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Optimizing Sourdough Process for the Production of Honey and Rose-Like Aromas in Breads

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Abstract

Background and Objective: Fermentation is one of the best methods for *in-situ* generation of aromas in breads. However, generating desirable flavors needs controlling several effective factors. In this study, optimal conditions for the production of honey and rose-like aromas were investigated by fitting a quadratic model using response surface method. To the best of the authors' knowledge, no study has used this method for the high-efficiency production of flavor compounds in sourdough.

Material and Methods: In the present study, headspace solid-phase microextraction-gas chromatography mass spectrometry was used to demonstrate ability of *Kluyveromyces marxianus* and *Leuconostoc mesenteroides* co-culture to produce 2-phenyl ethyl acetate and 2-phenyl ethyl alcohol in sourdough. Therefore, experiments were developed using response surface method and six parameters of dough yield, temperature, time, fructose, phenylalanine and bran proportions. Volatiles were collected from sourdough using headspace solid-phase microextraction method, followed by measuring the extracted volatile compounds using gas chromatograph connected to a mass selective detector.

Results and Conclusion: Results suggested that fermentation was optimum at 25 ℃ for 66.5 h with dough yield, 400; fructose, 6%w v^{-1}; phenylalanine, 0.3% w v^{-1} and bran, 20% w w^{-1} for the production of rose and honey-like aromas with high efficiency (2-phenyl ethyl alcohol 127.1 mg l^{-1} and 2-phenyl ethyl acetate 70.7 mg l^{-1}). Assessment of the baking and storage effects on the selected aroma compounds showed that although sharp decreases occurred in their concentrations due to the oven temperature, they were still detectable in the bread after 3 days of storage. Based on the optimized model, it can be concluded that increasing time and decreasing fermentation temperature led to the strengthening of aroma production. Furthermore, phenylalanine and fructose strongly affected development of the target aromas.

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1. Introduction

Fermentation is a well-known method for the creation of tastes and aromas without adding chemicals or expensive natural flavors. Sourdough is produced through the fermentation of cereal flours by yeasts and lactic acid bacteria (LAB), leading to increases in food nutritional content, textural property and shelf life [1-3]. Moreover, creating desirable flavors in breads is especially important [4]. During fermentation, yeasts can contribute to the

aromatic profile of the breads. *Kluyveromyces* species are within the most investigated strains for the production of aromatic compounds [5]. Various aroma compounds, including fruit esters, carboxylic acids, ketones, furanone, alcohols and aromatic hydrocarbons, have been reported in this microbial genus [6]. *Kluyveromyces marxianus* (*K.*) *marxianus* is an appropriate producer of 2-phenyl ethyl alcohol (PEA) and 2-phenyl ethyl acetate (PEAC) [7]. In addition, LAB include profound effects on the fermentation of sourdough and flavor quality of the final products. Production of aromatic compounds during fermentation is linked to their metabolic activities, not relying on acidification [8]. *Leuconostoc* spp., as heterofermentative LAB, can produce ethanol, CO_2, lactic acid and acetate during fermentation. It is noteworthy that these strains can be used as primary starters in cream and butter fermentations, or traditional cheeses due to their capacity for producing diacetyl,2,3-butanediol and acetoin [9]. Studies suggest that using mixed cultures of LAB and yeasts can result in several important benefits [10,11]. Use of mixed cultures result in better sensorial qualities, improved aromas and texture properties as well as longer shelf lives [12]. Physical, chemical and biological factors determine quantity and quality of the volatile compounds. These factors include pH, temperature, aeration, carbon and nitrogen sources and nutrient availability and concentration [13-15]. Optimization of the fermentation conditions is an essential step for high-efficiency production. The major aim of this study was production of sourdoughs with desirable aromas. Studies have been published on improvement of the texture and sensory characteristics of various sourdough breads [16,17]. Moreover, aroma-producing compounds in sourdoughs and breads have been investigated by various researchers [18-20]. However, to the best of the authors' knowledge, no particular study has been carried out on assessment of the optimization conditions for the production of aromas in sourdoughs. Therefore, the major aim of the current study was to optimize fermentation conditions and factors contributing to the production of aromas in sourdoughs as well as investigating effects of the baking process and storage time on the stability of aromas.

2. Materials and Methods

2.1 Materials and chemicals

Rye, oat and barley flour were purchased from a local market. These flour included protein contents of 9.8, 12 and 11.1 in 100 g, lipid contents of 3.6, 2.3 and 6.5 in 100 g and ash contents of 2.2, 3.6 and 3.7 in 100 g, respectively. Wheat bran was purchased from a local market. Further, fructose and phenylalanine (Phe) included analytical grades and were provided by Sigma-Aldrich, USA. In addition, PEA and PEAC (Sigma-Aldrich, USA) were used as standards.

