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# From solid dispersion to the simultaneous extraction of lipids and proteins: a bio-guided strategy to improve the nutritional and biological properties of almond milk

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ABSTRACT

Almond milk is a popular dairy alternative, but it is low in nutrients. To enhance its nutrient density, unroasted, light, and dark roasted almonds were ground to different particle sizes (flour, butter, paste) and subjected to aqueous (AEP) and enzyme-assisted extraction processes (EAEP). Dark roasting increased oil extractability from flour to 79 %, while reducing particle size to butter and paste further increased oil extractability up to 96 %. Roasting enhanced protein extractability from flour (62 % dark) but reduced it from butter and paste (81 to 71 % for AEP). EAEP increased protein extractability up to 92 % for butter and paste. Microscopy revealed that roasting increased oil body bursting, cellular disruption, and protein aggregation. Butter produced milks with higher nutrient density (1.51–1.91 % protein and 4.61–5.42 % oil), reduced energy requirements, and high *in vitro* protein digestibility (84–88.1 %). Proteolysis and roasting reduced the α-helix content and increased unordered structures of dark roasted milk proteins. Surface hydrophobicity was reduced for unroasted and light roasted EAEP milks but increased for dark roasted milk. EAEP milk proteins exhibited higher absolute zeta potential values than AEP milk proteins. These findings underscore the potential of AEP and EAEP as alternative methods for producing almond milks with enhanced nutritional content.

## 1. Introduction

Almond milk has emerged as a popular alternative to dairy milk, with an expected United States market value of $10.91B by 2030 (Mordor Intelligence, 2025). Non-dairy milks in general are growing in popularity, with almond milk as the category leader in the United States (Poinski, 2020). However, its nutritional composition lacks in comparison to dairy milk, with most containing ~0.5 % protein and ~1.25 % fat (Singhal et al., 2017). Most commercial almond milk production involves dispersing finely ground roasted almond paste in water under high shear mixing (Grant and Hicks, 2018). Emulsifiers and/or gums may be added to keep the solids in suspension, along with sweeteners and/or flavorants (McClements et al., 2019). This method limits the quantity of almond paste that can be used in the product, as high solid loadings would result in particle sedimentation and unpleasant gritty textures. Because of this, the protein and fat content of almond milk produced using solid dispersion methods are also limited. Most almond milks on the retail market in the United States are roughly 2 % almonds, leading to a final protein concentration of 0.5 g/100 g based on a market survey. Compared to bovine milk's roughly 3.3 g protein and 3.7 g fat per 100 g, this disparity precludes almond milk from serving as a true nutritional analog to bovine milk (Vanga and Raghavan, 2018).

Almonds are intrinsically a nutrient-dense food, with relatively high levels of protein (~20 %) and fat (~50–55 %) by weight (Yada et al., 2011, 2013). Therefore, almond milk has great potential for nutritional improvement. Although some protein- and fat-richer almond milks (up to 2.1 % protein and 4.6 % fat, based on a market survey) are commercially available, the use of more almond paste or larger particle size ground almonds in their manufacturing process often leads to sedimentation and an unpleasant grittiness on the tongue.

In the most popular almond milks, emulsifiers and gums such as lecithin, gellan gum, and xanthan gum are used to both create and

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stabilize the emulsion in the milk. These additives keep the insoluble almond solids in suspension and impart a desirable creamy mouthfeel. Though more research is needed in this area, food additives within this category have been linked to potential gut health issues such as increased inflammation and potential carcinogenesis, feeding negative publicity and consumer wariness of almond and other plant-based milks (Cani, 2015; Chassaing et al., 2015; Partridge et al., 2019; Viennois et al., 2017). These ingredients can also contribute to equipment fouling (i.e., deposition of unwanted material on heat exchangers) during thermal treatment (Huang and Goddard, 2015; Prakash et al., 2010), which can lead to increased equipment downtime.

To overcome the limitation in nutrient content of almond milk while maintaining a fluid texture without sedimentation, a new processing approach is necessary. Aqueous-based extractions present a valuable alternative to the current approach of commercial almond milk production. In aqueous (AEP) and enzyme-assisted aqueous extraction processes (EAEP), lipids and proteins are simultaneously extracted into the aqueous phase, while the fiber-rich insoluble fraction is removed through centrifugation. Therefore, a soluble protein- and lipid-enriched extract (i.e., almond milk) can be obtained (Campbell et al., 2011; Rosenthal et al., 1996, 1998). By tailoring the upstream processing conditions for the raw materials, i.e., degrees of roast and grind, as well as the extraction conditions (pH, temperature, extraction time, degree of hydrolysis), manufacturers can optimize the extraction step to meet their product specifications. Exploiting the natural emulsifying and gelation capacities of the proteins within the almonds can also potentially reduce the need of emulsifiers and/or hydrocolloids. To date, sustainable extraction approaches such as AEP and EAEP, along with the impact of key extraction parameters on the overall extractability and protein structural modifications that influence their functional and biological properties, have yet to be evaluated for almond milk production.

Given the significant influence of unit operations such as grinding, roasting, and extraction methods on both extraction yields and structural composition of the extracted compounds, this study was undertaken to elucidate the combined effects of roasting intensity, grinding methods, and enzyme use (aqueous vs. enzyme-assisted aqueous processes) on oil and protein extractability from almonds, as well as on the structure of the spent fiber-rich byproduct via confocal laser scanning microscopy. Importantly, this study investigated how these factors collectively influenced the nutritional composition, physicochemical properties (including secondary structure via circular dichroism, surface hydrophobicity, zeta-potential) and *in vitro* protein digestibility of almond milks produced via AEP and EAEP. By exploring how the structural and compositional modifications resulting from the upstream processing conditions impact the final product, a bio-guided process can be developed to produce milks with tailored nutritional composition and desired functional and biological properties. This innovative approach could provide almond milk producers with a valuable tool for enhancing product quality and better meet consumer preferences.

## 2. Materials and methods

### 2.1. Almond processing

Blanched deskinned sweet almonds (*Prunus dulcis*) were generously provided by Blue Diamond Growers (Sacramento, CA). Almonds were divided into three sets of 12 kg each and either left unroasted or roasted to "light" or "dark" color. Roasting was performed on aluminum sheet pans in a Turbofan convection oven (Moffat, Winston-Salem, NC) for 18 min at either 138 °C ("light" roast) or 148 °C ("dark" roast) according to industry guidelines (Huang, 2014), with almonds spread into a single layer on the sheet to ensure even roasting. Almonds were then ground to "flour" (large grind), "butter" (medium grind), or "paste" (fine grind) consistency on a Comitrol 1700 nut grinder (Urschel Laboratories, Chesterton, IN) that is commonly used for industrial almond paste manufacturing (Fig. 1).

