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ORIGINAL ARTICLE

Influence of Maillard reaction conditions and solvent extraction on the surface activity and foaming characteristics of black cumin protein concentrates

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Abstract Cold press manufacture of black cumin (BC) oil leads to the formation of BC press cakes that contain significant amounts of protein. Here, an attempt was made to enhance the functionality of BC protein concentrates obtained from cakes based on Maillard conjugation using 3 different of carbohydrates. Molecular weight distribution of the conjugates was determined via electrophoretic techniques. The extent of carbohydrate binding was measured by RP-HPLC–RID. Surface activity and elasticity was studied using drop shape tensiometry. The extent of glucose binding accounted for up to 85% for a protein:glucose ratio of 1:2. Foaming capabilities were moderately enhanced due to Maillard conjugation in the absence of solvent extraction, while due to solvent induced partial denaturation, further enhancement of foaming performance took place. Furthermore, sugar binding capabilities were enhanced upon solvent treatment, while surface pressure and foaming capacity were not necessarily improved. Adsorption rate at the air–water surface and dilational elasticity was highly dependent on molecular size of reacting sugars. In addition, oil remaining in the samples also had a bearing on the extent of Maillard conjugation. Consequently, tailoring of processing conditions could enhance foaming characteristics of BC proteins and ensure their utilization in food foams and other food dispersions.

Keywords Black cumin protein concentrates · Maillard conjugation · Surface activity · Foaming capacity · Drop shape tensiometry · FT-IR spectroscopy

Introduction

Most manufactured food products are made of heterogeneous mixtures where dispersed phase(s) are contained in an aqueous medium. The presence, composition, size, and concentration of dispersed particles, their charge characteristics, and interactions with the continuous medium determine the microstructural attributes of the food matrix (Dickinson 2012). Proteins are functional biopolymers that enable the stabilization of such dispersed systems including food foams thanks to their surface active characteristics. Although most of the commercial protein products utilized by the food industry have originated from animal resources (i.e., dairy proteins), plant proteins become increasingly available and their technical functionalities including foam stabilization have been investigated (Schmidt et al. 2018; Xiong et al. 2018).

Current availability of commercial plant protein products include proteins manufactured from legumes, cereals and oilseeds. According to Day (2013), the conversion efficiency of plant proteins to animal proteins is roughly 15%, which implies cost efficiency and improved

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sustainability upon plant protein usage. Since current demand of the food industry is sufficient to consume commercially available plant proteins (Day 2013), novel protein products could target further and novel uses.

Due to the deoiling processes, proteins become highly concentrated in oilseed cakes or meals, potentiating the manufacture of plant protein products. These by-products are mostly utilized in feed manufacture, which mostly turn out as low value products. Depending on the source, protein content in the deoiled meals could account for up to 60% (Radha et al. 2007).

Black cumin (*Nigella sativa*) is a commercially important medicinal plant from *Ranunculaceae* family (Baydar 2009). It is native to the Eastern Mediterranean regions, South Europe and Turkey (Baydar 2009; Baytop 1999). Black cumin seeds contain approximately 21% protein, 35% carbohydrates and 35 to 38% oil (Baydar 2009). Consequently, in the deoiled meals/cakes, one can expect to find a corresponding protein concentration $> 30\%$. Black cumin seeds have been traditionally utilized in medicinal applications and nutritional supplements, and as spice. Its direct consumption as a seed is relatively difficult. Therefore, industrially generated components could enhance its edibility.

In our previous studies, after aqueous extraction, black cumin protein concentrates were shown to possess relatively poor technical properties, while further hexane extraction enhanced the surface activity (Coşkun et al. 2019). The surface activity of proteins occurs thanks to their amphiphilic nature. Adsorption at the surface and consecutive molecular rearrangements lead to partial unfolding of the proteins (Hasenhuettl 2008). In the case of protein–sugar complexes or conjugates, in addition to the polar residues of the proteins, hydrophilic sugar molecules further protrude into the aqueous phases generating strong steric protection. These processes extend the stability of emulsions and foams (Dickinson and İzgi 1996). Protein–sugar conjugates that occur naturally or are prepared via Maillard reactions have both been shown to demonstrate significant foaming capabilities (Naji-Tabasi and Razavi 2016; Nooshkam et al. 2020). Here, an attempt was made to enhance the foaming characteristics of black cumin proteins based on Maillard conjugation.

Materials and methods

Materials

Cold press cakes of black cumin seeds were obtained from a local manufacturer (Neva Foods Ltd., İstanbul, Turkey). During cold press operation, the highest temperature observed by the samples was < 40 °C. All the laboratory reagents and chemicals were purchased from Sigma-Aldrich Corp.

Methods

Preparation of black cumin protein concentrates

Preparation of protein concentrates was based on an alkali extraction–isoelectric precipitation (AE-IP) technique (Boye et al. 2010). Firstly, 50 g of cake sample was dispersed in water (1:15, w/v) and to facilitate solubilization, medium pH was set to pH 9.5 using concentrated NaOH (1 N). The dispersions were kept stirred (500 rpm) for 1 h under ambient conditions (22 ± 1 °C). Immediately afterwards, the dispersions were centrifuged to remove any undissolved materials (13500xg, 15 min and 4 °C) using a CR22N high-speed centrifuge (Hitachi, Japan). The supernatant was collected and the medium pH was lowered to facilitate isoelectric precipitation (pH 4.5) using 1.0 N HCl. Finally, the precipitate was collected by centrifugation under identical conditions as before and lyophilized using a Teknosem TRS 2/2 V freeze drier (Teknosem, İstanbul, Turkey).

