High pressure processing for sea buckthorn juice with higher superoxide dismutase activity

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Summary
Optimization of high pressure processing (HPP) of sea buckthorn juice was carried out regarding total aerobic bacteria (*TAB*) and activity of superoxide dismutase (SOD, EC 1.15.1.1) using response surface methodology (RSM). The optimized HPP condition of 500 MPa for 6 min reduced *TAB* by over 3 log CFU·ml^{-1} and increased the SOD activity of the juice. Effects of HPP at optimum conditions on the quality of the juice were compared with high-temperature short time (HTST) processing (100 °C for 15 s). *TAB* counts in the juice were decreased by 3.26 log CFU·ml^{-1} and 2.28 log CFU·ml^{-1} by 500 MPa for 6 min and HTST, respectively, while yeasts and moulds were not detected. Moreover, HPP increased the activity of SOD by 17.1 %, and did not change concentrations of vitamin C, total phenols, carotenoids and the antioxidant capacity of the juice. In contrast, HTST decreased the activity of SOD by 48.9 %, concentration of vitamin C by 14.3 % and the antioxidant capacity, and increased concentration of total phenols by 6.6 %. Meanwhile, HPP had no influence on sugars and colour of the juice, while HTST decreased concentration of saccharose and changed the juice colour. HPP could be applied for processing of sea buckthorn juice to gain a product of high quality and higher SOD activity.

Keywords
sea buckthorn juice; high pressure processing; superoxide dismutase; bacteria; antioxidant

Sea buckthorn *(Hippophae rhamnoides* L.) is a functional plant, which grows in the temperate zone of Asia and Europe [1]. Its berries contain more than 180 compounds, including functional enzymes like superoxide dismutase (SOD, EC 1.15.1.1) [2]. Therefore, they have been used for medicinal and nutritional purposes in many countries [3]. Sea buckthorn juice is getting more and more popular among consumers because of abundant antioxidant compounds such as SOD, phenolic compounds (flavonoids and phenolic acids), ascorbic acid, carotenoids and nitrogen compounds (alkaloids, amino acids and amines). The bioactive compounds contribute to the nutritional and medicinal function of sea buckthorn juice.

SOD forms a class of closely related enzymes that catalyse breakdown of the superoxide anion into oxygen and hydrogen peroxide. SOD enzymes are present in almost all aerobic cells and in extracellular fluids. There are three major families of superoxide dismutase, depending on the metal cofactor: Cu/Zn type (which binds both copper and zinc), Fe and Mn types (which bind either iron or manganese), and finally Ni type (which binds nickel) [4, 5]. Recently, high SOD activity in sea buckthorn berries and juice was attached a great importance because it was proven that SOD catalyses the reaction converting reactive oxygen species (ROS) into less reactive species [6], reducing oxidative stress of body and the risk of many acute and chronic pathologies, such as ischemia-reperfusion injury [7]. Sea buckthorn juice was proven to be a remedy for the reduction of cardiovascular disease risk because of its abundant SOD and other bioactive compounds. It is also considered to be useful in treating arsenic poisoning, inflammatory diseases, tumours and cancer [8].

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Thermal processing has been widely applied in juice industry for ensuring microbiological safety and extending the shelf life. However, undesirable detrimental changes in juice quality occur during and after conventional thermal processing. In particular, SOD in many raw juices is susceptible to heat and inactivation of SOD leads to a greater loss of antioxidant capacity and functionality of the juices. It is well known that the raw sea buckthorn juice is characterized by a high activity of SOD, however, retention of high SOD activity after thermal processing is hard to achieve. Therefore, exploring a novel technology of pasteurization of sea buckthorn juice with high SOD activity is needed.

High pressure processing (HPP) treatment has emerged as a novel, additive-free food preservation technology. It was scientifically and commercially proven that HPP can produce microbiologically safe and stable products with improved quality characteristics [9, 10]. It could also retain the activity of some indigenous enzymes in raw juices. HUANG et al. found that HPP (300–500 MPa for 5–20 min) increased polyphenol oxidase (PPO, EC 1.10.3.1) and peroxidase (POD, EC 1.11.1.7) activities and had no effect on pectin methylesterase (PME, EC 3.1.1.11) activity in apricot nectars [11]. CLARIANA et al. found that the activity of SOD in sliced ham cut increased after treatment at 400 MPa for 6 min [12]. To our knowledge, application of HPP to sea buckthorn juice with high SOD activity was not reported, but HPP is possibly a promising alternative way to produce sea buckthorn juice with high SOD activity.

The objective of this work was to optimize HPP conditions for processing sea buckthorn juice using response surface methodology (RSM) on the total aerobic bacterial (*TAB*) counts and SOD activity. Microbial loads and quality of the juice after HPP and high temperature short time (HTST) treatment were also compared. This study explored the application of HPP in sea buckthorn juice as a method for preserving and even increasing SOD activity.

MATERIALS AND METHODS

Preparation of sea buckthorn juice

Fresh sea buckthorn berries were purchased from Jintudi (Inner Mongolia, China). Clean berries were mixed with purified water at a ratio of 1:1 by weight, an treated with a juice extractor LJY-C010 (Joyoung, Shandong, China) at 333 Hz for 2 min at room temperature. The juice was separated into two portions. One portion was filled into 100 ml polyethylene terephthalate (PET) bottles and then kept at 4 °C until treated by HPP within 3 h. The other portion was kept in a plastic bucket at 4 °C and treated by HTST within 3 h.

