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Effects of Fermentation Temperature and Time on Chickpea-Initiated Sourdough Production

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HIGHLIGHTS

- Fermentation temperature and time affect the production of chickpea-initiated sourdough production
- Chickpea particle size and water-chickpea ratio had rather limited effects on the FCL and CY properties

Abstract

Chickpea is a nutritious staple pulse with numerous consumption patterns. It is also used in bakery products, especially in bread, in the forms of chickpea flour, fermented-chickpea liquor (FCL), and chickpea yeast (FCL-fermented sourdough) in various countries. However, a large variation exists in the traditional fermentation of FCL and chickpea yeast (CY). In this study, fermentation parameters (cracked-chickpea size, water-chickpea ratio, fermentation temperature, and fermentation time) for the production of FCL and CY were sequentially optimized through separate RSM designs. Therefore, this study aimed at optimization of the fermentation conditions for the preparations of both FCL and CY. First, FCL production parameters of fermentation temperature, time, chickpea particle size, and water-chickpea ratio were optimized and determined to be 40°C, 26 h, 2-6 mm, and 4:1 ratio, respectively. Secondly, the CY process parameters (fermentation temperature and time) were optimized using the optimized FCL conditions and determined to be 38°C and 9 h. The validation studies proved that there is no statistical difference ($p>0.05$) between the RSM-model predicted and experimental responses. At the optimized fermentation conditions, the FCL and CY had pH values of 4.44 and 4.31, and LAB counts of 9.87 and 9.08 log cfu g-1. The optimum fermentation conditions determined in this study are somewhat comparable to those commonly employed in the traditional preparations of both FCL and CY. Such optimization could lead to the better utilization of FCL and CY in the food industry and improved consumer health outcomes.

Keywords: Sourdough; fermented-chickpea liquor; chickpea yeast; fermentation conditions; lactic acid bacteria

1. Introduction

Chickpea (*Cicer arietinum*) is a leguminous grain rich in proteins, dietary fiber, minerals, and health-promoting phytochemicals (Foschia et al. 2017). It is processed into various foods through decortications/dehulling, soaking, sprouting, fermenting, boiling, mashing, steaming, frying, and roasting

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(Deshpande and Damodaran 1990). Furthermore, chickpea is used in bakery products, especially in bread, in the forms of chickpea flour, fermented-chickpea liquor (FCL), and FCL-initiated sourdough (chickpea yeast - CY) in certain localities of Türkiye, Greece, Bulgaria, and Macedonia (Hatzikamari et al. 2007a; Sayaslan and Şahin 2018). The CY, also known as sweet dough to differentiate it from the conventional sourdough, is a traditional leavening practice to improve the flavor, texture, and nutritional properties of bakery products (Sayaslan and Şahin 2018; Durmaz et al. 2023).

Extensive investigations on the sourdough process (Crowley et al. 2002; Dal Bello et al. 2007; Moroni et al. 2009; Durmaz et al. 2023) revealed that sourdough usage improved dough processability, retarded staling and molding, enhanced taste and aroma, and improved nutritional quality of bakery foods. Considered a variant of the conventional sourdough practice, the FCL and CY were also shown to provide similar benefits (Hancıoğlu-Sıkılı 2003; Baykara 2006; Kefalas et al. 2009; Narlıoğlu 2013; Çebi 2014; Kasım 2014; Saad et al. 2015;
Hendek-Ertop and Coşkun 2018; Hendek-Ertop and Şeker 2018). Microbiological, enzymatic and hydrolytic reactions occur in the fermentation stages of the FCL and CY preparations (Hatzikamari et al. 2007), all of which positively contribute to the dough rheology and sensory properties of baked goods. During chickpea fermentation, mostly lactic acid bacteria (LAB) and, to a lesser extent, certain nonpathogenic species of *Bacillus* and *Clostridium* were found to increase in the fermentation medium (Katsaboxakis and Mallidis 1996; Hatzikamari et al. 2007a, b; Çebi 2009; 2014). Additionally, the activities of hydrolytic enzymes, including amylases, cellulase, α-galactosidase, invertase, and proteases; and the amounts of such hydrolysis products as free fatty acids, reducing sugars, and free amino acids were also elevated (Hatzikamari et al. 2007a). Furthermore, a vast number of taste and aroma compounds were generated during the chickpea fermentation (Hancıoğlu-Sıkılı 2003).

