



Corn and barley protein concentrates: effects of fractionation and micronization on the chemical, functional, and thermal properties

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Abstract

The present study intends to investigate the impact of fractionation and micronization on the compositional, functional, and thermal characteristics of two protein concentrates derived from corn and barley. Fractionation of protein concentrates (by sieving) resulted in three fractions, i.e., fine (< 100 µm), medium (100–300 µm), and coarse (> 300 µm), while micronization resulted in an average particle size of 30 µm. No specific pattern was observed between particle size and chemical composition of corn and barley protein concentrates due to the complex interplay between particle size, surface area, structural changes, and botanical origin. Protein solubility varied depending on the pH and particle size, showing that the coarse fraction exhibited higher solubility than the fine fraction. For both corn/barley protein concentrates, coarse fraction had higher water/oil-holding capacity. The barley fine fraction showed high foaming capacity, while all proteins lacked emulsification ability. Across all samples, a trend of increasing enthalpy with decreased particle size was observed, except for micronized barley, where enthalpy decreased, indicating possible protein structural changes. Thus, variations in properties among fractions highlight the importance of particle size in determining the proteins' functionality for potential applications in food systems.

Keywords Protein functionality · Particle size · Plant proteins · DSC · Protein solubility · Foaming capacity

Introduction

Plant-based diets have been on the rise in recent years, and they continue to gain popularity due to increased awareness and consciousness about human health, the environment, and animal welfare [1]. Research and international non-profit organizations such as the World Health Organization (WHO) promoted plant-based diets due to their association with

reduced risk of non-communicable diseases such as heart disease, diabetes, and certain types of cancers [2, 3]. These diets are often lower in saturated fats and cholesterol while being rich in vitamins, minerals, fiber, and antioxidants [4–6]. Concerns about the environmental impact of meat production, mainly greenhouse gas emissions, deforestation, and water usage, have also increased the transition toward plant-based diets [7–9]. Plant-based crops have been shown to have a low carbon footprint and consequently, they are considered more sustainable and environmentally friendly than animal farming [10–12]. Ethical and moral concerns about the treatment of animals in the food industry drive many people to reduce or remove meat and animal-derived products from their daily diet [13, 14]. Lately, the availability, versatility, and quality of plant-based food products have improved, which can further facilitate the transition to animal-free diets [15–17]. The market has seen a surge in a spectrum of plant-based meat substitutes, as well as dairy, egg, and seafood alternatives that can closely mimic the taste and texture of traditional animal-based foods [18, 19].

Plant proteins are essential components of plant-based products. They are available in various commercial forms including whole flours, isolates, hydrolysates, concentrates,

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and texturized [20]. The main conventional protein sources are soy and wheat, while recently peas have been gaining a lot of interest as a hypoallergenic source [21]. As the demand for plant-based protein continues to grow, researchers and food companies are exploring and developing new plant protein sources to meet these demands. The portfolio of legume proteins is expanding beyond pea and faba to encompass a diverse array of alternatives such as lupin, mung, and chickpea [22–24]. These proteins are incorporated in a variety of food products including bakery, snacks, pasta, and meat and milk alternative products [25–27]. The realm of proteins derived from cereals also keeps expanding with the inclusion of new sources (e.g., oats) beyond wheat proteins and gluten [25]. The valorization of side streams of the starch, oil, ethanol, and/or energy industries could contribute to creating new and sustainable proteins [28, 29]. Advanced extraction and separation technologies enabled the recovery of barley and corn protein ingredients [30–32].

Plant protein functionality is a crucial parameter for their application in various food products. Functionality refers to the specific properties and characteristics of a protein that determine how it interacts with other ingredients and how it contributes to the texture, structure, and overall quality of a food product [33]. Plant proteins were reported to have lower functionality compared to animal proteins. Therefore, several processing approaches were used for the functionalization of plant proteins including enzyme treatment, high pressure, extrusion, lactic acid bacteria fermentation, electrospraying, and ultrasound [20, 34–36]. In addition, particle size can have a significant impact on protein functionality including water- and oil-holding capacities, solubility, emulsification, foaming, and gelling [37–39]. The particle size of plant proteins can also influence the texture and mouthfeel of the food products. In beverages, the use of micronized proteins can result in smoother textures, whereas coarse particles may create a grittier or grainier textured mouthfeel [40, 41]. Modulating the particle size of plant proteins is an important variable to consider when designing a food product [42]. Manufacturers such as Cargill and Roquette sell

their proteins in different particle sizes to fit different food applications. To the authors' knowledge, while extensive research exists on the impact of particle size on flour quality [43, 44], its influence on purified forms such as protein concentrates remains less explored. This study aims to fill this gap by evaluating how dry-fractionation and micronization affect the functionality of corn and barley protein concentrates. The proteins were sieved to create fine, medium, and coarse particle sizes, with additional micronization to achieve particles around 30 μm . Through this research, we analyzed the chemical, functional, and thermal properties of these protein concentrates.

