Effects of selenium-containing exopolysaccharide extracted from *Armillaria luteo-virens* on physico-chemical and antioxidant properties of yogurt

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Summary

To improve the properties of yogurt, selenium-containing exopolysaccharide was added to milk. Orthogonal design was employed to optimize parameters of yogurt production based on physico-chemical indicators. Influence of the Se-containing exopolysaccharide on physico-chemical and antioxidant properties of yogurt were also studied. The optimum conditions were Se-containing exopolysaccharide 0.80 $g \cdot l^{-1}$, saccharose 80 $g \cdot l^{-1}$, inoculum 30 $ml \cdot l^{-1}$ (3.00×10^7 $CFU \cdot l^{-1}$) and culture time 8 h, which led to production of yogurt with desired sensory characteristics, water holding capacity (*WHC*), counts of lactic acid bacteria (LAB), selenium concentration, and titration acidity. At optimal conditions, the Se-containing exopolysaccharide significantly improved sensory properties, *WHC*, selenium and soluble protein concentrations ($P < 0.05$), and significantly reduced titration acidity ($P < 0.05$). While Se-containing exopolysaccharide at 0.20 $g \cdot l^{-1}$ significantly increased LAB counts, at 0.60–1.00 $g \cdot l^{-1}$ a significant reduction in counts was observed in comparison to the control ($P < 0.05$). When compared with the control group, significantly ($P < 0.05$) enhanced removal of free radicals H_2O_2, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) was observed at 0.80–1.00 $g \cdot l^{-1}$, 0.20–1.00 $g \cdot l^{-1}$ and 0.20–1.00 $g \cdot l^{-1}$ of Se-containing exopolysaccharide, respectively. Furthermore, significant correlations between physico-chemical and antioxidant properties were determined at $P = 0.05$ or $P = 0.01$.

Keywords

selenium-containing exopolysaccharide; yogurt; *Armillaria luteo-virens*; physico-chemical properties; antioxidant activity

Selenium is a critical micronutrient that plays a significant role in maintaining the normal growth and development of humans and animals, and exerts anticancer, immunity improvement and antioxidant activities [1]. The biological function of selenium depends on its form. In general, organic Se-containing compounds are more compatible with health and safer than inorganic selenium as a dietary supplement. Microorganisms, such as bacteria and fungi, are active at enrichment and transformation of inorganic selenium to the organic form [2–4]. Se-containing proteins, Se-containing polysaccharides and Se-containing nucleic acids are its main forms in Se-containing mushrooms [5, 6]. Se-containing polysaccharide extracted from mushrooms exhibits also antioxidant activities [7, 8]. Se-containing polysaccharides apparently have potential to be used as functional ingredients in food or medical industries.

Yogurt is a fermented food product with unique flavour, nutritional characteristics and health benefits. Selenium enrichment using lactic acid bacteria (LAB) has been employed to produce Se-enriched fermented milk products [9, 10]. However, LAB grown with high sodium selenite levels may reduce elemental selenium from inorganic sodium selenite [3]. Several studies were undertaken recently in an attempt to develop organic Se-enriched food products using plant, mushroom and yeast resources, including Se-enriched fermented milk products with novel functional properties produced by adding Se-enriched materials during fermentation [11, 12]. However, Se-containing exopolysaccharide from mushrooms has not been considered as a source of selenium for production of yogurt yet.

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Based on the previous study, selenium and polysaccharide concentration of the Se-containing exopolysaccharide extracted from cultivation of Se-enriched *Armillaria luteo-virens* and optimum formulation conditions of Se-enriched yogurt supplied with the Se-containing exopolysaccharide were investigated. The effects of Se-containing exopolysaccharide supplementation on physicochemical and antioxidant properties of yogurt were systematically investigated in the present study.

MATERIALS AND METHODS

Materials

A. luteo-virens strain from Key Laboratory of Medicinal Plant and Animal Resources of the Qinghai-Tibetan Plateau, Qinghai Normal University (Xining, China) was preserved on potato glucose agar (potato infusion 200 g, glucose 20 g, agar 20 g, distilled water 1 000 ml; Yongda Chemical Reagent, Tianjin, China). *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains were cultured in pure milk, which was obtained from a local milk producer (Tuolunbao, Xining, China) and autoclaved at 115 °C for 15 min. Na_2SeO_3 was prepared as a 800 mmol·l^{-1} stock solution in deionized water and sterilized by filtration through a 0.22 μm membrane filter (Millipore, Billerica, Massachusetts, USA).