In general, the FCL is traditionally prepared using coarsely ground or cracked chickpea. The cracked chickpea is then spontaneously fermented in several folds of water at 30-40°C for 15-20 h. The development of a foamy structure atop the fermentation medium is regarded as a sign of successful fermentation (Hatzikamari et al. 2007a; Sayaslan and Şahin 2018). Upon completion of the fermentation, the foamy FCL is sieved and used in the bakery formulations either directly (Kefalas et al. 2009; Kasım, 2014; Sayaslan and Şahin 2018, Şahin et al. 2018; Durmaz et al. 2023) or in the form of FCL-fermented sourdough, i.e., chickpea yeast (Hancıoğlu-Sıkılı 2003; Baykara 2006; Narlıoğlu 2013; Çebi 2009; 2014; Hendek-Ertop and Coşkun 2018; Hendek-Ertop and Şeker 2018). Although numerous preparation methods for the FCL and CY were reported in the literature, all extensively varied in the fermentation conditions. For instance, Kefalas et al. (2009) used coarsely-ground chickpea (>1.5 mm) for the FCL preparation, in which the crushed chickpea (100 g) was fermented in 300 ml of water at 35°C overnight. Kasım (2014) utilized 100 g of coarsely-ground chickpea (2-3 mm) and 1.5 g of salt to obtain the FCL through the fermentation in 550 ml of water at 42°C for 16 h. Sayaslan and Şahin (2018) and Şahin et al. (2018) also used coarsely-ground chickpea (>2.0 mm). The chickpea (100 g) together with 1.0 g of salt were fermented in 350 ml of boiled and cooled water at 40°C for 16 h. In those studies, the FCL was directly added to the bakery formulations in exchange of water. In the subsequent studies, however, CY was utilized in the bakery products instead of FCL. In this respect, Baykara (2006) and Narlıoğlu (2013) used 100 g of blender-ground chickpea (no mention of size distribution) and fermented it in 500 ml of salt-containing (1%) water at 35°C for 24 h. Çebi (2009) and Çebi (2014) also utilized coarsely-ground chickpea (100 g) together with 1.0 g of salt and fermented it in 350 ml of boiled and cooled water at 40°C for 16 h. Hendek Ertop and Coşkun (2018) and Hendek Ertop and Şeker (2018) followed somewhat a different FCL preparation approach. The process for preparing the chickpea mixture involved soaking 100g of whole chickpeas in 100ml of water for 20 hours. The mixture was blended until it reached a uniform consistency. Following this, 220ml of water, 2g of salt, 10g of sugar, and 50g of wheat flour were added, and the mixture was left to ferment for 12 hours at 26°C. The fermented slurry was then filtered through a fine screen (no mention of size) to obtain the FCL. The FCL was further fermented with wheat flour to produce CY. Finally, the CY was dried and incorporated into bread formulations.

The above-discussed literature on the preparations of FCL and CY indicates that rather a large variation exists in such fermentation parameters as cracked-chickpea size, water-chickpea ratio, fermentation

temperature, and fermentation time. Therefore, this study aimed at optimization of the fermentation conditions for the preparations of both FCL and CY.

2. Materials and Methods

The chickpea (variety Koçbaşı), straight-grade wheat flour, and non-iodized table salt were purchased from local suppliers in Karaman, Turkey. The de Man, Rogosa and Sharpe (MRS) agar and Anaerocult®A for anaerobic conditions were from Merck (Darmstadt, Germany). Other chemicals used in the analyses were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

Grinding and size classification of chickpea

The chickpea sample was coarsely ground using a pilot-scale custom-made hammer mill equipped with an 8.0-mm screen. The ground chickpea was sieved using a stack of sieves with successive apertures of 6.0, 4.0, 2.0, and 0.15 mm. The overs of sieves were utilized in the study as per the experimental design (Table 1).

Table 1. Independent and dependent variables of RSM design used in optimization of fermented-chickpea liquor (FCL) production

Run	Independent variable				Dependent variable (experimental response)			
	Temperature (°C)	Time (h)	Water-chickpea ratio	Chickpea particle size (mm)	Dry-matter content** (%)	Foam height (mm)*	pH**	LAB count (log cfu g^{-1})*
1	37.5	10	5	2-4	3.5	27	5.1	10.06
2	30.0	10	4	2-4	4.3	10	6.0	9.40
3	37.5	10	3	2-4	6.6	17	5.0	10.61
4	30.0	20	4	0.15-2	4.9	27	5.0	10.26
5	37.5	20	4	2-4	4.5	40	4.8	10.18
6	37.5	30	3	2-4	7.0	33	4.8	10.78
7	37.5	20	4	2-4	4.4	40	4.9	10.13
8	37.5	20	5	0.15-2	4.3	27	4.6	10.06
9	37.5	20	4	2-4	4.6	37	4.7	10.28
10	37.5	30	4	4-6	4.6	33	4.5	10.84
11	37.5	20	4	2-4	5.1	40	4.6	10.29
12	30.0	20	4	4-6	3.9	13	5.1	9.73
13	30.0	20	3	2-4	6.2	41	5.6	10.50
14	45.0	20	3	2-4	7.1	33	4.6	9.80
15	37.5	20	3	4-6	5.8	27	4.8	10.14
16	37.5	20	4	2-4	4.6	43	4.7	10.29
17	37.5	10	4	4-6	3.9	7	4.9	10.26
18	37.5	20	5	4-6	3.2	13	4.6	10.00
19	45.0	20	4	4-6	4.8	33	4.7	10.20
20	45.0	30	4	2-4	4.7	20	4.5	10.21
21	37.5	30	4	0.15-2	5.4	13	4.8	10.85
22	37.5	30	5	2-4	3.7	43	4.7	10.88
23	45.0	10	4	2-4	4.2	27	5.1	10.33
24	45.0	20	5	2-4	3.7	33	4.5	10.00
25	30.0	20	5	2-4	2.6	30	5.7	9.00
26	37.5	20	3	0.15-2	7.7	13	4.7	10.34
27	45.0	20	4	0.15-2	5.4	13	4.9	9.96
28	37.5	10	4	0.15-2	5.5	7	5.0	10.61
29	30.0	30	4	2-4	4.6	47	5.0	10.63

