Supplementary data for article:

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Supplementary material

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

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2.4. Electrophoretic analysis

Total CM powder proteins and insoluble fraction of CM powder proteins were dissolved in denaturing buffer and resolved on 14% SDS-polyacrylamide gel under reducing conditions (100 µg of milk powder/well) with constant cooling using Hoefer SE600 Electrophoresis unit (Amersham Biosciences). Soluble protein fractions (20 µg/well) were resolved in the same way under reducing, non-reducing and native (absence of SDS and β-mercaptoethanol) conditions. The gels were stained by Coomassie Brilliant Blue.

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

In-gel trypsin digestion of 1D protein bands of FD CM soluble fraction proteins was performed according to protocol previously described (Shevchenko, Tomas, Havlis, Olsen, & Mann, 2006). Identification of camel milk soluble fraction proteins from tryptic, semitryptic and non-tryptic peptides was performed with the LTQ Orbitrap XL mass spectrometer and EASY- nLC II system (Thermo Fisher Scientific Inc., Bremen, Germany) and the PEAKS Studio 8.5 software program (Bioinformatics Solutions Inc., Waterloo, ON, Canada). Briefly, signature MS/MS spectra were searched using PEAKS DB algorithm against a database consisting of a UniProtKB (tremble and reviewed) genus *Camelus* database (taxon identifier 9836, downloaded on August 17, 2018 from http://www.uniprot.org/ with more than 20 000 entries in tremble and Sprot sections) and contamination database cRAP (the common Repository of Adventitious Proteins, downloaded on October 18, 2016 from http://www.thegpm.org/crap/). Oxidation (Met) and deamidation (Gln, Asn) were considered as variables, with carbamidomethylation (Cys) set as fixed in the PEAKS DB algorithm. Up to two missed trypsin cleavages with non-specific cleavages at both ends of a peptide were allowed. Mass tolerances were set to ± 10 ppm for parent ions and ± 0.5 Da for

fragment ions. Protein filters were as follows: protein −10 lgP ≥ 30, proteins unique peptides ≥ 2. The resulting false discovery rate of the peptide sequence and protein identification was lower than 0.01%, and de novo alignment local confidence score was ≥ 60%. 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.

2.10. Circular dichroism spectroscopy

Secondary structures of CM proteins were monitored by recording far-UV CD spectra using a JASCO J-815 spectropolarimeter (JASCO, Tokyo, Japan) in a 0.1 mm path length quartz cell. Dialyzed soluble protein fractions were prepared at concentration 1.00 mg/ml in 20 mmol/L sodium phosphate buffer pH 6.8. Each spectrum was average of four accumulations. Since average protein molecular weight could not be estimated for CM protein mixture, proportion of secondary structures was determined using a mathematical model proposed by Raussens et al. (Raussens, Ruysschaert, & Goormaghtigh, 2003).

2.14. Determination of lipid peroxidation

For the quantification of malondialdehyde (MDA), CM powder samples (100 mg) were dissolved in denaturing buffer. After centrifugation (20 min, 12000g) at 4°C lipid fractions (about 20 mg) were separated and their mass was measured. Saturated trichloroacetic acid (300 µl) and TBA (200 µL, 14.4 mg/mL in 100 mmol/L NaOH) were added to lipid fractions. The reaction mixtures were incubated for 20 min at 80°C. After centrifugation (10 min, 10000g), the absorbance of the supernatant was monitored at 532 nm. Lipid peroxidation was expressed as content of MDA per gram of lipids using calibration curve for MDA.