Preparation of selenium-containing exopolysaccharide

Three pieces of mycelial agar plugs (4 mm diameter) removed from the edge of *A. luteo-virens* colony were placed into a 250 ml Erlenmeyer flask with 100 ml potato glucose broth medium (potato infusion 200 g, glucose 20 g, distilled water 1 000.0 ml; Yongda Chemical Reagent) and incubated for 4 days at 25 °C. Then, 15 ml of this culture was added to the final volume of 100 ml fermentation medium, with 40 g·l^{-1} saccharose, 1 g·l^{-1} beef extract (Aoboxing Biotechnology, Beijing, China), 1 g·l^{-1} KH_2PO_4 and 1 g·l^{-1} $MgSO_4$. This was incubated at 25 °C until the 4th day of fermentation, 100 μl Na_2SeO_3 stock solution was added and further incubated by shaking (2.16 Hz) at 25 °C for 8 days. After that, the cultures were centrifuged at 5 000 ×*g* for 20 min. The supernatants were collected and concentrated at 60 °C using a rotary evaporator to approximately 20 % of its initial volume, and precipitated overnight with three volume of ethanol at 5 °C. The precipitated polysaccharides were centrifuged at 5 000 ×*g* for 30 min and resuspended in distilled water. The protein in the supernatant was removed with Sevag reagent (chloroform : butyl alcohol, 4 : 1, v/v) [13].

Selenium and polysaccharide concentration determination

The selenium concentration of tested samples was analysed by East Allreach Analysis (Guangdong, China) according to GB5009.93-2017 [14]. The main apparatus for analysis was the atomic fluorescence spectrophotometer AFS-1201 (Haiguang Analytical, Beijing, China). Polysaccharide concentration was determined using the anthrone-sulfate method of Li [15].

Preparation of inoculum

A volume of 50 ml of pure milk was transferred into a 100 ml bottle and autoclaved at 115 °C for 15 min. A mixed culture was obtained by inoculation with *S. thermophilus* and *L. bulgaricus* at 1 : 1, and incubated statically at 40 °C for 5 h until the counts of LAB reached 10^6 CFU·g^{-1}. After incubation, the culture was stored at 5 °C for 1 day.

Yogurt production

Orthogonal experimental design without interaction was used to derive the optimum formulation conditions using four factors at three levels, which included 9 experimental runs. The parameters of orthogonal experiment are shown in Tab. 1. According to the experimental design, determined amounts of pure milk were supplemented with Se-containing exopolysaccharide and saccharose. Milk samples were autoclaved at 115 °C for 15 min, cooled to 40 °C, and then inoculated with 30, 40 and 50 ml·l^{-1} (3.00×10^7, 4.00×10^7 and 5.00×10^7 CFU·l^{-1}, respectively), the final volume of 50 ml being placed in a 100 ml bottle. After inoculation, milk samples were incubated at 40 °C for 7 h, 8 h or 9 h, and stored at 5 °C provide information on the duration of storage.

Validation of the derived optimum formulation related to sensory evaluation, selenium concentration, water holding capacity (*WHC*), titration acidity or count of LAB was verified by triplicate trials.

The control yogurt was prepared with 60 g·l^{-1} saccharose, 30 ml·l^{-1} (3.00×10^7 CFU·l^{-1}) inoculum and fermentation time of 7 h.

Fermentation conditions were selected based on the above results of orthogonal experimental design. In brief, Se-enriched yogurt was prepared by adding 0.20 g·l^{-1}, 0.40 g·l^{-1}, 0.60 g·l^{-1}, 0.80 g·l^{-1} or 1.00 g·l^{-1} Se-containing exopolysaccharide to pure milk, together with 80 g·l^{-1} saccharose. Then, samples were autoclaved for 15 min at 115 °C, cooled to 40 °C, inoculated with 30 ml·l^{-1} (3.00×10^7 CFU·l^{-1}) of the inoculum and incubated

statically at 40 °C for 8 h. Selenium-free yogurt was prepared in parallel as a control.

Water holding capacity

WHC was determined using the centrifugation method with a modification [16]. An amount of 5 g of each yogurt sample was centrifuged at 4500 ×*g* for 30 min. The supernatant was removed and the pellet weight was recorded. All assessments were done in triplicate. *WHC* was calculated using Eq. 1.

$$WHC = \left(1 - \frac{W_2}{W_1}\right) \times 100 \quad (1)$$

where *WHC* is water holding capacity (in percent), W_1 is original sample weight (in grams) and W_2 is the sample weight after removal of the supernatant by centrifugation (in grams).

Titration acidity

Titration acidity was determined by the procedure described in International Dairy Federation standard IDF 150 [17] with minor modifications. An amount of 5 g of each yogurt sample was mixed with 20 ml of distilled water (in advance boiled and cooled), then titrated with 0.1000 mol·l^{-1} NaOH using 5 g·l^{-1} phenolphthalein indicator to produce a faint pink colour. The measurements were done in triplicates. Results were expressed as NaOH volume per kilogram of sample.

Lactic acid bacteria in yogurt

Counts of LAB in yogurt samples were determined according to ISO 7889:2003 [18] with minor modifications. Modified Chalmers agar plates were prepared using soya peptone 5.0 g, beef extract 5.0 g, yeast extract 5.0 g, glucose 20.0 g, lactose 20.0 g, calcium carbonate 10.0 g, agar 15.0 g, neutral red 0.05 g, pH 6.0, distilled water 1000 ml.