Solvent extraction

Soxhlet extraction system was utilized for removing the remaining oil from cold press cakes (Behr Labortechnik, R106S, Germany). In this process, samples were treated with hexane for 7 h at a sample to hexane ratio of 1:50. Drying was carried out at 55 °C until constant weight was attained.

Preparation of Maillard conjugates

In order to prepare Maillard conjugates of black cumin proteins and glucose, 3 different protein:glucose ratios (1:1, 1:2, 1:4) were selected. For comparative purposes, lactose and maltodextrin was also used at a concentration ratio of 1:2. Protein concentrates were dispersed in 750 ml of deionized water in order to generate a final protein concentration of 1% and dispersion pH was adjusted to pH 12 using 10 M NaOH. The dispersion was kept stirred for 1 h to ensure hydration. Heating was carried out at 100 °C for 0–30 min using a water-bath. Immediately afterwards, the samples were cooled in an ice bath and thus formed conjugates were immediately lyophilized.

Native- or SDS-PAGE analysis

Native-PAGE electrophoresis was administered to analyze Maillard conjugates under non-reducing conditions (Laemmli 1970) using Bio-Rad Mini Protean Tetra Cell

System (Bio-Rad Laboratories Inc., USA). Firstly, conjugates were dispersed in sodium phosphate buffer (pH 7). Immediately afterwards, conjugate dispersions and 2 × sample buffer containing 1% Bromophenol blue, 25% glycerol and 62.5 mM Tris–HCl (pH 6.8) were mixed 1:1 in 1.5 ml tubes. All samples were loaded on a Mini-Protean TGX Stain-Free Precast Gel (12%), while Precision Plus protein standards from the same manufacturer were also utilized. Electrophoresis was carried out for 45 min using Tris/Glycine running buffer at 200 V (constant). Upon the completion of electrophoresis, imaging was carried out by transferring the gel to Gel Doc EZ System. Image analysis was based on Image Lab Software (Bio-Rad). For switching to SDS-PAGE, the same gel was treated to reducing conditions, SDS was added and samples were kept heated at 100 °C for 5 min.

Foaming capacity and foam stability

Foaming capacity and foam stability were determined according to Sathe and Salunkhe (1981). Briefly, 50 ml of 2% (w/v) conjugate dispersions were whipped for 3 min using a Waring lab blender at "high stir" setting and then poured into 100 ml graduated cylinders. To ensure pH stability, 100 mM sodium citrate or sodium phosphate buffers were used for pH 3–5 and pH 7 samples, respectively. % Change in sample volume was monitored as a function of pH and time.

RP-HPLC-RID analysis

Sugar analyses were performed on a Shimadzu LC-20AD HPLC system which consisted of a pump, thermostated column compartment and refractive index detector. Inertsil InertSustain NH_2 Column (4.6 mm ID × 250 mm, 5 µm pore size) was used, while the analysis was based on an application note published by the manufacturer (Inertsil application-Analysis of Sugars, Data No. LB180-0871). An isocratic flow was administered where the mobile phase was made of 85% acetonitrile and 15% HPLC water for 25 min. The column temperature was 40 °C, and eluent flow rate was 1 $ml.min^{-1}$. For all sugars analyzed, at least 5 concentration levels of all standards (0–2%) were injected. The sugar concentration in the samples was calculated based on calibration curves.

After the redispersion of Maillard conjugates, equilibrium dialysis (1:1 by volume) was carried out for 72 h using appropriate dialysis tubing (D977, Sigma-Aldrich Corp., 14 kDa cutoff) to determine the extent of unreacted sugars. The samples were withdrawn from the dialysis permeate and filtered through 0.45 µm PTFE membranes (Isolab, Germany) prior to injection into the HPLC system.

FT-IR analysis

FTIR absorption spectra were collected from 4000 to 650 cm^{-1} using an IRTracer-100 spectrophotometer (SHIMADZU, Kyoto, Japan) equipped with a DLATGS detector system, ATR module and 2 cm^{-1} resolution.

Drop shape tensiometry

Surface tension ($mN.m^{-1}$) at the air-aqueous dispersion interface was determined using Attension Theta Tensiometer (Biolin Scientific, Finland) (25 °C). For this purpose, an air bubble was automatically formed at the tip of an inverted syringe, which was immersed in a quartz cuvette containing the aqueous sample. Changes in droplet shape was analyzed over time, as the assembly was monitored by a charge coupled device camera (Gülseren et al. 2007). Surface tension was calculated using the software supplied by the instrument manufacturer (OneAttension version 2.6).

For the determination of surface elasticity, an equilibration duration of 3000 s was used prior to analysis. Once the equilibrium surface tension values were attained, dilational elasticity was determined at a strain amplitude range of 0–0.5 ($\Delta A/A = 0$ to 0.5, A being the bubble surface area) and a sinusoidal oscillation frequency (x = 100 MHz). This extent of dilation lies within the linear viscoelastic range (Gülseren and Corredig 2012). The measurements are based on automatically controlled, sinusoidal compression–expansion of the aqueous droplet at a defined oscillatory frequency and dilational amplitude. Dilational surface elasticity was calculated from the change in surface tension in proportion to the change in droplet surface area (Benjamins et al. 1996). To enable accurate measurements of elasticity, conjugate dispersions were treated with α-amylase (A1031, Sigma-Aldrich, 4 h, 20 °C) prepared in sodium phosphate buffer (100 mM, pH 7) and non-protein substrates were removed via extensive dialysis.