2.2 Sourdough preparation

To prepare the sourdough, flour and other ingredients were mixed with water to achieve the desired dough yield (DY), characterized as DY = quantity of flour + quantity of water × 100 / quantity of flour. For control sourdough, 10 g of each flour were mixed with 60 ml of water to achieve 90 g of sourdough with a DY of 300 without addition of other ingredients. For the other samples, proportions of flour, water and other ingredients (e.g., wheat bran, fructose and phenylalanine) were adjusted based on the design by response surface method (RSM). Before adding water, the prepared mixtures were pasteurized for 20 min at 70 ℃ using water bath to decrease microbial load and risk of mold contamination. This process decreased mold spore counts from 5×10^3 to less than 10^2 cfu g^{-1}. Moreover, inoculation was carried out using *Leuconostoc (L.) mesenteroides* to achieve formulation of sourdough. The *L. mesenteroides* subsp. mesenteroides PTCC 1663 and *K. marxianus* PTCC5195 were supplied by the Iranian Biological Resource Center, Tehran, Iran. The *L. mesenteroides* was inoculated into de Man, Rogosa and Sharpe broth (Ibresco, Iran) and incubated at 37 and 25 ℃ for 24 h, followed by inoculating *K. marxianus* into yeast mold broth (Ibresco, Iran) and incubating at 25 ℃ for 48 h under aerobic conditions. Culture media were centrifuged at 1792× g for 10 min. Then, isolated biomasses were washed twice with sterile physiological serum and integrated with the corresponding combination. Sizes of inocula for *L. mesenteroides* and *K. marxianus* were 10^6 and 10^4 cfu ml^{-1} (100:1), respectively. Control sourdough was incubated at 30 ℃ for 24 h and other samples were fermented at specific times and temperatures based on the RSM design. It is noteworthy that water temperature is important for making sourdough since the fermentation process is disrupted due to the temperature shock if the temperature is extremely hot or cold. Therefore, water temperature is close to room temperature in all laboratory projects at industrial scales. Conversely, the fermentation process is directly affected by temperature control during fermentation. Relatively, fermentation temperature was controlled in this study as well as previous studies.

2.3 Baking procedure

To produce the bread, 40% (w w^{-1} on flour basis) of the optimal sourdough were added to the bread formulation (Table 1) and mixed using mixer (Escher M120; Escher Mixers Lago di Vico, Italy). Furthermore, dough was kneaded at 25 ℃ for 10 min and then split into pieces with a constant weight of 50 g. Then, pieces of the dough were transferred to baking trays in a proofer (Enkomak, Ankara, Turkey) and baked at 35 ℃ for 90 min with a relative humidity of 85%. Eventually, samples were baked at 230 ℃ for 18 min.

Table 1. Ingredients used in baking of the breads

Bread type	Ingredients(g)					
	Wheat flour	Water	Salt	Bread yeast	Vegetable oil	Optimized sourdough
Bread without sourdough	5000±100	2800±100	50±0	75±5	50±5	0
Sourdough bread	3000±100	1900±100	50±0	75±5	50±5	2000±100

2.4 Extraction of the volatile compositions using headspace solid-phase microextraction

The volatile mixtures were extracted using headspace solid-phase microextraction (HS- SPME) method.. An SPME fiber (50/30 um DVB/Carboxen/PDMS Stable Flex) from Supelco, Bellefonte, PA, USA, was used to carry out the procedure. Before the extraction, fiber was inserted into the gas chromatography (GC) injection port for thermal conditioning to eliminate contaminants according to the manufacturer's instructions. To carry out the analyses, 2 g ($2.00 \pm 0.01 \times 10^{-1}$ g) of the sample were weighed in a crimp-neck 20-ml headspace vial. Furthermore, NaCl solution (15% w v^{-1}) was added to the samples to improve volatility in sourdough samples. Vials were sealed using aluminum cap and polytetrafluoroethylene septum. Then, vial magnetically stirred was transferred into a water bath (50 ℃) and the fiber was introduced to its headspace through piercing the silicone septum. For equilibrating volatile compounds in the headspace, fiber was removed after 30 min and manually inserted into the injection port of a GC for thermal desorption of the compounds.

2.5 Gas chromatography-mass spectrometry analysis

Extracted volatile compounds by SPME fiber were assessed using Hewlett-Packard 6850 GC coupled to an Agilent Technologies 5973 Mass Selective Detector (Agilent Technologies, USA). Then, compounds were separated using StabilWAX-DA Capillary Column (length, 30 m; film thickness, 0.25 µm and internal diameter, 0.32 mm) (RESTEK, USA). Helium (initial temperature, 250 ℃; pressure, 47.8 kPa; purge time, 2 min and purge flow, 50.0 ml/min) was used as the carrier gas. As the oven temperature program, the primary temperature was 40 ℃ for 5 min, then increased to 260 ℃ at a rate of 10 ℃/min and held at the final temperature for 3 min. Temperature of the injector was set at 250 ˚C for the thermal desorption of volatile compositions from the SMPE fiber. The electron impact mass spectra were acquired from 40 to 400 amu at an ionization potential of 70 eV. Similarity of the query spectra with those in the reference library (NIST14) was used for the detection of the compounds. All samples were analyzed in triplicate through this procedure.