Soya peptone, beef extract and yeast extract were purchased from Aoboxing Biotechnology, glucose and lactose from Yongda Chemical Reagent, calcium carbonate from Dengzhong Chemical Reagent (Tianjin, China), agar from Biotopped Science and Technology (Beijing, China) and neutral red from Beijing Chemical Works (Beijing, China).

Each yogurt sample (10 ml) was mixed with 90 ml of sterile physiological salt solution to produce 10^{-1} dilutions. From this suspension, serial dilutions were prepared in sterile physiological salt solution up to 10^{-6} and then 0.1 ml portions were used to spread-inoculate plates in triplicates. The plates were incubated at 37 °C for 24–36 h and colony forming units per millilitre of sample were recorded.

Tab. 1. Arrangement and results of orthogonal design.

Treatment	Orthogonal test factors				Results			
	A Se-enriched exopolysaccharide [g·l^{-1}]	B Saccharose [g·l^{-1}]	C Inoculum [ml·l^{-1}]	D Culture time [h]	Sensory evaluation	Selenium concentration [mg·l^{-1}]	Lactic acid bacteria counts [10^8 CFU·ml^{-1}]	Water holding capacity [g·kg^{-1}]	Titration acidity [ml·kg^{-1}]
1	0.40	60	30	7	83.83 ± 2.79	0.62 ± 0.02	2.65 ± 0.10	586.63 ± 14.40	1113.30 ± 30.90
2	0.40	70	40	8	82.00 ± 2.40	0.79 ± 0.05	2.88 ± 0.30	747.70 ± 22.46	1250.70 ± 38.20
3	0.40	80	50	9	86.17 ± 2.71	0.82 ± 0.04	2.55 ± 0.02	738.83 ± 8.82	1248.80 ± 30.00
4	0.60	60	40	9	80.00 ± 1.10	1.19 ± 0.08	2.99 ± 0.22	734.49 ± 22.44	1166.70 ± 26.90
5	0.60	70	50	7	80.67 ± 2.66	0.96 ± 0.04	3.16 ± 0.08	549.96 ± 12.74	1054.70 ± 29.50
6	0.60	80	30	8	90.67 ± 2.34	0.97 ± 0.06	1.74 ± 0.20	631.99 ± 27.32	1074.70 ± 4.60
7	0.80	60	50	8	85.00 ± 1.79	1.53 ± 0.10	2.27 ± 0.26	589.97 ± 9.82	1132.00 ± 22.30
8	0.80	70	30	9	83.67 ± 0.82	1.43 ± 0.08	2.13 ± 0.22	737.29 ± 7.00	1113.30 ± 38.20
9	0.80	80	40	7	87.83 ± 2.14	1.45 ± 0.03	2.21 ± 0.10	539.62 ± 11.70	1089.30 ± 36.30

Sensory evaluation

Sensory assessment of each yogurt sample was performed by eight trained voluntary panelists using a sensory score for three properties. The scale of 100 points included 20 points for whey separation, 40 points for flavour and 40 points for texture, as described by Xie et al. [12]. The panel of assessors was composed of food or pharmaceutical professionals. Water was provided to the panel members to cleanse their palates between samples.

Determination of soluble protein

For determination of soluble protein, 1 ml of homogenized yogurt was mixed with 2.5 ml Coomassie Brilliant Blue G-250 solution, which was obtained by dissolution of 100 mg Coomassie Brilliant Blue G-250 in 50 ml of 95% ethanol, added 120 ml of 85% H_3PO_4 and made up to a volume of 1 000 ml. The reaction took place for 10 min and then absorbance was determined at 595 nm using a spectrophotometer V-5100 (Yuanxi Instrument, Shanghai, China). Bovine albumin (Jinhui Biosciences, Shanghai, China) was used as a standard.

Analysis of fat

Fat was estimated by Rose-Gottlieb method according to Li [15]. A volume of 10 ml of a yogurt sample was mixed well with 2.5 ml of 25% ammonia solution and heated in a water bath at 60 °C for 5 min, and then shaken for 2 min. The mixture was added 10 ml of 95% ethanol and immediately placed in cold water to cool down. After cooling, the mixture was added 15 ml of petroleum ether, shaken for 1 min and followed by 15 ml of petroleum ether, shaken for 1 min and incubated statically for 30 min. The petroleum ether layer was taken out, evaporated in a water bath at 60 °C and dried at 100 °C until constant weight.