Experimental design

Response surface methodology (RSM) was employed to optimize HPP treatment parameters (pressure and pressure-holding time) on *TAB* counts and SOD activity. The experiment was carried out according to central composite face-centered design. Single factor experiment results showed that pressure and pressure-holding time were the dominating factors. Thus, five levels of each independent variable (pressure and pressure-holding time) were chosen (Tab. 1) according to the single-factor experiment results by using Design Expert 8.0 (Stat-Ease, Minneapolis, Minnesota, USA). Pressure scale 100–500 MPa and pressure-holding time scale 2–10 min were used to study their comprehensive effects on *TAB* counts and SOD activity in sea buckthorn juice.

Tab. 1. Factors and levels of response surface methodology test.

	Factor	Levels				
		–1.414	–1	0	1	1.414
X_1	Pressure [MPa]	17.16	100	300	500	582.84
X_2	Time [min]	0.34	2	6	10	11.66

Following the design, 13 selected processes of two variables were performed. Initial temperature of each treatment was approximately 25 °C. A second order polynominal equation was used to express the responses as a function of independent variables (pressure and pressure-holding time), which is given as:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_1X_2 + b_4X_1^2 + b_5X_2^2 \quad (1)$$

Y represents the dependent variable (estimated response); b_0, b_1, b_2, b_3, b_4 and b_5 represent the equation coefficients; X_1 and X_2 represent the independent variables studied, pressure and pressure-holding time, respectively.

High pressure processing treatment

The sea buckthorn juices were processed with a 30 l HPP machine (HPP 700; Baotou Kefa, Inner Mongolia, China) at ambient temperature (25 °C). The initial temperature in the vessel was 25 °C, and the highest temperature was estimated as lower than 40 °C after treatments. The pressurization rate was 120 MPa·min^{-1} and the de-pressur-

ization time was less than 3 s. The samples were processed by HPP at various pressures (100, 200, 300, 400 and 500 MPa) for different treatment duration (2, 4, 6, 8 and 10 min). All treatments were carried out in triplicate. Samples were stored at 4 °C after HPP for further research within 3 h.

High temperature short time treatment

Sea buckthorn juice was treated by a tubular heat exchanger unit (Armfield FT74 HTST Processing Unit; Ringwood, United Kingdom) at 100 °C for 15 s. The juice was then cooled down to 20 °C and aseptically transferred into aseptic 100 ml PET bottles. Samples were stored at 4 °C after HTST for further research within 3 h.

Microbiological analysis

The plate count method was used to count total aerobic bacteria (*TAB*) as well as yeasts and moulds (*YM*) in juice [13]. Untreated and treated juices were serially diluted in sterile 8.5 g·l^{-1} NaCl solution and 1.0 ml of each dilution was plated in duplicate. Plate count agar and Rose Bengal agar (Beijing Land Bridging Technology, Beijing, China) were used for counting *TAB* and *YM* colonies. *TAB* plates were incubated at 37 °C for 48 ± 2 h, while *YM* plates were incubated at 27 °C for 72 ± 2 h. After incubation, the colonies were counted.

Analysis of pH

Thermo Orion 868 digital pH meter (Thermo Fisher Scientific, Waltham, Massachusetts, USA), which was calibrated with buffers at pH 4.01, 6.86 and 9.18, was used to measure pH values.

Titratable acidity

The titratable acidity (*TA*) was determined via titration with a standardized 0.2 mol·l^{-1} NaOH solution to the end point with phenolphthalein (pH 8.1 ± 0.1) according to the method of Lisiewska and Kmiecik [14]. The results were expressed as grams of citric acid equivalents per litre.

Total soluble solids

Total soluble solids (*TSS*) of the juice were determined as degrees Brix at 20 ± 1 °C using a WAY-2S digital refractometer (Shanghai Precision and Scientific Instrument, Shanghai, China).

Saccharose, glucose and fructose determination

The determination method was according to Liu et al. with some modifications [15]. The HPLC system (Knauer, Berlin, Germany) was equipped with a K-1001 pump (Knauer) connected to a refractive index detector (RI-2301, Knauer), and a 20 μl injection loop. The column was a Waters sugar pak 1 column (4.6 mm × 250 mm, 5 μm particle size; Waters, Milford, Massachusetts, USA). A volume of 50 ml of sea buckthorn juice was mixed and homogenized with 100 ml distilled water, then centrifuged at 9000 ×g for 15 min at 4 °C, and the supernatant was then collected for the determination of saccharose, fructose and glucose. The mobile phase was 50 mg·l^{-1} calcium disodium ethylene diaminetetraacetate, the flow rate was 0.5 ml·min^{-1} at 90 °C. Quantification was carried out using external standards of HPLC-grade saccharose, glucose and fructose (Sigma Aldrich, St. Louis, Missouri, USA). Results were expressed as grams per litre of sea buckthorn juice.

Colour measurement

Colour assessment was conducted at 25 ± 2 °C using a Hunter Lab Colour Quest XE colorimeter (Hunter Laboratory, Reston, Virginia, USA) in the reflectance mode. Observer was $D/10^{o}$. Sample layer was a 10 mm glass cell. Colour was expressed as L^* (brightness), a^* (redness) and b^* (yellowness) values. A numerical total colour difference (ΔE) was calculated using the following equation,

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (2)$$

where L_0^*, a_0^* and b_0^* were values for untreated samples.

Activity of superoxide dismutase

The SOD activity was determined by water-soluble tetrazolium (WST) method using WST-1 reagent (Jiancheng, Nanjing, China) with a slight modification [16]. A volume of 20 ml of sea buckthorn juice was centrifuged at 10 000 ×g for 10 min at 4 °C and 0.2 ml of the supernatant liquid was mixed with 39.8 ml phosphate buffered saline (0.1 mol·l^{-1} sodium phosphate and 0.1 mol·l^{-1} sodium chloride, pH 7.2). Samples and reaction reagents were added into microliter 96-well flat-bottom plates according to Tab. 2. The plate was put into the Multiscan plate reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to be mixed thoroughly, incubated at 37 °C for 20 min, and absorbance to be measured at 450 nm.

