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Preliminary Characterization of Structural and Rheological Behavior of the Quinoa Hyperprotein-Defatted Flour

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Protein functional properties are related to physical and chemical parameters that influence protein behavior in food systems during processing, storage and consumption. The structural and rheological properties of three quinoa hyperprotein flours (without defatting, WD, chemically defatted, CD, and mechanically defatted, MD) were evaluated. The values of the fluidity index (n) were significantly different ($p < 0.05$), which was associated with changes in protein or starch structures due to solvent treatments or heating of the flour during pressing. In addition, a strong dependence of the consistency index (k) on the shear rate was observed. For dispersions with a concentration of 12% (w/v), CD and WD had a significantly lower setback value than MD. The viscosity peak was affected by the presence of lipid molecules. Greater changes were evident in the β-sheet (1,610 and 1,625 cm^{-1}) and β-spin (1,685 and 1,695 cm^{-1}) structures. The changes identified in these structures were associated with the defatting treatment. Consequently, the intensity ratio 2,920/1,633 cm^{-1} was more sensitive to changes in the fat content of the flours. It was shown that defatting conditions increase the protein adsorption kinetics and that the viscoelastic properties of the protein increase when the flour has a lower fat content. Hyperprotein quinoa flour could be used to improve the protein content of products such as snacks, pastas, ice cream, bakery products, meat extenders, among others, due to its foaming, gelling or emulsifying capacity. The objective of this work was to study the effect of two types of defatting of hyperprotein quinoa flour on its structural and rheological properties.

Keywords: functional food, flow behavior, interfacial properties, structural properties, protein

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INTRODUCTION

Nowadays, there is a great interest among the population and the food industry in proteins with high nutritional value and biological or technological functionality. For this reason, different types of functionality, such as solubility in protein hydrolysates, nutraceutical capacity, antioxidant and antihypertensive capacity, anti-inflammatory, immunological properties, antithrombotic, antitumor, hypocholesterolemic, antihypertensive, antiobesity, and antimicrobial properties have

been studied (Haque et al., 2016; Chatterjee et al., 2018; Ruan et al., 2020). This has led to the study of different types of biomass as protein sources, and the processing of them to obtain concentrates, isolates or functional peptides. Among the proteins that have emerged in recent years as high quality, quinoa is one of the most desirable, for its supply of essential amino acids (Hayes and Bleakley, 2018). In addition, an increase in the demand for plant-based proteins has been reported, due to different factors such as dietary restrictions, food preferences (veganism), occurrence of clinical conditions such as animal protein allergies or the beneficial health effects (Chakrabarti et al., 2018; Montesano et al., 2020). Furthermore, in chronic diseases such as type 2 diabetes and coronary heart disease, plant protein, unlike animal protein, has shown an inverse relationship with these types of diseases (Sluijs et al., 2010; Malik et al., 2016; Song et al., 2016).

An average daily protein intake of 1.0–1.2 g/kg/day is recommended at older ages and even higher for those with acute or chronic diseases, as protein intake generates benefits on muscle mass and strength, physical functioning and/or hip strengthening (Ortolá et al., 2020). For the food industry, the use of quinoa (*Chenopodium quinoa* Willd.), due to its nutritional potential is one of the preferred raw materials (Mota et al., 2016; Nowak et al., 2016; Wang and Zhu, 2016; Shi et al., 2019) as it contains 14–18% protein (d.b) which is concentrated in the middle part of the episperm (Navruz-Varli and Sanlier, 2016; López-Castejón et al., 2019; Roa Acosta et al., 2020b). Its high dietary fiber content (between 7 and 10%) as well as histidine (3.2% w/w) and lysine (6.1% w/w) makes it attractive for the development of functional foods (Kozioł, 1992; Abugoch et al., 2008; Nowak et al., 2016).

Previous studies had determined a methodology for obtaining hyper-protein quinoa flour (HP-QF) through an abrasive milling process. The abrasive mill polishes the external part of the quinoa grain (the embryo or protein part), however, as it is an abrasion process, part of the starchy perisperm (internal part) can also be polished together with the protein fraction (Roa Acosta et al., 2020b). This HP-QF reported protein values between (15.62 ± 0.23) and (34.85 ± 0.17) g/100 g (w.b. – wet basis), at different abrasion times. An alternative to concentrate the percentage of protein in the HP-QF is through a defatting process. Reported methods for this operation include the pressing (Melo et al., 2021) and treatment with organic solvents (Alcázar-Alay et al., 2017; Feyzi et al., 2017). On the other hand, there are currently no studies in which a sequential flow-pasting-flow test is performed on hyperprotein flour dispersions. Therefore, this test could constitute an interesting approximation of the rheological behavior of dispersions when subjected to shearing and heating, as for example in mixing and cooking processes of beverages fortified with protein-rich flours.

