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Impact of lipids on the functional, rheological, pasting and thermal properties of ultrasound-processed canary seed flours

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ABSTRACT

Canary seed, a gluten-free cereal, is renowned for its high content of fats, starch, and proteins. The extraction of oil is often necessary to extend flour shelf life or obtain purified starch and protein fractions. Similar to many gluten-free flours, it may require additional thermal treatments to enhance its suitability to formulate several food products. Thus, this study investigated the impact of ultrasound (US) treatment on the techno-functional and physicochemical properties of both whole (WCS) and defatted (DWCS) canary seed flours. The US treatment slightly increased water and oil absorption capacities in WCS. Lipids in the flour and treatment temperature affected the surfactant properties. Untreated and treated defatted DWCS flours exhibited higher emulsifying activity (up to +36%) and foaming capacity (up to +340%) than WCS. The presence of lipids and US treatment influenced protein extraction, with changes in albumins (WCS and DWCS), globulins (DWCS) and prolamins (WCS) recovery. US treatment altered pasting and rheological properties of all studied flours, impacting viscosity and starch-lipid complex formation. Rheological properties were lipid-dependent, and defatted samples formed stronger gels. US treatment decreased gelatinization enthalpy in WCS (up to −9.5%) and increased it in DWCS (up to +11.3%) flours. Moreover, lipids significantly influenced retrogradation, which was absent in untreated and treated WCS flours. US-treated flours displayed similar diffraction patterns to untreated samples, but with reduced intensities as temperature increased. The untreated defatted sample exhibited a 9% lower crystallinity. These findings highlight the benefits of US treatment and defatting for improving gluten-free food products formulated with canary seed flour.

1. Introduction

Canary seed (*Phalaris canariensis* L.), belonging to the family Poaceae, sub-family Pooideae, is a cereal grain crop The grain is cultivated in many regions with temperate climates, with Canada being the foremost producer of canary seed, accounting for 60% of global production and holding the position of the primary exporter with over 75% market share (Estrada-Salas, Montero-Morán, Martínez-Cuevas, González, & Barba de la Rosa, 2014; Mason, L'Hocine, Achouri, Pitre, & Karboune, 2020). Traditionally, canary seed has only been used as bird feed due to the presence of siliceous hairs that cover the seed hull, which are considered harmful to human health (Abdel-Aal, Hucl, Patterson, & Gray, 2010). However, through mutagenesis and breeding techniques, safe varieties have been developed for human consumption (Mason, L'Hocine, Achouri, & Karboune, 2018). In 2016, canary seed received novel food approval from Health Canada, indicating that it was recognized as safe for human consumption (Canada, 2016). Additionally, it has been granted GRAS (Generally Recognized as Safe) status by the U.S. Food and Drug Administration, further affirming its safety for consumption (Achouri, et al., 2020).

Canary seed flour is a rich source of essential nutrients, including proteins (21–23%), dietary fiber (5.9%) and ash (2.3%) (Chen, Yu, Wang, Gu, & Beta, 2016). The protein from canary seed flour is considered of excellent quality, containing substantial amount of the essential amino acid tryptophan (4.6 g/100g protein) (Abdel-Aal, 2021). Moreover, the flour is renowned for its high antioxidant properties, which can be attributed to a rich content of bioactive compounds such as peptides, phenolic compounds, flavonoids, and phytosterols (Abdel-Aal, 2021). These compounds play an important role in reducing oxidative stress and inflammation, linked to the development of chronic diseases

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like cancer (Olaiya, Soetan, & Esan, 2016). Additionally, some studies indicate that canary seed flour may have cholesterol-lowering effects, which can be beneficial in managing and preventing cardiovascular diseases (Mason, et al., 2018; Mason et al., 2020; Urbizo-Reyes, et al., 2022). Canary seed flours also has shown promising results in regulating blood sugar levels (Estrada-Salas, et al., 2014), making it a potential dietary option for individuals with diabetes or those at risk of developing the condition. An additional advantage of canary seed flour is that it is gluten-free, making it an excellent nutritional source for individuals adhering to a gluten-free diet (Achouri, et al., 2020).

The content of oil and starch in canary seed is reported to be 7.7–9% and 57–67%, respectively (Abdel-Aal, 2021). The high oil content can lead to poor storage properties and restrict its application due to the risk of lipid oxidation. To mitigate this issue and improve the shelf life of canary seed-based products, thermal and hydrothermal treatments can be applied, along with the removal of canary seed oil. The abundant starch in canary seed flour makes it an excellent raw material to produce versatile products like bread, cakes, pasta, flakes, and snacks, influencing factors such as thickness, creaminess, and viscosity. Both lipids and starch can interact during food processing, impacting the quality attributes of final food products (Cai, et al., 2021). This is mainly because the starch-lipid complex affects the crystalline structure, thermal properties, and gelatinization behavior of starch, ultimately influencing the nutritional, functional, digestibility, texture, and sensory attributes of cereal-based foods (Cai, et al., 2021; Eliasson, 1994; Lu et al., 2023; Tapia-Blácido, Mauri, Menegalli, Sobral, & Añón, 2007). The interaction between starch and lipids is a well-known phenomenon, but different types of complexes may form depending on the chosen food processing technology. One non-thermal food processing technology that has gained significant attention in recent years is ultrasound (US), as it has the potential to enhance mass and energy transfer processes (Astráin-Redín, Alejandre, Raso, Cebrián, & Álvarez, 2021; Náthia-Neves, Santana, Viganó, Martínez, & Meireles, 2021). Additionally, US is considered part of the "Green Food Processing" concept, as defined by Chemat et al. (2017), which aims to promote more sustainable and environmentally friendly food processing methods by reducing energy and water consumption. The use of US technology has emerged as a promising approach to enhance bread making performance by modifying the physical and biochemical properties of flours (Vela, Villanueva, & Ronda, 2021, 2023; Vela, Villanueva, Náthia-Neves, & Ronda, 2023). These modifications include influencing hydration and surfactant properties, rheological properties, starch gelatinization, crystallinity and protein structure and functionality. The US application also affects the particle size distribution, resulting in improved texture and flowability of gluten-free flours, thereby enhancing dough handling properties and final product quality (Vela, Villanueva, Náthia-Neves, & Ronda, 2023). Operating beyond audible frequencies (above 20 kHz), US generates compressions, rarefactions, and turbulence (Bhargava, Mor, Kumar, & Sharanagat, 2021). In liquid media, US induces acoustic cavitation, leading to microbubble rupture, high-pressure conditions, and microturbulence (Bhargava, et al., 2021; Kumar et al., 2023). As sonic waves pass through liquid medium, usually water, they create oscillations that generate air/vapor bubbles due to reduced surface tension (Kumar, et al., 2023). Within flours, US-induced cavitation produces expanding bubbles that subsequently collapse, resulting in high shear forces, erosion, and material modification. The extent of modification depends on factors such as treatment time, temperature, frequency, solid-liquid ratio, and botanical source (Vela, Villanueva, & Ronda, 2021).

To date, no studies evaluating the effect of lipids on the processing of canary seed flours have been found in the available literature. However, given the increasing demand for nutritious gluten-free products, there is a natural tendency for the consumption of this flour to rise in the coming years. Understanding the interactions between lipids and starch in cereal grains is crucial, as they can significantly impact the physicochemical properties of processed cereal-based goods.

Therefore, the aim of this study was to investigate the influence of lipids on the US-assisted processing of canary seed flour. The main focus was on evaluating its influence on functional properties, protein fractions and their molecular weight distribution, thermal properties, crystallinity, pasting characteristics and the rheological behavior of gels made with sonicated flours. We believe that the knowledge generated through this research will offer valuable guidance for the development of novel food processing approaches and formulations, aimed at enhancing the nutritional value and functional characteristics of cereal-based products. By examining the impact of lipids on the processing of canary seed flour, we aim to provide valuable insights into the potential incorporation of this raw material into bakery products, which will meet the demands of health-conscious consumers seeking gluten-free options with enhanced nutritional benefits.