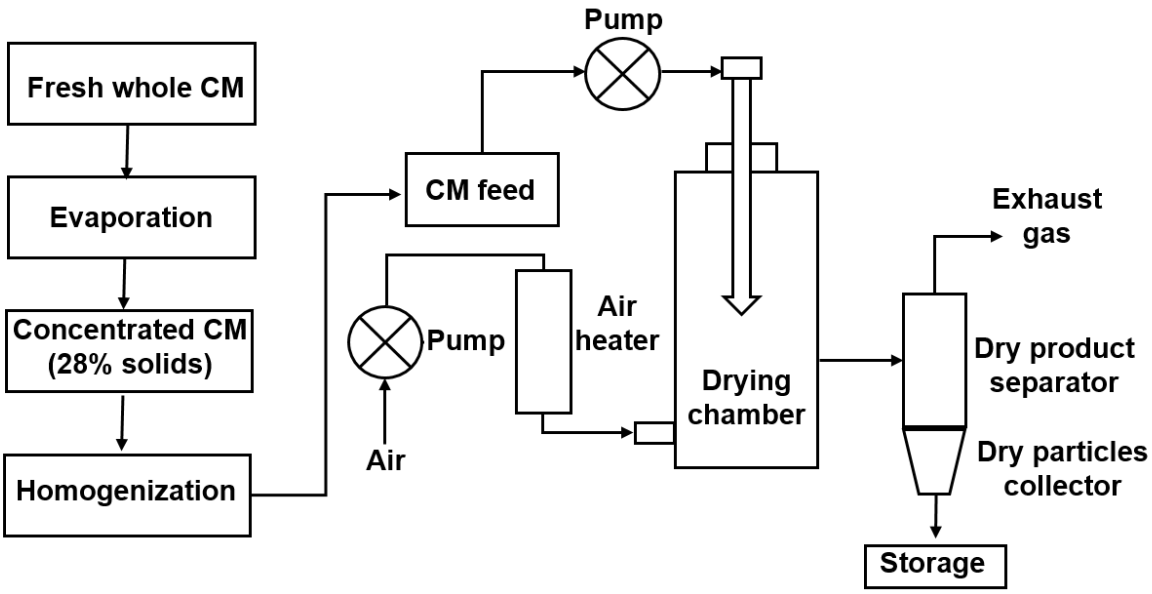

Figure S1. The graphical scheme for spray drying process of camel milk.

Table S1. The moisture, protein, ash and fat content in fresh and experimentally dried camel milks.

Milk Powder	Moisture (%)	Ash (%)	Protein (%)	Fat (%)
Fresh milk	87.09 ± 3.85^{a}	3.16 ± 0.04^{b}	2.0 ± 0.05^{b}	2.0 ± 0.05^{b}
SD 190°C	2.06 ± 0.90^{b}	7.32 ± 0.73^{a}	25.12 ± 0.36^{a}	20.9 ± 0.2^{a}
SD 200°C	1.58 ± 0.36^{b}	7.01 ± 0.45^{a}	25.67 ± 0.23^{a}	n.d.
SD 210°C	1.52 ± 0.40^{b}	8.28 ± 0.58^{a}	27.19 ± 0.34^{a}	n.d.
SD 230°C	1.94 ± 0.17^{b}	8.04 ± 0.66^{a}	27.17 ± 0.77^{a}	n.d.
SD 240°C	1.75 ± 0.94^{b}	7.96 ± 0.39^{a}	27.16 ± 0.20^{a}	n.d.
SD 250°C	2.20 ± 0.02^{b}	8.02 ± 0.54^{a}	26.58 ± 0.22^{a}	n.d.
Freeze-dried	1.85 ± 0.24^{b}	7.52 ± 0.18^{a}	26.51 ± 0.22^{a}	n.d.

