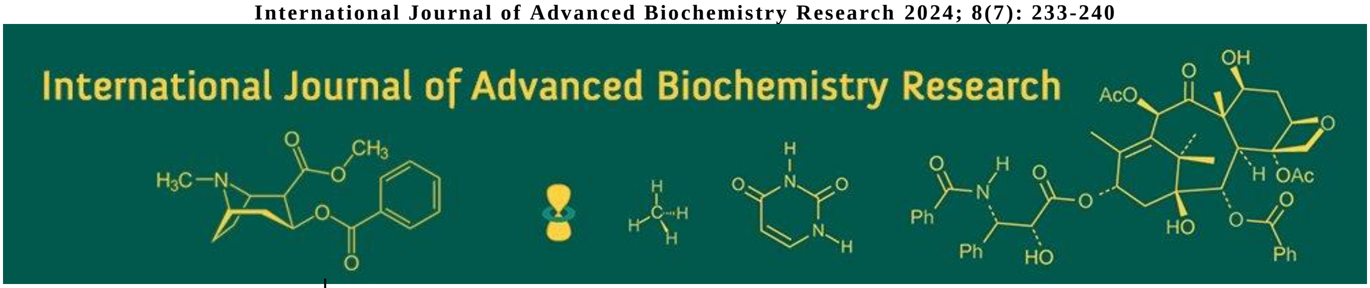
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Combined effects of hydrocolloids, frozen storage and thawing on proximate composition of frozen dough bread using response surface methodology

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Abstract

Present study is about investigation of combined effects of hydrocolloids, frozen storage duration and thawing temperature on the proximate composition of frozen dough bread. Frozen dough bread formulations were prepared, varying hydrocolloid type, frozen storage duration, and thawing temperature. Proximate composition analysis revealed significant variations in moisture, fiber, ash, fat, carbohydrate, and protein content across experimental setups. In bread development, moisture ranged 36.87-43.43%, fiber 1.37-1.95%, ash 0.39-0.99%, fat 1.59-2.79%, carbohydrate 41.42-42.34%, and protein 9.72-17.92%. Hydrocolloids notably impacted fiber, ash, and carbohydrate content. Thawing temperature and storage time influenced moisture, protein, and carbohydrate levels. The study underscores the complex interplay between formulation components and freezing parameters in shaping bread composition, offering insights for quality control and optimization of bread production processes.

Keywords: Hydrocolloids, frozen storage, thawing, frozen dough, bread, gluten

Introduction

The study on the combined effect of hydrocolloids, thawing temperature, and storage time on the proximate composition of frozen dough bread is a crucial exploration in the realm of food science and technology. As consumers increasingly seek convenient food options without compromising on quality, frozen dough bread has emerged as a popular choice. However, ensuring its nutritional integrity and sensory attributes after freezing and thawing processes poses a significant challenge for manufacturers.

Hydrocolloids, such as gums and gels derived from plants, seeds, or seaweeds, play a pivotal role in modifying the rheological properties of dough and improving its structural stability during freezing and thawing. Their incorporation into bread dough formulations for frozen storage can potentially enhance the texture, water-holding capacity, and gluten network formation, and gas retention properties during freezing and thawing of dough and overall quality of bread.

The storage time of frozen dough bread post-thawing can significantly impact its proximate composition and quality attributes. The moisture content of the dough affects its texture and shelf life, with higher moisture levels contributing to a softer crumb and shorter shelf life. Protein content influences dough elasticity and structure formation during baking, impacting bread volume and texture. Additionally, the presence of additives such as hydrocolloids and emulsifiers can enhance dough stability, texture, and overall quality (Rosell *et al.*, 2001) [12]. Prolonged storage durations may induce lipid oxidation, protein denaturation, and staling reactions, leading to changes in flavour, colour, and texture. Therefore, monitoring the evolution of proximate composition parameters over time is essential for optimizing the shelf life and nutritional security of frozen dough bread products.