The technological functionality of proteins is an important property to analyze during food development, due to its relationship with the stabilizing capacity in different types of dispersed systems, foams, gels and emulsions (Liu and Yu, 2016). Temperature is a frequent variation factor during food processing, which affects the foaming and emulsifying capacity of protein. In addition, pH has a significant effect on the physicochemical, structural and functional properties of proteins (Cerdán-Leal et al., 2020) and these depend significantly on the enzymatic, chemical or physical modification methods (Dakhili et al., 2019). Consequently, the difference in the functional properties of proteins is directly related to their structure, concentration, processing history and interactions with other molecules such as carbohydrates and lipids (Martínez and Añón, 1996; Lichtenstein et al., 2006; Tatulian, 2013).

The chemical nature of quinoa protein contributes to the reduction of interfacial tension by adsorbing at the air/water interface, possibly preventing destabilization of emulsions and foams. This behavior is defined by structural properties, which depend on pH (Mäkinen et al., 2015; Ruíz et al., 2016). Currently, there are few studies related to the interfacial properties of defatted hyperprotein flour from quinoa. Besides, several studies (Princen, 1986; Chen and Dickinson, 1998; Dickinson and Casanova, 1999; Liu and Yu, 2016; Dickinson, 2019), have demonstrated the relationship of protein solubility, pH and temperature to the ability to form emulsions, foams and gels (Kaspchak et al., 2017; López-Castejón et al., 2019). The aim of this work was to study the effect of two types of defatting of hyperprotein quinoa flour on the physicochemical and rheological properties.

MATERIALS AND METHODS

Materials

Quinoa grain of the Tunkahuan variety from the District of Los Milagros Bolivar, Cauca, Colombia was used. The quinoa samples were provided by the company SEGALCO S.A.S.

Flours Production

The hyperproteic quinoa flour (HP-QF) was produced by abrasive milling in a continuous flow abrasive mill (MAVIMAR, Popayan, Colombia) with a capacity of 30 kg/h. This process allowed the production of a protein-rich fraction from the germ (HP-QF) and a starch-rich fraction from the perisperm of the grain (Roa Acosta et al., 2020b).

Protein and Fat Determination

The protein and fat content of the HP-QF was determined by AOAC methods. Protein was determined according to AOAC 955.04 method and was expressed as g protein/100 g (d.b.). Fat was determined gravimetrically after a cyclic extraction process with petroleum ether in a Soxhlet apparatus (AOAC 920.39C method). The results were expressed as expressed g fat/100 g d.b.

Defatting Flour

The flour referred to as chemically defatted flour was obtained after fat determination. Briefly, reflux extractions were performed for 6 h at 60°C, using a Soxhlet apparatus and petroleum ether as extractant. The fat content was determined and the defatted flour was left overnight under a fume hood to evaporate the solvent. On the other hand, Mechanical defatting was performed in an automatic oil press machine (Cgoldenwall, K28, Shanghai China). Briefly, the machine was programmed to heat up to 120°C during the compression and shearing

process. Subsequently, the fat content was determined to verify the defatting percentage. Thus, flours analyzed were: chemically defatted (CD), mechanically defatted (MD), and hyperprotein flour without defatting (WD).

Rheological Characterization

A sequential flow—pasting—flow rheological analysis was performed using an AR 1500 rheometer, TA Instruments, New Castel, USA, equipped with a starch pasting cell and a 5,000 μm GAP. The method was programmed to perform a flow test at constant temperature, then a pasting test and finally, another flow test at constant temperature, with the aim of studying the rheology of the dispersions before and after heating. For the analysis of the flours, aqueous dispersions were prepared at a concentration of 12 g/100 mL (Polo et al., 2021).

Flow Assays

Flow assays were carried out at a constant temperature of 30°C in the 0.01 and 200 s^{-1} shear rate range. The experimental data were fitted to the Power law equation (Ostwald model) (Eq. 1).

$$\tau = k\dot{\gamma}^{n} \quad (1)$$

where τ represents the shear stress, k the consistency index, $\dot{\gamma}$ the shear rate, and n is the flow index. A Newtonian flow type was determined for values of $n = 1$, pseudoplastic for $n < 1$ and dilatant for $n > 1$.

Pasting Assay

The pasting assay of the dispersions was carried out by programming a temperature gradient, with an initial temperature of 30°C for 40 s, then heating at a rate of 10°C/min up to 90°C, maintained at this temperature for 4 min, then cooling (10°C/min) to 30°C and finally maintained at this temperature for 2 min. The values of Peak viscosity [Pa.s], Peak time [s], Trough [Pa.s], Final viscosity [Pa.s], Pasting temperature [°C], Setback [Pa.s] were recorded (Roa Acosta et al., 2020a).

