


Optimisation of stabiliser usage conditions in oat milk production using response surface methodology

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ABSTRACT

Present study aimed to optimise stabiliser concentration and swelling time of oat in the production of oat milk using response surface methodology. The effects of independent variables (stabiliser concentration, swelling time) on dependent variables (rheological and sensory properties) were studied using a central composite rotatable design of experiments. Physico-chemical properties and colour values of samples were not significantly ($P > 0.05$) correlated to stabiliser concentration and swelling time. While stabiliser concentration affected the consistency index ($P < 0.05$), swelling time had no effect on the rheological properties. The stabiliser ratio affected the sensory texture of samples ($P < 0.05$). The sensory scores did not increase with increasing K value, on the contrary, the samples with the highest K value had lower sensory scores ($P < 0.05$). Considering the K value and sensory scores, the optimum stabiliser concentration and swelling time were determined as 0.102%, and 51.2 min, respectively.

KEYWORDS

oat milk, stabiliser, swelling time, rheology, response surface methodology, optimisation

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1. INTRODUCTION

In recent years, global food consumption paradigms have been changing rapidly (Patterson et al., 2020). Since the production of milk proteins is relatively costly and its possible negative effects on the environment, there has been a clear tendency in recent years to benefit from plants as an alternative protein source (Khalesi and FitzGerald, 2021). Dairy-free diet also offers alternatives to individuals who suffer from lactose intolerance and cow milk protein allergy. In addition, increasing veganism, flexitarianism, and vegetarianism have led to rise in demand toward plant-based products (Feng et al., 2021). This trend has triggered the efforts to develop dairy-alternative innovative plant-based foods by scientific community and today, a great number of plant protein sources from cereals, legumes, nuts, and seeds are incorporated into food formulations, and such products in various forms are now enjoying market success (Tangyu et al., 2019; Sa et al., 2020).

Oat (*Avena sativa*) is a grain variety that is becoming increasingly important in human nutrition as well as animal nutrition. Oats are good sources of protein, fat, soluble fibre, phytochemicals, and also of β -glucans (Wang et al., 2022). Considering the nutritional characteristics and plantation performances, oat milk has drawn consumers' and food industry's attention as non-dairy milk substitutes (Watson, 2020).

Oat milk production protocol includes basically obtaining oat slurry by mixing oat flour and water, hydrolysis of the starch by glucoamylase and α -amylase, filtration, homogenisation, and heat treatment. Colloidal stability of oat milk is achieved by means of stabilising agents. The gelatinisation temperature of oat starch, which amount to almost half of oat compounds, is between 44.7 and 73.7 °C (Tester and Karkalas, 1996). This eventually carries a heat-induced gelatinisation risk during heat treatment (Deswal et al., 2014b). Therefore, conversion of starch into maltodextrins *via* chemical (Hoover, 2000) or enzymatic (Tester et al., 2006) hydrolysis is of critical importance in keeping oat milk in liquid form after heat treatment. Among many advantages of enzymatic hydrolyses of oat starch over chemical hydrolysis, it is noteworthy that it increases filtration efficiency and thus yield by lowering viscosity (Aiyer, 2005). There are many factors that affect the rheological and sensory properties of oat milk, including enzyme concentration, slurry concentration, liquefaction time, stabiliser concentration, and swelling time of stabiliser-oat mixture. Optimisation of these parameters is important in terms of end product quality and production efficiency. Deswal et al. (2014b) optimised the slurry concentration, enzyme concentration, and liquefaction time in oat milk based on the rheological characteristics and yield parameters by applying response surface methodology (RSM) as 27.1% (w/w), 2.1% (w/w), and 49 min, respectively.

To the best of our knowledge, no optimisation study has been carried out on the concentration of stabilisers used in oat milk formulation and swelling time of oat-stabiliser mixture. The present study aimed to optimise these variables using RSM based on physico-chemical, rheological, and sensory characteristics of oat milk.