### 2.2. Characterization of almond starting materials

#### 2.2.1. Particle size analysis

The particle size distribution of almond butters and pastes was determined using laser diffraction with a Mastersizer 3000E particle analyzer (Malvern Panalytical, Malvern, United Kingdom) using isopropanol (99.9 % pure, Fisher Chemical, Waltham, MA) as a dispersant. This selection was made due to the large amount of free oil, and it aligns with previous methods described for almond butter, which has a refractive index of 1.59 (Gebauer et al., 2016). Each type of raw material (i.e., unroasted flour, light roasted flour, etc.) was prepared in triplicate (three separate samples), and each sample was analyzed 10 times, resulting in an average value based on 30 measurements per sample. Particle size was reported as the De Brouckere [4,3] volume-weighted mean diameter using Mie theory calculations, as well as d10, d50, and d90 percentiles. Due to their larger particle size, almond flours were measured using the same method on a Mastersizer 3000 (generously provided by Malvern Panalytical).

#### 2.2.2. Starting material proximate analyses

The total oil content of the starting materials was determined using the acid hydrolysis method (AOAC Method 922.06 for Fat in Flour) (Min and Ellefson, 2010). Total protein content (%) was quantified using the Dumas method (Vario Max Cube, Elementar America, Ronkonkoma, NY, USA), and calculated as total nitrogen (%) × conversion factor of 5.18 (Souza et al., 2019). Total solids were quantified by drying the samples in a vacuum oven at 65 °C until a constant weight was achieved (AACC, n.d.). Ash was determined via combustion in muffle furnace for 4 h at 600 °C (American Oil Chemists' Society, 2017). Each sample replicate was analyzed in triplicate.

### 2.3. Almond milk extraction using the AEP and EAEP

For each roasting and grinding condition, almonds were processed using AEP and EAEP at a 1:10 solids-to-liquid ratio with deionized water (50 g ground almond:500 g water) at pH 9.0 for 60 min. Extractions were carried out in 1 L glass beakers in a 50 °C recirculating water bath under constant stirring of 120 rpm. For the EAEP, the same extraction conditions as the AEP were employed, with the addition of 0.5 % w/w (weight of enzyme/weight of ground almonds) FoodPro® alkaline protease (Danisco, Palo Alto, CA, USA). Following extraction, the slurry was centrifuged at 25 °C for 30 min at 3280 × *g* and the supernatant ("milk") was decanted to separate it from the remaining insoluble pellet fraction ("insolubles"). All extractions were performed in triplicate.

### 2.4. Quantifying oil and protein extractability

Oil and protein content of the almond milks and insoluble fractions was measured using the methods described in section 2.2. Total protein and oil extractability (TPE/TOE), which quantify the total amount of all protein/oil extracted from the original starting material, were calculated according to Eqs. (1) and (2), respectively:

$$TPE\ (\%) = \left[1 - \left(\frac{Protein\ (g)\ in\ the\ insoluble\ fraction}{Protein\ (g)\ in\ the\ almond\ material}\right)\right] \times 100 \quad (1)$$

$$TOE\ (\%) = \left[1 - \left(\frac{Oil\ (g)\ in\ the\ insoluble\ fraction}{Oil\ (g)\ in\ the\ almond\ material}\right)\right] \times 100 \quad (2)$$

### 2.5. Confocal laser scanning microscopy

Confocal laser scanning microscopy (CLSM) was employed to image the cellular structure of starting materials and insoluble fractions, providing insights into pre- and post-extraction morphology. Fresh

**Fig. 1.** Almond starting materials of different roasts and grinds used to produce AEP and EAEP milks. $U$ = unroasted, $L$ = light roasted, $D$ = dark roasted: $F$ = flour, $B$ = butter, $P$ = paste.

extractions were performed for each starting material to prevent cellular damage from freezing of insolubles. Samples were applied to slides immediately following extraction and centrifugation. For slide preparation, small amounts of the starting material or insolubles were placed in the well of a concave depression slide, then stained with fluorophores. Proteins were stained with fluorescein isothiocyanate (FITC, Sigma-Aldrich [St. Louis, MO], 1 mg/mL in acetone, diluted 1:100 v/v in deionized water, with 20 μL pipetted onto slide). Lipids were labeled with Nile red (Sigma-Aldrich, 1 mg/mL in dimethylsulfoxide [Alfa Aesar, Ward Hill, MA], further diluted to 1:100 v/v in deionized water, with 20 μL pipetted onto slide). Cell walls were stained with Calcofluor white (Sigma-Aldrich), with 20 μL pipetted directly onto slide. After staining, the slide was sealed with a fluoroprotectant (ProLong Gold Antifade, Thermo Fisher Scientific, Waltham, MA), and a glass coverslip was applied. The slides were allowed to cure in the dark at ambient temperature for 24 h. Subsequently, the edges of the coverslips were sealed with clear nail polish. Slides were stored at 4 °C until imaging. Imaging was performed using an Olympus Fluoview FV1000 laser scanning confocal microscope (Olympus Optical Co., Tokyo, Japan) equipped with excitation lasers in the following lines: 488 nm for FITC,

515 nm for Nile red, and 405 nm for Calcofluor white using a 40x magnification oil immersion objective lens.

### 2.6. Starting material selection and almond milk compositional, nutritional, and physicochemical characterization

Due to its comparable protein and oil extractability to the paste, along with less demanding grinding requirements, the butter was selected as the starting material for producing milks for subsequent comprehensive compositional, nutritional, and physicochemical characterization. These almond milks were analyzed in triplicate for protein, oil, moisture, and ash composition using methods described in Section 2.3. The carbohydrate content was calculated by difference.

#### 2.6.1. Impact of almond butter roasting on color of AEP and EAEP milks

The impact of roasting on color was assessed using *CIE L*a*b** colorimetry. Triplicate measurements were performed on a Hunter 1-UV–Vis colorimeter (HunterLab, Reston, VA) for both the almond butter starting materials (UB, LB, DB) and the corresponding milks produced with them.

