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Propionic Acid Bio-Fortification of Yogurts by Adjunct Culture of *Propionibacterium freudenreichii*

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Abstract

Background and Objective: Propionic acid bacteria are useful microorganisms that can produce beneficial food compounds**.** These bacteria mainly produce propionic acid that decrease the microbial population and growth along with increasing the shelf life. *Propionibacterium freudenreichii* was applied to produce propionic acid in yogurt in this study.

Material and Methods: First, the process variables like inoculum percentage, strain type, milk fat and inulin amount, fermentation temperature, sunflower oil quantity, and refrigerated duration on propionic acid production by *Propionibacterium freudenreichii* was evaluated using Plackett-Burman design as a screening method. Next the acid production was optimized by a central composite design with 3 major factors of seed size, concentration of inulin, and refrigerated storage.

Results and Conclusion: Analysis of variance showed that the models have been significant ($p \leq 0.05$). They represented that propionic acid production was influenced by three main factors. Optimized propionic acid production in yogurts by *Propionibacterium freudenreichii* ssp. *shermanii* (10^8 CFU. ml^{-1}) was observed in 21 days after of refrigeration of skim-milk 2% (w v^{-1}), inulin (3% w v^{-1}) and incubation temperature of 43 °C. Reconfirmation test showed that the highest produced propionic acid was 12.53 ± 0.24 mg l^{-1} in yogurt, which increased production up to 6.2 time. Results showed that *Propionibacterium freudenreichii* ssp. *shermanii* increases propionic acid contents in synbiotic yogurts containing inulin.

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1. Introduction

Today's consumers are more aware than ever before and want a variety of foods, better quality, and looking for foods rich in nutrients and rich in vitamins. These foods should have a longer shelf life with minimal processing and without the need for additives (synthetic and artificial materials should not be used in the structure and production of food) [1,2]. PAB (propionic acid bacteria) are well recognized and accepted as useful microorganisms in the food industry that can produce and biosynthesize some beneficial food compounds [3]. PAB, in addition to being used as a potential probiotic in many fermented dairy products, have extensive antimicrobial activity and prohibit the gram-positive and some gram-negative bacteria's growth (like *Pseudomonas* and *Salmonella*), as well as fungi and yeasts and their presence in the food environment, increases shelf life [4].

PAB are Gram-positive, nonsporulating, nonmotile, short, bacilli that can produce and biosynthesize a large number of beneficial food compounds. These bacteria can produce important industrial products like propionic acid [5] and conjugated linoleic acid (CLA) [6], vitamins of the B group, and K [7-9] and bacteriocins [5] in commercial and industrial scopes [10,11].

PAB are also applied in dairy products, in addition to meeting nutritional needs and creating satiety, also creates other benefits in the food industry. These benefits include improving the viscosity of products like yogurt (by producing exopolysaccharides) and inhibiting the undesirable microorganisms' growth (by producing propionic acid and bacteriocin) and thus increasing its shelf life. In addition, PAB growth does not intervene the lactic acid bacteria growth in dairy products like yogurt [12,13].

It was reported that the treatment of PA (propionic acid) may prolong the food's shelf life by reducing the microbial populations as PA makes the conditions unfavorable for the growth and survival of bacteria [14]. PAs are able to pass via the cell membrane into the cytoplasm and release protons by intracellular alkaline media. So the pH gradient of cell membrane is disconcerted and affected the nutrients transfer and inhibited the cells growth [15]. SCFAs (short-chain fatty acids) are like acetic, butyric, lactic and propionic acids [16]. Also SCFA would improve colonic motility and enhance the foods transit rate in 3 various ways: mucosal receptors stimulation to vagal or enteric nerves, colonic smooth muscle direct stimulation and gastro-intestinal regulatory peptides release like peptide YY in gut. YY may also suppress the appetite [17,18] show some inhibitory impacts on gastric emptying [19].

Some studies mentioned to the main variables on propionic acid production and other products by PAB, including microorganisms strain (*Propionibacterium* sp, *Selenomonas* sp, *Veillonella* sp, *Megasphaera* sp, *Clostridium homopropionicum*, *Bacteroides* sp. and *Fusobacterium necrophorum* [20-23], pH [24], seed size [25], fermentation temperature and incubation temperature [26], prebiotics [27], refrigerated storage [28] and dissolved oxygen [29]. The optimization of conditions is so important for production of biomass and propionic acid by PAB [30].

This study evaluates the effects of some factors on PA production in yogurts by *P. freudenreichii* ssp. *shermanii* and *P. freudenreichii* ssp. *freudenreichii* by Plackett-Burman design (PBD).

To the best of our knowledge this is the first comprehensive study on evaluation of all process variables affecting on propionic acid production in coculture of yogurt starters and *P. freudenreichii*. The variables including concentration of milk fat, bacterial strains, content of inulin, inoculum size, oil quantity, storage time, and fermentation temperature on production of PA were evaluated in this project. The response surface methodology design (RSM) was used for optimizing the affecting factors on PA production in yogurt containing *P. freudenreichii* ssp. *shermanii*.

2. Materials and Methods

2.1. Materials

Skim-milk and cream (40% w w^{-1}) were provided by Pak Dairy Company (Tehran, Iran). Inulin (polymerization degree ≥ 25) was prepared from Ava Salamat Javid Company (Tehran, Iran). The *P. freudenreichii* ssp. *freudenreichii* (PTCC 1674) was prepared from Iran Research and Science and Technology (Tehran, Iran). The propionic acid standard was bought from Sigma (St. Louis, USA). Other chemicals were purchased from Merck (Darmstadt, Germany). All of the solvents used within this project were HPLC grade.