Attenuated Total Reflectance-Fourier Transform-Infrared Spectroscopy

Spectra were obtained on an FT-IR spectrometer model IRAFFINITY-1S (Shimadzu, Inc., Shelton CT, Japan) with a MIRacle 10 attenuated total reflectance (ATR) accessory (Shimadzu, Inc., Shelton CT, Japan) with a single reflection diamond crystal at an incidence angle of 45°. Measurements were obtained by taking the average of 45 scans with a resolution of 4 cm^{-1} at 25°C. Happ-Genzel apodization was used, with a magnitude phase correction. A flat tip was used to obtain an intimate contact between sample and crystal, without pressure control. A background spectrum was recorded in air (without sample) prior to each measurement. Spectra were acquired between 500 and 4,000 cm^{-1} and the mean of the replicates for each sample was reported. Spectral analysis was performed using OriginPro version 2016. Spectra baselines were corrected and normalized between 0 and 1. Moreover, the deconvolution process of the peaks in the 800–1,200 cm^{-1} and 1,600–1,800 cm^{-1} regions was performed. Finally, defatting, protein and starch indexes were calculated using the intensity ratio of the peaks 2,920/1,633 cm^{-1}, 1,740/1,633 cm^{-1}, and 1,047/1,018 cm^{-1}, respectively (Roa Acosta et al., 2020a).

Surface Tension Measurements

Solutions for interfacial studies were prepared by dissolving samples in Milli-Q ultrapure water. Samples were diluted at a concentration of 1% (w/v) in a solution (phosphate buffer) at pH 7 and ionic strength (0.05 M). For surface pressure (π) and surface dilatational property measurements of adsorbed protein films at the air-water interface, an automatic drop tensiometer (TRACKER H, Teclis, France) was used. The diffusion (k_1), penetration (k_2), and rearrangement (k_3) rates of samples in the air-water interface were calculated according to the methodology described by Carranza-Saavedra et al. (2021).

Statistical Analysis

Two-way analysis of variances (ANOVA) at the 95% confidence level was used to determine significant differences between properties and samples. Tukey's test was used to determine which mean results were significantly different from others. Besides, one-way ANOVA at the 95% confidence level was used to determine significant differences in structural and rheological properties.

RESULTS AND DISCUSSION

Flow Behavior

The results found during the flow analysis of quinoa flour dispersions, defatted by treatments (chemical and mechanical) and without defatting, are summarized in **Table 1**. It was found that all the data obtained from the flow assay fitted well to the power law model, obtaining R^2 values higher than 0.99 before heating and 0.989 after heating. Results were analyzed in terms of the variation of the parameters flow consistency index (k) and flow index (n), due to the different concentrations of the dispersion before and after heating. Thus, before heating, values >1 for the parameter "n" were found in all treatments, denoting a dilatant flow behavior, which means an increase in viscosity with increasing shear rate (Metzner and Whitlock, 1958). Dilatant behavior is an indication that the applied force causes the material to adopt a more ordered structure or a greater interactions between particles (Mleko and Foegeding, 1999). Moreover, it was found that k values, in general, were statistically equal ($p < 0.05$) before heating in most of the dispersions analyzed, only when MD 12% flour was used the k value was higher (comparison made for statistically equal n values, $p < 0.05$). After heating, it was found that in some treatments the value of n was <1 (treatments CD and MD 12%), indicating a change toward a pseudoplastic flow. Pseudoplastic flows have a different characteristic from dilatants, a reduction in apparent viscosity with increasing shear rate (Ağar et al., 2016). It could be observed that when the concentration of the flours was increased, after heating, the values of n were lower ($p < 0.05$) in the dispersions. Therefore, it could be inferred that the reduction of n values could be related to the increase of molecules such as protein or starch mainly present in the flours. Also, the heating process carried out during the pasting assay allows

TABLE 1 | Flow properties of hyperprotein flour-added dispersions before and after heat treatment.

	Before heating			After heating		
Treatment	**K [Pa.s^n]**	***n***	**R^2**	**K [Pa.s^n]**	***n***	**R^2**
CD-6%	0.00196 ± 0.00006^{b}	1.768 ± 0.008^{a}	0.99945 ± 0.00007	$0.004043 \pm 0.000004^{d}$	$1.6415 \pm 0.0007^{a,b,c}$	0.99855 ± 0.00007
CD-9%	0.00242 ± 0.00001^{b}	$1.735 \pm 0.002^{a,b}$	0.99965 ± 0.00007	$0.025 \pm 0.001^{c,d}$	$1.294 \pm 0.007^{e,f}$	0.9958 ± 0.0003
CD-12%	0.0033 ± 0.0001^{b}	$1.69 \pm 0.01^{a,b,c}$	0.996 ± 0.005	0.18 ± 0.02^{b}	0.97 ± 0.02^{g}	0.995 ± 0.007
MD-6%	0.0029 ± 0.0006^{b}	$1.71 \pm 0.05^{a,b,c}$	0.9993 ± 0.0001	0.009 ± 0.003^{d}	$1.50 \pm 0.08^{c,d,e}$	0.9958 ± 0.0008
MD-9%	0.008 ± 0.001^{b}	$1.53 \pm 0.05^{b,c,d}$	0.9970 ± 0.0003	0.071 ± 0.008^{c}	1.11 ± 0.03^{f}	0.9936 ± 0.0001
MD-12%	0.023 ± 0.003^{a}	1.2 ± 0.2^{f}	0.99 ± 0.01	0.36 ± 0.02^{a}	0.81 ± 0.07^{g}	0.989 ± 0.005
WD-6%	0.0025 ± 0.0002^{b}	$1.71 \pm 0.02^{a,b,c}$	0.9996 ± 0.0001	0.005 ± 0.002^{d}	$1.68 \pm 0.03^{a,b,c}$	0.9989 ± 0.0006
WD-9%	0.0033 ± 0.0002^{b}	$1.63 \pm 0.04^{a,b,c}$	0.9992 ± 0.0005	0.0073 ± 0.0001^{d}	$1.531 \pm 0.003^{b,c,d}$	0.9975 ± 0.0007
WD-12%	0.0068 ± 0.0008^{b}	$1.55 \pm 0.02^{b,c,d}$	0.9994 ± 0.0002	$0.026 \pm 0.002^{c,d}$	$1.335 \pm 0.008^{d,e,f}$	0.99655 ± 0.00007