Material and methods

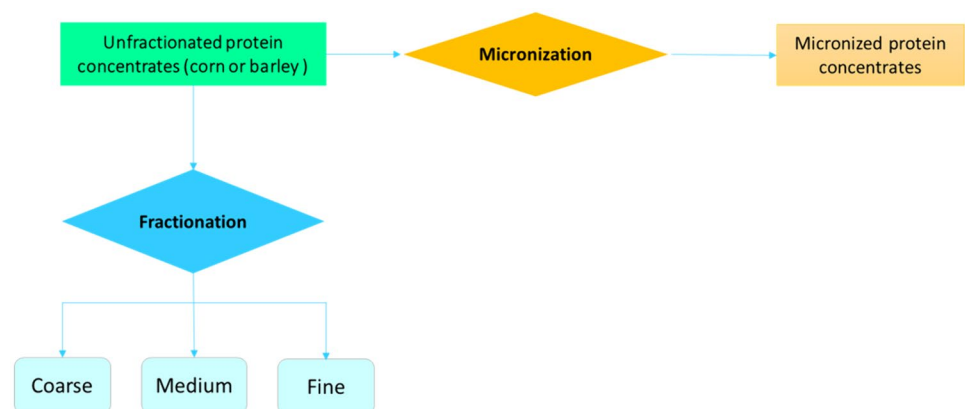
Fractionation and micronization

Corn and barley protein concentrates (Pannonia Bio Zrt, Hungary) have an average particle size of 825 and 487 μm , respectively. Then, these proteins were subjected to two physical dry processes (micronization and dry-fractionation) to produce fractions with different particle sizes (Fig. 1). Using an electric sieve shaker (Retsch GmbH, Haan, Germany) with two sieves (100 and 300 μm), protein concentrates were dry fractionated into three fractions, i.e., fine (< 100 μm), medium (100–300 μm), and coarse (> 300 μm). The reasons behind the use of these sieves were based on a market search conducted to identify the particle size of the currently available plant proteins on the market. Regarding the dry micronization process (Mills series MP, STM, Varese, Italy), protein concentrates were ground to obtain a particle size of 30 μm .

Chemical composition

The nitrogen percentage of protein samples was assessed using a carbon/hydrogen/nitrogen (CHN) determinator (CHN 628 Series, Leco Corporation, St. Joseph, MI, USA).

Fig. 1 Material preparation method



Each sample (100 mg) was added to a specific aluminum foil which was accurately folded and then placed inside the instrument's sample holder. The combustion temperature was set at 1050 °C [45]. Nitrogen data were used to calculate the protein percentage as N percentage $\times$ 6.25. Moisture, fat, and ash contents were analyzed and calculated according to AACC Approved Methods of Analysis. Total carbohydrates were calculated by subtracting the moisture, ash, lipid, and protein contents from 100.

Functional properties

Protein solubility

Protein solubility was determined using the method of Burger et al. [46]. Protein samples (200 mg) were suspended in 20 mL of deionized water. The resulting protein suspensions were adjusted to pH 2, 4, 5, 7, and 9 with 0.1N HCl or NaOH before being magnetically stirred for 1 h and then centrifuged at 7000 g for 10 min. The protein content of the residue was determined by CHN (N to protein = 6.25). Protein solubility (%) was calculated using the following equation:

$$\text{Protein solubility (\%)} = \left(1 - \frac{\text{Protein content of the residue}}{\text{Total protein in the sample}} \right) \times 100\%$$

Water/oil-holding capacity

The water- and oil-holding capacities (WHC/OHC) were evaluated using the method of Wang et al. [47]. Protein samples (0.5 g) were dispersed in 10 mL of deionized water or sunflower oil with a vortex for 30 s. After standing for 10 min, the dispersion was centrifuged (10,000 g for 20 min at 20 °C). The WHC/OHC of samples was expressed as grams of retained water/oil per gram of the tested samples.

Foaming capacity/stability

The foaming properties were estimated using the method described by Wang et al. [47] with slight modifications. In brief, 35 mL of protein solutions (3%) was homogenized at 24,000 rpm for 2 min using an Ultra-Turrax T25 (IKA Labortechnik, Janke & Kunkel, Staufen, Germany) equipped with an 18-mm stainless steel dispersing element. Then, the contents were immediately poured into a 100-mL measuring cylinder. The volume was recorded before and after homogenization. Foaming capacity was expressed as volume (%) increase due to homogenization. For the determination of foaming stability, foam volume changes were recorded after 30 min of storage.