*Results were given as mean of dublicate measurements.** Results were given as mean of triplicate measurements

Preparation and optimization of fermented-chickpea liquor (FCL)

In the first stage of the study, important fermentation variables in the FCL production stage were optimized through the response surface methodology (RSM) with a four-factor and three-level Box-Behnken design (Table 1) (Montgomery 2017). The independent variables of the design were fermentation

temperature (30, 37.5, 45°C), fermentation time (10, 20, 30 h), chickpea particle size (0.15-2.0, 2.0-4.0, 4.0-6.0 mm), and water-chickpea ratio (3:1, 4:1, 5:1) (Table 1). The dependents variables (responses) of the RSM design were soluble plus suspended solids (dry-matter), foam height, pH, and LAB count in the FCL.

The fermentation process for the coarsely-ground chickpea was adapted from Sayaslan and Şahin (2018). For this purpose, the ground chickpea (100 g), salt (1.0 g) and boiled-cooled warm (about 50°C) distilled water (amount as per the experimental design) were placed in a 1-liter graduated glass bottle with an air-tight cap. The bottle content was subjected to spontaneous fermentation in an incubator (WiseCube, Daihan Scientific, Seoul, South Korea) without shaking (temperature and time as per the experimental design). Once the fermentation was over, the height of the foam atop the fermentation medium was recorded. The content of the bottle, including the foam, was then sieved through a 0.2-mm screen to obtain the FCL. The FCL was sampled and used for the determination of dry-matter, pH, and LAB count.

Preparation and optimization of chickpea yeast (FCL-fermented sourdough)

In the second stage of the study, the best fermentation conditions for the CY production were studied through the RSM Central Composite Design with two independent variables at three levels (fermentation temperatures of 30, 35, 40°C; fermentation times of 3, 6, 9 h). The CY was prepared using the FCL at the optimized conditions (40°C, 26 h, 2-6 mm of chickpea size, 4:1 water-chickpea ratio) that were determined in the first stage of the study. For this purpose, the FCL (100 g) was mixed with 100 g of wheat flour at 200 rpm for 2 min using a mixer (RW20, IKA GmbH, Staufen, Germany) to give a slack dough with a yield of 200% (Chavan and Chavan 2011). A certain amount of that dough (150 g) was then placed in a 1-liter glass bottle with an air-tight cap fitted with a monometer and subjected to fermentation as per the RSM experimental design (Table 2). At each h of the fermentation, the generated pressure (gassing power) was recorded, the system was zeroed, and restarted for the regeneration of the gas. When the fermentation was over, the CY was sampled for pH measurement and LAB count.

Table 2. Independent and dependent variables of RSM design used in optimization of chickpea yeast (CY) production

Run	Independent variable				Dependent variable (experimental response)		
	Coded value		Actual value				
	Factor-1	Factor-2	Temperature (°C)	Time (h)	pH**	Gassing power (mmHg)*	LAB count (cfu g-1)*
1	0	0	35	6	4.69	102	$3.1x10^8$
1	0	0	35	6	4.69	102	$3.1x10^8$
2	1	-1	40	3	4.83	78	$6.0×10^8$
3	0	1.41	35	10.2	4.26	130	$2.9×10^8$
4	0	0	35	6	4.70	89	$6.7×10^8$
5	-1.41	0	27.9	6	4.89	38	$1.4×10^9$
6	1.41	0	42.1	6	4.65	152	$1.8×10^9$
7	0	0	35	6	4.72	92	$1.1×10^8$
8	0	0	35	6	4.7	86	$6.6×10^8$
9	-1	-1	30	3	5.21	42	$3.5×10^8$
1	-1	1	30	9	4.65	75	$8.4×10^8$
11	0	-1.41	35	1.8	5.28	58	$3.3×10^7$
12	0	0	35	6	4.78	92	$8.3×10^8$
13	-1	1	40	9	4.32	125	$1.2×10^9$

*Results were given as mean of dublicate measurements.** Results were given as mean of triplicate measurements

Chemical and microbiological analysis

The moisture content of the wheat flour was determined using a moisture analyzer (ATS-120, Axis, Gdansk, Poland). The height of the foam (foam height) atop the FCL container was manually measured. The dry-matter (solubles plus suspended solids) content of the FCL was measured on a refractometer (RA-600, Kyoto Electronics, Tokyo, Japan). The pH of the FCL was read directly, while that of the CY was measured upon homogenization of the CY (10 g) in 90 ml of distilled water for 1 min (Çebi 2009). The gassing power (gas production capacity) of the CY samples was measured in a 1-liter glass bottle with an air-tight cap fitted

with a monometer during fermentation. For the LAB count, the FCL was sampled and used directly for inoculation. In the case of CY, however, the CY (25 g) was first homogenized in the physiological saline water (225 ml) for 1 min and used for inoculation at appropriate dilutions. The LAB count was performed using the De Man, Rogosa and Sharpe (MRS) agar as described by Çebi (2009).

