

EFFECT OF SUBSTRATE COMPOSITION ON YIELD AND ANTIOXIDATIVE ACTIVITY OF EXOPOLYSACCHARIDES FROM *LACTOBACILLUS FERMENTUM* B62

– Research paper –

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Abstract: Exopolysaccharides (EPS) can not only give food a unique texture but also has antioxidant capacities. To select the medium composition that influences the yield and antioxidative activity of EPS, Plackett–Burman (PB) design was employed to appraise the effects of carbon sources, nitrogen sources, and inorganic salts on yield and DPPH free radical scavenging (DPPH-FRS) rate of EPS in MRS medium fermented by *Lactobacillus fermentum* B62. The result indicated that sucrose ($p < 0.01$), peptone ($p < 0.01$), and KH_2PO_4 ($p < 0.001$) had the most distinguishing comprehensive effects on yield and DPPH-FRS rate of EPS, and fructose also had a noticeable effect on the two factors ($p < 0.05$, $p < 0.001$, respectively). Additionally, glucose ($p < 0.05$), soy protein ($p < 0.001$), yeast extract ($p < 0.01$), KH_2PO_4 ($p < 0.001$) and $\text{Ca}(\text{H}_2\text{PO}_4)_2$ ($p < 0.001$) significantly positive affect the yield of EPS. And inulin ($p < 0.05$), tryptone ($p < 0.001$), beef extract powder ($p < 0.001$), NaH_2PO_4 ($p < 0.01$) and $\text{C}_2\text{H}_3\text{NaO}_2$ ($p < 0.05$) significantly positive affect the DPPH-FRS rate of EPS. Within the test ranges, sucrose, fructose, peptone and KH_2PO_4 all showed significant positive relativity to the yield and anti-oxidative activity of EPS.

Keywords: *Lactobacillus fermentum* B62, exopolysaccharides, DPPH, medium optimization, Plackett-Burman design

INTRODUCTION

Exopolysaccharides (EPS) usually refers to the metabolites produced by microorganisms during the growth process, mainly composed of glucose, fructose, galactose, etc. Most EPS are beneficial to the human body and biodegradable (Pourjafar et al., 2022). In recent decades, EPS has been widely used in food, petrochemical, agriculture, medicine, and other fields because of its rheological properties, thermal stability, and immune regulation properties. A large number of articles reported that the microorganisms producing EPS are widely distributed and have many kinds, which can be divided into the following three categories in general (Tiwari et al., 2020). (1) Bacteria: *lactic acid bacteria* (LAB) (Yang et al., 2019), *Agrobacterium* (Matthysse et al., 2018), *Pseudomonas* (Yang et al., 2016), etc.; (2) Fungi:

Aureobasidium, *Rhodotorula*, *Candida*, etc. (Saadat, et al., 2021); (3) Algae: *Dictyosphaerium sp.* (Wang et al., 2020), *Planococcus* (Finore et al., 2014), *Parachlorella sp.* (Ms et al., 2020).

EPS from LAB are widely used as stabilizers, emulsifiers, and gelling agents, in the food industry (Saadat, et al., 2019). *Lactobacillus* is a member of LAB and is generally considered to be Generally Recognized As Safe (GRAS) or Qualified Presumption of Safety (QPS) status (Caggianiello et al., 2016). Ale et al. (2016) added EPS extracted from *Lactobacillus fermentum* LF2 to yogurt and milk respectively, reduced the level of Interleukin-6 (IL6) in the small intestine, increased the level of Secretory Immunoglobulin A (IgA), and alleviated the intestinal inflammation of Salmonella infected mouse model. Zhang et al. (2013) showed that the EPS which isolated from *Lactobacillus plantarum* C88 has the excellent antioxidant activity in vitro. And this additive can improve antioxidant enzyme activity, eliminate oxygen free radicals and reduce lipid peroxidation. In addition, EPS (crude and

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purified EPS from *Lactobacillus rhamnosus* RW-9595M fermentation in whey-based medium) can be used as the food additive to improve the rheological properties of yogurt and help to produce new functional yogurt with better rheological texture (Doleyres, et al., 2005). Therefore, adding these EPS into the food can improve the quality of food and promote human health. Compared with plant, animal, and algae polysaccharides, the LAB of producing EPS is more stable, reliable, reproducible and has large market demand. However, the production cost is higher than such polysaccharides. Hence, optimizing the economic low-cost LAB medium and improving the

production of EPS will be critical developing techniques (Daba, et al., 2021).

In previous work, five strains (B55, B62, B30, 7830, and K2) were screened and identified with high EPS yield and strong antioxidant activity from 66 strains preserved in our lab. Among the 66 strains, B62 has demonstrated the preliminary identification as *Lactobacillus fermentum* with the highest EPS yield and the stronger ability of removing DPPH free radicals (Shangguan et al., 2019). In line with that research, the Plackett-Burman (PB) design method is adopted to further optimize the medium composition of B62 in order to obtain higher EPS and stronger ability of removing DPPH free radicals.