2.6 Experimental design

Extensive screening was carried out in an authors' previous study [21]. The best combinations of five types of flours (wheat, barley, oat, soy and rye) and various types of LAB and yeasts were selected using D-optimal algorithm based on sensory assessment results. These types of LAB and yeasts included *Saccharomyces cerevisiae, K. marxianus, L. mesenteroides, Lactobacillus rhamnosus, L. sakei, L. plantarum, L. sanfranciscensis, L. amylovorus and L. delbrueckii*. The assessments were carried out by 45 assessors (18 men and 27 women) using five-point hedonic scale. Table 2 summarizes results of these sensory screenings. Based on the results, a mixture of rye, oat and barley (1:1:1), which was fermented using a co-culture of *L. mesenteroides* and *K. marxianus*, included the greatest aroma score. Thus, this combination was chosen for the optimization purposes. Moreover, most of the panelists described aroma of the selected sourdough as a rose/honey-like aroma. The PEA and PEAC represented rose and honey-like aromas [22]. Since the chemical concentrations were significant in the selected sourdough, they were chosen as the target for the optimization. The recent study included assessment of the effects of fermentation circumstances (e.g., time, DY and temperature), amino acids (e.g., phenylalanine, asparagine and leucine), sugars (e.g., fructose, sucrose, glucose and sorbitol) and enrichments using other parts of the grains (e.g., starch, bran and embryo) using the novel method of electronic nose. The most effective parameters on aroma intensity were selected accordingly. For the optimization purposes, the current experiments were designed using RSM with six factors of time (24, 48 and 72 h), temperature (37, 31 and 25 ℃), DY (200, 300 and 400), fructose percentage (2, 4 and 6% w/flour), phenylalanine (0.1, 0.2 and 0.3%) and bran (10, 15 and 20%w w^{-1} flour).

2.7 Assessment of aroma stability during proofing, baking and storage

To investigate aroma stability, volatile compounds were extracted and analyzed using HS-SPME method immediately after proofing.

Table 2. Sensory screening results based on D-optimal design

Group□	Selected species	wheat	barley	oat	rye	soya	Sensory scale by model	Sensory scale
A	*L. sanfransiscensis*	0	50	0	50	0	4.46	4.5
B	*B. subtilis+ S. cerevisiae*	0	42.85	34.73	22.41	0	3.87	3.75
C	*L. delbruekii*	0	92.88	0	7.11	0	3.46	3.25
D	*L. plantarum*	8	63.01	28.98	0	0	3.35	3.25
E	*L. mesenteroides+K. marxianus*	0	33.33	33.33	33.33	0	4.68	4.6
F	*L. amylovorus+K. marxianus*	0	41.47	10.61	47.90	0	3.9	4
G	*L. sakei+K. marxianus*	0	20	0	80	0	4.11	4
H	*L. rhamnosus +S. cerevisiae*	0	0	70	30	0	3.61	3.5

□Each group contains 65 experiments which fermented by LAB, LAB+ *Kluyveromyces marxianus and lactic acid bacteria +saccharomyces. cerevisiae* **,**
Abbreviations: L: lactobacillus; B: bacillus; S: Saccharomyces; k: kluyveromyces, LAB: lactic acid bacteria

After the baking process, breads were cooled down to room temperature and 2 g of the crumb were isolated from the center of each bread for the aroma analysis. Then, effects of storage were studied on Days 1 and 3 of baking. All samples were packed in polyethylene bags and stored at 25 ℃ using incubator. All experiments were carried out in triplicate.

2.8 Statistical procedures

Regarding optimization, effects of independent factors on the responses (PEA and PEAC) were modeled using the second-order polynomial equation generated by a central composite design that included independent parameters. Then, interaction variables were chosen based on the lack of fit, model analysis and R^2 analyses. The experiments were designed using Design Expert Software v.11 (Stat-Ease, USA). The proposed quadratic mathematical model is shown in Eq. (1).

$$Y = \beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\Sigma\beta_{ij} x_i x_j \quad \text{(Eq.1)}$$

Where, Y was the response and xi and x_j were independent variables. Moreover, β_0 was the intercept and βi, βii and βij were the linear, square and interaction regression coefficients, respectively. One-way analysis of variance was used to assess the model significance ($p \leq 0.05$). Furthermore, effects of independent parameters on the responses were statistically computed using F-test. Then, response surface curves were drawn based on the experimental results to show effects of the interaction of these factors on the responses. Numerical optimization method was used to assess the best level of each factor to produce the maximum quantity of the selected compounds as responses. The generated model was validated by testing the proposed optimal points.

