

Study on the physicochemical/ microbial properties and gas chromatography profile of synbiotic yogurt

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Abstract

A synbiotic yogurt was provided by adding aerobic (*Lactobacillus acidophilus*) and anaerobic (*Bifidobacterium bifidum*) probiotics and inulin to the yogurt. The effects of aerobic and anaerobic probiotics, storage time, and inulin on the physicochemical/microbial properties of synbiotic yogurt in terms of acidity, viscosity, syneresis, and microbial count were studied. A head-space solid phase microextraction-gas chromatography (HS-SPME-GC) method was used to extract and detect of VOCs profile (total peak area and total peak height) of yogurt sample by nano-sized polyaniline fiber. The D-Optimal Combined Design (DOCD) was used to analyze the effect of probiotics type (aerobic and anaerobic), inulin percent (W/W %), and storage time of yogurt (day) on the physicochemical/microbial properties of synbiotic yogurt. Results showed that the aerobic and anaerobic probiotics, inulin, and storage time of yogurt affect the yogurt physicochemical/microbial property and there were relations between the physicochemical/microbial properties of yogurt and VOCs gas chromatography profile (total peak area and total peak height). The current research also enables us to obtain microbial count by total peak area and total peak height of the VOCs GC-profile of yogurt sample.

Keywords: Synbiotic Yogurt, Physicochemical property, Microbial property, Nano-sized, Gas chromatography.

Introduction

Yogurt is a type of fermented milk by which lactose converts to lactic acid by the starter microorganisms consist of a mixture of *Lactobacillus delbrueckii ssp. bulgaricus* (*Lactobacillus bulgaricus*) and *Streptococcus salivarius ssp. thermophilus* (*Streptococcus thermophilus*) [1 and 2]. Some other starters such as *Lactobacillus delbrueckii ssp. Lactis* and *Lactobacillus helveticus* have also been used [1 and 2]. Probiotic yogurts due to their advantages like health benefits gained high popularity during the last two decades. Probiotic bacteria improve intestinal microbial balance in the host animal and were used as live microbial feed supplements. Two famous probiotics that are used in dairy industry include *Lactobacillus acidophilus* and *Bifidobacterium spp.* These probiotics produce acetic acid and lactic acid that decrease the pH of the colonic content so the lower pH inhibits the development of *E. coli*. The probiotics are believed to decrease the uptake of nutrients and space for putrefactive and pathogenic bacteria in the gut (Ziemer *et al.*, 1998)

The high molecular-weight polymers composed of saccharide subunits are called polysaccharides. Several monosaccharide residues by chemical reactions are joined together by glycosidic linkages and polysaccharides are formed (Degeest *et al.*, 2001). The extracellular polysaccharides and intracellular polysaccharides are two types of polysaccharides that synthesized by organisms. Inulin as an intracellular polysaccharide is produced by plant and glycogen is produced by microorganisms and animals. Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/ or activity of one or a limited number of bacteria in the colon (Degeest *et al.*, 2001). This selectivity has been demonstrated for *Bifidobacterium*, whose growth may be promoted by the uptake of substances such as fructo- oligosaccharides, transgalactosylated oligosaccharides and soybean oligosaccharides (Degeest *et al.*, 2001). Beside their prebiotic properties, certain oligosaccharides have shown a number of functional effects on the GIT physiology

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including reduced fat and cholesterol absorption, modulation of microbial proliferation, thus subsequently reducing intestinal disturbances, cardiovascular diseases and intestinal cancer (Degeest *et al.*, 2001).

A synbiotic product is the combination of probiotics and prebiotics. A synbiotic enhances survival and activity of the organism, for example FOS in conjunction with a *Bifidobacterium* strain or lactitol in conjunction with *Lactobacillus* (Degeest *et al.*, 2001). The synbiotic has synergistic effects due to promotion the growth of existing strains of beneficial bacteria in the colon. Synbiotics also act to improve the survival, implantation and growth of newly added probiotic organisms. The synbiotic concept has been widely used by yogurt manufacturers in the world.

D-optimal designs are one form of design provided by algorithm. These types of computer-aided designs are particularly useful when classical designs do not apply. Unlike standard classical designs such as factorials and fractional factorials, D-optimal design matrices are usually not orthogonal and effect estimates are correlated (Gladitz *et al.*, 1982). These types of designs are always an option regardless of the type of model the experimenter wishes to fit (for example, first order, first order plus some interactions, full quadratic, cubic, etc.) or the objective specified for the experiment (for example, screening, response surface, etc.). D-optimal designs are straight optimizations based on a chosen optimal criterion and the model that will be fit. The optimality criterion used in generating D-optimal designs is one of maximizing $|X'X|$, the determinant of the information matrix $X'X$ (Gladitz *et al.*, 1982).

Some polymers like polyaniline, polypyrrole, polythiophene as conducting polymers have some interesting properties like conductivity, nano-size, high temperature resistance and etc. These polymers have an increasing number of applications in various fields like solid phase extraction agent, electronic devices, chemical sensors, and separation filters. Among these polymers, polyaniline (PANI) has been studied most extensively in recent years since it can be

synthesized easily, is comparatively stable in air, is relatively cheap and exhibits a number of interesting properties such as chemical sensitivity (Stejskal *et al.*, 2002). Conducting polymer composites have attracted considerable interest in the recent years because of their numerous applications in a variety of chemical sensors, chemical filters and electric and electronic devices. It has been found that such composites can exhibit some novel properties such as high surface area, positive temperature coefficient of resistance (PTC), and chemical separation ability (Stejskal *et al.*, 2002). A recent and very successful application of conducting polymers is the application of these polymers in the solid-phase microextraction (SPME). Pawliszyn and co-workers in 1989 invent the SPME as a type of SPE method that uses micro area solids compounds extraction from different phases. The SPME method by a single step dose all extraction process, including extraction, separation, concentration and sample introduction. In the SPME by using a fiber, analytes are extracted and concentrated from sample (Pawliszyn, 2003). The SPME method has some advantage compared to the SPE method like lower detection limit, lower cost and higher speed. Some techniques like gas chromatography (GC), HPLC, GC/ mass spectrometry (GC/ MS), LC/ MS and super critical fluid chromatography (SFC) are combined with SPME method and are used successfully for the extraction, detection and analysis of VOCs and semi-VOCs in the different samples like biological, environmental, industrial and food samples (Pawliszyn, 2003).

