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Influence of orange fiber addition on quality of orange fruit yogurt using response surface method

Afsaneh Azimi[1], Shahin Zomorodi[2], Ali Mohamadi Sani[1]*

[1]Department of Food Science & Technology, Quchan Branch, Islamic Azad University, Quchan, (IRAN)

[2]Department of Agricultural Engineering Research, Agriculture Center, Urmia, (IRAN)

E-mail: mohamadisani@yahoo.com

ABSTRACT

Response surface methodology (RSM) was employed to investigate the combined effects of orange fiber (0, 0.2, 0.6, 1 and 1.2 g/100 ml) and storage time (3, 7, 16, 25, 29 days) on physicochemical, rheological and sensory properties of orange fruit yogurt. Results showed that by increasing the fiber amount, the viscosity and total solids increased and syneresis decreased significantly ($P<0.05$), but perceived acidity and pH were not affected significantly ($P>0.05$). The amount of syneresis and acidity significantly increased during storage, but pH and viscosity decreased ($P<0.05$). Also, increasing the amount of fiber content led to increasing the yellowish spectrum (+b* parameter) of yogurt while the rate of lightness (L*) decreased significantly ($P<0.05$). Sensory evaluation results showed that higher flavor, color and overall acceptance score was obtained at lower fiber amount. However, hedonic tests showed that almost all assayed yogurts have color, flavor and overall acceptance scores at least 4.25 on a seven-point scale, considered in the present work as the acceptable value. Analysis of variance revealed that the quadratic models are well adjusted to predict the experimental data. Finally, the optimum conditions for production of orange yogurt containing orange fiber were obtained using 1.2% fiber after storage for 16 days.

KEYWORDS

Orange fiber;
Orange yogurt;
Rheology;
Syneresis.

INTRODUCTION

Dietary fiber (DF) is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine[1]. Coronary disease, hypertension, diabetes, hypercholesterolemia and gastrointestinal disorders may decrease or be prevented by consuming higher quantity of fiber in the diet[2]. The recommended daily intake of fiber is about 38 g for men and 25 g for women[3]. Despite dietary guidelines (DG), dietary fiber intakes of the general public are well below the recommended levels. In the United States, the average American adult consumes only 14 to 15 grams of dietary fiber a day. Approximately 75% of Americans do not have adequate dietary fiber intake. Dietary fiber intake levels in the Asia-Pacific region and in most industrialized nations in Europe are also far below the recommended levels[4,5]. Production of orange in 2010 in FAO countries was 64 million tons[6]. One-third of the citrus fruits are processed to obtain several products, mainly juice[7]. The amount

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of residue obtained from citrus fruits accounts for 50% of the original amount of whole fruit[8]. The residue remaining after juice and essential oil extraction is mainly used to obtain pectin and also for animal feed with little economic value. The accumulation of these residues is an environmental problem. However, these by-products are rich in fiber[9] and citrus fruits have better quality than other sources of dietary fibers due to the presence of associated bioactive compounds (flavonoids and vitamin C) with antioxidant properties[10]. The possibility of successfully including these by-products in the human food industry would help in enhancing the economic development of citrus producers and processors[9]. Yogurt is one of the dairy products, which should continue to increase in sales due to diversification in the range of yogurt-like products, including reduced fat content yogurts, probiotic yogurts, yogurt shakes, drinkable yogurts, yogurt mousse, yogurt ice-cream, etc[11]. For a long time, yogurt by itself has been recognized as a healthy food, due to the beneficial action of its viable bacteria that compete with pathogenic bacteria for nutrients and space[12]. Dairy products, as yogurt, can provide major opportunities for the development of fiber enriched foods. Their acceptability by the consumers is mainly based on satisfactory textural and sensory attributes[13]. Since consumer concerns are related to both nutritional and sensory aspects, several authors studied texture characteristics of yogurts, containing the addition of different type of dietary fibers : oat, rice, soy and maize fibers[14], apple, wheat, bamboo and inulin[2], b-glucan in yogurt[15], orange fiber in yogurt[13], inulin in yogurt ice cream[16]. Dello Staffolo et al.[2] observed that the type of fiber significantly affected the rheological properties of the yogurts. Wheat and bamboo fiber fortification increased yogurt compression force and texture sensory scores; consumer's preferred firmer yogurts, probably, resulting from the insoluble nature of these fibers. According to Sendra et al.[13], Citrus fiber from orange by-products is a novel ingredient that can be successfully used in yogurt production. Yogurts behave as shear thinning fluids and very weak gels. Orange fiber addition modifies yogurt rheology. The aim of the present study was to model and evaluate the combined effects of orange fiber and storage time on sensory, physicochemical and rheological properties of orange fruit yogurt by applying the RSM.

MATERIAL AND METHODS

Materials

Cow's milk (2.5% fat, 3.1% protein, 11.6% TS and pH 6.6-6.7), Commercial starter cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus* (DSM Food Specialities Australia, Moorebank, NSW, Australia), skim milk powder, orange fruit, sugar, pectin, citric acid and microbial mediums of Nutrient Agar, Yeast Glucose Cloranfenicol Agar and Potato Dextrose Agar (Merk, Germany) were used in this study.

Methods

Fiber preparation

Fiber powder was processed following Larrauri[17] and Lario et al.[18] recommendations for orange fiber preparation. Orange peels from Oranges were grinded to a high particle size (15 mm) and washed in hot water (90ºC for 5 min, peels: water 1:4). Afterwards the residue was pressed to reduce excess moisture and dried at 65ºC for 24 h to maximum 2 percent moisture. The dried orange fiber extract is then grinded to the desired particle size. The obtained fibers were vacuum-packed in vacuum pouches (2 kg) and pouches were placed at 4ºC. Powders with one particle size (0.701–0.991) were obtained.