SOD inhibition rate (I) expressed in percent was calculated according to Eq. 3.

$$I = \frac{(A_1 - A_2) - (A_3 - A_4)}{(A_1 - A_2)} \times 100 \quad (3)$$

where A_1 is the absorbance of the control, A_2 is the absorbance of blank of the control, A_3 is the ab-

sorbance of the sample, and A_4 is the absorbance of blank of the sample.

The SOD activity (E) expressed as enzyme units per millilitre was calculated according to Eq. 4.

$$E = \frac{I}{50} \times D_1 \times D_2 \quad (4)$$

where I is the SOD inhibition rate, D_1 is the system dilution factor, D_2 is the sample dilution factor, number 50 represents inhibition rate of one unit SOD.

Tab. 2. Reaction system of superoxide dismutase.

Reagents	1	2	3	4
Sea buckthorn supernatant liquid [μl]	–	–	20	20
Double distilled water [μl]	20	20	–	–
Xanthine oxidase working solution [μl]	20	–	20	–
Buffer [μl]	–	20	–	20
WST-1 solution [μl]	200	200	200	200

1 – control, 2 – blank of the control, 3 – sample, 4 – blank of the sample, WST – water-soluble tetrazolium.

Activity of invertase

Invertase assay was performed using saccharose as a substrate according to Liu et al. with some modifications [17]. A volume of 5 ml of juice was added into the dialysis tube (cutoff value 8000–12000 Da; Solarbio, Beijing, China) and dialysed overnight in 50 mmol·l^{-1} tris(hydroxymethyl)aminomethane (Tris)-HCl buffer, pH 4.0, at 4 °C, and then transferred to a 10 ml volumetric flask. A volume of 0.5 ml of the enzyme solution was mixed with 4.5 ml of 200 mmol·l^{-1} sodium phosphate–citric acid buffer, pH 5.2, containing 100 mmol·l^{-1} saccharose, and shaken in a water bath at 40 °C for 8 h. The reaction was quenched by placing tubes in boiling water for 15 min and immediately cooling in an ice bath. 3,5-Dinitrosalicylic acid (DNS) solution was also added into the tube before the boiling process. The released glucose was determined spectrophotometrically at 540 nm. One unit of invertase activity was defined as the amount of enzyme required to liberate 1 μmol of glucose per millilitre of substrate per hour under assay conditions.

Vitamin C

Determination method was according to Liu et al. [15] with some modifications. A volume of 20 ml of sample was mixed with 20 ml of 2.5% metaphosphoric acid and incubated at 4 °C for 2 h. Then, the mixture was centrifuged at 10000 ×g for 10 min at 4 °C, and the supernatant was subsequently used for HPLC analysis. The HPLC system RF-10AXL (Shimadzu, Kyoto, Japan) was equipped with a system controller (CBM-20A), an autosampler (SIL-20A), two pumps (LC-20AT), a UV–visible detector (SPD-20AV) and a column oven (CTO-20A). The analytical column was a C18 (4.6 mm× 250 mm, 5 μm particle size) from Waters. The mobile phase was 1 mol·l^{-1} meta-phosphoric acid. The flow rate was 1 ml·min^{-1} at 30 °C. The detection was carried out at 254 nm. Calibration curves were prepared with external standards (L-ascorbic acid diluted in 2.5% meta-phosphoric acid to a concentration series of 20–120 mg·l^{-1}). Results were expressed as milligrams per litre of sea buckthorn juice.

Carotenoids

The extraction procedure of carotenoids was based on that of Teleszko et al. [18] with some modifications. Extraction was performed by mixing 10 ml of juice, 3 ml of methanol, 7 ml of water, 0.2 g ascorbic acid, 0.1 ml of acetic acid and 10 ml of hexane. Samples were mixed and sonicated for 15 min, placed for 1 h at 4 °C, sonicated again for 15 min, and centrifuged at 10000 ×g for 10 min at 4 °C. The hexane layer was collected and transferred to a 10 ml volumetric flask. The whole procedure was carried out under subdued light, covering all samples with aluminium foil to avoid light exposure.

Total carotenoids

The absorbance of extraction samples was measured at 450 nm using hexane as a blank with a spectrophotometer T6 (PG General, Beijing, China). Concentration of total carotenoids (M) expressed in milligrams per litre was calculated according to Eq. 5

$$M = \frac{A}{V} \times 1000 \quad (5)$$

where A is sample absorbance; V is sample volume (10 ml) and 1000 is conversion coefficient.

All-trans β-carotene

HPLC analysis was carried out on the HPLC system (Shimadzu) equipped with an Agela carotenoid C30 column (250 mm × 4.6 mm, 5 μm particle size). The injection volume was 20 μl and the flow rate was 1 ml·min^{-1}. The mobile phase consisted of two different solvent mixtures: A, methanol, methyl *tert*-butyl ether (MTBE) and water

(81 : 15 : 4, v/v/v), and B, the same solvents but in the ratio 6 : 90 : 4 (v/v/v). The linear gradient was from 1 % to 100 % B in 60 min. The column was equilibrated for 10 min prior to each analysis. The detection was carried out at 445 nm at 30 °C. Calibration curves of the standards were made on the basis of diluting standards in hexane to yield 1–20 mg·l^{-1} (all-trans β-carotene).