MATERIALS AND METHODS

Microbial strains and culture conditions

Lactobacillus fermentum B62 (Shaanxi University of Science & Technology, Xi'an, China) was isolated from the inner Mongolia dairy products. The strain was incubated (37°C, 24 h) in the MRS at an inoculum size of 2%.

Preparation of crude EPS

The MRS medium composition was varied according to PB experiment design method to achieve the best production and anti-oxidative activity of EPS. In short, various types of carbon sources, nitrogen sources, and inorganic salts were added into MRS, respectively. Then, the 3% activated *Lactobacillus fermentum* B62 was added to the modified MRS medium and incubated at 37 °C for 48 h to prepare EPS.

The cell supernatant was collected by centrifugation (8000 rpm, 15 min, 4°C), and then, 10% Trichloroacetic acid solution (TCA) and double volume-chilled ethanol were added into supernatant, respectively, and storage overnight at 4°C. The sediments (crude EPS) were harvested by centrifugal mixture (8000 rpm, 15 min) (Savadogo et al., 2003). After dissolved in distilled water, the crude EPS was dialyzed for 24h.

Phenol-Sulphuric Acid Method for EPS quantification

EPS was measured according to the phenol-sulphuric acid method (Dubois et al., 1956). 1 mL 5% (w·v) phenol was added to the sample (1 mL) and next added 5 mL concentrated sulfuric acid (keep sample tube in ice), then keeping the sample at room temperature (20 min). Then, the absorbance (at 490 nm) was measured (Shangguan et al., 2019).

Standard curve is shown in formula (1):

$$y = 0.0034x + 0.0134 (R^2 = 0.9934) \quad (1)$$

DPPH free radical scavenging (DPPH-FRS) rate

DPPH-FRS rate was determined by the method of (Wang et al., 2014) with some modifications. DPPH solution (0.1 mM in 95% ethanol) was mixed with 3.0 mL sample solutions, meanwhile at room temperature in the dark (30 min). Then, the absorbance (517 nm) was measured. As a control group, the sample was mixed with 95% ethanol solution, while the blank group was 95% ethanol instead of the sample (Shangguan et al., 2019).

The DPPH-FRS rate was obtained by the following formula (2):

$$\text{DPPH radical scavenging rate(\%)} = \frac{[A_0 - (A_i - A_j) \times 100\%]}{A_0} \quad (2)$$

with A_0 – the absorbance of the blank group;

A_i – the absorbance of experimental group;

A_j – the absorbance of the control group.

Plackett-Burman design

Based on the PB design, each factor was divided into two levels of trials: the low level was "-1", and the high level was "+1" (Plackett et al., 1946). In this experiment, PB design was used to screen 8 influencing factors and generated a set of 12 experimental designs. Based on the results of our previous research, the carbon sources (glucose, lactose, sucrose, fructose, inulin, galactose, fructooligosaccharides, raffinose), nitrogen sources (peptone, yeast extract, beef extract powder, tryptone, casein, soy protein, bovine serum albumin, whey protein;), and inorganic salts (K_2HPO_4 , $(NH_4)_2SO_4$, Na_2CO_3 , KH_2PO_4 , $CaCl_2$, NaH_2PO_4 ,

C₂H₃NaO₂, Ca(H₂PO₄)₂) were tested at a lower level (addition amount: 0.5%, 0.5%, 0.1%, respectively) and higher level (addition amount: 1%, 1%, 0.2%, respectively).

The tests were performed in three groups in parallel, the 11 variables in each group contained 3 dummy terms (carbon sources: C, E, J; nitrogen sources: B, E, H; inorganic salts: B, E, J) for estimating standard deviation, and the EPS yield and DPPH-FRS rate were the test response values (Dong Y et al., 2020). The PB test design was based on a first-order polynomial model:

$$Y = \beta_0 + \sum \beta_i X_i \quad (3)$$

where: Y – Response value (EPS yield and DPPH radical scavenging rate); β_0 – Model Intercept; β_i –

Linearity coefficient; X_i – Independent variable level.

As an efficient analysis tool, PB design can be used for the influence of various factors in the preliminary selection experiment, to reliably list the degree of influence of related factors (Chen et al., 2017).

Statistical Analysis

The statistical analysis was performed by (Version, 8.0.6) to determine the important variables and corresponding coefficients. The significant differences among the values were evaluated by variance (ANOVA).

RESULTS AND DISCUSSION

Effect of the carbon source on EPS yield and DPPH-FRS rate of *L. fermentum* B62

PB experimental design matrix and data results with carbon sources were showed in Table 1. EPS yield and the DPPH-FRS rate were used as the response values.

The variance analysis of several factors on EPS yield and DPPH-FRS rate is displayed in Table 2 showing that the model of the EPS yield ($p=0.0325$) and the DPPH-FRS rate ($p=0.0124$) are significant. The degree of influence on EPS yield analysis are: D ($p=0.0040$) > A($p=0.0310$) > F ($p=0.0391$) > L ($p=0.2553$) > B ($p=0.3924$) > K ($p=0.4041$) > G ($p=0.5507$) > H ($p=0.6841$), where symbols used to describe the parameters were: A, glucose; B, lactose; D, sucrose; F, fructose; G, inulin; H, galactose; K,

fructooligosaccharides; L, raffinose.