Statistical analysis and study design

The data obtained were analyzed by using SPSS statistical software (version 25, SPSS Inc., Chicago, USA). Data were expressed as sample mean ± standard deviation. In all cases, at least three replicates for the samples were analyzed. Where necessary, representative runs were shown. The primary parameters in the study included protein: carbohydrate ratio, carbohydrate type, heating duration, and presence or absence of hexane extraction. All studies were primarily based on glucose conjugation. While lactose or maltodextrin replaced glucose for comparison, no further optimization was carried out.

Results and discussion

Electrophoretic analysis

The molecular weight distribution of proteins in the black cumin protein concentrates and their Maillard conjugates was investigated using native- and SDS–PAGE (Fig. 1). Previously, SDS–PAGE and 2D-electrophoretic analysis of the protein concentrates were carried out (Coşkun et al. 2019). Firstly, on Lane 1, SDS-PAGE analysis of untreated concentrates were shown. The major bands appeared between 15 and 25 kDa, around 37 kDa (Lane 1). The current results were coherent with our earlier findings (Coşkun et al. 2019). In the case of Maillard conjugates, although there was smearing and some faint spots were observed throughout the gel, the major bands were shown to lie between 75 and 250 kDa in all cases (Lanes 2–4) for protein:glucose ratios of 1:1 to 1:4. For the 1:4 samples, the size of the major band was considerably smaller as most other detectable bands were concentrated at molecular weights < 150 kDa. These findings simply implied that sugar: protein ratio could tailor the size of the conjugates. At a protein: glucose ratio of 1:2, the distribution of molecular weights was also investigated as a function of pH (Lanes 5–7). Smearing was once again the case at Lanes 5 and 6 between 25 and 37 kDa, 20 kDa and 10–15 kDa. At pH 7 (Lane 7), while a broad band was observed between 75 and 250 kDa, it did not exist at pH 3 (Lane 5) or pH 5 (Lane 6). This observation could partly be related to the isoelectric precipitation of proteins at pH 3 or 5. Based on 2D-electrophoresis data, black cumin proteins mostly demonstrated neutral or acidic pI values which in turn supported this observation (Coşkun et al. 2019). Similarly, in the previous literature, the major bands for Maillard complexes appeared primarily at pH 7, whereas the likelihood of Maillard reaction taking place was considerably less at lower pH in model systems (Guan et al. 2010; Lertittikul et al. 2007). Conjugate formation results in a decreased band intensity compared to pure protein (Kato 2002) rendering the molecular size evaluation of conjugates difficult. Since the conjugate bands appeared at higher molecular weight spots, these findings indicated covalent binding between proteins and glucose. Especially for high molecular weight proteins, conjugate bands could appear at the border between the stacking gel and the resolving gel (Kato et al. 1988), which was mostly the case for pH 7 samples (Lane 7).

RP-HPLC-RID analysis

Using an HPLC method, glucose binding characteristics of black cumin protein concentrates were determined. A representative HPLC run and a sample calibration curve were depicted on Figure S1 and S2 of Supplementary Data, respectively. As shown on Table 1, there was a tiny amount of unbound fructose (approx. 0.013%) and a higher concentration of unbound glucose (approx. 1.3%) in the concentrates. As heating was carried out, a significant amount of glucose was isomerized to fructose (Suzuki and Tsumura 1972). Meanwhile a certain portion of glucose (Table 1)

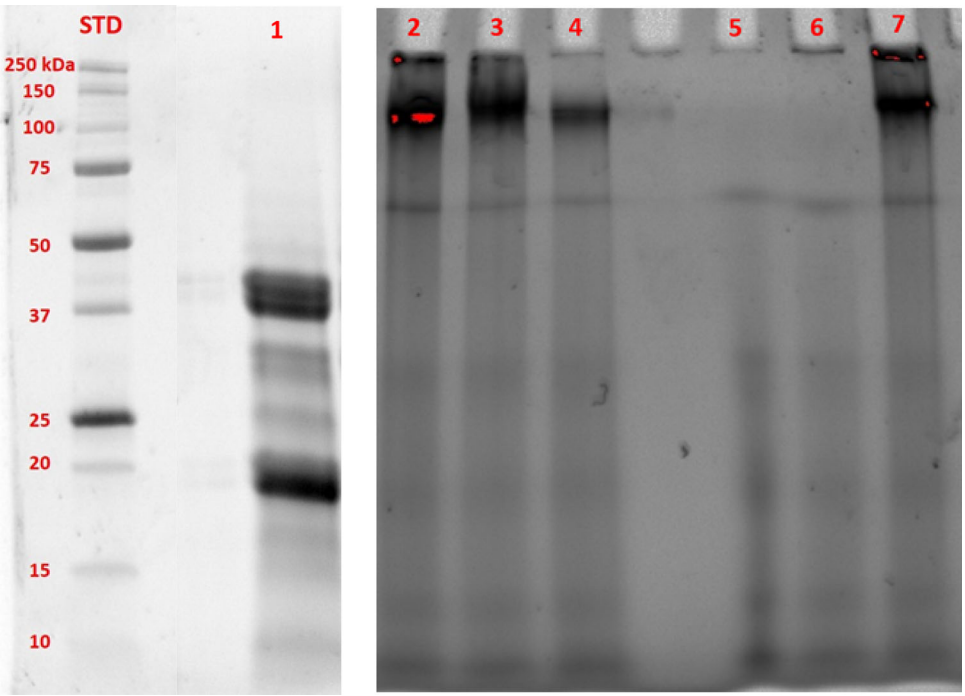

Fig. 1 SDS-PAGE analysis of black cumin protein concentrates (2%) (Lane 1). Native-PAGE analysis of black cumin-glucose Maillard reaction products (30 min, pH 7 at 100 °C) as a function of protein: glucose ratio of 1:1, 1:2, and 1:4 (Lanes 2–4, respectively). Lane 5–7: 1:2 protein: glucose ratio 30 min at pH 3, 5 and 7 respectively