CD, chemical defatting; MD, mechanical defatting; WD, without defatting. Different lower case letters mean significant differences ($p < 0.05$) among values of k or n, before or after of heating or among treatments.

TABLE 2 | Pasting properties of hyperprotein flour dispersions.

	Peak viscosity [Pa.s]	**Peak time [s]**	**Trough [Pa.s]**	**Final viscosity [Pa.s]**	**Pasting temperature [°C]**	**Setback [Pa.s]**
CD-6%	0.0184 ± 0.0003^{e}	357 ± 4^{c}	0.0202 ± 0.0001^{d}	$0.0311 \pm 0.0003^{e,f}$	$86 \pm 2^{a,b}$	$0.0329 \pm 0.0006^{c,d}$
CD-9%	0.0607 ± 0.002^{d}	369 ± 3^{b}	$0.053 \pm 0.001^{c,d}$	$0.077 \pm 0.002^{c,d}$	81.4 ± 0.6^{b}	$0.070 \pm 0.001^{c,d}$
CD-12%	0.139 ± 0.003^{b}	370 ± 2^{b}	0.136 ± 0.002^{b}	0.220 ± 0.008^{b}	$85 \pm 3^{a,b}$	0.22 ± 0.02^{b}
MD-6%	0.033 ± 0.007^{e}	378 ± 1^{b}	$0.033 \pm 0.005^{c,d}$	$0.052 \pm 0.008^{c,d,e}$	88.3 ± 0.3^{a}	$0.052 \pm 0.007^{c,d}$
MD-9%	0.084 ± 0.002^{c}	$381 \pm 1^{a,b}$	0.081 ± 0.005^{c}	0.07 ± 0.08^{f}	75.15 ± 0.07^{c}	0.0117 ± 0.0008^{d}
MD-12%	0.38 ± 0.01^{a}	379 ± 3^{b}	0.35 ± 0.03^{a}	0.64 ± 0.02^{a}	75.8 ± 0.3^{c}	0.60 ± 0.04^{a}
WD-6%	0.017 ± 0.002^{e}	389 ± 5^{a}	0.014 ± 0.001^{d}	$0.0205 \pm 0.0002^{e,f}$	$84.1 \pm 0.2^{a,b}$	$0.018 \pm 0.003^{c,d}$
WD-9%	0.0256 ± 0.0008^{e}	388 ± 2^{a}	0.023 ± 0.004^{d}	$0.044 \pm 0.004^{d,e,f}$	$83.9 \pm 0.3^{a,b}$	$0.042 \pm 0.001^{c,d}$
WD-12%	$0.0601 \pm 0.0006^{c,d}$	389 ± 4^{a}	$0.054 \pm 0.002^{c,d}$	0.085 ± 0.003^{c}	81.6 ± 0.3^{b}	0.079 ± 0.005^{c}

CD, chemical defatting; MD, mechanical defatting; WD, without defatting. Different lower case letters mean significant differences ($p < 0.05$) among treatments.

the processes of gelatinization, gelification and retrogradation of starch. In this way, the 12% dispersions, with defatted flours, are more concentrated in protein but also in starch. During heating there is a swelling and breaking of the starch granules. This process releases amylose into the aqueous medium, which subsequently on cooling, aligns with the movement to form viscous suspensions (Ratnayake and Jackson, 2008). Therefore, the higher the concentration of the dispersion, the more amylose is released into the medium to form such suspensions. In **Table 2**, it was found that the value corresponding to the setback of these two dispersions (CD and MD 12%) was also higher than in other dispersions. This parameter is also associated with a higher starch retrogradation (Gao et al., 2021). Another effect that can be verified is a slight but significant ($p < 0.05$) reduction in the value of *n*, after heating for the same system, as for example in the CD and MD 9 and 12% dispersions.

