Maillard reaction products formation and antioxidative power of spray dried camel milk powders increases with the inlet temperature of drying

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ABBREVIATIONS ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); **ALA**, α-lactalbumin; **ANS**, 1-anilino-8-naphthalenesulfonate; **CD** circular dichroism; **CM**, camel milk; **CSA**, camel serum albumin; **FD**, freeze drying; **GLYCAM 1**, glycosylation-dependent cell adhesion molecule 1; **HPLC**, high performance liquid chromatography; **MR**, Maillard reaction; **SD**, spray drying; **SDS**, sodium dodecyl sulfate; **UHPLC**, ultra high performance liquid chromatography; **PAGE,** polyacrylamide gel electrophoresis

ABSTRACT

Demand for camel milk (CM) is increasing worldwide, due to its high nutritious value and health benefits. In this study, whole CM powders were produced by spray drying (SD) at six inlet temperatures (190°C - 250°C) and by freeze drying (FD). Physicochemical and functional properties of CM powder proteins were investigated. SD at higher inlet temperatures (230°C - 250°C) resulted in higher extent of Maillard reaction (MR), in comparison to lower temperatures (190°C - 200°C) and FD treatment. Both treatments had negative effect on casein solubility, while whey proteins remained soluble and slightly increased its solubility with the extent of MR. The CM powders obtained at higher inlet temperatures demonstrated improved antioxidant activity. Secondary structure of whey proteins did not differ among the samples, while surface hydrophobicity of whey proteins was higher in all SD than in FD samples, suggesting only limited denaturation of camel whey proteins at higher inlet temperatures of drying. Thus, the effects of SD under the conditions applied in our study did not decrease camel whey protein solubility, while drying procedure itself regardless of temperature decreased solubility of camel milk caseins. MR generated during CM processing could be an important means of compensating for the lack of antioxidant protection normally associated with β-lactoglobulin but happens to be absent from this milk.

Key words:

Camel milk; camel milk powder; spray drying; inlet temperature; Maillard reaction

1. INTRODUCTION

Camel milk (CM) is gaining increasing popularity among consumers worldwide. Traditionally, it is consumed in arid and semi-arid regions as fresh or soured milk. Gross composition of CM is similar to that of ruminant species with slightly lower content of total solids, fat, protein and lactose, and higher content of minerals and vitamin C (Yadav, Kumar, Priyadarshini, & Singh, 2015; Yoganandi, Mehta, Wadhwani, Darji, & Aparnathi, 2014). CM protein composition differs from the ruminant milk. β-lactoglobulin is absent from CM, and α-lactoglobulin is the major whey protein similarly to human milk. The lack of β-lactoglobulin makes CM hypoallergenic (Ehlayel, Bener, Abu Hazeima, & Al-Mesaifri, 2011). CM has higher whey protein to caseins ratio compared to cow's milk, which is responsible for the formation of soft and easily digestible coagulum (Berhe, Seifu, Ipsen, Kurtu, & Hansen, 2017; Shamsia, 2009). CM has the smallest milk-fat globules, in comparison to ruminants, which do not naturally aggregate due to the absence of agglutinin (Khalesi, Salami, Moslehishad, Winterburn, & Moosavi-Movahedi, 2017). Together with the absence of β-lactoglobulin this results in better digestibility of CM in the human gastrointestinal tract (Meena, Rajput, & Sharma, 2014). High content of lysozyme, lactoferrin, lactoperoxidase and immunoglobulins confer to CM high antimicrobial activity (El Agamy, Ruppanner, Ismail, Champagne, & Assa, 1992). Bioactive peptides from CM were found to exert inhibitory activity towards key metabolic enzymes related to diabetes and obesity (Mudgil, Kamal, Yuen, & Maqsood, 2018). Many other therapeutic properties have been reported for CM such as antihypertensive, antithrombotic, anticancer and antiviral activity (Berhe et al., 2017; El Agamy et al., 1992; Mati et al., 2017). Apart from numerous health promoting effects, milk consumption possess certain health risks which are primarily associated with milk contamination by aflatoxins, secondary metabolites of some *Aspergillus spp*. members

(Ketney, Santini, & Oancea, 2017). Interestingly, CM has been reported to contain lower levels of aflatoxins compared to ruminant milk (Hussain, Anwar, Asi, Munawar, & Kashif, 2010; Rahimi, Bonyadian, Rafei, & Kazemeini, 2010).

Milk dehydration and production of milk powders is commonly used to stabilize milk constituents for their storage and to facilitate transportation. Cow's milk powders are highly utilized in food industry as food additives to improve color, flavor, texture, and nutritional value of dairy and non-dairy products, but also to improve emulsifying, gelling and foaming characteristics of food products. The most frequently employed technique to produce milk powders is spray drying (SD). It involves SD of milk into a current of hot gas where water from the fine droplets is rapidly evaporated (Schuck et al., 2016). SD parameters influencing physicochemical characteristics of milk/dairy powders are feed solids concentration, milk flow rate, inlet temperature, outlet temperature, and nozzle air pressure. Drying air temperature has significant effects on water activity, glass transition temperature, color properties, and particle morphology of final milk powders (Habtegebriel, Edward, Wawire, Sila, & Seifu, 2018; Ogolla et al., 2019; Zouari et al., 2018). It has been observed that higher inlet temperatures give rise to bigger particles with lower surface free fat, which reduces lipid peroxidation in bovine milk powders resulting in improved flavor quality (Nijdam & Langrish, 2006; Park, Bastian, Farkas, & Drake, 2014; Park, Stout, & Drake, 2016).

During drying, particles usually reach temperature considerably lower than inlet temperature, since the thermal energy is consumed for evaporation (Schuck, le Floch-Fouere, & Jeantet, 2013). Still, SD process creates conditions under which Maillard reaction (MR) may take place, i.e. water evaporation, high temperature, high concentration of lactose and lysine-rich proteins, reduced water activity. In dairy products, lysyl residues of milk proteins react with carbonyl

groups of lactose to initiate MR. Lactosylation of cow's milk proteins *via* MR upon SD treatment has been documented in the literature (Mehta & Deeth, 2016). Extensive lactosylation was found on skim bovine milk powder proteins when SD at inlet temperature 185°C (90°C outlet temperature) (Guyomarc'h, Warin, Muir, & Leaver, 2000). SD outlet temperature exerted strong effect on browning of bovine cheese powder *via* MR (Koca, Erbay, & Kaymak-Ertekin, 2015).

Effects of SD on MR products of CM were rarely studied. The increase in drying air temperature and a decrease in flow rate caused reduction of lightness of CM powder indicating occurrence of MR and caramelisation (Ogolla et al., 2019). Sulieman et al. found SD CM powders to be lighter in color compared to cow's milk powders suggesting lower degree of MR (Sulieman, Elamin, Elkhalifa, & Laleye, 2014). Most of the studies investigating MR of dried dairy products were done on cow's milk, and data about MR products and antioxidant capacity of SD CM in relation to varying drying conditions are still scarce.