Table 1 Glucose binding (%) and fructose formation (%) characteristics in black cumin protein concentrates due to Maillard conjugation (100 °C) as a function of time at a protein:glucose ratio of 1:2

Time (min)	Concentration after HPLC		Binding (%)
	Fructose (%)	Glucose (%)	
0	0.013 ± 0.01	1.319 ± 0.02	34.0
15	0.345 ± 0.04	0.491 ± 0.07	75.5
30	0.230 ± 0.04	0.289 ± 0.04	85.5

was in all cases conjugated to proteins decreasing the extent of unbound glucose in the medium. Conjugation efficiency was as high as 85.5% after 30 min of processing which was considerably higher than that at 15 min (approx. 75.5%). In the hexane treated samples, once again, the increase in fructose concentration in conjugates 1:2 and 1:4 showed that glucose partly isomerized to fructose during the heat treatment (data not shown). The absence of free fructose or glucose concentration in the 1:1 sample indicated that the black cumin protein bound 100% of all glucose during the heat treatment. The extent of binding accounted for 96 and 88%, respectively, for the 1:2 and 1:4 samples after 30 min of heating. As it will be demonstrated in the later sections, the increase in bound sugar clearly affected foaming capacity.

FT-IR analysis

Spectroscopic analysis of proteins is complicated by various molecular vibrations (Cooper and Knutson 1995), while the most prominent spectral properties for proteins are amide I (between 1700 and 1600 cm^{-1}) and amide II bands (between 1580–1510 cm^{-1}) (Oliver et al. 2009). Amide I band shows stretching vibrations of C = O (70–85%) and C–N (10–20%) groups, and its corresponding components are highly correlated to the secondary structural elements of the proteins (Kong and Yu 2007). For carbohydrates, the saccharide band in the region of 1200–950 cm^{-1} originates from the bending vibration modes of the C–C and C–O–C and C–H bonds. In order to demonstrate the structural changes upon Maillard conjugation, FT-IR spectroscopy was utilized. While Fig. 2 summarizes the findings on conjugation with respect to the amide I and surrounding regions, Fig. 3 summarizes the influence of Maillard conjugation on the formation of glycoprotein peaks.

Various absorbance peaks in the IR spectrum (for example, 1700–1350 cm^{-1}) were affected by the extent of conjugation, since the peak heights for the conjugates were significantly lower compared to the data for unheated protein concentrates (Fig. 2a). Amide I peaks were smaller in the case of glucose conjugated samples, once again demonstrating that Maillard conjugation took place. Comparable results were obtained when hexane treatments were carried out prior to glucose conjugation (Fig. 2b), or when Maillard reaction was carried out using other carbohydrates (lactose and maltodextrin) (Fig. 2c).

Furthermore, second derivative spectra for the Amide I bands were studied (data not shown). The extent of β-turn and β-sheet elements in the samples increased in the conjugate samples as exemplified by 1683, 1662, 1635, and 1616 cm^{-1} peaks for the secondary derivative spectra (Kong and Yu 2007). While reaction with lactose and maltodextrin caused reduction in the intensities of structural element peaks, after hexane treatments, conjugation with glucose enhanced the peak intensities (data not shown).

For sugars or glycoproteins (for example, mucin), carbohydrate region band, tends to be pronounced (Lewis et al. 2013). Consequently, remarkable differences were observed in the band intensity around 1200–1000 cm^{-1} due to the absorption of carbohydrate bound proteins (Fig. 3) (Kacurakova and Wilson 2001; Natalello et al. 2005). In all cases, conjugation with carbohydrates as well as increasing extent of sugar binding accounted for pronounced increases in peak intensity (Fig. 3a, b and c).

Based on FT-IR, HPLC, native- and SDS–PAGE, Maillard conjugation was shown to take place between glucose, lactose or maltodextrin and black cumin proteins, while a variety of structural and molecular size related changes were identified. In the following sections, the influence of these processes on surface activity and foaming capacity is being discussed.

Foaming capacity and stability

Foaming capacity and stability for untreated protein concentrates were studied and compared to that of glucose conjugated proteins (Fig. 4). In addition, the influence of carbohydrate type (Figure S3 of Supplementary Data) and hexane treatments on foaming characteristics were investigated (Figure S4 of Supplementary Data).

At the time of preparation, foaming capacity of pH 5 or pH 7 samples were higher than pH 3 samples (Fig. 4). As time passed, the stability of the foams rapidly decreased in most cases. Especially, in the case of pH 5 samples, the collapse was rapid, whereas for pH 7 samples, approx. 90% of the foam volume was lost after 2 h.

