3^{b}	6.9 ± 0.1^{b}	32.5 ± 0.9^{b}	49.6 ± 0.2^{c}	32.5 ± 0.9^{b}	50.0 ± 0.2^{c}	86.3 ± 0.4^{b}	82.1 ± 0.1^{b}
	300	79.7 ± 0.2^{cA}	60.1 ± 0.9^{cA}	2.2 ± 0.1^{bA}	8.0 ± 0.0^{aA}	33.0 ± 0.5^{abB}	53.5 ± 0.2^{aA}	33.1 ± 0.5^{abB}	54.1 ± 0.2^{aA}	86.1 ± 0.2^{bB}	81.5 ± 0.1^{cA}

L*,lightness value; a*,green-red; b*,blue-yellow; C*, chroma; h*, hue angle. 0, refers to pretreatments without MEF application (Conventional pretreatments, CV). Values are presented as average ± SD. Different lowercase letters in columns indicate significant differences between voltages within each solvent ($p < 0.05$). Different uppercase letters in rows indicate significant differences between solvents (water and ethanol-water) at 0 and 300 V ($p < 0.05$).

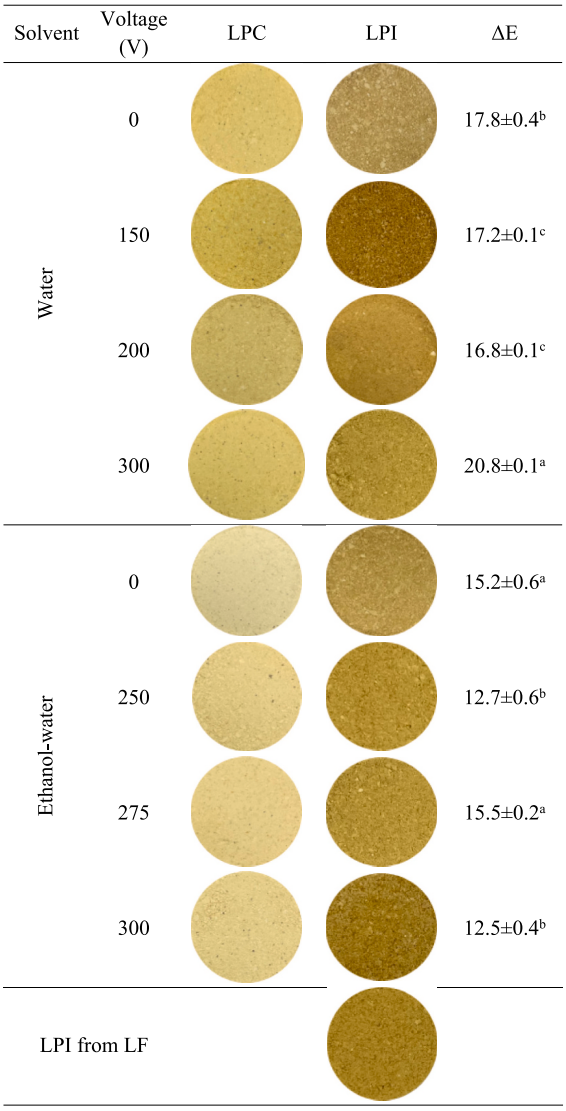

Fig. 4. Images of lupin protein isolates (LPI) and lupin protein concentrates (LPC) from pretreated lupin flours (PLF). Total color differences (AE) between LPI from PLF and LPI from LF. Different lowercase letters indicate homogeneous groups ($p < 0.05$). 0, refers to pretreatments without MEF application (Conventional pretreatments, CV).

Further research is essential to explore the impact of thermal processing or cooking on color changes, as these transformations can greatly influence the product's visual appearance.

4. Conclusions

This study suggests that moderate electric field-assisted (MEF) pretreatment could be an effective strategy for enhancing the quality of lupin protein by reducing anti-nutritional (ANF) and anti-technological factors (ATF), while improving protein yield and techno-functional properties of lupin protein isolate (LPI). Furthermore, MEF may facilitate the production of lupin protein concentrate (LPC), a high-protein fraction with promising functional properties, highlighting its potential as a sustainable plant-based ingredient for the food industry. The observed reduction in ANF and ATF content could be attributed to MEF-induced electroporation and ohmic heating, which might disrupt cell structures and enhance mass transfer. This, in turn, may facilitate the release of undesirable compounds such as polyphenols, alkaloids, and saponins, while also promoting fat removal. Notably, minimal differences were observed across voltage levels, suggesting that full permeabilization may occur at the lowest voltage for each solvent.

Additionally, MEF may influence the structural properties of proteins, leading to modifications that could enhance the techno-functional characteristics of LPI and LPC. Changes in protein conformation might improve water and fat absorption capacities, foaming ability, and emulsification properties by increasing solubility, surface activity, and stability. These improvements could make lupin protein fractions more suitable for various food applications, including plant-based meat and dairy alternatives.

Overall, MEF presents a potentially scalable, eco-friendly approach aligned with circular economy principles, reducing the need for organic solvents and offering a sustainable alternative for plant-based protein extraction and utilization.

CRediT authorship contribution statement

Paola Navarro-Vozmediano: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **José V. García-Pérez:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision, Project administration. **Rubén Domínguez-Valencia:** Conceptualization, Writing – review & editing. **José Benedito:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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