Regarding the consistency index (*k*) after heating, for WD dispersions, no significant differences ($p < 0.05$) were found among the treatments studied. It was also found that between the CD 9 vs. MD 9% dispersions after heating, there were no significant differences ($p < 0.05$), while for the 12% dispersions the consistency was higher ($p < 0.05$) in the mechanically defatted flour dispersions than in the chemically defatted ones. For the other dispersions, since in general the "n" values were significantly different ($p < 0.05$), the corresponding k values are not in the same units and therefore it is not correct to compare them. Differences in flow indexes may be associated with changes in protein or starch structures due to solvent treatment or heating of the flour during pressing. Finally, it can be said that there was a strong dependence of the consistency of flour dispersions on the shear rate.

Pasting Properties

Figure 1 shows the viscosity profiles of the dispersions analyzed. **Figure 1A** illustrates an example of the concentration effect of the chemical defatted on dispersion viscosity. It could be seen that the higher the concentration, the higher the viscosity profile. This trend was the same for the dispersions with the other types of flour. On the other hand, dispersions with MD flour showed significantly higher viscosity values than CD and WD flours for the three concentrations analyzed (**Figures 1B–D**). The viscosity peak was found between 347 and 388 s, reaching values between 0.017 (WD6%) and 0.38 (MD12%) Pa.s, for all the dispersions analyzed (**Table 2**). Peak time values (**Table 2**) were on the order of those reported by Lu et al. (2020) and Polo et al. (2021), who reported values between 340 and 422 s for starches heated

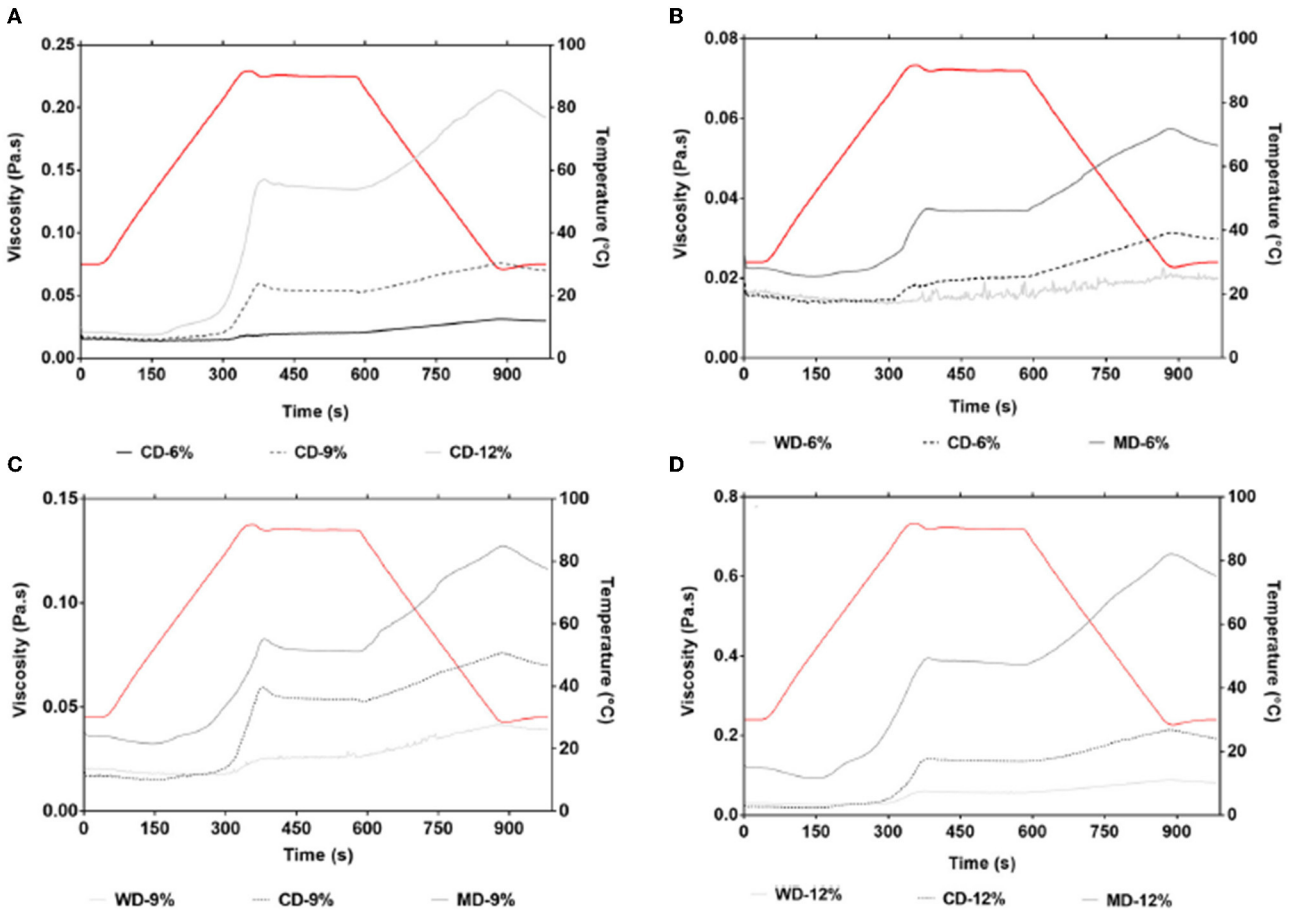

FIGURE 1 | Effect of concentration and defatting on the viscosity profiles of hyperprotein quinoa flour dispersions. Effect of chemical degreasing concentration on viscosity **(A)**. Dispersions with flour at concentrations 6% **(B)**, 9% **(C)** and 12% **(D)** of chemically defatted (CD), mechanically defatted (MD) and hyperprotein flour without defatting (WD).