3. Results and Discussion

3.1 Identification of the volatile compounds using headspace solid-phase microextraction-gas chromatography mass spectrometry

A list of volatile compounds identified in this study is reported in Table 3. The most frequent categories were aldehydes, alcohols and acids. These volatile compounds are responsible for the production of favorable aromas such as 2,3-butanediol (butter), benzaldehyde (almond), benzene acetaldehyde (honey and sweet), PEA (honey and rose), nonanal (citrus), decanal (orange peel), PEAC (flower and honey), ɣ-nonalactone (peach and coconut), 2-undecenal (sweet), 2-octenal and 2-butyl (fruity and pineapple) [22]. The final aroma perception is affected by a complex mixture of volatile components" in the aroma profile. Therefore, effects of each volatile compound depend on its concentration and detection threshold. In general, PEAC includes important positive effects on the sensory perception because of its high odor activity value, which is defined as the ratio of the concentration of aroma compound and its odor threshold. Also, a very high concentration of PEA makes it an important volatile compound.

3.2 Model fitting and response surface plotting

As stated earlier, effects of the independent factors on PEA and PEAC were modeled using the following quadratic mathematical models generated by the central composite design:

Table 3. List of identified compounds using headspace solid-phase microextraction-gas chromatography mass spectrometry in sourdough

Sourdough					
Identified Compound	Rt	Identified Compound	Rt	Identified Compound	Rt
Carbon dioxide	1.4	2,4-Nonadienal	14.68	Naphthalene, 1,7-dimethyl-	17.67
Acetic acid	2.71	Benzaldehyde, 2,5-dimethyl-	14.73	Tetradecane, 2,6,10-trimethyl-	17.92
2,3-Butanediol	6.49	Furan, 3-phenyl-	14.83	Octadecane, 6-methyl-	18.52
Oxime-, methoxy-phenyl-_	8.99	Phenylethyl alcohol	15.27	Dodecanoic acid	19.27
Benzaldehyde	10.11	Benzene, pentamethyl-	15.65	Hexadecane	19.8
Benzene, 1,4-dichloro-	11.22	1H-Indene, 1-ethylidene-	15.97	Tetradecane, 2,6,10-trimethyl-	19.88
Benzeneacetaldehyde	11.79	2-Methoxy-4-vinylphenol	16.09	Tetradecanal	19.99
2-Octenal, (E)-	12.04	2-Phenylethyl pivalate	16.63	Tetradecanoic acid	21.6
Ethanone, 2,2-dihydroxy-1-phenyl-	12.43	ɣ-nonalactone	16.78	2-Pentadecanone, 6,10,14-trimethyl-	22.53
Nonanal	12.87	2-Undecenal	16.82	Butyl octyl phthalate	22.74
2-Phenylethyl acetate	13.04	2-Butyl-2,7-octadien-1-ol	16.9	2-Heptadecanone	23.15
Benzene, 1,2,3,4-tetramethyl-	13.66	Biphenyl Lemonene	17.13	Methyl palmitate	23.39
Octanoic Acid	13.87	Tetradecane	17.27	n-Hexadecanoic acid	23.78
Naphthalene	14.27	Dodecanal	17.42	Ethyl palmitate	24.07
Decanal	14.52	Naphthalene, 2,7-dimethyl-	17.49	Linoleic	25.44

Retention Time (minute)

$PEA = 1.155 \times 10^8 + 1.933 \times 10^7 A - 2.025 \times 10^7 B + 9.039 \times 10^6 D + 1.51 \times 10^7 E - 4.993 \times 10^6 AB + 6.495 \times 10^6 AD + 3.049 \times 10^6 AE - 8.629 \times 10^6 BE + 6.569 \times 10^6 DE + 9.577 \times 10^6 D^2 - 1.023 \times 10^8 E^2$

$PEAC = 1.562 \times 10^7 + 2.279 \times 10^7 A - 1.248 \times 10^7 B + 3.905 \times 10^6 D + 2.879 \times 10^7 E + 2.696 \times 10^6 AD + 4.134 \times 10^5 BD - 7.055 \times 10^6 BE + 5.443 \times 10^5 CF + 2.608 \times 10^6 DE + 7.565 \times 10^6 A^2 + 1.303 \times 10^6 B^2 + 1.713 \times 10^7 E^2 - 9.047 \times 10^6 F^2$

Where, A, B, C, D, E and F were time, temperature, DY, fructose, phenylalanine and bran (as individual effects), respectively. Moreover, A^2, B^2, C^2, D^2, E^2 and F^2 were the quadratic effects and AB, AC, AD, AE, AF, BC, BD, BF, BE, CD, CE, CF, DE, DF and EF were the interaction effects. Model terms were investigated using *p*-values (probability) with 95% confidence level. Figures 1 and 2 illustrate interaction effects of the factors on the production of target compounds using response surface curves.

3.3 Model validation and experimental verification

Numerical optimization method was used to assess validity of the model. This method offers solutions to achieve the maximum desirability. The lower and the upper limits for each factor were used within the range to produce the maximum quantities of the target compounds. The proposed conditions were used to compare the predicted results with the experimented results (Table 4). In general, experimental data were similar to predicted data by the model.