Total phenols

Total phenols were determined using the Folin-Ciocalteu method described by Cao et al. [19]. A volume of 10 ml of juice was mixed with 10 ml of 80% methanol, then sonicated for 10 min and centrifuged at 10 000 ×g at 4 °C for 10 min. A volume of 0.4 ml of the 40-fold diluted supernatant liquid was mixed with 2 ml of the Folin-Ciocalteu reagent (Solarbio) and incubated for 1 h in the dark at room temperature. Then, 1.8 ml of sodium carbonate (105.99 g·mol^{-1}) was added to the mixture and left to react for 15 min. The mixture was then immediately measured at 765 nm by a spectrophotometer T6. Results were expressed as milligrams of gallic acid per kilogram of sample.

Antioxidant capacity

The antioxidant capacity was studied by evaluating the free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and by evaluating the ferric reducing antioxidant power (FRAP) [20].

DPPH assay

The reaction was started by adding 120 μl of 3-fold diluted sea buckthorn juice to the cuvette containing 4 ml of methanol solution (0.14 mol·l^{-1}) of the DPPH free radical (Solarbio). The mixture was incubated in the dark for 45 min at room temperature and then the decrease in the absorption at 517 nm was measured using the spectrophotometer T6. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, Solarbio) solutions within the concentration range of 100–1 000 μmol·l^{-1} were used for calibration. Methanol was used for the baseline correction.

Radical-scavenging activity (R) of DPPH expressed in percent was calculated according to Eq. 6.

$$R = \frac{(A_1 - A_2)}{A_1} \times 100 \quad (6)$$

where A_1 is the absorbance at 517 nm of the control, and A_2 is the absorbance in presence of sea buckthorn juice.

Antioxidant activity is expressed as millimoles of Trolox equivalents per kilogram of sample.

FRAP assay

Freshly prepared FRAP solution contained 25 ml of 0.3 mol·l^{-1} acetate buffer (pH 3.6), 2.5 ml of 10 mmol·l^{-1} 2, 3, 5-triphenyltetrazolium chloride (TPTZ) (dissolved in 40 mmol·l^{-1} HCl), and 2.5 ml of 20 mmol·l^{-1} ferric chloride. A volume of 4 ml of FRAP solution was mixed with 100–1 000 μmol·l^{-1} Trolox solution or 10-fold diluted sea buckthorn juice at 37 °C. The ferric-reducing ability of samples was determined by monitoring the increase in absorbance at 593 nm by using the spectrophotometer T6 after 10 min.

Radical-scavenging activity (R) of FRAP expressed in percent was calculated according to Eq. 6, where A_1 is the absorbance at 593 nm of the control, and A_2 is the absorbance in presence of sea buckthorn juice.

Antioxidant activity is expressed as millimoles of Trolox equivalents per kilogram of sample.

Statistical analysis

The data were analysed using SPSS 21.0 software (IBM, New York, New York, USA) for analysis of variance and Duncan's test. The significance was established at $p < 0.05$. Relationships between variables were examined using Pearson's correlation coefficients. The experiments were performed in triplicate.

RESULTS AND DISCUSSION

Regression models of response

The values of the responses (*TAB* counts and SOD activity) obtained under different experimental conditions are summarized in Tab. 3.

Multiple regression analysis of the data gave the following second-order polynomial equation among Y_1 (*TAB*), Y_2 SOD activity), X_1 (pressure) and X_2 (pressure-holding time):

$$Y_1 = 2.20 + 1.22X_1 + 0.36X_2 + \\ + 0.13X_1X_2 - 0.29X_1^2 - 0.08X_2^2 \quad (7)$$

$$Y_2 = 1556.97 + 14.91X_1 + 30.82X_2 + \\ + 2.47X_1X_2 - 35.20X_1^2 - 12.54X_2^2 \quad (8)$$

A summary of the analysis of variance for the selected quadratic model is shown in Tab. 4. Statistical testing of the model was done in the form of ANOVA, which is required to test the adequacy and significance of the model. Coefficient R^2 was used for testing the goodness of fit of the regression equation. For *TAB* counts, R^2 was greater than 0.9700, indicating a high degree of correlation between the observed and predicted values. ANOVA of the regression model demonstrated

that the model was highly significant, as was evident from the calculated *F*-value (85.29) and a very low probability value ($p < 0.0001$). The model also showed statistically insignificant lack of fit, as was evident from the low calculated *F*-value (2.74) and relatively high probability value ($p > 0.05$). For SOD activity, the model was found to be adequate for prediction within the range of variables employed. ANOVA of the regression model demonstrated that the model was highly significant, as was evident from the calculated *F*-value (5.78) and a low probability value ($p < 0.05$). The model also showed statistically insignificant lack of fit, which was evident from the low calculated *F*-value (0.73) and relatively high probability value ($p > 0.05$).

The *p* values are used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. The smaller the *p* value, the more significant the corresponding coefficient [21]. For *TAB* counts and SOD activity, it was seen (Tab. 4) that all the linear coefficients, one quadratic term (X_1^2) being very small ($p < 0.05$) and interactive effects were not significant. The coefficients for quadratic effect of the pressure-holding time (X_2) and interactive effects were not significant, meaning the pressure and pressure-holding time had no interactive effects in our study.

Tab. 3. Results of response surface methodology test.