Marmalade preparation

Orange fruit was washed after the fruit skin was removed manually and then the pulp was obtained from the crushed fruit. The 50% ratio fruit pulp and 50% ratio sugar; with 0.15% pectin and 0.2% citric acid was mixed and boiled for 10 min, and filled into cleaned glass jars. The marmalade was stored at room temperature until used in yogurt production.

Yogurt production

Whole milk was fortified with 2 % skim milk powder. The yogurt base was supplemented with 0, 0.2, 0.6, 1 and 1.2% (*w/w*) of orange fiber. Each mix was then pasteurized at 85°C for 30 min and cooled to approximately 44°C followed by inoculation with yoghurt starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp *bulgaricus*) and incubated at 42°C until the pH reached 4.60. Yogurts were immediately cooled after fermentation. Then orange marmalade were added at a ratio of 20% (*w/w*) in to each yogurt mix and gently mixed for 20 s. The mixtures filled into 50 mL sterile plastic cups and stored at 4°C for 29 days. Viscosity behavior, color, pH, syneresis and microbial quality and sensory characteristics were tested at 3, 7, 16, 25 and 29 days of storage.

Physico-chemical analyses

Total solids, titratable acidity and pH of yogurt samples were determined following standard methods[19]. Total solids was determined using a drying oven at 105°C (Memmert, Germany). Titratable acidity was expressed in terms of % lactic acid. The pH was measured with a pH meter (Metrohm 691, Swiss). Color of yogurts was determined in a tristimulus colorimeter (Minoleta CR-400, Osaka, Japan); the L* (lightness), a* (redness/ greenness) and b* (yellowness/ blueness) parameters of the Hunter scale were analyzed. To determine syneresis, one hundred grams of yogurt sample was placed on a filter paper resting on a top of a funnel. After 2 h of drainage at 7°C, the quantity of whey collected in a 50 ml graduated cylinder was used as an index of syneresis[20].

The orange fiber was analyzed for pH, moisture, ash, total dietary fiber and water holding capacity. Ash content was measured by heating a 5g sample in a muffle furnace at 100°C for 1 hour, 200°C for 2 hours and 550°C overnight[19]. Moisture was determined using a drying oven at 105°C[19]. Titratable acidity was expressed in terms of % citric acid[19]. The pH was measured with a (Metrohm 691, Swiss) pH meter. Dietary fiber was analysed by the enzymic gravimetric method[21]. Samples (0.5 g) were only treated with pepsin (40°C, 1 h) because of their low starch content, filtered, and the residue washed successively with 2 × 10 mL of distilled water, 2 × 10 mL of 95% ethanol and 2 × 10 mL of acetone. The residue corresponded to insoluble dietary fiber (IDF), four volumes of 95% ethanol were added to the filtrate and washing. After 1 h, the mixture was filtered and the precipitate, soluble dietary fiber (SDF) was washed with 2 × 10 mL of 78% ethanol, 2 × 10 mL of 95% ethanol and 2 × 10 mL of acetone. Both residues were dried at 105°C and weighed, total dietary fiber (TDF) was the sum of SDF and IDF. For measurement of water-holding capacity (WHC) in the experimental orange fiber, 250-mg dry sample, 25 mL of distilled water was added, stirred and held at room temperature for 1 h. After centrifuging, the residue was weighed and WHC was calculate as g water per g of dry sample[22]. Total soluble solid content of orange marmalade (°Brix) was determined by using a hand refractometer (Tajiri Ind. Co., Japan).

Measurement of viscosity

The viscosity of the yogurt batches was measured during storage at 7°C and all samples were treated at a constant shear rate[14]. Samples were tested using a 64 spindle coupled to an LVTD digital viscometer (Brookfield DV11, USA). The viscometer was set at constant revolutions of 30 rpm. The yoghurt was gently stirred for 20 s (20 continuous sweeps) before analysis.

Microbial analyses

Testing for total count, yeast and mold was according to standard methods for the examination of dairy products, using the Nutrient Agar (NA) and Yeast Glucose Cloranfenicol Agar (YGCA) respectively[23].

To aerobic mesophilic bacteria count, yeast and mold determination of orange fiber and marmalade, using the Nutrient Agar (NA) and Potato Dextrose Agar (PDA) respectively.

Sensory evaluation

For sensory evaluation, twenty assessors from the Agricultural Research Center of West Azerbaijan (Urmia, Iran) were selected. During evaluation, the panelists were situated in private booths under incandescent light. Drinking water was provided between samples to cleanse the palate. The samples were served 40 ml in white plastic cups with a consumer sensory evaluation questionnaire. The consumers were asked to evaluate sensory characteristics such as flavor, color and texture of all yogurt samples. The sensory evaluation was based on seven-point hedonic scale (1= unacceptable, 2 = dislike extremely, 3 = dislike moderately, 4 = neither like nor dislike, 5 = like moderately, 6 = like extremely, and 7 = excellent). The overall acceptability was calculated as sum of the scores of the parameters judged[24, 25].

Experimental design and statistical analysis

The experiments were designed using a Central Composite Rotary Design (CCRD). Five different levels, two factors (fiber amount and storage time) factorial central composite rotary design and five replicates

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at the center points leading to 13 runs was employed. Coded and actual levels and experimental design are given in TABLE 1.

TABLE 2 shows experimental results and the predicted responses of viscosity, sensory and physicochemical properties of orange fruit yogurt.