In this work, the effects of aerobic and anaerobic probiotics, storage time, and inulin on the physicochemical/microbial property of synbiotic yogurt were studied. The PANI fiber was used to extract and detect VOCs of yogurt by HS-SPME-GC method.

Material and methods

Low-fat milk (1.5% fat) was prepared from the Pegah factory from Urmia, Iran. The yogurt starter (set 1 type) containing *S. thermophilus*

and *Lactobacillus spp.*, probiotic microorganism starter namely *Lactobacillus acidophilus* (L-10) were purchased from industrial enzymes company, Tehran, Iran. The probiotic starter (BB-12) *Bifidobacterium bifidum* was purchased from Chr. Hansen Denmark. Frutafit HD inulin with an average chain length was purchased from SENSUS Netherlands. The culture medium, including: MRS-Agar (Biolife), RCA (Merk), Oxgall-bile (Sigma), and Peptone Water (Merk) were used. Aniline purchased from Fluka, Switzerland and stored in a refrigerator in the dark prior to use. Potassium dichromate ($K_2Cr_2O_7$) was used as oxidant from Aldrich. All analytical reagents were purchased from Merck.

Apparatus

The PANI fiber was prepared by a chemical polymerization. An SPME fiber holder for manual sampling was designed and fabricated by Pirsá *et al.* (2016). The GC apparatus used in this study was from Agilent 7890 A, Wilmington, DE, USA. The scanning electron micrographs (SEM) using an SEM instrument (Philips XL30, Holland) was used to evaluate the morphology of PANI fiber.

Brookfield viscometer (Brookfield DVII+, USA), Incubator (Heraeus D6450 Hanau-type SI 6120), Mixer (NO.HA Model 3020, Japan), Autoclave (WEBO GmbH-bad Schwartau, Germany), Anaerobic Jar (Anaerocult, Merk, Darmstadt, Germany), Gas pack (Anaerocult A, Merk, Darmstadt, Germany) were used to test physicochemical and microbial analysis.

Yogurt preparation

Preparation of yogurt samples was done as follow: 10 Kg milk (pasteurized, homogenized and 1.5% fat) was heated to 45°C. Inulin hydrocolloid (0, 2, 3, and 4%Wt) was added to the milk according to the experimental design (Table 1). The mix was cooled (to 37°C) and yogurt starter, as well as probiotics *Lactobacillus acidophilus*, and probiotic *Bifidobacterium bifidum* (BB-12) were added to the mix. Then the samples were packed and transported to the incubator (40-37°C). The sample pH was controlled during incubation. The samples in the pH=4.6 were transferred to

the fridge (5°C). At 1, 11 and 21 days of storage, the physicochemical/ microbial properties of yogurt and VOCs gas chromatography profile (total peak area and total peak height) were analyzed (Ozer *et al.*, 2005)

Apparent viscosity measurement

The apparent viscosity was measured by using a Brookfield viscometer (Brookfield DVII+, America, LV2) after 30 seconds rotation. Yogurt samples were stirred for 1 minute before measurement (Ghasempour *et al.*, 2012)

Acidity determination

Acidity was determined by titration of 10 g yogurt sample (5 g sample and 5 g of distilled water) by NaOH solution (0.1 N) in the presence of phenolphthalein.

Syneresis measurement

25g of sample was poured onto the filter paper. The sample was placed in the refrigerator for 2 hours. Finally the syneresis was calculated as a percentage of the volume of separated clear liquid phase to the initial weight of the yogurt (Sahan *et al.*, 2008)

Survival of probiotics

To investigate changes in the *Lactobacillus acidophilus* and *Bifidobacterium bifidum* count, 1 gram of yogurt sample was well mixed with 9 ml peptone water (0.1%). The diluted suspended samples were cultured. For this purpose, 1 ml of diluted samples was transferred to the plates. Then, 15 ml of the appropriate culture medium was added to each plate (Kailasapathy, 2006).

Fiber preparation, chromatographic conditions and extraction procedure

The PANI nano-fiber was fabricated by chemical polymerization method at room temperature under atmospheric condition, according to our previous work (Pirsá *et al.*, 2013). HS-SPME extraction procedure was done as previously reported (Pirsá *et al.*, 2016). A gas chromatography instrument (Agilent 7890 A, Wilmington, DE, USA) with flame

ionization detector (GC-FID) at our previous research work condition was used (Pirsa *et al.*, 2016). The gas chromatographic profile of

yogurt VOCs at the optimum condition is shown in Fig. 1.