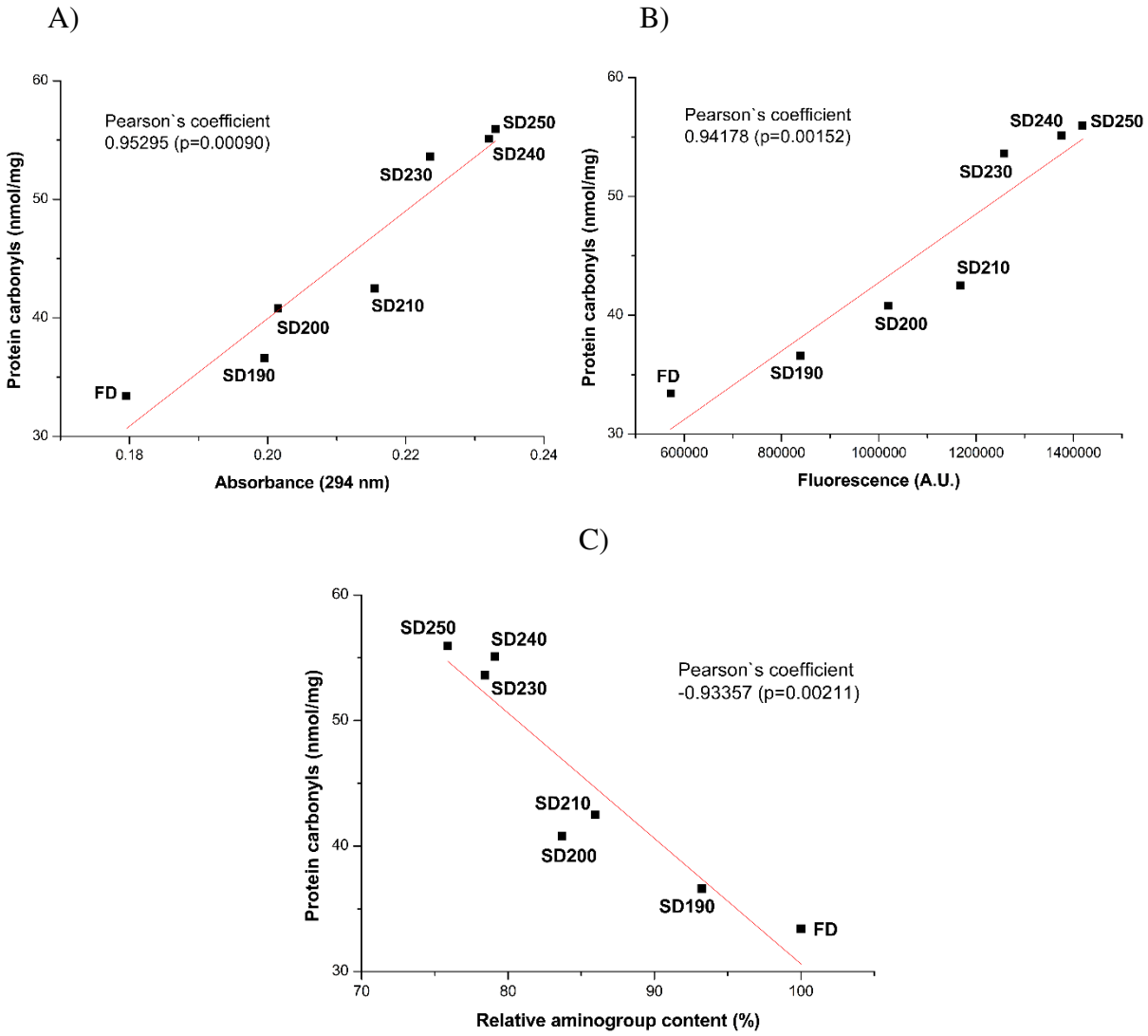

Figure S2. Correlation between extent of Maillard reaction (MR) and protein carbonyl content in soluble protein fraction of camel milk powders obtained by freeze-drying (FD) and spray drying at six different inlet temperatures, 190°C, 200°C, 210°C, 230°C, 240°C and 250°C (SD190, SD200, SD210, SD230, SD240 and SD250). A) Extent of MR monitored by absorbance at 294 nm; B) Extent of MR monitored by fluorescence intensity at 425 nm (excitation at 350 nm); C) Extent of MR monitored by content of remained free amino groups.

Table S2. Percentages of the secondary structures were estimated according to (Raussens, Ruysschaert, & Goormaghtigh, 2003). [a]denote no significant differences ($p < 0.05$).

	α-helix	β-sheet	β-turn	Random coil
FD	22.46 ± 0.41^{a}	18.48 ± 0.64^{a}	12.53 ± 0.00^{a}	37.70 ± 0.06^{a}
SD190	22.59 ± 0.42^{a}	18.94 ± 0.55^{a}	12.53 ± 0.00^{a}	37.89 ± 0.09^{a}
SD200	22.04 ± 1.39^{a}	19.16 ± 0.76^{a}	12.53 ± 0.00^{a}	38.01 ± 0.43^{a}
SD210	21.28 ± 0.39^{a}	20.34 ± 0.09^{a}	12.53 ± 0.00^{a}	38.31 ± 0.1^{a}
SD230	21.13 ± 0.8^{a}	20.00 ± 0.83^{a}	12.53 ± 0.00^{a}	38.38 ± 0.13^{a}
SD240	21.68 ± 1.07^{a}	19.77 ± 0.78^{a}	12.53 ± 0.00^{a}	38.15 ± 0.28^{a}
SD250	22.50 ± 1.01^{a}	19.17 ± 1.13^{a}	12.53 ± 0.00^{a}	37.91 ± 0.36^{a}