Multiple regression analysis was performed in order to fit a second order polynomial equation, described below, to the data:

$Y= \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2$

Where Y is the response, $^2_0$ constant, β_1 and β_2 are the linear coefficient, β_{11} and β_{12} the quadratic coefficient, and β_{12} the interaction coefficient. X_1 and X_2 are the independent variables. The quality-of-fit of the quadratic model was expressed by determination coefficient (R^2), and its statistical significance was examined by F value.

The analysis was made using coded units. SAS (Version 6.0, SAS Institute Inc., Cary, NC27513, USA) was employed for response surface analysis and mapping of plot. All experiments were carried out in triplicate and data were expressed as average values.

TABLE 1 : Coded and uncoded values of independent variables used in central composite rotary design with two responses: fiber content (X_1, g/100 ml) and storage time (X_2, day)

Run	Fiber content (X_1, g/100ml)		Storage time(X_2, day)	
	Un coded value	Coded Value	Un coded value	Coded Value
1	0.2	-1	7	-1
2	0.2	-1	25	1
3	1	1	7	-1
4	1	1	25	1
5	0	-1.414	16	0
6	1.2	1.414	16	0
7	0.6	0	3	-1.414
8	0.6	0	29	1.414
9	0.6	0	16	0
10	0.6	0	16	0
11	0.6	0	16	0
12	0.6	0	16	0
13	0.6	0	16	0

RESULTS AND DISCUSSION

Fiber and marmalade characteristics

Fiber was obtained from orange fiber by-products by a procedure described by Larrauri[17] and Lario et al.[18]. According to Lario et al.[18], processing conditions of the residue affect fiber composition and properties. Washing previous to drying yields an orange fiber with the highest dry matter. WHC is enhanced by washing and high fiber particle size, whereas. Drying induces browning of the fiber; washing previous to drying prevents fiber browning, probably due to the removal of sugars. Washing water is a good potential source of soluble sugars and carotenoids.

Physicochemical properties of orange fiber and orange marmalade were determined in TABLES 3, 4.

Checking of the fitted models

Experimental results and predicted values are given in TABLE 2. Results showed experimental values were very close to the predicted values. ANOVA showed that the second-order regressions were statistically significant (P<0.05) and the quadratic polynomial models are well fitted to the experimental data (TABLES 5, 6 and 8).

The lack-of-fit was not significant indicating that the models are adequately accurate for predicting syneresis, viscosity, total count, texture, flavor, color score and overall acceptability for any combination of independent factors in the ranges studied. The coefficients of determination (R^2) were high and varied between 0.83 and 0.95. The values of the adjusted determination coefficient (adjusted R^2) were also high to confirm a high significance of the models[26]. This suggested that second-order terms were sufficient and higher-order terms were not necessary. Therefore, obtained models can be used to determine the relative effect of the factors, to find an optimum parameter combination for desirable responses, and to predict experimental results for other conditions. The quadratic polynomial models for total solids, pH,acidity, syneresis, viscosity, total count, texture, flavor, color score and overall acceptability stated as equations 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 respectively. Where X_1 is fiber amount (g/100 mL) and X_2 is storage time (day).

$$Y = 23.05059 + 1.670712X_1 \quad (1)$$

$$Y = 4.446858 - 0.043276$$

$$X_2 + 0.001389\,X_2^2 \quad (2)$$

$$Y = 0.640017 + 0.045392$$

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TABLE 2 : Experimental results and predicted values of viscosity, sensory and physicochemical properties of orange fruit yogurt

Run	Total solids (%)		Acidity (%)		Syneresis (%)		pH		Viscosity (cP)	
	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
1	23.66	23.91	0.83	0.99	33.51	33.30	4.26	4.10	1420	1662.72
2	23.17	24.17	0.73	1.01	33.57	36.82	4.29	4.10	1220	1883.94
3	24.18	23.34	0.84	0.84	28.17	30.46	4.21	4.10	2739	1398.82
4	24.72	24.77	0.72	0.97	32.75	38.91	4.25	4.22	1980	2526.75
5	23.03	24.04	0.99	1.01	38.10	36.13	4.05	4.10	1380	1765.00
6	25.35	25.60	0.89	0.98	27.13	29.15	4.15	4.13	2940	2810.01
7	24.16	23.63	0.80	0.94	35.53	33.21	4.3	4.10	1600	1507.81
8	24.15	24.46	0.77	1.00	38.73	37.95	4.31	4.14	1500	2171.94
9	24.06	24.82	0.97	0.99	35.21	32.64	4.11	4.11	1825	2287.50
10	24.41	24.82	0.97	0.99	34.45	32.64	4.12	4.11	1700	2287.50
11	24.02	24.82	1.03	0.99	36.9	32.64	4.13	4.11	1820	2287.50
12	23.86	24.82	1.07	0.99	37.07	32.64	4.09	4.11	1860	2287.50
13	23.85	24.82	0.99	0.99	37	32.64	4.06	4.11	1620	2287.50

Run	Total counts (log CFU/g)		Texture score		Flavor Score		Color score		overall acceptability score	
	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted	Observed	Predicted
1	4.17	3.99	4.5	4.89	5.75	4.52	5.25	4.88	5.16	4.83
2	4.14	4.08	4.33	5.06	5.58	4.69	5.33	4.96	5.08	4.88
3	4.81	3.81	5.34	4.53	4.75	4.45	5.32	4.69	5.13	4.77
4	4.36	4.51	5	5.36	4.67	5.31	4.62	5.17	4.76	5.01
5	3.69	4.03	4.33	4.98	5.66	4.59	5.22	4.92	5.07	4.85
6	4.04	4.20	5.5	5.57	4.60	4.50	4.61	4.55	4.92	4.75
7	4.77	3.91	4.9	4.72	5.17	4.44	5.1	4.79	5.05	4.80
8	4.39	4.25	4.55	5.21	5	4.96	5	5.04	4.85	4.93
9	4.2	4.11	4.84	5.27	4.75	4.54	4.93	4.73	4.84	4.80
10	4.2	4.11	5.17	5.27	4.75	4.54	4.9	4.73	4.94	4.80
11	3.84	4.11	5	5.27	4.25	4.54	5	4.73	4.75	4.80
12	3.84	4.11	4.75	5.27	4.25	4.54	5	4.73	4.86	4.80
13	4.11	4.11	4.16	5.27	5	4.54	4.8	4.73	4.90	4.80