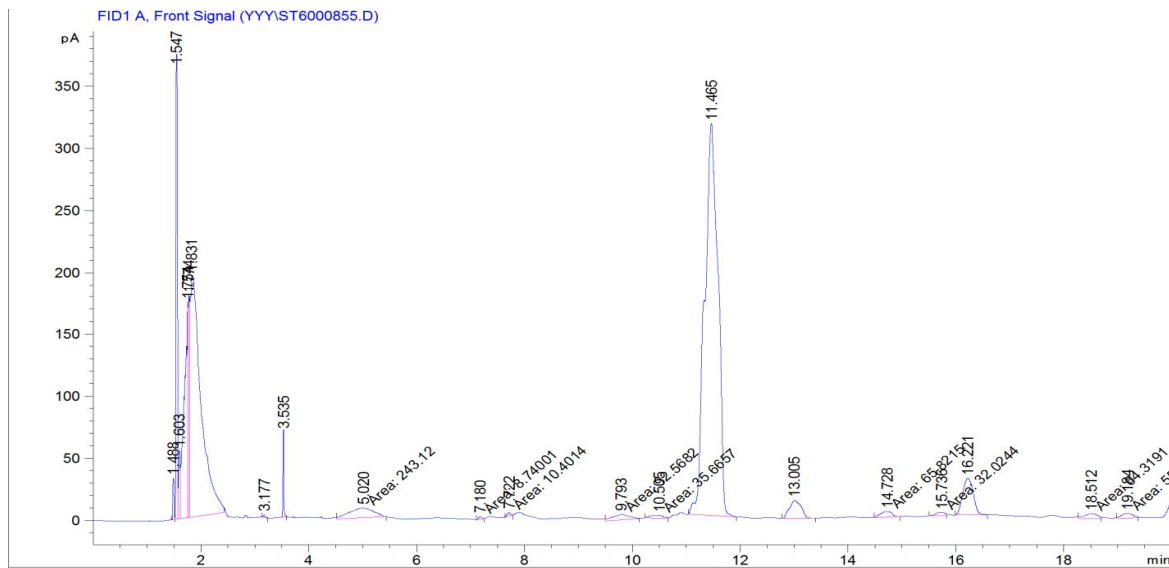


Fig.1. GC-FID chromatogram of synbiotic yogurt VOCs extracted by PANI fiber

Results and Discussion
Morphology

The morphology, size and porosity of the synthesized PANI film were studied by scanning electron microscopy (SEM). Fig. 2

shows the SEM micrographs of the PANI fiber. Results show that PANI particles are seed like and in the nano-size between 40- 100 nanometers.

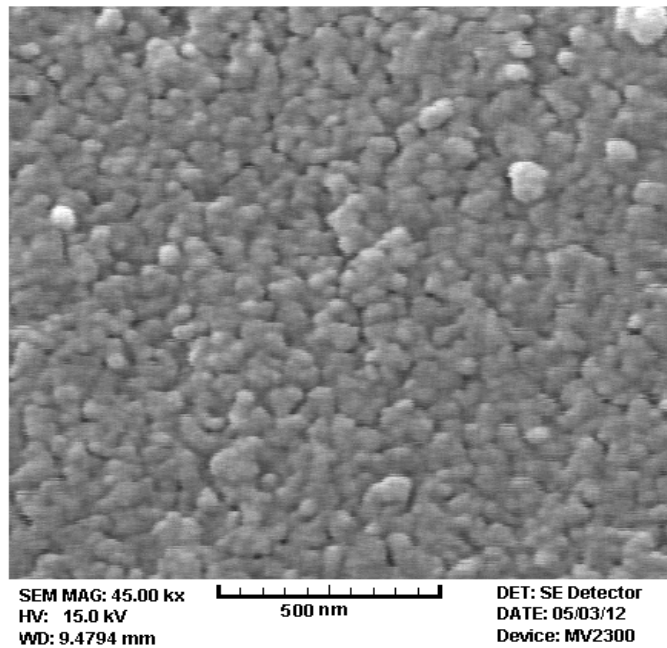


Fig.2. SEM images of PANI fiber

Experimental design

Effects of variables on the physicochemical/microbial properties

The probiotic type (aerobic and anaerobic), storage time (day), and inulin level (% Wt) were three variables that affect physicochemical and microbial properties of synbiotic yogurt including acidity, viscosity, syneresis, and microbial count. To study the effect of these variables on the responses, a D-Optimal Combined Design (DOCD) was used.

Three variables, inulin percent (Wt %) (F1) (In four levels), storage time (day) (F2) (in 3 levels) and probiotic bacteria type (aerobic and anaerobic) as a categorical factor (F3) were investigated. In Table 1, the 3 processing variables as factors, levels and experimental design are given. Table 1 also presents the evaluated responses including, acidity, syneresis, viscosity, microbial count, total peak area, and total peak height.

Table 1- List of experiments in the DOCD and the responses of each run

Run	Factors			Physicochemical/microbial properties				GC chromatogram	
	Inulin %	Storage (day)	Probiotic type	Acidity	Syneresis (%)	Viscosity (mPas)	Microbial count(cfu/g)	TPA*	TPH**
1	2	1	anaerobic	0.75	40.8	1632	4.70E+08	1127.8	314.8
2	0	1	aerobic	0.829	35.2	1896	2.00E+05	705.7	22.3
3	4	1	anaerobic	0.72	38.4	1724	5.60E+07	1134.2	102.8
4	4	21	anaerobic	0.98	29.8	1996	1.24E+09	656.9	28.5
5	4	1	anaerobic	0.72	39.2	1900	4.90E+07	1060.4	175.6
6	4	21	aerobic	0.98	31.48	1908	1.00E+07	768.6	79.1
7	4	1	aerobic	0.756	36.4	2192	1.00E+07	861	160.5
8	3	11	anaerobic	0.81	44	1164	2.00E+08	945.5	98.5
9	0	11	aerobic	0.972	43.2	1488	2.00E+05	1276	158.9
10	0	21	anaerobic	1.031	32.4	1732	1.00E+09	246	23.1
11	0	21	anaerobic	1.001	34	1396	2.00E+09	803.9	91.6
12	2	1	aerobic	0.738	39.2	2128	1.00E+05	918	153.9
13	0	21	aerobic	1.067	31.2	1376	2.00E+05	870.7	78
14	2	11	anaerobic	0.954	42	1716	5.00E+08	1170.3	122.4
15	0	1	anaerobic	0.828	32.8	1984	3.60E+08	1394	61
16	0	21	aerobic	1.026	32	1532	4.20E+06	490.4	70.4
17	4	21	anaerobic	1.04	29.12	1600	5.00E+08	3212	359.9
18	0	11	anaerobic	0.9	44	1472	7.00E+08	1501.4	185.6
19	4	21	aerobic	1.074	30.16	1568	1.00E+07	790.5	64.8
20	3	11	aerobic	0.846	40	1448	1.00E+05	2012.8	145.7