2.2. Cultures preparation

L. delbrueckii ssp. *bulgaricus* (LB) and *Streptococcus thermophilus* (ST) were applied as yogurt starter culture due to the mild acid-producing activity on PAB and the common starter culture containing *P. freudenreichii* ssp. *shermanii*. The condition of culture cultivation has been described elsewhere [31,32]. The probiotics (10^8 CFU ml^{-1}) was mixed with 60 ml reconstituted milk and dissolved completely before use.

2.3. Milk preparation

The solution of skim milk in distilled water was autoclaved and cooled down to 35 °C. The Pearson square method was used to prepare different samples of milk fats as described elsewhere [32].

2.4. Fermentation

All the variables and their levels were chosen after pre-experience and literature review [27,28,31,32]. The method of yogurt preparation has been described elsewhere [32]. Yogurt samples were used to compare propionic acid productions by *P. freudenreichii* ssp. *shermanii*. An example of control yogurt that only had the highlighted yogurt starter culture (LB and ST). Other yogurt samples included starter culture and *P. freudenreichii* ssp. *shermanii*, and the third yogurt samples included traditional starter culture, *P. freudenreichii* ssp. *shermanii* and inulin (3% w v^{-1}). Fermentation temperature of all yogurt samples was 43 °C. Propionic acid produced by *P. freudenreichii* ssp. *shermanii* was measured after 24 h of yogurt samples storage and after 7, 12 and 21 days of storage (4 °C).

2.5. Sample preparation for propionic acid determination and analyses

After homogenizing the yogurt products, 6 ml of yogurt samples and 5 mL of 0.5 N sulfuric acid were thoroughly mixed. The samples were then centrifuged at 5000×g for 15 min and the supernatant was filtered and kept at -20 ° C until injected into the HPLC [5]. A liquid chromatographer with

Table 1. The levels of independent variables and 8-trial of Plackett-Burman Design to evaluate the effect of 7 factors on propionic acid production in yogurt

Run No	Independent variables							Response	
	A strains	B milk fat %w w^{-1}	C inulin %w v^{-1}	D sunflower oil g l^{-1}	E Seed size %	F temperature °C	G storage time d	Experimented propionic acid g l^{-1}	Predicted propionic acid g l^{-1}
1	PFF**	0	1	0.1	1	43	14	8.6 ±0.5	8.9
2	PFF	2	1	0	2	30	14	9.1 ±0.9	9.2
3	PFF	2	3	0	1	43	1	6.8 ±0.2	7.1
4	PFS*	2	3	0.1	1	30	14	7.1 ±0.6	7.0
5	PFF	0	3	0.1	2	30	1	6.5±0.5	6.7
6	PFS	2	1	0.1	2	43	1	6.9 ±0.4	6.8
7	PFS	0	3	0	2	43	14	10.2 ±0.4	10.5
8	PFS	0	1	0	1	30	1	6.1 ±0.3	5.7

* PFS: *Propionibacterium freudenreichii ssp. shermanii*
** PFF: *Propionibacterium freudenreichii ssp. Freudenreichii*

an autosampler, a vacuum degasser, and a quaternary pump equipped with a C18 ODS column (Agilent, CA, USA) at 210 nm, was applied for determination of propionic acid production by PAB in yogurt. The mobile phases used were water (set by sulfuric acid 5×10^{-4} M) (pH 3) (eluent A) and pure methanol (eluent B). The ratio of A to B was 70:30 under a flow rate of 1 ml min^{-1}.

2.6. Experimental design

This research was conducted progressively in three steps. As previously stated, all possible process variables which may have an impact on the production of propionic acid in yogurts by PAB were selected. The screening step followed by the main variables identification and conducted by Plackett-Burman design. After that the selected factors were investigated in three levels by central composite design (CCD) under RSM designations. Then at a reconfi-rmation step, the best-predicted situation for propionic acid production by PAB were examined. Response of the system, the quantities of produced propionic acid in yogurts were compared with predicted values. Blank or control yogurts, only contained starter cultures.

2.7. Plackett-Burman design

The significant factors were chosen after the literature review. The selected variables: milk fat, strain type, quantity of sunflower oil, inoculum and inulin amount and environm-ental factors like fermentation and incubation temperatures and storage time are represented in Table 1. The characters of + (high level) and - (low level) show different variable levels.

2.8. Response surface methodology design

RSM is a statistical method to design experiments, making models, evaluating factors effects and assessing their optimal conditions for optimal response. Generally, RSM is a significant tool for optimizing conditions via involved factors in the production of a product [24,30,31]. The factors that produce optimal response can be identified by RSM and design factor [32]. For a better predictions of optimum conditions of propionic acid bioproduction and test sets minimization, CCD under RSM was used. All factors were applied at three levels in this study.

2.9. Statistical analysis

The statistical analysis and design of experiments in bothe PBD and CCD were carried out by MINITAB statistical software (Version 16.0) and response surface plots. All experiments have been trireplicated. Data were treated statistically by analysis of variance (ANOVA) and showed by mean value ±SD in various days. The p-values ≤0.05 were significant statistically.