TABLE 3 : Physicochemical properties of orange fiber

Water holding capacity (WHC) (g water/ g dry sample)	Soluble fiber (%)	Total dietary fiber (%)	Acidity (%)	pH	Moisture (%)	Ash (%)	Aerobic mesophilic bacteria count (log CFU/g)	Mold count (log CFU/g)	Yeast count (log CFU/g)
12±0.2	21.4±1.0	71.5±1.2	0.14±0.02	4.53±0.01	1.43±0.2	3.44±0.2	5.89±0.04	2±0.05	3.77±0.07

Presented values are the means (±SD) of three replicate trials

TABLE 4 : Physicochemical properties of orange marmalade

pH	Acidity (%)	Total soluble solid (°Brix)	Aerobic mesophilic bacteria count (log CFU/g)	Mold count (log CFU/g)	Yeast count (log CFU/g)
3.82±0.01	0.46±0.02	62±3	0	0	0

Presented values are the means (±SD) of three replicate trials

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X_2 - 0.001533 X_2^2 (3)

Y = 33.15512 + 7.977453

X_1 + 0.196862 X_2 - 13.59206X_1^2 (4)

Y = 1597.098 - 222.4315

X_1 - 15.28365X_2 + 1301.29 X_1^2 (5)

Y = 4.969007 + 0.42343X_1 - 0.138318

X_2 + 0.003881X_2^2 (6)

Y = 4.17122 + 0.988947X_1 + 0.037787

X_2 - 0.001617 X_2^2 (7)

Y= 6.819066 - 3.202841 X_1 - 0.110515 X_2 + 1.781255

X_1^2+ 0.003241 X_2^2 (8)

Y = 5.27661 - 0.054759

X_1 - 0.024269 X_1 X_2 (9)

Y = 5.338662 - 0.742245 X_1 - 0.010178

X_2+ 0.472148X_1^2 (10)

Chemical and physical characteristics

TABLE 5 shows that for total solids content the linear effects of fiber (X_1) and for pH and acidity the quadric effects of storage time (X_2) were significant (P< 0.05). figure 1 shows that higher total solids were obtained at higher fiber content. It can be related to water-binding properties of fruit fiber[27]. This observation is consistent with the findings of Sahan et al.[28] who reported that addition of b-glucan composite into the milk increased the total solids content in the yogurts. Also figure 1 shows pH values decreased by passing time during storage and acidity increased. pH value increased and acidity decreased after 16th day. Microorganism's activity caused pH decrease in fruit yoghurt. Yeasts also used sugar and organic acids and so pH value decreased. As sugar sources finish, microorganisms begin to consume proteins and producing some products by microorganisms, will result in pH increase[29, 30]. These results are in agreement with the findings of Vahedi et al.[31].

Syneresis

TABLE 5 shows that for syneresis values both the linear and quadratic effects of fiber (X_1) were significant, whereas storage time (X_2) only had linear effect (P< 0.05).

Figure 2 demonstrates the combined effects of fiber content and storage time on syneresis. It can be seen from figure 2 that lower syneresis was obtained at higher fiber content and shorter storage time. Without a doubt, the key property of fruit fiber is the hydration. Hydration summarizes the ability to swell, bind water, enhance the viscosity, and prevent syneresis. Especially those fruit fibers that are produced carefully without collapse of the cell wall architecture are able to swell in a very short time and form a sponge-like network. This matrix is able to immobilize water to a high degree. The high water-binding is a technological as well as a physiological benefit[27]. Garcý´a-Pe´ rez et al.[32] reported that addition of 1 g/100 ml orange fiber reduced syneresis and improved the creaminess sensory scores, together with increased gel firmness and stickiness.

Dello Staffolo et al.[2] found that yogurt fortified with wheat, bamboo, inulin and apple fibers did not show syneresis even after 21 day storage time at 4°C. Supavititpatana, et al.[33] also indicated that syneresis of the corn–milk yogurt was affected by the addition of gelatin. Increased levels of gelatin significantly reduced the extent of syneresis (P< 0.05).

Syneresis increased in all of the samples during storages significantly. The syneresis behavior during stor-

TABLE 5 : Analysis of variance for the experimental response of chemical composition and viscosity of fruit yogurt samples

Source	df	pH		Acidity		total solids		Syneresis		Viscosity	
		Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value
Model	5	0.0183	8.468**	0.0282	7.406*	0.7679	10.850**	29.5173	12.306**	583560.7	32.180***
Liner	2	0.0006	0.280	0.0055	1.448	1.7865	25.240***	56.9976	23.762***	1223353	67.46***
Quadratic	2	0.0452	20.885**	0.0649	17.04**	0.0008	0.012	16.7675	6.990*	196488.4	10.835**
Fiber(X_1)	1	0.0003	0.153	0.0024	0.632	3.5728	50.477***	88.8819	37.055***	2295335	126.573***
Time(X_2)	1	0.0009	0.408	0.0086	2.264	0.0002	0.003	25.1130	10.469*	151365.5	8.347*
X_1^2	1	0.0009	0.425	0.0207	5.441	0.0016	0.023	32.5197	13.557**	260972	14.39**
X_1 X_2	1	0.0001	0.011	0.0002	0.054	0.2650	3.744	0.0562	0.023	78120.25	4.309
X_2^2	1	0.0904	41.732***	0.1198	31.478***	0.000012	0.001	0.0651	0.027	86194.05	4.753
Lack of fit	3	0.0040	5.231	0.0067	4.091	0.0979	1.939	3.6325	2.466	28680.44	2.805