3.4 Effects of various parameters on the aroma production

Time includes important effects on the fermentation process. Based on the data, formation of PEA and PEAC was significantly affected by time ($p \leq 0.05$). Results of a study by Hazelwood et al. demonstrated that the Ehrlich pathway included a growth-associated activity [23]. It is noteworthy that the pathway was active under non-growing circumstances at the end of the process. The aggregation of PEAC was terminated by increasing production of PEA while the product formation was inhibited until 48 h. Based on a previous study, PEA and ethanol were reported as toxic to various yeast strains [24]. However, further increases in the PEA concentration were observed after 48 h, indicating that although bioconversion of L-phenylalanine to PEA was active, cells did not grow further (Fig. 3). Moreover, increases in the PEA concentration during this phase could be due to the cleavage of PEAC into PEA and acetate; from which, the latter was used by the cells.

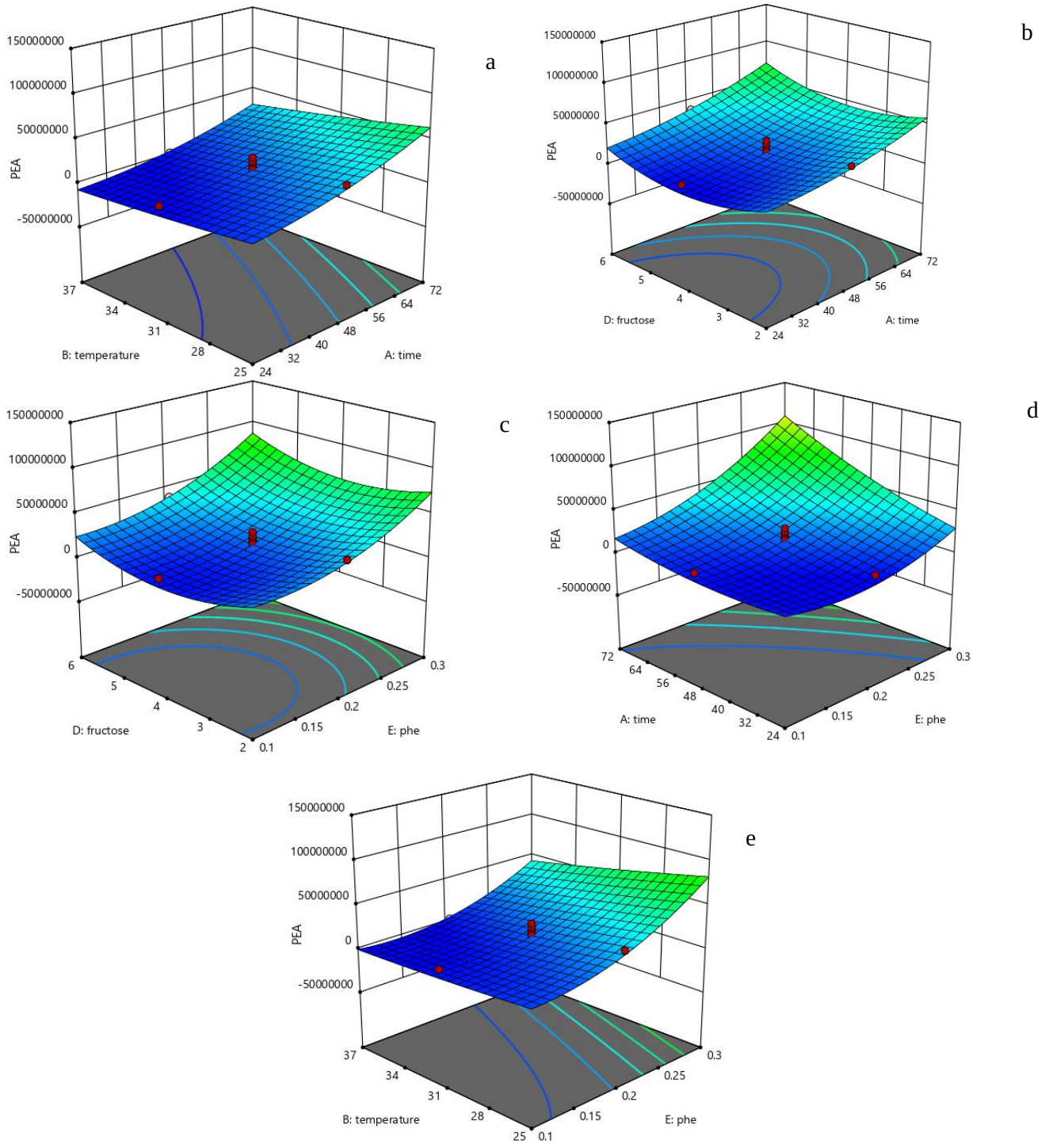

Figure 1. Response surface curves. Interactions between the factors affecting 2-phenyl ethyl alcohol (PEA) formation. a) Temperature × time, b) fructose × time, c) fructose × phe, d) time × phe and e) temperature × phe

This could explain decreases in the concentration of PEAC in prolonged fermentation; similar to that reported by Wittmann et al. [25]. Temperature is considered one of the most essential factors for alcoholic fermentation development since it can affect the fermentation rate and the product final quality as well as kinetics of the process with regards to duration [4].