#### 2.6.2. Influence of almond butter roasting and extraction methods on in vitro protein digestibility of almond milk

The *in vitro* protein digestibility (IVPD) of AEP and EAEP milks produced with UB, LB, and DB was determined using the INFOGEST 2.0 method (Brodkorb et al., 2019) with a modified pancreatin loading as described by Yang et al. (2025a). Enzyme activities were determined and simulated digestion fluids were prepared according to the INFOGEST protocol. Briefly, 2.5 mL of each milk extracted from "butter" materials was subjected to simulated salivary, gastric (2000 U/mL of pepsin), and intestinal (20 TAME U/mL of pancreatin) digestion. Immediately after digestion, 24 % trichloroacetic acid solution was added in a 1:1 (v/v) proportion to stop digestion and the samples were incubated at 4 °C overnight to promote protein precipitation. Samples were then centrifuged at 3280 × *g* for 30 min at 4 °C. The supernatant was decanted, and the pellet was washed with 10 mL cold acetone (Thermo Fisher Scientific), followed by incubation at −20 °C for 60 min. The samples were again centrifuged (3280 × *g*, 4 °C, 30 min), the supernatant decanted, and the residual acetone was evaporated in a fume hood overnight. The remaining pellets were then quantified for total nitrogen by the Dumas method as described in Section 2.3.

An enzyme blank was prepared by performing an empty digest with 2.5 mL of RO water instead of sample. IVPD was calculated based on a mass balance of nitrogen using Eq. (3):

$$IVPD\% = \frac{N_{milk} - \left(N_{pellet} - N_{blank}\right)}{N_{milk}} \times 100 \quad (3)$$

Where $N_{milk}$ is the mass of nitrogen (mg) of the milk prior to digestion, $N_{pellet}$ is the mass of nitrogen (mg) of the pellet after digestion, and $N_{blank}$ is the mass of nitrogen (mg) of the pellet of the enzyme blank.

#### 2.6.3. Almond milk protein characterization via SDS-PAGE

To characterize changes in protein molecular weights between extraction methods, AEP and EAEP milks produced from UB, LB, and DB were analyzed for molecular weight via SDS-PAGE. The protein profile was determined using the method described by Laemmli (1970) with some modifications. Fifty μg of each of the proteins were loaded onto a precast 12 % acrylamide Criterion TGX Precast gel, along with a low range SDS-PAGE standard (14.4–97.4 kDa) as a molecular mass marker. Following staining with Coomassie blue dye, gels were imaged using a Gel Doc EZ Imager system and Image Lab software.

#### 2.6.4. Defatting of almond milk

Almond milk produced from the AEP and EAEP was refrigerated overnight and then centrifuged at 3280 × *g* for 30 min at 4 °C to separate the aqueous and cream fractions. The cream layer (oil-rich fraction) was manually removed via transfer pipet to isolate the defatted milk, which was subsequently freeze-dried for circular dichroism spectroscopy and physicochemical analyses.

#### 2.6.5. Circular dichroism (CD) spectra

CD spectroscopy was utilized to determine the secondary structure of the almond milk proteins obtained from UB, LB, and DB. Freeze-dried defatted milk samples were diluted to a final protein concentration of 0.5 mg/mL in 0.005 M sodium hydroxide (pH 11) and filtered with a 22 μm paper filter to reduce scattering. The CD spectra were scanned at the far-UV range (190–250 nm) with a CD spectropolarimeter (Jasco J-715, Jasco Corp., Tokyo, Japan) in a 0.1 cm quartz CD cuvette (Hellma, Muellheim, Baden, Germany) at 25 °C. The scanning speed, response, and bandwidth used were 10 nm/min, 8 2, and 1.0 nm, respectively. The CD data were expressed as delta epsilon (Δε) and the proportions of the secondary structures present were determined by the BeStSel online software (Micsonai et al., 2018) with a scale factor of 0.05. The secondary structures quantified were α-helix, β-sheet, β-turns, and unordered structures. Each defatted milk sample was measured in triplicate.

#### 2.6.6. Surface hydrophobicity ($H_0$) of defatted almond milk proteins

The $H_0$ of the proteins in the milks was determined with the ANS (1-anilino-8-naphthalenesulfonate) fluorescence probe method described by Zhang et al. (2013) and Dias et al. (2020) with some modifications. Freeze-dried defatted almond milk samples were suspended in a sodium phosphate buffer (PBS, 10 mM, pH 7.0) to achieve protein concentrations from 0.02–0.70 mg/mL. A 250 μL aliquot of the sample and 1.25 mL of ANS solution (8.0 mM in 10 mM PBS, pH 7.0) were then pipetted into a 96-well black plate and shaken at 300 rpm for 30 min. Fluorescence intensity was measured in a SpectraMax iD5 plate reader (Molecular Devices, San Jose, CA, USA) using an excitation wavelength of 390 nm and emission wavelength of 470 nm. The $H_0$ of the protein sample was defined as the slope of the fluorescence intensity versus the protein concentration curve as determined by linear regression. Each extraction replicate ($n = 3$) was analyzed in duplicate and the $H_0$ was calculated as the average of six measurements for each condition (roasting and extraction method).

#### 2.6.7. Zeta potential of defatted almond milk proteins

To assess the impact of roast and grind on surface charge of proteins, the zeta potential of the almond milk proteins was measured using dynamic light scattering with a Zetasizer Nano (Malvern Instruments Ltd., United Kingdom). Freeze-dried defatted almond milk samples were diluted to 0.05 % (w/v) with 0.01 M pH 7 phosphate buffer to achieve a mean count rate over 100 kcps. Each extraction replicate ($n = 3$) was analyzed in triplicate at 25 °C.

### 2.7. Statistical analysis

Results are presented as means ± one standard deviation. Statistical analyses were conducted using two-way ANOVA for extractability and proximate composition, and one-way ANOVA for all other experiments. ANOVA was followed by Tukey's tests for mean differences using a significance of $p < 0.05$ using Statistica™ Software (TIBCO Software Inc, Palo Alto, CA, US).