2. MATERIALS AND METHODS

2.1. Materials

The rolled oats were purchased from Eti Gıda A.S. (Eskisehir, Turkey). Food grade amyloglucosidase (glucan 1,4- α -glucosidase) (AMG[®]300L, Novozyme, Denmark) with an activity of 300



AGU/mL and α -amylase (BAN[®]480L, Novozyme, Denmark) with an activity of 480 KNU-B/g in a liquid form were used for hydrolysis of maltose into glucose. Sodium alginate was used as stabiliser (Hamulsion[®] Stabiliser Systems, Tate & Lyle PLC, London, UK). Vegetable oil (sunflower oil) and table salt were purchased from the local market. Chemicals used were of analytical grade and supplied from Merck Co. (St. Louis, USA) unless otherwise stated.

2.2. Methods

2.2.1. Production of oat milk. Oat milk slurry was prepared by shredding oat:water mixture (1:9) with a blender for 1 min at 25 °C. Afterwards, the slurry was subjected to heat treatment at 95 °C for 3 min using a temperature-controlled water bath. AMG (0.05%) and BAN (0.03%) were added to the slurry at 65 °C simultaneously. The mixture was left for 30 min to allow enzymes to take effect and then filtered through a double layer muslin cloth. Vegetable oil (1.5%), table salt (0.1%), and stabiliser mixture were added into the liquefied oat slurry, and the mixture was rested for swelling. The mixture was stirred using an Ultraturrax at 13,000 r.p.m. for 3 min and then heat treated at 95 °C for 3 min. The oat milk samples were cooled and stored at 4 °C until analysis. A total of 13 samples (8 samples with different stabiliser concentrations-swelling time levels and 5 control samples with 0.10% stabiliser concentration and 50 min swelling time) were produced as determined by response surface methodology-central composite rotatable design. All productions were made in triplicate. Oat milk production protocol is given in Fig. 1.

2.2.2. Physicochemical analysis. The pHs of the samples were measured using a combined electrode pH-meter (Hanna Instruments Inc., Germany), and titratable acidity was determined by Soxhlet-Henkel method (AOAC, 1990). Total solids and ash were determined by gravimetric method at 105 °C and 600 °C, respectively. The colour values were determined by Chroma meter (Konica Minolta CR 410, Sensing Inc., Osaka, Japan). ΔE values were calculated using the following formula:

$$\Delta E = \sqrt{(L - L_{ref})^2 + (a - a_{ref})^2 + (b - b_{ref})^2}$$

The average of the control samples (Sample No: 9–13, see Table S2) was taken as the reference value of the L^* , a^* , and b^* values.

2.2.3. Dynamical rheological tests. Dynamic rheological assessments of the samples were carried out using a small deformation dynamic rheometer (Kinexus Pro + model, Malvern, Worcestershire, UK). The rheometer was attached with a 40 mm stainless steel 4° conical geometry spindle with a 2 mm gap. Shear stress was measured at 31 logarithmically spaced shear rates in the range of 0.1–100 s⁻¹ at 4 °C.

The flow curves were fitted to the Herschel–Bulkley fluid model using non-linear regression. The consistency index (K) and consistency coefficient values were calculated according to the following equation:

$$\eta = \eta_0 + K\dot{\gamma}^n$$

where η is shear stress (Pa), η_0 is yield stress, K is a consistency index (Pa.s), $\dot{\gamma}$ is the shear rate (s⁻¹), and n is the flow behaviour index.