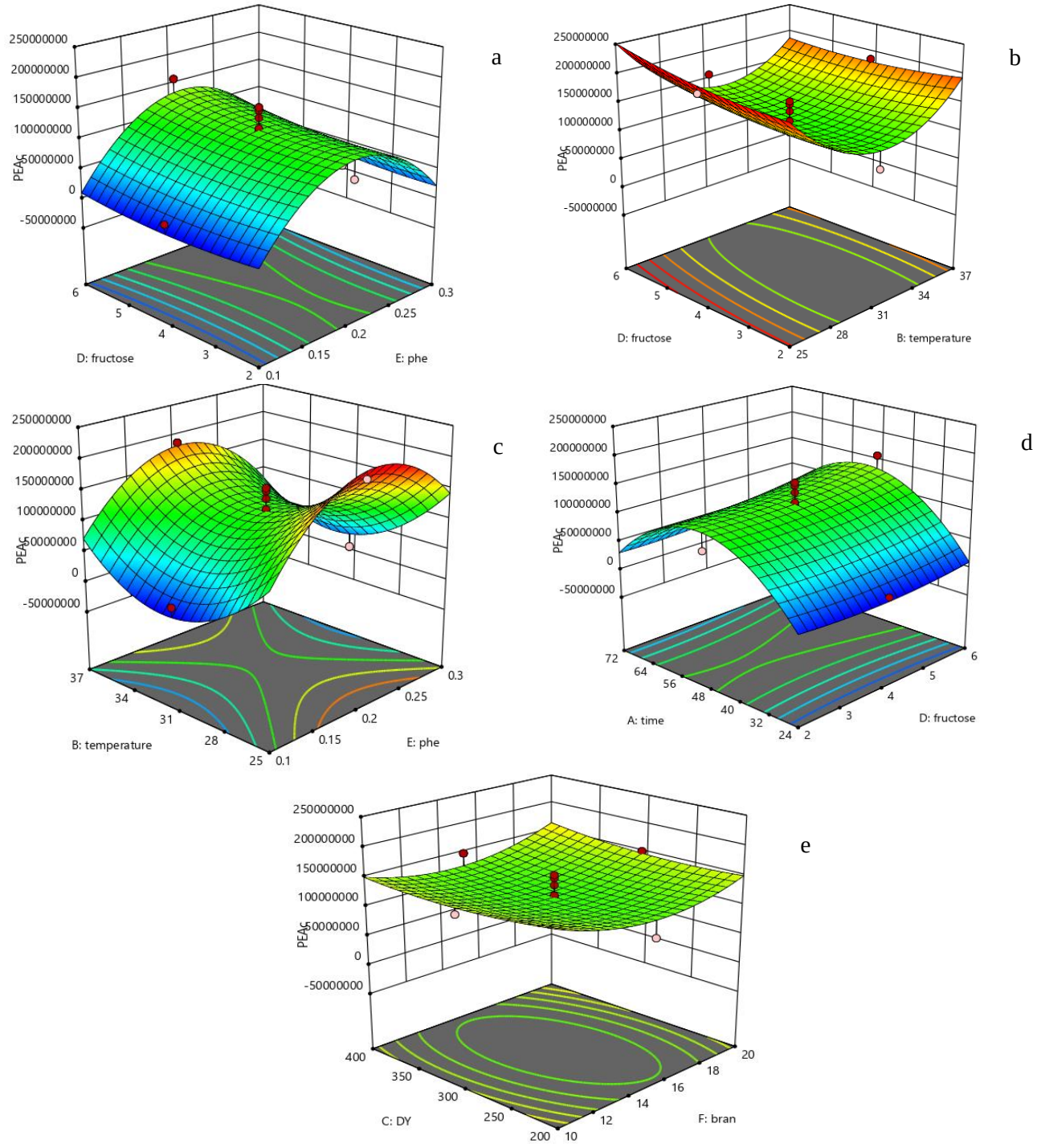

Figure 2. Response surface curves. Interactions between the factors affecting 2-phenyl ethyl acetate (PEAC) formation. a) fructose × phe, b) fructose × temperature, c) temperature × phe, d) time × fructose and e) DY × bran

Based on the present findings, temperature included a strong negative effects ($p \leq 0.05$) on the formation of PEA and PEAC. The negative effects of temperature on PEA and PEAC could be due to their decreased stability and thus increased evaporative losses at high temperatures. Additionally, yeasts include adaptable responses to low-temperature fermentations through modifying fatty acid biosynthesis in the membrane due to the higher concentration of medium-chain fatty acids with lower concentrations of oleic acid. Relatively, they can yield optimal fluidity of the cell membrane. Therefore, volatile compounds can easily be excreted through the plasma membrane [26]. It has already been reported that cell stress can motivate ester production [27]. Various types of flour include a wide range of water absorption capacity; thus, the value of DY reflects the quantity of water used in the formulation. Furthermore, higher quantities of DY can lead to further acidifications.