The degree of influence on DPPH-FRS rate analysis is: D ($p=0.0016$) > F ($p=0.0068$) > G ($p=0.0249$) > B ($p=0.0849$) > H ($p=0.4083$) > A ($p=0.5981$) > L ($p=0.8475$) > K ($p=0.9349$). Therefore, comprehensive the above two response values, sucrose (D) was chosen as the carbon source in the follow-up test. In addition, F had a significant effect on the two factors.

The impacts of D and F on the two response values are both positive, as shown in Figure 1. D was greater than F. Figure 2 further showed that A had a favorable impact on EPS yield and G had a positive effect DPPH-FRS activity. In future experiments, we could consider increasing the amount of D-sucrose.

Table 1. PB experimental design matrix and data results for carbon sources

RUN	A	B	C	D	E	F	G	H	J	K	L	R1	R2
1	1	0.5	-1	0.5	1	0.5	1	1	-1	1	1	95.04	21.21
2	0.5	1	1	1	-1	0.5	0.5	1	-1	1	1	135.52	27.63
3	1	1	-1	0.5	-1	1	0.5	1	1	0.5	1	102.30	22.18
4	0.5	1	-1	1	1	0.5	1	1	1	0.5	0.5	132.23	30.16
5	1	0.5	1	1	1	0.5	0.5	0.5	1	0.5	1	137.42	29.75
6	1	0.5	1	1	-1	1	1	1	-1	0.5	0.5	205.41	44.98
7	0.5	1	1	0.5	1	1	1	0.5	-1	0.5	1	90.10	26.63
8	0.5	0.5	-1	1	-1	1	1	0.5	1	1	1	143.47	44.49
9	0.5	0.5	1	0.5	1	1	0.5	1	1	1	0.5	85.35	22.96
10	1	1	-1	1	1	1	0.5	0.5	-1	1	0.5	215.28	36.07
11	1	1	1	0.5	-1	0.5	1	0.5	1	1	0.5	95.81	20.63
12	0.5	0.5	-1	0.5	-1	0.5	0.5	0.5	-1	0.5	0.5	48.56	18.63

A, glucose; B, lactose; C, dummy factor; D, sucrose; E, dummy factor; F, fructose; G, inulin; H, galactose; J, dummy factor; K, fructooligosaccharides; L, raffinose; R1, EPS yield, mg/L; R2, DPPH-FRS rate, %.

Table 2. ANOVA of carbon source on EPS yield and DPPH-FRS rate

Source	EPS yield				DPPH-FRS rate		
	DF	MS	F	P-Value	MS	F	P-Value
Model	8	3170.98	12.06	0.0325*	107.74	23.75	0.0124*
A-glucose	1	3889.22	14.79	0.0310*	1.57	0.35	0.5981
B-lactose	1	261.24	0.99	0.3924	29.19	6.43	0.0849
D-sucrose	1	17037.63	64.79	0.0040**	544.66	120.08	0.0016**
F-fructose	1	3244.57	12.34	0.0391*	202.66	44.68	0.0068**
G-inulin	1	118.10	0.45	0.5507	79.44	17.51	0.0249*
H-galactose	1	52.94	0.20	0.6841	4.17	0.92	0.4083
K- fructo -oligosaccharides	1	246.84	0.94	0.4041	0.04	0.01	0.9349
L-raffinose	1	517.32	1.97	0.2553	0.20	0.04	0.8475
Residual	3	262.95			4.54		
Cor Total	11						

**p<0.01, highly significant; *p<0.05, significant

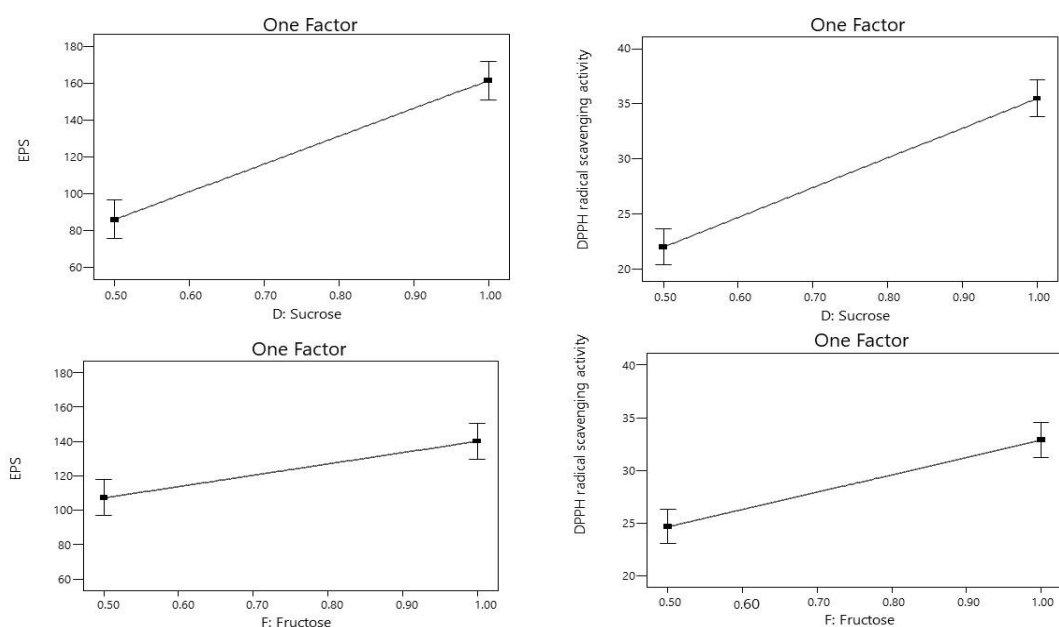


Figure 1. Confidence intervals of sucrose and fructose on EPS yield and DPPH-FRS rate