Thawing temperature serves as a critical parameter in the frozen dough bread manufacturing process, as it directly affects the rate of ice crystal dissolution and subsequent dough hydration. Rapid thawing at higher temperatures may lead to enzymatic and microbial degradation, resulting in undesirable changes in the nutritional composition and sensory attributes of bread. Conversely, slow thawing at lower temperatures can promote uniform water distribution and minimize textural defects in the final product.

This research aims to investigate how the interplay between hydrocolloids, thawing temperature, and storage duration impacts the proximate composition of frozen dough bread. Proximate composition refers to the quantitative analysis of major constituents in food, including moisture, protein, fat, carbohydrates, and ash content. Understanding changes in these components is essential for evaluating the nutritional profile and textural attributes of bread.

Furthermore, the investigation into the combined effect of hydrocolloids, thawing temperature and storage time on the proximate composition of frozen dough bread offers valuable insights for food scientists, manufacturers, and consumers alike. By elucidating the underlying mechanisms governing these interactions, this research contributes to the development of innovative strategies for enhancing the nutritional quality, sensory attributes, and shelf stability of frozen dough bread in the modern food industry.

Material and methods

The experiment was conducted in the Grain Science and Crop Quality Laboratory of Department of Food Science and Technology, JNKVV Jabalpur (MP). Raw materials required for the experiment i.e., common salt (Sodium chloride), instant dry yeast (Saccharomyces Cerevisiae), powdered sugar, and oil were obtained from Vipin Trading, Napier Town, Jabalpur, while refined wheat flour was procured from Priyadarshani Suvidha Sahaseva Kendra, Civic Centre Jabalpur. The food grade hydrocolloids i.e, HPMC (Hydroxy propyl methyl cellulose) and Guar gum for the preparation of dough were ordered online from amazon. The potable water in the department was used to make the dough from refined wheat flour, hydrocolloids and other materials for bread development.

Preparation of Frozen Dough Bread

Frozen bread dough was prepared using a method adapted from Ali Asghar (2006) [5]. Briefly, frozen bread dough was prepared using the formulation shown in Table 3.2. For each formulation (1 kg), the ingredients were mixed in a laboratory dough mixer for 8 minutes, low speed to obtained optimal dough development. The dough was moulded which were frozen in the ultra-low temperature freezer with temperature of -30 °C, until the temperature at the centre of the dough was -18 °C. The frozen dough were removed from the ultra-low freezer and immediately stored in polyethylene bags in a domestic freezer at -18 °C for 60 days. After preparing the dough, unfrozen dough was taken for study. Further samples were removed after 15 days interval, thawed at five different temperatures i.e., 20, 30, 40, 50 and 60 °C, fermented and proofed at 30 °C for 1 hour and 35 minutes and finally baked at 200 °C for 20 minutes. The breads obtained from frozen dough were cooled at room temperature for at least one hour and evaluated.

Table 1: Standard recipe for preparation of bread

Ingredients	Refined Wheat flour	Sugar	Salt	Dry Yeast	Bread improver	Oil	Water
Quantity	100 g	10 g	1 g	3 g	0.1 g	5 ml	20 ml

Proximate composition of developed bread

The nutritional evaluation of developed breads with respect to different variables was carried out by the standard procedures. Moisture content was determined using standard AOAC (2000) [4] method. Samples were oven dried at 105 °C until a constant weight was achieved. The protein content in sample was determined by using conventional Micro-Kjeldhal digestion and distillation procedure as given in AOAC (2000) [4] using Pelican's Kel Plus digestion and distillation assembly. The fat content of the sample was determined by the procedure as described in AOAC (2000) [4] using Pelican's Sox plus automatic fat analysis system. The ash content present in the sample was determined according to the procedure given in AOAC (2000) [4] using Muffle furnace. Fibre content was determined by sequential boiling with sulphuric acid and sodium hydroxide following AOAC (2000) [4] method. Carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, ash, and crude fibre from 100 (AOAC 2000) [4].