Therefore, the objective of the study was to compare the effects of two methods for milk powder preparation on CM proteins and MR: SD, as high temperature-based method favoring MR, and freeze drying (FD), as low temperature-based method. SD CM powders were produced at six different inlet temperatures (190°C - 250°C) using a pilot spray dryer. Protein profile, protein structure and MR in SD CM powders, in relation to inlet temperature, were compared to FD CM powder. Functional properties such as solubility and antioxidant activity were assessed.

2. MATERIALS AND METHODS

2.1. Materials

All the chemicals were of analytical grade, purchased from Sigma-Aldrich (St. Louis, MO, USA). Milli-Q water (Millipore, France) was used for all experiments.

2.2. Spray drying of camel milk

The spray drying experiments were conducted in the food pilot plant, Department of Food Science, College of Food and Agriculture, UAE University. Fresh raw CM was supplied by Al-Ain Farms (UAE), one of the largest camel farms in UAE with around 1,500 lactating camels, and immediately stored at +4°C. Whole milk was concentrated to 28% solids (wt/wt) using a pilot unit vacuum evaporator (Model FT22, Armfield Ltd, UK) with boiling temperature set at 70°C. The concentrated CM was homogenized by pressure to emulsify the free fat that may release during evaporation. The concentrated and homogenized CM was dried using a pilot model spray dryer (Model FT80, Armfield Ltd, UK), and the spray drying process is presented in Fig. S1. The counter current configuration of the spray dryer was used with a maximum water evaporation rate of 3 l/h. In this study, the twin fluid pressure nozzle atomizer was used. Shearing between high velocity air and low velocity liquid disintegrates the liquid stream into droplets, producing a high velocity spray. The inlet temperature was set at six levels: 190°C, 200°C, 210°C, 230°C, 240°C and 250°C, and the corresponding outlet temperatures were as follow: 71°C, 74°C, 78°C, 84°C, 88°C and 92°C. FD (lyophilized) CM was prepared with a vertical freeze-dryer Telstar, Cryodos – 80 model (Terrassa, Spain). During the process, the chamber temperature was maintained at approximately -80°C and 0.05 mbar. The freeze drying process took 4 weeks to remove water from milk samples and reach the equilibrium. After drying all powders (SD and FD) were immediately stored at -21°C for 2 months until analyses, and at

+4°C during analysis (6 months). All powder samples were produced from the same batch of fresh milk. The moisture, protein and ash content are given in the Table S1.

2.3. Reconstitution of camel milk powders

Camel milk powders were reconstituted in 20 mmol/L sodium phosphate buffer pH 6.8 to a final concentration of 50 mg/ml with gentle stirring at room temperature for 1h. Insoluble protein fractions were removed by centrifugation at 10000 x g for 10 min. Supernatants, containing phosphate buffer soluble proteins of CM powders, are termed as soluble protein fractions in further text. Soluble protein fractions were defatted by tetrachloroethylene extraction (2:1 v/v) and dialyzed against 20 mmol/L sodium phosphate buffer pH 6.8. Protein concentration was determined by bicinhoninic acid assay (Pierce, Amsterdam, The Netherlands).

For analysis of total proteins of CM powders, milk powders were dissolved in denaturing buffer (8 mol/L urea, 2 mol/L thiourea, 2% SDS, 20 mmol/L sodium phosphate pH 6.8) at concentration 200 mg of powder/ml, and diluted in 20 mmol/L sodium phosphate buffer pH 6.8 for further analyses.

2.4. Electrophoretic analysis

Experimental details on electrophoretic analysis are described in Supplementary material.

2.5. Proteomic identification of camel milk proteins from soluble fraction of freeze dried camel milk powder

The method is described in Supplementary material. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD023290 and 10.6019/PXD023290.

2.6. Size exclusion chromatography

Size exclusion chromatography was performed on UHPLC workstation Nexera XR (Shimadzu Corporation, Kyoto, Japan) using column ACQUITY UPLC Protein BEH SEC 125Å (4.6×150 mm I.D., Waters, Milford, MA, USA). UV spectra were recorded at 220nm. Samples were applied to column at concentration 0.35 mg/ml. BEH125 SEC Protein Standard Mix (Waters) was used for column calibration. The data acquisition was performed using LabSolutions CS Analysis Data System (Shimadzu Corporation).

2.7. Monitoring of Maillard reaction

Fluorescent spectra of CM proteins were recorded on Horiba Scientific Fluoromax-4 spectrofluorimeter (Horiba, Kyoto, Japan) in a 10 mm path length cell. Dialyzed soluble protein fraction was diluted to 0.5 mg/ml, and total CM proteins to 2 mg of powder/ml, in 20 mmol/L sodium phosphate buffer pH 6.8. The samples were excited at 340 nm and emission spectra were recorded in 350-600 nm range. The absorbance at 294 nm and 420 nm were measured by NanoDrop 2000c spectrophotometer (Thermo Scientific, USA). Protein concentration in soluble fraction was 3 mg/ml for A420 and A294, while for absorbance monitoring of total proteins 200 mg of powder/ml for A420 and 10 mg of powder/ml for A294.

2.8. Determination of protein carbonyls

Carbonyls were determined by 2,4-dinitrophenylhydrazine assay in soluble protein fraction (3 mg/ml) and in total CM proteins (12 mg of powder/ml) as described in (Perusko, Al-Hanish, Cirkovic Velickovic, & Stanic-Vucinic, 2015).

2.9. Free amino group content

The content of free amino groups was determined by ortho-phthalaldehyde method (Guan, Qiu, Liu, Hua, & Ma, 2006), except that 50 µl of dialyzed soluble fractions (0.5 mg/ml) were

incubated with 200 µl of ortho-phthalaldehyde reagent. The content of remained free amino groups was expressed in percentages relative to FD sample which was taken as 100%.

2.10. Circular dichroism spectroscopy

Secondary structures of CM proteins were monitored by recording far-UV CD spectra. Experimental details are described in Supplementary material.

2.11. Determination of surface hydrophobicity

Surface hydrophobicity was determined based on the binding of the fluorescence probe 1-anilino-8-naphthalenesulfonate (ANS). Fluorescence spectra of dialyzed soluble protein fraction (0.4 mg/ml), saturated by ANS (80 µmol/L) in 10 mmol/L sodium phosphate buffer pH 7.2 were recorded at excitation wavelength 350 nm, after incubation in the dark for 30 min.