Table 4. Results predicted by the model and actual results achieved using headspace solid-phase microextraction-gas chromatography mass spectrometry under optimal conditions. Real concentrations were calculated using standards

Sourdough type	Target compound	Predicted result [a] (peak area)	Experimental result [a] (peak area)	Real Concentration [a] (ppm)
Optimized sourdough	PEAC	$1.30\times10^{8}\pm 8.37\times10^{6}$	$1.22\times10^{8}\pm8.6\times10^{6}$	70.74±1.01
	PEA	$2.09\times10^{8}\pm2.05\times10^{7}$	$1.86\times10^{8}\pm9.7\times10^{5}$	127.14±8.9
Control sourdough	PEAC	-	$0.11\times10^{8}\pm9.11\times10^{5}$	8.04±1.03
	PEA	-	$0.20\times10^{8}\pm6.89\times10^{6}$	13.69±1.82

[a] Value (Mean ± standard deviation)

Overall, higher DY supports, homogeneous distributions and better diffusions of nutrients are the major factors controlling the microbial concentration [28]. In sourdough with a higher DY, there is a less microbial competitive pressure since use of a smaller quantity of flour could decrease flour-derived bacterial loads. However, a low DY suggests that the sourdough includes a higher concentration of free amino acids. Therefore, proteases derived from flour, free amino acid metabolism by yeasts and secondary proteolysis by LAB can develop flavors in breads [28]. Based on the present results, the highlighted compounds were not affected by DY. Data revealed that fructose could include mild effects on producing rose aromas in sourdoughs ($p \leq 0.05$). However, other studies reported that PEA and PEAC were exclusively produced from the supplied L-phenylalanine and sugars were not converted to these compounds. This was due to the efficient feedback restriction of prephenate dehydratase in the L-phenylalanine biosynthetic pathway [24]. Positive effects could be attributed to the effects of fructose on exopolysaccharide production by *L. mesenteroides*. These compounds led to enhanced viscosity and thereby affected the flavor retention, release and perception. It is well-known that salting-out effects of the carbohydrates can increase volatility of the flavor compounds relative to water [29]. Formation of PEA and PEAC included a significant positive correlation ($p \leq 0.05$) with the quantity of the added phenylalanine to the sourdough. These findings are in contrast to those of Hernandez-Orte et al., reporting that the addition of amino acids decreases contents of PEA in wines [30]. Moreover, Garde-Cerdan and Ancin-Azpilicueta (2008) and Lee et al. (2011) concluded that the formation of PEA and its corresponding ester was positively linked to the quantity of amino acids [31,32]. This positive link could be explained by studying the Ehrlich pathway in *K. marxianus*. In this pathway, PEA is produced from phenylpyruvate via the catabolism of phenylalanine. Then, PEAC is formed through the reaction of alcohol acetyltransferases in presence of acetyl-CoA [33]. Availability of PEA and acetyl-CoA, expression and activity of the alcohol acetyltransferase(s) and reverse activity of the esterases determine the quantity of PEAC production (Fig. 3). Bran affects the total volatile compound since it is rich in ash content. Sourdoughs with increased ash contents are distinguished by a higher total concentration of the volatile compounds. In this study, addition of bran did not significantly affect rosy aromas in sourdoughs. After creating the optimum production conditions, PEA and PEAC increased respectively from 13.6921 and 8.04292 ppm in control sourdough to 127.1412 and 70.7429 ppm in optimized sourdough (Table 4).

3.5 Effects of baking and storage time on the aroma

Consumer acceptability of breads is highly affected by its flavor. Furthermore, sensory attribute must last during storage. The final flavor of breads depends on the ingredients, fermentation and baking conditions. Enzymatic activity and fermentation control the aroma production during mixing and proofing stages. Grosch and Schieberle have reported that prolonging dough fermentation increases concentration of PEA [34]. In the current study, concentration of the target compounds in doughs was less than that in sourdoughs. This difference was because the current study used 40% sourdough to prepare doughs. Regarding use of this dough proportion, it could be concluded that the quantity of the two compounds increased in the proofing stage (Fig. 4). When dough is transferred into the oven, complex physicochemical phenomena occur. Consequently, porous crumb and crispy crust are formed. The Maillard reaction and sugar caramelization are the major reactions that occur during this stage, resulting in the final bread aroma. During the early stages of baking, heat can accelerate reactions such as esterification; therefore, concentration of volatile compounds may increase within the first few minutes [35]. Generally, increased temperature of the oven inactivates enzymatic activity and fermentation. Moreover, evaporation of the volatile compounds sharply decreases the chemical concentrations (Fig. 4). As soon as the crust is formed, evaporation loss of the volatile compounds decreases because the crust can act as a barrier. Furthermore, the rate of crust formation is affected by the baking temperature and bread recipe. An interesting finding of this study included preserved quality of the breads through the distribution chain. Use of sourdough is a strategy to control the staling rate and hence further stability of flavor [36]. Studies have shown that contents of the aromatic compounds are responsible for decreased fresh-bread flavor during the storage [37-40]. For the highlighted

