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OPTIMIZATION OF ENSET FERMENTATION IN THE PRODUCTION OF KOCHO USING RESPONSE SURFACE METHODOLOGY

– Research paper –

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Abstract: Response surface methodology (RSM) was used for optimization of enset fermentation process. Two numerical (time and amount of starter culture) and one categorical variable (types of starter strain) was used for evaluation of sensory quality of kocho. The physicochemical properties, proximate composition and color of kocho product were also analyzed. It was found that the coefficient of determination (R^2) of the response variables were greater than 80% described that high percentage of the variability was defined by the model. These findings revealed that fermentation time, amount of starter culture and types of starter strain affected the sensory attributes of kocho. The preferred sensory quality of kocho was produced using 2% *L. plantarum* as starter strain at 6 days of fermentation time.

Keywords: D-optimal design, sensory analysis, physicochemical properties, color analysis

INTRODUCTION

Enset (*Enseteventricosum* (Welw.)Cheesman) is the perennial banana-like plant that belongs to order *Scitaminae* and family *Musaceae*. Geographically it distributed in most part of Asia and Sub-Saharan Africa. However, it is considered as one of the oldest cultivated plants and a unique food resources in Ethiopia (Olango et al., 2014). Over 70% of its part is composed of pseudostem and corm (Nurfeta et al., 2012), and is used for the preparation of the most important food products: Kocho, Bulla, and Amicho. Kocho is a starchy fermented food obtained from scraping pseudostem and grated corm of enset plant (Nurfeta et al., 2012); fiber is obtained as a by-product during scraping of the pseudostem and used for making bags, ropes, cleaning materials and mats (Tsegaye and Struik, 2000). Enset fermentation techniques and length differ among regions (Hunduma& Ashenafi, 2011). Pit fermentation, surface fermentation and surface fermentation followed by pit fermentation as a two-steps process are the common fermentation techniques used in different parts of a country.

Enset is a potential high yielding plant in Ethiopia. Currently, about 123 million enset plants are harvested all over the country with the yield of nearly 32 milion quintals of kocho (CSA, 2017). Tsegaye and Struik, (2001) reported that the yield of kocho is high as compared with others food crops in terms of edible dry matter and energy, its, nutritive value is also comparable to potato (Bosha et al., 2016). This indicates that enset cultivation can considerably improve food security at household and at the national level.

Thus, kocho is a very important source of food, energy and industrial raw material (Olango et al., 2014), in Ethiopia. Traditionally, it is ranked as the first important staple and co-staples food crop in the central, south and southwestern part of Ethiopia. The possible reasons to the limited cultivation of enset and consumption of kocho in others parts of the country could be related to strong odor for non-consumers and the long fermentation period. The odor of kocho is not acceptable by most of the inhabitants of nonenset cultivating areas.

The proximate composition of enset products differs among studies (Yirmaga 2013; Bosha et al., 2016). Enset products is rich in carbohydrates and some minerals (Ca and Zn) compared to other foodstuffs and contains

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comparable concentration of Cu, Fe, and Mn. It is however the protein and fat contents are very low as similar to potato and others starch rich food products (Bosha et al., 2016). Therefore, the food prepared from enset need to complement with protein and fat.

To the best of our knowledge, so far, no study has been carried out in the optimization of kocho fermentation. Response surface methodology (RSM) has been applied in the optimization of ingredient amount, processing condition and formulation of different foods such as, sausage, snacks food, cassava cake, chocolate flavored beverage, biscuit dough, and yogurt from coconut milk (Colmenero et al., 1995; Thakur & Saxena, 2000; Gallagher et al., 2003; Gan et al., 2007; Deshpande et al., 2008). Meanwhile, RSM has been carried out in the optimization of functional foods (Chen et al., 2016; Li et al., 2018). RSM combined both statistical and mathematical techniques. It is used for modeling and analysis of a response of interest which is influenced by several variables, and to optimize this response (Ei-Gendy et al., 2013).

Thus, the aim of this study was to optimize fermentation time, and concentration and types of starter culture of kocho with highest sensorial values. Meanwhile, the physicochemical properties of kocho produced were analysed.