Data analysis and model fitting

The experimental data collected through the RSM designs were analyzed using the Design-Expert 7.0 software (Stat-Ease Inc., Minneapolis, USA). Appropriate models were selected for each response using the degree of significance (p), regression coefficient (R^2), and lack of fit test.

3. Results

3.1. Optimization of fermented-chickpea liquor (FCL) production

The optimization process for the FCL aimed to maximize foam height and LAB count while minimizing pH. Table 1 presents the responses to the independent variable combinations used in the FCL optimization. The experimental design resulted in different dry-matter contents for the FCL treatments, ranging from 2.63% to 7.70%, and foam heights varying between 7 and 43 mm. The pH values and LAB counts of the FCL samples ranged from 4.5 to 6.0 and from 9.00 to 10.88 log cfu g-1, respectively. The quadratic models were well-suited for predicting all responses, including dry-matter content, foam height, pH, and LAB count, as indicated by the ANOVA results presented in Table 3. All models were significant ($p<0.05$), and their lack of fit tests was insignificant ($p>0.05$), indicating their soundness and validity for predicting the responses.

Table 3. Model type and significance (*p*) values of RSM optimization for fermented-chickpea liquor (FCL) production*

Models type and terms		Significance (*p*) value by response			
		Dry-matter content (%)	Foam height (mm)	pH	LAB count (log cfu g-1)
Model type	Quadratic	0.0075	<0.0001	0.0035	< 0.0001
Lack of fit		0.3691**	0.0507**	0.1246**	0.0691**
Model		< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*
Terms	A	0.0028*	0.5554	< 0.0001*	0.0733
	B	0.0535	< 0.0001*	0.0004	< 0.0001*
	C	< 0.0001*	0.5554	0.6903	0.0007*
	D	< 0.0001*	0.1028	0.5961	0.0943
	AB	0.7798	0.0001	0.3634	0.0004*
	AC	0.7798	0.2217	0.6458	< 0.0001
	AD	0.5134	0.0016*	0.4926	0.0183*
	BC	0.7424	1.0000	0.6458	0.0408
	BD	0.1858	0.0447*	0.6458	0.2582
	CD	0.2012	0.0070*	0.8177	0.6350
	A^2	0.1589	0.0521	0.0008*	< 0.0001*
	B^2	0.7806	0.0002*	0.1422	< 0.0001*
	C^2	0.0003*	0.1887	0.6191	0.2190
	D^2	0.0529	< 0.0001*	0.2748	0.2908

A: Fermentation temperature, B: Fermentation time, C: Water-chickpea ratio, D: Chickpea particle size *p<0.05; significant,**Lack of fit should be non-significant at p<0.05

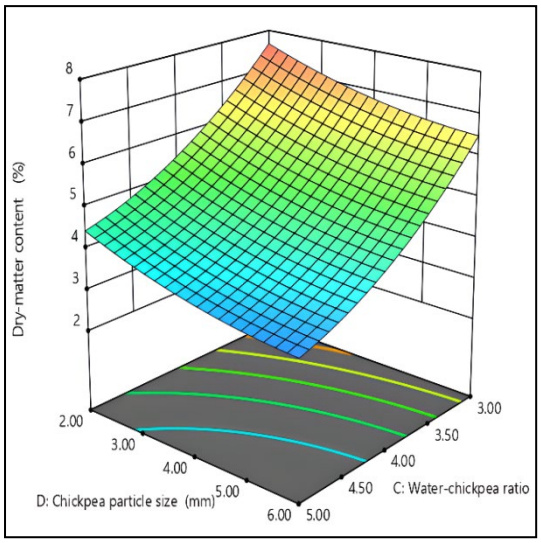
The dry-matter contents of the FCL samples were significantly impacted by the water-chickpea ratio and chickpea particle size, as evidenced by Table 3. A 3D-contour plot in Figure 1a illustrates the correlation between the water-chickpea ratio and chickpea particle size to the dry-matter contents of the FCL. Increasing water content and chickpea particle size reduced the amount of solid substances transferred to the water. However, as temperature increased, more chickpea components were dissolved, increasing the amount of water-soluble substances transferred to the water. A significant success criterion in the fermentation process is generating a considerable amount of foam atop the chickpea liquor's surface, as Hatzikamari *et al.* (2007a)

and Sayaslan and Şahin (2018) noted. The ANOVA results in Table 3 indicated that time and temperature-time interaction had a significant impact on foam height, as shown in Figure 1b. The chickpea is a source of saponins (Kerem et al. 2005), which are amphiphilic in nature, containing lipid-soluble glycone and water-soluble sugar chains. They act as surface-active compounds possessing wetting, emulsifying, and foaming properties (Güçlü-Üstündağ and Mazza 2007). According to Çabuk et al. (2018), foaming capacity is related to pH and fermentation time. Shi et al. (2015) soaked various pulses at room temperature for 12 to 18 hours to release saponins by simple diffusion. They discovered that saponin levels in the slurry increased with extended soaking time and soaking-cooking combinations. Our study revealed that chickpea particle size had a positive interaction with fermentation temperature and time but a negative interaction with the water-chickpea ratio, as shown in Table 4. The negative quadratic terms indicated an excessive increase in those variables reduced foam height. During fermentation, as the amount of chickpea remains constant, increasing the water-chickpea ratio leads to reduced concentrations of saponins in the water, restricting foam formation. Furthermore, the temperature-time interaction was negatively correlated with foam height because high-temperature short-time treatments promote the degradation of saponins. In contrast, low-temperature long-time treatments have a limited impact (Shi et al. 2004).