Statistical Analysis

In this study, a central composite rotatable design was employed, comprising four independent variables across 30 experimental runs. The ranges of variables were selected taking into consideration the maximum and minimum values used for control samples of preparation. Responses surface methodology (Mayer's, 1976) was used to reduce the number of experiments, without affecting the accuracy of result. Six central point experiments were conducted to assess method repeatability. The variables included guar gum, HPMC, thawing temperature, and frozen storage time, each ranging from low to high levels: 0.5-2.5% for guar gum and HPMC, 20-60 °C for thawing temperature, and 0-60 days for frozen storage time. The analysis of variance (ANOVA table) revealed at significance at $p<0.05$ level.

Results and Discussion

Proximate composition of developed breads

On the basis of Response Surface Methodology analysis, data is obtained and presented as result in the form of table and figures which is in line with various previous studies. Proximate composition indicated the major nutrition profile of the product which is important for human nutrition. In this research effect of different studied variables on proximate profile was studied and discussed in this section as below-

Moisture content

The moisture content of the developed breads ranged from 36.87% to 43.43% as shown in table 2. The maximum moisture content i.e., 43.43% in the developed bread was found in Experiment 23 which exhibited the combination of variables as 1.5 grams of Guar Gum, 1.5 grams of HPMC at 0 days (fresh dough). While the minimum value i.e., 36.87% was reported in the Experiment 24 exhibiting the combination of variables as 1.5 grams of Guar Gum, 1.5 grams of HPMC, with a thawing temperature of 40 °C after 60 days of frozen storage. The Model F-value of 19.53, presented in ANOVA table, indicates that the model is statistically significant, with terms A, B, C, and D being significant ($p<0.05$). The R^2 value of the model is 0.7576, indicating that it can explain 75.76% of the variability in the experiments. Table 5 also provides the probability values of coefficients for the linear model, showing that the levels of GG and HPMC had a significant positive linear effect at the 1% confidence level. Conversely, increased thawing temperature and storage time had a significant negative linear effect at the same confidence level on the moisture content of the developed frozen dough bread. Similar observations were reported by Maleki and Milani (2013) [10], who found that the moisture content of bread crumb ranged between 34.34% and 46.11%. Mandala (2005) [11] also showed that fresh samples exhibited an average moisture content of 42%, which decreased to 40-41% after frozen storage.

Fiber content

The fiber content of the developed breads ranged from 1.37% to 1.95%. The maximum fiber content, 1.95%, was found in Experiment 28, which combined 1.5 grams of Guar Gum and 1.5 grams of HPMC, with a thawing temperature of 40 °C after 30 days of frozen storage. The minimum fiber content, 1.37%, was found in Experiment 13, which combined 1 gram of Guar Gum and 1 gram of HPMC, with a thawing temperature of 50 °C after 45 days of frozen storage. The Model F-value of 7.02, presented in ANOVA table 3, indicates that the model is statistically significant, with terms A and B being significant ($p<0.05$). The R^2 value of the model is 0.5291, indicating that it can explain 52.91% of the variability in the experiments. The fiber content of the developed breads showed significant differences concerning Guar Gum and HPMC, both of which had a significant positive linear effect at the 1% confidence level. Conversely, thawing temperature and frozen storage time had no significant effect on the fiber content of the developed breads. These findings are in tune with the findings of Schiraldi *et al*. (2019) [15] and Smith *et al*. (2018) [16] who reported that the addition of hydrocolloids to bread formulations led to increased fiber content.

