Bovine collagen rheological properties as a function of high pressure treatment

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The aim of this research was to verify the hypothesis about the influence of high pressure treatment on rheological properties of bovine collagen due to protein partial coagulation or denaturation [1], [2].

Material and methods

Bovine collagen material was gained from factory preparing natural sausage casing. Specific skin layer rich for collagen was chipped, calcinated by $Ca(OH)_2$ and acidified by diluted hydrochlorid acid, washing by tap water, mixed and homogenized. Temperatures during this treatment were under 30°C.

Chilled samples were prepared from large dose having weigt about 5 kg. Each sample with mass about 150 g was placed into PE/PA plastic pouch and vacuum closed. High pressure treatment was made in tap water compressed in the vessel of unit type CYX 6/0103 (ŽĎAS join stock co.) – having chamber volume 2 litres and maximum pressure 500 MPa. Parameters of high preessure treatment and pH of samples are given in Table 1.

Table 1 Parameters of high pressure processing and samples pH

Sample number	Pressure (MPa)	Pressure holding time (min)	pH*) (-)
1	0	0	2.58
2	200	5	2.45
3	200	10	2.44
4	300	5	2.40
5	300	10	2.29
6	350	5	2.24
7	350	10	2.29
8	400	5	2.37
9	400	10	2.33
10	400	15	2.58

*) Remark: mean pH ± standard error = 2.40 ± 0.12

Dry matter of collagen was predicted by drying for 24 hours at 105°C resulting the value 9.78 ± 0.06 %. Sample temperatures during high pressure treatment ranged between 16 and 25°C. Rheological properties of all samples were measured rheometer Haake RS-150 Rheostress (Thermo Scientific, Germany) with geometry plate/plate using oscillatory measurement mode in linear viscoelastic range using relative deformation amplitude 0.05, plate diameter 35 mm and gap between plates 2 mm. Collagen temperature during measurement was kept 10°C. Frequency f ranged between 0.1 to 1.47 Hz. Each sample was measured using three replicates. All received data of G´, G´´ were statistically analyzed by ANOVA using QC Expert 3.1 software (Czech Republic) and linearly correlated on process parameters using Datafit software version 6.1.10 software (Oakdale Engineering, USA). We tested how parameters G´, G´´ are influenced by frequency, pressure and holding time.

Results and their discussion

Anova analysis results for G´and G´´ are given in Tables 2 and 3. It is clear that all process parameters have statistically significant influence both on G´and G´´ because parameters p are much lower than cricical value 0.05.

Table 2 ANOVA for G´ as influenced by process parameters

Parameter	sum of squares	F-statistics	p. 10^9	significance
x_1 Pressure (MPa)	22460880.9	12.401	3.15	significant
x_2 time (min)	21981325.95	16.178	1.18	significant
x_3 f (Hz)	70333521.16	37.992	3.32	significant

Table 3 ANOVA for G´´ as influenced by process parameters

Parameter	sum of squares	F-statistics	p	significance
x_1 Pressure (MPa)	937152.0	9.236	5.55×10^{-7}	significant
x_2 time (min)	960792.3	12.723	8.98×10^{-8}	significant
x_3 f (Hz)	4567024.5	59.419	5.60×10^{-50}	significant

Having all parameters significantly influencing storage and loss moduli we tested the specific correlation equations that can be used for quantification of these dependencies. We tested linear equations having three parameters

$$G' = a*x_1+b*x_2+c*x_3+d \quad (1)$$

$$G'' = a*x_1+b*x_2+c*x_3+d \quad (2)$$

We found that they are statistically reliable. The real values of coefficients a, b, c and d are given in the Tables 4 and 5. It is clear from both tables that reliability limits at 95% confidence intervals do not cover the zero value. These facts confirm that received coefficients are reliable and equations valid. Reliability of equation (1) can be confirmed by the coefficient of multiple determination R = 0.767 compared with critical value for freedom rate = experimental data – number of equation coefficients = 255 - 4 = 251. This value can be read from statistical

tables [3] for freedom rate =100 R_{crit} = 0.195. Coefficient of multiple determination R = 0.847 is valid for equation (2). The same value R_{crit} = 0.195 is valid for this equation. Comparing R_{crit} with actual values of multiple determination coefficients can anybody conclude that both equations are statistically significant and can be used for description of dependency of G´ and G´´ on frequency, pressure level and pressure holding time.

Table 4 Coefficients for equation (1) valid for G´

coefficient	Value	95% (+/-)	Lower limit	Upper limit
a	1.17	0.61	0.55	1.78
b	28.99	18.16	10.83	47.15
c	1116.7	130.8	985.9	1247.5
d	3831.9	159.6	3672.4	3991.5

Table 5 Coefficients for equation (2) valid for G´´

coefficient	Value	95% (+/-)	Lower limit	Upper limit
a	0.23	0.12	0.11	0.35
b	6.69	3.49	3.20	10.18
c	296.9	25.2	271.7	322.0
d	772.9	30.7	742.2	803.6

Conclusions

Presented results confirmed that collagen rheological properties characterized in linear viscoelastic range by storage and loss moduli are dependent on frequency of oscillation, pressure level and pressure holding time. Correlation equations confirmed that dependencies are positive i. e. the higher values of frequency, pressure and holding time the higher values of G´and G´´ are reached.

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