The influence of protein:glucose ratio (1:1–1:4) and heating duration (0–30 min at 100 °C) was monitored (pH 7) on foaming characteristics (Fig. 4). Especially at a ratio of 1:2 and 15 min of treatment, foaming capacity was significantly enhanced. For 1:1 and 1:2 ratios, 15 min treatments were generally more effective than the 30 min

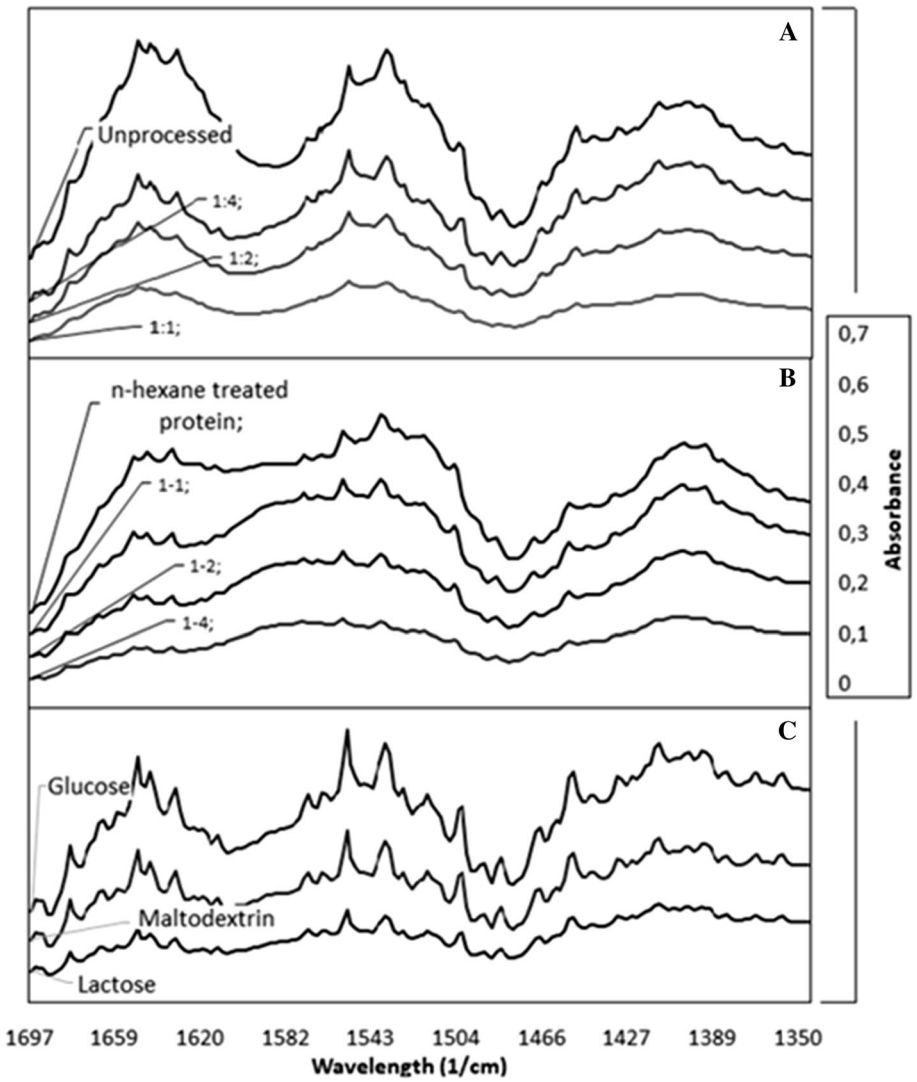

Fig. 2 FT-IR analysis (1700–1350 cm^{-1} spectra) of black cumin protein concentrates and their corresponding Maillard conjugates (**a, b**) at protein:glucose concentration ratios of 1:1, 1:2, 1:4. (**a**) In the absence, or (**b**) in the presence of solvent extraction. (**c**) Similar spectra were also presented as a function of the carbohydrate type at a protein:carbohydrate concentration ratio of 1:2

treatments based on the foam stability. At the highest glucose concentration, sample viscosity is slightly higher than the other samples which in turn could positively affect the foam stability. However, that was not the case. The maximum improvement among the samples took place at a protein:glucose ratio of 1:2 for 15 min process at pH 7, which represented a 23% increase in foaming capacity. These findings were coherent with the previous data (Dickinson and Izgi 1996). Since proteins demonstrate pronounced surface activity and ability to form thick viscoelastic layers and also ability to be strongly solvated by the aqueous medium, they become efficient stabilizers of foams.

Based on a similar protein:carbohydrate ratio (1:2), the influence of thermal treatment duration on Maillard conjugation was studied for lactose and maltodextrin (Figure S3). The foaming capacity of the samples processed at a protein:lactose ratio of 1:2 was 70%, which represented a 16% increase compared to the unprocessed sample, whereas maltodextrin conjugation under similar conditions did not significantly improve the foaming performance. The foaming capacities of lactose or maltodextrin conjugates were considerably lower compared to glucose counterparts. In the literature, reaction mechanisms of monosaccharides and disaccharides with proteins were found to be clearly different (Kato et al. 1988). A previous report indicated a linear correlation between DS and saccharide size. The lower reactivity of maltodextrin and dextran were related to steric hindrance effects (Kato 2002). Possibly due to the larger molecular weights of these molecules, reaction rate was considerably lower (Li et al. 2009).