*total peak area

**total peak height

The Design-Expert software (version 7) and Minitab version 17 were used to perform statistical analysis. Initially, the full term second order polynomial response surface models were fitted to each of the response variables, according to the following equation:

$$Y = b_0 + b_1 \times F_1 + b_2 \times F_2 + b_3 \times F_3 + b_4 \times F_1 \times F_1 + b_5 \times F_2 \times F_2 + b_6 \times F_3 \times F_3 + b_7 \times F_1 \times F_2 + b_8 \times F_1 \times F_3 + b_9 \times F_2 \times F_3 \quad (1)$$

Where Y is the responses (acidity, syneresis, viscosity, and microbial count); F1, F2 and F3 are inulin percent (% Wt), storage time (day) and probiotic type respectively. For inulin four levels (0, 2, 3, and 4 %), storage time three levels (1, 11, and 21 days), and probiotic type (aerobic and anaerobic) were chosen to construct a design. b0 to b9 are the coefficient values obtained through multiple linear regressions.

Table 2- Some characteristics of the constructed models and analysis of variance for responses

Acidity						
Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	0.26786	2	0.13393	77.077	< 0.0001	Significant
A-inulin	0.011709	1	0.011709	6.7388	0.0188	
B-Time	0.247916	1	0.247916	142.67	< 0.0001	
Residual	0.029539	17	0.001738			
Lack of Fit	0.022031	12	0.001836	1.22	0.4404	Not Significant
Pure Error	0.007509	5	0.001502			
Cor Total	0.2974	19				
R ² =0.900675, Adj R ² =0.888989, Pred R ² =0.866264						
Syneresis						
Model	436.5174	4	109.1294	32.40407	< 0.0001	Significant
A-inulin	0.113234	1	0.113234	0.033623	0.8570	
B-Time(days)	130.2021	1	130.2021	38.66125	< 0.0001	
AB	23.63183	1	23.63183	7.017063	0.0182	
B ²	259.182	1	259.182	76.95961	< 0.0001	
Residual	50.51651	15	3.367767			
Lack of Fit	47.49411	10	4.749411	7.857019	0.0173	Significant
Pure Error	3.0224	5	0.60448			
Cor Total	487.0339	19				
R ² =0.896277, Adj R ² =0.868618, Pred R ² =0.814185						
Viscosity						
Model	668828.2	2	334414.1	7.554622	0.0045	Significant
B-Time(days)	300661.5	1	300661.5	6.792131	0.0184	
B ²	390296.5	1	390296.5	8.817041	0.0086	
Residual	752524.6	17	44266.15			
Lack of Fit	532212.6	12	44351.05	1.006551	0.5385	not Significant
Pure Error	220312	5	44062.4			
Cor Total	1421353	19				
R ² =0.470557, Adj R ² =0.40827, Pred R ² =0.399						
Microbial count						
Model	37.34429	2	18.67214	45.65753	<0.0001	Significant
B-Time(days)	2.501241	1	2.501241	6.116089	<0.0242	
C-probiotic type	35.90213	1	35.90213	87.78867	0.0001	
Residual	6.952334	17	0.408961			
Lack of Fit	5.953415	12	0.496118	2.483274	0.1622	Not significant
Pure Error	0.998919	5	0.199784			
Cor Total	44.29662	19				
R ² =0.84305, Adj R ² =0.824586, Pred R ² =0.774708						

Where possible, stepwise deletion of terms was applied to remove the statistically non-significant terms in order to simplifying the model. However, when the exclusion of such terms from the model decreases R² (adjusted)

increases the estimator of the variance S, the term was included in the model. The statistically non-significant linear terms also remained in the model when the respective quadratic or interactive effects were statistically

significant. The quadratic polynomial models for all response functions accompanied by F values and corresponding R^2 were used. The analysis of variance and estimated regression coefficients are summarized in Table 2.

Response Surface and three-dimensional plot to study variables effect on the physicochemical/microbial properties

The three-dimensional (3D) plots, linear regression interaction curve and contour plots based on the model function were used to predict responses to survey influence of each variable on the analyzed physicochemical/microbial properties.

Effects of variables on the syneresis

Syneresis is one of the important factors in determining the quality of yogurt. Syneresis in yogurt is due to the shrinkage of a three-dimensional network of protein structures, which leads to a reduction in the binding of whey proteins and leads to the outflow of water from yogurt (Lucey, 2005).

Fig. 3 shows the three-dimensional plot of syneresis versus inulin level and storage time. Results show that inulin level and storage time have affected the yogurt syneresis, but probiotic type doesn't have significant effect on the syneresis. At the initial sample storage time (1 day) yogurt syneresis is increased by increasing of the inulin percent, but at the end of sample storage time (21 days) yogurt syneresis is decreased by increasing the inulin percent.

The reason for this behavior can be due to the acidity of yogurt. By increasing the concentration of hydrogen ions, the forces of repulsion are reduced and in casein micelles, aggregation occurs, and a stronger protein network is created and syneresis is decreased.

Production of exopolysaccharides is another reason for reduction of the syneresis. Researchers such as Kailasapathy (2006), Souza and Saad (2009) have reported in probiotic yogurt a decrease in syneresis with increasing acidity.