## 3. Results and discussion

### 3.1. Impact of roasting and grinding on particle size

The mean [4,3] volume-weighted diameter of all almond starting materials was reported along with the d10, d50, and d90 values (Table 1). The mean diameter of flours exhibited a wide standard deviation among all roasts, indicating a broad range of particle sizes. Though the standard deviation of the mean particle size of the light

**Table 1**
Influence of roasting and griding on almond particle size distribution as measured by dynamic laser scattering.

| Starting Material | d10 | d50 | d90 | d[4,3] |
|---|---|---|---|---|
| UF | 20.93 ± 6.03[a] | 453.65 ± 60.15[a] | 849.35 ± 109.00[a] | 440.15 ± 62.88[a] |
| LF | 5.55 ± 0.23[bc] | 380.0 ± 106.51[b] | 382.7 ± 38.43[c] | 270.5 ± 145.49[b] |
| DF | 7.38 ± 0.27 [b] | 318.75 ± 21.20[c] | 556.4 ± 106.62[b] | 250.75 ± 56.51[b] |
| UB | 5.58 ± 0.05[bc] | 23.33 ± 00.83[d] | 103.33 ± 10.45[d] | 39.11 ± 2.44[c] |
| LB | 4.80 ± 0.10[c] | 12.96 ± 0.71[d] | 69.31 ± 37.91[de] | 25.09 ± 2.00 [c] |
| DB | 4.88 ± 0.13[c] | 12.70 ± 0.82[d] | 54.75 ± 15.91[de] | 22.83 ± 1.75 [c] |
| UP | 5.10 ± 0.03[c] | 17.96 ± 0.26[d] | 52.96 ± 2.22[de] | 24.08 ± 0.68 [c] |
| LP | 4.75 ± 0.02[c] | 11.95 ± 0.58[d] | 38.69 ± 9.84[e] | 18.54 ± 0.83 [c] |
| DP | 4.63 ± 0.05[c] | 11.13 ± 0.32[d] | 33.78 ± 3.04[e] | 18.27 ± 1.24[c] |

Unroasted (U), light (L), and dark (D) almond flour (F), butter (B), paste (P). Different letters within each column indicate statistically significant differences by two-way ANOVA followed by Tukey's test for HSD ($p < 0.05$) to discriminate means.

roasted flour was too large to be significantly different from that of unroasted flour, both the light and dark roasted flours tended to be smaller in diameter than the unroasted flour. This is likely due to the release of oil during roasting, along with the shrinking and hardening of cell walls, which contribute to easier grinding during processing (Gorrepati et al., 2015). A similar reduction in particle size with roasting, compared to unroasted material, was also observed in peanuts crushed by a roller mill by Li et al. (2016b).

### 3.2. Impact of roasting, grinding, and extraction methods on oil and protein extractability

The protein bodies and oleosomes (oil bodies) contained within almond cells are bound by an intracellular cytoplasmic network (Pascual-Albero et al., 1998). When almonds are ground, this network is destroyed, the protein and oil bodies rupture, and oil becomes a continuous phase (Gorrepati et al., 2015). Roasting can also lead to oleosome rupture and protein body distortion (Perren and Escher, 2013). Due to these and other desirable sensory changes incurred in roasting (i.e., enhanced flavor and color (Huang, 2014)), almonds are commonly roasted before grinding, as it facilitates the size reduction process. The finely ground roasted almonds used in almond milk processing serve as an ideal substrate for AEP and EAEP, as these processes leverage the upstream disruption of plant cell walls and the strategic selection of extraction medium pH and solids-to-liquid ratio to enhance protein solubilization and diffusion out of the cellular matrix, further facilitating oil body or free oil release (Rosenthal et al., 1996).

Although roasting can improve extraction yields, it also has the potential to denature proteins, leading to aggregation and change in functionality that may impact protein solubility and/or extractability (Li et al., 2016b; Liu et al., 2016; Perren and Escher, 2013). Similarly, reducing particle size can improve extractability by increasing the surface area to volume ratio (Gao et al., 2024; Li et al., 2016b; Rosenthal et al., 1996). A thoroughly disrupted cellular wall is necessary to maximize contact between the aqueous medium and the target components for extraction. However, depending on the magnitude of the increase in extractability, this may not justify the additional energy input. Importantly, the addition of proteolytic enzymes to assist the extraction can increase oil and protein extractability by hydrolyzing proteins into smaller and more soluble peptides and disrupting the oil body membrane (Campbell et al., 2016; Dias and de Moura Bell, 2022). Furthermore, proteolysis has the potential to modulate protein functionality depending upon the enzyme's activity (Campbell and Glatz, 2009; Souza et al., 2019), necessitating careful processing optimization to generate proteins with desired functional, biological, and nutritional properties for specific applications. The addition of enzymes to extraction can increase processing costs, but the improvements in extractability and functionality can increase efficiency enough to overcome this cost and increase profitability (Yang et al. 2025b).

AEP and EAEP were used to produce almond milks with nine different almond starting materials with varying degree of roast and grind (UF, LF, DF, UB, LB, DB, UP, LP, and DP). The effects of upstream roasting and particle size reduction during grinding on total oil (TOE) and protein extraction (TPE), which are crucial to control the nutrient profile of the final milk, are shown in Fig. 2. In general, both the degree of roasting and particle size significantly impacted total oil extraction (TOE) (Fig. 2A). Oil extractability from the flour was favored by higher levels of roasting in the AEP, although no statistically significant differences were observed between TOE of dark and light roasted flours. However, for the more finely ground butter and paste, TOE was similar or slightly lower than that of unroasted flour. The increase of oil extraction with roast in flour can be attributed to the release of intracellular oil in roasting, but the lack of increase or even the small reduction observed in TOE for the roasted butters and pastes imply that this is not the case with finer particle size. This result is in agreement with the findings by Li et al. (2016b), who reported that AEP oil extraction from crushed peanut pastes decreased with increased temperature of roasting. The reduction in oil extractability is likely due to the distortion of intracellular protein bodies brought on by roasting (Perren and Escher, 2013). During roasting, the denaturation and aggregation of proteins may lead to oil entrapment within the cell wall matrix, thereby reducing oil and protein extraction yields. This phenomenon is evident when comparing the lower oil extractability from the dark roasted paste, which was significantly lower than that of the unroasted (89.42 % vs. 96.39 %), highlighting the substantial impact of roasting. These effects, combined with the fine particle size of the paste, can lead to particle aggregation and emulsion formation within the extraction slurry. This could further hinder the separation of the extracted oil into the liquid phase during centrifugation, thus decreasing oil yield (De Moura et al., 2011). These findings align with those reported by Lamsal et al. (2006) who observed a decrease in oil extraction recovery from 75 % to 50 % when the extrusion temperature of soybeans increased from 100 °C to 120 °C, attributing this reduction to increased protein denaturation, which may bind to the oil and hinder its recovery.