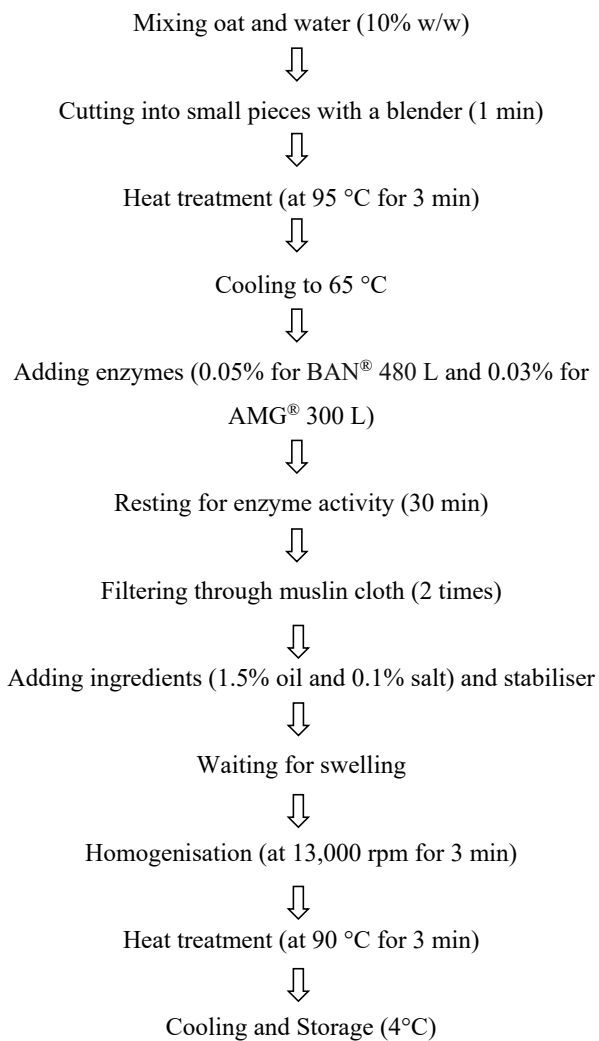


Fig. 1. Flow diagram of oat milk production

2.2.4. Sensorial evaluation. Sensory evaluation of the oat milk samples was performed by five trained panellists from the Department of Dairy Technology, Ankara University, according to Clark and Costello (2016). The panellists were requested to evaluate the samples for appearance, texture, and taste & flavour. Mean scores were used for comparison of the samples. The samples were randomly coded with three-digit numbers and served at 4 ± 1 °C.

2.2.5. Experimental design and statistical analysis. The central composite rotatable design (CCRD) was used for experimental designing. Stabiliser concentration (x_1) and swelling time of stabiliser (x_2) were used as independent variables. To determine the effects of these variables on rheological and sensory properties of oat milks and the level of interactions between these variables, response surface methodology-central composite rotatable design (RSM-CCRD) was applied (Table S1 in supplement, available on the server of the Publisher).

The consistency index values and sensory scores of the samples were chosen as responses (y) separately for this study. In order to estimate the response, an empirical model composed of a second-order polynomial equation was constructed, which is given below.

$$y_i = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i < j} \beta_{ij} X_i X_j + \varepsilon$$

where y is the predicted response; β_0 is the model constant, β_i are the coefficients of the linear effects, β_{ii} are the coefficients of the quadratic effects, β_{ij} are the coefficients of interaction between the factors, X_{ij} and X_j are the independent coded variables, ε is the error, k is the number of the variables considered, i and j are the coded factors of the system.

The coefficients were calculated by regression analysis, and their significance was verified using ANOVA procedure of Minitab statistical package (Minitab® 16.1.1, State College, PA).

Central composite design with the two independent variables, their levels, and their experimental responses are given in Table S2.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical analysis

Gross compositions and acidity values of oat milk samples are given in Table 1. Overall, stabiliser concentrations and swelling times had no significant effect on the gross composition and acidities of the samples ($P > 0.05$). The colour values of oat milk samples are given in Table 2. As with gross composition and acidity values, the colour characteristics of the samples did not differ significantly ($P > 0.05$). The sensorial characterisation of foods is of critical importance for meeting consumers' demands for tasty food. Among the sensory properties, the colour of the food gives the most distinctive visual information. For this reason, it is essential to determine the colour in studies with the purpose of developing a new product or improving the properties of the existing product.