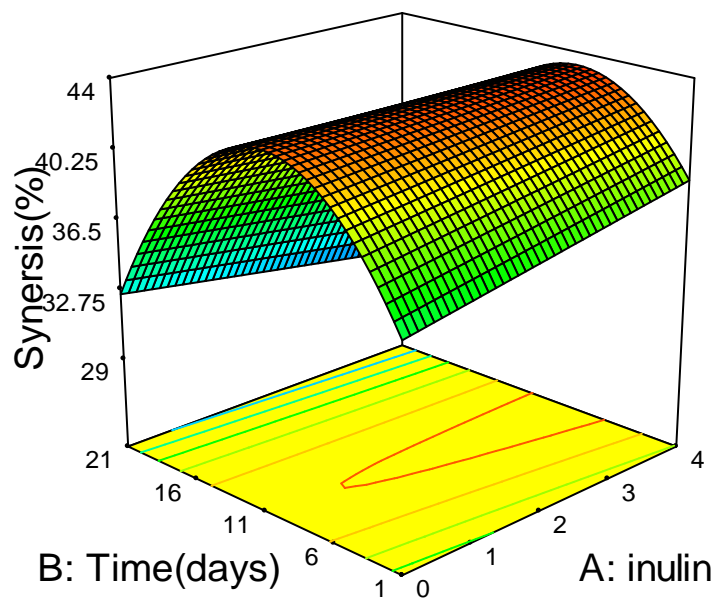


Fig.3. Three-dimensional plot of syneresis versus inulin percent and storage time

Linear regression curve of acidity based on variables

Fig. 4 (A) shows the linear regression curve of acidity versus storage time and fig. 4 (B)

shows the linear regression curve of acidity versus inulin levels. Results show that probiotic type doesn't have a significant effect on the

acidity and storage time and inulin levels have an effect on the acidity, but there is not any interaction between storage time and inulin

percent. According to the linear curves, yogurt acidity is increased by storage time and decreased by inulin level increasing.

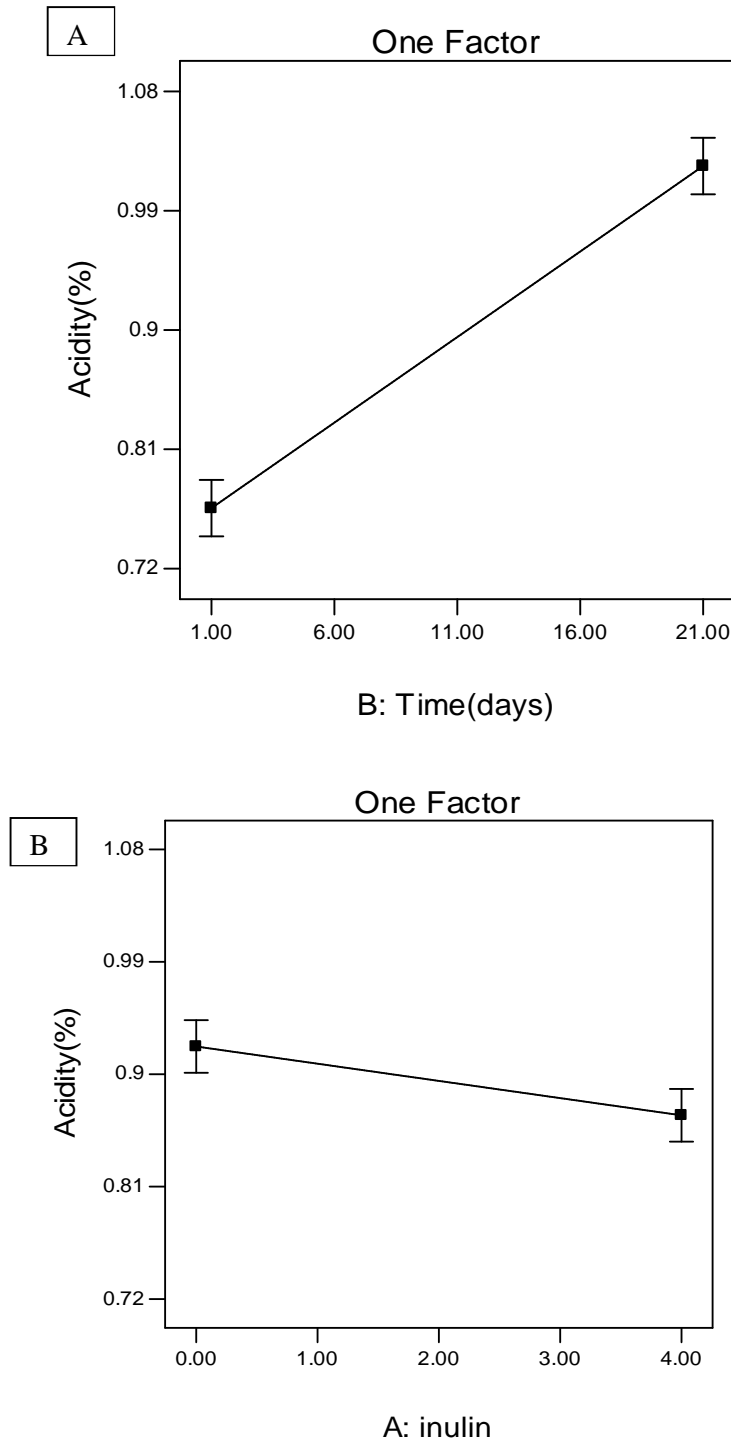


Fig.4. Linear regression curve of acidity versus (A) storage time and (B) inulin percent

Increasing acidity by increasing storage time can be due to the acidification phenomenon that results from the lactose fermentation process. Lactose fermentation is affected by traditional starter of yogurt and probiotics. Lactose fermentation produces lactic acid and increases the acidity of yogurt.

Acidity reducing in the presence of inulin can be due to the ability of inulin to control the acidification process. Balthazar *et al.* (2015) examined the effect of different inulin concentrations on sheep's yogurt acidity, and concluded that yogurt containing inulin had less acidity than the control yogurt. Therefore it can be said that inulin can be used as a control agent for acidity in yogurt.

Linear interaction curve of microbial count based on variables

Fig. 5 shows the linear regression interaction curve of microbial count versus storage time and probiotic type. Results show that the inulin level doesn't have an effect on the microbial count. The microbial count is increased by increasing storage time in the presence of both aerobic and anaerobic probiotics. Also result showed that the probiotic type had significant effect on the microbial count, anaerobic probiotic showed more microbial count than aerobic probiotic. Inulin as a prebiotic promotes the growth and metabolic activity of probiotics. Ozer *et al.* (2005) successfully used inulin as an agent for the growth of *Lactobacillus acidophilus* and anaerobic *Bifidobacterium bifidum*. Sadek *et al.* (2004) demonstrated the increasing of viability of probiotics in the presence of inulin as a prebiotic.