2.12. Solubility of spray dried camel milk proteins

50 mg of milk powders was mixed with 1 ml of 20 mmol/L sodium phosphate buffer pH 6.8 at room temperature with gentle mixing at rocker for 1h. The CM samples were centrifuged at 10000 x g for 20 min. The supernatants were withdrawn, defatted by tetrachloroethylene extraction (2:1 v/v) and protein concentration was determined.

2.13. Antioxidant activity

$ABTS^{\bullet+}$ solution was generated in a reaction of 14 mmol/L ABTS with 5 mmol/L potassium persulfate for 24 hours protected from the light and subsequently was diluted to give absorbance approximately 0.9 at 670 nm. The aliquots of soluble protein fractions (30 µl, 2.00 mg/ml) were added to 120 µl of $ABTS^{\bullet+}$ reagent and allowed to react for 6 min. Absorbance was measured at ELISA reader with 670 nm filter. Reducing power of soluble protein fractions (2.5 mg/ml) was determined according to (Perusko et al., 2015).

2.14. Determination of lipid peroxidation

Lipid peroxidation was estimated by determination of malondialdehyde. Experimental details are described in Supplementary material.

2.15. Statistical analysis

The data are presented as mean ± standard deviation for experiments done in at least duplicates. Differences between the variables were tested for significance by one-way ANOVA accompanied with Tukey's post-hoc test using Origin Pro 8.5.1 (OriginLab, Northampton, MA). Differences at $p < 0.05$ were significant.

3. RESULTS

3.1. Characterization of spray dried camel milk powder proteins by electrophoresis

In the present study we have produced whole CM powders by FD and SD treatment at six inlet temperatures, 190°C, 200°C, 210°C, 230°C, 240°C and 250°C, and the samples were termed FD, SD190, SD200, SD210, SD230, SD240 and SD250, respectively. Physicochemical properties of CM proteins were compared and related to the degree of MR product formation. FD or SD whole CM powders, reconstituted in 20 mmol/L phosphate buffer pH 6.8, were centrifuged (10000 x g for 10 min) and defatted. The obtained supernatants were termed soluble protein fraction, and the term insoluble fraction was used for pellet. Soluble fraction contained only about 25% of total CM powder proteins. Total CM powder proteins were obtained by complete dissolving of CM powders in denaturing buffer (8 mol/L urea, 2 mol/L thiourea, 2% SDS, 20 mmol/L sodium phosphate pH 6.8), and this solution was termed total proteins.

Total proteins, soluble fractions and insoluble fractions were analyzed by SDS PAGE under reducing conditions (Fig. 1A, 1B and 1C). The major protein bands were identified by LC-

MS/MS (Fig. 1D and Excel tables E1). The most abundant proteins of total protein samples were α_{s1}- and β-casein with electrophoretic mobility corresponding to 30-35 kDa, as camel caseins are known for their lower electrophoretic mobility, in comparison to their bovine counterparts, depending on their phosphorylation patterns (Saliha, Dalila, Chahra, Saliha, & Abderrahmane, 2013). In soluble fractions caseins were dramatically under-represented, especially α_{s1}-caseins (Fig. 1B), suggesting that soluble fraction contains mostly camel whey proteins (about 85%). In contrast, insoluble fraction contained mostly caseins, with very low level of whey proteins (Fig. 1C). Since CM caseins showed low solubility in both FD and SD samples, it seems that evaporation process, rather than high temperature, influenced their solubility. Caseins are known as proteins with no well-defined secondary and tertiary structures, and during evaporation casein micelles interact through casein molecules, mainly through hydrophobic interactions to form aggregates. The main factor in stabilization of casein micelles is the presence of κ-casein at their surface, and its glycosylated forms confer them electrostatic repulsion due to a negative charge, steric hindrance, and increased hydrophilicity (Broyard & Gaucheron, 2015). In contrast to bovine milk, CM casein micelles are known to contain higher content of hydrophobic β-casein, and also they are sparsely covered by κ-casein which is predominantly present in non-glycosylated form (Kappeler, Farah, & Puhan, 1998). Therefore, CM micelles might be more prone to aggregation by hydrophobic interaction and show decreased heat stability in comparison to bovine milk micelles. During storage, the casein micelles associate more firmly resulting in further decrease of milk powder solubility (Bansal, Truong, & Bhandari, 2017; Fang, Rogers, Selomulya, & Chen, 2012; Felix da Silva, Ahrné, Ipsen, & Hougaard, 2018). In general, CM proteins are known to be considerably less soluble than bovine milk proteins at neutral and alkaline pH (Maqsood et al., 2019).

Soluble fractions were also analyzed by SDS PAGE under non-reducing conditions (Fig. 1E) and by native electrophoresis (Fig. 1F). Although the protein profiles of SD samples and FD sample were the same under reducing as well as non-reducing conditions (Fig. 1A, 1C and 1D), SD at 200°C, or higher inlet temperatures, induced smear of the major protein bands, α-lactalbumin (ALA), glycosylation-dependent cell adhesion molecule 1 (GLYCAM 1) and camel serum albumin (CSA) towards higher molecular weights (Fig. 1B and 1E). The native electrophoresis (Fig. 1F) showed pronounced band smearing towards more acidic position in all SD samples, due to decreased protein pI values. The native electrophoresis (Fig. 1F) also showed high molecular weight constituents in the stacking gel in all samples. These soluble aggregates were of non-covalent nature, since denaturing reducing, and non-reducing conditions evidenced no disulfide or other covalent polymers. In insoluble fraction (Fig. 1C), smearing of α_{s1}- and β-caseins could be observed, which was in paralel to smearing of whey proteins, and this suggests that the most dominat proteins of both fractions are modified. Thus, electrophoretic analysis evidenced indiscrete gradual increase in protein molecular weights (SDS-PAGE) and acidification (native electrophoresis), indicating covalent modification of CM proteins *via* MR, which is promoted at high temperatures.

3.2. Characterization of proteins of soluble fraction by gel filtration

Proteins of soluble fraction were analyzed by gel filtration, and their overlaid chromatograms are shown in Fig. 2. All samples contained soluble noncovalent aggregates with mass of about 200 kDa (Rt 3.00 min, box I) and 112 kDa (Rt 3.44 min, box II), also noticed in native PAGE (Fig 1F). However, Rt of the later (box II) in SD samples is shifted up to 3.42 min, implying that they are modified up to mass of about 115 kDa. In FD sample the peak of CSA was eluted at about 65 kDa (box III, Rt 3.89 min), and in SD samples it was modified up to about 68.5 kDa (box III, Rt

3.85 min). Similarly, the mass of ALA in FD sample was about 12.5 kDa (box VII, Rt 12.5 min), while in SD samples ALA is modified up to 13.5 kDa (box VII, Rt 5.16 min). In addition to Rt shift, broadening of ALA peak, especially at higher SD inlet temperatures, can be observed. This implies intensive modification of ALA via MR, which was also observed in electrophoresis (Fig. 1B, 1E and 1F). The peaks in box IV, box V and box VI originate from αS1 -casein (Rt 4.44, 33 kDda), β-casein/κ-casein (Rt 4.65, 25.5 kDa) and GLYCAM 1 (Rt 4.75, 22.5 kDa), respectively.