compounds of this study, the compound concentrations did not dramatically decrease after 3 days of storage. These findings are similar to those of Jensen et al. , who reported that the concentration of alcohol did not change significantly during storage [41] (Fig. 4). A comparison between the doughs/breads prepared without sourdough and those prepared with sourdough can demonstrate effects of using optimal sourdough on aroma improvement (Fig. 5).

4. Conclusion

In the present study, optimal conditions for producing rose/honey-like (PEA and PEAC) aroma in the selected formulation of fermented sourdough were determined using a co-culture of *L. mesenteroides* and *K. marxianus*. Individual, quadratic and interaction effects of variables were optimized based on a quadratic mathematical model generated using central composite design. Experimental results showed good agreements with the predicted results from the model. Correlation coefficients for R^2 and R^2-adj were 0.97 and 0.95, and 0.89 and 0.85 for PEA and PEAC, respectively. Non-significant lack of fit was good for the optimized models, supporting validation of the models. Therefore, provided models were sufficiently accurate. This study demonstrated that the selected microbial strains for the preparation of sourdoughs were capable of producing desirable aromas during fermentation. Furthermore, analysis of the aroma stability after baking and storage showed that although contents of the target compounds strongly decreased by the baking process, their quantities in fresh breads were still detectable and the decrease rates were low within three days of storage.

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6. Conflict of Interest

The authors report no conflicts of interest.

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بهینه سازی فرایند خمیر ترش برای تولید برای تولید رایحه مشابه عسل و رز در نان‌ها

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چکیده

سابقه و هدف: تخمیر یکی از بهترین روش‌ها برای تولید درجای رایحه در نان‌هاست. تولید عطر و طعم مطلوب نیازمند کنترل بسیاری از عوامل موثر است. در این مطالعه، شرایط بهینه برای تولید رایحه رز و عسل با استفاده از مدل درجه دوم و روش سطح پاسخ تعیین شدند. .بر اساس اطلاعات نویسندگان، هیچ مطالعه‌ای از این روش برای تولید ترکیبات عطر و طعم با راندمان بالا در خمیر ترش استفاده نکرده است.

مواد و روش ها: در مطالعه حاضر، برای نشان دادن توانایی کشت همزمان *کلویورومایسس مارکسیانوس* و *لوکونوستوک مزنتروئیدس* در تولید ۲- فنیل اتیل استات و ۲- فنیل اتیل الکل از روش ریز استخراج فاز جامد از فضای فوقانی کروماتوگرافی گازی- طیف سنج جرمی استفاده شد. بنابراین، آزمایشات با به‌کارگیری روش سطح پاسخ و شش عامل شامل راندمان خمیر ، درجه حرارت، زمان ، فنیل آلانین ، فروکتوز و سبوس گندم طراحی شدند. ترکیبات فرار از خمیر ترش با روش ریز استخراج فاز جامد از فضای فوقانی استخراج و توسط کروماتوگرافی گازی متصل به آشکارساز طیف سنج انتخابی جرمی اندازه‌گیری شدند.

یافته‌ها و نتیجه‌گیری: نتایج نشان دادند که تخمیر در ۲۵ °C برای۶۶/۵ ساعت با راندمان خمیر ۴۰۰ ، فروکتوز $\%wv^{-1}$ ۶ فنیل آلانین $\%\ w\ v^{-1}$ ۰/۳ و سبوس $\%\ w\ w^{-1}$ ۲۰ برای تولید رایحه رز و عسل با راندمان بالا مناسب است.(۲- فنیل اتیل الکل $mg\ l^{-1}$ ۱۲۱/۱و ۲- فنیل اتیل استات $mg\ l^{-1}$ ۷۰/۷). ارزیابی اثرات پخت و نگهداری بر روی ترکیبات منتخب نشان داد که هرچند به دلیل دمای فر کاهش شدیدی در غلظت آنها رخ می دهد، اما این ترکیبات هنوز پس از سه روز نگهداری قابل تشخیص هستند. بر اساس مدل بهینه شده می توان نتیجه گرفت که افزایش زمان و کاهش درجه حرارت تخمیر منجر به تقویت تولید رایحه می شوند. همچنین فنیل آلانین و فروکتوز اثر قوی بر توسعه ترکیبات رایحه هدف داشتند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- ۲- فنیل اتیل استات
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