Emulsification capacity

Protein samples were dispersed in distilled water (5% w/v) and centrifuged (9000 $\times$ g speed for 15 min). The supernatant was then mixed with sunflower oil (1:1 v/v) and homogenized (24,000 rpm for 1 min) using an Ultra-Turrax T25 (IKA Labortechnik, Janke & Kunkel, Staufen, Germany) equipped with an 18-mm stainless steel dispersing element. Aliquots (15 mL) of the emulsion samples were centrifuged (3000 $\times$ g for 5 min), and the volume of the individual layers was read using a 50-mL scaled tube. The emulsifying activity is expressed as the percentage of the volume of the emulsion divided by the initial volume.

Thermal properties

Thermal properties were studied using a differential scanning calorimeter (DSC-Q200, TA Instruments Waters, USA). Samples (8–10 mg) were weighed and sealed into hermetic stainless-steel pans (PerkinElmer, Waltham, MA, USA). The instrument was calibrated with indium (melting point: 156.6 °C, melting enthalpy: 28.71 J/g) and mercury

(melting point: – 38.83 °C, melting enthalpy: 11.44 J/g). Samples were equilibrated at 25 °C, kept for 5 min at that temperature, and then heated from 25 °C to 130 °C at a rate of 5 °C/min. DSC curves were analyzed with Universal Analysis Software (Version 3.9A, TA Instruments) to obtain the enthalpy change for transition (ΔH , J/g), onset and offset temperatures of transition (T_{on} and T_{off} , °C), and peak temperature at the maximum (T_p , °C).

Statistical analysis

All experimental data were statistically analyzed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Analysis of variance (ANOVA) with a confidence interval of 95% ($p \leq 0.05$) was used to determine whether the studied samples were statistically different. Significant differences among the mean values were calculated using Duncan's test. Three determinations were performed for each sample. Data were expressed as means $\pm$ SD.

Results and discussion

Effect of fractionation and micronization on the chemical composition

The chemical composition of unfractionated and micronized corn and barley protein concentrates, along with their respective fractions, is presented in Table 1. For corn protein concentrates, the effect of fractionation was found to be significant on moisture content, specifically, the coarse and medium fractions exhibited statistically similar values, both lower than the fine fraction. The moisture content of micronized corn protein concentrates was significantly higher than that of unfractionated corn protein concentrates. Probably, this can be attributed to the smaller particle size ability to trap moisture more effectively [48]. The content of protein of fractionated proteins (coarse, medium, and fine) exhibited the opposite trend to fat content. Coarse samples showed the lowest protein content, while medium and fine fractions showed similar values. A similar trend was observed in previous studies investigating the dry fractionation of legume flours [49]. Micronization did not have a significant effect on protein content, consistent with previous results [50, 51] reporting that the protein content of flours was not altered by micronization. When considering fat content, the coarse corn proteins exhibited higher content than that of unfractionated corn protein concentrates followed by medium and fine fractions. This could be due to the distribution of fat-containing components within the corn kernel. Notably, micronization significantly reduced fat content to almost half compared to the corn protein concentrate. This reduction may occur due to the physical processing involved,

which can break down the fat globule membrane or cause fat to be distributed differently within the material [52]. Ash content increased significantly by decreasing particle size. This increase suggests that the process of milling before fractionation might have concentrated or exposed the inorganic mineral content, leading to a higher measured ash content in finer fractions. However, micronization did not impact the ash content, implying that the minerals and inorganic components in the corn protein concentrates were not altered or concentrated during the micronization process. Regardless of carbohydrates, the three corn protein fractions (i.e., fine, medium, and coarse) were found statistically similar but higher than those of the starting material (unfractionated corn proteins). Micronization significantly increased the carbohydrate content, suggesting that the concentration of carbohydrates, such as sugars and starches, in the starting material became more prominent or accessible after micronization. This observation aligns with similar findings reported in a study that employed thermal micronization methods on corn grain [53].

Regarding barley concentrates, coarse and medium fractions showed the highest moisture content followed by the fine fraction. Micronization significantly reduced the moisture content, differently from what was observed in corn protein concentrates. This suggests that besides particle size, the actual moisture content is influenced by various intrinsic and extrinsic factors. It might be hypothesized that the decrease could be ascribed to the intense mechanical force leading to increased temperature which might cause water evaporation easily [54, 55]. As observed in corn protein concentrates, the protein content of barley fractions showed an increase with decreased particle size. Micronization was found to reduce protein content unlike what was found in the case of corn protein concentrates. This reduction might be attributed to

Table 1 Effects of fractionation and micronization on the chemical composition of corn and barley protein concentrates