Trial No.	Levels of independent variables		Dependent variables	
	X_1	X_2	SOD activity [U·ml^{-1}]	*TAB* [log CFU·ml^{-1}]
1	0.000	0.000	1548.16	2.25
2	0.000	0.000	1532.63	2.12
3	–1.414	0.000	1464.33	0.05
4	0.000	–1.414	1486.27	1.51
5	0.000	0.000	1557.57	2.05
6	1.000	1.000	1540.56	3.38
7	0.000	1.414	1589.63	2.78
8	1.414	0.000	1520.92	3.40
9	0.000	0.000	1563.48	2.30
10	–1.000	1.000	1515.98	0.59
11	1.000	–1.000	1485.41	2.58
12	0.000	0.000	1583.02	2.30
13	–1.000	–1.000	1470.73	0.31

X_1 – pressure, X_2 – time, SOD – superoxide dismutase, *TAB* – total aerobic bacteria.

Localization of optimal conditions

Graphical representations of the regression equation Eq. 7 and Eq. 8, called response surfaces and contour plots, were obtained using the Design Expert. As shown in Fig. 1, *TAB* counts decreased with the increase of the pressure and pressure-holding time. However, the *TAB* counts reduction did not change after it reached 3.2 log CFU·ml^{-1}. Thus, 3.2 log CFU·ml^{-1} reduction was chosen as the inactivation criterion of *TAB* counts after HPP, and the process parameters for this *TAB* counts reduction were optimal in this investigation. It was seen that reaching a 3.2 log CFU·ml^{-1} reduction needs over 400 MPa of pressure and over 6 min of the pressure-holding time. As shown in Fig. 2, SOD activity increased with the increase of the pressure-holding time, however, it did not increase at ≥ 400 MPa. This suggests that it would be better to use an optimized HPP process at a relatively shorter treatment time to achieve efficiency in the industry. Thus, when the pressure-holding

Tab. 4. Variance analysis results of total aerobic bacterial counts and superoxide dismutase activity.

Source	Total aerobic bacterial counts					SOD activity				
	Sum of squares	Degree of freedom	Mean square	*F*	Prob > *F*	Sum of squares	Degree of freedom	Mean square	*F*	Prob > *F*
X_1	12.00	1	12.00	372.97	< 0.0001**	1778.77	1	1778.77	5.78	0.0472*
X_2	1.03	1	1.03	32.14	0.0008**	7599.42	1	7599.42	24.69	0.0016**
X_1X_2	0.068	1	0.068	2.10	0.1905	24.50	1	24.50	0.080	0.7860
X_1^2	0.60	1	0.60	18.75	0.0034**	8620.92	1	8620.92	28.01	0.0011**
X_2^2	0.050	1	0.050	1.54	0.2540	1094.21	1	1094.21	3.55	0.1014
Model	13.72	5	2.74	85.29	< 0.0001**	1778.77	1	1778.77	5.78	0.0472*
R^2	0.9723					0.8209				
Lack of fit	0.17	3	0.058	4.52	0.0896	763.22	3	254.41	0.73	0.5850

SOD – superoxide dismutase, X_1 – pressure, X_2 – time, Prob – probability, *F* – F-value of variance, R^2 – correlation coefficient.
* – means variance is significant ($p < 0.05$), ** – means variance is highly significant ($p < 0.01$).

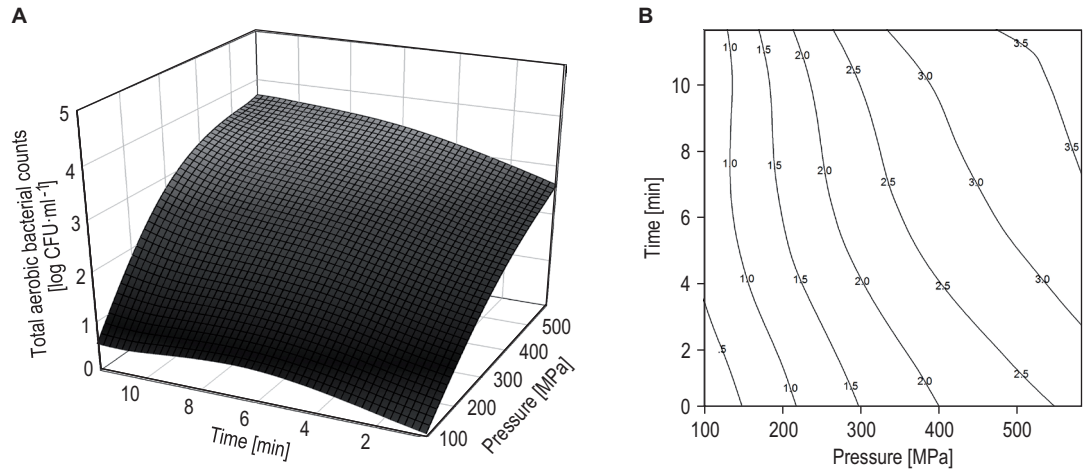

Fig. 1. Effects of the pressure and time on total aerobic bacterial counts in sea buckthorn juice.
A – response surface plot, B – contour plot.