With the addition of protease in the EAEP, small but significant increments in oil extractability were observed in some roast/grind conditions (light/dark butter and paste) compared to the AEP. This underscores the potential role of the endoprotease in hydrolyzing the denatured protein bodies into more soluble protein fragments and disrupting the oil body membrane, which could in turn favor the release of more oil (Souza et al., 2019; Dias and de Moura Bell, 2022). Notable exceptions were observed for the unroasted flour (65.08 % for AEP vs. 53.36 % for EAEP), unroasted butter (94.36 % for AEP vs. 92.06 % for EAEP), and the EAEP unroasted and light roasted pastes (95.97 and 95.92 %, respectively), whose values were not statistically different. The fine particle size of the butter and paste and lower roasting intensity may explain the high levels of oil extraction in both AEP and EAEP. However, at higher levels of roasting and griding (i.e., dark butter and paste) the observed reduction in oil extractability due to roasting was overcome with the addition of a protease. Protein denaturation and more extensive grinding or cell wall disruption can increase the availability of protein sites for enzyme action, while also enhancing the exposure of the material to both the enzyme and the extraction medium. These effects collectively enhance the extractability of both proteins and lipids. As more proteins are extracted, the material develops a more porous structure, which facilitates the release and washing out of lipids into the

**Fig. 2.** Impact of roasting, particle size, and extraction methods (AEP vs. EAEP) on total (A) oil and (B) protein extractability. $U$ = unroasted, $L$ = light roasted, $D$ = dark roasted. Different letters indicate statistically significant difference between means by two-way ANOVA followed by Tukey's test ($p < 0.05$).

extraction medium (De Souza et al., 2020; Lamsal et al., 2006; Souza et al., 2019)

When assessing the impact of roasting and grinding on protein extractability, a decrease in protein extractability was evident when moving from unroasted to dark roasted butter and paste in the AEP (Fig. 2B). TPE for both light and dark roasted butter and paste was significantly lower (70.40–75.14 %) than that for unroasted butter and paste (80.99–81.99 %). Heat treatment significantly impacts the secondary and tertiary structures of proteins (Rahaman et al., 2016; Vanga et al., 2016; Zhou et al., 2021), inducing conformational changes such as unfolding, structural loss and rearrangement into new conformations. Almond proteins have been found to denature when exposed to temperatures as low as 96 °C (Amirshaghaghi et al., 2017; Zhang et al., 2016), well below the roasting temperatures (138 °C for light and 148 °C for dark roasting) employed in this study. This denaturation and aggregation could explain the observed decrease in protein extractability for dark roasted butter and paste compared with their unroasted counterparts. These findings align with those reported by Lamsal et al. (2006), who observed a decrease in protein extractability from 50.6 to 32.9 % when the extrusion temperature of soybeans increased from 100 °C to 120 °C, attributing this decline to increased protein denaturation at higher temperatures.

Similar to what was observed for TOE, the use of protease in the EAEP significantly enhanced protein extractability from all almond starting materials compared to the AEP. For butter and paste, protein extraction in the EAEP was statistically similar among all roasts (88.84–91.76 %), but higher than all their AEP counterparts (maximum TPE in the AEP was 81.99 % for unroasted paste). This is consistent with previous research that demonstrated increased TPE with protease-assisted extraction for almond flour and almond oil press cake (de Almeida et al., 2019; Dias and de Moura Bell, 2022; Niu et al., 2021; Souza et al., 2019). Proposed mechanisms for higher extraction yields in the EAEP include proteases' ability to disrupt and loosen the protein bodies from the cellular matrix, releasing polypeptides that are more soluble in the extraction medium than native proteins (Campbell et al., 2011). However, improvements in protein extractability in the EAEP depend on several factors, including enzyme specificity, extent of cell wall disruption, heat treatments, and degree of protein denaturation. While protein denaturation might enhance the availability of sites for hydrolysis, extensive denaturation can lead to protein aggregation or binding with lipids (de Moura et al. 2011; Li et al., 2016b). This could impact almond milk produced by this method if the processor wishes to separate the protein and lipid streams to tailor to a specific nutritional goal. For example, Zhang et al. (2011) observed a reduction of peanut protein hydrolysate extractability after roasting at 220 °C for 20 minutes, likely due to the excessive denaturation and subsequent decrease in protein solubility from intense heat treatment.

However, while enzymes improve extractability, care must be taken

to avoid the formation of bitter-tasting peptides in the resulting almond milk due to protein hydrolysis (Liu et al., 2022; Seo et al., 2008). A balance of extraction efficiency with consumer preferences is necessary to prevent excessive protein hydrolysis leading to an unpalatable product.

### 3.3. Confocal laser scanning microscopy (CLSM)

The microstructure of the almond starting materials and insoluble fractions (extraction byproduct) were illustrated using CLSM (Fig. 3). The fluorescence dyes (red, green, and blue colors indicate lipids, protein bodies, and cell walls/background light, respectively) displayed the effects of grinding and roasting on the release of free oil as well as extractability of oil and protein components. Fig. 3A illustrates the microstructure of almond flour and insoluble fractions. In the unroasted flour, intact cells are visible with entrapped oil and lipid bodies. In the roasted flours, greater cell disruption and distortion of some cell walls were observed. Upon roasting, oil bodies likely burst and coalesced into a continuous oil phase both outside and within intact cells. Changes in oil body size and distribution after roasting were also observed by Altan et al. (2011) and Li et al. (2016b) using CLSM. AEP and EAEP insoluble fractions of the unroasted almond flour demonstrate the extraction of a portion of the oil and protein from the almond flour, although a significant amount remained entrapped within the cells. The oil bodies appear to have ruptured in the EAEP insoluble fraction, underscoring the hydrolysis of oleosins in the oil body membrane by the protease used. As the degree of roast increased from unroasted to dark roasted flour, there was an increase in free oil release in the starting materials, leading to higher oil extraction as depicted in the insoluble fractions. Intact cells, however, still prevented the release of contained oil and protein bodies. Finer grinds such as butter (Fig. 3B) and paste (Fig. 3C) led to even greater cellular disruption, with increased coalescence of oil bodies and aggregation of protein bodies. The CLSM images of the insoluble fractions highlight how grinding and proteolysis favor the removal of lipids and proteins, in agreement with the TOE and TPE results. The paste grind (Fig. 3C) left virtually no cells intact, and EAEP insolubles of pastes exhibited extremely few protein and oil bodies remaining. This indicates that physical disruption is a critical upstream processing step in AEP/EAEP for the improvement of extraction efficiency.