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity of the yogurt samples was analysed by the method described by Blois [19] with slight modifications. The yogurt samples were centrifuged at 5 000 ×g for 20 min. The supernatants were collected. A volume of 1 ml of yogurt supernatant or ascorbic acid at 10 mg·ml^{-1} concentration was mixed with 5 ml of ethanolic solution of DPPH (0.04 mg·ml^{-1}) in a test tube. The reaction mixture was incubated at room temperature for 30 min in dark. The absorbance of the solution was determined spectrophotometrically at 517 nm using a spectrophotometer V-5100. Ascorbic acid was used as a positive control. The experiments were carried out in triplicates. DPPH radical-scavenging activity (*DRSA*) was calculated by using Eq. 2 and expressed in percent.

$$DRSA = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100 \quad (2)$$

where A_0 is the absorbance of the mixture of 1 ml ethanol and 5 ml DPPH solution, A_1 is the absorbance of the mixture of 1 ml yogurt sample supernatant (or ascorbic acid) and 5 ml DPPH solution, and A_2 is the absorbance of the mixture of 1 ml yogurt sample supernatant (or ascorbic acid) and 5 ml ethanol.

Hydroxyl radical-scavenging activity of the yogurt samples was determined by a method of Smirnoff and Cumbes [20] with slight modifications. The supernatants of yogurt were obtained by centrifugation (5 000 ×g, 20 min). Volumes of 1 ml of $FeSO_4$ (9 mmol·l^{-1}), 1 ml of salicylic acid in ethanol (9 mmol·l^{-1}), 1 ml of each sample supernatant (or ascorbic acid) and 1 ml of H_2O_2 (8.8 mmol·l^{-1}) were added into the tube. The mixture was incubated at 37 °C for 30 min. Positive controls were prepared using 10 mg·ml^{-1} of ascorbic acid. The absorbance was read at 510 nm using a spectrophotometer V-5100. Assays were carried out in triplicate. The percentage of the hydroxyl radical-scavenging activity (*HRSA*) of each extract was calculated from Eq. 3.

$$HRSA = \left(1 - \frac{B_1 - B_2}{B_0}\right) \times 100 \quad (3)$$

where B_0 is the absorbance of the mixture of 1 ml $FeSO_4$, 1 ml of salicylic acid in ethanol, 1 ml distilled water and 1 ml H_2O_2; B_1 is the absorbance of the mixture of 1 ml $FeSO_4$, 1 ml of salicylic acid in ethanol, 1 ml of each sample supernatant (or ascorbic acid) and 1 ml distilled water; and B_2 is the absorbance of the mixture of 1 ml $FeSO_4$, 1 ml of salicylic acid in ethanol, 1 ml sample supernatant (or ascorbic acid) and 1 ml H_2O_2.

2,2-Azinobis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) radical-scavenging activity of the yogurt samples was assessed using the method described by Re et al. [21] with slight modifications. The supernatants of yogurt were established similarly as for DPPH assay. The radical cation was prepared by mixing 7.4 mmol·l^{-1} ABTS with 2.6 mmol·l^{-1} potassium persulfate (1 : 1, v/v) and leaving the mixture for 24 h until the reaction was completed and the absorbance was stable. The ABTS radical solution was diluted with ethanol to an absorbance of 0.7 (± 0.02) at 732 nm. A volume of 1 ml of yogurt supernatant or ascorbic acid at 10 mg·ml^{-1} was mixed with 4 ml of diluted ABTS

radical solution in a test tube. Measurements were taken after 6 min at 734 nm using a spectrophotometer V-5100. The antioxidant activity of the samples was calculated by determining the decrease in absorbance. A positive control was established by ascorbic acid. Assays were carried out in triplicate. Percentage of ABTS radical-scavenging ability (*ARSA*) was calculated by Eq. 4.

$$ARSA = \left(1 - \frac{C_1 - C_2}{C_0}\right) \times 100 \quad (4)$$

where C_0 is the absorbance of the mixture of 1 ml ethanol and 4 ml diluted ABTS radical solution, C_1 is the absorbance of the mixture of 1 ml yogurt supernatant (or ascorbic acid) and 4 ml diluted ABTS radical solution, and C_2 is the absorbance of the mixture of 1 ml yogurt supernatant (or ascorbic acid) and 4 ml ethanol.

Statistical analysis

The data were processed using SPSS software, version 16.0 (SPSS, Chicago, Illinois, USA). The results were presented as mean ± standard deviation. Fisher's least significant differences (LSD) test was used to determine whether differences between means were statistically significant ($P < 0.05$). Correlation analysis was conducted between physico-chemical and antioxidant properties using Spearman's rho method.

RESULTS AND DISCUSSION

Selenium-containing exopolysaccharide

Some mushrooms have been used as effective carriers of selenium, selenium being incorporated into their polysaccharide components [4, 5, 7]. For example, Se-containing polysaccharides extracted from Se-enriched mushrooms grown substrate with various selenium concentrations, such as *Ganoderma lucidum*, displayed different selenium concentrations and constant polysaccharide concentrations [7]. Selenium-polysaccharide from Se-enriched *Grifola frondosa* contained mannose, glucose and galactose in the ratio of 3.3 : 23.3 : 1.0 [8]. The polysaccharide of *A. luteo-virens* is composed of arabinose and xylose [22]. However, studies on exopolysaccharide sugar composition of *A. luteo-virens* is rather limited. To the best of our knowledge the concentrations of selenium and polysaccharide of *A. luteo-virens* have not been reported till date. In our study, the concentrations of selenium and polysaccharide in Se-containing exopolysaccharide extracted from the fermentation liquid of *A. luteo-virens* cultured with 0.8 mmol·l^{-1} Na_2SeO_3 were (2.84 ± 0.23) g·kg^{-1} and (562.70 ± 3.50) g·kg^{-1}, respectively. These findings indicate that mushrooms have different abilities of enriching and transforming selenium.