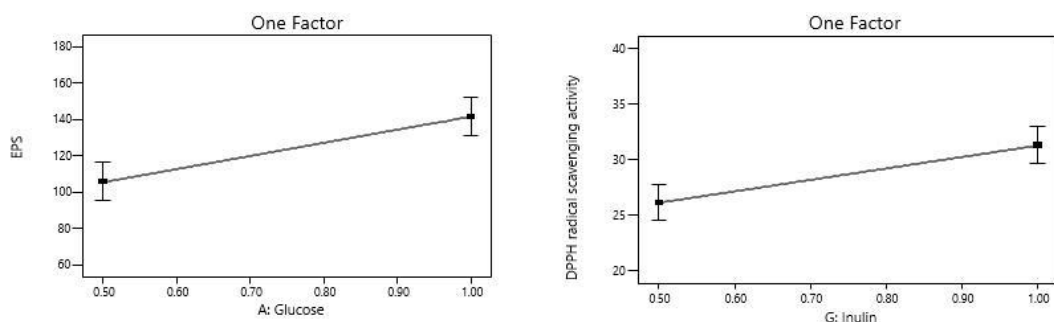


Figure 2. Confidence intervals of glucose on EPS production and inulin on DPPH-FRS rate

Effect of the nitrogen source on EPS yield and DPPH-FRS rate of *L. fermentum* B62

PB experimental design data results for screening of nitrogen sources are displayed in Table 3. R1 and R2 are the EPS yield and DPPH-FRS rate response values, respectively.

The ANOVA of different nitrogen sources for the EPS yield and DPPH-FRS rate by *Lactobacillus fermentum* B62 is shown in Table 4. The model of the EPS yield ($p=0.0046$) and the DPPH-FRS rate ($p=0.0014$) can be significant. The degree of influence on EPS yield analysis is: J ($p=0.0007$) > C ($p=0.0027$) > A ($p=0.0053$) > G ($p=0.0503$) > D ($p=0.1183$) > K ($p=0.1232$) > F ($p=0.2178$) > L ($p=0.3500$), with A, Peptone; C, Yeast extract; D, Beef extract powder; F, Tryptone; G, Casein; J, Soy

protein; K, Bovine serum albumin; L, Whey protein. The degree of influence on DPPH-FRS rate analysis is: F ($p=0.0002$) > D ($p=0.0008$) > A ($p=0.0033$) > J ($p=0.0500$) > K ($p=0.0553$) > G ($p=0.2398$) > C ($p=0.2747$) > L ($p=0.3779$). Therefore, comprehensive the above two response values, peptone (A) was chosen as the nitrogen source in the follow-up test.

Figure 3 shows the effects of A-peptone on the two response values are both positive. Furthermore, it can be observed (Figure 4) that the effect of J-soy protein and C-yeast extract on EPS yield and the effect of F-tryptone and D-beef extract powder on DPPH-FRS activity are positive. In future experiments, we could consider increasing the amount of peptone.

Table 3. PB experimental design matrix and data results for nitrogen sources

RUN	A	B	C	D	E	F	G	H	J	K	L	R1	R2
1	1	-1	0.5	0.5	1	0.5	1	1	0.5	1	1	87.08	24.05
2	1	1	0.5	0.5	-1	1	0.5	1	1	0.5	1	107.15	30.30
3	0.5	-1	0.5	1	-1	1	1	-1	1	1	1	103.77	34.90
4	1	-1	1	1	-1	1	1	1	0.5	0.5	0.5	103.51	37.60
5	0.5	1	0.5	1	1	0.5	1	1	1	0.5	0.5	98.75	24.10
6	0.5	-1	0.5	0.5	-1	0.5	0.5	-1	0.5	0.5	0.5	64.50	20.25
7	0.5	1	1	0.5	1	1	1	-1	0.5	0.5	1	89.33	28.50
8	1	1	1	0.5	-1	0.5	1	-1	1	1	0.5	124.27	23.50
9	0.5	1	1	1	-1	0.5	0.5	1	0.5	1	1	91.38	25.35
10	0.5	-1	1	0.5	1	1	0.5	1	1	1	0.5	108.83	27.80
11	1	1	0.5	1	1	1	0.5	-1	0.5	1	0.5	86.38	38.90
12	1	-1	1	1	1	0.5	0.5	-1	1	0.5	1	118.12	26.65