at 140, 160, and 180°C. The viscosity peak is associated with the starch swelling process during heating prior to their physical breakdown and can be affected by the presence of molecules such as lipids (Raphaelides and Georgiadis, 2006). The swelling of the starch granules is related to the entrapment of water within their structure, this process is called gelatinization (Lu et al., 2020). The increase in viscosity may be associated with the interaction of water with proteins and starch. During heating there is a denaturation and unfolding of the proteins present, which can form gel-like networks with the water and amylose fragments released after starch gelatinization (check denaturation in the defatting process) (Raphaelides and Georgiadis, 2006). On the other hand, the final viscosity found after the cooling stage ranged from 0.0205 ± 0.0002 (WD 6%) to 0.64 ± 0.02 (MD 12%) Pa.s, the latter being significantly higher than the other dispersions. The final viscosity reflects association or retrogradation of starch molecules after the cooling period, simulating industrial processes. The increase in viscosity of dispersions during cooling may denote short-term retrogradation of starch, which may be reflected in the setback value (Vamadevan and Bertoft, 2015). For the dispersions at a concentration of 12%, CD and WD had a significantly lower setback value than MD, which may indicate that short-term degradation could be reduced in CD and WD flours. Among the other concentrations, in general, no significant differences were found in the setback value. Regarding the pasting temperature, it was observed that there were no significant differences between the 6% dispersions. For the 9% and 12% dispersions, MD flour dispersions had a significantly lower value (**Table 2**) than CD and WD ($p < 0.05$). The degree of swelling of starch granules and leached amylose largely depends on factors such as the amount of water available, the temperature and time of heating, the presence of other substances, for example lipids, surfactants, sugars or salts.

Chemical and FTIR Analysis

The results for protein content (dry basis), for the WD, MD, and CD hyperprotein flours was 31.5, 41.5, and 46.4 g/100 g, respectively, while the lipid content was 19.7, 10.0, and 2.2 g/100 g, respectively. These results show that chemical defatting produced a higher ($p < 0.05$) defatting, which in turn, allowed obtaining flours with a higher ($p < 0.05$) protein concentration.

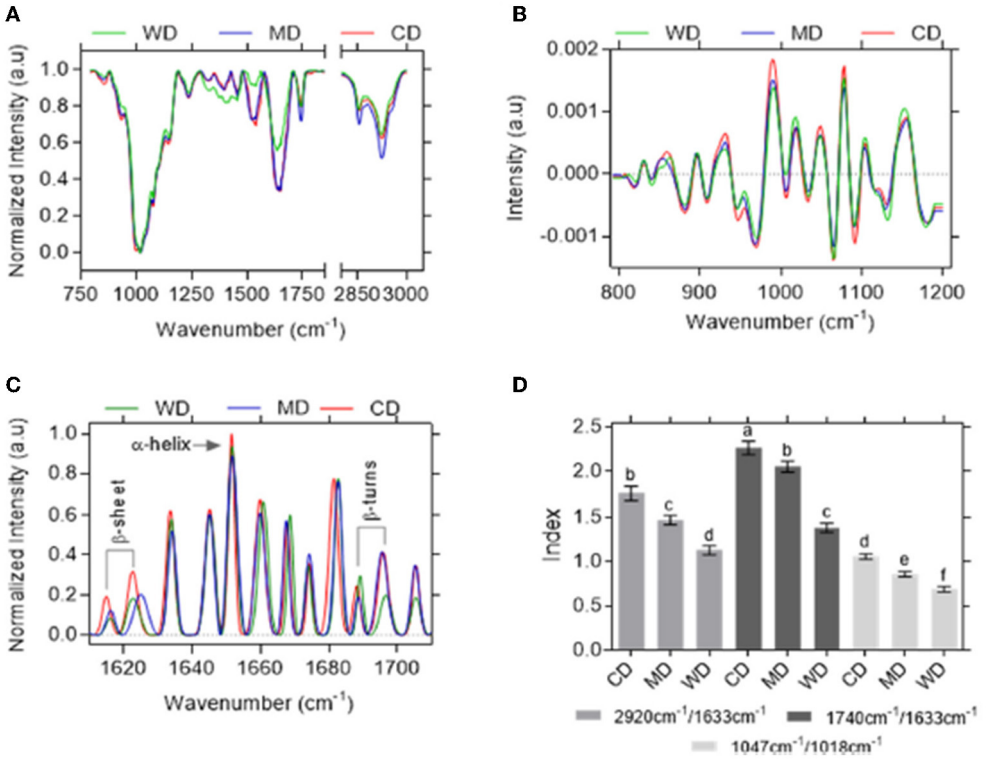

FIGURE 2 | Structural analysis spectra of defatted and without defatting hyperprotein flours. Protein-associated peaks **(A)**, deconvolution of peaks in the bands between 800–1,200 cm^{-1} **(B)**, deconvolution of peaks in the three types of flour in the bands between 1,600–1,800 cm^{-1} **(C)**, protein index for CD, MD and WD flour **(D)**.