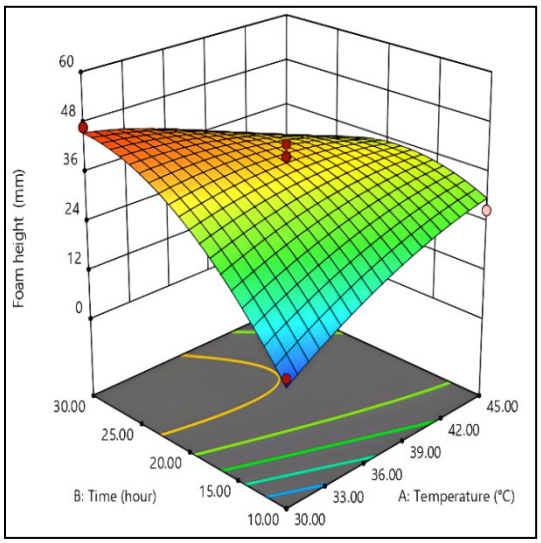
Table 4. Reduced best models for fermented-chickpea liquor (FCL) production and their coefficient of variation (R^2) values

Response	Reduced best model	R^2	Adjusted R^2	Predicted R^2	Adequate precision*
Dry-matter Content (%)	$4.68+0.2833\times A-1.61\times C-0.5858\times D+0.4383\times C^2$	0.94	0.93	0.90	32.36
Foam height (mm)	$36.77+7.83\times B-11\times A\times B+8.5\times A\times D+5\times B\times D-7\times C\times D-8.02\times B^2-15.77\times D^2$	0.89	0.85	0.76	19.08
pH	$4.78-0.3417\times A-0.2333\times B+0.2819\times A^2$	0.73	0.70	0.61	14.86
LAB count (log cfu g-1)	$10.23+0.0817\times A+0.2433\times B-0.1808\times C-0.0758\times D-0.3375\times A\times B+0.425\times A\times C+0.1925\times A\times D+0.1625\times B\times C-0.3399A^2+0.3401\times B^2$	0.92	0.87	0.72	18.41

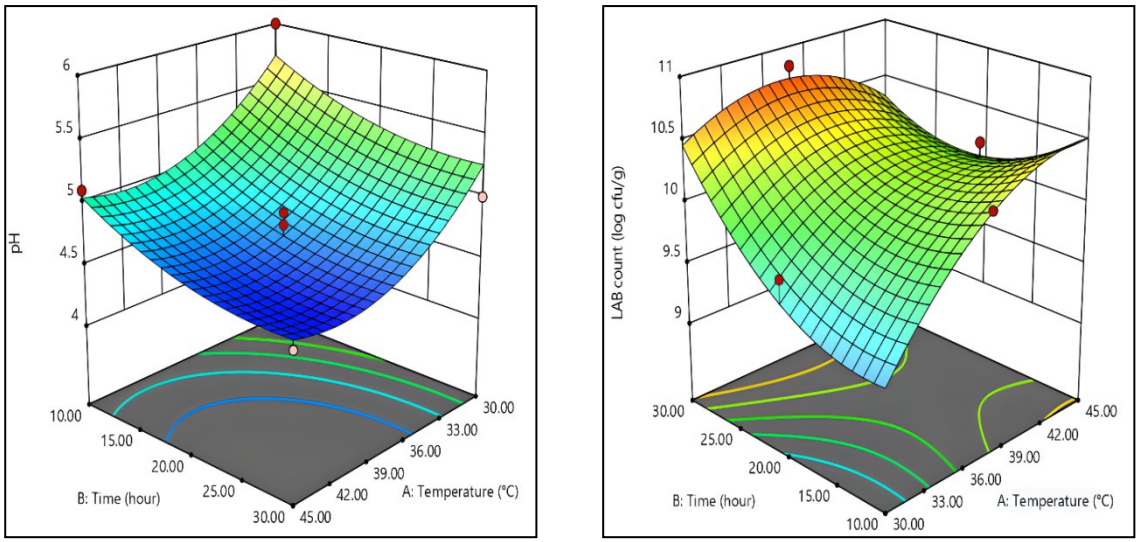
A: Fermentation temperature, B: Fermentation time, C: Water-chickpea ratio, D: Chickpea particle size *A ratio greater than 4 is desirable



(a)



(b)



(c) **(d)**

Figure 1. 3D-contour plots for optimization of FCL production - effects of chickpea particle size x water-chickpea ratio on dry matter content (a), chickpea particle size x fermentation time on foam height (b), fermentation time x fermentation temperature on pH (c), and fermentation time x fermentation temperature on LAB count (d)