2. Material and methods

2.1. Raw material preparation and characterization

The hulled canary seed kernels were provided by the Fitopal Company (Palencia, Spain) and were dehulled and ground in a Chopin CD1Laboratory Mill (Chopin, France) to obtain whole canary seed flour (WCS), which consisted as the first raw material to be investigated in this study. To obtain the second raw material, the ground WCS was defatted twice with hexane (1:6 wt/vol.) for 20 h each time (resulting in a total extraction time of 40 h) using a shaker equipment at 30 °C and 225 rpm. The resulting defatted WCS was sieved to ≤500 μm and named by DWCS. Both flours (WCS and DWCS) were stored at - 18 °C until further use.

The chemical composition of WCS and DWCS flours was characterized, and the results, on a dry basis (d.b.), is presented in Table 1. Moisture content was determined using the method 931.04 (AOAC, 1997); lipids were analyzed using the method 963.15 (AOAC, 1997); proteins (N x 6.25) were measured by elemental analysis (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 900 °C, using pure oxygen (250 mL/min) as the combustion gas and pure helium as the carrier gas (140 mL/min); ash content was determined by the method 972.15 (AOAC, 1997); apparent amylose content and total starch were assessed using the assay kit K-AMYL from Megazyme (Megazyme Bray, Ireland).

2.2. Ultrasound (US) treatment

WCS and DWCS were treated with a UP400St system (Hielscher Ultrasonics, Germany) equipped with S24d22D titanium tip operating at constant frequency of 24 kHz with a maximum output power of 180W. The 22 mm diameter probe was submerged to a depth of 2 cm below the surface of the flour suspensions. The ultrasonic energy was applied at 100% amplitude with an 80% on/off pulse (0.8 s on/0.2 s off) to a water-flour dispersion (345g) at a ratio of 1:7 (flour:water, w/w) for 40 min, and the effect temperature (20, 30, and 40 °C) was evaluated during the sonication process. These temperature conditions were determined from DSC assays based on the peak left limit, established from the first temperature where the signal of the of gelatinization peak started to separate from the baseline in the untreated samples (50.13 °C for WCS and 46.33 °C for DWCS), while the sonication time and the flour

Table 1
Chemical composition (% w/w) of the WCS and DWCS flours.

Parameters	WCS	DWCS
Moisture (%)	12.3 ± 0.3	10.80 ± 0.01
Lipids (%)[a]	6.43 ± 0.04	0.23 ± 0.01
Proteins (%)[a]	15.2 ± 0.5	19.9 ± 0.2
Ash (%)[a]	2.5 ± 0.1	2.6 ± 0.1
Apparent amylose (%)[a]	23 ± 1	23.61 ± 0.03
Total starch (%)[a]	76 ± 3%	78 ± 3%

[a] Results expressed on a dry basis.

concentration were selected based on preliminary experiments and previous US treatments conducted by our research group (Vela, Villanueva, & Ronda, 2021; Vela, Villanueva, Solaesa, & Ronda, 2021). The studied temperatures were maintained at a constant level during treatments by using recirculating water from a LAUDA RA12 water bath (LAUDA, Lauda-Königshofen, Germany). All experiments were carried out in duplicate. Temperature was continuously monitored using an RS-1316 dual data logger thermometer (RS-1316, Radionics, Dublin, Ireland), and the data were recorded using DL Thermo® software. The temperature profiles of all experiments are presented in the Supplementary Material (Fig. S1). After sonication, the mixture of canary seed flour and water was lyophilized using a freeze dryer (SP Industries Inc, Warminster, USA). All the extraction conditions applied in this study as well as the nomenclature used throughout the text are summarized in Table 2.

2.3. Functional properties

The functional properties of untreated and treated WCS and DWCS flours were determined following protocols proposed by Abebe, Collar, and Ronda (2015).

For water and oil absorption capacity (WAC and OAC), 2 g (d.b.) of sample was stirred into 20 mL of distilled water or refined corn oil for 30 s, stirring 3 times, with a rest of 10 min after each stirring. The samples were then centrifuged at 3000×*g* for 30 min at 25 °C, and the released water or oil was drained. WAC and OAC was expressed as grams (g) of water or oil held per 100 g of flour.

To determine water absorption index (WAI), water solubility index (WSI), and swelling power (SP), the aqueous dispersions (1% w/v d.b.) of the flour were homogenized in a vortex (30 s) and then cooked in boiling water for 15 min. After cooling down to room temperature, the dispersions were centrifuged at 3000×*g* for 10 min. The supernatant was collected for WSI determination (expressed as g of dissolved solids in supernatant/100 g of flour d.b.), and the sediment was weighed to calculate WAI (g of sediment/g of flour d.b) and SP (g of sediment/g of insoluble solids in flour d.b.).

For foaming capacity, aqueous dispersions (5% w/v d.b.) of the flours were manually stirred for 5 min to produce foam. Foaming capacity was calculated by measuring the increase in volume (in mL) within the flour dispersion. The foam stability (FS) was determined by measuring the foam volume after 60 min and expressed as percentage of initial foam volume.

Emulsifying activity (EA) and emulsifying stability (ES) were determined by mixing the flours (7g) with distilled water (100 mL) and corn oil (100 mL). The mixture was homogenized using an Ultra-Turrax T25 homogeniser (IKA, Staufen, Germany) at 1000 rpm for 1 min and then centrifuged at 1300×*g* for 5 min. EA was calculated as the ratio of the volume of the emulsified layer to the total initial volume, and expressed as a percentage. ES was determined after heating the emulsion at 80 °C for 30 min in a water-bath, then cooled down to room temperature and centrifuged at 1300×*g* for 5 min. ES was expressed as the ratio of the emulsified layer to the total initial volume, and expressed as a percentage. All functional properties were measured in triplicate.

2.4. Protein fractionation, quantification and characterization

Before fractionation, all untreated and treated samples were defatted with hexane according to procedure described in section 2.1. The protein fractionation of all studied flours was carried out according to the method described by Osborne (1924), with modifications proposed by Náthia-Neves, Calix-Rivera, Villanueva, and Ronda (2023). A mixture of flour and the corresponding solvent (1:20 ratio, w:v) was vortexed vigorously in an Eppendorf tube. The albumins, globulins, prolamins and glutelins fractions were then extracted from the flours using distillated water, 50 mM Tris HCl (pH 7.8), 50% aqueous 1-propanol and 50% aqueous 1-propanol containing 1% of DTT (Dithiothreitol), respectively. After the fractionation, each protein fraction was quantified using the Bradford (1976) method with bovine serum albumin standard (43–426 μg/mL; $R^2 = 0.992$) in water, to evaluate albumins and globulins, and wheat gliadins standard in 50% aqueous 1-propanol (102–810 μg/mL; $R^2 = 0.997$) to evaluate prolamins and gliadins. The protein content of each fraction extracted from the flours was expressed as mg of protein fraction/g of flour. All flours were analyzed by SDS-PAGE according to Laemmli (1970) with some modifications. Prior to SDS-PAGE analysis, the samples containing lipids were defatted with hexane, following the procedure described above. The protein fractions were run in 12% separating gel and 5% stacking gel. The same amount of protein (45 μg) was analyzed under reducing conditions and the complete protocol used for sample preparation, running conditions, protein bands staining and distaining can be found in our previous study (Náthia-Neves, et al., 2023).

2.5. Pasting properties

The pasting properties of the gels made from all the studied canary seed flours were measured using a Rapid Visco Analyzer (RVA-4500, PerkinElmer, Australia) following the AACC (Intl, 1999) Standard 1 protocol. Briefly, an aqueous dispersion of flour at 14% moisture basis (3g of flour into 25 g water) was equilibrated at 50 °C for 1 min and heated to 95 °C at a heating ratio of 12.2 °C/min. The samples were held at 95 °C for 2.5 min and then cooled down to 50 °C at cooling rate of 11.8 °C/min. Pasting temperature (PT), peak viscosity 1 (PV1) obtained in the heating zone, peak time (Pt), final viscosity (FV), breakdown viscosity (BV), peak viscosity 2 (PV2) obtained in the cooling zone, and setback viscosity (SV) were recorded from the pasting curves. All samples were evaluated in triplicate.