MATERIALS AND METHODS

Enset pulps and selection of starter strain

Approximately 20 kg of the scraping pulps of enset was purchased from Wolkite area, Ethiopia. Wolkite was selected because kocho is a staple diet around Wolkite. Lactic acid bacteria were isolated from kocho at different fermentation stages and characterized for both phenotypic and genotypic properties. *Lactobacillus plantarum* (n=1) and *Lactobacillus brevis* (n=1) were selected as a possible starter strain due to their predominance, fast pH reduction, high microbial counts and biomass production. The pulps of enset and freeze-dried starter strains were stored at 4 ºC until used.

Starter culture preparation

The freeze-dried starter strains were rehydrated in sterile distilled water (27 °C) for 10 minutes. 500g of enset pulp roughly sterilized on water bath was inoculated with starter strains in amount of 2% (ca. 7 log CFU/g) after rehydration. After incubation for 48h at room temperature (20-25°C), it was used as a starter culture. Tereafter, 500g of kocho pulp was prepared according to a certain suggested level of RSM (Table 1) and fermented at room temperature using traditional surface fermentation method (Zerihun and Brihanu, 2015).

Experimental design and statistical analysis

The statistical analysis for generated test formulation was done by using design expert (version 7.0, Stat-Ease Inc., Minneapolis, MN, USA) software. D-optimal was used to study the interaction of variables by applying RSM. There are two numerical variables; fermentation time (days) and amount of starter culture (%) and one categorical variable; types of starter culture. The three coded (uncoded) levels of fermentation days: -1 (2), 0 (4), +1(6), amount of starter culture (%v/v): -1 (1), 0 (2), +1 (3) and a single and mixed types of strain were incorporated and given a total of 22 experimental runs (Table 1). Sensory descriptive characteristics of kocho were taken as the responses of the design experiment. Experimental data were analysed by using JMP 13 software (SAS) by means of one-way analysis of variance (ANOVA). Tukey's test at the significance $P \leq 0.05$ was used to show the significance of differences. RSM was used to derive equations to estimate the response variables as follows:

$$Y= \beta_o+ \beta_1X_1+ \beta_2X_2+ \beta_3X_3+ \beta_{11}X_1^2+ \beta_{22}X_2^2+ \beta_{12}X_1 X_2+ \beta_{13}X_1 X_3+ \beta_{23}X_2 X_3 \quad (1)$$

where, β_o is constant ; β_1, β_2, β_3 are the linear regression, β_{11} and β_{22} are the quadratic regression, $\beta_{12,}$ $\beta_{13,}$ β_{23} are interaction regression, and $X_{1,}$ $X_{2,}$andX_3 are variables.

Physicochemical analysis

Samples of 10 g of kocho were homogenized with 90 ml of distilled water and their pH was measured using a digital pH meter (Hanna Instrument, HI2020, Romania). The pH meter was calibrated prior to each reading with standard buffers according to manufacturer's instruction. A titratable acidity of the sample was determined as described by Oyedeji et al (2013).

The proximate composition (moisture, ash, fat and protein content) of kocho samples was determined according to the standard methods of the Association of Official Analytical Chemists (AOAC, 2000). Total carbohydrate was calculated by difference.

Descriptive sensory analysis
The sensory evaluation of fermented kocho samples was tested by trained Addis Ababa University students according to Einstein (1991). The selected panels contained 10 individuals peoples as recommended by Stone and Sidel (1985). Each panellist evaluates all samples using 5 point hedonic scales (1= very poor; 5= excellent) for the selected descriptive characteristics such as odor, color, texture, appearance, and overall acceptability (Meilgaard et al., 1991), using score sheets.

Color
The colors of the fermented kocho samples were determined using a benchtop spectrophotometer (CM- 600d, Konica Minolta, Japan). Three measurements were made on each sample.