3. Results and Discussion

3.1. Main affecting factors selection by Plackett-Burman design

The aim of screening experiments is to choose main affecting factors (Table 1). A student *t*-test was applied to show the significant factors in this study [33]. The tabulated *t*-value (freedom degree 6) was 1.94 at $p \leq 0.05$. The factors that linked to *t*-value was higher than tabulated-*t* (1.94 for $p \leq 0.05$) was significant. The statistical calculations for propionic acid production in yogurts by PAB are shown in Table 2.

Table 2. Statistical analysis of propionic acid of production by propionic acid bacteria [a] in yogurt

Factors	Coefficient	t-value
A (strains)	0.421	1.50
B (milk fat (% w w^{-1})	-0.241	0.85
C (Inulin (% w v^{-1})	1.262	4.50
D (sunflower oil g.l^{-1})	0.298	1.03
E (inoculum size %)	1.037	3.67
F (temperature °C)	0.312	1.10
G (storage time d)	2.612	9.32

[a] A_0 = 7.6 (mean of the experimental propionic acid), standard error, S_b = 0.28, estimated error, S^2_e=0.49, tabulated t-value (degree of freedom 6) at $p \leq 0.05$ is equal to 1.94.

Results of Table 2 represented that storage time and inoculum size at 4 °C, and prebiotic (inulin) concentration were significant.

Results presented in Table 3 show that adding 3% (w v^{-1}) inulin to yogurts enhanced the propionic acid production (Table 3). This enhance could be via inulin role in maintenance and growth of probiotics and also *P. freudenreichii* longer survival during refrigeration storage with higher propionic acid production in yogurt. Inulin is a well-known source of prebiotics used in functional foods [34]. It was reported that inulin addition (1%) to cheese improved the growth and survival of probiotics [35]. Also it was claimed that adding 1 to 3% prebiotics like inulin promote the viability of *Propionibacterium* strains in milk [36].

Table 2 indicates that the storage of yogurts with *P. freudenreichii* at 4 °C for 14 days improved propionic acid production. The PABs would survive at pH=2 and adapt to the colonic environment [6]. Since the samples with *P. freudenreichii* possess pH levels greater than 2; hence, *P. freudenreichii* would produce propionic acid within storage. Furthermore, results reported in Table 2 indicates inoculum size is another influential factor in propionic acid production by *P. freudenreichii* in yogurt (*t*-value = 3.67). The results showed that regression for production of propionic acid by *P. freudenreichii* ssp. *shermanii* was significant. The lack-of-fit test was insignificant (p = 0.149), 1.2 % of variations did not described (R^2=98.8%).

3.2. Optimization of propionic acid production by RSM

After selecting the main affecting factors, RSM and CCD were applied to optimize the three factors (inoculum size, prebiotic amount and storage period at 4 °C) (Table 3). The RSM results are represented in Table 4. The produced model for production of propionic acid in coculture of *P. freudenreichii and yogurt starter culture* was obtained as the Equation 1:

$$Y = 3.12 + 3.19X_1 - 3.88X_2 + 0.47X_3 - 0.77(X_1)^2 + 0.92(X_2)^2 - 0.01(X_3)^2 + 0.2X_1X_2 + 0.02X_1X_3 + 0.08X_2X_3 \quad \text{Eq. 1}$$

where Y, X_1, X_2, and X_3 are equivalent experimental responses, inulin, inoculum size, and storage period at 4 °C, respectively. The effect of variables on production of propionic acid by *P. freudenreichii* ssp. *shermanii* are calculated by the above equitation. Table 4 represents that inoculum size, storage period at 4 °C, and concentration of inulin significantly influenced production of propionic acid ($p \leq 0.05$).

3.3. Effects of inoculum size and inulin on propionic acid production

Fig. 1 refers to effects of seed and inulin content on propionic acid production by this probiotic. The presence of inulin plays significant role in the probiotics survival and also production of propionic acid in yogurts [28,37]. Fig. 1A indicates that increases of inoculum size from 1 to 2.8 %, caused increased propionic acid in yogurt by *P. freudenreichii* ssp. *shermanii*. Anyway, the content of produced acid decreased with increased inoculum. The higher biomass could be the reason for increased production of propionic acid.

Table 3. The 17-trials of CCD to assess the effect of main and interaction effects on optimization of propionic acid production in synbiotic yogurt by propionic acid bacteria

Run	inoculum size %	inulin (%w v^{-1})	Storage time (d)	Propionic acid (mg l^{-1})
1	1	3	1	4.0
2	3	3	1	4.6
3	1	1	21	6.9
4	3	1	21	7.8
5	1	3	21	10.0
6	3	3	21	13.0
7	1	2	11	6.5
8	3	2	11	8.9
9	2	1	11	8.3
10	2	3	11	10.5
11	2	2	1	3.9
12	2	2	21	9.1
13	2	2	11	8.8
14	2	2	11	8.5
15	2	2	11	8.4
16	1	3	1	4.0
17	3	3	1	4.6

Table 4. Analysis of variance results for propionic acid production in yogurt with *P. freudenreichii ssp. shermanii*

Source of variation	Degree of freedom	Sum of squares	Mean square	F-value	P-value
Regression	9	118.03	13.11	65.99	0.000
Linear	3	93.22	3.74	18.84	0.001
Square	3	17.75	5.91	29.78	0.004
Interaction	3	7.05	2.35	11.83	0.017
Lack of fit	5	1.30	0.26	6.02	0.149
Pure error	2	0.08	0.04		
Total	16	119.42			