	Moisture (g/100 g)	Protein (g/100 g of dry matter)	Fat (g/100 g of dry matter)	Ash (g/100 g of dry matter)	Calculated carbohydrates
Corn protein concentrates					
Unfractionated	5.48 ± 0.14 ^{bc}	55.50 ± 0.10 ^c	14.73 ± 0.13 ^d	2.98 ± 0.15 ^{bc}	21.33 ± 0.02 ^a
Coarse	5.31 ± 0.14 ^{ab}	51.49 ± 0.61 ^a	15.01 ± 0.07 ^e	2.62 ± 0.05 ^a	25.58 ± 0.45 ^b
Medium	5.07 ± 0.07 ^a	53.94 ± 0.33 ^b	12.78 ± 0.05 ^c	3.11 ± 0.00 ^c	25.12 ± 0.30 ^b
Fine	5.71 ± 0.03 ^c	53.59 ± 0.60 ^b	11.61 ± 0.00 ^b	3.33 ± 0.03 ^d	25.77 ± 0.59 ^b
Micronized	6.92 ± 0.13 ^d	54.89 ± 0.91 ^{bc}	7.12 ± 0.06 ^a	2.89 ± 0.04 ^b	28.19 ± 0.89 ^c
Barley protein concentrates					
Unfractionated	7.54 ± 0.03 ^b	68.75 ± 0.03 ^c	3.50 ± 0.16 ^b	1.36 ± 0.02 ^b	18.86 ± 0.18 ^a
Coarse	8.61 ± 0.09 ^d	62.59 ± 0.49 ^a	4.75 ± 0.02 ^d	1.21 ± 0.05 ^a	22.86 ± 0.57 ^b
Medium	8.78 ± 0.03 ^d	66.19 ± 0.24 ^b	3.58 ± 0.04 ^{bc}	1.47 ± 0.06 ^b	19.99 ± 0.18 ^a
Fine	8.32 ± 0.03 ^c	68.60 ± 0.80 ^c	3.12 ± 0.01 ^a	1.86 ± 0.08 ^c	18.11 ± 0.86 ^a
Micronized	7.31 ± 0.11 ^a	65.36 ± 1.33 ^b	3.77 ± 0.12 ^c	1.34 ± 0.03 ^b	22.23 ± 1.35 ^b

For each protein concentrate, means with the same letter are not statistically different by Duncan's test for $p < 0.05$

protein denaturation during the crushing process in relation to its surface area and structural changes. A similar trend was observed in previous studies [54]. Fat content was significantly reduced with reduced particle size. Conversely to corn proteins, micronization increased the fat content. This could be related to several factors associated with smaller particle sizes, specifically higher specific surface areas and a more damaged structure [54]. As observed in corn protein concentrates, ash content increased with decreasing the particle size, while micronization did not induce any effect. Coarse fraction showed the highest carbohydrate content, similar to micronized proteins. Fine and medium fractions showed similar values to the unfractionated barley concentrates. This finding implies that the distribution of carbohydrates within the starting material is not uniform, and the larger particles in the coarse fraction contain a higher proportion of carbohydrates. As for the case of corn, the micronization process employed in this study seems to improve starch availability. These consistent results underscore the impact of micronization on enhancing the detection and availability of carbohydrates within the processed material, shedding light on the potential mechanisms at play in this transformative process.

Regarding the effect of botanical origin, barley protein concentrates exhibited higher protein content compared to corn protein concentrates. In fact, the barley grains contained around 10 g/100 g of proteins, while the corn grains contained around 8 g/100 g of proteins (Table 1 in the supplementary materials). Conversely, fat content was found significantly higher in corn protein concentrates, reflecting the higher fat content in corn grains (4 g/100 g) in comparison to barley grains (2.76 g/100 g) (Table 1 in the supplementary materials). However, the carbohydrate content of corn proteins was found higher than that of barley, yet the carbohydrate content in both crops was comparable

(73.52 g/100 g in corn and 73.79 g/100 g in barley) (Table 1 in the supplementary materials). Ash content was found higher in corn protein concentrates but followed an inverse trend in grains (1.45 g/100 g in corn and 2.42 g/100 g in barley). This variability can be attributed to the impact of processing steps and structural properties of grains on the purity of proteins and the retention of other nutrients such as carbohydrates and ash.