RESULTS AND DISCUSSION

Model fitting for RSM
Two numerical variables, fermentation period (X_1) and starter culture amount (X_2), and one categorical variable, the types of starter culture (X_3), were used in the evaluation of Enset fermentation. The experimental results on the effects of those variables in sensory descriptive of kocho are shown in Table 1.
The experimental data obtained from variables (independent and dependent) were fitted to the quadratic model and examine for the goodness of fit. The analysis of variance (ANOVA) was also done to define the lack of fit and the significance of the independent variables on the dependent variables (Table 2). The lack of fit test is used to measure the failure of a model to signify experimental data at the point of excluding in the regression (Gan et al.,2007). The goodness of model fit was recognized by the coefficient of determination (R^2) and should be ≥ 80% (Yaakob, et al., 2012). The R^2 values for all response variables showed more than 80% and also the lack of fit is insignificant. Therefore, high quantity of variables is well described by the model.

Table 1. Arrangement of D-optimal and results of response variables of kocho

Run	Variables[1]			Responses [2]			
	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	1	-1	B[3]	3.2	3.2	3.1	3.0
2	-1	1	P	2.8	3.0	2.3	2.5
3	-1	-1	PB	2.3	2.3	2.2	2.0
4	1	1	B	3.0	3.2	3.4	3.4
5	1	-1	P	3.3	3.9	3.7	4.0
6	-1	1	B	3.0	2.8	2.2	2.2
7	-1	1	PB	2.5	2.1	1.7	2.2
8	1	-1	PB	3.2	3.8	3.8	3.8
9	-1	1	PB	2.7	2.4	2.0	2.4
10	-1	-1	P	2.6	2.8	1.9	2.3
11	0	-1	PB	3.0	3.2	2.9	3.0
12	1	1	P	3.3	3.7	3.8	4.0
13	1	1	P	3.5	3.6	4.0	4.2
14	1	1	PB	3.4	3.7	3.7	3.6
15	0	1	B	2.6	2.7	3.0	3.0
16	1	-1	B	3.2	3.2	3.2	3.0
17	0.5	0	B	3.0	3.1	3.0	3.0
18	0	0	PB	3.1	3.0	3.4	3.2
19	0	-0.5	P	2.9	3.1	3.0	3.0
20	-0.5	-0.5	B	2.4	2.8	2.8	2.8
21	-1	1	P	2.8	2.8	2.4	2.5
22	1	1	PB	3.7	4.0	4.0	4.0

[1] X_1 (time), X_2 (amount of starter), and X_3 types of starter; [2] Y_1(odor), Y_2 (color), Y_3 (appearance), Y_4 (overall acceptability) ; [3]B (*L.brevis*), P (*L.plantarum*), PB (*L.plantarum* + *L.brevis*)

Table 2. Analysis of variance of the response variances

Source	Df	Response variables							
		Odor		Color		Appearance		Overall Acceptability	
		SS	F	SS	F	SS	F	SS	F
Model	11	2.36	4.87**	5.38	20.19***	10.3	25.5***	8.89	22.2***
Linear	4	2.14	46.4***	4.75	185.4***	10.0	268.3***	9.19	240.1***
Interaction	5	0.19	2.87^{ns}	0.76	16.0^{ns}	0.55	7.74^{ns}	0.32	4.45^{ns}
Quadratic	2	0.0015	0.39^{ns}	0.021	0.93^{ns}	0.039	1.33^{ns}	0.016	0.47^{ns}
Lack of fit	5	0.35	4.18^{ns}	0.13	1.10^{ns}	0.25	2.06^{ns}	0.24	2.03^{ns}
Pure error	5	0.085		0.11		0.12		0.12	
R^2		84.3%		95.6%		96.5%		96.1%	
Adj- R^2		66.9%		90.9%		92.8%		91.8%	

Df= degree of freedom;***significant at P ≤ 0.001; **significant at P ≤ 0.01; *Significant at P ≤ 0.05; ^{ns} not significant; F = ratio of variance estimates; SS = Sum of squares

Physicochemical properties

The two largest families of lactic acid bacteria; *L. brevis* and *L. plantarum* were selected as a starter culture in the fermentation of kocho. The starter strains were selected based on their predominance in traditional kocho fermentation as well as a desired physicochemical characteristic (result not show). Similarly, different study reported on the use of *L. brevis* and *L. plantarum* as starter culture in fermentation food such as cheese, sauerkraut, sourdough, borde, cassava, and sorghum flour fermentation (Abegaz, 2007; Ali & Mustafa 2009; Edward et al., 2010; Lim et al., 2018), so considered are a "generally regarded as safe" organism. The pH values of kocho samples ranged from 3.31 to 5.75, and also the production of acidity ranges from 0.22 to 0.45% (Table 3).