2.6. Rheological properties

The rheological properties of the gels made from all the studied canary seed flours were determined by using a Kinexus Pro + rheometer (Malvern Instruments Ltd., Malvern, UK) with a serrated parallel plate geometry (40 mm diameter, 1 mm gap). Gelatinized WCS and DWCS gels were prepared following the same program as described in the

Table 2
Ultrasound treatment conditions and samples identification.

Samples Identification	Lipid Content (%)	Flour/water (w/w)	Probe diameter (mm)	Temperature (°C)	Time (min)	Amplitude (%)	On/Off (%)
WCS (untreated)	6.43	–	–	–	–	–	–
WCS-20	6.43	1/7	22	20	40	100	80
WCS-30	6.43	1/7	22	30	40	100	80
WCS-40	6.43	1/7	22	40	40	100	80
DWCS (untreated)	0.23	–	–	–	–	–	–
DWCS-20	0.23	1/7	22	20	40	100	80
DWCS-30	0.23	1/7	22	30	40	100	80
DWCS-40	0.23	1/7	22	40	40	100	80

WCS refers to whole canary seed flours with their natural lipid content, and DWCS refers to defatted canary seed flours with reduced lipid content.

pasting properties' analysis (Section 2.5). After the formation of the gels, the samples were cooled to 25 °C and held for 5 min on the plate to allow relaxation before rheology tests. Strain sweeps were performed from 0.1 to 1000% strain at a constant frequency of 1 Hz to determine the linear viscoelastic region (LVR). Frequency sweep tests were performed from 10 to 1 Hz at 1% and 25 °C within the LVR and the data were fitted to the power law model described by Ronda, Villanueva, and Collar (2014). All samples were analyzed in triplicate.

2.7. *Differential scanning calorimetry (DSC)*

Thermal properties of all samples were analyzed using a differential scanning calorimeter (DSC3, STARe-System, Mettler-Toledo, Switzerland) equipped with a thermal analysis data station. 6 mg of flour were weighed into a 40 μl capacity aluminium pan, and 15 μl of distilled water was added with a micropipette (Eppendorf® Research® Plus Pipettes, Hamburg, Germany). Before heating, the pans were hermetically sealed and kept at room temperature for 30 min to allow moisture equilibration. The samples were then heated from 0 to 110 °C at a rate of 5 °C/min, using an empty sealed aluminum pan as the reference. Thermal parameters such the onset (T_O), peak (T_P), and endset (T_E) temperatures and the enthalpies of gelatinization and amylose-lipid dissociation (ΔH) (J/g flour d.b.) were recorded from the DSC curves. After heating (gelatinized), the pans were refrigerated at 4 ± 2 °C for 7 days and subsequently analyzed again by DSC using the same procedure to determine their retrogradation properties. All samples were analyzed in triplicate.

2.8. *X-ray diffraction (XRD)*

The diffractograms of all untreated and treated flours were collected using a Bruker-D8-Discover-A25 diffractometer (Bruker AXS, Rheinfelden, Germany) under the conditions of CuKα radiation at 40 kV, 30 mA, with the scanning angle 2θ from 5° to 40° at a scanning rate of 1.2°/min. The relative crystallinity was quantitatively estimated based on the relationship between the total peaks area and the reduced peaks area assigned to the crystalline part of the sample and was expressed as a percentage.

2.9. *Statistical analysis*

All data reported are averages and standard deviation of the observations. Analysis of variance (ANOVA) and Fisher test was applied to determine the differences ($p < 0.05$) between means using the commercial Minitab v.16® software (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. *Functional properties*

The functional properties of untreated and treated WCS and DWCS flours are presented in Table 3. The hydration properties refer to flour's capacity to associate with water, being influenced mainly by hydrophilic parts in carbohydrates and proteins. No significant difference was found in WAC and OAC values among the untreated flours, eliminating the matrix effect on this property. However, the US treatment led to an increase in the WAC (up to +16%) and OAC (up to +110%) of all treated samples. Higher WAC values are desirable flour characteristic as this property enables the retention of texture, nutrients, and bioactive compounds by preventing liquid loss during food manufacturing. Higher WAC determined in both WCS and DWCS treated samples could be due to starch structure modification as well as changes in proteins structure (Kumar, Sharma, & Singh, 2017). Moreover, these results might be attributed to disrupted hydrogen bonds between amorphous and crystalline regions and slight expansion of the amorphous region, improving the hydrophilic tendency of starch molecules (Vela, Villanueva, & Ronda, 2021). The observed increase in the OAC indicates that US treatment may induce changes in proteins, the hydrophobic domains of macromolecules, and the amount of polar amino acids (Batariuc, Coţovanu, & Mironeasa, 2023). The WAI, WSI, and SP properties were more influenced by the composition of the studied flours rather than the employed US treatment conditions. In general, the presence of lipids led to a decrease in these properties, with the most pronounced decrease observed in the WSI property.

Regarding the surfactant properties, which depend on the proteins as well as other components like carbohydrates, a significant effect of the presence of lipids in the flours and the interaction between flour lipid content and treatment temperature was observed on EA, ES, and FC properties. The treatment temperature was also found to have an impact on ES and FC properties, while FS was affected only by the presence of lipids in the flour. The EA represents the proteins' capacity to quickly adsorb at the oil/water interface during emulsion formation, while ES indicates the proteins' ability to sustain a stable emulsion. FC is associated with the proteins' capability to rapidly diffuse to the interface air/water, reorient themselves, and create a viscous film without excessive aggregation or coagulation. On the other hand, foaming stability is impacted by the intermolecular cohesiveness and viscosity of the

Table 3

Functional properties of untreated and treated WCS (whole canary seed flour) and DWCS (defatted canary seed flour) flours.

Samples	WAC (g/g)	OAC (g/g)	WAI (g/g)	WSI (g/100g)	SP (g/g)	EA (g/100g)	ES (g/100g)	FC (mL)	FS (g/100g)
WCS (untreated)	0.73^{d}	1.04^{e}	11.6^{de}	3.4^{c}	11.9^{cd}	38^{f}	21^{a}	7.2^{cd}	62^{bc}
WCS-20	0.83^{b}	2.18^{a}	$10.8\ ^{e}$	2.2^{d}	11.08^{d}	38^{f}	ND	8.0^{c}	81^{a}
WCS-30	0.79^{c}	2.09^{bc}	12.5^{bcd}	2.6^{d}	12.9^{bc}	39^{ef}	ND	7.0^{cd}	61^{bc}
WCS-40	0.84^{ab}	2.12^{abc}	12.1^{cd}	2.5^{d}	12.4^{c}	43^{de}	ND	6.2^{d}	67^{b}
DWCS (untreated)	0.74^{d}	1.03^{e}	11.6^{de}	5.2^{b}	12.2^{c}	48^{bc}	10^{cd}	23.0^{b}	52^{c}
DWCS-20	0.84^{ab}	2.06^{cd}	13.4^{ab}	5.0^{b}	14.2^{a}	51^{ab}	16^{b}	24.0^{b}	60^{c}
DWCS-30	0.86^{a}	1.99^{d}	12.8^{abc}	6.1^{a}	13.6^{ab}	53^{a}	12^{c}	27.0^{a}	61^{bc}
DWCS-40	0.85^{ab}	2.18^{ab}	$13.6\ ^{a}$	5.2^{b}	14.3^{a}	46^{cd}	9^{d}	27.5^{a}	64^{bc}
SE	0.01	0.04	0.4	0.3	0.4	2	1	0.6	6
Analysis of variance and significance (p-values)									
Flour (F1)	**	ns	***	***	***	***	***	***	*
Temperature (F2)	ns	*	ns	*	ns	ns	**	**	*
F1 x F2	*	*	**	ns	*	**	**	***	ns

WAC: water absorption capacity, OAC: oil absorption capacity, WAI: water absorption index, WSI: water solubility index, SP: swelling power, EA: emulsion activity, ES: emulsion stability, FC: foaming capacity and FS: foaming stability. All values refer to sample dry matter. The different letters in the same column indicate statistically significant differences between means at $p < 0.05$.