Source	df	pH		Acidity		total solids		Syneresis		Viscosity	
		Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	*F* Value	Mean Square	F Value
Pure Error	4	0.0008		0.0016		0.0505		1.4733		10225	
Total	12										
R^2			0.858		0.841		0.885		0.897		0.958
R^2 (adj)			0.756		0.727		0.804		0.824		0.928
Coefficient Variation(CV)			1.113		6.875		1.106		4.443		7.417

*** *P*<0.05; ** *P*<0.01; *** *P*<0.001**

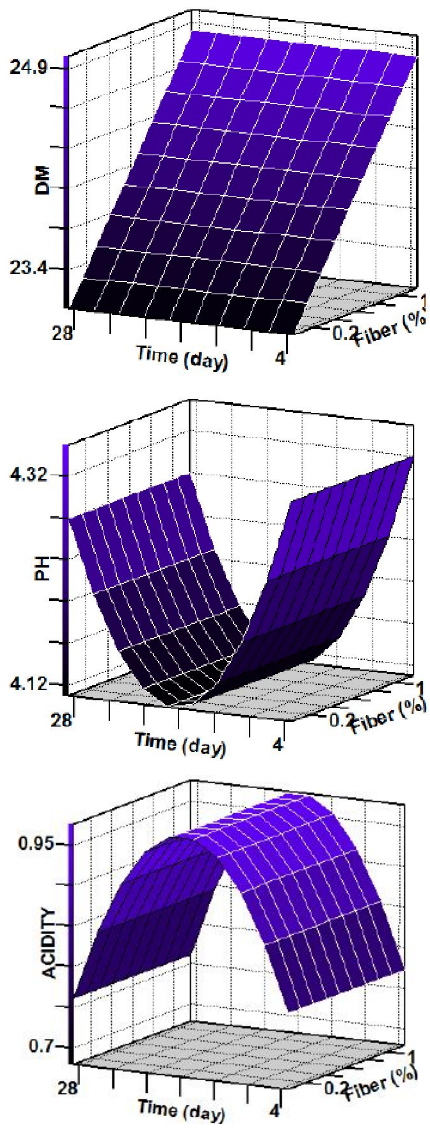

Figure 1 : Response surface plots for total solids (%), acidity (%) and pH as a function of fiber content and storage time

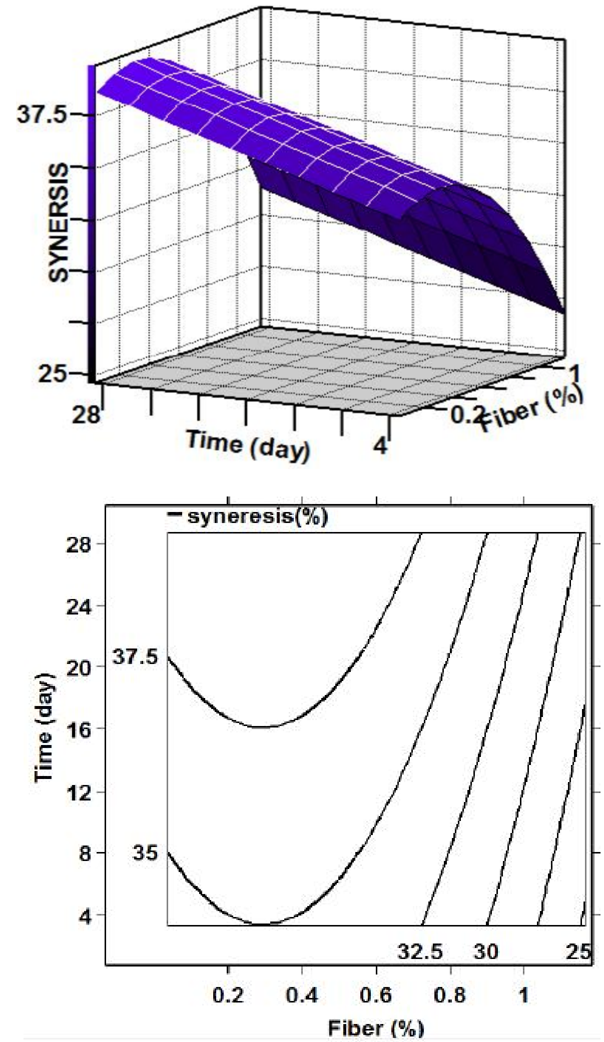

Figure 2 : Response surface and contour plot for syneresis (%) as a function of fiber content and storage time

age time was similar to the results of Tarakçi & Küçüköner[20]. Similar results were reported for fruit-flavored yogurt containing Kiwi Marmalade[34].