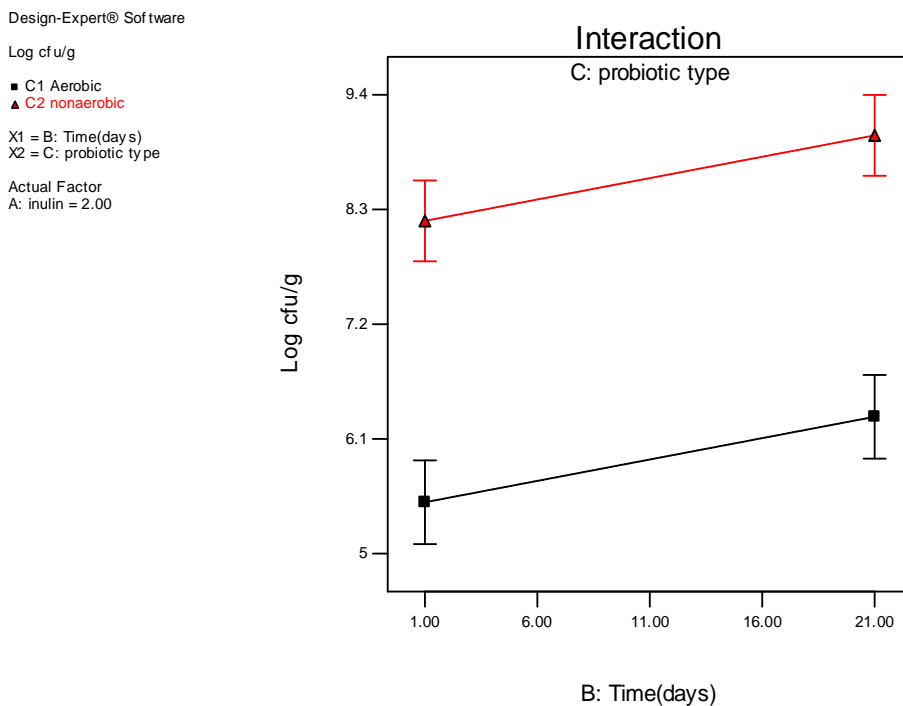


Fig. 5. Linear regression interaction curve of microbial count versus storage time

Regression plot of viscosity based on variables

Viscosity is resistance to flow and one of the important factors on the quality of food products. Viscosity is affected by factors such

as particle size, dry matter, and compression of the protein network.

Fig. 6 shows the regression plot of viscosity versus storage time. Results show that only the

yogurt storage time affects the viscosity. The probiotic type and inulin level don't have a significant effect on the viscosity. The viscosity is decreased by increasing storage time to 11 days and then is increased.

In general, during storage period, the yogurt viscosity may be reduced due to changes in the compression of the protein network and the

formation of pores in yogurt. The increase in viscosity after the eleventh day may be due to an increase in the hydration of inulin colloid, or creation of protein-protein interaction.

Toneli *et al.* (2007) reported a positive effect of inulin on the viscosity of low-fat or high-fat foods such as salads, chocolate and yogurt.

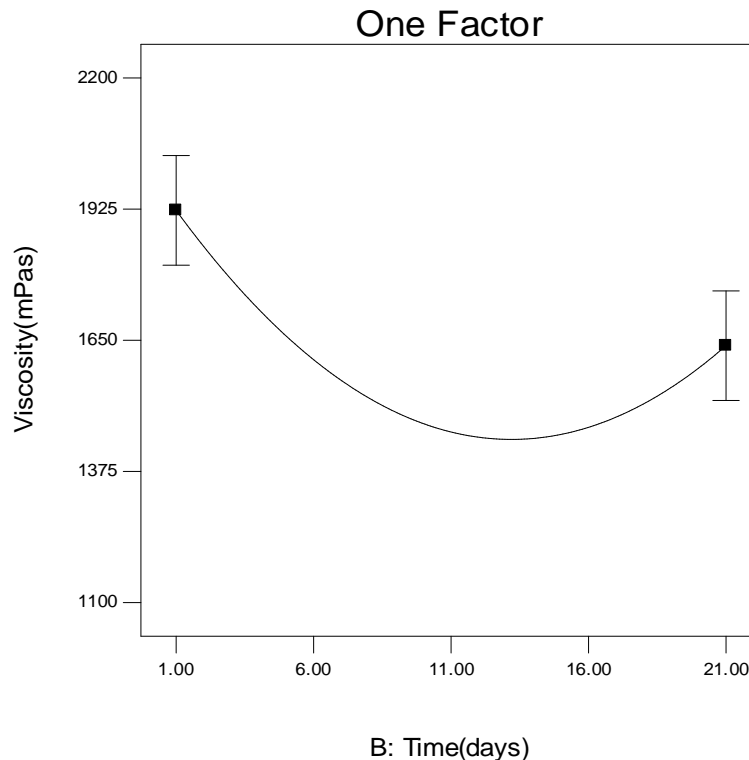


Fig. 6. Regression plot of viscosity versus storage time

Relation between physicochemical/ microbial properties of yogurt and volatile compounds

For evaluation of relation between physicochemical/ microbial properties of yogurt and volatile compounds, GC chromatograms, the acidity, viscosity, syneresis, and microbial count of yogurt were considered as variables and total peak area and total peak height are considered as responses. So the full term second order polynomial response surface models were fitted to each of the response variables, according to equation (1). Where Y is the responses (total peak area and total peak height); factors (F) are acidity,

syneresis (%), viscosity (mPas), and microbial count (cfu/g), and b values are the coefficient values obtained through multiple linear regressions. The quadratic polynomial models for two response functions accompanied by F values and corresponding R^2 was used, the estimated regression coefficients summarized in Table 3.

Fig. 7 and Fig. 8 show the surface plot and counter plot of the total peak area and total peak height based on variables (acidity, syneresis (%), viscosity (mPas), and microbial count (cfu/g)) respectively.