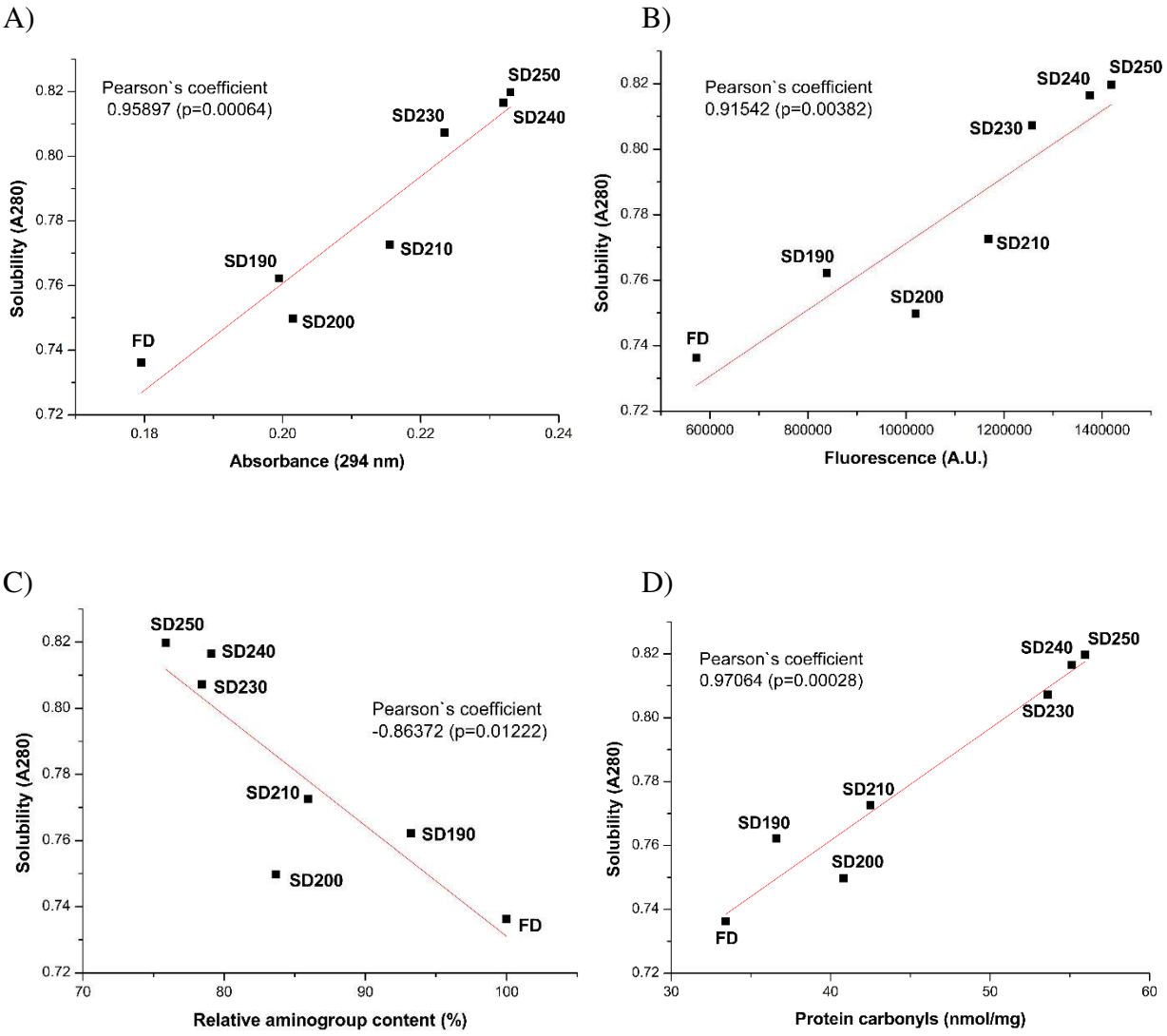

Figure S3. Correlation between extent of Maillard reaction (MR) and solubility of camel milk powders obtained by freeze-drying (FD) and spray drying at six different inlet temperatures, 190°C, 200°C, 210°C, 230°C, 240°C and 250°C (SD190, SD200, SD210, SD230, SD240 and SD250). A) Extent of MR monitored by absorbance at 294 nm; B) Extent of MR monitored by fluorescence intensity at 425 nm (excitation at 350 nm); C) Extent of MR monitored by content of remained free amino groups; D) Extent of MR monitored by formation of protein carbonyls.