Factors	Degree of freedom	Coefficient estimate	Standard error	t-value	P-value
Intercept	1	3.12	1.33	2.33	0.052
X_1	1	3.19	1.15	2.76	0.028
X_2	1	-3.88	1.15	-3.36	0.012
X_3	1	0.47	0.07	6.24	0.000
X_1^2	1	-0.77	0.27	-2.84	0.025
X_2^2	1	0.92	0.27	3.39	0.011
X_3^2	1	-0.01	0.00	-7.25	0.000
X_1X_2	1	0.20	0.15	1.26	0.245
X_1X_3	1	0.02	0.01	1.74	0.125
X_2X_3	1	0.08	0.01	5.55	0.001

In a few studies, the effect of inoculum size for production of propionic acid has been tested and similar results have been reported. In a study conducted by [13], it was reported that increasing the different percentages of PAB addition to yogurt starter culture (*L. delbrueckii* ssp. *Bulgaricus and Streptococcus thermophilus*) increases propionic acid production in this product. For example, in yogurt production in which 1% of *Propionibacterium jensenii* B1264 were added to the yogurt, the production of propionic acid was 67.17 mg kg^{-1}, while the production in yogurt containing traditional starter by the end of storage time at 4 ° C was 37.30 mg kg^{-1}. In another study, it was reported that enhancing the different ratio of adding 2 bacteria *Lactobacillus acidophilus* and *Propionibacterium freudenreichii* ssp. *shermanii* increases propionic acid production in the fermented dairy beverages [38]. In this way, propionic acid production in the ratio of 1:2 was equal to 0.35 (%w w^{-1}), and this amount of production increases in the ratio of 1:4 to 0.77 (%w w^{-1}).

Fig. 1A shows production of propionic acid by this probiotic in yogurts enhanced with increasing amounts of inulin to nearly 3% (w v^{-1}). There is great interest in using prebiotics to increase the survival and colonization of probiotics [27].

It can be concluded that supplementation of prebiotics can stimulate growth but other properties e.g. enhanced production of propionic acid depends on strain. It is reported that adding inulin as prebiotic in Labneh cheese resulted in more probiotics survival and enhanced volatile fatty acids like propionic acid, acetyl and acetaldehyde [35].

3.4. Effects of inoculum size and refrigerated storage on production of propionic acid

Fig 1B represents that propionic acid production increased in yogurts by enhancing storage time at 4 °C at constant inulin amount. Increases of propionic acid amount (12.2 mg l^{-1}) continued up to the 21d of storage. Naturally, the optimum pH for growth of PAB is 6-7 and PAB strains can survive at pH=2 [6]. Since the pH of the yogurts were higher than 4 during the storage, PAB produce propionic acid. In the storage time, the samples at 4 °C, propionic acid concentration increased.

As shown in Fig. 1B, the concentration of propionic acid in yogurt decreased after 21 days of refrigerated storage due to decreased PAB viability.

In several studies, it has been stated that the concentration of propionic acid by PAB increased during the storage time. During 28 days storage, the concentration of produced acid increased by the ratio of 1 to 3 in both *L. acidophilus* and *P. freudenreichii* ssp. *shermanii*, increase at 30 °C. Acid content reach from 0.75 to 1.20 w w^{-1} [38].

In another study the propionic acid concentration increased during the 28-day storage of fiber-enriched yogurt samples [39]. PAB needs more time to grow than yogurt starter culture (ST, LB), and its peptidase activity is much more than protease activity [4].