Table 3. Physicochemical and color properties of kocho products

Run	Variables			Physicochemical analysis							Color [1]		
	X_1	X_2	X_3	pH	TA	Moisture (wb)[2]	Ash	Protein	Fat	T. Ch. (db)[3]	L	a*	b*
1	6	1	B	4.25^{l*}	0.32^{d}	79.1^{h}	0.98^{e}	4.7^{b}	0.5^{e}	85.3^{h}	69.2^{i}	3.6^{j}	19.9^{e}
2	2	3	P	5.14^{f}	0.29^{e}	84.4^{a}	1.04^{b}	4.3^{c-f}	0.35^{f}	90.1^{a}	67.0^{m}	5.6^{e}	18.0^{k}
3	2	1	PB	5.75^{a}	0.23^{hi}	81.3^{de}	1.03^{bc}	5.2^{a}	0.5^{e}	88.0^{c}	66.6^{n}	5.3^{f}	15.9^{l}
4	6	3	B	4.02^{o}	0.32^{d}	78.8^{l}	0.99^{de}	4.0^{e-g}	0.5^{e}	84.3^{kl}	75.0^{d}	3.1^{k}	22.5^{a}
5	6	1	P	4.15^{m}	0.32^{d}	77.0^{o}	0.99^{de}	4.4^{b-e}	0.5^{e}	82.9^{n}	71.9^{f}	2.5^{mn}	15.6^{m}
6	2	3	B	5.15^{f}	0.26^{f}	80.1^{f}	0.99^{de}	3.9^{fg}	0.5^{e}	85.5^{h}	66.1^{o}	3.7^{j}	13.3^{p}
7	2	3	PB	5.18^{e}	0.26^{f}	81.4^{d}	0.99^{de}	4.5^{b-d}	0.75^{d}	87.6^{cd}	67.3^{l}	5.1^{g}	18.5^{i}
8	6	1	PB	3.97^{p}	0.35^{c}	75.3^{p}	0.99^{de}	4.7^{b}	1.0^{c}	82.0^{o}	78.8^{b}	2.6^{lm}	11.4^{s}
9	2	3	PB	5.10^{g}	0.26^{f}	81.2^{e}	1.06^{a}	4.5^{b-d}	0.75^{c}	87.5^{d}	64.4^{r}	7.0^{b}	18.5^{i}
10	2	1	P	5.51^{b}	0.25^{fg}	83.8^{b}	1.04^{b}	4.3^{c-f}	0.25^{fg}	89.4^{b}	65.7^{p}	5.1^{g}	19.2^{h}
11	4	1	PB	5.03^{h}	0.25^{fg}	80.1^{f}	1.02^{c}	5.3^{a}	0.5^{e}	86.9^{e}	70.0^{h}	4.5^{i}	18.5^{i}
12	6	3	P	3.50^{q}	0.43^{b}	79.0^{h}	0.98^{e}	4.3^{c-f}	1.5^{a}	85.8^{g}	71.2^{g}	2.4^{n}	14.3^{o}
13	6	3	P	3.37^{s}	0.45^{a}	77.6^{m}	0.99^{de}	4.1^{d-g}	1.2^{b}	83.9^{l}	75.1^{d}	2.7^{l}	15.4^{n}
14	6	3	PB	3.42^{r}	0.43^{b}	78.1^{l}	0.99^{de}	4.4^{b-e}	0.5^{e}	84.0^{kl}	79.4^{a}	1.7^{o}	12.0^{r}
15	4	3	B	4.72^{k}	0.30^{e}	79.2^{g}	1.02^{c}	4.4^{b-e}	0.2^{gh}	84.8^{ij}	72.3^{e}	4.7^{h}	18.3^{j}
16	6	1	B	4.12^{n}	0.32^{d}	78.4^{k}	0.98^{e}	4.5^{b-d}	0.5^{e}	84.4^{jk}	72.4^{e}	3.2^{k}	13.2^{p}
17	5	2	B	4.25^{l}	0.30^{e}	80.0^{f}	0.99^{de}	4.2^{d-g}	1.2^{b}	86.4^{f}	68.6^{j}	2.6^{lm}	21.6^{c}
18	4	2	PB	4.84^{j}	0.29^{e}	78.2^{l}	1.03^{bc}	4.1^{d-g}	0.1^{h}	83.4^{m}	67.1^{m}	6.7^{c}	19.7^{f}
19	4	1.5	P	4.96^{i}	0.34^{c}	78.6^{j}	1.02^{c}	4.4^{b-e}	0.25^{fg}	84.3^{kl}	67.9^{k}	4.7^{h}	22.0^{b}
20	3	1.5	B	5.32^{c}	0.24^{gh}	77.4^{n}	1.06^{a}	3.8^{g}	0.75^{d}	83.0^{mn}	63.3^{s}	8.5^{a}	19.4^{g}
21	2	3	P	5.30^{d}	0.22^{i}	83.5^{c}	1.00^{d}	4.1^{d-g}	0.5^{e}	89.1^{c}	65.2^{q}	6.4^{d}	20.6^{d}
22	6	3	PB	3.31^{t}	0.42^{b}	79.2^{g}	1.04^{b}	4.4^{b-e}	0.5^{e}	85.1^{l}	77.0^{c}	0.9^{p}	12.2^{q}