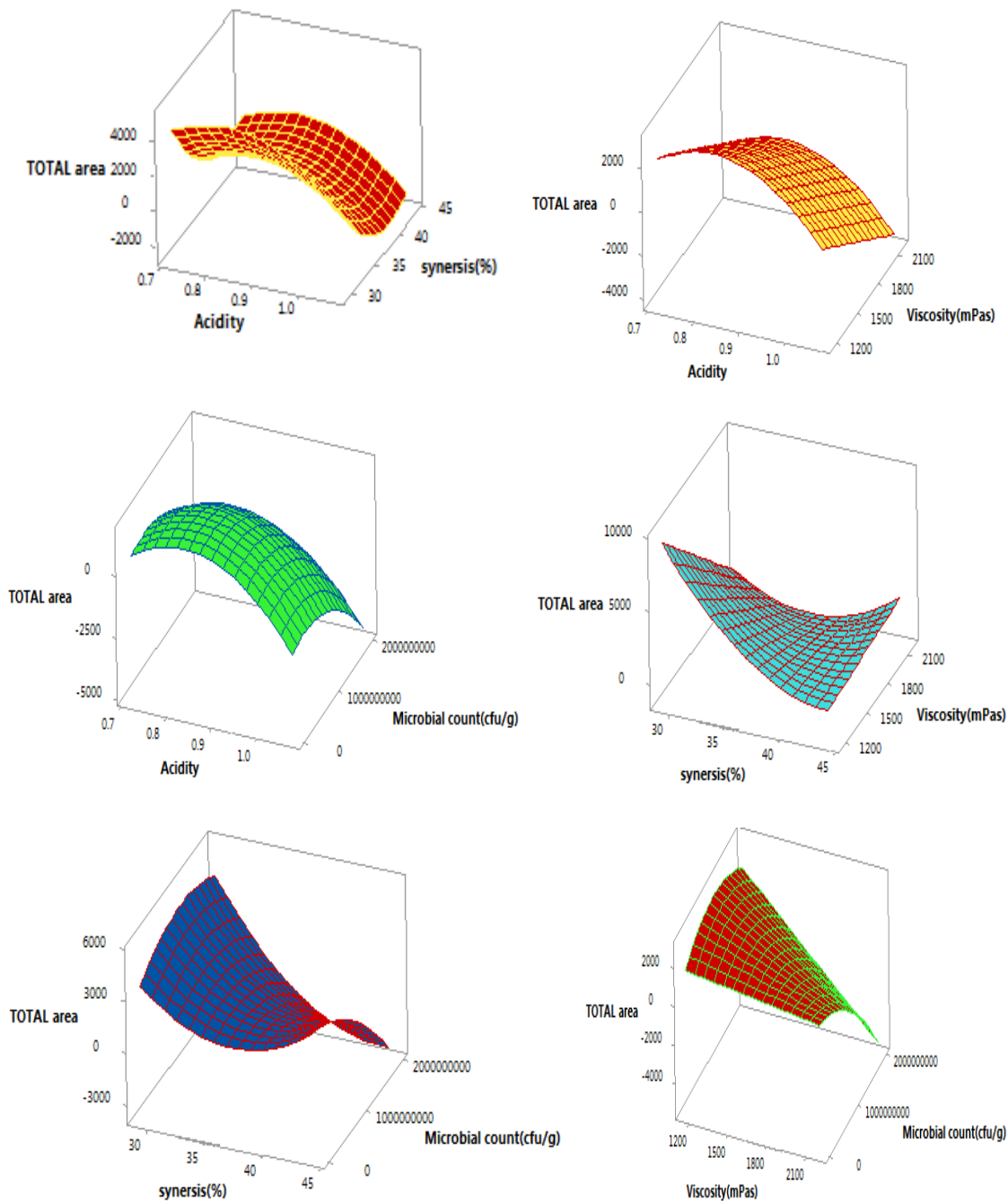


Fig. 7. Surface plots of total peak area based on variables

Results show that the total peak area and total peak height of yogurt VOCs are affected by physicochemical/ microbial properties of yogurt and there are good relation between

physicochemical/ microbial properties of yogurt and volatile compound peaks in GC that can help to suggest and determine microbial count, viscosity, acidity and syneresis of yogurt.

Table 3- Some characteristics of the constructed models for responses (total peak are and total peak height)

Regression equation	Model Summary
Total peak area = 52683 + 95755 Acidity - 3827 syneresis(%) - 23.67 Viscosity(mPas) + 0.000015 Microbial count(cfug) - 57231 Acidity*Acidity + 37.5 syneresis(%)*syneresis(%) - 0.000000 Microbial count(cfug)*Microbial count(cfug) + 0.624 syneresis(%)*Viscosity(mPas) - 0.000000 syneresis(%) *Microbial count(cfug) - 0.000000 Viscosity(mPas)*Microbial count(cfug)	R-sq= 79.33% R-sq(adj)= 56.37%
Total peak height = 5990 + 10 Acidity - 272 syneresis(%) - 1.583 Viscosity(mPas) + 0.000005 Microbial count(cfug) + 2.82 syneresis(%)*syneresis(%) - 0.000000 Microbial count(cfug)*Microbial count(cfug) - 0.000002 Acidity*Microbial count(cfug) + 0.0467 syneresis(%)*Viscosity(mPas) - 0.000000 syneresis(%)*Microbial count(cfug) - 0.000000 Viscosity(mPas) *Microbial count(cfug)	R-sq= 78.18% R-sq(adj)= 53.93%