3.3. Monitoring of Maillard reaction

Spectrophotometric and fluorescence measurements, as indicators of MR, were done to compare the degree of MR among the samples. The formation of fluorophores, with a maximum emission at about 425 nm when excited at 350 nm, indicating early MR stages (Jing & Kitts, 2002), were monitored. Fluorescence intensity of SD samples obtained by higher inlet temperatures (SD230 – SD250) were significantly ($p < 0.05$) then in FD sample and SD samples dried at lower temperatures (SD190 – SD210), in both total protein and soluble protein fraction (Fig. 3A, 3B and 3C). This suggests that extent of MR was dependent on drying temperature, but also that extent of MR of soluble protein fraction could represent the extent of MR of whole CM powders.

The UV absorbance at 294 nm, which indicates the formation of intermediate products (Ajandouz, Tchiakpe, Dalle Ore, Benajiba, & Puigserver, 2001), was employed to follow the progress of MR in CM samples. Similarly to fluorescence intensity, A294 of samples dried at highest temperatures (SD240 and SD250) was significantly ($p < 0.05$) higher than of the samples obtained by the lowest drying temperatures (SD190 and SD200) and FD sample, in both total protein and soluble protein fraction (Fig. 3D and 3E). The browning intensity (A420 nm), as the late phase MR indicator, was almost not detectable in any of SD samples (Fig. 3D) of soluble

fraction, due to their low concentration. However, in total protein samples browning was observed (Fig. 3E), being significantly ($p < 0.05$) higher at higher drying temperatures than at lower ones and in FD sample, therefore again showing dependence of MR extent on drying temperature.

The MR was further monitored in soluble fraction by the comparison of remained available – NH_2 groups for both type of treatments (Fig. 3F). Higher inlet temperatures (SD230 – SD250) resulted in significantly ($p < 0.05$) lower content of free amino groups in comparison to FD and SD190 samples. In the sample SD250 remained amino group content was about 25% lower than in FD sample.

These results suggest that higher SD inlet temperatures result in higher degree of MR in comparison to lower temperatures, while absence of significant difference in MR extent between SD at lower inlet temperatures and FD indicates that SD at lower inlet temperatures are not able to notably accelerate MR. Park et al. (Park et al., 2016) also observed higher degree of MR in nonfat bovine milk powder after SD with inlet temperature of 260°C than with 160°C or 210°C. Domination of early stages of MR seems to be the result of very short exposure to high temperatures during drying (Maltesen & van de Weert, 2008).

The reactive α-dicarbonyls, oxidation products of reducing sugars formed during MR in food systems, lead to oxidative deamination of basic amino acids and hence, cause protein carbonylation (Luna & Estévez, 2018; Villaverde & Estévez, 2013). Therefore, we have investigated formation of protein carbonyls and their relation to MR extent. Fig. 4A showed that in soluble fraction protein carbonyl content in samples SD at higher inlet temperatures (SD230 – SD250) was significantly higher ($p < 0.05$) than FD and SD190 samples. Similarly, protein

carbonyl content in total CM proteins was significantly higher ($p < 0.05$) after high-temperature drying than after FD and low-temperature drying (Fig. 4B). Moreover, protein carbonyls content strongly correlated ($p < 0.005$) with all parameters for extent of MR (Fig. S2), suggesting that their generation is highly dependent on MR onward. Therefore, in addition to protein glycation due to MR, oxidative modifications of proteins, influencing their structural and functional properties, should also be taken into account for monitoring of MR. In the last few years, several studies demonstrated formation of food protein carbonyls during MR, especially of milk proteins (Choudhary, Arora, Kumari, Narwal, & Sharma, 2017; Oh et al., 2016; Perusko et al., 2015). This study, by showing the dependence of protein carbonyls content on MR extent, implies that their formation can be used as additional parameter for monitoring of MR in the future studies.

3.4. Protein structure analysis

To compare the secondary structures of soluble protein fraction of CM powders obtained by different methods, far-UV CD spectra were recorded (Fig. 5A). All SD and FD samples showed very similar spectra with peak minimum around 209 nm originating from whey proteins. Indeed, calculation of secondary structure fractions using mathematical model published by Raussens et al. (Raussens, Ruysschaert, & Goormaghtigh, 2003), revealed that there were no significant ($p < 0.05$) differences in the percentages of secondary structures among SD samples, neither between FD and SD samples (Table S2). These results suggest that high inlet temperatures up to 250°C are not a factor influencing camel whey protein secondary structures. Also, presence of sugar (lactose) exerted thermoprotective effect on the protein secondary structure (Haque, Chen, Aldred, & Adhikari, 2015).

Some insight into tertiary structure differences between SD and FD samples were examined by binding of hydrophobic probe ANS, whose fluorescence intensity increases upon its non-

covalent binding to hydrophobic patches on protein surfaces. All SD samples in 190°C – 250°C range showed higher quantum yield after ANS addition, compared to FD sample (Fig. 5B), evidencing higher surface hydrophobicity. The highest hydrophobicity was observed in SD190 sample due to partial protein unfolding and exposure of hydrophobic regions. In SD200 and SD210 samples hydrophobicity slowly decreased because of increased covalent binding of hydrophilic saccharide moieties *via* MR. However, under higher inlet temperatures during drying (SD230 – SD250) protein unfolding and hydrophobic regions exposure exceeds introduction of hydrophilicity, resulting in higher hydrophobicity than in sample SD210. Therefore, changes in protein surface hydrophobicity are result of hydrophobic region exposure, depending on protein unfolding, and extent of protein modification by hydrophilic species, depending on progression of MR. In addition, it should be mentioned that in all samples only small part of caseins remained, whose surface hydrophobicity is the most sensitive to high temperatures.

These results imply that short exposure time to high temperatures during SD was long enough to induce transient partial unfolding and higher surface hydrophobicity then in FD sample, but too short to induce differences in protein secondary structure of SD milk compared to FD milk.