Viscosity

TABLE 5 shows that for viscosity values both the linear and quadratic effects of fiber (X_1) and the linear effects of storage time (X_2) were significant (P< 0.05). Figure 3 shows that higher viscosity was obtained at higher fiber amount and shorter storage time. It can be explained by the fact that in fruits or fruit-based products, the cell wall matrix is the principal structural component and the water-binding properties of fruit fiber can be used to control the texture and the rheological

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behavior of food thereof[27]. This observation is consistent with the findings of Dello Staffolo et al.[2] who reported that fiber type and storage time were intervening factors in the apparent viscosity. Sahan et al.[28] also indicated that addition of b-glucan to yoghurt samples increased the viscosity. Also according to Sendra et al.[13], citrus fiber from orange by-products is a novel ingredient that can be successfully used in yogurt production which modifies texture properties.

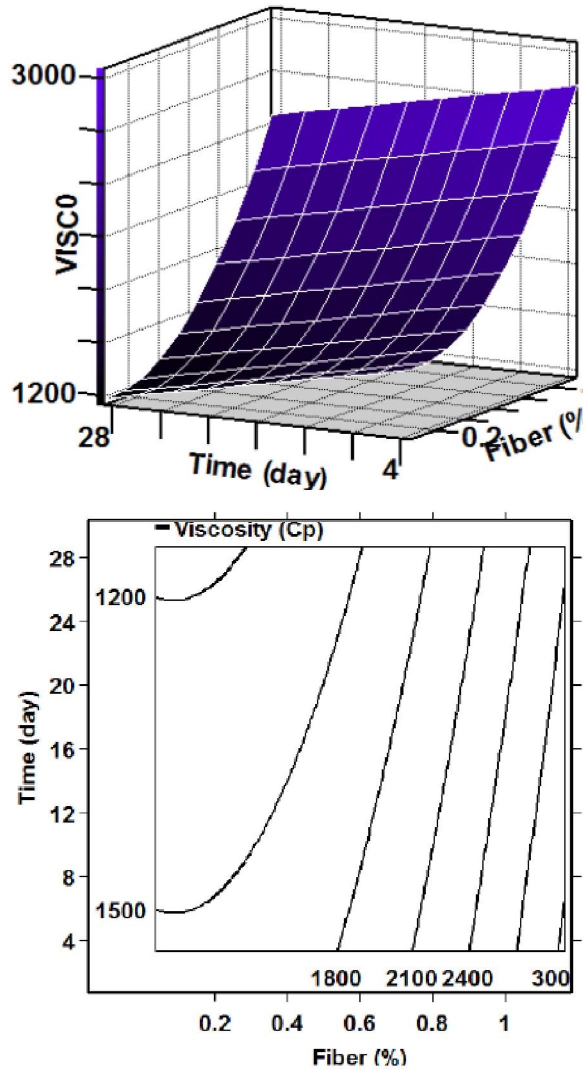

Figure 3 : Response surface and contour plot for viscosity (cP) as a function of fiber content and storage time

Microbiological quality

The statistical data presented in TABLE 6 indicate that total count was significantly affected by linear effects of fiber content and quadratic effects of storage time, whereas for yeast and mold count both linear and quadratic effects of storage time were significant (*P*<

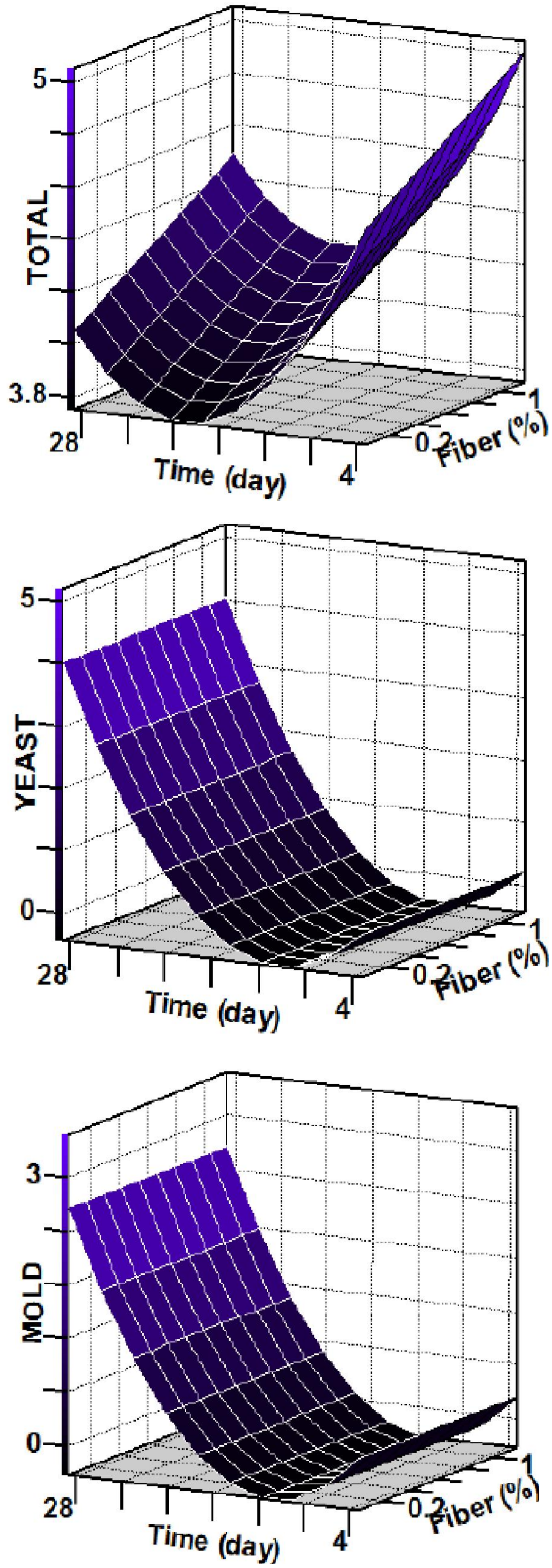

Figure 4 : Response surface plots for microbial counts (*log cfu/g*) as a function of fiber content and storage time color characteristics