The components/ingredients of the grain-based material have a great influence on the colour shades of the product. The oat species used in beverage formulations also affect the colour properties of the oat-based beverages as reported by Taner et al. (2012). Components such as phenolic substance, flavonoids (Shen et al., 2009), and fatty acids (Lee et al., 2017) are the major compounds affecting the colour of cereals. In the present case, since the oat milk samples were



Table 1. Gross composition and acidity values of oat milk samples*

Samples	Total solids (%)	Ash (%)	Protein (%)	pH value	Titrateable acidity (LA%)
1	7.39 ± 0.379	0.208 ± 0.060	0.459 ± 0.065	6.44 ± 0.064	0.051 ± 0.005
2	7.38 ± 0.377	0.174 ± 0.008	0.467 ± 0.073	6.48 ± 0.066	0.049 ± 0.003
3	7.25 ± 0.284	0.221 ± 0.056	0.504 ± 0.015	6.46 ± 0.045	0.048 ± 0.003
4	7.42 ± 0.345	0.179 ± 0.030	0.486 ± 0.014	6.47 ± 0.048	0.053 ± 0.007
5	7.27 ± 0.369	0.174 ± 0.016	0.474 ± 0.058	6.48 ± 0.036	0.046 ± 0.009
6	7.48 ± 0.434	0.220 ± 0.029	0.487 ± 0.010	6.47 ± 0.057	0.050 ± 0.004
7	7.12 ± 0.164	0.173 ± 0.009	0.447 ± 0.076	6.49 ± 0.054	0.047 ± 0.008
8	7.37 ± 0.397	0.184 ± 0.020	0.447 ± 0.074	6.48 ± 0.062	0.048 ± 0.003
9	7.37 ± 0.337	0.173 ± 0.053	0.488 ± 0.078	6.49 ± 0.053	0.048 ± 0.003
10	7.32 ± 0.286	0.178 ± 0.038	0.478 ± 0.099	6.47 ± 0.036	0.045 ± 0.007
11	7.46 ± 0.149	0.172 ± 0.041	0.460 ± 0.015	6.50 ± 0.050	0.045 ± 0.007
12	7.50 ± 0.097	0.200 ± 0.050	0.464 ± 0.015	6.47 ± 0.039	0.046 ± 0.007
13	7.36 ± 0.188	0.190 ± 0.043	0.455 ± 0.017	6.46 ± 0.034	0.046 ± 0.007

*Non-lettered columns indicate that differences between scores are not significant ($P > 0.05$).

prepared from the same oat species and the samples were subjected to similar treatments except for stabiliser concentration and swelling time of stabiliser-oat slurry mixture, there was no clear difference in colour properties of the samples ($P > 0.05$). The ΔE values, which show the colour differences among the samples, were also quite low, indicating that the colour differences could not be detected by naked eye.

3.2. Dynamical rheological tests

Figure 2 shows the effects of stabiliser concentration (X_1) and swelling times (X_2) on the rheological properties of oat milk samples. Consistency index could be defined as the value of viscosity or stress at a shear rate of 1.0 s^{-1} (Deswal et al., 2014a). For a Newtonian fluid ($n = 1$), the K value is equal to the viscosity of the fluid. While the consistency index (K) values were significantly affected by the stabiliser concentration, the swelling time had no effect on rheological properties of the samples. The increase in the concentration of stabiliser resulted in a significant increase in the K value, which is the viscosity indicator. In particular, the K value of the samples containing 0.15% stabiliser was remarkably higher than the rest of the samples. It is an expected phenomenon that K values increase with the increase in the use of stabilisers due to the decrease in the mobility of free water as a result of increased water-binding by stabilisers or *vice versa* (Gómez-Díaz and Navaza, 2004). Therefore, samples containing lower stabiliser concentrations (Samples 1, 3, and 5) had relatively lower K values.

Similarly, time vs. shear viscosity graph plotted using rheological data shows that samples with high stabiliser concentration (Samples 2, 4, and 6) had higher viscosity values (Fig. S1B in Supplement file, available on the server of the Publisher). However, the shear viscosity values of all samples showed a similar trend over time (Fig. S1). In addition, Fig. S1A shows that sample 5, which had the lowest stabiliser concentration, could not remain stable during the time of the experiment.