A, Peptone; B, Dummy factor; C, Yeast extract; D, Beef extract powder; E, Dummy factor; F, Tryptone; G, Casein; H, Dummy factor; J, Soy protein; K, Bovine serum albumin; L, Whey protein; R1, EPS yield, mg/L; R2, DPPH-FRS rate, %

Table 4. ANOVA of nitrogen sources on EPS yield and DPPH-FRS rate

Source	EPS yield				DPPH-FRS rate		
	DF	MS	F	P-Value	MS	F	P-Value
Model	8	353.65	46.40	0.0046**	47.48	105.39	0.0014**
A-Peptone	1	407.84	53.51	0.0053**	33.67	74.72	0.0033**
C-Yeast extract	1	642.55	84.30	0.0027**	0.80	1.78	0.2747
D-Beef extract powder	1	35.95	4.72	0.1183	91.30	202.64	0.0008***
F-Tryptone	1	18.42	2.42	0.2178	243.90	541.33	0.0002***
G-Casein	1	76.78	10.07	0.0503	0.96	2.14	0.2398
J-Soy protein	1	1603.85	210.42	0.0007***	4.56	10.13	0.0500
K-Bovine serum albumin	1	34.53	4.53	0.1232	4.20	9.32	0.0553
L-Whey protein	1	9.30	1.22	0.3500	0.48	1.07	0.3779
Residual	3	7.62			0.45		
Cor Total	11						

*** $p<0.001$, extremely significant; ** $p<0.01$, highly significant; * $p<0.05$, significant

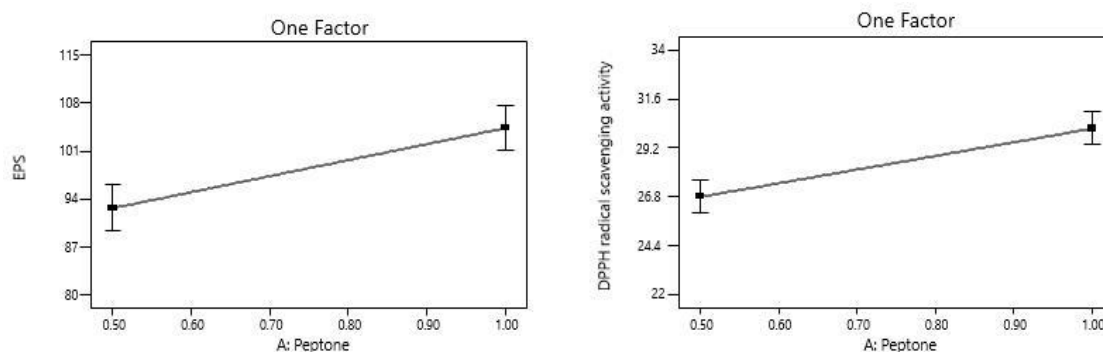


Figure 3. Confidence intervals of peptone on EPS yield and DPPH-FRS rate

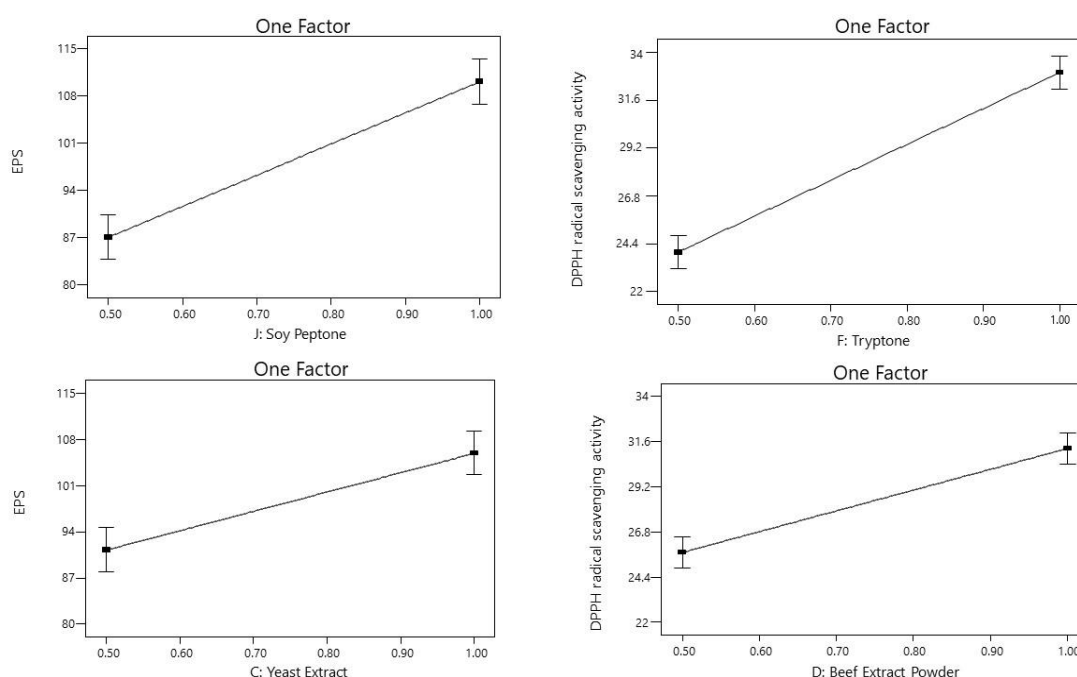


Figure 4. Confidence intervals for EPS yield by soy protein and yeast extract and DPPH-FRS rate by tryptone and beef extract powder