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TABLE 6 : Analysis of variance for the experimental response of microbial counts of fruit yogurt samples

Source	df	Total count		Yeast		mold	
		Mean Square	F Value	Mean Square	F Value	Mean Square	F Value
Model	5	0.2239	7.969**	7.357	23.36***	3.2785	168.115***
Liner	2	0.1794	6.387*	12.4195	39.434***	5.2349	268.43***
Quadratic	2	0.3582	12.751**	5.9539	18.905**	2.9565	151.6***
Fiber(X_1)	1	0.2295	8.169*	0.019	0.060	0.005	0.256
Time(X_2)	1	0.1294	4.605	24.8199	78.808***	10.4647	536.601***
X_1^2	1	0.0171	0.61	0.374	1.188	0.0133	0.683
X_1 X_2	1	0.0441	1.57	0.038	0.121	0.01	0.513
X_2^2	1	0.6594	23.471**	11.8812	37.725***	5.872	301.1***
Lack of fit	3	0.0202	0.594	0.7349	6.248***	0.0455	6.923***
Pure Error	4	0.034		1.18E-15		6.57E-16	
Total	12						
R^2			0.965		0.943		0.991
R^2 (adj)			0.941		0.903		0.985
Coefficient of Variation(CV)			2.413		59.265		23.577

*** P<0.05; ** P<0.01; *** P<0.001**

0.05). Figure 4 illustrates the combined effects of fiber content and storage time on microbial analysis. It can be seen from figure 4 that yogurts containing higher amounts of fiber had higher total microbial count. Aerobic mesophilic bacteria count in the orange fiber was high (5.89 log CFU/g) which led to higher total count in yogurt samples while increasing fiber content. Furthermore, total count decreased until 16th day and then increased, but yeast and mold count during storage significantly increased. Increase of mold and yeast during storage time can be attributed to sanitary conditions during production of yogurt, contamination from air and the type of fruit marmalade used in yogurt manufacture. These results are in agreement with the finding of Tarakçi & Küçüköner[20] who observed that in the fruit yogurt samples yeast and mold count increased progressively during storage.

Experimental results showed that the coefficients of determination (R^2) and (adjusted R^2) were high, but the lack-of-fit of mold and yeast was significant. Therefore, obtained models of yeast and mold count cannot be used to predict experimental results for other conditions.

Color characteristics

The color characteristics of the yogurts are shown in TABLE 7. The L* (lightness) values of yogurts decreased by fiber addition, but the b* (yellowness/blueness) values significantly increased (P<0.05). These results are in agreement with the findings of Dello Staffolo et al.[2] who reported that apple fiber gave a distinctive brownish color and lower lightness (L*) values than the control yogurt sample. The brownish color associated with this fiber would make it necessary to add flavor components to modify yogurt formulation to match consumer preferences. Also according to Sanz et al.[35] asparagus fibers diminished the clarity and imparted a yellow-greenish color to the yogurt.

TABLE 7 : Effect of orange fiber on color values of yogurt

Fiber content (%)	b*	a*	L*
0	23.43±2.2c	5.85±1.59ab	56.19±2.01a
0.2	24.91±2.36b	5.93±1.56ab	55.91±2.29ab
0.6	19.83±2.49d	4.42±1.8c	55.06±1.99abc
1	29.50±2.42a	6.61±1.46a	54.75±1.9bc
1.2	23.72±3.57bc	4.98±1.80bc	54.44±2.93c

Sensory evaluation

Statistical analysis showed significant effects of variables on sensory properties (P< 0.05). Figure 5 shows higher flavor, color score was obtained at lower fiber amount, whereas the higher texture score was obtained at higher fiber amount. Also overall acceptability decreased with increasing fiber amount to 0.6gr/100 ml, then remained constant. Hedonic tests showed that almost all assayed yogurts have color, flavor and overall acceptance scores at least 4.25 on a seven-point scale

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TABLE 8 : Analysis of variance for the experimental response of sensory evaluation of fruit yogurt samples

Source	df	Texture		Flavor		Color		Acceptability	
		Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value
Model	5	0.3037	14.615**	0.4818	6.97*	0.109	8.152**	0.0388	8.098**
Liner	2	0.689	33.156***	0.7414	10.724**	0.1619	12.101**	0.05331	11.124**
Quadratic	2	0.0666	3.207	0.4622	6.685*	0.0347	2.594	0.0331	6.914*
Fiber(X_1)	1	1.2519	60.237***	1.4527	21.014**	0.2513	18.784**	0.0395	8.242*
Time(X_2)	1	0.1262	6.075*	0.03	0.435	0.0724	5.418	0.0671	14.007**
X_1^2	1	0.0119	0.573	0.565	8.173*	0.0126	0.945	0.0485	10.12*
X_1 X_2	1	0.0072	0.348	0.002	0.029	0.1521	11.37*	0.021	4.387
X_2^2	1	0.1294	6.225*	0.4793	6.934*	0.0629	4.706	0.0259	5.401
Lack of fit	3	0.0014	0.039	0.0113	0.1	0.022	3.203	0.0043	0.851
Pure Error	4	0.0353		0.1125		0.0069		0.0051	
Total	12								
R^2			0.912		0.832		0.853		0.852
R^2 (adj)			0.85		0.713		0.748		0.747
Coefficient of Variation(CV)			2.957		5.326		2.308		1.399