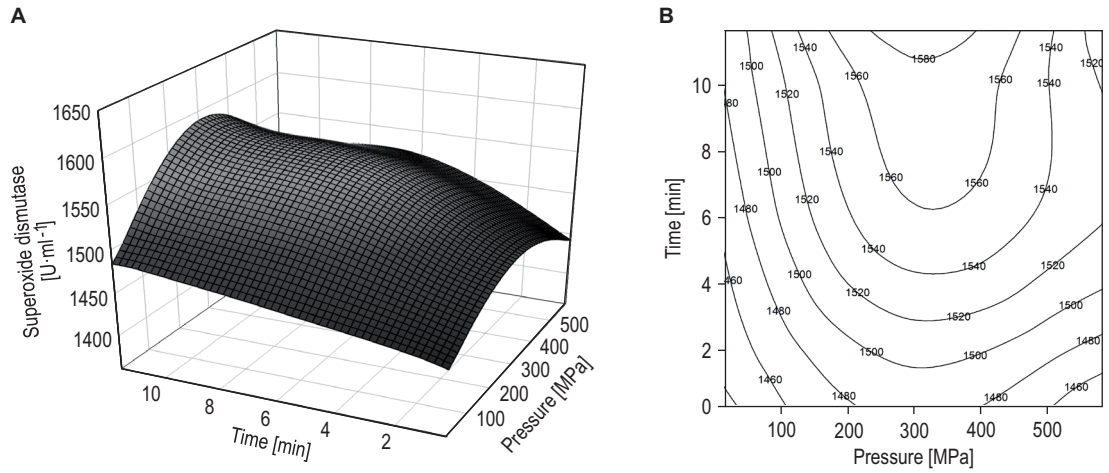

Fig. 2. Effects of the pressure and time on superoxide dismutase activity in sea buckthorn juice.
A – response surface plot, B – contour plot.

time was 6 min, the pressure was approximately 500 MPa according to the equation when using 3.2 log CFU·ml^{-1} reduction in *TBA* counts as the criterion.

Verification of the model

The conditions optimized by contour plot were tested for verification by conducting the experiments under 500 MPa for 6 min. The results showed that the experimental and predicted reduction values of *TAB* counts were 2.86 ± 0.21 log CFU·ml^{-1} and 2.65–3.61 log CFU·ml^{-1}, respectively, and the experimental and predicted values of SOD activity were 1667.66 ± 46.90 U·ml^{-1} and 1589.95–1683.41 U·ml^{-1}, respectively. The experimental values were found to be in good agreement with the predicted ones, proving that this model was reasonable for conditions of HPP of juice.

Microbial loads

Based on RSM study on sea buckthorn juice processing conditions, HPP at 500 MPa for 6 min was selected to treat the samples in this study, while 300 MPa for 6 min and HTST were chosen as controls of a relatively lower pressure and thermal processing conditions. As shown in Tab. 5, *TAB* counts in sea buckthorn juice after HPP at 300 MPa for 6 min were reduced by 2.22 log CFU·ml^{-1}, while after HPP at 500 MPa for 6 min and HTST thez were significantly reduced by 3.26 log CFU·ml^{-1} and 2.98 log CFU·ml^{-1}, respectively, indicating that HPP at 500 MPa for 6 min and HTST exhibited

similar pasteurization effects. Similar results were reported for *TAB* counts of mango pulps after HPP (500 MPa for 5 min) and HTST (110 °C for 8.6 s), being decreased by 4.39 log CFU·ml^{-1} and 4.75 log CFU·ml^{-1}, respectively [22]. The counts of the *YM* (initial load 2.87 ± 0.02 log CFU·ml^{-1}) were below 1.0 CFU·ml^{-1} after HPP at 500 MPa for 6 min and HTST, indicating that *YM* were more sensitive to HPP and HTST [23]. Inactivation of microorganisms by HPP is known to mainly result from membrane structure changes under high pressure and displacement of the equilibrium related to food pH and temperature [24].

pH, titratable acidity, total soluble solids

The values of pH, *TSS* and *TA* are shown in Tab. 5. Sea buckthorn berries contain high concentrations of acids such as malic, quinic and ascorbic acids, leading to a low pH value and relatively high acidity [25]. There was no significant change after HPP and HTST, indicating that the processes did not influence these parameters. Similar results were also found in studies with orange juice, carrot juice and pineapple purée [13, 26, 27].

Colour parameters

The values of L^* and b^* increased significantly after HPP treatments, and increased with the increase of the pressure, while there were no significant differences in a^* value. Wang et al. [28] also reached a similar observation that HPP-treated purple sweet potato nectars became brighter (higher L^* values) and more yellow (higher b^* values). This was caused by the extraction effect on pigments by HPP. The values of L^* and b^*also increased significantly, while a^* value decreased significantly after HTST treatment, which correlated with the decrease of the carotenoids produced by thermal treatment.

ΔE values in HTST-treated sea buckthorn juice were higher than those in HPP-treated ones, indicating that HPP protected better the original colour. Our results were similar to those of Patras et al. [29], who found that ΔE value was higher in thermally processed (70 °C for 2 min) carrot juice than in HPP-treated juices (400–600 MPa for 15 min). Marszalek et al. [30] also found ΔE value of thermally processed (90 °C for 15 min) strawberry to be higher than that of HPP-treated products (500 MPa for 5 min). Yi et al. [31] also found ΔE value to be higher in case of thermally processed (85 °C for 5 min) apple juice than in case of HPP-treated juices (600 MPa for 3 min). ΔE value of 2.00 would be a noticeable visual difference for a product. The colour of HTST-treated sea buckthorn juice significantly changed while HPP-treated juices did not change.

Fructose, glucose and saccharose

Natural sugars in sea buckthorn juices are glu-

Tab. 5. Changes of basic properties of sea buckthorn juices after various treatments.