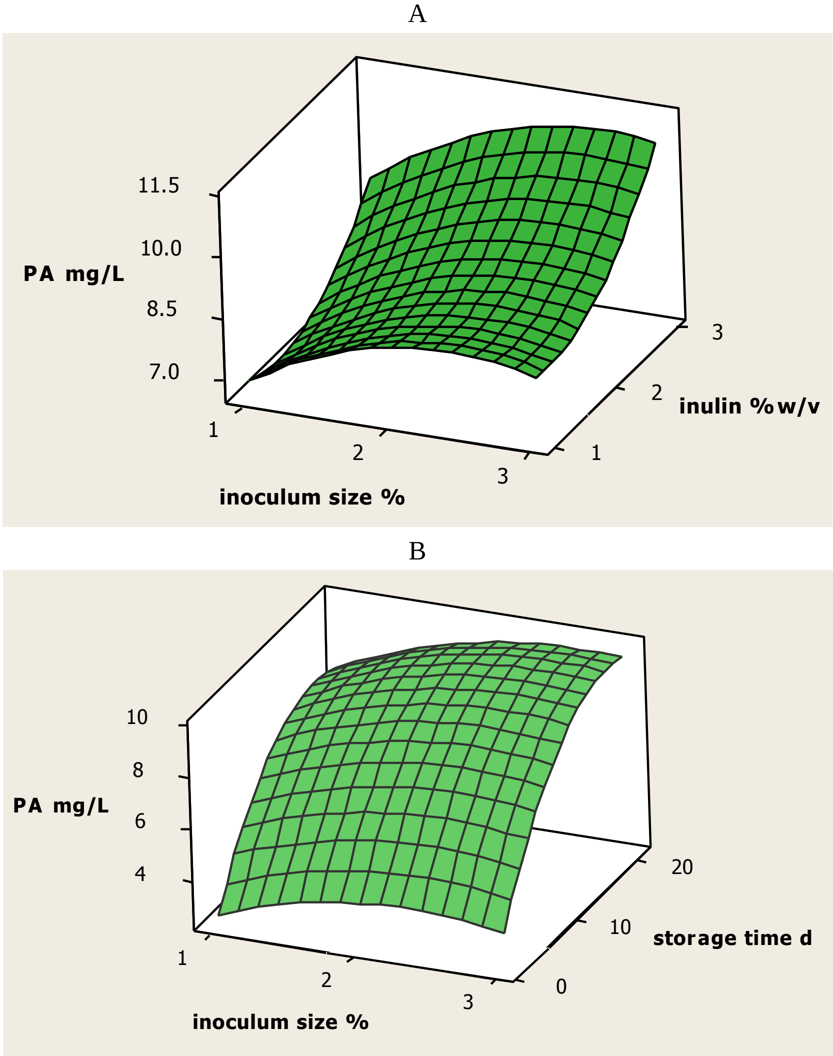

Figure 1. Surface plot of interactive effect on the propionic acid production in yogurt by *P. freudenreichii* ssp. *shermanii*; Inulin and inoculum size (A); storage time at 4°C and inoculum size (B).

On the other hand, PAB strain has more growth in a carbonate source containing lactate (yogurt) than carbon source rich in lactose (milk), so the yogurt starter culture are predominant in the first days of storage and by consuming milk lactose and producing more lactic acid than other acids (acetic and propionic) [4]. PAB, in the continuation of storage days' increase in lactate and peptides due to the growth of yogurt starter culture and also the reduction of dissolved oxygen due to the activity of *Streptococcus thermophilus* and adaptation to new conditions of yogurt samples (low temperature and high acidity) grow and multiply [13]. According to previous studies, PAB in yogurt samples produces propionic acid (2 moles) and acetic acid (1 mole) and carbon dioxide (1 mole) by consuming lactic acid (3 moles) [15]. Fig 2 demonstrates the optimum conditions for propionic acid production in yogurts by *P. freudenreichii* ssp. *shermanii*. The best results for inoculum size (X_1), inulin level (X_2), and refrigerated storage (X_3) were 2.81%, 3 % (w v^{-1}), and 21 days, respectively.

3.5. Model verification

Triplicated samples were made under optimal condition: inoculum size (2.81 %), inulin concentration (3.0 % w v^{-1}), and refrigerated storage (~21 days). The propionic acid amount in yogurts samples containing *P. freudenreichii* ssp. *shermanii* was compared with two samples. The highest propionic acid amount was 12.53 ± 0.24 mg l^{-1}. Model predicted values of production of propionic acid by *P. freudenreichii* ssp. *shermanii* in yogurts (13.11 mg l^{-1}) and there was not significant lack of fit among experiments (Fig. 3).

Fig. 3 shows the highest propionic acid production amount in samples with *P. freudenreichii* ssp. *shermanii*, in comparison with control one during the 21 days of storage. The rate of samples containing *P. freudenreichii* ssp. *shermanii* and with or without inulin was 12.53 ± 0.24 and 9.23 ± 0.38 mg l^{-1}, respectively.

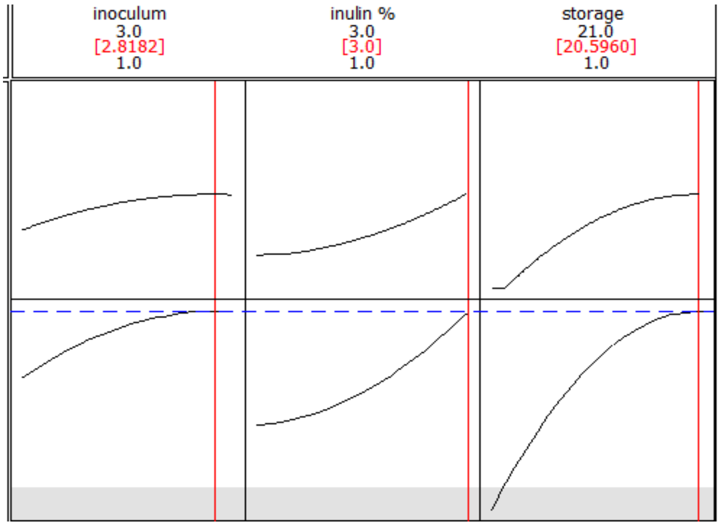

Figure 2. Optimization plot of the production of propionic acid in yogurt by *P. freudenreichii* ssp. *shermanii*

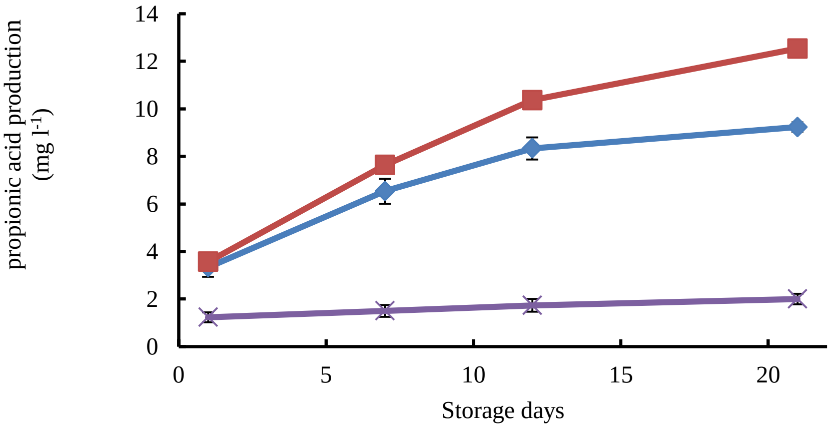

Figure 3. Production of propionic acid within storage time at 4 °C. Yogurt samples: Yogurt starter cultures (×), Yogurt starter cultures +PS4 (♦), Yogurt starter cultures+PS4+Inulin (■)