### 3.4. Starting material selection and almond milk compositional, nutritional, and physicochemical characterization

Given that butter and paste achieved similar TOE and TPE, butter was chosen as the starting material to produce milks for further analyses. By not grinding materials as finely as a paste, this method can potentially reduce energy consumption during upstream processing for almond milk production. The milks extracted from the almond UB, LB, and DB were analyzed for proximate composition (Table 2). No significant difference in oil content was found between AEP and EAEP milks regardless of roast (average 4.84 %). However, EAEP milks tended to have higher protein content than AEP (1.90 % vs. 1.63 %), which is consistent with the TOE and TPE. The ash content was significantly higher in EAEP milks than in AEP milks due to the increased NaOH added during extraction to keep the pH elevated, since pH decreases as proteolysis occurs due to the exposure of acidic end groups (Wouters et al., 2016). All milks were significantly higher in oil and protein content than a leading commercial almond milk (calculated from its Nutrition Facts panel). As most commercial almond milks are considerably lower in oil and protein than dairy milk (McCarthy et al., 2017; McClements et al., 2019), this provides a promising method for the development of a nutritionally superior almond milk. Additional adjustment of the solids-to-liquid ratio and other extraction parameters could increase protein levels further, allowing for the potential to meet the protein content in dairy milk.

**Fig. 3.** Confocal laser scanning microscopy images of starting materials, AEP insolubles, and EAEP insolubles from almond (A) flour, (B) butter, and (C) paste materials. Red stains indicate lipids, green indicate protein bodies, and blue indicate cell walls or background light. Images were captured using a 40x oil immersion lens.

**Table 2**
Impact of roasting and extraction methods on the macronutrient composition of almond milks produced via AEP and EAEP compared to an average commercial almond milk.

| Extraction | Lipids (%) | Protein (%) | Ash (%) | Moisture (%) | Carbohydrate (%) |
|---|---|---|---|---|---|
| AEP UB | 4.61 ± 0.36[a] | 1.74 ± 0.07[bc] | 0.12 ± 0.01[a] | 92.29 ± 0.25[ab] | 1.24 ± 0.22[a] |
| AEP LB | 4.68 ± 0.29[a] | 1.63 ± 0.04[cd] | 0.13 ± 0.01[a] | 92.36 ± 0.14[ab] | 1.20 ± 0.08[a] |
| AEP DB | 4.45 ± 0.61[a] | 1.51 ± 0.08[d] | 0.12 ± 0.01[a] | 92.53 ± 0.55[a] | 1.38 ± 0.13[a] |
| EAEP UB | 5.42 ± 0.60[a] | 1.88 ± 0.02[ab] | 0.20 ± 0.01[b] | 91.51 ± 0.43[b] | 1.00 ± 0.24[a] |
| EAEP LB | 5.16 ± 0.45[a] | 1.87 ± 0.06[a] | 0.19 ± 0.01[b] | 91.67 ± 0.44[ab] | 1.09 ± 0.62[a] |
| EAEP DB | 4.73 ± 0.30[a] | 1.91 ± 0.01[a] | 0.21 ± 0.02[b] | 92.20 ± 0.23[ab] | 0.96 ± 0.06[a] |
| Commercial Almond Milk | 1.25 | 0.42 | — | — | 0.83 |

Unroasted (U), light roasted (L) and dark roasted (D) almond butter. Different superscript letters within the same column indicate statistically significant difference of means by one-way ANOVA followed by Tukey's test ($p < 0.05$).

### 3.5. Impact of almond butter roasting on color of AEP and EAEP milks

The colorimetry data indicated lower $L^*$ values for the roasted almond butters compared to the unroasted almond butter, which was expected due to Maillard browning occurring during roasting (Huang, 2014) (Supplementary Table 1). The $a^*$ and $b^*$ values also increased, which is consistent with previous studies (Ünver, 2016; Zhang et al., 2011). Some degree of roast is desirable due to the aforementioned improvement of flavor and color qualities of almonds, but extensive roasting can lead to the formation of dangerous acrylamide products, thus a moderate roast is safer for consumers (Amrein et al., 2005; Zhang et al., 2011). More intense roasting also requires higher energy inputs and can lead to extensive denaturation of proteins, resulting in decreased protein in solubility and extractability (De Moura et al., 2011; Yan et al., 2021).

### 3.6. Influence of almond butter roasting and extraction methods on in vitro protein digestibility of almond milk

Overall, the *in vitro* protein digestibility of all milks was very high (84.4–88.1 %) (Fig. 4A), with no significant impact from roasting conditions employed or extraction processes. The overall *in vitro* protein digestibility values in this study were higher than those previously reported in almond milk protein digestibility studies. Martínez-Padilla et al. (2020) reported 25.19 % protein digestibility in a commercial almond drink, and Vanga et al. (2020) found 75.53 % protein digestibility in a milk made from whole almonds blended with water. Previous studies (Vanga et al., 2016; Zhou et al., 2021) on peanut and tree nut protein digestibility have found that protein digestibility increased with roasting; however, this could be due to differences in roast time/temperature utilized (170 °C for 10 min/50–75 C° for 15–45 min, respectively) and the specific proteins present in the matrix. However, these changes in protein digestibility of the staring materials with roasting were not reflected in the milk proteins produced by the AEP. In addition, the EAEP has been demonstrated to significantly increase almond protein digestibility compared to the AEP (88.5 % vs. 79.1 %), as the proteolysis that occurs during the extraction process acts as a "pre-digestion" step (Dias et al., 2023). However, in the present study, it is possible that the more dilute nature of the almond milk sample allowed for improved digestibility. It is also important to note that different proteases were used in both studies, which could have influenced the digestibility of the hydrolysates. Variations in unit operations employed for almond milk production, including those applied to the starting material, can significantly influence the digestibility of the final milk. Consequently, direct comparisons of our data with the existing literature may be limited due to process-related differences.

### 3.7. Almond milk protein characterization via SDS-PAGE

SDS-PAGE (Fig. 4B) revealed a protein profile for the almond milks characterized by prominent bands between 31.0 and 45.0 kDa and 21.5 and 31.0 kDa, corresponding to the acidic (42–46 kDa) and basic (20–22 kDa) subunits of amandin protein (Sathe et al., 2002), respectively. Additional protein bands were observed within a broader molecular weight range for the AEP samples. The LB and DB lanes for AEP appear to show a higher concentration of high-molecular weight proteins near the top of the gel compared to the unroasted AEP lane, which was similarly reported by Devnani et al. (2020) for almond milk proteins treated at 75−95 °C for 30 min. This suggests denaturation and aggregation in the roasted butters, which could have impacted TPE as discussed in Section 3.2. EAEP samples exhibited significantly lower molecular weight proteins/polypeptides compared with the AEP.