[1]Values are the means of triplicate analysis; wb= wet base, [3] db= dry base; *Values in the same column with different superscripts for each type of analysis are significantly different

The length of fermentation time and the amount of starter culture showed the effect on the reduction of pH. Run 22 (6 days, 3% mixed starter culture) produced the lowest value of pH at 3.31 and run 3 (2 days, 1% mixed starter culture) produced the highest value of PH at 5.75. The metabolic activity of those strains might be caused by the pH value differences. The pH values were significantly different for the different formulations of kocho products ($P \leq 0.05$). The titratable acidity of kocho increased when the pH was decreased. Increase titratable acidity and reduction of pH related to the production of organic acid during fermentation of vegetables including kocho fermentation (Andeta et al., 2018).

The moisture content of kocho samples ranged from 75.3-84.4%. The moisture content relatively reduced when the fermentation time was increased (Table 3). Similarly, Gashe (1987) reported that the moisture content of kocho samples reduced from 84% to 66% at day 6. This could be due to leakage or/and evaporation at a time of fermentation. Moreover, the reduction of the moisture contents has been related to the use of water by microbes for metabolic and growth activity, and due to a function of factors such as temperature, time and humidity (Karssa, et al., 2014).The reduction of moisture would help to avoid the growth of unwanted microorganisms like clostridia (Andeta et al., 2018). There were significant differences ($P \leq 0.05$) in the moisture content of different formulations of kocho products (Table 3). The ash content of the samples ranged from 0.98 to 1.06%.

However, Bosha et al (2016) and Yirmaga (2013) reported relatively higher amount of ash content, an average of 3.81 and 3.44%, respectively. This differences could be due to enset cultivars variances and the length of fermentation time. Yirmaga (2013) also stated that the ash content is highly affected by fermentation time of kocho. The protein and fat content of the samples ranged from 3.8 to 5.3% and 0.1 to 1.5 %, respectively. Bosha et al (2016) reported that the protein content of kocho is low, like other starchy food such as potato. Therefore, food prepared from kocho need to complement with other protein rich food such as meat, peas and beans. In this study, the amount of protein was comparable to the study by Yirmaga (2013), and higher than that reported by Bosha et al (2016). The average fat content obtained in this study was similar to Bosha et al (2016). Protein and fat content were significantly different for the samples at different formulation ($P \leq 0.05$). Total carbohydrate content of the samples ranges from 82 to 90.1% (db). The food products prepared from enset are rich in carbohydrate (Bosha et al., 2016). The carbohydrate content was significantly different ($P \leq 0.05$) for different kocho samples.
The color of kocho samples was measured with a spectrophotometer in addition to panelist's evaluation. The tri-stimulus color parameters L-A-B values are routinely used for describing the influence of process variables on product color (Teamir et al., 2018). The lightness of kocho (L) ranged from 63.3 to 79.4. The lightest sample was run 14 (6 days, 3% mixed starter culture) the darkest was run 20 (3 days, 1.5% mixed starter culture). The length of fermentation duration relatively increased the lightness value of kocho. The main reason could be the limited oxygen availability during lactic acid fermentation (Vatansever et al., 2017), which reduced the effects on the color changes. Similarly, other research indicated that kocho developed dark discoloration when loosely wrapped or taken off from the pit (Hunduma& Ashenafi, 2011). The color of kocho product at different formulation showed that a significant difference ($P \leq 0.05$).