This value was 2.10 ± 0.23 mg l^{-1} in control yogurts. In this study, the control sample included only yogurt starter cultures (ST, LB) that are known as propionic acid producers [40]. Fig. 3, indicates that at the first days of storage (until 7 days), significant differences were observed in propionic acid production by *P. freudenreichii* ssp. *shermanii* in yogurts (containing inulin or not) with nearly 5 times increases in propionic acid production compared to control yogurts. On 12 days of storage, the amount of propionic acid in samples with inulin reached to 10.3 ±0.31 mg l^{-1}, increasing 6 times compared to control sample. In samples with no inulin, the raise was 4.8 times. In the samples with *P. freudenreichii* and inulin, increases were observed until 21d; the same as Fig. 2.

4. Conclusion

The purpose of this project was to evaluate the production of propionic acid in yogurt by *P. freudenreichii* ssp. *shermanii*. It is the first time that the propionic acid production by *P. freudenreichii* in yogurt is evaluated. Results from PBD showed that the inoculum size, storage period at 4 °C and inulin concentration significantly influen-ced propionic acid production in yogurts containing *P. freudenreichii* ssp. *shermanii* by the help of RSM design and optimizing the conditions for 3 significant factors. Results showed that *Propionibacterium freudenreichii ssp. shermanii* increases propionic acid contents (nearly 5 times) in synbiotic yogurt samples containing inulin (3% w v^{-1}) under the optimal conditions of these three factors, compared to control yogurts during 21 days. It is suggested to evaluate the other probiotics function on the properties of different types of yogurt and also other dairy products. Also sensory evaluation of yogurt enriched with propionic acid is recommended for future research. Although it was in road map of this research (and also has been confirmed in previous work of team), but it could not been carried out due to beginning of Corona pandemic in Tehran (Feb 2020), serious lock down of university and necessities of considering health protocols.

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6. Conflict of Interest

The authors report no conflicts of interest.

7. Authors Contributions

Conceptualization, KKD, and OZ; methodology, OZ, AMM, and AM.; software, OZ, and KKD; validation, OZ, AM, AMM, KKD, RM; formal analysis, RM; investigation, OZ; resources, KKD; data curation, OZ; writing-original draft preparation, OZ, KKD; writing-review and editing, OZ, RM, KKD; supervision, KKD; project administration, KKD; funding acquisition, KKD.

8. References

1. Hernandez E. Enrichment of Baked Goods with Omega-3 Fatty Acids, In: Food Enrichment with Omega-3 Fatty Acids, Elsevier, Amesterdam, 2013: pp. 319-335. doi:10.1533/9780857098863.3.319

2. Dibazar P, Khosroshahi Asl A, Zomorodi S.Optimization grape fiber and chitosan amounts in fruit yoghurt using response surface methodology (RSM). Food Sci Tech. 2016; 13:75-88.

3. Ouwehand AC, Salminen S, Von Wright A, Ouwehand A. The Probiotic Potential of Propionibacteria, In: Food Science and Technology, New York-Marcel Dekker, 2004: pp. 159-174.

4. Moslemi M, Mazaheri Nezhad Fard R, Hosseini S, Homayouni-Rad A, Mortazavian AM. Incorporation of propionibacteria in fermented milks as a probiotic. Crit Rev Food Sci Nutr. 2016; 56: 1290-1312. doi:10.1080/10408398.2013.766584

5. Ahmadi N, Khosravi-Darani K, Zarean-Shahraki S, Mortazavian AM, Mashayekh S. Fed-batch fermentation for propionic acetic and lactic acid production. Orient J Chem. 2015; 31:581-588. doi:10.13005/ojc/310174

6. Van Wyk J, Morkel RA, Dolley L. Metabolites of Propionibacterium:Techno-and Biofunctional Ingredients, In: Alternative and Replacement Foods, Elsevier, Amesterdam, 2018: pp. 205-260. doi:10.1016/B978-0-12-811446-9.00008-3

7. Abou Ayana IA, El-Deeb AM, Ibrahim AE. Research article using of dairy propionibacteria as bio-preservative in kareish cheese. Int J Dairy Sci. 2016; 11:114-123. doi:10.3923/ijds.2016.114.123

8. Massoud R, Khosravi K, Mortazavian AM. Vitamin B12: From deficiency to biotechnological solution. Cur Nutr Food Sci. 2018; 14:1-9. doi:10.2174/1573401314666171207145429

9. Massoud R, Khosravi K, Golshahi M, Mortazavian AM. Assessment of process variables on vitamin B12 production in fermented dairy product including propionic acid. Cur Nut Food Sci. 2020; 16: 155-161(7). doi:10.2174/1573401315666181204105601