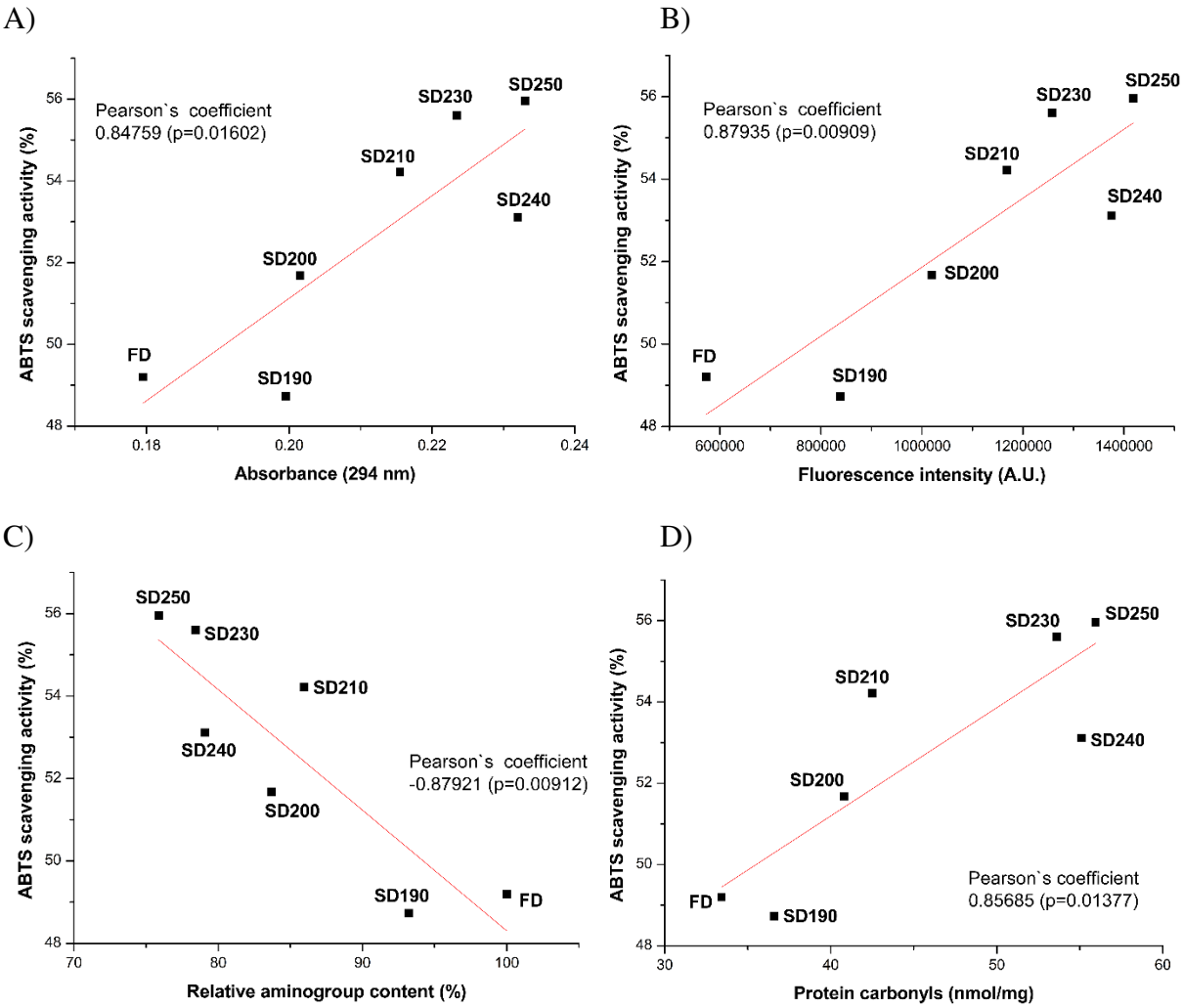

Figure S4. Correlation between extent of Maillard reaction (MR) and ABTS scavenging activity of soluble protein fraction of camel milk powders obtained by freeze-drying (FD) and spray drying at six different inlet temperatures, 190°C, 200°C, 210°C, 230°C, 240°C and 250°C (SD190, SD200, SD210, SD230, SD240 and SD250). A) Extent of MR monitored by absorbance at 294 nm; B) Extent of MR monitored by fluorescence intensity at 425 nm (excitation at 350 nm); C) Extent of MR monitored by content of remained free amino groups; D) Extent of MR monitored by formation of protein carbonyls.