Optimization of yogurt containing selenium-enriched exopolysaccharide

Several important factors have to be considered at development of a functional food. Many researchers reported that Se-enriched yogurt was obtained through adding sodium selenite or Se-enriched materials to milk, then fermented by adding a mixture of LAB [9, 12], or by adding Se-enriched LAB inoculum to milk [10]. The optimum production conditions of Se-enriched yogurt were determined through statistical tests [23]. In this study, orthogonal experimental design was utilized to optimize the production of yogurt containing Se-enriched exopolysaccharide based on evaluation indicators (Tab. 1). The extreme difference analysis results of orthogonal experiments are given in Tab. 2.

As shown in Tab. 2, we found that the effect on sensory score of yogurt decreased in the order of: saccharose > inoculation > culture time > Se-containing exopolysaccharide according to the extreme difference analysis. The optimal regarding sensory properties were achieved at 0.80 g·l^{-1} Se-containing exopolysaccharide, 80 g·l^{-1} saccharose, 30 ml·l^{-1} (3.00×10^7 CFU·l^{-1}) inoculum, 8 h culture time (Tab. 2). Similarly Xie et al. [12], who extracted selenoprotein from Se-enriched *Pleurotus ostreatus*, also reported the order of the four factor effects and the higher sensory scores of yogurt through the orthogonal test.

With respect to selenium concentration of yogurt, the order of the four factor effects was given as Se-containing exopolysaccharide > inoculation = culture time > saccharose (Tab. 2). The optimal conditions for low concentration of selenium were 0.40 g·l^{-1} Se-containing exopolysaccharide, 70 g·l^{-1} saccharose, 30 ml·l^{-1} (3.00×10^7 CFU·l^{-1}) inoculum and 7 h culture time, while the optimal conditions for high selenium concentration of yogurt were 0.80 g·l^{-1} Se-containing exopolysaccharide, 60 g·l^{-1} saccharose, 40 ml·l^{-1} (4.00×10^7 CFU·l^{-1}) inoculum and 9 h culture time. The optimal conditions for high selenium concentration were obtained by Box-Behnken design, with the order of the factor effects being inoculum amount > Se^{4+} concentration > pH [23].

Based on titration acidity range analysis (Tab. 2), the order of the four factors from strong to weak was Se-containing exopolysaccharide > culture time > inoculation > saccharose. The values of titration acidity from each sample were

Tab. 2. Extreme difference analysis of physico-chemical indicators.

Factor		L Low level	M Medium level	H High level	*R*	Order of levels	Order of factors
Sensory evaluation							
A	Se-enriched exopolysaccharide	252.00	251.34	256.50	5.16	HLM	BCDA
B	Saccharose	248.83	246.34	264.67	18.33	HLM	
C	Inoculum	258.17	249.83	251.84	8.34	LHM	
D	Culture time	252.33	257.67	249.84	7.83	MLH	
Selenium concentration [mg·l^{-1}]							
A	Se-enriched exopolysaccharide	2.23	3.12	4.41	2.18	HML	ACDB
B	Saccharose	3.34	3.18	3.24	0.16	LHM	
C	Inoculum	3.02	3.43	3.31	0.41	MHL	
D	Culture time	3.03	3.29	3.44	0.41	HML	
Lactic acid bacteria counts [10^8 CFU·ml^{-1}]							
A	Se-enriched exopolysaccharide	8.08	7.89	6.61	1.47	LMH	BCAD
B	Saccharose	7.91	8.17	6.50	1.67	MLH	
C	Inoculum	6.52	8.08	7.98	1.56	MHL	
D	Culture time	8.02	6.89	7.67	1.13	LHM	
Water holding capacity [g·kg^{-1}]							
A	Se-enriched exopolysaccharide	2073.16	1916.44	1866.88	206.28	LMH	DACB
B	Saccharose	1911.09	2034.95	1910.44	124.51	MLH	
C	Inoculum	1955.91	2021.81	1878.76	143.05	MLH	
D	Culture time	1676.21	1969.66	2210.61	534.40	HML	
Titration acidity [ml·kg^{-1}]							
A	Se-enriched exopolysaccharide	3612.80	3296.10	3334.60	316.70	LHM	ADCB
B	Saccharose	3412.00	3418.70	3412.80	6.70	MHL	
C	Inoculum	3301.30	3506.70	3435.50	205.40	MHL	
D	Culture time	3257.30	3457.40	3528.80	271.50	HML	

Values represent sum of experimental results of the low, medium and high level of factor.
R – differential value. Order of levels – effect of factor level on physico-chemical indicator sorted in descending order. Order of factors – effect of factor on physico-chemical indicator sorted in descending order.