3.5. Solubility of freeze dried and spray dried camel milk powders

Protein solubility of CM powders was estimated by measuring protein concetration in the supernatants obtained after reconstitution of milk powders. The samples obtained at higher inlet temperatures (SD230 – SD250), showed significant ($p < 0.05$) increase in solubility compared to samples dried at lower temperatures (SD190 – SD210) or by FD (Fig. 6A). Similarly, SD of egg white at inlet temperature of 180°C did not alter protein solubility (Katekhong & Charoenrein, 2017). Also, there was not much effect of inlet air temperature on solubility of goat milk powder obtained by SD in the range 160 – 180°C (Reddy et al., 2014). Higher solubility can be

359 explained by higher degree of attached lactose units to compact whey proteins *via* MR, providing
360 steric hindrance and making CM proteins more hydrophilic, thus reducing aggregation through
361 hydrophobic interactions. Indeed, solubility of the samples highly correlated with extent of MR
362 (Fig. 6D and S3). Thus, higher extent of MR may slightly overcame formation of insoluble non-
363 covalent aggregates at higher temperatures, resulting in increased protein solubility. Different
364 outcomes have been observed in different studies. Previously published studies demonstrated
365 that MR may improve protein solubility, as well as thermal stability (Liu & Zhong, 2012;
366 Perusko et al., 2015). During storage/aging of milk protein powders, MR is actually decreasing
367 the solubility, presumably by an increased cross-linking of proteins (Fan et al., 2018). Other
368 authors demonstrated that operating at lower spray drying temperature ranges (140°C) is
369 important for minimizing loss of the solubility of camel milk proteins (other studied inlet
370 temperatures were 170 and 200°C). The authors also observed some loss of proteins solubility
371 and extensive protein denaturation at elevated inlet/outlet temperature (250°C inlet (120°C
372 outlet)) (Anandharamakrishnan, Rielly, & Stapley, 2007). Thus, the effect of spray drying on
373 protein solubility is very complex and may depend on the variety of factors, such as extent of
374 MR, degree of protein cross-linking and protein denaturation and sample itself. In our study, no
375 significant structural changes/protein denaturation was observed and the extent of MR seems not
376 to be high (no observable cross-linking and denaturation), minimizing negative effects of applied
377 drying process on the solubility of proteins.

378 **3.6. Antioxidant activity of soluble fraction of camel milk powders**

379 Antioxidant properties of CM originate from its high content of vitamin C (Hailu et al., 2016)
380 and CM proteins such as β-casein, α-lactalbumin and lactoferrin (Berhe et al., 2017). Here, we
381 examined relation between the inlet temperature and antioxidant activity of soluble CM proteins

(Fig. 6B and C). Soluble fraction demonstrated general trend of increased $ABTS^{\bullet+}$ scavenging ability (Fig. 6B), and reducing power (Fig. 6C), with increase of inlet temperature. The samples dried at inlet temperatures 210°C to 250°C were significantly more powerful $ABTS^{\bullet+}$ scavengers than FD and SD190 samples. $ABTS^{\bullet+}$ scavenging capacity correlated with the extent of MR (Fig. 6D and S4). The reducing power of samples obtained at inlet temperatures SD230 to SD250 was significantly higher ($p < 0.05$) compared to FD and SD190 to SD210 samples. The reducing power correlated ($p < 0.005$) with all parameters for extent of MR (Fig. 6D and S5).

Early MR products of bovine milk proteins in milk powder and sweetened condensed milk were shown to possess antioxidant properties (Cortés Yáñez, Gagneten, Leiva, & Malec, 2018). Among industrial heat treated bovine milk (UHT, Microfiltered and High Quality Pasteurized), UHT treated milk showed the highest antioxidant capacity as a result of severe heat-treatment which induced formation of antioxidant compounds *via* MR (Manzi & Durazzo, 2017). Higher antioxidant activity of CM samples dried at higher inlet temperatures, could contribute to improved CM powder shelf-live. Antioxidant activity derived from MR products have important role during storage of dried diary-based products, retarding lipid peroxidation and formation of volatile off-flavors (Giroux, Houde, & Britten, 2010; McGookin & Augustin, 1997). Indeed, determination of lipid peroxidation in lipid fraction of CM powder samples (Fig. 6E) demonstrated significantly ($p < 0.05$) lower peroxidation in SD samples dried at lower temperatures (190°C and 200°C) than in FD sample, due to antioxidant effects of MR products. However, with increase of inlet temperatures lipid peroxidation was more intense, indicating that at higher temperatures lipid peroxidation overwhelmed protecting effect of generated MR products. Regardless of that, lipid peroxidation in samples obtained at inlet temperatures up to 240°C is not higher than its level in FD samples due to antioxidant effects of MR products. Park

et al. demonstrated that during SD of bovine WPC higher inlet temperatures resulted in lower extent of lipid peroxidation in comparison to lower inlet temperatures (Park et al., 2014). This was explained by larger particle sizes encapsulating more fat during the drying process, and thus decreasing the amount of free fat. However, it should be mentioned that half of protein content in their samples was β-lactoglobulin, significantly contributing to antioxidant activity due to its free thiol group, which is lacking in CM. The results of this study suggest that higher antioxidant activity, induced by higher inlet temperatures, could also contribute to counteract lipid peroxidation.

Many antioxidants, which are natural components of food, are sensitive to processing conditions (such as temperature, pH, UV radiation) and storage, and thus are mostly lost. In contrast, processing and storage conditions mainly increase the content of MRPs, resulting in antioxidant enrichment in foods. Also, milk-based product "dulce de leche", in addition to its pleasant aroma and color, have exceptional antioxidant capacity due to MRPs (Cortés Yáñez et al., 2018). In bovine milk, preheat temperatures trigger the release of free -SH groups (primarily from β-lactoglobulin) which provide some antioxidant protection during storage of the resulting whole milk powder. However, as camel milk is lacking β-lactoglobulin, generation of MRPs could be important compensation of antioxidant protection not only in camel milk, but also in other β-lactoglobulin–free milks, such as BLG-free milk produced by BLG bi-allelic knockout cow (Sun et al., 2018). On the other hand, the price for processing/storage-induced MRP-derived antioxidant enrichment is loss of lysine, and decreased nutritional value. Therefore, in milk-based products, especially β-lactoglobulin–free milks, MR should not be completely avoided, but antioxidant formation and loss of nutritional value should be balanced by controlled extent of Maillard reaction during processing/storage.