Finally, glucose conjugation was carried out using hexane treated protein concentrates and their foaming characteristics were studied for different reaction pH (pH 3–7) values and protein:glucose ratios (1:1–1:4) (Figure S4). Once again, foaming capacities of pH 5 and pH 7 samples was higher than pH 3 counterparts. The stability of

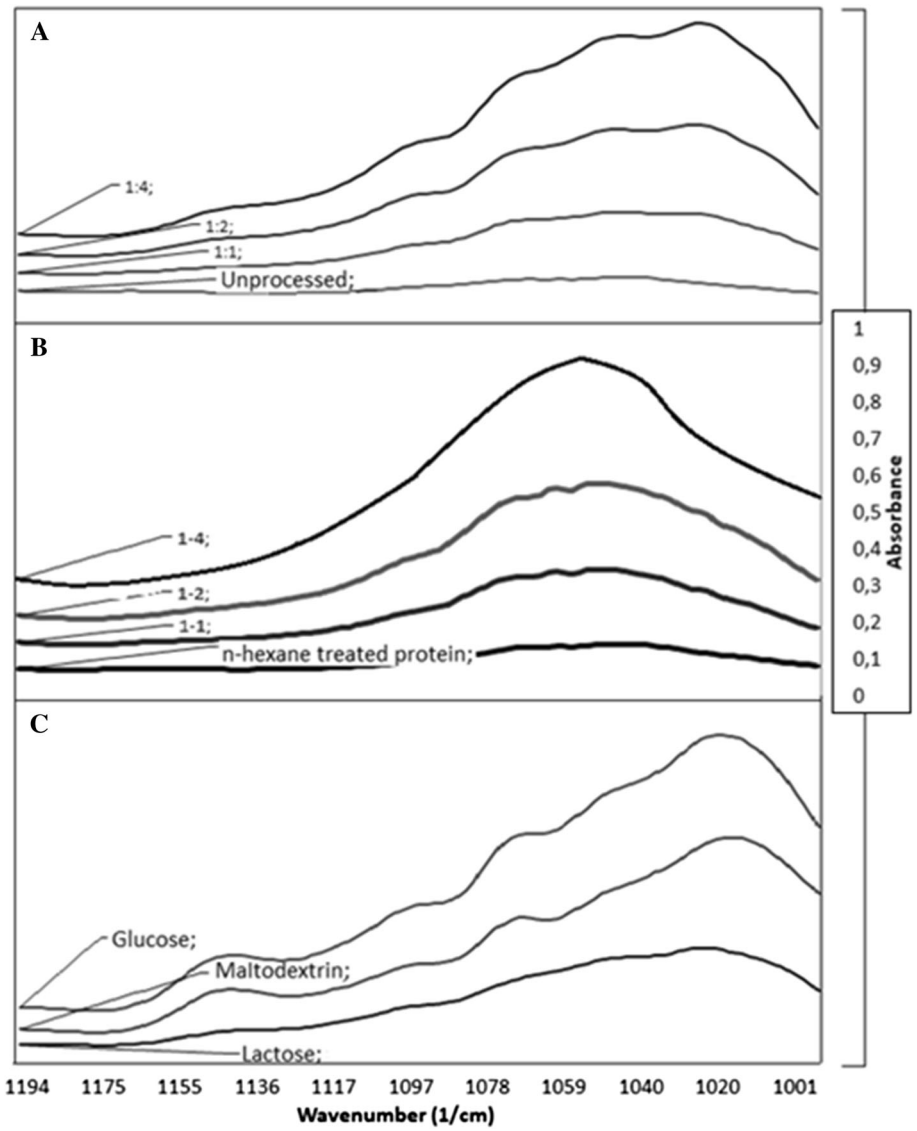

Fig. 3 FT-IR analysis (1200–1000 cm^{-1} spectra) of black cumin protein concentrates and their corresponding Maillard conjugates (**a, b**) at protein:glucose concentration ratios of 1:1, 1:2, 1:4. (**a**) In the absence, or (**b**) in the presence of solvent extraction. (**c**) Similar spectra were also presented as a function of the carbohydrate type at a protein:carbohydrate concentration ratio of 1:2

foams decreased rapidly in most cases, although volume retention was more pronounced compared to the samples that were not hexane treated. pH dependence of foaming capacity was weaker for hexane deoiled samples (Figure S4). After 2 h, foam stability ranged mostly between 40–50%, which was significantly higher compared to both unprocessed protein concentrates and glucose conjugated samples that were not treated by hexane (Fig. 4).

When increasingly more glucose was conjugated to the samples (i.e., beyond 1:2), this process decreased the foaming efficiency, possibly since interfacial packing characteristics of glucose molecules had to be altered at the air–water surface. The increasingly hydrophilic (i.e., glucose bearing) molecular surfaces could have a difficulty at penetrating the surface rendering foams less stable. Similarly, molecular size of the conjugated carbohydrate moiety and remaining oil molecules had a bearing on the surface activity. When remaining oil was present, proteins in part lost their capabilities to interact with and stabilize air phase. Consequently, drop shape tensiometry was utilized to clarify the adsorption characteristics.