Independent variables effect on the sensory qualities of Kocho

The effect of fermentation time, amount and types of starter culture on the sensory attribute of kocho is represented by the quadratic model. Sensory attributes analysis were carried out to examine the application of traditional surface fermentation practice, and a relatively shorter fermentation time with an immediate addition of starter culture during fermentation of enset. Yaakob et al (2012) stated that the sensory evaluation helps to describe product appearances in respect to customer acceptance. Figures1-4 show the effects of each variable on the sensory quality of kocho.
The response surface scheme for the odor of kocho is shown in Figure 1.
The linear and quadratic effect of fermentation time were positive for all strain types except *L. brevis*. Moreover, the linear effect of the amount of starter culture was positive and quadratic effect was negative for all strain types (Table 4). The samples fermented for short time were relatively less acceptable than others. The odor of kocho depends on the fermentation time, amount of starter culture and types of strains. Kocho has an unpleasant odor, which is unpleasnt for individuals from non-consumer areas. Gashe (1987) stated that the activity of the clostridial species during the first 20 to 30 days of kocho fermentation might be the possible reason for a strong butyrous odor of kocho.

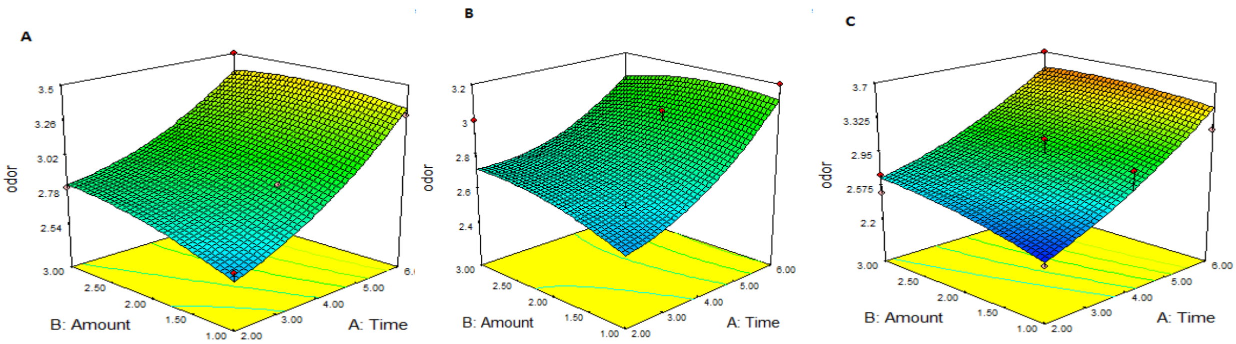

Figure 1. Response surface scheme of the effect of fermentation period and starter culture on the odor of kocho with different types of starter strains. (A) *L. plantarum* , (B) *L. brevis* and (C) Mixed starter culture

The poor and very variable sensory attribute of kocho could lead to a lower market price than other crops (Ashenafi, 2006; Brandt et al., 1997). Reduction of fermentation time with an immediate addition of starter culture probably inhibited the availability of unwanted microbes due to fast pH reduction. In this study, the pH reduction of kocho samples ranged from 5.75 to 3.31. The activity of spore-forming microbes which caused off-flavor may be limited due to lowering of the pH below ≤ 4.4 (Gashe, 1987). The response of odor by the panelists ranged from 2.3 to 3.7. This showed that most panelists accept the odor of kocho.