F1: Flour lipid content; F2: Temperature (°C); * for $p < 0{,}05$; ** for $p < 0{,}01$; *** for $p < 0{,}001$; ns: not significant; ND: not detectable.

interfacial film, along with a specific level of elasticity that allows localized contact deformation (Shevkani, Singh, Kaur, & Rana, 2014).

Untreated and treated DWCS exhibited a significant increase in both EA and FC compared to their WCS counterparts. This may be attributed to the higher protein content in DWCS (19.9%) as compared to the counterpart WCS flour (15.2%). In addition, the absence of lipids in the flours treated by US was found to enhance both EA and FC properties. As presented in Table 3, the EA increased up to 36% (30 °C) and FC increased more than 340% in the defatted flours compared to the WCS flours. Furthermore, the US treatment, combined with the presence of lipids, negatively affected the ES of WCS flours. As shown in Table 3, no ES was found in these flours after the US treatment, indicating that the treatment may have altered the native state of the proteins, leading to the loss of this property (T. Wang et al., 2022). Additionally, the cavitation generated during the treatment may lead to coalescence of lipid droplets, affecting the ES (Gao & Meng, 2022). Samples with more lipids may have a more fragile and less stable interfacial layer (Szterk, Roszko, & Górnicka, 2013), and the additional modifications caused by US may negatively impact the ES. In contrast, defatted treated samples maintained or, in some cases, increased the ES (samples treated at 20 °C).

Interestingly, the data suggested that untreated and treated WCS flours were not as effective in forming foams compared to their respective defatted ones, although they showed good stability once formed. Only the WCS flour treated at 20 °C presented significant improvements in FS (with a 30% increase), while all treated DWCS samples showed significant improvements in this property, ranging from a 15% to a 23% increase. These findings indicate that the applied US conditions might have increased viscosity, which, in turn, imparted rigidity to the interfacial film for foam stabilization (Shevkani, et al., 2014). Moreover, conformational changes in proteins induced by unfolding and subsequent interactions, which seemed to favor the FS, could have occurred during US treatments. The increase in surfactant properties in defatted samples was also reported by Damodaran and Parkin (2017) and Galves et al. (2019), who suggested that the defatting process may lead to the solubilization of non-polar side chains of amino acid residues, weakening the hydrophobic interactions in the protein.

3.2. *Protein fractionation and characterization by SDS-PAGE*

As discussed earlier, proteins play a crucial role in the functional properties of flours. Therefore, to investigate the effects of treatment conditions and the presence of lipids in the flour during treatment on the proteins, we performed protein fractionation (Fig. 1a and b) using the Osborne solubility method and evaluated the protein structure via SDS-PAGE (Fig. 1c). All analyzed flours in this section were previously defatted with hexane (see Section 2.1).

The presence of lipids in the flour had a significant effect on all protein fractions. Furthermore, both temperature and the interaction between the presence of lipids and temperature showed a significant influence on the extraction of albumins, prolamins, total protein, and protein recovery (see Table S1). According to literature, the main storage protein fraction in canary seed flour is prolamins, followed by the glutelins (Abdel-Aal, 2021; Perera et al., 2022), which is in agreement with the findings in this study. The content of prolamins and glutelins is widely recognized as a crucial factor influencing the techno-functionality of protein ingredients. For instance, in the case of wheat, it is well-established that glutelins contribute to the dough's elastic properties, while the alcohol-soluble fraction of prolamins mainly influences the dough's viscous properties (Achouri, et al., 2020). The proteins from canary seed flour were efficiently extracted using the

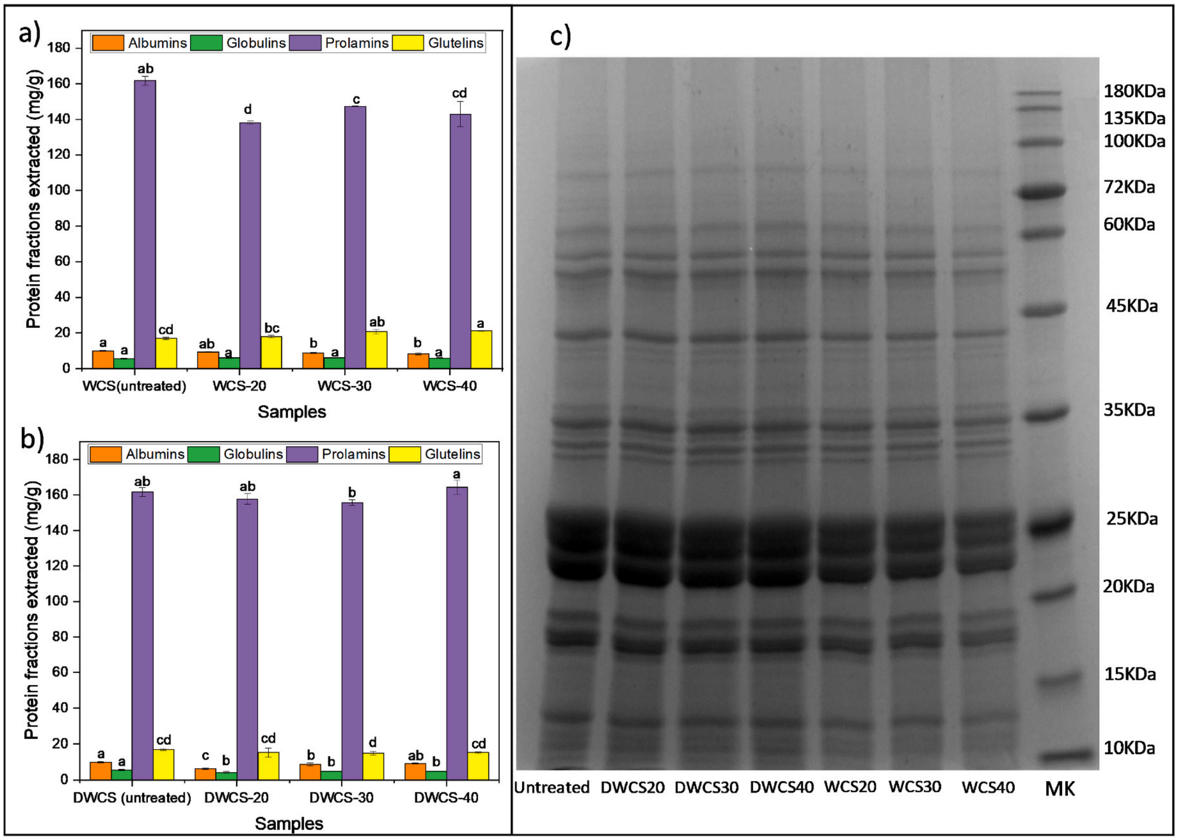

Fig. 1. Protein fractions of untreated and treated flours: a) whole canary seed flours; b) defatted whole canary seed flours, and c) SDS-PAGE under reduced conditions from all untreated and treated flours. WCS refers to whole canary seed flours with their natural lipid content. DWCS refers to defatted canary seed flours with reduced lipid content. MK stands for molecular weight marker. The different letters in both representations (a) and (b) for the same protein fraction indicate statistically significant differences between the means of the studied flours at $p < 0.05$.

employed fractionation method, as a substantial amount of protein was recovered from the tested samples (86–97%, Table S1). The US treatment led to a significant reduction in the content of albumins in WCS-30 (12%), WCS-40 (16%), DWCS-20 (37%), and DWCS-30 (12%) samples. This finding is consistent with Náthia-Neves, et al. (2023), Nugdallah and El Tinay (1997) and Sashikala, Sreerama, Pratape, and Narasimha (2015), who indicated that the albumin fractions of tef, cowpea and green gram seeds, respectively, are the most heat-sensitive protein fraction. The content of globulins was only affected by the US treatment in defatted samples (DWCS-20, DWCS-30, and DWCS-40), clearly indicating that the flour composition significantly influenced the extraction of this protein fraction after US treatment. The prolamins recovered from treated WCS were significantly lower (up to - 15%, 20 °C) compared to those extracted from the untreated WCS. The temperature of the US treatment did not affect to this fraction. Interestingly, the reduction in the prolamins fraction was accompanied by an increase in the glutelins fractions in the WCS flours, rising from 18 mg/g (at 20 °C) to 21 mg/g (at 30 °C and 40 °C). This suggests that non-polar lipids in the samples were likely associated with the prolamins fraction, causing changes in their solubility (McCann, Small, Batey, Wrigley, & Day, 2009). Furthermore, the additional modifications caused by US may contribute even more to the loss in solubility of this protein fraction, which could be solubilized further in the reducer agent used for glutelins extraction. In contrast, no significant difference was observed in the prolamins and glutelins extracted from treated defatted flours.