4. CONCLUSIONS

Camel milk powder represents an attractive ingredient for food industry, and better understanding of physicochemical properties of proteins of CM powders, arisen during CM processing is needed for industrial manufacture of high quality powder. In this work, effects of SD, and inlet temperature as an important SD parameter, on CM proteins and MR were compared to effects of FD. CM caseins either from FD or SD powders demonstrated poor solubility. Inlet temperature of SD exerted significant effect on the MR of CM proteins, and higher temperatures promoted MR to a higher extent, in comparison to lower SD temperatures applied in this study and FD. Whey proteins, which remained soluble at all tested inlet temperatures, were modified *via* MR, and this was the most pronounced for ALA. Promoted MR resulted in formation of protein carbonyls, where carbonyl content strongly correlated with the extent of MR. Higher degree of MR, observed in samples treated at higher SD temperatures, also strongly correlated with improved functional properties, such as stronger antioxidant power. As CM lacks important antioxidant β-lactoglobulin, certain extent of MR could be desirable as compensation. Therefore, the controlled level of MR during processing/storage of CM-based systems would provide a balance between antioxidant enrichment and loss of nutritional value.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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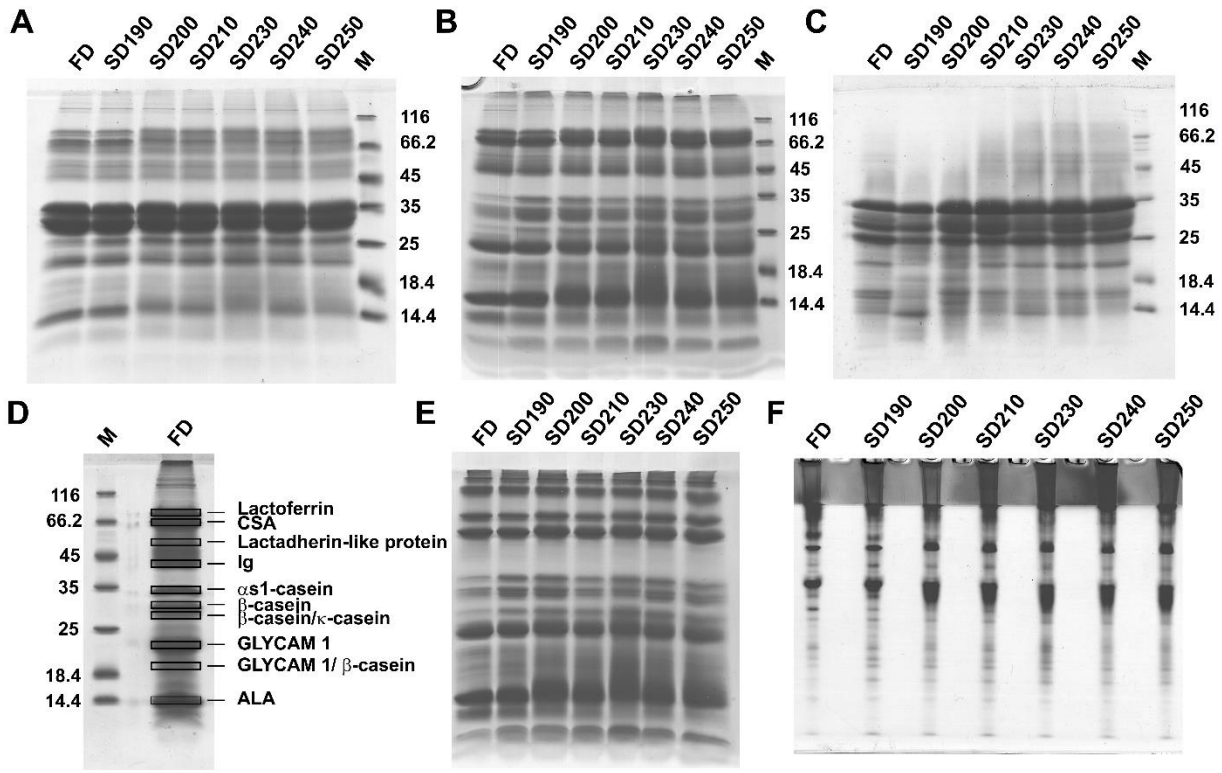
610 dried camel milk powder. *Drying Technology, 37*(13), 1615-1624. doi:
611 10.1080/07373937.2018.1526189