Table 2. Colour parameters and rheological properties of oat milk samples *

Sample	Colour parameters				Rheological properties		
	L*	a*	b*	ΔE	Yield stress	K	n
1	51.47 ± 1.351	1.08 ± 0.130	7.89 ± 0.247	0.96 ± 0.02	0.008 ± 0.006	0.017 ± 0.007	0.92 ± 0.028
2	51.22 ± 1.701	1.06 ± 0.174	7.90 ± 0.492	0.65 ± 0.09	0.560 ± 0.058	0.327 ± 0.061	0.31 ± 0.021
3	50.28 ± 1.752	1.12 ± 0.151	7.93 ± 0.445	0.40 ± 0.02	0.004 ± 0.001	0.033 ± 0.014	0.72 ± 0.068
4	50.97 ± 1.650	0.95 ± 0.62	7.83 ± 0.370	0.71 ± 0.03	0.852 ± 0.056	0.366 ± 0.048	0.36 ± 0.057
5	51.09 ± 1.715	1.08 ± 0.146	7.93 ± 0.389	0.91 ± 0.04	0.004 ± 0.001	0.016 ± 0.009	0.71 ± 0.099
6	50.32 ± 1.906	1.08 ± 0.185	7.98 ± 0.481	0.41 ± 0.03	0.765 ± 0.062	0.675 ± 0.050	0.19 ± 0.047
7	51.12 ± 1.730	1.04 ± 0.175	7.73 ± 0.398	0.79 ± 0.03	0.009 ± 0.008	0.034 ± 0.006	0.62 ± 0.057
8	51.30 ± 1.475	1.06 ± 0.160	7.66 ± 0.414	0.78 ± 0.01	0.012 ± 0.010	0.041 ± 0.003	0.54 ± 0.014
9	50.37 ± 1.641	1.11 ± 0.189	7.99 ± 0.477	-	0.018 ± 0.009	0.042 ± 0.003	0.55 ± 0.022
10	50.46 ± 1.799	1.12 ± 0.179	7.77 ± 0.509	-	0.014 ± 0.007	0.041 ± 0.007	0.59 ± 0.061
11	50.71 ± 1.502	1.09 ± 0.167	7.83 ± 0.369	-	0.006 ± 0.002	0.038 ± 0.013	0.59 ± 0.032
12	50.73 ± 1.724	1.09 ± 0.167	7.83 ± 0.408	-	0.006 ± 0.001	0.030 ± 0.007	0.60 ± 0.044
13	50.61 ± 1.314	1.09 ± 0.160	7.85 ± 0.401	-	0.010 ± 0.005	0.035 ± 0.001	0.59 ± 0.045

*Non-lettered columns indicate that differences between scores are not significant ($P > 0.05$).



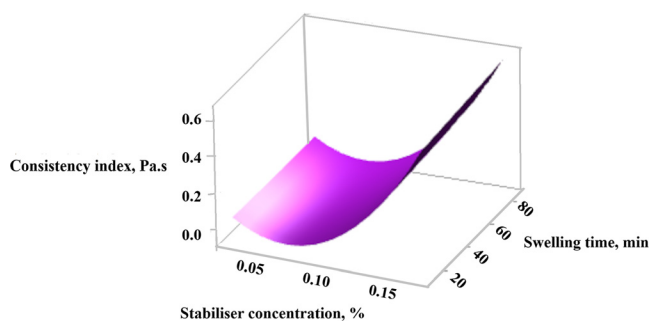


Fig. 2. Effect of stabiliser concentration (X_1) and swelling time (X_2) on the consistency index (K) values of the samples

Also, the lack of fit value was found to be insignificant ($P > 0.05$) (Table S3). Ideally, the lack of fit should be insignificant and hypothesis of significance of regression is rejected (Myers et al., 2016). This shows that the model is satisfactory as a prediction equation.