10. Kouya T, Tobita K, Horiuchi M, Nakayama E, Deguchi H, Tanaka T, Taniguchi M. Production of extracellular bifidogenic growth stimulator (BGS) from *Propionibacterium shermanii* using a bioreactor system with a microfiltration module and an on-line controller for lactic acid concentration. J Biosci Bioengin. 2008; 105: 184-191. doi:10.1263/jbb.105.184

11. Farhadi S, Khosravi-Darani K, Mashayekh M, Mortazavian AM, Mohammadi A, Shahraz F. Effect of incubation temperature and inoculation ratio of starter culture on propionic acid production in dairy beverage fermented with Propionibacterium. Iran J Nutr Sci Food Technol. 2012; 7: 41-50. doi:10.1111/1471-0307.12004

12. Gorret N, Maubois J, Ghoul M, Engasser J. Exopolysaccharide production by *Propionibacterium acidipropionici* on milk microfiltrate. J Appl Microbiol. 2001; 90:779-787. doi: 10.1046/j.1365-2672.2001.01306.x

13. Ekinci F, Gurel M. Effect of using propionic acid bacteria as an adjunct culture in yogurt production. J Dairy Sci. 2008; 91: 892-899. doi:10.3168/jds.2007-0244

14. Piwowarek K, Lipinska E, Hac-Szymanczuk E, Kieliszek M, Scibisz I. *Propionibacterium* spp. source of propionic acid, vitamin B12, and other metabolites important for the industry. Appl Microb Biotech. 2018;102(2): 515-538. doi:10.1007/s00253-017-8616-7.

15. Zhang A, Yang ST. Propionic acid production from glycerol by metabolically engineered *Propionibacterium acidipropionici*. Process Biochem. 2009; 44: 1346-1351. doi: 10.1016/j.procbio.2009.07.013

16. Ruijschop RM, Boelrijk AE, Te Giffel MC. Satiety effects of a dairy beverage fermented with propionic acid bacteria. Int Dairy J. 2008; 18: 945-950. doi:10.1016/j.idairyj.2008.01.004

17. Ayan E, Baylan N, Cehreli S. Optimization of reactive extraction of propionic acid with ionic liquids using central composite design. Chem Eng Res Des. 2020; 153: 666-676. doi:10.1152/ajpgi.1998.275.6.G1415

18. Batterham RL, Heffron H, Kapoor S, Chivers JE, Chandarana K, Herzog H, Le Roux CW, Thomas EL, Bell JD, Withers DJ. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab. 2006; 4: 223-233. doi:10.1016/j.cherd.2019.11.015

19. Liljeberg H, Bjorck I. Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: Studies in humans and rats using test products with added organic acids or an organic salt. Am J Clin Nutr. 1996; 64: 886-893. doi:10.1093/ajcn/64.6.886

20. Dahiya S, Lakshminarayanan S, Mohan SV. Steering acidogenesis towards selective propionic acid production using co-factors and evaluating environmental sustainability. Chem Eng J. 2020; 379:122-135. doi:10.1016/j.cej.2019.122135

21. Yang ST, El-Ensashy H, Thongchul N. Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels Chemicals and Polymers. John Wiley and Sons, Inc. 2013: 319-335.

22. Cibis KG, Gneipel A, Konig H. Isolation of acetic propionic and butyric acid-forming bacteria from biogas plants. J Biotechnol. 2016; 220: 51-63. doi:10.1016/j.jbiotec.2016.01.008

23. Sun J, Liu C, Wang Y, Martin K, Venkitasubramanian P. Processes for Making Methacrylic Acid, available form: https://patents.google.com/patent/US7253307B1/en (Accessed 2007-08-07)

24. Cousin FJ, Jouan-Lanhouet S, Theret N, Brenner C, Jouan E, Le Moigne-Muller G, Dimanche-Boitrel MT, Jan G. The probiotic *Propionibacterium freudenreichii* as a new adjuvant for trail-based therapy in colorectal cancer. Oncotarget 2016; 7: 61-71. doi:10.18632/oncotarget.6881

25. Yang B, Gao H, Stanton C, Ross RP, Zhang H, Chen YQ, Chen H, Chen W. Bacterial conjugated linoleic acid production and their applications. Prog Lipid Res. 2017; 68:26-36. doi:10.1016/j.plipres.2017.09.002

26. Khan MM, Mir NA. Production of vitamin B12 by improved strains of *Propionibacterium freudenreichii*. Biotechnol Bioinf Bioeng. 2011; 1:19-24.

27. Effat B, Ibrahim MK, Tawfik NF, Mehanna NS, Soliman NR. Effect of different prebiotics on survival and viability of some dairy propionibacteria. Int J Adv Res Biol Sci. 2019; 6: 99-104. doi:10.22192/ijarbs.2019.06.08.014

28. Akalın A, Tokusoglu O, Gonc S, Aycan S. Occurrence of conjugated linoleic acid in probiotic yoghurts supplemented with fructooligosaccharide. Int Dairy J. 2007; 17: 1089-1095. doi:10.1016/j.idairyj.2007.02.005

29. Ammar EM, Martin J, Brabo-Catala L. Propionic acid production by *Propionibacterium freudenreichii* using sweet sorghum bagasse hydrolysate. Appl Microbiol Biotechnol. 2020; 104, 9619-9629. doi:10.1007/s00253-020-10953-w