The production of FCL is subject to pH changes, which have been found to be negatively correlated with temperature and time, as indicated in Table 3 and Figure 1c. Specifically, the first-order coefficients for temperature and time are negative, signifying that pH levels decrease as these variables increase. However, the positive quadratic terms suggest that extreme increases in these variables may actually enhance pH levels during FCL production. Based on our data analysis, the pH changes during FCL production are not significantly influenced by variations in the water-chickpea ratio or the particle size of chickpeas. During the fermentation of chickpea liquor, various bacteria and yeast develop over time, leading to the generation of a number of biochemical substances including ethanol, hydrogen peroxide, lactic acid, acetic acid, and CO_2. These substances are produced as a result of hydrolytic enzymes such as cellulase, galactosidase, invertase, amylase, and proteinase. In turn, the presence of these enzymes is likely to reduce pH levels in the FCL. It is worth noting that multiple instances of invertase and amylase were observed throughout fermentation, which could further contribute to decreased pH levels (Galal et al. 1978; Hancıoğlu-Sıkılı 2006; Hatzikamari et al. 2007b).

The LAB count in FCL samples was found to be significantly affected by the fermentation temperature and time, as indicated by the linear and quadratic effects observed in Table 3, Table 4, and Figure 1d. Additionally, the water-chickpea ratio and chickpea particle size were found to have negative linear effects on the LAB count, with increasing values of these variables leading to decreased LAB counts. These findings suggest that using medium-sized chickpea particles (2-6 mm) and a water-to-chickpea ratio of approximately four-fold could maximize the LAB count. Moreover, it was observed that temperature and water-chickpea ratio, temperature and chickpea particle size, and time and water-chickpea ratio showed positive correlations, except for the temperature and time interaction. Previous studies (Hancıoğlu-Sıkılı 2006; Hatzikamari et al. 2007b; Çebi 2009) have reported the development of various bacteria and yeast during chickpea fermentation, including LAB such as *Lb. plantarum, Lb. pentosus, Lb. bifermantans, Str. thermophilus, Lc. ssp. lactis, Lb. brevis, Lb. plantarum, Lb. pentosus, Weisella confuse*, and the yeast *S. cerevisiae*. However, nonpathogenic *Bacillus spp.* (specifically *B. cereus, B. thuringiensis*, and *B. licheniformis*) and *Clostridium spp*. (especially *Cl. Perfringens* and *Cl. beijerinckii*) were also identified in FCL prepared in Greece (Hatzikamari et al. 2007b). These findings highlight the importance of carefully selecting fermentation conditions to achieve optimal LAB count in FCL samples. Selecting the appropriate fermentation

temperature, time, water-chickpea ratio, and chickpea particle size may increase the LAB count and produce high-quality FCL.

Table 4 displays the coded equations, or reduced models, derived from the RSM design. These models serve as a valuable tool for identifying the relative impacts of the factors under analysis by comparing the factor coefficients. After removing nonsignificant terms ($p>0.05$) from the equations, the reduced models (Table 4) accurately describe the effects of significant process variables on the responses. The values of R2, adjusted R2, predicted R2, and adequate precision for the responses range between 0.69-0.94, 0.66-0.93, 0.59-0.90, and 13.99-32.16, respectively. The difference between the predicted and adjusted R^2 values is suggested to be less than 0.2. Table 4 shows that all coefficients are reasonable; thus, the RSM models are deemed acceptable. Generally, a model can be considered significant if the lack of fit test is insignificant, there is a satisfactory agreement between the adjusted and predicted R2, the adequate precision is over four, and the residuals are suitably distributed. In these cases, the model is considered a good predictor of the responses, and the desirability value of the model is closest to 1.0 (Anonymous 2007).

This study aimed to determine the optimal conditions for producing FCL with maximum foam height, LAB count, and minimum pH. Experimentation showed that the highest desirability value of 0.92 was achieved at a fermentation temperature of 40 °C for 26 hours, with a four-fold water-chickpea ratio and 2-6mm chickpea particle size. Validation studies showed no statistical difference between the experimentally obtained values and those predicted by the RSM model ($p > 0.05$). At the optimized fermentation conditions, the FCL had a pH value of 4.44 and a LAB count of 9.87 log cfu g-1 (Table 5). These results demonstrate the successful optimization of FCL production under specific conditions and may have important implications for future research.