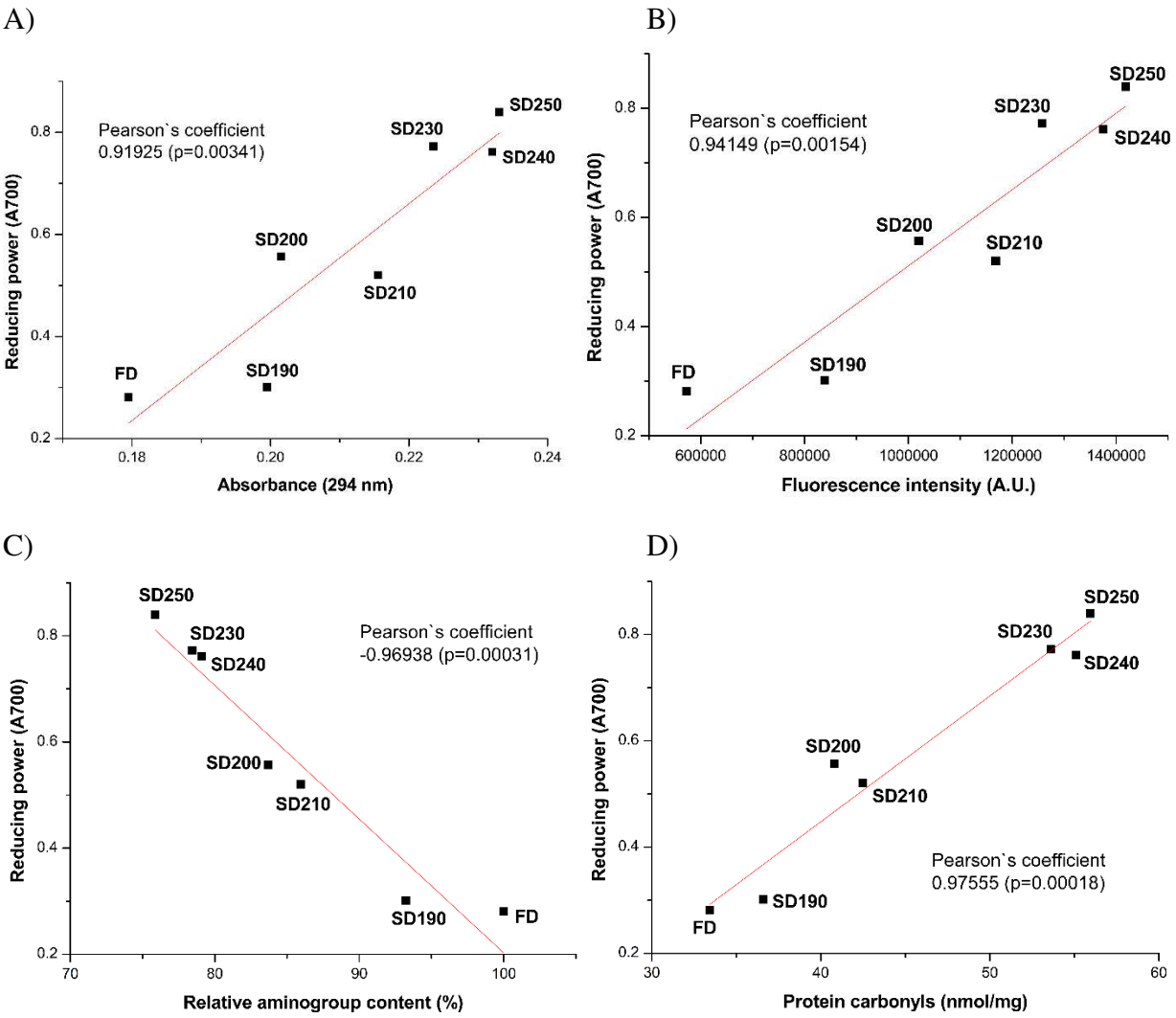

Figure S5. Correlation between extent of Maillard reaction (MR) and reducing power of soluble protein fraction of camel milk powders obtained by freeze-drying (FD) and spray drying at six different inlet temperatures, 190°C, 200°C, 210°C, 230°C, 240°C and 250°C (SD190, SD200, SD210, SD230, SD240 and SD250). A) Extent of MR monitored by absorbance at 294 nm; B) Extent of MR monitored by fluorescence intensity at 425 nm (excitation at 350 nm); C) Extent of MR monitored by content of remained free amino groups; D) Extent of MR monitored by formation of protein carbonyls.

References

Raussens, V., Ruysschaert, J.-M., & Goormaghtigh, E. (2003). Protein concentration is not an absolute prerequisite for the determination of secondary structure from circular dichroism spectra: a new scaling method. Analytical Biochemistry, 319(1), 114-121. https://doi.org/10.1016/s0003-2697(03)00285-9