	Control	HPP (300 MPa, 6 min)	HPP (500 MPa, 6 min)	HTST (100 °C, 15 s)
TAB counts [log CFU·ml^{-1}]	3.77 ± 0.23^{a}	1.55 ± 0.04^{b}	0.51 ± 0.21^{c}	0.79 ± 0.12^{c}
YM counts [log CFU·ml^{-1}]	2.87 ± 0.02^{a}	1.50 ± 0.02^{a}	ND	ND
pH	2.99 ± 0.01^{a}	2.98 ± 0.03^{a}	2.99 ± 0.01^{a}	2.99 ± 0.01^{a}
TA [g·l^{-1}]	1.04 ± 0.01^{a}	1.06 ± 0.01^{a}	1.07 ± 0.01^{a}	1.07 ± 0.02^{a}
TSS [°Brix]	11.10 ± 0.06^{a}	11.10 ± 0.11^{a}	11.10 ± 0.15^{a}	11.00 ± 0.15^{a}
L^*	34.19 ± 0.48^{a}	35.09 ± 0.19^{b}	35.26 ± 0.50^{b}	36.62 ± 0.55^{c}
a^*	6.18 ± 0.17^{a}	6.37 ± 0.23^{a}	6.37 ± 0.18^{a}	5.86 ± 0.11^{b}
b^*	15.53 ± 0.57^{a}	16.39 ± 0.23^{b}	16.89 ± 0.39^{b}	18.10 ± 0.61^{c}
ΔE	–	1.26	1.74	3.55
Fructose [g·l^{-1}]	11.95 ± 0.33^{a}	12.22 ± 0.45^{a}	11.46 ± 0.29^{a}	11.64 ± 0.53^{a}
Glucose [g·l^{-1}]	0.92 ± 0.01^{a}	0.93 ± 0.03^{a}	0.91 ± 0.03^{a}	0.90 ± 0.04^{a}
Saccharose [g·l^{-1}]	62.33 ± 1.39^{a}	63.85 ± 2.66^{a}	61.05 ± 3.72^{a}	56.80 ± 1.31^{b}
Total sugar [g·l^{-1}]	83.49 ± 1.81^{a}	85.41 ± 3.42^{a}	81.65 ± 2.32^{a}	77.43 ± 2.20^{b}

All data were the mean ± standard deviation, $n = 3$. Different letters in superscript represented a significant difference within the same rank in columns ($p < 0.05$).
HPP – high pressure processing, HTST – high-temperature short time treatment, *TAB* – total aerobic bacteria, *YM* – yeasts and moulds, *TA* – titratable acidity (expressed as grams of citric acid per litre equivalents), *TSS* – total soluble solids, ΔE – overall colour difference, ND – not detected.

cose and fructose, while saccharose was added artificially in this study in order to adjust *TSS* of the juice to be more acceptable. As shown in Tab. 5, compared with untreated juice, the concentration of glucose, fructose and saccharose in HPP-treated juice, and the concentration of glucose and fructose in HTST-treated juice showed no difference, but the concentration of saccharose in HTST-treated juice decreased significantly ($p < 0.05$). This reduction was attributed to hydrolysis of saccharose into glucose and fructose under heat and in acid environment (pH ≈ 3) [32]. Consequently, the concentrations of glucose and fructose should have increased after HTST, but they remained stable in our observation, which was possibly due to their anticipation in the Maillard reaction. The amount of reduced sugar participating in the reaction was approximately equal to that produced by hydrolysis of saccharose [32].

Activity of superoxide dismutase and invertase

As shown in Fig. 3A, the activity of SOD in sea buckthorn juice did not change after 300 MPa for 6 min treatment, while 500 MPa for 6 min treatment increased its activity significantly by 17.1 %, indicating that optimized HPP treatment conditions activated SOD in sea buckthorn juice. According to previous research, SOD spatial structure is relatively stable even under 1 000 MPa, thus it maintains high activity after HPP treatment [33]. The increase in SOD activity was possibly attributed to SOD extraction induced by HPP. Clariana et al. [12] also found that HPP at 400 MPa increased the activity of SOD in sliced ham. In contrast, SOD activity significantly decreased by 48.9 % after HTST, indicating that thermal treatment inactivated SOD.

Invertase is an enzyme that converts saccharose into fructose and glucose in juice products. As shown in Fig. 3B, HTST almost fully inactivated invertase, while HPP at 300 MPa for 6 min did not influence its activity. HPP at 500 MPa for 6 min decreased its activity by 30.8 %, indicating that HPP did not fully inactivate invertase in sea buckthorn juice in this study, and the remaining invertase in juices possibly affected the sugar composition and concentration during storage. Liu et al. [15] also found that the activity of invertase in mango pulps significantly increased by 8.6 % after HPP at 600 MPa for 1 min.

Vitamin C, total polyphenols, carotenoids and antioxidant capacity

Sea buckthorn juice is rich in vitamin C, which is considered as the most important compound contributing to the antioxidant activity [34]. As shown in Tab. 6, concentration of vitamin C in sea buckthorn juice did not change after HPP at 300 MPa for 6 min and 500 MPa for 6 min as compared with that in untreated sea buckthorn juice. In contrast, it decreased by 14.3 % after HTST. These results indicated that HPP retained vitamin C in sea buckthorn juice. Zhao et al. [35] found that concentration of vitamin C in korla pear juice remained stable after HPP at 500 MPa for 10 min, while it decreased by 13.4 % after HTST.