Effect of fractionation and micronization on functional properties

Table 2 summarizes the effects of fractionation and micronization on protein solubility under different pH conditions. Relative to the pH effect, protein solubility of barley and corn protein proteins was higher at acid and basic pH conditions. Values of pH between 4 and 5 led to low protein solubility because the isoelectric points of the studied proteins were in this pH range [56]. As for particle size, coarse corn proteins had higher solubility followed by medium and fine. This trend is in contradiction with previous studies showing that the lower the particle size, the higher the plant protein solubility [57–59]. For instance, it was reported that decreasing particle size of soy protein isolates increased the surface area and improved solubility from 30 to 40% [58]. The micronization effect was not significant on corn protein solubility, except at pH 7, where micronized proteins had higher values than those of the unfractionated counterparts. This finding is most likely a result of the mechanical forces involved in the micronization process, causing modifications in the size and shape of particles. These modifications can enhance the interactions between proteins and water, thereby leading to an increase in solubility [52]. For barley protein concentrates, fine and coarse fractions showed similar protein solubility values at pH 2 which were higher than those

Table 2 Effects of fractionation and micronization on the protein solubility of corn and barley protein concentrates

Protein solubility	pH2	pH4	pH5	pH7	pH9
Corn protein concentrates					
Unfractionated	32.42 ± 0.04 ^{c/D}	21.34 ± 0.47 ^{b/C}	13.06 ± 0.13 ^{a/A}	13.90 ± 0.17 ^{a/A}	16.77 ± 0.28 ^{a/B}
Coarse	39.81 ± 0.00 ^{d/B}	38.84 ± 2.74 ^{c/B}	36.42 ± 3.43 ^{c/B}	29.62 ± 0.69 ^{d/AB}	29.62 ± 0.69 ^{c/A}
Medium	23.64 ± 0.66 ^{b/A}	23.64 ± 0.66 ^{b/A}	23.17 ± 0.00 ^{b/A}	22.71 ± 0.65 ^{c/A}	23.18 ± 1.31 ^{b/A}
Fine	14.00 ± 1.32 ^{a/A}	14.93 ± 1.32 ^{a/A}	14.47 ± 0.68 ^{a/A}	15.40 ± 0.66 ^{b/A}	15.40 ± 1.97 ^{a/AB}
Micronized	33.71 ± 0.26 ^{c/D}	22.49 ± 0.40 ^{b/C}	14.02 ± 0.21 ^{a/A}	15.60 ± 0.38 ^{b/AB}	17.10 ± 0.18 ^{a/B}
Barley protein concentrates					
Unfractionated	9.76 ± 0.29 ^{c/C}	5.72 ± 0.08 ^{a/A}	7.87 ± 0.13 ^{a/B}	9.67 ± 0.30 ^{a/C}	14.49 ± 0.35 ^{bc/D}
Coarse	9.99 ± 0.57 ^{c/C}	8.79 ± 0.00 ^{e/B}	7.99 ± 0.00 ^{a/A}	8.39 ± 0.57 ^{a/AB}	9.19 ± 0.57 ^{a/BC}
Medium	7.55 ± 0.00 ^{a/B}	6.80 ± 0.00 ^{d/A}	7.18 ± 0.53 ^{a/AB}	10.20 ± 4.81 ^{a/C}	15.49 ± 0.53 ^{c/CD}
Fine	9.48 ± 0.00 ^{bc/C}	6.56 ± 0.00 ^{c/A}	8.39 ± 1.55 ^{a/B}	11.30 ± 0.52 ^{a/D}	13.85 ± 0.00 ^{b/E}
Micronized	8.83 ± 0.06 ^{b/C}	5.95 ± 0.06 ^{b/A}	7.18 ± 0.09 ^{a/B}	9.65 ± 0.18 ^{a/D}	14.63 ± 0.06 ^{bc/E}

For each column, means with the same lowercase letter are not statistically different by Duncan's test for $p < 0.05$

of the medium fraction, while at pH 4, increasing particle size increased protein solubility. At pH 5 and 7, no significant differences were observed among the different samples. At pH 9, medium fraction had the highest value followed by fine and coarse fractions. A similar trend was observed in a previous work [56]. Regarding the effect of micronization, at pH 2, unfractionated proteins showed a higher value than that of micronized proteins, in contrary to the trend observed at pH 4. For pH 4, 7, and 9, micronization did not affect protein solubility. Overall, corn protein concentrates showed higher solubility than that of barley proteins probably due to a higher surface hydrophobicity. Another possible explanation might be the higher polyphenol content in barley as compared to corn grains. The binding of phenolic compounds to barley protein molecules might contribute to decreased protein solubility [60].

For each row, means with the same capital letter are not statistically different by Duncan's test for $p < 0.05$.

As illustrated in Table 3, the water-holding capacity (WHC) of coarse corn protein concentrates tends to absorb more water than the fine, medium, and unfractionated fractions. This pattern can be influenced by several factors related to the particle size as well as the structure and three-dimensional arrangement of proteins. Coarse fractions have larger particles, which can create more surface area for water absorption, leading to higher WHC [61]. Coarse corn fractions often have high porosity, which means they can hold more water due to their larger interstitial spaces [62]. The WHC of barley protein concentrates followed the same trend as corn proteins. Values of WHC of barley proteins align with previous studies [63, 64]. Furthermore, micronization reduced WHC in both proteins probably due to the degradation of protein's structure with consequent disruption of its interaction with water. This observation aligns with the

finding by Ye et al. studying the effect of mechanical micronization on insoluble dietary fibers [65].