Figure 2 shows the spectra obtained from the FTIR analysis. In panel A, the spectrum in the bands of interest associated with the composition of carbohydrates, lipids and proteins, are presented. The peaks associated with lipids in the spectrum are located at 2,920 and 2,850 cm^{-1} associated with asymmetric and symmetric stretching, respectively, of the C-H bond. In **Figure 2D**, the defatting, protein and crystallinity indexes are reported. The defatting index (2,920/1,633 cm^{-1}) showed a higher defatting ($p < 0.05$) in the CD flours than in the MD and WD flours. This result helps to explain the shorter peak times reported in **Table 2** for the CD flours.

On the other hand, peaks associated with proteins appear in the spectrum at 1,650 cm^{-1}, mainly due to the stretching of the C=O bonds, and at 1,565 cm^{-1}, related to the vibration of the N-H bond and stretching of the C-N bond. In this regard, also in **Figure 2A**, it can be verified that in these bands, the peaks mentioned above were more intense in the defatted flours due to a higher concentration of proteins after the removal of lipids. In addition, in **Figure 2D**, it was observed that the protein index was higher for the CD flour than for the MD and WD flours, which corroborates a higher protein concentration. The deconvolution process of the peaks in the region between 1,600 and 1,700 cm^{-1} made it possible to identify the changes in the secondary structures of the proteins (Carbonaro and Nucara, 2010) due to the defatting process of the flours. In this region, the bands between 1,650 and 1,653 cm^{-1} are assigned to α-helix structures (Barth and Zscherp, 2002; García-Parra et al., 2021). In addition, intense bands between 1,612 and 1,640 cm^{-1} and weak bands between 1,680 and 1,700 cm^{-1} are associated with antiparallel β-sheet structures (Carbonaro and Nucara, 2010; Guerrero et al., 2014). α-helix structures were found at 1,651 cm^{-1} in the three types of flours (**Figure 2C**), in this band it could be verified that the CD flour presents a greater peak than the other flours, denoting a greater change in the structure. Likewise, in the 1,610 and 1,625 cm^{-1} bands, a greater change in the β-sheet structure was observed for CD flour. For the β-turns structures, at 1,685 cm^{-1} and 1,695 cm^{-1} differences in these structures were observed for these types of flours. The changes identified in these structures could be associated with the defatting treatment.

Interfacial Properties

Figure 3 shows the kinetics of adsorption interfacial pressure (π) with time of protein adsorption at the air/water interface of the flours (MD, CD, WD). The interfacial pressure (π_{180}) tends to a plateau of constant value. It is observed that defatted flours show a higher surface activity with respect to non-defatted flour. The behavior of adsorbed protein films can be interpreted in terms of monolayer coverage. In general, there is a time-dependent increase in protein surface activity, which differs by about 2 mN/m, at π_{180}. This behavior may be related to the different degree of denaturation, since more compact molecules could more effectively reduce the interfacial tension. Consequently,