612

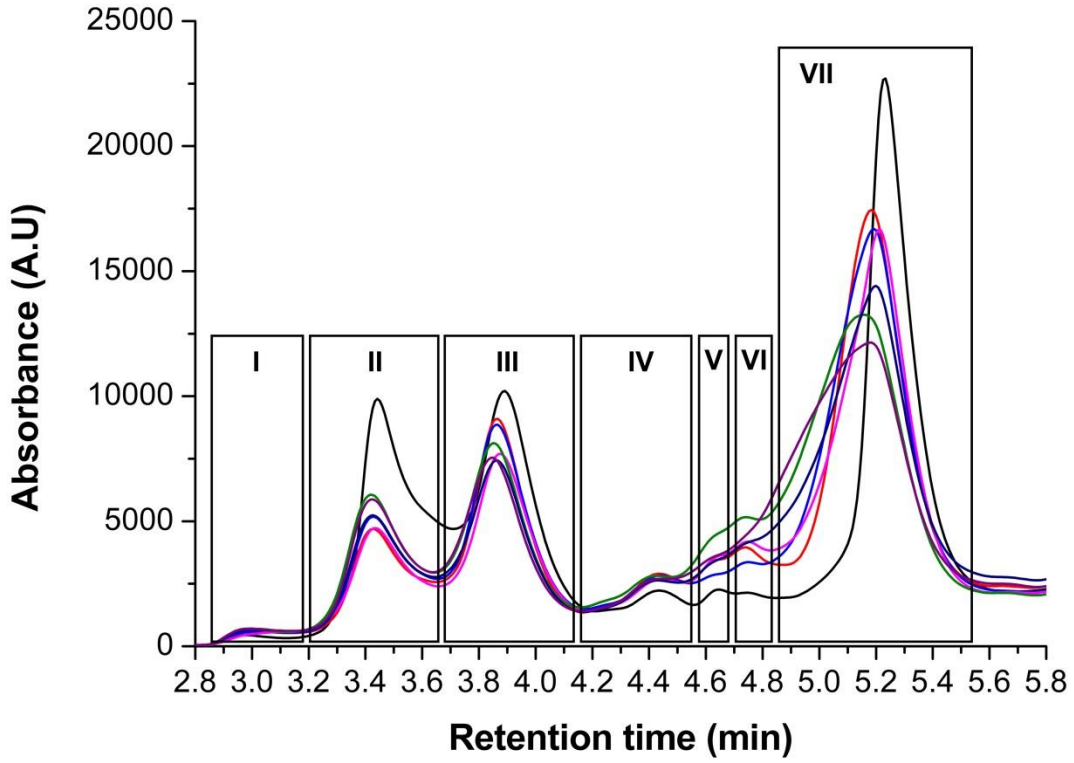
613 **FIGURE LEGENDS**



614

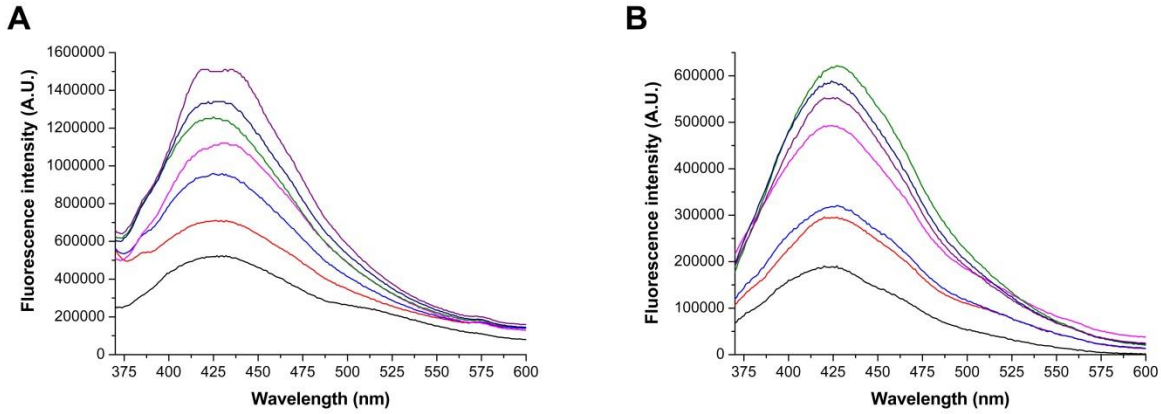
615 **Fig. 1.** Electrophoretic analysis of CM powder proteins obtained by SD and FD. Reducing SDS
616 PAGE of total CM powder proteins, dissolved in denaturing buffer (A), CM powder proteins
617 soluble (B) and insoluble (C) in 20 mM phosphate buffer pH 6.8. The major CM powder protein
618 bands identified by LC-MS/MS (D). CM powder proteins soluble in 20 mM phosphate buffer pH
619 6.8 resolved on non-reducing SDS PAGE (E) and native PAGE (F). M – molecular weight
620 markers.

621



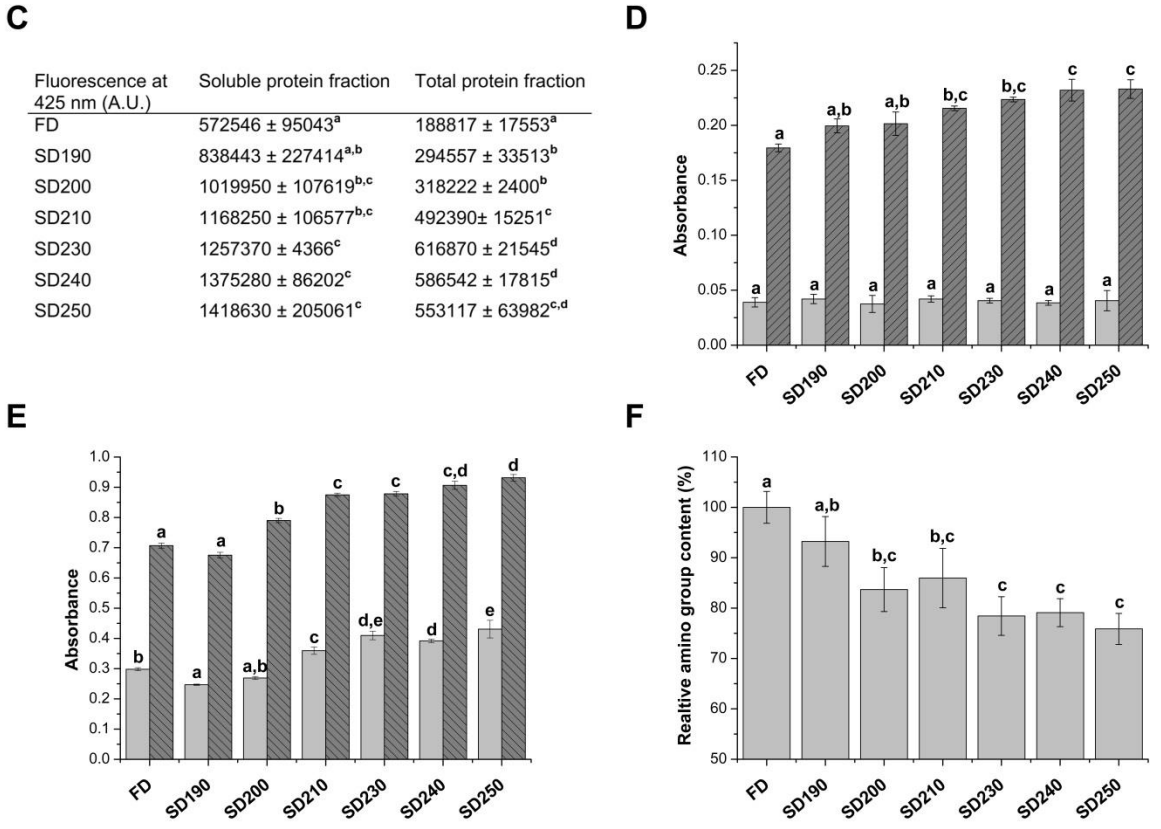
622

623 **Fig. 2.** Overlaid gel filtration chromatograms of soluble protein fraction proteins SD at six inlet
624 temperatures (190°C - 250°C) and FD. The chromatograms are normalized to the same total peak
625 area. —— FD, —— SD190, —— SD200, —— SD210, —— SD230, —— SD240, ——
626 SD250



C

Fluorescence at 425 nm (A.U.)	Soluble protein fraction	Total protein fraction
FD	572546 ± 95043[a]	188817 ± 17553[a]
SD190	838443 ± 227414[a,b]	294557 ± 33513[b]
SD200	1019950 ± 107619[b,c]	318222 ± 2400[b]
SD210	1168250 ± 106577[b,c]	492390± 15251[c]
SD230	1257370 ± 4366[c]	616870 ± 21545[d]
SD240	1375280 ± 86202[c]	586542 ± 17815[d]
SD250	1418630 ± 205061[c]	553117 ± 63982[c,d]