Effect of the inorganic salts on EPS yield and DPPH-FRS rate of *L. fermentum* B62

Table 5 demonstrates the experimental design matrix for PB and the data results with inorganic salts. R1 and R2 are used as the response values. Variance was used to evaluate the impact of different inorganic salts on EPS yield and DPPH-FRS rate (Table 6). The model on the EPS yield ($p=0.0006$) and the DPPH-FRS rate ($p=0.0026$) can be shown to be significant. The degree of influence on EPS yield analysis: A ($p=0.0001$) > L ($p=0.0003$) > F ($p=0.0007$) > G ($p=0.0048$) > H ($p=0.0080$) > D ($p=0.0503$) > K ($p=0.0904$) > C ($p=0.3096$). The

degree of influence on DPPH-FRS rate analysis: F ($p=0.0002$) > H ($p=0.0031$) > K ($p=0.0279$) > G ($p=0.1763$) > C ($p=0.3015$) > D ($p=0.3419$) > L ($p=0.7852$) > A ($p=0.8491$). Therefore, comprehensive the above two response values, KH_2PO_4 (F) was chosen as the nitrogen source in the follow-up test.

Figure 5 shows the effects of F on the two response values are both positive. Furthermore, it can be seen from Figure 6 that the effect of A and L on EPS yield and the effect of H and K on DPPH-FRS activity are positive. In future experiments, we could consider increasing the amount of F.

Table 5. PB experimental design matrix and data results for inorganic salts

RUN	A	B	C	D	E	F	G	H	J	K	L	R1	R2
1	0.1	1	0.2	0.2	-1	0.1	0.1	0.2	-1	0.2	0.2	59.27	27.85
2	0.2	-1	0.2	0.2	-1	0.2	0.2	0.2	-1	0.1	0.1	84.01	35.14
3	0.2	1	0.2	0.1	-1	0.1	0.2	0.1	1	0.2	0.1	79.45	25.48
4	0.1	-1	0.2	0.1	1	0.2	0.1	0.2	1	0.2	0.1	58.75	36.61
5	0.1	1	0.2	0.1	1	0.2	0.2	0.1	-1	0.1	0.2	86.96	32.45
6	0.2	1	0.1	0.1	-1	0.2	0.1	0.2	1	0.1	0.2	99.06	36.07
7	0.2	-1	0.1	0.1	1	0.1	0.2	0.2	-1	0.2	0.2	90.59	28.67
8	0.1	-1	0.1	0.1	-1	0.1	0.1	0.1	-1	0.1	0.1	53.22	22.85
9	0.2	1	0.1	0.2	1	0.2	0.1	0.1	-1	0.2	0.1	83.15	33.49
10	0.1	1	0.1	0.2	1	0.1	0.2	0.2	1	0.1	0.1	53.90	27.52
11	0.1	-1	0.1	0.2	-1	0.2	0.2	0.1	1	0.2	0.2	83.49	33.69
12	0.2	-1	0.2	0.2	1	0.1	0.1	0.1	1	0.1	0.2	89.20	21.60

A, K_2HPO_4 ; B, dummy factor; C, $(NH_4)_2SO_4$; D, Na_2CO_3 ; E, dummy factor; F, KH_2PO_4 ; G, $CaCl_2$; H, NaH_2PO_4 ; J, dummy factor; K, $C_2H_3NaO_2$; L, $Ca(H_2PO_4)_2$; R1, EPS yield, mg/L; R2, DPPH-FRS rate, %

Table 6. ANOVA of inorganic salts on EPS yield and DPPH-FRS rate

Source	EPS yield				DPPH-FRS rate		
	DF	MS	F	P-Value	MS	F	P-Value
Model	8	349.37	187.83	0.0006***	36.45	68.13	0.0026**
A- K_2HPO_4	1	1405.66	755.71	0.0001***	0.02	0.04	0.8491
C- $(NH_4)_2SO_4$	1	2.77	1.49	0.3096	0.83	1.55	0.3015
D- Na_2CO_3	1	18.74	10.07	0.0503	0.68	1.27	0.3419
F- KH_2PO_4	1	406.07	218.31	0.0007***	238.39	445.52	0.0002***
G- $CaCl_2$	1	106.42	57.21	0.0048**	1.66	3.10	0.1763
H- NaH_2PO_4	1	74.38	39.99	0.0080**	41.42	77.41	0.0031**
K- $C_2H_3NaO_2$	1	11.30	6.08	0.0904	8.58	16.03	0.0279*
L- $Ca(H_2PO_4)_2$	1	769.60	413.75	0.0003***	0.05	0.09	0.7852
Residual	3	1.86			0.54		
Cor Total	11						