Drop shape tensiometry

Dynamic surface tension values of Maillard conjugate dispersions were investigated by means of drop shape tensiometry (Fig. 5a). In the case of the untreated reference, the equilibrium surface tension was approx. 39.6 $mN.m^{-1}$ after 3000 s of adsorption, while for all carbohydrate conjugated samples, the corresponding value was approx. 32 $mN.m^{-1}$. However, adsorption kinetics of these samples were quite different. Essentially, time to reach equilibrium increased with the molecular weight of the conjugated carbohydrate. Glucose and lactose behaved

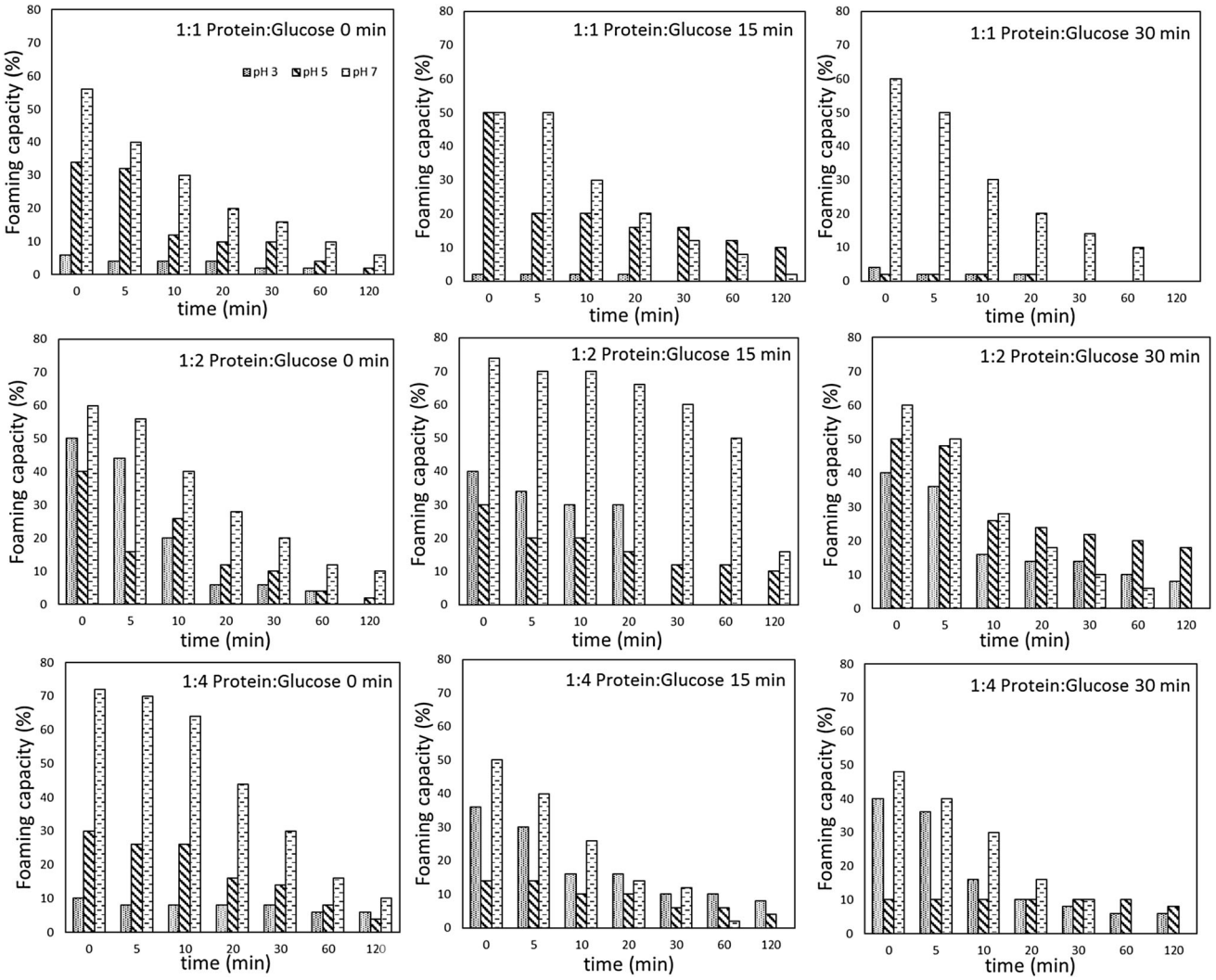

Fig. 4 Foaming capacity (%) and stability of foams prepared with black cumin protein concentrates (2%) and glucose as a function of protein:sugar ratio (1:1, 1:2 and 1:4), pH (3–7), reaction duration (0–30 min) and storage time (0–120 min)

roughly similar, whereas in the first few hundred seconds, tension values for maltodextrin conjugates were considerably higher. In all cases, however, surface pressure increased more rapidly compared to the reference. While conjugate characteristics had a bearing on the adsorption rate, Maillard conjugation enhanced the surface activity of protein concentrates regardless of the type and concentration of the carbohydrate moeity.

Surface elasticity of the conjugates were investigated by the alteration of bubble volume at a constant frequency (0.1 or 0.5 Hz) (Table S1 of Supplementary Data). In the case of glucose, although the adsorption was the fastest, surface elasticity was moderate and found to be frequency dependent. Decreasing surface elasticity with frequency indicated possible deformation and/or displacement of glucose conjugates from the surface which could affect the long-term foam stability.

Lactose samples demonstrated the highest surface elasticity values. Since the adsorption kinetics were reasonably fast and elasticity was pronounced, for delicate foam systems, it could be advantageous to utilize lactose conjugates. Finally, maltodextrin conjugates demonstrated the slowest rate of adsorption and at neither frequency, surface elasticity was ideal. Possibly the large hydrophilic groups of maltodextrin decreased its rate of adsorption and due to the steric effects at the surface, the packing was limited.