The polypeptide molecular weight profiles of all studied flours, both untreated and treated WCS and DWCS flours, did not exhibit significant differences, ranging from 10 to 100 kDa (Fig. 1c). These profiles showed major polypeptide bands representative of low molecular weight (LMW) globulins and glutelins (10–18 kDa) and high molecular weight (HMW) globulins and glutelins (30–100 kDa). Additionally, a highly dense protein blot corresponding to prolamins proteins can be observed in all studied flours at the ~18–25 kDa region. Similar profiles were previously reported by Perera et al. (2022) and Achouri et al. (2020). The intensity of the bands in the sonicated WCS flours exhibited a slight decrease, corroborating the earlier discussion that the presence of lipids in the flours, during US treatment, altered the content of the distinct Osborne fractions. To the best of our knowledge, there is no study addressing the effect of the lipid content of cereals on protein fractions submitted to US treatment.

The hexane-based oil extraction process may induce structural alterations in proteins by impacting hydrophobic interactions, hydrogen bonding, and electrostatic interactions, potentially modifying their inherent functional properties (Damodaran et al., 2017; Griebenow & Klibanov, 1996). The use of organic solvents is often associated to protein denaturation, as they solubilize non-polar amino acid side chains, weakening hydrophobic interactions within proteins (Perera, et al., 2022). Perera et al. (2022) investigated the impact of hexane and ethanol on canary seed flour polypeptide patterns using SDS-PAGE, finding no discernible influence of the used solvents on the polypeptide profile. The authors concluded that solvent de-oiling did not denature canary seed protein, suggesting potential improvements or no changes in the functional and nutritional properties. It is important to note that the extraction performed by Perera et al. (2022) was only for 1 h, while in our study, a more prolonged oil extraction using hexane was carried out. In this present study, the combined effects of defatting and US treatment showed a positive impact on protein solubility. Enhanced protein recovery in defatted samples and more pronounced protein bands in SDS-PAGE analyses indicate the effectiveness of hexane solvent in extracting oil from canary seed flour for preparing protein products. Further research is recommended to evaluate possible effects of the hexane oil removal process on the native state of canary seed proteins.

3.3. Pasting properties

The pasting properties of all studied flours are presented in Table 4,

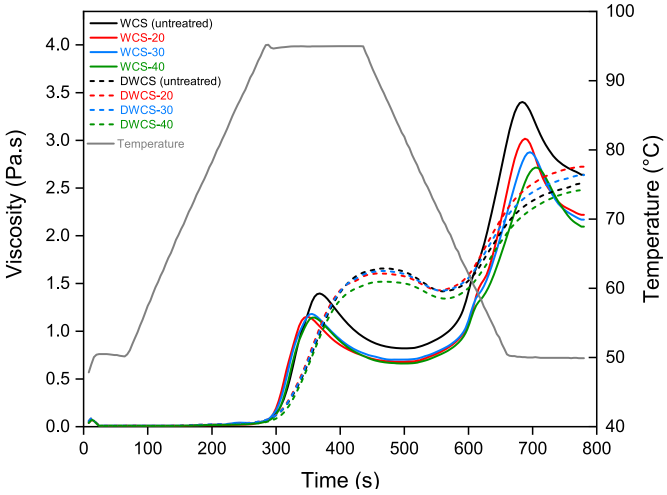

Fig. 2. Pasting profile of the studied flours. WCS refers to whole canary seed and DWCS refers to defatted canary seed flour.

Table 4
Pasting parameters of untreated and treated WCS (whole canary seed flour) and DWCS (defatted canary seed flour) flours.

Samples	PT (°C)	PV1 (Pa·s)	Pt (min)	TV (Pa·s)	BV (Pa·s)	FV (Pa·s)	SV (Pa·s)	PV2 (Pa·s)
WCS (untreated)	94.7^{ab}	1.41^{c}	6.10^{b}	0.82^{bc}	0.58^{ab}	2.6^{a}	1.82^{a}	3.35^{a}
WCS-20	94.9^{ab}	1.15^{d}	5.83^{c}	0.68^{d}	0.47^{d}	2.2^{cd}	1.54^{bc}	2.99^{b}
WCS-30	95.0^{a}	1.20^{d}	5.90^{c}	$0.69b^{cd}$	0.49^{cd}	2.2^{d}	1.47^{cd}	2.88^{c}
WCS-40	94.7^{b}	1.14^{d}	6.03^{b}	0.66^{d}	0.48^{cd}	2.1^{d}	1.43^{d}	2.72^{d}
DWCS (untreated)	94.7^{ab}	1.55^{a}	7.00^{a}	0.91^{ab}	0.64^{a}	2.5^{ab}	1.62^{b}	ND
DWCS-20	94.6^{b}	1.52^{ab}	7.00^{a}	0.97^{a}	0.55^{bc}	2.7^{a}	1.76^{a}	ND
DWCS-30	94.7^{ab}	1.45^{bc}	7.00^{a}	0.94^{ab}	0.51^{cd}	2.4^{bc}	1.47^{cd}	ND
DWCS-40	94.8^{ab}	1.44^{bc}	7.00^{a}	0.90^{ab}	0.56^{bc}	2.5^{ab}	1.63^{b}	ND
SE	0.1	0.04	0.03	0.05	0.03	0.1	0.04	0.03
Analysis of variance and significance (p-values)								
F1	ns	***	***	***	*	***	***	***
F2	ns	ns	*	ns	ns	ns	**	***
F1 x F2	ns	ns	*	ns	ns	ns	**	***

PT: pasting temperature, PV1: peak viscosity 1, Pt: peak time, TV: trough viscosity, BV: breakdown viscosity, FV: final viscosity, SV: setback viscosity, PV2: peak viscosity 2.

The different letters in the same column indicate statistically significant differences between means at $p < 0.05$.

F1: Flour lipid content; F2: Temperature (°C); * for $p < 0,05$; ** for $p < 0,01$; *** for $p < 0,001$; ns: not significant; ND: not detectable.

and the corresponding pasting curves are shown in Fig. 2. The defatting process only significantly changed the PV1, Pt, SV, and PV2 parameters of the untreated flours. On the other hand, the US treatment led to a reduction in all pasting parameters for WCS flours, except for PT, and also resulted in reduced PV1, BV, and SV values in treated DWCS flours. Among these parameters, treatment temperature emerged as a significant factor specifically for the SV and PV2 parameters. A similar decrease in the viscosity profile was reported in ultrasonicated quinoa (Zhu & Li, 2019), tef (Vela, Villanueva, Ozturk, Hamaker, & Ronda, 2023) and rice flours (Vela, Villanueva, Solaesa, & Ronda, 2021). These studies reported that lower PV values indicate a weakened granule structure, which is typically linked to an increased ratio of amylose to amylopectin in starch and a reduction in granule size resulting from the treatment. Lower BV values indicate an improved capacity of the treated samples to withstand stress and heating compared to the untreated samples, while lower SV values indicate reduced amylose retrogradation. It is important to note that these authors applied the US treatment to flours with their original lipid content intact, and no second viscosity peak on the pasting curves was observed. Regarding the PT, the literature reports inconsistent observations in sonicated samples. For Vela, Villanueva, Solaesa, and Ronda (2021), an increase in PT was observed in rice flour samples treated by US, while Yang et al. (2019) found a decrease in PT of rice starch as a results of the US application. In this study, no statistically significant difference was observed, indicating that the impact of US on this property may be compensated by the interactions between lipids, starch, and proteins.