The color is one of major parameters in the assessment of sensory acceptability of food products; it plays an important role for marketing purpose (Teamir et al., 2018; Yaakob et al., 2012). A positive linear effect and a negative quadratic effect of the amount of starter culture were also found. The acceptability of color of kocho samples ranged from 2.1 to 4 as shown in Figure 2. Similarly to the spectrophotometer results kocho samples which were fermented for a long period of time were relatively more acceptable than others. Table 4 shows that the color of kocho samples fermented with *L. plantarum* and mixed starter strain types is affected by the fermentation time, with positive liner and quadratic effects. Yaakob et al (2012) also described that the fermentation period, the amount of starter culture and temperature could affect the color of the yoghurt.

A positive liner effect of both fermentation time and amount of starter culture on the appearance of kocho are presented in Table 4. However, the quadratic effect was negative in both cases. Fermentation appearance of fermented kocho samples is shown in Figure 3. The appearance of kocho increased when the fermentation period increased. This could be due to the digestibility of kocho starch with the microbes. The appearance of kocho ranged from 1.7 to 4.0. Run 7 and 10 relatively shows the lowest score. Samples which were fermented for a long period of time with the high amount of a mixed starter culture relatively shown the highest score (run 13 and 22).

The overall acceptability of the kocho samples ranged from 2 to 4.2. The liner effect of fermentation period and amount of starter culture were positive, and the quadratic effects were negative (Table 4, Figure 4).

Table 4. The regression coefficient of the actual factor for the response variables

Term	Estimated coefficients											
	L.plantarum				*L.brevis*				Mixed			
	[1]Y1	Y2	Y3	Y4	Y1	Y2	Y3	Y4	Y1	Y2	Y3	Y4
β_o	2.1	2.6	0.31	0.87	2.25	2.7	0.96	1.2	1.73	1.75	0.51	0.81
β_1	0.03	7E-3	0.52	0.61	-0.02	-0.1	0.36	0.43	0.11	0.18	0.58	0.61
β_2	0.38	0.16	0.87	0.27	0.34	0.17	0.76	0.27	0.41	0.14	0.65	0.23
β_{11}	0.02	0.03	-0.02	-0.03	0.02	0.03	-0.02	-0.03	0.02	0.03	-0.02	-0.03
β_{12}	-0.05	-0.03	-0.2	-0.04	-0.05	-0.03	-0.2	-0.04	-0.05	-0.03	-0.2	-0.04

[1]Y_1(odor), Y2 (color), Y3 (appearance), Y4 (overall acceptability)

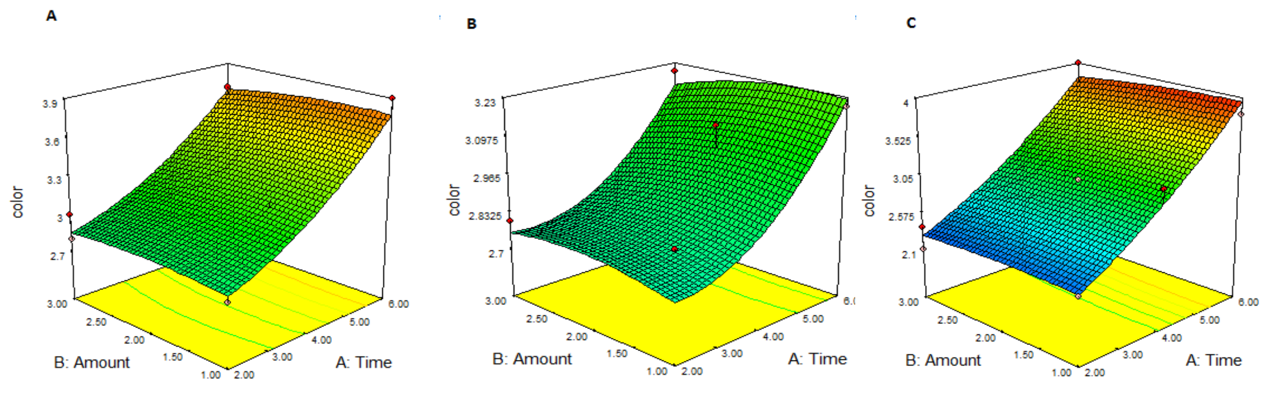

Figure 2. Response surface scheme of the effect of fermentation period and starter culture on the color of kocho with different types of starter strains. (A) *L. plantarum*, (B) *L. brevis* and (C) Mixed starter culture