***p<0.001, extremely significant; **p<0.01, highly significant; *p<0.05, significant

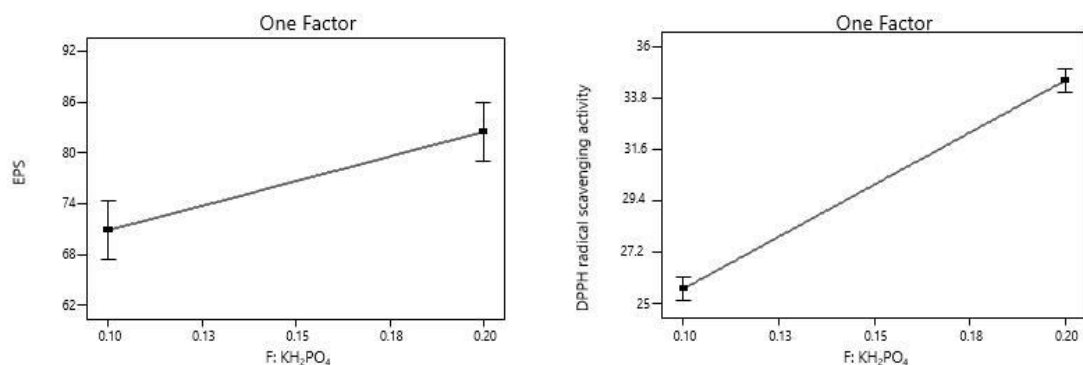


Figure 5. Confidence intervals of KH_2PO_4 on EPS yield and DPPH-FRS rate

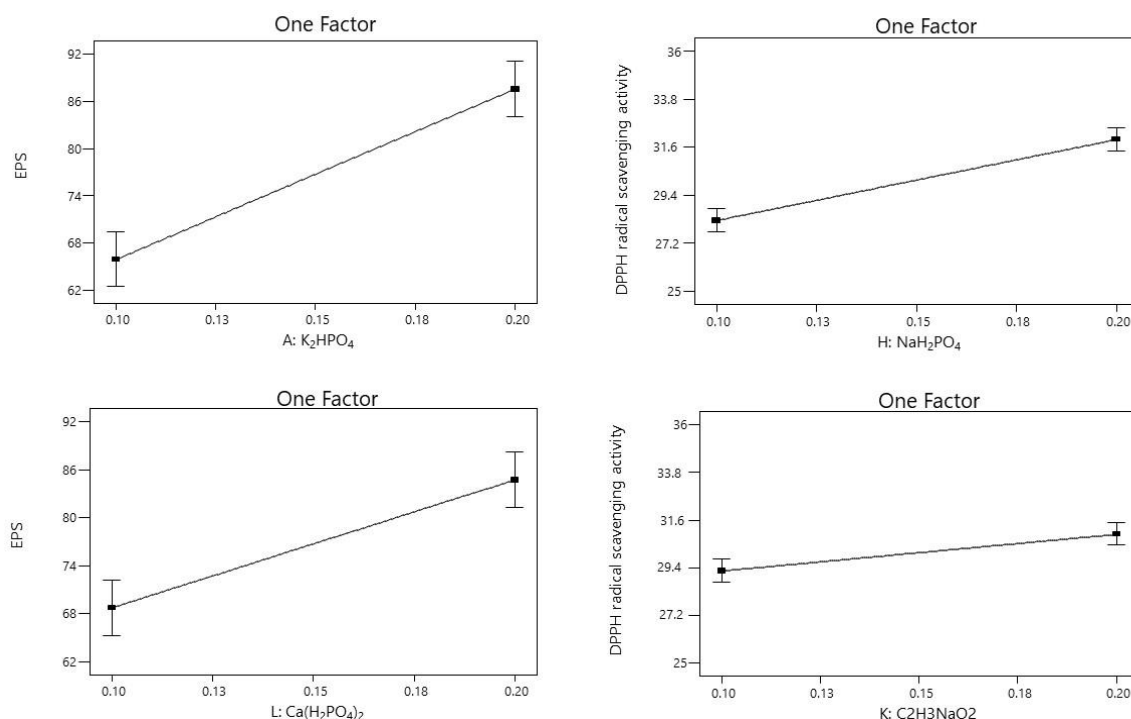


Figure 6. Confidence intervals for EPS yield by K₂HPO₄ and Ca(H₂PO₄)₂ and DPPH-FRS rate by NaH₂PO₄ and C₂H₃NaO₂

DISCUSSION

In our study, sucrose as an important carbon source may play a significant role in promoting the metabolic synthesis pathway of EPS produced by *Lactobacillus fermentum* B62, and the structural characteristics of the EPS make it have strong antioxidant activity. In the future, we can also mix the selected sucrose and fructose with strong influence as a carbon source. Improving yield and anti-oxidative activity of EPS by LAB is important for industrial production and the yield and structure of EPS are affected by changing the composition of the medium and culture conditions (Wang et al., 2017). Cheng et al. (2019) reported that antioxidant activity and yield of EPS are higher in *Lactobacillus plantarum* LPC-1 when sucrose and a mixture of sucrose and glucose are used as carbon sources than glucose. Further metabonomic analysis shows that the difference of carbon sources has a great influence on basal metabolism. When sucrose was used as the medium carbon source, the phosphofructokinase (PFK), pyruvate dehydrogenase (PDH), and lactate dehydrogenase (LDH) activities were lower. Therefore, the way to synthesize EPS has been significantly improved, which can improve the metabolic fusion of sugar synthesis of EPS, and a higher yield of EPS was achieved. Nevertheless, the effects of different