To date, the effect of US on the pasting properties of defatted flours has not been reported in the current literature. However, some studies have described the effects of lipids on the development of paste viscosity when starch is heated in water. These effects are reported to be dependent on various factors, such as the starch source, type of lipid, pasting conditions, and water content of the system (Blazek & Gilbert, 2011; Wang et al., 2020; Zhang, Huang, Luo, & Fu, 2012). The main lipids present in canary seed flour are monounsaturated (280 g/kg) and polyunsaturated fatty acids (546 g/kg) (Abdel-Aal, Hernandez, Rabalski, & Hucl, 2020). According to a review written by Wang et al. (2020), the addition of fatty acids decreased the PV of wheat, maize, and tef starches. The extent of this decrease depended on the chain length and polarity of the fatty acids and was attributed to the formation of starch-lipid complexes, which restricted water uptake and swelling by the granules. From the defatting process performed in this study, it can be concluded that the lipids in canary seed flour are mainly non-polar lipids, as 96% of lipids were removed by using hexane.

The application of US had a significant impact on PV1 in WCS flours, reducing it by up to 19% compared to the untreated sample (Table 4). A similar, trend toward PV1 reduction was observed in DWCS flours as a result of US treatment. This observation may be attributed to the surface morphology of the starches. Previous research has indicated that the pores and channels present on the surface of cereal starches may help mitigate the inhibitory effects of starch-lipid complexes on granule swelling (Cai, et al., 2021). Additionally, the presence of lipids significantly influenced Pt (Table 4). Untreated WCS initiated PV1 earlier than untreated DWCS, as Pt for WCS was 6.1 min, while for DWCS, it was 7 min. The treatment conducted at 20 and 30 °C significantly reduced Pt for WCS flours, but no significant effect of the treatment was observed for DWCS samples.

The FV, which relies heavily on the amylose composition, crystalline structure rigidity, and the ability of swollen amylose to return to its original shape after cooling, was observed to be higher in untreated WCS compared to the treated samples. This difference indicates the inclination of amylose in untreated WCS flour to undergo retrogradation, as it remains in its native state and is consequently more susceptible to this phenomenon (Dongmo, et al., 2020). The decrease in this parameter following the US treatment could be attributed to the rupture of amylose into low molecular weight molecules. The BV of a starch paste is defined as the difference between the peak viscosity and the viscosity after holding for a certain period of time at 95 °C (Lawal, 2004). It serves as an indicator of the starch's sensitivity to changes in viscosity, thereby reflecting its fragility. BV showed slightly higher values in defatted samples compared to WCS ones, which may be attributed to the ability of the lipids to interact with the amyloid, thereby limiting its swelling and reassociation capacity (Dongmo, et al., 2020). Conversely, the reduction of this parameter as a result of the US treatment would be linked to a significant rupture of the amylose following the sonication process (Vela, Villanueva, Li, Hamaker, & Ronda, 2023).

As shown in Table 4 and Fig. 2, the presence of lipids led to the formation of a second viscosity peak (PV2), indicative of the formation of starch-lipid complexes during the setback phase of RVA protocol (Cai, et al., 2021). All WCS flours presented an evident second peak in the cooling phase, which was not observed for DWCS samples. Additionally, the PV2 was found to decrease with increasing treatment temperature. The intensity of the viscosity peak observed during the RVA setback phase, as well as FV, provides valuable insights into both the quality and quantity of starch-lipid complexes formed (Cai, et al., 2021). SV, which is commonly used to reflect the retrogradation of starch paste, indicates the reassociation of amylose molecules released during gelatinization (Dongmo, et al., 2020). The defatting process significantly reduced the SV of the untreated flour (1.82 Pa for WCS and 1.62 Pa for DWCS). However, all sonicated WCS samples presented lower SV values compared to all DWCS flours. This finding suggests that the US treatment allows for an enhancement in the re-association of amylose when lipids content is decreased (Li, Obadi, Shi, Xu, & Shi, 2021). These results are consistent with previous research, where the addition of corn oil to normal corn starch resulted in larger BV and smaller SV (Li, et al., 2020).

3.4. Rheological properties of gels

The results from rheological tests applied on the gels prepared with untreated and treated WCS and DWCS flours are presented in Table 5. The strain sweeps assays showed that both the US treatment and the matrix composition used significantly changed τ_{max} and crossover parameters. Interestingly, the combination of the matrix effect with the US treatment had a remarkable impact on τ_{max}, while the untreated samples exhibited nearly identical values for both native flours studied. After applying the US treatment, a 62% reduction in τ_{max} was observed in the WCS-20 sample, while an increase of over 32% was observed in this parameter in the defatted samples treated at 20 °C. This indicates that sonicated defatted samples, in contrast to whole flours, developed stronger gels capable of withstanding higher stress before their structural disruption (Vela, Villanueva, Solaesa, & Ronda, 2021). Regarding the crossover point ($G' = G''$), an increase was observed in all WCS treated samples in comparison to the untreated one. It's worth mentioning that defatted samples presented higher crossover values for both untreated and treated flours compared to the whole flours. A similar increasing in crossover point due to the sonication process was previously reported by Vela, Villanueva, Ozturk, et al. (2023) in tef flours. Moreover, gels prepared from defatted flours exhibited a predominant and persistent elastic behavior (with lower values of $(\tan \delta)_1 < 1$), which remained consistent even at higher deformation levels. Regarding the τ_{max} results, a significant reduction in this parameter was observed for WCS samples when compared to the untreated one, while a significant increase in τ_{max} was observed for DWCS samples compared to the untreated sample. Higher values of τ_{max} values indicate increased gels stability against shear, as they require higher stress to disrupt their structure and transition to a predominantly viscous behavior (Calix-Rivera, Villanueva, Náthia-Neves, & Ronda, 2023).

Oscillatory tests showed that all properties were more significantly influenced by the presence of lipids in canary seed flours (except for b and c coefficients) than by US treatment. Flours containing 6.45% lipid content formed gels with lower G_1' and G_1'' moduli, indicating a softer consistency. Not only does the lipid content in flours influence

Table 5
Rheological parameters of untreated and treated WCS (whole canary seed flour) and DWCS (defatted canary seed flour) flours.

Samples	G_1' (Pa)	a	G_1'' (Pa)	b	$(\tan \delta)_1$	c	τ_{max} (Pa)	Crossover (Pa)
WCS (untreated)	308^{c}	0.039^{ab}	20^{c}	0.342^{a}	0.066^{a}	0.30^{ab}	73^{c}	28^{e}
WCS-20	269^{c}	0.039^{ab}	18^{c}	0.344^{a}	0.066^{a}	0.30^{ab}	28^{e}	62^{d}
WCS-30	266^{c}	0.041^{a}	18^{c}	0.319^{c}	0.069^{a}	0.27^{c}	28^{e}	65^{d}
WCS-40	273^{c}	0.037^{b}	18^{c}	0.335^{abc}	0.067^{a}	0.29^{ab}	21^{e}	60^{d}
DWCS (untreated)	505^{a}	0.025^{c}	28^{ab}	0.327^{abc}	0.055^{b}	0.29^{ab}	74^{d}	128^{bc}
DWCS-20	455^{b}	0.027^{c}	29^{a}	0.320^{bc}	0.057^{b}	0.29^{bc}	98^{a}	139^{ab}
DWCS-30	463^{ab}	0.024^{c}	25^{b}	0.335^{ab}	0.054^{b}	0.32^{a}	80^{c}	129^{c}
DWCS-40	496^{ab}	0.026^{c}	27^{ab}	0.338^{ab}	0.055^{b}	0.31^{ab}	87^{b}	146^{a}
SE	21	0.001	1.0	0.01	0.001	0.01	3	8
Analysis of variance and significance (p-values)								
F1	*	***	***	ns	**	ns	***	***
F2	ns	ns	ns	ns	ns	ns	**	ns
F1 x F2	ns	ns	ns	*	ns	*	*	ns