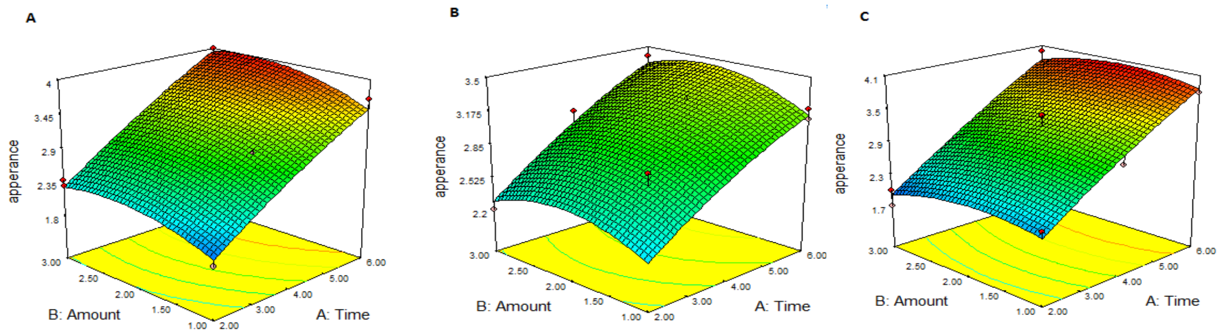

Figure 3. Response surface scheme of the effect of fermentation period and starter culture on the appearance of kocho with different types of starter strains. (A) *L. plantarum* , (B) *L. brevis* and (C) Mixed starter culture

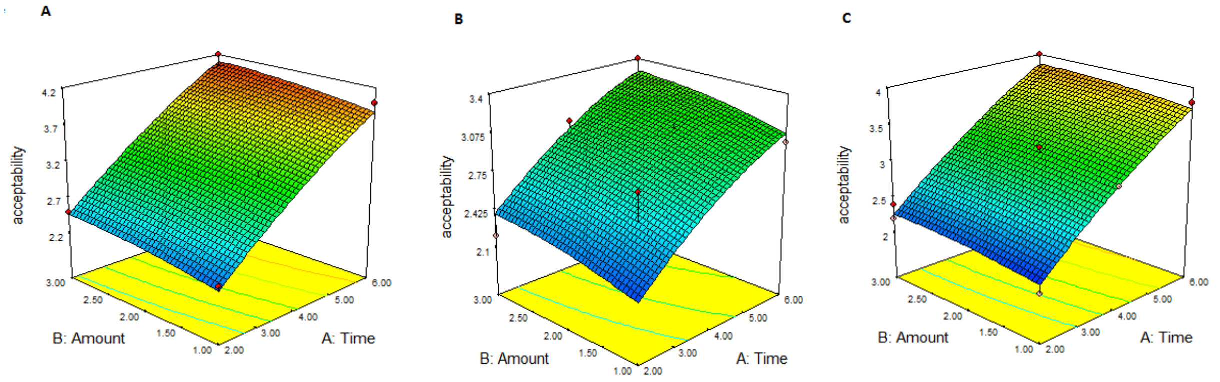

Figure 4. Response surface scheme of the effect of fermentation period and starter culture on the overall acceptability of kocho with different types of starter strains. (A) *L. plantarum*, (B) *L. brevis* and (C) Mixed starter culture

The optimum overall acceptability of kocho was 4.0 at 6 days of fermentation time and 2% *L. plantarum* strain types. However, lower overall acceptability was shown in samples fermented with mixed strain (3.8) and *L.brevis* strain (3.3). Kocho samples which were fermented for long period of time, with high amount of starter culture and *L. plantarum* type of strain appeared to be more acceptable. The predicted values of the dependent variables resulting from the equation ranged from 2.5 to 4.0. Therefore, the optimized region for overall acceptability of kocho ranged between "neutral" and "like".

CONCLUSION

The optimized value obtained from RSM found at the same ranges to the experimental results. The RSM model successfully optimized fermentation condition of kocho with the selected types of starter strains. Fermentation time, amount of starter culture and types of starter strain affected the sensory attributes of kocho. Good quality of kocho could be produced at 6 days of fermentation period with 2% of *L. plantarum* starter strain.

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