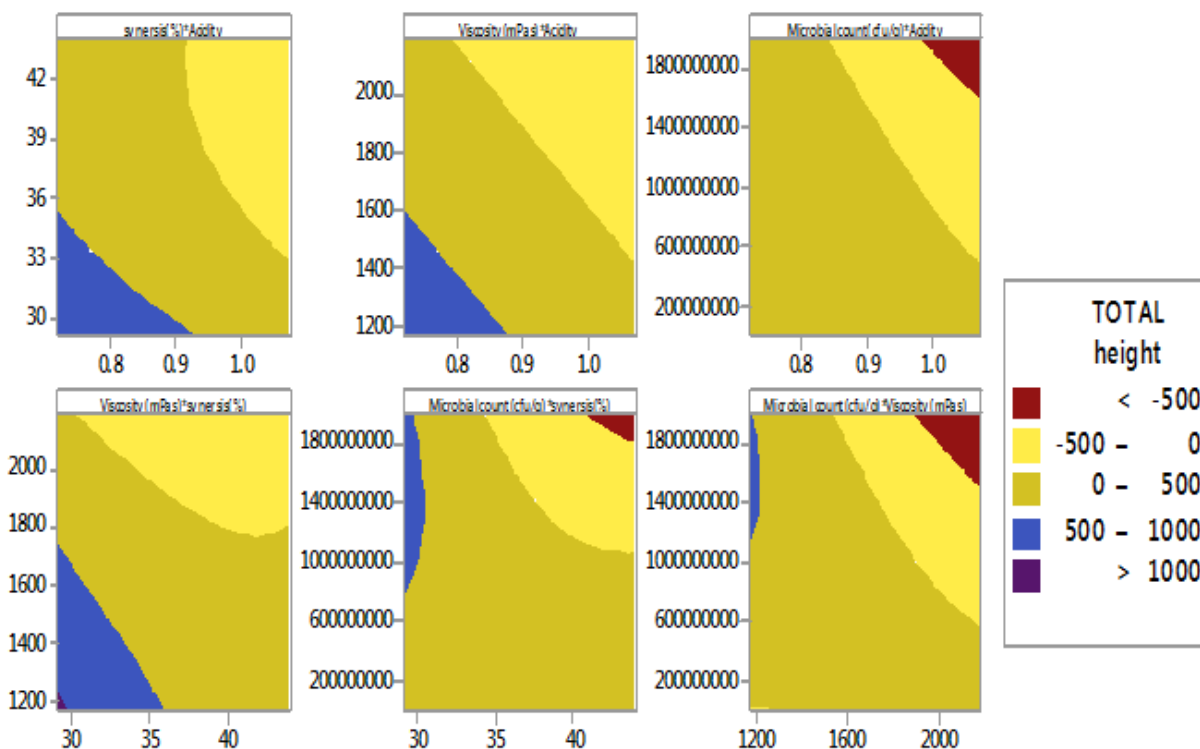


Fig. 8. Counter plots of total peak height based on variables

Conclusion

The physicochemical and microbial properties of yogurt are affected by probiotics type (aerobic and anaerobic), inulin level and storage time of yogurt. Physicochemical and microbial properties of yogurt have a strong effect on the VOCs of yogurt. The chemically synthesized nano- size Polyaniline was used to extract and analyze yogurt VOCs by HS-SPME- GC method. The D- Optimal Combined Design (DOCD) was used to study the effect of probiotics type (aerobic and anaerobic), inulin

level (W/W %), and storage time of yogurt (day) on the physicochemical/microbial properties of synbiotic yogurt and the relation between physicochemical/ microbial properties and VOCs GC- characters (total peak area and total peak height). According to the results: 1- Inulin level and storage time have affected the yogurt syneresis, but probiotic type doesn't have significant effect on the syneresis, 2- Inulin level and storage time have effect on the sample acidity, but there is not any interaction between storage time and inulin level, 3- The microbial

count is increased by increasing storage time in the presence of both aerobic and anaerobic probiotics, and 4- total peak area and total peak height of yogurt VOCs are affected by physicochemical/ microbial properties of yogurt and there are good relation between physicochemical/ microbial properties of

yogurt and volatile compounds peaks in gas chromatography.

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مطالعه ویژگی‌های فیزیکوشیمیایی / میکروبی و پروفیل کروماتوگرافی گازی ماست سین بیوتیک

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چکیده

ماست سین بیوتیک با افزودن پروبیوتیک‌های هوازی (لاکتوباسیلوس اسیدوفیلوس) و بی‌هوازی (بیفیدوباکتریوم بیفیدوم) و اینولین به داخل ماست تهیه شد. اثرات پروبیوتیک‌های هوازی و بی‌هوازی، زمان نگهداری و اینولین بر خواص فیزیکوشیمیایی / میکروبی ماست سین بیوتیک از نظر اسیدیته، ویسکوزیته، سینرزیس و میزان بار میکروبی مورد بررسی قرار گرفت. برای استخراج و شناسایی پروفیل مواد فرار (مساحت سطح کلی پیک‌ها و ارتفاع کلی پیک‌ها) نمونه‌های ماست از روش ریز استخراج از فضای فوقانی با فاز جامد - کروماتوگرافی گازی (HS-SPME-GC) به وسیله فیبر نانوساختار پلی‌آنیلین استفاده شد. برای بررسی اثر نوع پروبیوتیک (هوازی و بی‌هوازی)، درصد اینولین و زمان نگهداری ماست بر روی خواص فیزیکوشیمیایی / میکروبی ماست سین بیوتیک از طرح آماری مرکب بهینه استفاده شد. نتایج نشان داد که پروبیوتیک‌های هوازی و بی‌هوازی، اینولین و زمان نگهداری ماست بر ویژگی‌های فیزیکوشیمیایی ماست تاثیر می‌گذارند و ارتباط معنی‌داری بین خواص فیزیکوشیمیایی ماست و پروفیل مواد فرار کروماتوگرافی گازی (مساحت سطح کلی پیک‌ها و ارتفاع کلی پیک‌ها) وجود دارد. تحقیق ارائه شده، محاسبه بار میکروبی از طریق بررسی مساحت سطح کلی پیک‌ها و ارتفاع کلی پیک‌های نمونه‌های ماست را امکان‌پذیر می‌سازد.

واژه‌های کلیدی: ماست سین بیوتیک، خواص فیزیکوشیمیایی، خصوصیات میکروبی، نانومواد و کروماتوگرافی گازی

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