* P<0.05; ** P<0.01; *** P<0.001

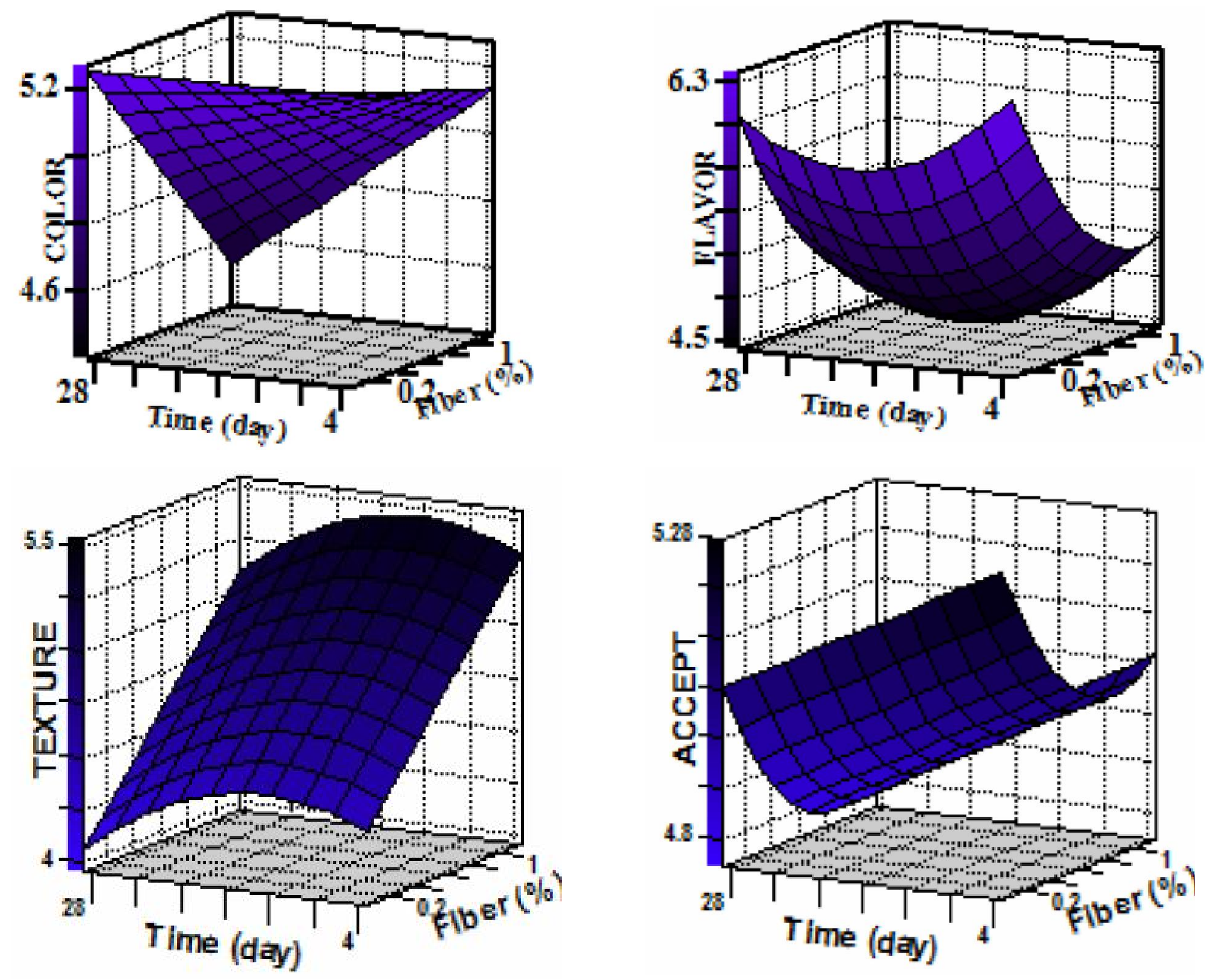

Figure 5 : Response surface plots for sensory scores as a function of fiber content and storage time

(TABLE 2).

This observation is consistent with the findings of Ferna´ndez-Garcý´a & McGregor[14] who evidenced that fiber addition led to a significant decrease of the overall flavor score for yogurts. Also texture score increased with increasing fiber content. These findings are in agreement with the results of viscosity test showing that increasing fiber amount, increased apparent viscosity. Furthermore, flavor score decreased until 16th day and then remained constant, but texture and color score and overall acceptability during storage significantly decreased. This observation is according to the findings of Tarakçi[34] who reported that when pH decreased in the yogurt samples during storage, flavoring characteristics decreased because of increased acidic taste.

Optimization

Analysis of variance revealed that the quadratic models are well adjusted to predict the experimental data. For optimization, the contour plots are put on each other and the overlapping area is considered as optimized. The goal was to maximize total solids, viscosity and acceptability and minimizing syneresis. The selected viscosity, syneresis, total solids and overall acceptability were respectively as follows: 2861 cP, 28.49%, 24.99%, 4.95 counts of 7.

From the analysis of the contour plots, the optimal conditions for production of orange yogurt containing orange fiber to achieve the abovementioned criterion were obtained using 1.2% fiber after storage for 16 days.

CONCLUSION

RSM was useful to study the individual and interactive effects of fiber amount and storage time on syneresis, total solids, overall acceptability and rheological properties of yogurt. Yogurt fortified with 1.2% orange fiber did show lower syneresis and higher viscosity. The results of sensory evaluation showed that overall acceptability was decreased by increasing the amount of fiber. Despite of decreasing sensory properties of samples with increasing the amounts of fiber, hedonic tests showed that gained scores for color, flavor and overall acceptance were acceptable (score of 4 or higher).

Therefore, the orange fiber can be used successfully in the production of fruit yoghurt at 1.2% concentration.

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