medium composition on enhanced EPS yield often vary depending on each LAB strain (Mdk et al., 2020). A study reported that maltose was used to replace glucose as the carbon source with yeast nitrogen base as nitrogen source was added (Chen et al., 2022). After optimizing the culture temperature, initial pH, culture time, carbon source, and the dosage of nitrogen source, it was found that the EPS yield of *Lactocaseibacillus rhamnosus* ZFM216 was 76.70% higher than that of before optimization. These differences may be due to the diverse regulation of the EPS of LAB synthesis pathway by various carbon sources (Imran et al., 2016). The antioxidant activity of microbial exopolysaccharides may be attributed to the hydrogen-donating ability, various electron-donating and electron-withdrawing functional groups, and the enriched reduced monosaccharide (Andrew et al., 2019).

Soy peptone, yeast extract and peptone are usually selected as nitrogen sources to promote the EPS yield by lactic acid bacteria. Xin et al. (2017) found that soybean peptone as a nitrogen source in *Lactobacillus plantarum* KX041 medium had a more significant effect on enhancing the yield of EPS than tryptone or ordinary peptone, and this is in agreement with our experimental results. The

study showed that the DPPH-FRS capacity of the EPS was mainly due to lower molecular weight and the consisted of arabinose, mannose, glucose, and galactose. A study also reported that by selecting yeast extract as a nitrogen source and increasing its addition (30 g/L) while optimizing the fermentation temperature (35.6°) and fermentation time (22 h) resulted in 280.105 mg/L of the EPS production of *Lactobacillus plantarum* SP8 (Zhang et al., 2020). Moreover, the DPPH-FRS capacity of the EPS is mainly due to the presence of hydroxyl and other functional groups in EPS, which can donate electrons or react with free radicals to terminate free radical chain reactions (Imran et al., 2016). In future studies, we can improve the EPS production by increasing the addition of nitrogen source and also optimizing the culture temperature, culture conditions and initial pH.

Phosphate is an inorganic salt in the medium that can increase the yield and antioxidant activity of EPS. Suryawanshi et al. (2019) through response surface methodology and fitting with artificial neural networks modified the MRS media of *Lactobacillus sp.* The results suggested that the EPS yield was the highest, when the medium composition consists of (g/L) dextrose 15,

potassium dihydrogen phosphate 2.5, sodium dihydrogen phosphate 3, magnesium sulfate 0.25, and tri-ammonium citrate 1.5. Another study reported that the best culture conditions for *L. acidophilus* to produce EPS are: Tween 80 0.6 mL, dipotassium hydrogen phosphate (K_2HPO_4) 3.6 g, and trisodium citrate ($C_6H_5Na_3O_7$) 4.1 g, and further analysis indicated that K_2HPO_4 and $C_6H_5Na_3O_7$ affected EPS production, possibly because they could act as buffers to change the pH of fermentation culture medium (Qi et al., 2016). Ricciardi et al. (2002) found that different pH had an impact on the yield of EPS by *Streptococcus thermophilus* SY, and the yield of EPS was the highest with pH 6.4.

The above discussion may indicate that when the composition of the medium changes, the production of EPS and the free radical scavenging rate will change, but the high yield of exopolysaccharide does not indicate that it has a strong free radical scavenging rate, and the antioxidant activity of EPS is mainly in its structural features. Thus, in our study, three influencing factors are screened by considering the yield and the DPPH-FRS rate of EPS.

CONCLUSION

The effects of medium composition on the yield and anti-oxidative activity of EPS are comprehensively assessed by PB design. In summary, the eight types of carbon source, nitrogen source, and inorganic salts are screened and among them sucrose ($p<0.01$), peptone ($p<0.01$) and KH_2PO_4 ($p<0.001$) are found to have a very significant comprehensive impact on increasing yield and DPPH-FRS rate of EPS by *Lactobacillus fermentum* B62. And fructose also had a noticeable effect on the two factors ($p<0.05$, $p<0.001$, respectively). Furthermore, glucose ($p<0.05$), soy peptone ($p<0.001$), yeast extract ($p<0.01$), K_2HPO_4 ($p<0.001$) and $Ca(H_2PO_4)_2$ ($p<0.001$) had noticeable positive effects on the EPS

yield, while the effects on free radical scavenging rate are not significant. And inulin ($p<0.05$), tryptone ($p<0.001$), beef extract powder ($P<0.001$), NaH_2PO_4 ($P<0.01$) and $C_2H_3NaO_2$ ($p<0.05$) had noticeable positive effects on the free radical scavenging rate of EPS, while the effects on the EPS yield are not significant. Within the test ranges, sucrose, fructose, peptone and KH_2PO_4 all showed significant positive relativity to the yield and anti-oxidative activity of EPS. This study may provide some technical support for the future industrial production of EPS and improvement of goat milk fermentation.

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