The values of corn and barley protein concentrates are consistent within the same range as other plant proteins, typically ranging from 2 to 5 g/g [66]. For both corn and barley proteins, a decrease in OHC was observed with decreased particle size (coarse > medium > fine). A higher OHC was reported in association with higher solubility and was attributed to an increase in porosity [52, 67]. In addition, another reason might be that coarse fraction had an intact internal structure of the protein matrix compared to fine fraction. A similar effect was observed after micronization due to structure alteration including porosity disruption. Proteins with high OHC can result in improved food stabilization and reduced loss during food processing [67].

Foaming capacity was statistically similar among fine, medium, and coarse protein concentrates derived from corn. However, for barley proteins, the fine fraction showed significantly the highest value, which was within the same range measured for barley proteins extracted from brewer's grain spent [66], followed by medium and coarse fractions. This result can be attributed to their higher surface area and the increased accessibility of active sites to interact with water and air [68]. Noteworthy, the use of fine barley protein concentrates can be of interest to achieve the desired foam quality for products such as meringues, whipped creams, or foamy beverages. Micronization did not affect the foaming capacity of corn protein while it significantly reduced that of barley concentrates. This reduction can be attributed to the structural vulnerability of barley protein concentrates resulting in a higher degree of alteration due to the mechanical forces and heat generated during micronization [69]. It was reported that the loss of foaming capacity was correlated to the increase in the surface hydrophobicity and cross-linking

Table 3 Effects of fractionation and micronization on water/oil-holding and foaming stability/capacities of corn and barley protein concentrates

	Water-holding capacity (WHC, g/g)	Oil-holding capacity (OHC, g/g)	Foaming capacity (%)	Foaming stability (%)
Corn protein concentrates				
Unfractionated	2.16 ± 0.17 ^b	2.55 ± 0.03 ^e	11.00 ± 1.00 ^b	95.67 ± 0.58 ^b
Coarse	2.68 ± 0.01 ^c	1.65 ± 0.02 ^c	8.57 ± 0.00 ^a	98.25 ± 1.52 ^c
Medium	2.17 ± 0.03 ^b	1.55 ± 0.01 ^b	8.57 ± 0.00 ^a	93.42 ± 0.00 ^a
Fine	2.07 ± 0.07 ^{ab}	1.39 ± 0.03 ^a	8.57 ± 0.00 ^a	98.25 ± 1.52 ^c
Micronized	1.98 ± 0.04 ^a	1.72 ± 0.03 ^d	11.33 ± 1.15 ^b	97.33 ± 1.15 ^{bc}
Barley protein concentrates				
Unfractionated	3.26 ± 0.08 ^d	3.11 ± 0.41 ^c	24.00 ± 0.00 ^c	95.67 ± 0.58 ^c
Coarse	3.68 ± 0.09 ^e	1.90 ± 0.03 ^b	18.09 ± 1.65 ^b	95.16 ± 0.07 ^c
Medium	3.02 ± 0.12 ^c	1.77 ± 0.01 ^{ab}	42.86 ± 0.00 ^d	74.00 ± 0.00 ^b
Fine	2.47 ± 0.06 ^b	1.50 ± 0.03 ^a	113.34 ± 1.65 ^e	55.36 ± 0.67 ^a
Micronized	2.28 ± 0.15 ^a	1.86 ± 0.09 ^b	15.33 ± 0.58 ^a	95.67 ± 1.53 ^c

For each protein concentrate, means with the same letter are not statistically different by Duncan's test for $p < 0.05$

of the protein [69]. Foaming stability did not follow a specific trend among the corn protein fractions, while it was significantly reduced in barley proteins. This can suggest that particle size is not the only factor impacting this parameter but also protein structure and its ability to interact with air–water interfaces to stabilize foams [64]. Despite its high forming capacity, fine barley proteins showed low stability, similar to a previous study [66]. To valorize the high foaming capacity of fine barley fraction, stabilizers such as gums can be added to ensure better stability in applications such as egg replacements [19, 70]. Micronization did not show any effect on the foam stability of corn and barley protein concentrates. This can be related to the limited solubility of the studied proteins remaining unchanged.

All analyzed samples did not show emulsification properties. In a previous study, proteins extracted from brewer's spent grains showed very low emulsification properties [66, 71]. However, in the realm of protein concentrates, innovative post-treatments such as ultrasound and enzymatic hydrolysis, emulsions were formed and remained stable [63, 72]. These post-treatments can lead to protein unfolding and thus more hydrophobic group exposure, allowing faster adsorption onto the oil–water interface [72].