Tab. 3. Verification of optimum formulation conditions of yogurt with Se-containing exopolysaccharide.

Treatment of yogurt	Sensory evaluation	Selenium concentration [mg·l^{-1}]	LAB counts [10^8 CFU·ml^{-1}]	*WHC* [g·kg^{-1}]	Titration acidity [ml·kg^{-1}]
Control	90.83 ± 1.83^{bc}	0.01 ± 0.00^{d}	5.40 ± 0.17^{cd}	529.04 ± 14.54^{d}	988.70 ± 30.60^{b}
High *WHC*	90.83 ± 1.60^{bc}	0.71 ± 0.03^{c}	2.91 ± 0.11^{e}	598.56 ± 12.76^{bc}	940.70 ± 3.10^{c}
Low titration acidity	89.50 ± 1.52^{cd}	1.02 ± 0.10^{b}	5.78 ± 0.35^{c}	559.99 ± 28.91^{cd}	916.00 ± 16.00^{c}
High LAB counts	91.83 ± 2.32^{b}	0.65 ± 0.03^{c}	5.20 ± 0.14^{d}	628.35 ± 17.27^{ab}	918.00 ± 11.10^{c}
High sensory score	94.67 ± 1.75^{a}	1.42 ± 0.12^{a}	9.73 ± 0.53^{a}	667.16 ± 32.27^{a}	919.30 ± 9.00^{c}
Low selenium concentration	88.50 ± 2.07^{d}	0.67 ± 0.02^{c}	7.77 ± 0.25^{b}	650.13 ± 29.84^{a}	1018.00 ± 15.60^{a}
High selenium concentration	88.83 ± 1.33^{cd}	1.45 ± 0.14^{a}	5.78 ± 0.20^{c}	459.73 ± 6.95^{e}	1018.70 ± 3.10^{a}

Different small letters indicate a significant difference among treatments ($P < 0.05$).
LAB – lactic acid bacteria, *WHC* – water holding capacity.

greater than 1000 ml·kg^{-1} (Tab. 1), therefore, the Se-containing exopolysaccharide, saccharose, inoculum and culture time under the optimum conditions for low titration acidity were 0.60 g·l^{-1}, 60 g·l^{-1}, 30 ml·l^{-1} (3.00×10^7 CFU·l^{-1}) and 7 h, respectively. The same approach was successfully used by JIA [24], who used yeasts for enriching and transforming selenium, and optimized production conditions of Se-enriched yogurt regarding titration acidity using a four-factor five-level orthogonal design.

According to *WHC* range analysis (Tab. 2), we found that the influencing degree of the four factors was culture time > Se-containing exopolysaccharide > inoculation > saccharose. Se-containing exopolysaccharide, saccharose, inoculum and culture time under the optimum conditions were 0.40 g·l^{-1}, 70 g·l^{-1}, 40 ml·l^{-1} (4.00×10^7 CFU·l^{-1}) and 9 h, respectively.

The order of the four factor effects on counts of LAB was found to be saccharose > inoculation > Se-containing exopolysaccharide > culture time (Tab. 2). The optimum conditions were 0.40 g·l^{-1} Se-containing exopolysaccharide, 70 g·l^{-1} saccharose, 40 ml·l^{-1} (4.00×10^7 CFU·l^{-1}) inoculum and 7 h culture time regarding the concentration of LAB in yogurt. A similar approach was previously used by FANG et al. [25], who based on sensory evaluation and levels of LAB found the optimum production conditions for mushroom polysaccharide-containing yogurt.

These studies showed considerable variations in optimum technology conditions in different fermentation conditions. ZHAO et al. [7] studied Se-enriched polysaccharide extracted from mushrooms, but did not apply it to yogurt. We extracted exopolysaccharide from cultivation of Se-enriched *A. luteo-virens* and optimized the conditions of production of Se-enriched yogurt based on several indicators. Six optimum conditions of yogurt were obtained for sensory evaluation, selenium concentration, counts of LAB, *WHC* and titration acidity. Four factors were different for response variables (Tab. 2).

Verification of fermentation conditions of yogurt

A validation test was conducted using separate experiments at optimum conditions. The experimental values for counts of LAB, sensory evaluation, *WHC*, titration acidity and selenium concentration were determined in optimum and control groups (Tab. 3). The results of complementary experiment confirmed that the practical optimal conditions of yogurt were well consistent with the predicted values. Furthermore, the high sensory evaluation condition of yogurt had higher

Tab. 4. Effects of Se-containing exopolysaccharide on physico-chemical properties of yogurt.