$G_{1'}$ (elastic modulus), G_1'' (viscous modulus), and $(\tan \delta)_1$ (loss tangent) are the coefficients obtained from fitting the frequency sweeps data to the power law model and represent the moduli and loss tangent values at a frequency of 1 Hz. The a, b, and c exponents quantify the dependence degree of dynamic moduli and the loss tangent with the oscillation frequency. τ_{max} represents the maximum stress tolerated by the sample in the LVR.
The different letters in the same column indicate statistically significant differences between means at $p < 0.05$.
F1: Flour lipid content; F2: Temperature (°C); * for $p < 0,05$; ** for $p < 0,01$; *** for $p < 0,001$; ns: not significant.

rheological properties, but it is primarily the composition of these lipids that plays a significant role. For example, monoacyl lipids have been reported to play a major role in the paste and gel behavior of wheat and corn starches (Takahashi & Seib, 1988). The research conduct by Shah, Zhang, Hamaker, and Campanella (2011) suggests that the higher the degree of unsaturation of the free fatty acids, the higher the complexation, and hence the order of the amylose-lipid complex formed. As previously mentioned, canary seed flour is mainly composed by monounsaturated and polyunsaturated fatty acids. G' and G'' of maize-derived amylose were also negatively influenced by the formation of complexes with lipids (Li, Shi, Dong, & Yu, 2021). The results obtained in this study are also in agreement with Li, Obadi, et al. (2021), who observed an increased tendency to form a gel network from oat flours with 1.4% lipids compared to the same samples containing 6.7% lipids. Georgopoulos, Larsson, and Eliasson (2006) also reported that defatting wheat flour, enhanced G' and G'' to a greater extent. All the gels exhibited an increase in their viscous behavior with frequency, as the exponent "a" was consistently lower than the exponent "b", which accounts for the positive values of the exponent "c". The improvement of gel structure after defatting is also evident from the lower tan δ values obtained. The tan δ of defatted sample was always lower than the values from whole canary seed flours, suggesting that the gels from defatted flours had a higher solid-like rather than liquid-like character. According to Biliaderis and Tonogai (1991), granular lipids may inhibit extensive exudation of amylose from the granules and thereby reduce the extent of interchain associations between linear starch molecules; noncovalent intermolecular cross-linking of amylose is pivotal to the development of a three-dimensional network. These findings concur with the lower SV observed in the untreated defatted samples (Section 3.3) and agree with the data of Takahashi et al. (1988), who found reduced amylogram consistencies and setbacks for low-lipid cereal starches.

Table 6
Thermal properties of untreated and treated WCS (whole canary seed flour) and DWCS (defatted canary seed flour) flours.

Samples	First scan							Second scan					
	ΔHgel (J/g d.b)	To-gel (°C)	Tp-gel (°C)	Te-gel (°C)	ΔT (°C)	ΔH am-lip (J/g d.b)	Tp-am-lip (°C)	ΔH ret (J/g d.b)	To-ret (°C)	Tp-ret (°C)	Te-ret (°C)	ΔH am-lip (J/g d.b)	Tp-am-lip (°C)
WCS (untreated)	6.3^{bc}	61.8^{a}	69.3^{a}	74.1^{a}	12.2^{bc}	3.3^{b}	92.9^{c}	ND	ND	ND	ND	4.25^{a}	96.2^{c}
WCS-20	5.7^{e}	62.1^{a}	69.1^{ab}	73.9^{ab}	11.8^{c}	3.3^{b}	92.1^{c}	ND	ND	ND	ND	4.23^{a}	94.2^{d}
WCS-30	5.7^{e}	61.9^{a}	69.1^{ab}	73.7^{bc}	11.8^{c}	3.3^{b}	93.3^{c}	ND	ND	ND	ND	4.22^{a}	95.5^{c}
WCS-40	5.8^{de}	61.7^{a}	69.1^{ab}	73.9^{ab}	12.1^{bc}	2.9^{b}	93.2^{c}	ND	ND	ND	ND	4.15^{a}	95.7^{c}
DWCS (untreated)	6.2^{cd}	61.7^{a}	68.9^{b}	73.8^{abc}	12.2^{bc}	3.0^{b}	96.9^{b}	0.8^{c}	36.8^{a}	53.1^{a}	60.5^{b}	3.2^{b}	100.5^{b}
DWCS-20	6.9^{a}	60.7^{b}	67.9^{d}	72.9^{d}	12.3^{bc}	4.1^{a}	99.3^{a}	1.9^{b}	36.9^{a}	51.7^{a}	59.9^{d}	3.6^{ab}	101.3^{ab}
DWCS-30	6.4^{bc}	60.6^{b}	68.1^{d}	73.2^{d}	12.6^{ab}	3.1^{b}	99.2^{a}	2.1^{ab}	37.8^{a}	48.7^{b}	60.9^{a}	3.7^{ab}	101.9^{a}
DWCS-40	6.5^{b}	60.8^{b}	68.6^{c}	73.5^{c}	12.7^{a}	3.2^{b}	99.2^{a}	2.2^{a}	35.7^{a}	48.9^{b}	60.2^{c}	3.1^{b}	101.8^{a}
SE	0.1	0.3	0.1	0.1	0.2	0.2	0.5	0.2	2.3	0.6	0.1	0.4	0.5
Analysis of variance and significance (p-values)													
F1	**	**	***	***	**	ns	***	***	***	**	***	*	***
F2	ns	ns	*	*	ns	*	ns	ns	ns	***	**	ns	ns
F1 x F2	*	ns	*	*	ns	ns	ns	ns	ns	**	**	ns	ns

ΔHgel = Enthalpy of gelatinization. T_O-gel, T_P-gel, T_E-gel: Onset, peak and endset temperatures of gelatinization. ΔT = (T_E-gel – T_O-gel). ΔHam-lip = Enthalpy of the amylose-lipid dissociation. T_O-am-lip = Onset temperature of the amylose-lipid complex dissociation. ΔHret = Enthalpy of melting of retrograded amylopectin. T_O-ret, T_P-ret, T_E-ret: Onset, peak and endset temperatures of melting of retrograded amylopectin. ΔHgel, ΔHret, ΔHam-lip are given in J/g dry basis (d.b.).
The different letters in the same column indicate statistically significant differences between means at $p < 0.05$.
F1: Flour lipid content; F2: Temperature (°C); * for $p < 0,05$; ** for $p < 0,01$; *** for $p < 0,001$; ns: not significant; ND: not detectable.

3.5. *Thermal properties*

The gelatinization (first scan) and retrogradation (second scan measured after 7 days) properties of the treated and untreated canary seeds flours are presented in Table 6.

DSC thermograms of all samples exhibited two endothermic peaks, with the primary peak (~67–69 °C) corresponding to the starch gelatinization related to the melting of starch crystallites and a smaller peak appearing at a higher temperature (~92–99 °C) attributed to the dissociation of the amylose-lipid complex. The obtained results indicate that the effect of the US treatment on thermal properties is strongly influenced by the presence of lipids. As shown in Table 6, the properties evaluated in the first scan showed similar results between the two untreated samples (except for Tp-gel and Tp-am-lip properties), whereas different trends were observed upon applying the US treatment. For example, the US treatment led to a decrease tendency in the ΔHgel in all WCS samples, while an increase tendency in ΔHgel was observed in all treated DWCS flours. According to the literature, the US treatment lead to a decrease of the gelatinization enthalpy due to different alignments of hydrogen bonds within the starch molecules, as a consequence of a preferential disruption of amorphous regions after the sonication process (Jambrak, et al., 2010). On the contrary, the removal of lipids along with US treatment resulted in a slight increase tendency in the ΔHgel for samples treated at 20 °C and 40 °C. Carmona-García, et al. (2016) also reported a positive effect of the US treatment on ΔHgel in sonicated starches, attributing this enhancement to a rearrangement of the molecular packing within the granule microstructure. This suggests that the organization of the starch components (amylose and amylopectin) and their interaction with other components such as lipids and proteins of flours plays an important role in the gelatinization properties of sonicated samples.