**Fig. 4.** (A) *In vitro* protein digestibility (%) and (B) reducing SDS-PAGE of almond milks produced by different roasting and extraction methods. AEP = aqueous extraction process, EAEP = enzyme-assisted aqueous extraction process, UB = unroasted butter, LB = light roasted butter, DB = dark roasted butter. Different superscripts in (A) indicate statistically significant difference between means by one-way ANOVA followed by Tukey's test ($p < 0.05$).

Although some bands were observed between 21.5 and 31.0 kDa, most of the protein fragments were below 14.4 kDa, indicating significant hydrolysis of proteins. This is in agreement with previous findings that proteolysis did not affect the 22 kDa acidic subunit of amandin (Dias et al., 2023). This subunit's resistance to hydrolysis could be a limiting factor for protein digestibility and its immunoreactivity; however, as mentioned in Section 3.2, excessive hydrolysis is undesirable due to the generation of bitter peptides. This warrants careful consideration in the use of enzymes to tailor hydrolysis to the desired outcome. Despite the higher protein content in EAEP milks, the bands are not demonstrably denser than those of the AEP milks; perhaps indicating that a great deal of proteins in the EAEP were so hydrolyzed as to have washed out of the gel.

### 3.8. Circular dichroism (CD) spectra

CD was used to estimate the secondary structure of the AEP and EAEP almond milks (Table 3). The relative proportions of these four structures (α-helix, β-sheet, β-turn, and unordered structures) remained stable in all AEP milk skims regardless of roast. These findings align with previous research on extracting whole proteins from unroasted almond products (i.e. flour and milk) (Devnani et al., 2021; Dias and de Moura Bell, 2022). Albillos et al. (2009) found that native amandin secondary structure was relatively heat-stable, while ordered structures in amandin's acidic polypeptide decreased with increased temperature during CD measurements, indicating the importance of covalent links in stabilizing the native protein. Ma et al. (2022) found higher processing temperatures (150 and 180 °C for 5–30 min) actually increased α-helix structures in sesame seed proteins as opposed to 120 °C for unhydrolyzed proteins.

In contrast, the structural composition of the EAEP proteins differed significantly from that of the AEP and among the various roasting conditions within the EAEP, highlighting the combined effects of both roasting and proteolysis in the EAEP protein structure. The α-helix content of the EAEP proteins was reduced to 1.50 % for the unroasted and light roast milks and to 4 % for the dark roasted milk, compared with 7.8 % for the AEP proteins. Albillos et al. (2009) theorized that the instability of α-helices in almond protein in the absence of tertiary structures contributed to an observed decrease with thermal processing, as released polypeptides were no longer available to stabilize these structures, and helices unwound. Perhaps this instability was further impacted by hydrolysis. The decrease in the α-helix content of the EAEP proteins was accompanied by an increment of the β-sheet content for the unroasted and light roast milk proteins, while it decreased for dark roast milk proteins. The β-turns content was relatively unchanged and unordered structures of the EAEP milk proteins were higher for the dark roast milk proteins.

**Table 3**
Secondary structure composition of almond "butter" milk proteins as determined by circular dichroism in the far UV region (190–250 nm).

| Extraction | Roast | α-helix (%) | β-sheet (%) | β-turns (%) | Unordered (%) |
|---|---|---|---|---|---|
| **AEP** | **UB** | 7.67 ± 0.40$^{a}$ | 27.93 ± 0.15$^{bc}$ | 16.97 ± 1.69$^{a}$ | 47.43 ± 1.69$^{b}$ |
| | **LB** | 7.77 ± 0.42$^{a}$ | 27.03 ± 0.76$^{c}$ | 16.33 ± 0.42$^{ab}$ | 48.87 ± 0.76$^{ab}$ |
| | **DB** | 7.87 ± 1.08$^{a}$ | 28.47 ± 1.10$^{abc}$ | 15.87 ± 0.38$^{ab}$ | 47.83 ± 0.21$^{b}$ |
| **EAEP** | **UB** | 1.47 ± 0.38$^{c}$ | 34.00 ± 1.93$^{ab}$ | 16.20 ± 0.36$^{ab}$ | 47.37 ± 2.15$^{b}$ |
| | **LB** | 1.50 ± 0.44$^{c}$ | 35.33 ± 5.28$^{a}$ | 15.93 ± 0.97$^{ab}$ | 47.20 ± 4.78$^{b}$ |
| | **DB** | 3.87 ± 0.47$^{b}$ | 25.20 ± 1.25$^{c}$ | 15.17 ± 0.32$^{b}$ | 54.97 ± 2.25$^{a}$ |

Unroasted (UB), light roasted (LB), and dark roasted (DB) almond butter materials. Different superscript letters within the same column indicate statistically significant difference of means by one-way ANOVA followed by Tukey's test ($p < 0.05$).

Overall, despite no change in the α-helix content of the AEP milk proteins across roasting conditions, our data suggest that the protein modifications (i.e., denaturation and/or aggregation) induced by darker roasting, in alignment with reduced protein extractability at these conditions (e.g. 80.96 % TPE for UB vs. 73.97 and 70.40 % TPE for LB and DB, respectively), might have enhanced the enzyme's ability to alter these structures. This becomes evident from the reduced α-helix and β-sheet contents and increased quantity of unordered structures of dark roasted milk proteins compared to lighter roasts and unroasted almond butters. Additionally, the unordered structures of the EAEP proteins were similar to that of AEP but were significantly higher in the dark roast, suggesting that the higher degree of roasting caused conformational changes that enhanced the protease activity. The observed decrease in α-helix content and increase in unordered structures in EAEP proteins, compared to AEP, align with previous work in almond flour and peanuts, indicating that proteolysis disrupts these structures (Dias and de Moura Bell, 2022; Li et al., 2016a).