As shown in Tab. 6, concentration of total phenols in sea buckthorn juice did not change after HPP, while it significantly increased by 6.61 %

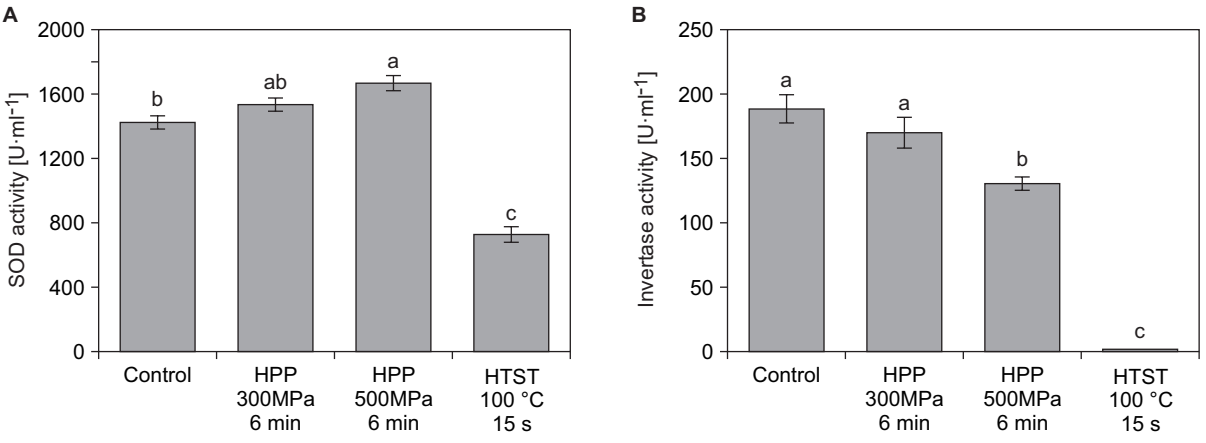

Fig. 3. Effects of high pressure processing on enzyme activity in sea buckthorn juices.

A – superoxide dismutase activity, B – invertase activity.
Different superscripted letters represent a significant difference within the same indicator ($p < 0.05$).
HPP – high pressure processing, HTST – high-temperature short time treatment.

Tab. 6. Changes of antioxidant properties of sea buckthorn juices after various treatments.

		Control	HPP (300 MPa, 6 min)	HPP (500 MPa, 6 min)	HTST (100 °C, 15 s)
Vitamin C [g·l^{-1}]		6.50 ± 0.01^{a}	6.65 ± 0.02^{a}	6.58 ± 0.02^{a}	5.57 ± 0.02^{b}
Total polyphenols [g·l^{-1}]		1.38 ± 0.04^{a}	1.42 ± 0.01^{ab}	1.44 ± 0.01^{ab}	1.47 ± 0.03^{b}
Carotenoids [mg·l^{-1}]	All-β-carotene	54.93 ± 0.60^{a}	55.31 ± 0.66^{a}	53.96 ± 2.14^{a}	43.12 ± 1.08^{b}
	Total carotenoids	60.76 ± 0.56^{a}	59.68 ± 2.37^{a}	60.13 ± 2.53^{a}	48.33 ± 0.41^{b}
Antioxidant capacity [mmol·kg^{-1}]	DPPH assay	4.66 ± 0.12^{a}	4.66 ± 0.14^{a}	4.67 ± 0.10^{a}	4.53 ± 0.10^{b}
	FRAP assay	9.65 ± 0.13^{a}	9.64 ± 0.12^{a}	9.75 ± 0.20^{a}	9.32 ± 0.16^{b}

All data are mean ± standard deviation, $n = 3$. Different letters in superscript represent a significant difference within the same rank in columns ($p < 0.05$).
HPP – high pressure processing, HTST – high-temperature short time treatment, DPPH – 1,1-diphenyl-2-picrylhydrazyl, FRAP – ferric reducing antioxidant power. Antioxidant capacity is expressed as millimoles of Trolox per kilogram equivalents.

after HTST. Both treatments were beneficial for the retention of phenols in sea buckthorn juice. Landl et al. [36] reported that HPP at 400 MPa had no effect on total phenols in apple purée. The increase in concentration of total phenols was attributed to plant cell disruption caused by HPP or HTST, leading to a higher extractability of these compounds [37].

Carotenoids are natural pigments and functional compounds in sea buckthorn juice. As shown in Tab. 6, there were no differences in total carotenoids and all-trans β-carotene between untreated and HPP-treated juices, while both of them decreased significantly ($p < 0.05$) after HTST. Zhang et al. found that concentration of β-carotene in carrot juice decreased significantly after HTST [13]. According to previous studies, the pressure higher than 300 MPa may cause irreversible protein denaturation [38], while treatments over 350 MPa increased extractable carotenoids due to protein-carotenoid complex denaturation [39]. In HTST-treated juice, crystalline carotene was suspended and mainly covered by polar lipids, forming the core structure of cloud particles. However, thermal treatment would destroy this protective matrix and cause degradation of carotenes [40].

Antioxidant capacity of sea buckthorn juices determined using DPPH and FRAP assay did not change after HPP, but showed a significant decrease after HTST (Tab. 6). This was in agreement with the change of values of antioxidant compounds including vitamin C, carotenoids and SOD. These results are in agreement with previous research of other authors. Plaza et al. [41] found that HPP-treated (400 MPa at 40 °C for 1 min) orange juice had higher antioxidant capacity comparing with thermally-treated (70 °C for 30 s) juice due to higher concentration of carotenoids and phenols. Zhao et al. [20] found that HPP-treated (500 MPa at 25 °C for 6 min) fresh apple juice had higher antioxidant capacity because of higher retention of vitamin C and polyphenols. The higher antioxidant capacity in HPP-treated juice may be also due to the fact that the treatment can better retain anthocyanins [42].

CONCLUSIONS

High pressure processing kept the microbiological safety and increased SOD activity of sea buckthorn juice. The optimized HPP condition was 500 MPa for 6 min. TAB counts of the juice decreased by 3.26 log CFU·ml^{-1} and yeasts and moulds were not detected. HPP increased the activity of SOD by 17.1 %. Meanwhile, HPP did not change the concentration of vitamin C, total phenols, carotenoids, antioxidant capacity, sugars and colour of the juice compared to HTST. This study demonstrated that HPP is a good alternative to thermal pasteurization to produce high-quality sea buckthorn juice with a higher SOD activity.

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