Table 2: Proximate composition of developed breads

Experiments	Moisture (%)	Fiber (%)	Ash (%)	Fat (%)	Carbohydrate (%)	Protein (%)
1.	37.99	1.6	0.7	1.94	41.7	16.07
2.	40.43	1.92	0.99	2.12	42.05	12.49
3.	40.55	1.8	0.85	2.61	42.3	11.89
4.	41.79	1.84	0.86	2.25	42.04	11.22
5.	39.81	1.49	0.53	2.67	41.57	13.93
6.	40.52	1.79	0.86	1.86	41.86	13.11
7.	39.72	1.78	0.82	2.42	42.28	12.98
8.	39.82	1.8	0.82	1.78	41.87	13.91
9.	37.97	1.42	0.46	1.62	41.49	17.04
10.	38.42	1.87	0.94	2.05	41.94	14.78
11.	38.71	1.8	0.85	2.72	41.8	14.12
12.	39.19	1.69	0.71	2.16	42.09	14.16
13.	37.28	1.37	0.39	1.59	41.45	17.92
14.	38.09	1.79	0.86	2.79	41.87	14.6
15.	37.68	1.7	0.75	2.03	42.2	15.64
16.	38.34	1.94	0.96	2.38	42.34	14.04
17.	37.92	1.37	0.39	1.73	41.42	17.17
18.	39.42	1.88	0.95	2.34	41.95	13.46
19.	38.29	1.42	0.46	2.51	41.92	15.4
20.	39.9	1.92	0.94	2.48	42.3	12.46
21.	39.32	1.9	0.92	2.64	41.98	13.24
22.	37.09	1.85	0.9	2.17	41.85	16.14
23.	43.43	1.9	0.92	1.83	42.2	9.72
24.	36.87	1.87	0.89	2.32	42.27	15.78
25.	38.19	1.67	0.71	1.68	42.17	15.58
26.	39.49	1.89	0.93	2.43	41.96	13.3
27.	39.17	1.87	0.94	2.2	42.05	13.77
28.	38.7	1.95	0.97	1.91	42.1	14.37
29.	38.78	1.93	0.95	2.78	42.28	13.28
30.	38.86	1.92	0.96	2.69	42.32	13.25

Table 3: ANOVA for second order regression model for chemical composition of developed breads

Source	Moisture (%)	Fiber (%)	Ash (%)	Fat (%)	Carbohydrate (%)	Protein (%)
Model SS	42.76	0.5039	0.4828	0.2077	0.8194	66.77
Model MS	10.69	0.1260	0.1207	0.0519	0.2049	16.69
Model DF	4	4	4	4	4	4
Error SS	0.9738	0.0529	0.0490	0.9439	0.0947	4.22
Error MS	0.1948	0.0106	0.0098	0.1888	0.0189	0.8447
Error DF	5	5	5	5	5	5
F Ratio	19.53	7.02	6.22	0.35	4.13	15.50
F Table	5.19	5.19	5.19	5.19	5.19	5.19
$R^{2\%}$	75.76	52.91	49.86	5.26	39.76	71.26
Std.Dev	0.7398	0.1339	0.1394	0.3867	0.2228	1.04
Mean	39.06	1.76	0.8060	2.22	41.99	14.16
C.V.%	1.89	7.59	17.29	17.39	0.5307	7.33

Table 4: Regression coefficient of full second order model and significant terms for chemical composition of developed breads

Coefficient	Moisture (%)	Fiber (%)	Ash (%)	Fat (%)	Carbohydrate (%)	Protein (%)
Constant	39.06	1.76	0.8060	2.22	41.99	14.16
Linear terms						
HPMC	0.4121	0.1125	0.1154	0.0421	0.0971	-0.7792
Guar gum	0.3546	0.0875	0.0771	0.0687	0.1562	-0.7442
Thawing	-0.3438	-0.0158	-0.0171	-0.0371	-0.0096	0.4233
Frozen storage t	-1.17	-0.0208	-0.0238	0.0279	-0.0146	1.20

Ash content

The ash content of the developed breads varied from 0.39% to 0.99%. The maximum (0.99%) and minimum (0.39%) values were reported in the Experiment 2 and Experiment 13 having the combination of variables as 2 gram of Guar Gum, 1 gram of HPMC, with a thawing temperature of 30 °C after 15 days of frozen storage and 1 gram of Guar Gum, 1 gram of HPMC, with a thawing temperature of 50 °C after 45 days of frozen storage respectively. The Model F-value of 6.22, presented in ANOVA table 3, indicates that the model is statistically significant, with terms A and B being significant ($p<0.05$). The R^2 value of the model is 0.4986, indicating that it can explain 49.86% of the variability in the experiments. The ash content of the developed breads showed significant differences concerning Guar Gum and HPMC, both of which had a significant positive linear effect at the 1% confidence level. Conversely, thawing temperature and frozen storage time had no significant effect on the ash content of the developed breads. Studies by Chen *et al.* (2017) [6] and Kim *et al.* (2020) [7] have reported similar observation as they found higher ash content in bread formulations containing hydrocolloids compared to those without. Although the actual ash content remains unchanged, changes in moisture content can affect its concentration within the bread (Sabanis & Tzia, 2011) [13].