Effect of fractionation and micronization on thermal properties

Figure 2 reports the thermal curves of the fractionated and micronized corn and barley proteins.

In the context of corn proteins (Fig. 2a), it is possible to observe, for all the samples, an endothermic phenomenon within the temperature range of 59 and 85 °C. Sánchez Del Angel et al. [73] reported that, among the corn protein fractions, albumins and globulins, which represent the more polar ones, show denaturation temperatures around 62–67 °C; on the contrary, prolamin and glutelin, the less polar types, reach denaturation temperatures around 80–92 °C. This process occurs cooperatively and depends on several factors such as protein nature, concentration, a_w , temperature, pH, and ionic force [74]. These changes can affect protein functionality and their eventual use in food systems. Denaturation may already occur during the different stages of the extraction process (extraction steps and drying). In this specific study, other than the endothermic event, a step change in heat capacity is observed (Fig. 2a), which suggests an endothermic event superimposed with a glass transition phenomenon. This endothermic relaxation peak has been associated with the release of stresses built into the material as a result of processing and maintained by weak interactions, especially by hydrogen bonds. When the material is heated, the molecule goes from a rigid to a flexible structure [75]. The unfractionated corn proteins showed a less pronounced phenomenon (lower

enthalpy), occurring at significantly lower temperatures, than the other samples (Table 4). Specifically, enthalpy was inversely related to the powder's dimension (micronized $\geq$ fine > medium > coarse > unfractionated). This result suggests that a more uniform structural distribution of the molecules leads to a more cooperative phase transition, resulting in a more intense phenomenon.

Examining the thermal behavior of the barley proteins (Fig. 2b), a very similar thermal phenomenon was observed to the one already described for corn samples, occurring in a similar temperature range (Table 4). The melting enthalpies recorded for the barley samples were higher than those observed for corn (Table 4); this result was expected, considering the higher protein percentage found in barley samples in comparison to the corn ones (Table 4). The study of Silventoinen et al. [76] focused on the thermal properties of barley protein-enriched fractions obtained by air classification using DSC and detected the presence of an endothermal transition with a peak around 90–95 °C, attributable to protein denaturation. The lower temperature observed in our study may be attributable to different barley varieties, extraction conditions, and environmental factors. Unfractionated and micronized samples showed a very similar thermal behavior (Fig. 2b; Table 4). The highest enthalpies were observed for the fine and medium fractions, while lower enthalpies were observed for the unfractionated, coarse, and micronized samples. The pattern of higher enthalpies for the fine barley protein fraction in comparison to the coarse fraction was also observed by Silventoinen et al. [76]. The lower enthalpies observed for the unfractionated and coarse samples may signify a less uniform particle distribution, as previously suggested for corn proteins. In addition, for the micronized samples, the reduction in enthalpy may be attributed to the effect of protein structural alteration during the particle size reduction process [69].

Conclusion

In summary, the chemical composition analysis of corn and barley protein concentrates and their fractions revealed significant effects of fractionation, micronization, and the botanical origin. Regarding the functionality, the solubility of corn and barley protein concentrates was influenced by pH, enhanced under both acidic and basic conditions, and by particle size. Corn concentrates exhibited higher solubility than that of barley concentrates. Micronization reduced WHC and particle size reduced OHC, revealing the intricate interplay between particle size and functional properties. Foaming capacity was highest in fine barley concentrates, though micronization reduced both foaming capacity and stability. While foaming stability varied for corn proteins, it was significantly reduced in barley proteins. All samples