Shear rate *vs.* viscosity and shear rate *vs.* shear stress profiles of the oat milk samples are presented in Fig. 3. The n values lower and higher than 1.0 indicate shear-thinning and shear-thickening fluid behaviours, respectively. In our study, shear viscosity values of all samples decreased with increasing shear rate, indicating a shear thinning flow behaviour in all samples. Plant-based milks are colloidal dispersions of various particles and components. The pseudoplastic behaviour of oat milk can be attributed to its colloidal structure. The shear thinning flow of cereal beverages stems from the breakdown of hydrocolloids due to hydrodynamic forces during shearing (Iskakova et al., 2019). This is further confirmed by the flow behaviour index values (n) of the samples (Table 2). Additionally, the samples with higher viscosity had less n value, pronouncing non-Newtonian properties. Oat milks samples with higher stabiliser concentrations (Samples 2, 4, and 6) had higher shear viscosity values at low shear rates.

Shear stress values of oat milk showed a non-linear trend (Fig. 3B). Silva et al. (2020) also reported a similar trend in plant-based beverages. Figure 3B also shows that samples 2, 4, and 6 had higher shear stress values than other samples, as was in K values.

3.3. Sensory properties

The effects of stabiliser concentration and swelling time of stabiliser-oat milk slurry mixture on sensory properties of oat milk samples are given in Fig. 4. Overall, the samples with high K value received lower texture and aroma & flavour scores ($P < 0.05$). On the other hand, the stabiliser concentration and swelling time did not affect the appearance of the samples significantly ($P > 0.05$). The most liked oat milk samples were those containing 0.1% stabiliser. The results of ANOVA analysis showed that while the stabiliser concentration had significant quadratic effect on sensory properties of oat milk samples (Table S4 and Fig. 5), the effects of swelling time was insignificant ($P > 0.05$). Lack of fit values were found to be insignificant ($P > 0.05$).



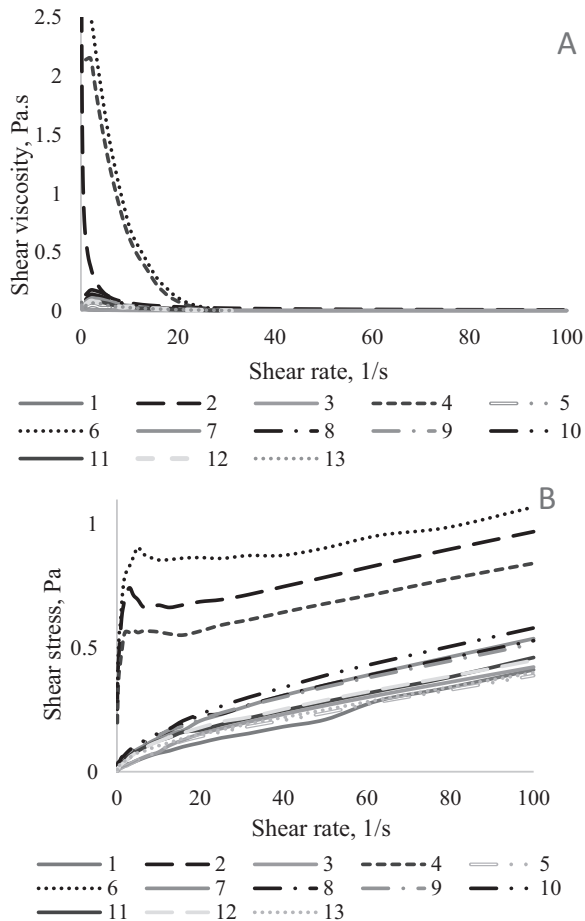


Fig. 3. Shear rate vs. viscosity (A) and shear rate vs. shear stress (B) profiles of oat milk samples

It is stated that the basic taste of plant-based milks is characterised by the plant from which milk is obtained. Especially oat-based milks were reported to have higher bitterness and after-taste values (Vaikma et al., 2021).