Table 5. Verification of RSM-optimized model for fermented-chickpea liquor (FCL) production

Response	Fermentation parameter				RSM-model predicted value	Experimental value	Error (%)
	Temperature (°C)	Time (h)	Water-chickpea ratio	Chickpea particle size (mm)			
Dry-matter content (%)	40	26	4:1	2-6	4.41	4.57	3.63
Foam height (mm)					29.81	33.00	10.70
pH					4.54	4.44	2.20
LAB count (log cfu g-1)					10.41	9.87	5.19
Desirability value of model 0.92							

3.2. Optimization of chickpea yeast (CY) production

The second stage of the study involved optimizing the process conditions that resulted in the highest LAB count and gas production capacity, with the lowest pH in the CY, based on the optimized FCL conditions. The RSM design consisted of two independent variables (fermentation temperature and time) with five levels each. The responses measured were pH, gassing power, and LAB count. Based on the experimental runs, the pH and gassing power of the CY samples varied from 4.32 to 5.28 and from 38 to 152 mmHg, respectively. The LAB counts of the samples ranged from 3.3x10^7 to 1.8x10^9 cfu g-1. Linear models were found to be the best fit ($p<0.01$) for predicting the pH and gassing power of the CY, while a quadratic model was appropriate ($p<0.01$) for the LAB count. All models developed for the responses of pH, gassing power, and LAB count passed the lack-of-fit tests ($p>0.05$) with acceptable R2 (0.86-0.95), adjusted R2 (0.79-0.94) and predicted R2 (0.69-0.90) values, as shown in Table 6.

The findings of the ANOVA test, as presented in Table 6, indicate that temperature and time exhibit significant negative linear effects on the pH of the CY. The observed negative coefficient suggests a negative relationship between the two variables. The pH reduction due to the increased LAB count is a critical quality parameter for the CY, as with the FCL. Previous research studies (Chavan and Chavan 2011; Şahin et al. 2018) have reported the pH values of fermented dough/sourdough and FCL/CY in the 4.0-4.5 and 4.5-5.0 range, respectively. Our present study confirms that fermentation temperature and time influence pH

reduction in FCL and CY, as illustrated in Table 6. The linear decline in pH from 5.2 to 4.2 of CY with an increase in fermentation temperature and time is evident from Figure 2a. The pH range of traditional sourdoughs is typically between 3.5 and 4.3, which satisfies the growth requirements of the dominant sourdough microorganisms (*30*). Among the *Lactobacillus spp., L. plantarum's* growth during fermentation produces weak acids that lower pH from 7.5 to 4.3 in 11 hours of fermentation (Chandra-Hioe et al. 2016; Çabuk et al. 2018).

Table 6. Model types, significance (*p*) values and reduced best models of the RSM optimization for chickpea yeast (CY) production

Response	Model type and terms	F value	p value	R^2	Adjusted R^2	Predicted R^2
pH	Linear	96.02	<0.0001	0.96	0.95	0.90
	A	28.53	0.0003			
	B	163.51	<0.0001			
	Lack of fit	5.42	0.062			
	Reduced best model	pH = 4.74 - 0.131×A - 0.315×B				
Gassing power (mmHg)	Linear	40.28	<0.0001	0.89	0.87	0.77
	A	52.50	<0.0001			
	B	28.06	0.0003			
	Lack of fit	5.70	0.0569			
	Reduced best model	Gassing power (mmHg) = 89.05+30.92×A+22.77×B				
LAB count (cfu g-1)	Quadratic	12.01	0.0018	0.86	0.79	0.69
	A	3.04	0.1194			
	B	4.84	0.059			
	A^2	30.94	0.0005			
	B^2	5.31	0.0475			
	Lack of fit	0.26	0.8755			
	Reduced best model	LAB count (cfu g-1) = $5.154 \times 10^8 + 1.444 \times 10^8 \times A + 1.835 \times 10^8 \times B + 4.916 \times 10^8 \times A^2 - 2.082 \times 10^8 \times B^2$				

*A: Fermentation temperature, B: Fermentation time -Lack of fit should be non-significant at $p<0.05$

An increase in fermentation temperature and time has been found to correspond with an increase in the gassing power of CY, as demonstrated by Table 6 and Figure 2b. During the processing of FCL and CY, the yeast *S. cerevisiae* produces CO_2 gas naturally within the fermentation medium, thereby supporting the leavening of dough. This is in accordance with previous studies by Chavan and Chavan (*29*), Hendek Ertop and Coşkun (2018), and Sayaslan and Şahin (2018). Additionally, *Lactobacillus brevis* and *Weisella confuse*, both gas-producing heterofermentative LAB, were identified in the FCL and CY and were determined to be responsible for dough leavening (Çebi 2009). It is widely accepted that yeast and heterofermentative LAB synergistically contribute to the leavening of dough in FCL and CY (Çebi 2009; Hendek-Ertop and Şeker 2018). The findings of this study indicate that fermentation temperature and time are important parameters in determining the LAB count of CY, as evidenced by a quadratic model (Table 6 and Figure 2c). Previous studies have also demonstrated that traditional wheat sourdough's breadmaking performance is closely related to sourdough incubation temperature and time, inoculum level, and proof time (Göçmen et al. 2007; Minervini et al. 2014). The LAB count in a typical sourdough and CY is expected to be around 10^7-10^8 cfu g-1 (De Vuyst and Neysens 2005; Sayaslan and Şahin 2018). In this study, the LAB count of the CY at the optimized conditions was found to be at an adequate level of 9.08 log cfu g-1.