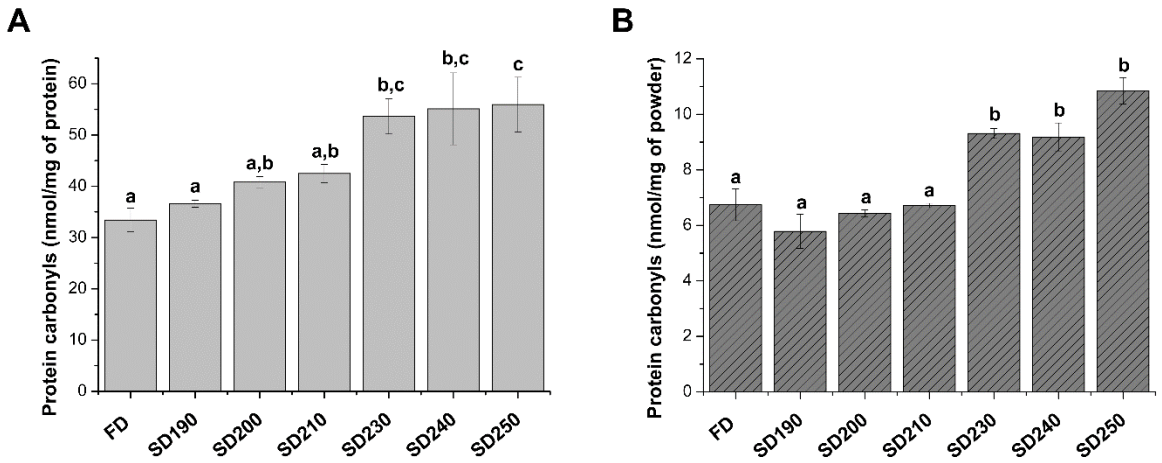


627

628 **Fig. 3.** Effects of SD and FD treatment on the extent of Maillard reaction. Fluorescence spectra
629 of CM powder proteins soluble in 20 mM phosphate buffer pH 6.8 (A) and total CM powder
630 proteins (B). Fluorescence intensity at 425 nm after excitation on 350 nm (C). Absorbance at 294
631 nm (striped dark grey bars) and 420 nm (light grey bars) of soluble protein fraction (D) and total
632 CM powder proteins (E). The relative free amino group content of soluble protein fraction (F).

633 Different small superscripts (a,b,c) denote significant differences ($p < 0.05$). FD,

634 SD190, SD200, SD210, SD230, SD240, SD250.

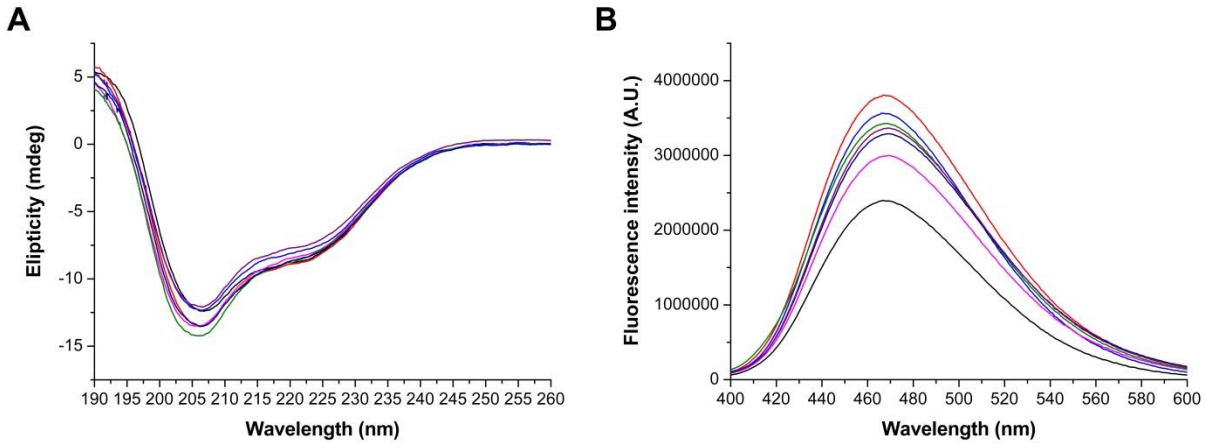


635

636 **Fig. 4.** Protein carbonyl content in soluble protein fraction (A) and total CM powder proteins

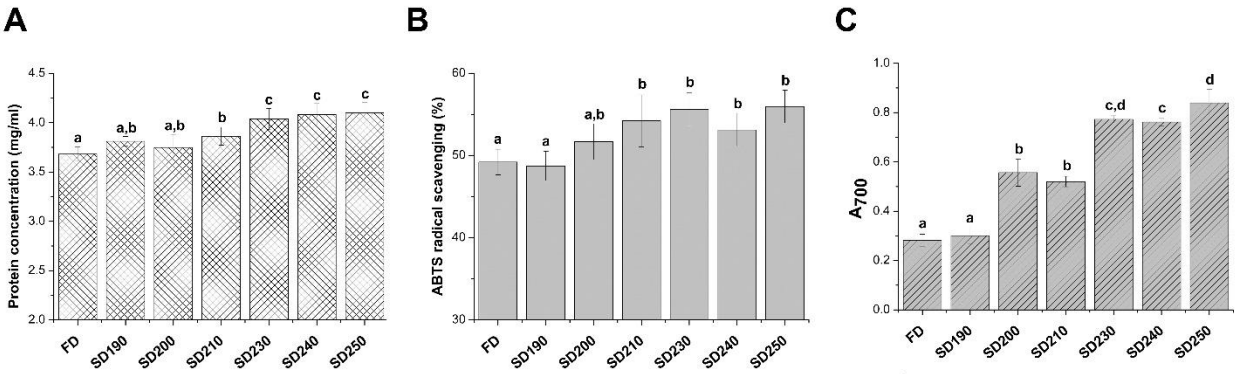
637 (B). Different small superscripts (a,b,c) denote significant differences ($p < 0.05$). Figure legend is

638 according to Fig. 1.



639

Figure 5. (A) Far-UV CD spectra of soluble camel milk proteins after freeze or spray drying in 190°C - 250°C temperature range. (B) ANS binding to soluble CM proteins upon FD and SD at different temperatures. —— FD, —— SD190, —— SD200, —— SD210, —— SD230, —— SD240, —— SD250.



Pearson`s correlation coefficients	Solubility (A280)	ABTS scavenging (%)	Reducing power (A700)
A294	0.95897 (p=0.00064)**	0.84759 (p=0.01602)*	0.91925 (p=0.00341)**
Fluorescence (A.U.)	0.91542 (p=0.00382)**	0.87935 (p=0.00909)*	0.94149 (p=0.00154)**
Protein carbonyls (nmol/mg protein)	0.97064 (p=0.00028)**	0.85685 (p=0.01377)*	0.97555 (p=0.00018)**
Relative amino group content (%)	-0.86372 (p=0.01222)*	-0.87921 (p=0.00912)*	-0.96938 (p=0.00031)**

*Correlation is significant at the 0.05 level (two-tailed)
**Correlation is significant at the 0.005 level (two-tailed)

Fig. 6. (A) Solubility of CM powders expressed as protein concentration of supernatants of CM powders reconstituted in 20 mM sodium phosphate buffer pH 6.8 (50 mg/ml); ABTS radical scavenging capacity (B) and reducing power (C) of soluble protein fraction; (D) Correlation between extent of MR and solubility and antioxidant activity. (E) Lipid peroxidation in lipid fractions of CM powders. Different small superscripts (a,b,c) denote significant differences ($p < 0.05$).