### 3.9. Surface hydrophobicity and zeta-potential of almond butter milk proteins

Roast intensity had a significant effect on the $H_0$ of extracted proteins in AEP-extracted milks (Fig. 5A). Overall milk protein hydrophobicity decreased as roast intensity increased, indicating that protein denaturation and aggregation induced by roasting led to conformational changes that hid the protein's hydrophobic sites. Roasting has been shown to reduce $H_0$ of proteins in the presence of carbohydrates, leading to hydrophilic sugar molecules forming aggregates and polymers with hydrophobic amino acids, thus reducing detectable $H_0$ (Hiller and Lorenzen, 2008). These results were similar to those of Rao et al. (2016) in which raw peanut protein exhibited higher surface hydrophobicity than that of roasted peanut protein isolate, which they attributed to intermolecular association of the protein during thermal treatment hiding these hydrophobic amino acids. Interestingly, Li et al. (2016b) inferred from confocal scanning microscopy that roasting led to protein unfolding and exposure of hydrophobic amino acid groups in AEP-extracted peanut insolubles due to their increased oil-holding capacity. The interaction between these exposed proteins can lead to aggregation, reducing hydrophobicity (Jung et al., 2005; Yang et al., 2024). The proteins extracted in the milk likely differ from those remaining in the insoluble fraction, which could explain this discrepancy.

The $H_0$ of proteins in EAEP unroasted and light roasted milks was lower than their AEP milks, but the $H_0$ of the EAEP DB milk was higher than that of AEP DB milk. Previous work in almond flour and almond oil press cake has shown a similar trend in AEP versus EAEP protein $H_0$ (de Souza et al., 2020; Dias and de Moura Bell, 2022). Proteolytic enzymes can cleave the extracted proteins and expose sites that otherwise would be hidden by roasting as in the AEP. The higher $H_0$ of the EAEP DB milk compared with the EAEP LB milk highlights the extensive conformational changes induced by the more intense roasting, which may have exposed sites that favored proteolysis, thereby increasing the exposure of hydrophobic sites (Jung et al., 2005) compared with other EAEP.

This is corroborated by the secondary structure results described in Table 3, specifically the increase in unordered structures observed for the EAEP DB milk. Such changes in secondary structure have been correlated with variations in $H_0$ (Hemung et al., 2008; Rahaman et al., 2016; Yan et al., 2021).

The overall surface charge of all butter milk proteins was negative at pH 7.0 (Fig. 5B), which is expected, as the isoelectric point of most almond proteins is 5.0 (Kshirsagar et al., 2011). The surface charge of all EAEP milk proteins exhibited higher absolute values (more highly negative) than those of AEP milk proteins, regardless of roast. This increase of net charge after proteolysis is consistent with previous work with EAEP almond proteins (de Souza et al., 2020; Dias and de Moura

**Fig. 5.** Impact of roasting and extraction methods on (A) surface hydrophobicity and (B) zeta-potential (mV) of defatted proteins at pH 7.0. AEP = aqueous extraction process, EAEP = enzyme-assisted aqueous extraction process, UB = unroasted butter, LB = light roasted butter, DB = dark roasted butter. Different letters within each graph indicate significant difference of means by one-way ANOVA followed by Tukey's test ($p < 0.05$).

Bell, 2022) and highlights the role of enzyme in protein surface properties, which could affect functionality (e.g., solubility, foaming, emulsification). The higher negative zeta potential of the EAEP milk proteins indicates stronger electrostatic repulsive forces between protein molecules, likely due to the exposure of ionizable groups (e.g., carboxyl and amino groups). Additionally, the exposure of hydrophobic regions upon hydrolysis, could lead to the reorientation of the hydrolyzed fragments, resulting in the subsequent exposure of previously hidden ionizable groups (Dias et al., 2020; Dias and de Moura Bell, 2022).

## 4. Conclusion

This study demonstrated the impact of different roasting and grinding degrees on the extraction of almond proteins and lipids, as well as on the nutritional and physicochemical characteristics of almond milks produced by the aqueous and enzyme-assisted aqueous extraction processes. Particle size reduction significantly improved extractability, while roasting had a variable effect. Higher roasting intensities reduced oil and protein extraction in the AEP, likely due to protein denaturation and aggregation, despite potential benefits to flavor and grindability. However, this limitation was overcome in the EAEP by using an alkaline endoprotease, achieving comparable extraction yields (86.76–91.76 %) for butter and paste grinds across all roast levels. Confocal laser scanning microscopy revealed that roasting led to bursting and coalescence of oil bodies, increased cellular disruption, and protein aggregation. Conversely, increased grinding consisted enhanced extractability. Butter was selected as the starting material for milk production due to its reduced energy requirement and high extractability. The resulting almond milks (UB, LB, and DB) had protein and lipid contents ranging from 1.51–1.91 % to 4.61–5.42 %, respectively, surpassing the nutritional profile of most commercial almond milks. This highlights the potential for further processing optimization to increase nutrient density. Although the secondary structure of the AEP milk proteins was unaffected by roast, the combined impact of roasting and proteolysis resulted in fewer α-helix and β-sheet structures and a higher proportion of unordered structures in the DB milk proteins, which could affect their stability and functionality. Increased roast intensity decreased $H_0$ in AEP proteins due to protein unfolding and exposure of hydrophilic sites that enhanced protein-water interactions. Proteolysis also reduced protein $H_0$, except for DB milk proteins, where denaturation followed by proteolysis likely enhanced exposure of hydrophobic sites. Similarly, EAEP proteins exhibited higher absolute ζ-potential compared to AEP, likely due to increased hydrolysis and subsequent exposure of additional ionizable groups. Although proteolysis led to a small increase in the *in vitro* digestibility of LB milk proteins, digestibility was high across all samples.

Overall, this study demonstrated that roasting and proteolysis significantly influenced protein properties as well as the final almond milk composition. Considering the reduced energy requirements, similar extraction yields, and aroma and flavor benefits due to roasting, the selection of light roast butter as the starting material for producing almond milks with enhanced nutritional properties warrants further processing optimization. These findings provide valuable insights for optimizing extraction parameters to improve almond's milk nutritional profile, stability, and consumer acceptance, highlighting its potential for commercial applications.

## Ethics in publishing statement

I testify on behalf of all co-authors that our article submitted followed ethical principles in publishing.

Title: From solid dispersion to the simultaneous extraction of lipids and proteins: a bio-guided strategy to improve the nutritional and biological properties of almond milk

All authors agree that:

This research presents an accurate account of the work performed, all data presented are accurate and methodologies detailed enough to permit others to replicate the work.

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All authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

## CRediT authorship contribution statement

**Jessica Ruiz Hallstrom:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Fernanda Furlan Gonçalves Dias:** Writing – review & editing, Methodology. **Jasmin S. Yang:** Writing – review & editing, Methodology, Formal

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## 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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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100681.

## Data availability

Data will be made available on request.

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