30. Khodaiyan F, Razavi SH, Mousavi SM. Optimization of canthaxanthin production by *Dietzia natronolimnaea* HS-1 from cheese whey using statistical experimental methods. Biochem Eng J. 2008; 40: 415-422. doi:10.1016/j.bej.2008.01.016

31. Gaucher F, Rabah H, Kponouglo K. Intracellular osmoprotectant concentrations determine *Propionibacterium freudenreichii* survival during drying. Appl Microbiol Biotechnol. 2020; 104: 3145-3156. doi:10.1007/s00253-020-10425-1

32. Zahed O, Khosravi-Darani K, Farsani SAM, Mohammadi A. Bacterial conjugated linoleic acid bio-fortification of synbiotic yogurts using *Propionibacterium freudenreichii* as adjunct culture. Ital J Food Sci. 2021; 33:1-11. doi:10.15586/ijfs.v33iSP1.1961

33. Khosravi-Darani K, Zoghi A. Comparison of pretreatment strategies of sugarcane baggase: Experimental design for citric acid production. Bioresour Technol. 2008; 99: 6986-6993. doi:10.1016/j.biortech.2008.01.024

34. Mohanty D, Misra S, Mohapatra S, Sahu PS. Prebiotics and synbiotics: Recent concepts in nutrition. Food Biosci. 2018; 26:152-160. doi:10.1016/j.fbio.2018.10.008

35. Salem MM, El-Gawad A, Hassan FA, Effat BA. Use of synbiotics for production of functional low fat labneh. Polish J Food Nutr Sci. 2007; 57: 151-159.

36. Effat BA, Ibrahim MK, Tawfik NF, Mehanna NS, Soliman NR. Effect of different prebiotics on survival and viability of some dairy propionibacteria. Int J Adv Res Biol Sci. 2019b; 6:99-104. doi:10.22192/ijarbs.2019.06.08.014

37. Xu S, Boylston TD, Glatz BA. Conjugated linoleic acid content and organoleptic attributes of fermented milk products produced with probiotic bacteria. J Agr Food Chem. 2005; 53: 9064-9072. doi:10.1021/jf051030u

38. Farhadi S, Khosravi-Darani, K, Mashayekh M, Mortazavian AM, Mohammadi A, Shahraz F. Production of propionic acid in a fermented dairy beverage. Int J Dairy Technol. 2013; 66: 127-134. doi:10.1111/1471-0307.12004

39. Fernandez-Garia E, McGregor JU, Traylor S. The addition of oat fiber and natural alternative sweeteners in the manufacture of plain yogurt. J Dairy Sci.1998; 81: 655-663. doi:10.3168/jds.S0022-0302 (98)75620-6

40. Laino JE, Del Valle MJ, De Giori GS. Development of a high folate concentration yogurt naturally bio-enriched using selected lactic acid bacteria. LWT- Food Sci Tech. 2013; 54: 1-5. doi:10.1016/j.lwt.2013.05.035

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زیست‌غنی‌سازی اسید پروپیونیک در ماست با کشت همزمان *پروپیونی‌باکتریوم فرودنریچی*

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چکیده

سابقه و هدف: باکتری‌های اسید پروپیونیک ریزاندامگان مفیدی هستند که می‌توانند ترکیبات مغذی مفیدی تولید کنند. این باکتری‌ها عمدتاً اسید پروپیونیک تولید می‌کنند که باعث کاهش شمارش و رشد میکروبی و افزایش ماندگاری ماست می‌شود. در این مطالعه از دو زیرگونه مختلف *پروپیونی‌باکتریوم فرودنریچی* برای تولید اسید پروپیونیک در ماست استفاده شد.

مواد و روش‌ها: ابتدا متغیرهای فرآیند مانند درصد تلقیح، نوع سویه، چربی شیر، مقدار اینولین، دمای تخمیر، مقدار روغن آفتابگردان و مدت زمان نگهداری در یخچال بر میزان تولید اسید پروپیونیک توسط *پروپیونی‌باکتریوم فرودنریچی* با استفاده از طرح پلاکت برمن به عنوان روش غربالگری ارزیابی شد. سپس تولید اسید توسط یک طرح مرکب مرکزی با ۳ فاکتور اصلی اندازه تلقیح، غلظت اینولین و زمان نگهداری در یخچال بهینه‌سازی شد.

یافته‌ها و نتیجه‌گیری: تجزیه واریانس نتایج نشان داد که مدل‌های حاصله از معنی‌داری بالایی برخوردار بودند (p≤۰/۰۵) و نشان داد که تولید اسید پروپیونیک تحت تأثیر هر سه عامل قرار دارد. تولید بهینه اسید پروپیونیک توسط *پروپیونی‌باکتریوم فرودنریچی* زیرگونه *شرمانی* در شیر بدون چربی (دو درصد w/v) ، اینولین و دمای انکوباسیون ۴۳°C و پس از ۲۱ روز نگهداری در یخچال به دست آمد. تست تاییدی نشان داد که بیشترین میزان پروپیونیک اسید تولیدی ۰/۲۴ ± ۱۲/۵۳ میلی‌گرم در لیتر در ماست بود که تا ۶/۲ برابر نسبت به شاهد افزایش یافت. نتایج نشان داد که *پروپیونی‌باکتریوم فرودنریچی* محتوای اسید پروپیونیک را در ماست سین‌بیوتیک حاوی اینولین را افزایش می‌دهد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافعی وجود ندارند.