Se-containing exopolysaccharide [g·l^{-1}]	Sensory evaluation	Selenium concentration [mg·l^{-1}]	Lactic acid bacteria counts [10^8 CFU·ml^{-1}]	Water holding capacity [g·kg^{-1}]	Titration acidity [ml·kg^{-1}]	Soluble protein [μg·ml^{-1}]	Fat [mg·ml^{-1}]
0	82.00 ± 3.58^{c}	0.01 ± 0.00^{f}	1.98 ± 0.09^{b}	503.88 ± 9.71^{d}	1 114.70 ± 9.20^{a}	96.87 ± 5.38^{f}	53.92 ± 1.38^{b}
0.20	84.83 ± 2.99^{bc}	0.36 ± 0.03^{e}	2.48 ± 0.34^{a}	647.70 ± 15.48^{b}	1 083.30 ± 4.20^{b}	154.08 ± 0.86^{e}	53.90 ± 2.13^{b}
0.40	86.17 ± 2.71^{b}	0.73 ± 0.05^{d}	1.78 ± 0.03^{b}	666.15 ± 9.09^{a}	1 070.70 ± 15.50^{c}	179.95 ± 2.28^{d}	55.17 ± 0.50^{b}
0.60	88.33 ± 1.75^{ab}	1.14 ± 0.08^{c}	1.36 ± 0.32^{c}	671.63 ± 4.56^{a}	1 055.30 ± 13.60^{c}	236.67 ± 12.43^{c}	58.73 ± 1.14^{a}
0.80	89.50 ± 1.87^{a}	1.51 ± 0.08^{b}	1.15 ± 0.06^{c}	680.93 ± 8.62^{a}	1 019.30 ± 1.20^{d}	292.39 ± 3.95^{b}	49.98 ± 1.78^{c}
1.00	86.17 ± 2.79^{b}	1.90 ± 0.10^{a}	1.17 ± 0.11^{c}	545.87 ± 16.63^{c}	1 010.70 ± 9.20^{d}	361.49 ± 7.00^{a}	50.56 ± 0.56^{c}

Different small letters indicate a significant difference among treatments ($P < 0.05$).

WHC, counts of LAB and selenium concentration, in addition to having lower titration acidity than other optimal conditions.

Physico-chemical properties of yogurt

Exopolysaccharide may have great effect on physico-chemical properties of yogurt. The exopolysaccharide produced by LAB could improve the texture of yogurt because of interacting with the free water in the gel-like structure, and reduce whey separation, which is the phenomenon previously observed by CARTASEV and RUDIC [26] and MENDE et al. [27]. Moreover, the concentration of polysaccharide and the properties of yogurt had no linear correlation, and the use of polysaccharides from mushrooms at certain concentration levels displayed higher *WHC*, lower whey separation and better mouthfeel when compared to the control yogurt without added polysaccharide [28]. Therefore, these polysaccharides could effectively replace some stabilizers in food. To examine the Se-containing exopolysaccharide effect, *WHC*, titration acidity, sensory evaluation, selenium concentration, counts of LAB, soluble protein and fat were measured and these results are shown in Tab. 4. The yogurt supplied with 0.60–0.80 g·l^{-1} Se-containing exopolysaccharide had higher *WHC* and soluble protein, lower acidity, lower counts of LAB and better sensory properties than the control yogurt without added Se-containing exopolysaccharide ($P < 0.05$).

Our results also suggest that 0.40–0.80 g·l^{-1} Se-containing exopolysaccharide significantly increased the sensory score ($P < 0.05$), whereas 0.20 g·l^{-1} Se-containing exopolysaccharide had no significant effect. The results support the hypothesis that higher concentration of selenium affects the sensory properties because of selenium flavour and colour. Consistent with this notion, ALZATE et al. [9] produced Se-enriched fermented milk by addition of sodium selenite and found that selenium content below 2 mg·kg^{-1} had no effect on sensory features until the 4th week after fermentation. Similarly, sensory score of yogurt was increased slightly by increasing Na_2SeO_3 content from 40 μg·kg^{-1} to 70 μg·kg^{-1}, then decreased sharply with addition greater than 70 μg·kg^{-1} [28].

Titration acidity of normal yogurt products is within the range of 700–1 200 ml·l^{-1}. The acidity was found to vary slightly at Na_2SeO_3 addition between 40 μg·kg^{-1} and 80 μg·kg^{-1} [28]. We found that 0.60–0.80 g·l^{-1} Se-containing exopolysaccharide decreased the titration acidity. This may be due to the polysaccharide type and its decomposition [29, 30].

By comparison of *WHC* of the control (503.88 g·kg^{-1}) and of the samples with Se-containing exopolysaccharide (545.87–680.93 g·kg^{-1}), addition of Se-containing exopolysaccharide increased significantly *WHC* of yogurt ($P < 0.05$).

The counts of LAB in yogurt might be inhibited by high concentrations of selenium [31, 32], which accords with our findings that the addition of 0.20 g·l^{-1} Se-containing exopolysaccharide facilitated the growth of LAB but 0.60–0.80 g·l^{-1} Se-containing exopolysaccharide significantly decreased the counts of LAB ($P < 0.05$).