The lipid content of the untreated flours only influenced Tp-gel and Tp-am-lip parameters. In WCS flours, Tp-gel was higher than in DWCS, while an opposite observation was done for Tp-am-lip, which presented higher values in DWCS compared to WCS untreated flour. This is in agreement with Y. Li, Obadi, et al. (2021), who observed that the amylose–lipid complexes dissociation peak area in the oat starch samples increased as the amount of added lipids decreased. The US treatment did not significantly change the To-gel, Tp-gel, ΔT, ΔH am-lip, and Tp-am-lip in WCS samples, but it produced a significant change in Tp-gel, T_E-gel, ΔH am-lip (20 °C), and Tp-am-lip parameters when defatted flours were treated, indicating that these parameters are not exclusively related to the starch. In general, the US treatment led to a reduction on To-gel, Tp-gel, Te-gel and ΔT parameters in DWCS flours, suggesting that the lower lipid amount in the samples the more cavitation phenomenon disrupted the most ordered double-helical structures and reduced the amount and stability of the better packed crystallites, thus lowering the dissociation temperature (Vela, Villanueva, Li, et al., 2023).

Interestingly, the presence of lipids had a significant impact on the retrogradation of the canary seed flours. The retrogradation endotherm was absent in both untreated and treated WCS samples, indicating that the retrogradation process did not occur by applying the analysis conditions. As a result, parameters such as ΔH ret, To-ret, Tp-ret, and Te-ret could not be detected for these samples. This suggests that the lipid presence in the canary seed flours might play a role in preventing or inhibiting the retrogradation phenomenon after 7 days. This is in line with results reported by Cai et al. (2021) who observed no retrogradion endotherm in potato starch added with lauric acid. However, these findings are not consistent with the pasting properties results, which showed that untreated WCS exhibited a higher tendency for retrodegradation compared to DWCS that presented lower SV values. This inconsistency may be attributed to the fact that in RVA assays, retrogradation primarily involves amylose (short-term retrogradation), whereas in DSC analysis conducted after 7 days (long-term retrogradation), the quantified retrogradation primarily pertains to amylopectin. Therefore, further investigation with a more detailed thermal including longer cooling storage times is necessary to better understand the underlying mechanisms and implications of this observation.

The ΔH am-lip value of the dissociation of the amylose–lipid complex of canary seed flour samples was higher in WCS samples after heating and storage at 4 °C, which may be associated to the release of amylose from inside the starch granules. The US treatment did not affect ΔH am-lip in both studied flours and Tp-am-lip in WCS flours, but it produced a slight increase in Tp-am-lip in defatted samples.

3.6. *X-ray diffraction and crystallinity*

XRD analyses were carried out to investigate the effect of the US treatment on the X-ray pattern and degree of crystallinity and the results are shown Fig. 3. Both untreated and treated samples showed typical A-type diffraction patterns for cereal samples, with characteristic peaks at scattering angles (2θ) of 15°, 23 and 26°. Additionally, an unresolved doublet appeared around 17° and 18°, along with a reflection at 20°, which is representative of amylose-lipid complexes. As observed in Fig. 3, treated samples exhibited similar diffraction patterns to the untreated ones, but with a slight reduction in diffraction intensities as the treatment temperature increased. This reduction in the diffraction intensities due to the US treatment, as well as the pronounced effect of the intensity of the applied treatment has been reported by several authors in different matrices, for example, in tef flour (Vela, Villanueva, Li, et al., 2023), taro and plantain starches (Carmona-García, et al., 2016) and rice starch (Yang, et al., 2019). According to these authors, the sonication process can cause more damage to the amorphous regions rather than the crystalline regions.

The presence of lipids resulted in a higher relative crystallinity (48.7%) in untreated samples compared to untreated defatted flour (44.6%), exhibiting more intensity peaks at 15°,17°, 18° and 20°. This suggests that a larger amount of crystalline complexes was formed between the free lipids and the starch (Wang, Zheng, Yu, Wang, & Copeland, 2017). Pasting essays also showed a higher PV1 tendency for DWCS (1.55 Pa s) compared to WCS (1.41 Pa s). Other authors have reported similar findings with the addition of lauric acid in wheat, maize, potato and wax maize starch on starch crystallinity (Cai, et al., 2021). A consistent trend in crystallinity was observed across all sample, with higher intensity peaks observed in sonicated WCS samples compared to sonicated defatted ones. The relative crystallinity of the flours exposed to US treatments significantly reduced in WCS flours whereas no significant reduction was observed in DWCS flours. This

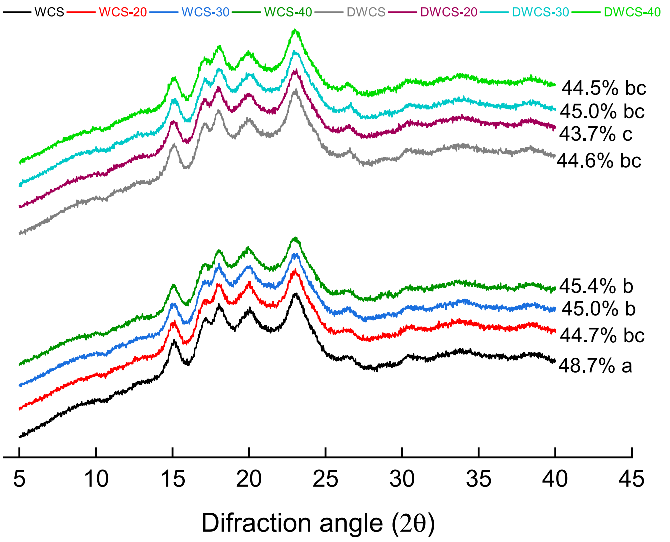

Fig. 3. X-ray diffraction (XRD) patterns of the studied flours. WCS refers to whole canary seed and DWCS refers to defatted canary seed flour.

once again confirms that not only does the treatment cause changes in the crystalline structure and other properties related to starch, but it also highlights the significant combined effect between the treatment conditions employed and the composition of the matrix to be treated. The reductions in the extent of crystallinity in WCS sonicated fours were consistent with the results obtained from DSC, as a reduction tendency in ΔH values was observed when these samples were sonicated. Physical treatments may cause reorientation of crystallites, reduction of large particle crystals, and/or destruction of crystalline regions, leading to a decrease in the number of double helices within the starch, ultimately resulting in reduced crystallinity (Tang, Wang, Cheng, Wu, & Ouyang, 2019). Similar reductions in the degree of crystallinity were also reported for sonicated oat, cassava, maize and potato (Falsafi, et al., 2019). Neither US treatment nor defatting process caused changes in the position of the feature diffraction peak of canary seed flours, indicating the crystal type of this raw material remained intact after these treatments.

4. Conclusions

This study presents the potential benefits of synergistically combining ultrasound treatment and defatting processes to enhance the surfactant properties of canary seed flours, contributing to the innovation of cereal-based products. The lipid presence of the flour and the temperature during ultrasonication significantly influenced the recovery of protein fractions, indicating changes in their solubility. Furthermore, the interaction between the flour's lipid presence and ultrasound treatment influenced the viscosity profile and the formation of starch-lipid complexes in canary seed flours. Defatted samples did not show a second viscosity peak and formed stronger gels with higher viscoelastic moduli. The presence of lipids seemed to retard retrogradation in both untreated and treated WCS samples after a 7-day storage period at 4 °C.

Therefore, it can be concluded that, WCS samples could be advantageous in formulating long shelf-life products rich in essential fatty acids. From a technological perspective, sonicated defatted canary seed flours are versatile ingredients suitable for a wide range of applications, offering desirable surfactant attributes. These attributes are particularly valuable in the production of items like cakes, muffins, and bread, where texture enhancement, stability, and consistency are crucial. Moreover, flours yielding stable batters and coatings are ideal for products such as tempura, fried chicken, and other fried foods. Due to their rheological properties, these samples with strong gelling properties may be used in producing jams, jellies, and fruit preserves, and could serve as alternatives to gelatin in vegetarian and vegan products.

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CRediT authorship contribution statement

Grazielle Náthia-Neves: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. **Marina Villanueva:** Methodology, Visualization, Conceptualization. **Felicidad Ronda:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodhyd.2024.109727.

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