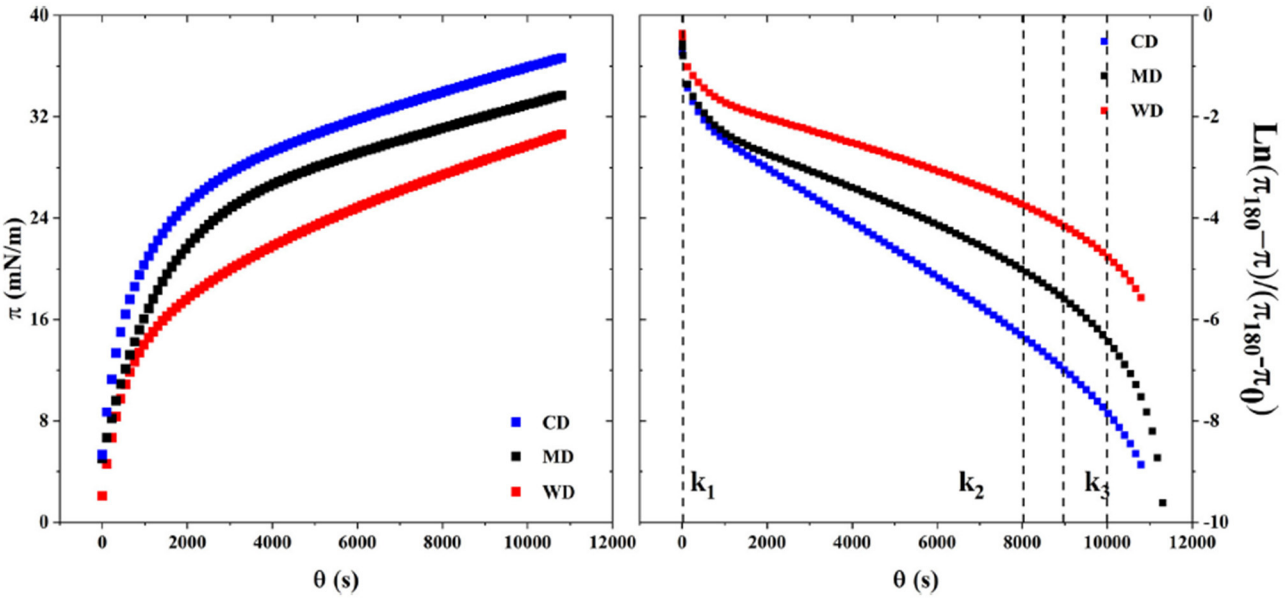

FIGURE 3 | Time evolution of surface pressure for quinoa hyperprotein-defatted flour adsorbed at the air/water interface at pH 7 and I 0.05M.

TABLE 3 | Kinetic parameters of protein adsorption at different quinoa concentrations at the air/water interface.

Deffating	K_d mN·m^{-1}s$^{-0.5}$	$K_p \cdot 10^4$ (s^{-1})	$K_r \cdot 10^4$ (s^{-1})	π_{180}
WD	0.7 ± 0.1	2 ± 1	10 ± 1	30.6 ± 0.2
MD	-	3 ± 1	12 ± 2	33.3 ± 0.6
CD	-	3 ± 1	15 ± 1	36.6 ± 0.7

Linear Regression coefficient (LR).

proteins in aqueous solution tend to adopt a configuration in which non-polar groups may aggregate at the center of the molecule and polar groups at the surface. In this way, the energy of the system is minimized, reducing the interaction between the non-polar groups and the water molecules. This allows an interfacial denaturation that consequently leads to a non-constant interfacial area during the process. In this case, cereal proteins usually have a high content of hydrophobic residues which causes complex oligomer structures (Conde et al., 2005a,b). The kinetic parameters of the WD, CD, and MD flours correspond to the initial diffusion stage that were fitted to the modified Ward and Tordai equation (MacRitchie, 1990; Xu and Damodaran, 1994). In the kinetic properties it was observed that WD is mainly controlled by a diffusion process (**Table 3**). The diffusion process occurs more rapidly in CD and MD. However, it is difficult to detect it with the experimental method used in the present study. In general, it was observed that the interfacial pressure is higher in CD and MD, behavior that could be related to the increased interfacial activity of the protein present in the defatted flour, where it controls the penetration adsorption kinetics presenting a higher rearrangement. Therefore, the value of the adsorption kinetics of the penetration constant (Kp) was dependent on the nature and defatting of the flour. These observations are consistent with the structural changes (FTIR-ATR) and rheological behavior previously discussed (Flow and pasting). Likewise, the value of the adsorption kinetics of the rearrangement constant (Kr) is higher as a function of the defatting process. This behavior has been associated with a higher degree of denaturation that contributes to the rearrangement of protein molecules adsorbed at the interface (Graham and Phillips, 1979; Carranza-Saavedra et al., 2016). This study supports the results of the present manuscript. The different adsorption mechanisms of CD and MD increase as a function of the defatting process due to higher protein richness. This shows that protein rearrangement and transition time between both adsorption kinetics increase with increasing protein richness, independent of pH (Miller et al., 1998; Bos and van Vliet, 2001; Qin et al., 2018; Felix et al., 2019, 2021).

CONCLUSIONS

The rheological study, carried out on dispersions of defatted and non-defatted quinoa hyperprotein flours, verified a dilatant flow behavior for all the dispersions before heating and a low influence of the concentration or type of flour on the consistency of the dispersion. On the other hand, the application of a heat treatment produced differences in the consistency index and flow type of the dispersions when the concentration and/or type of hyperprotein flour was modified.

On the other hand, it can be concluded that the mechanical defatting process produces hyperprotein flours that allow obtaining dispersions with a higher viscosity profile than those produced by chemically defatted or non-defatted flours. These differences could largely be explained by both the composition and the structural changes in proteins and starch. It is concluded

that the studied interfacial properties show the importance of the behavior of the protein in the formulation of emulsion. The protein denaturation contributes to gel formation, where the protein-protein interaction allows a better-structured network due to the formation of protein aggregates as protein. Finally, the rheological and interfacial properties studied in hyperprotein flours denote an interesting potential for the food industry, since (i) they have a high protein or lipid content; (ii) they could be used to improve the protein content in the formulation of new functional foods; (iii) they could also contribute to the stabilization of different dispersed systems (emulsions, foams, gels) in processed products.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

AUTHOR CONTRIBUTIONS

VO-G, JN-C, DR-A, and JS-D: methodology, formal analysis, writing—original draft, and investigation. JB-G: conceptualization, investigation, supervisión, content, and data curation. All authors contributed to the article and approved the submitted version.

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