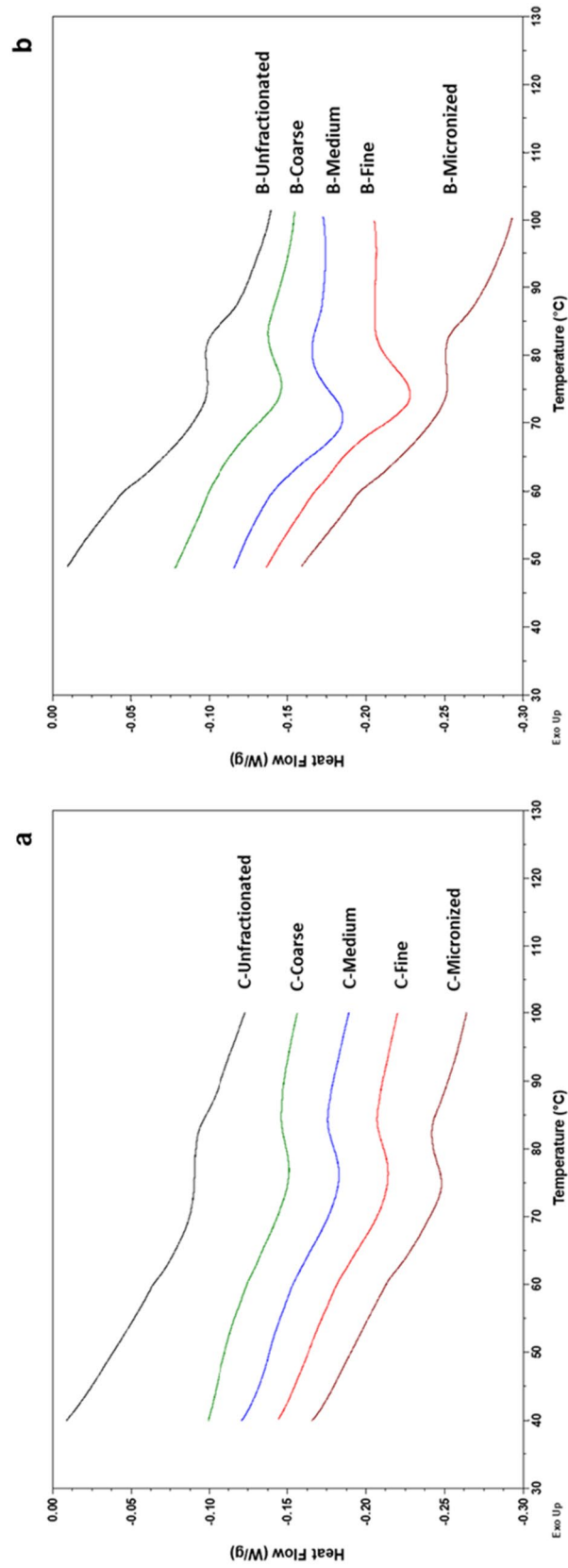


Fig. 2 Thermal curves of fractionated and micronized **a** corn and **b** barley protein concentrates

Table 4 Effects of fractionation and micronization on the thermal properties of corn and barley protein concentrates

	Onset temperature (Ton, °C),	Offset temperature (Toff, °C)	Range (°C)	Peak temperature (Tp, °C)	Enthalpy (W/g)
Corn protein concentrates					
Unfractionated	59.43 ± 0.09 ^a	81.73 ± 0.05 ^a	22.30 ± 0.14 ^c	69.14 ± 0.98 ^a	1.40 ± 0.38 ^a
Coarse	61.16 ± 0.00 ^c	83.46 ± 0.11 ^b	22.30 ± 0.11 ^c	74.50 ± 0.02 ^c	1.90 ± 0.07 ^b
Medium	62.95 ± 0.16 ^d	84.06 ± 0.00 ^b	21.12 ± 0.16 ^a	74.48 ± 0.10 ^c	2.16 ± 0.17 ^b
Fine	63.10 ± 0.26 ^d	85.01 ± 1.12 ^c	21.91 ± 0.86 ^{bc}	74.74 ± 0.76 ^c	2.20 ± 0.08 ^{bc}
Micronized	60.32 ± 0.06 ^b	81.62 ± 0.05 ^a	21.30 ± 0.11 ^{ab}	73.31 ± 0.38 ^b	2.34 ± 0.15 ^c
Barley protein concentrates					
Unfractionated	59.74 ± 0.23 ^a	80.87 ± 0.04 ^b	21.13 ± 0.27 ^c	72.13 ± 1.08 ^b	2.70 ± 0.58 ^a
Coarse	63.18 ± 0.20 ^b	82.94 ± 0.40 ^c	19.76 ± 0.21 ^b	73.73 ± 0.54 ^c	2.54 ± 0.04 ^a
Medium	59.86 ± 0.07 ^a	79.58 ± 0.06 ^a	19.72 ± 0.01 ^b	69.58 ± 0.67 ^a	3.87 ± 0.08 ^b
Fine	64.41 ± 0.61 ^c	83.42 ± 0.13 ^d	19.01 ± 0.74 ^c	73.42 ± 0.17 ^c	3.97 ± 0.19 ^b
Micronized	59.80 ± 0.05 ^a	80.80 ± 0.09 ^b	21.00 ± 0.04 ^c	71.90 ± 0.35 ^b	3.07 ± 0.01 ^a

For each protein concentrate, means with the same letter are not statistically different by Duncan's test for $p < 0.05$

lacked emulsification properties. Thermal analyses revealed an inverse relationship between enthalpy and particle size, suggesting that a more uniform structural distribution might enhance the cooperativity of phase transitions. Barley proteins showed higher enthalpies than those of corn concentrates in association with their higher protein content. These findings highlight the significant impact of fractionation/micronization on the properties of corn and barley protein concentrates. Further post-processing techniques may be explored to enhance the functionality of these plant proteins for various food applications.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article [and/or] its supplementary materials.

Declarations

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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