Finally, surface tension characteristics were also measured for hexane treated Maillard conjugates (Fig. 5b). In the case of the hexane treated reference, equilibrium surface tension was approx. 30.8 mN.m $^{-1}$ after 3000 s of adsorption, which was considerably lower than that of untreated reference (Fig. 5a). Potential changes occurring during hexane treatment might include partial denaturation, since the denaturation temperature and enthalpy values

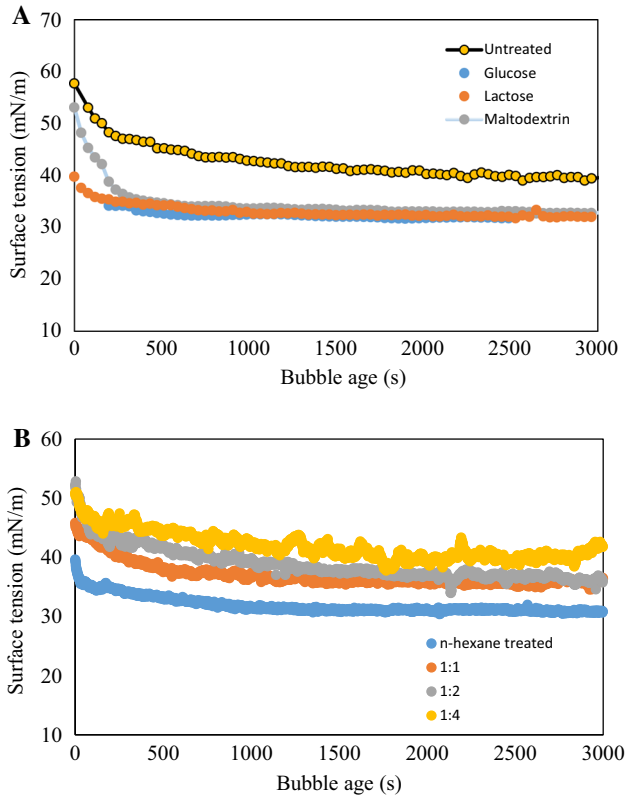

Fig. 5 Dynamic surface tension of black cumin protein Maillard conjugates and the corresponding untreated sample as a function of time (i.e., bubble age) at the air-conjugate dispersion interface. (**a**) Heat treatment was carried out at 100 °C for 15 min at pH 7 and a protein:carbohydrate concentration ratio of 1:2 for glucose, lactose or maltodextrin. (**b**) Based on similar treatments, hexane deoiled black cumin protein concentrates were conjugated with glucose as a function of protein:sugar ratio (1:1, 1:2 and 1:4). Representative runs

decreased for black cumin protein concentrates after hexane extraction (Coşkun et al. 2019). The equilibrium surface tension for the 1:1, 1:2 and 1:4 (protein:glucose) conjugates were observed approximately to be 36 $mN.m^{-1}$, 37 $mN.m^{-1}$, 40 $mN.m^{-1}$, respectively, demonstrating increased equilibrium surface tension values compared to the hexane treated reference. Consequently, while conjugate size affected the adsorption rate, Maillard conjugation increased the surface activity of black cumin protein concentrates primarily in the samples that were not hexane treated. Partly denatured black cumin proteins lost some of their surface activities as more hydrophilic groups were bound to the denatured protein molecules. The changes in surface activity were clearly reflected in the foaming behavior of black cumin proteins.

Consequently, these findings summarized the potential influence of Maillard conjugation and hexane extraction treatments on the foaming performance of black cumin protein concetrates and their Maillard conjugates. As increasingly more air is being incorporated into a food foam upon preparation and mechanical forces are being applied, surface active moieties (in this particular case, proteins and conjugates) are anticipated to rapidly adsorb to the freshly generated bubble surfaces. Adsorption kinetics is a reliable indicator of what might potentially happen in real life settings when foaming is taking place. Materials with superior surface activity may enable the stabilization of foams in the short term. For extended stability, viscoelastic characteristics such as surface elasticity are also critical, since how various surfaces react to destabilization may be affected by this parameter. Consequently, both foam texture and shelf-life may be manipulated through the administered processing techniques.

Conclusion

Valorization of industrial byproducts is a sustainable strategy to generate plant protein products. Plant proteins are natural ingredients and their Maillard conjugates can be manufactured in the absence of toxic chemicals or organic solvents, therefore they can be utilized in many food, cosmetic and pharmaceutical products as foam stabilizers or emulsifiers. Here, Maillard conjugation was exploited to generate foaming agents with enhanced performance. The influence of Maillard conjugation was distinctly different between the samples that were hexane treated or not. The conjugate performance was highly dependent on molecular size of carbohydrates, processing and extraction conditions which in turn determined the consequent structural characteristics. The current research investigated the potential mechanisms that affect foaming capacity and foam stability for Maillard conjugated and/or solvent treated black cumin proteins. Enhanced surface activity or dilatational elasticity of black cumin proteins may lead to enhanced stabilization capabilities in food foams or other food dispersions. These attributes could both enhance foam texture and shelf-life in the final food products. While the results were obtained for low protein concentration samples, due to the current mechanistic approach taken here, the findings are potentially relevant for more concentrated foams as well.

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Compliance with ethical standards

Conflict of interest The authors have declared that they have no conflict of interest.

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