Soluble protein concentrations of yogurt were observed to increase significantly ($P < 0.05$) as the addition of Se-containing exopolysaccharide increased (Tab. 4).

As for the fat concentration, the highest concentration of fat was observed in 0.60 g·l^{-1} Se-containing exopolysaccharide-added yogurt (Tab. 4). There was a marked decrease on fat in yogurt treated with 0.80–1.00 g·l^{-1} Se-containing exopolysaccharide ($P < 0.05$) compared with control yogurt.

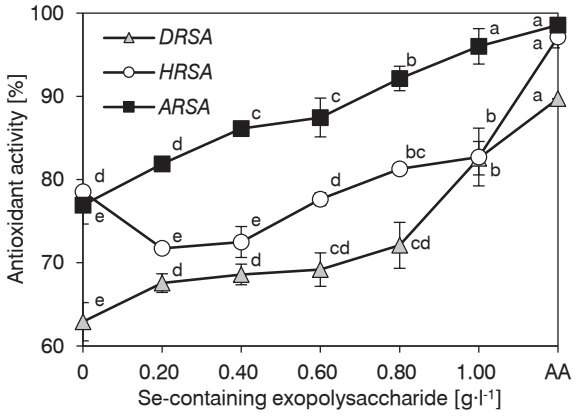

Fig. 1. Antioxidant activity of yogurt.

Different small letters indicate a significant difference among treatments ($P < 0.05$).
DRSA – 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity, *HRSA* – hydroxyl radical-scavenging activity, *ARSA* – 2,2-azinobis(3-ethylbenzthiazoline)-6-sulfonic acid radical-scavenging activity, AA – ascorbic acid.

Antioxidant activities of yogurt

The DPPH, hydroxyl (OH) and ABTS radical-scavenging potential of the yogurt samples are presented in Fig. 1. All the yogurt samples demonstrated significant scavenging activities, which varied depending on the concentration of Se-containing exopolysaccharide supplement in yogurt, while showed lower scavenging effect than ascorbic acid.

The physiological functions of yogurt have ge-

Tab. 5. Correlation of physico-chemical and antioxidant properties of yogurt.

Parameter	*DRSA*	*HRSA*	*ARSA*	Selenium concentration	LAB count	Soluble protein	Fat
DRSA	1	0.587	0.920**	0.907*	–0.655	0.939**	–0.523
HRSA		1	0.582	0.663	–0.808	0.676	–0.528
ARSA			1	0.990**	–0.816*	0.985**	–0.451
Selenium concentration				1	–0.866*	0.994**	–0.417
LAB count					1	–0.826*	0.278
Soluble protein						1	–0.471
Fat							1

Correlation analysis was conducted using Spearman's rho method (* – correlation is significant at the 0.05 level, ** – correlation is significant at the 0.01 level).
DRSA – 2,2-diphenyl-1-picrylhydrazyl radical-scavenging activity, *HRSA* – hydroxyl radical-scavenging activity, *ARSA* – 2,2-azinobis(3-ethylbenzthiazoline)-6-sulfonic acid radical-scavenging activity, LAB – lactic acid bacteria.

nerated increasing attention among researchers [9, 33]. Some researchers successfully prepared yogurts with antioxidant and antimicrobial activities by adding polysaccharides, or oligosaccharides, which has also been shown to enhance the antioxidant and antimicrobial activities of the yogurt [8, 34]. Some polysaccharide-added yogurts could effectively remove hydroxyl, DPPH and ABTS free radicals, their scavenging capabilities being positively correlated with the amount of added polysaccharides within a certain concentration range [8]. For instance, LASRADO and GUDIPATI [34] applied xylooligosaccharides from wheat bran in fermented milk, which led to a significant ($P < 0.05$) increase in radical-scavenging activity. Se-containing polysaccharides derived from Se-enriched mushrooms or from chemosynthesis have shown increased activities of scavenging DPPH and hydroxyl radicals, selenium playing a key role in offering the antioxidant capacity to the polysaccharide [7, 35]. Our results confirmed that Se-containing polysaccharides enhanced the *DRSA* and *ARSA* of the yogurt.

Correlation of physico-chemical and antioxidant properties of yogurt

Data on correlation of physico-chemical and antioxidant properties of yogurt are presented in Tab. 5. There were significant positive correlations between *ARSA* and *DRSA*, and between *ARSA* and selenium concentration ($P < 0.01$), as well as between *DRSA* and selenium concentration ($P < 0.05$). However *ARSA*, selenium concentration and concentration of soluble protein negatively correlated with counts of LAB ($P < 0.05$).

CONCLUSIONS

The optimum formulation of yogurt with Se-containing exopolysaccharide was recommended based on a prediction using orthogonal design. Compared to the control, yogurt with Se-containing exopolysaccharide showed better sensory, higher soluble protein and greater radical-scavenging activities. The yogurt with Se-containing exopolysaccharide could potentially serve as a dietary supplement. However, further studies regarding its flavour are needed to develop a viable product.

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