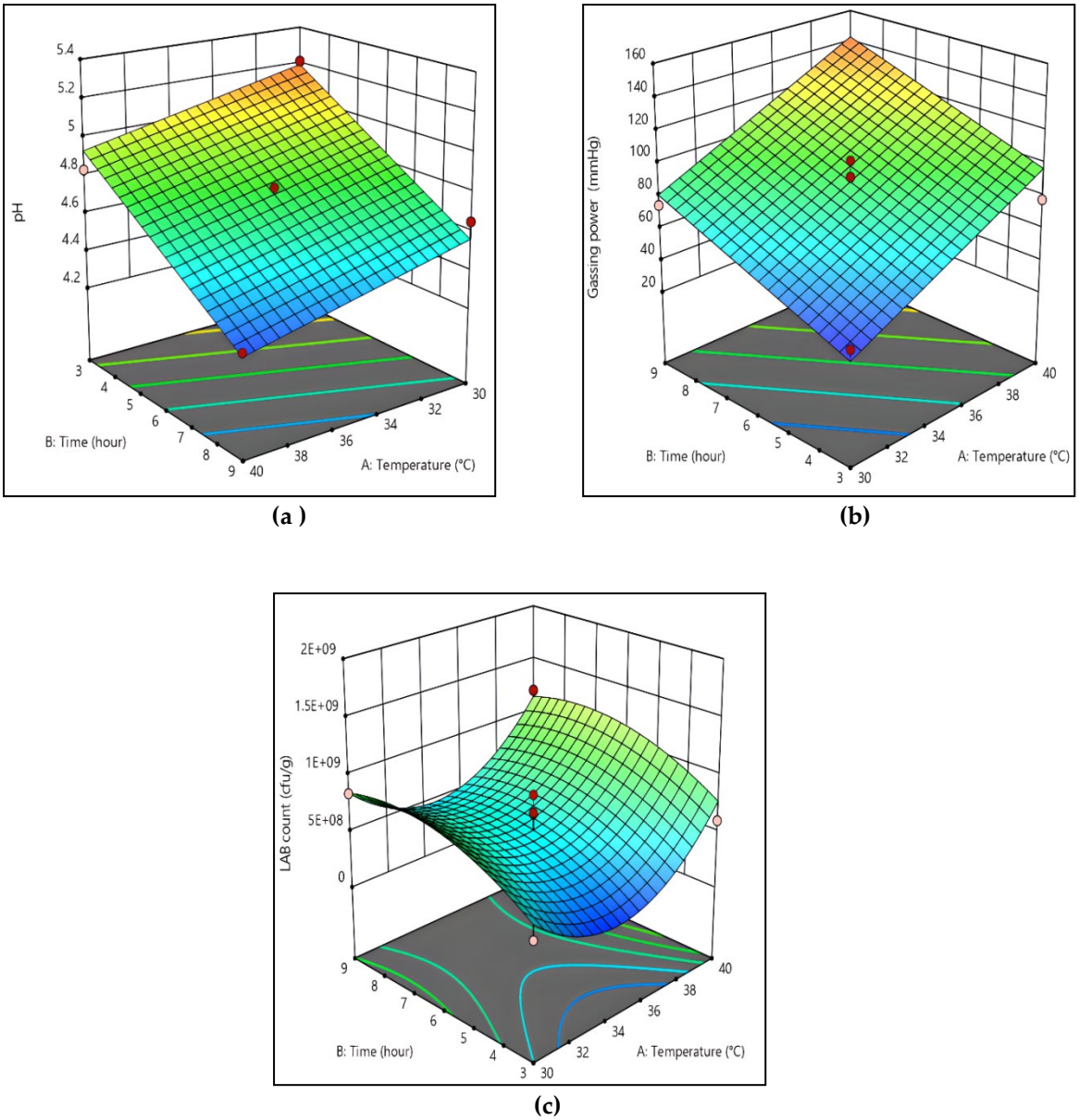


Figure 2. 3D-contour plots for optimization of chickpea yeast production - effects of fermentation temperature x fermentation time on pH (a), gassing power (b), and LAB count (c)

The results of the validation study conducted on the CY production under optimized conditions of 38 °C and 9 hours indicated that the RSM model's predicted values, including pH at 4.35, gassing power at 130.4 mmHg, and LAB count at 8.87 log cfu g-1, were not statistically different from the experimental data. The measured values, including pH at 4.31, gassing power at 136 mmHg, and LAB count at 9.08 log cfu g-1, were in line with the model's predictions. These findings suggest that the optimized conditions are effective for producing CY that the RSM model accurately predicts the values of key parameters.

4. Conclusions

The study's results have indicated that the production of FCL and CY is predominantly influenced by the fermentation temperature and duration, with the chickpea particle size and water-chickpea ratio having only minor contributions. The best temperature and duration for making FCL are 40°C and 26 hours, with chickpea particles that are 2-6 mm in size and a water-chickpea ratio of 4:1. For making CY, the best temperature is 38°C, and the best duration is 9 hours. When made under these conditions, FCL has a pH of

4.44 and LAB counts of 9.87 log cfu g-1, while CY has a pH of 4.31 and LAB counts of 9.08 log cfu g-1. These results are similar to traditional FCL and CY productions. This study underscores the potential to optimize fermentation conditions for producing FCL and CY with consistent quality and nutritional value. Such optimization could lead to the better utilization of CY in the food industry and improved consumer health outcomes.

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