3.4. Optimisation of stabiliser conditions

To determine the optimum concentration of stabiliser, the average consistency index values of the samples with the highest sensory texture scores were taken into account, and 0.04 Pa.s was determined as the target value. In terms of sensory properties, the total scores of all sensory properties were studied and the maximum score was targeted. Based on these evaluations, the optimum stabiliser concentration and swelling time were determined as 0.102% and 51.2 min, respectively. The composite desirability value for this calculation was found to be 0.987. As



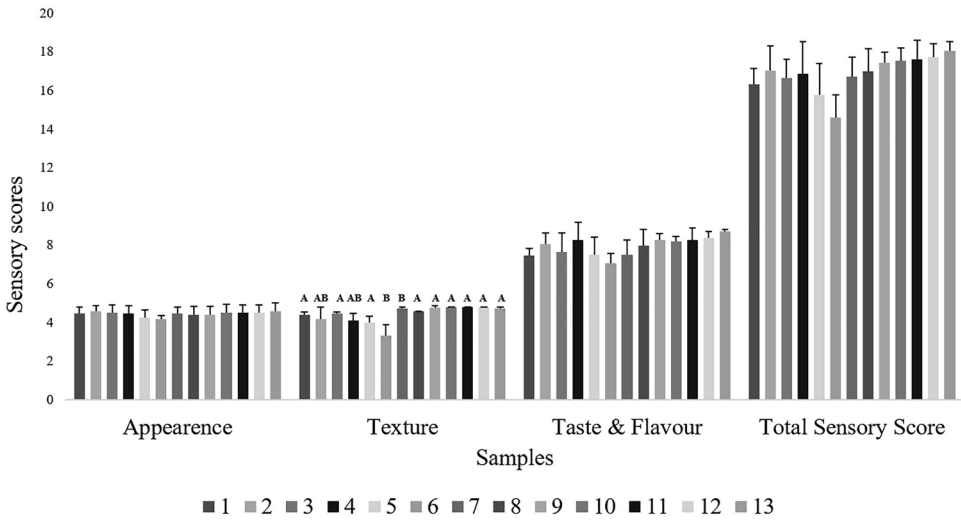


Fig. 4. Sensory properties of oat milk samples*. * Non-lettered areas indicate that differences between scores are not significant ($P > 0.05$). Means with different uppercase letters mean significant differences between values of samples ($P < 0.05$)

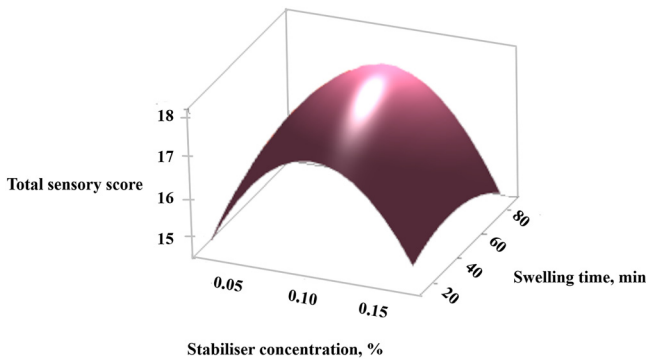


Fig. 5. Effect of stabiliser concentration and swelling time on total sensory scores of oat milk sample

predicted responses values, 0.04% for consistency index value and 17.67 values for total sensory scores were determined.

4. CONCLUSIONS

The popularity of plant-based dairy milk alternatives is increasing steadily. As the production volumes of these products increases, extra care should be taken to reduce production costs and optimise product quality with a satisfactory shelf-life. Therefore, parameters that affect the



quality of the final product should be selected with care. In the present study, the concentration of sodium alginate and swelling time of oat grains were optimised based on RSM. Future studies are recommended to focus on developing strategies to obtain minimally processed oat milk products with extended shelf-life and to produce oat milk with improved functional properties.

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SUPPLEMENTARY MATERIAL

Supplementary data to this article can be found online at <https://doi.org/10.1556/066.2022.00263>.

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