Fat content

The fat content of the developed breads varied from 1.59% to 2.79%. The maximum (2.79%) and minimum (1.59%) values were reported in the Experiment 14 and Experiment 13 having the combination of variables as 2 gram of Guar Gum, 1 gram of HPMC, with a thawing temperature of 50 °C after 45 days of frozen storage an 1 gram of Guar Gum, 1 gram of HPMC, with a thawing temperature of 50 °C after 45 days of frozen storage respectively. The Model F-value of 0.35, presented in ANOVA table 3, indicates that the model is statistically significant, with no significant model terms ($p<0.05$). The R^2 value of the model is 0.0526, indicating that it can explain only 5.26% of the variability in the experiments. The addition of hydrocolloids to frozen dough bread formulations typically does not significantly affect fat content. Hydrocolloids are low in fat and primarily used for their water-binding properties and dough rheology modification. Consistently, Chen *et al.* (2017) [6] and Kim *et al.* (2020) [7] also discovered no significant differences in fat content between bread formulations with and without hydrocolloids. These findings are in contrast with the results of Sudha and Rao (2009) [17], who found that the moisture and fat contents of puri increased marginally with the addition of hydrocolloids.

Carbohydrate content

The carbohydrate content of the bread ranged from 41.42% to 42.34%. The maximum carbohydrate content, 42.34%, was found in Experiment 16, which combined 2 grams of Guar Gum and 2 grams of HPMC, with a thawing temperature of 50 °C after 45 days of frozen storage. The minimum value, 41.42%, was reported in Experiment 17, which combined 0.5 grams of Guar Gum and 1.5 grams of HPMC, with a thawing temperature of 40 °C after 30 days of frozen storage. The Model F-value of 4.13, presented in ANOVA table 3, indicates that the model is statistically significant, with terms A and B being significant ($p<0.05$). The R^2 value of the model is 0.3976, indicating that it can explain 39.76% of the variability in the experiments. The carbohydrate content of the developed breads showed significant differences concerning Guar Gum and HPMC, both of which had a significant positive linear effect at the 1% confidence level. Conversely, thawing temperature and frozen storage time had no significant effect on the carbohydrate content of the developed breads. Sabanis *et al.* (2008) [14] and Lu *et al.* (2009) [9] found that the addition of various hydrocolloids to gluten-free bread formulations improved the retention of carbohydrate content by preventing the breakdown of starch granules. A similar observation was reported by Lazaridou *et al.* (2007) [8].

Protein content

Regarding the protein content of the developed breads, it varied between 9.72% and 17.92%. The maximum (17.92%) and minimum (9.72%) values were reported in the Experiment 13 and Experiment 23 having the combination of variables as 1 gram of Guar Gum, 1 gram of HPMC, with a thawing temperature of 50 °C after 45 days of frozen storage and 1.5 grams of Guar Gum, 1.5 grams of HPMC at 0 days (fresh) respectively. The Model F-value of 15.50, presented in ANOVA table 3, indicates that the model is statistically significant, with terms A, B and D being significant ($p<0.05$). The R^2 value of the model is 0.7126, indicating that it can explain 71.26% of the variability in the experiments. Table 4 also provides the probability values of coefficients for the linear model, showing that the levels of GG and HPMC had a significant negative linear effect at a 1% confidence level. In contrast, increased thawing temperature and storage time had a positive linear effect at the same confidence level on the protein content of the developed frozen dough bread. While freezing does not typically alter the protein content of dough significantly, it can affect the functionality of proteins, especially gluten. The formation of ice crystals during freezing may disrupt the gluten network, potentially impacting bread texture and volume. Therefore, employing proper freezing techniques is crucial to maintain the integrity of the gluten network and ensure consistent protein functionality.

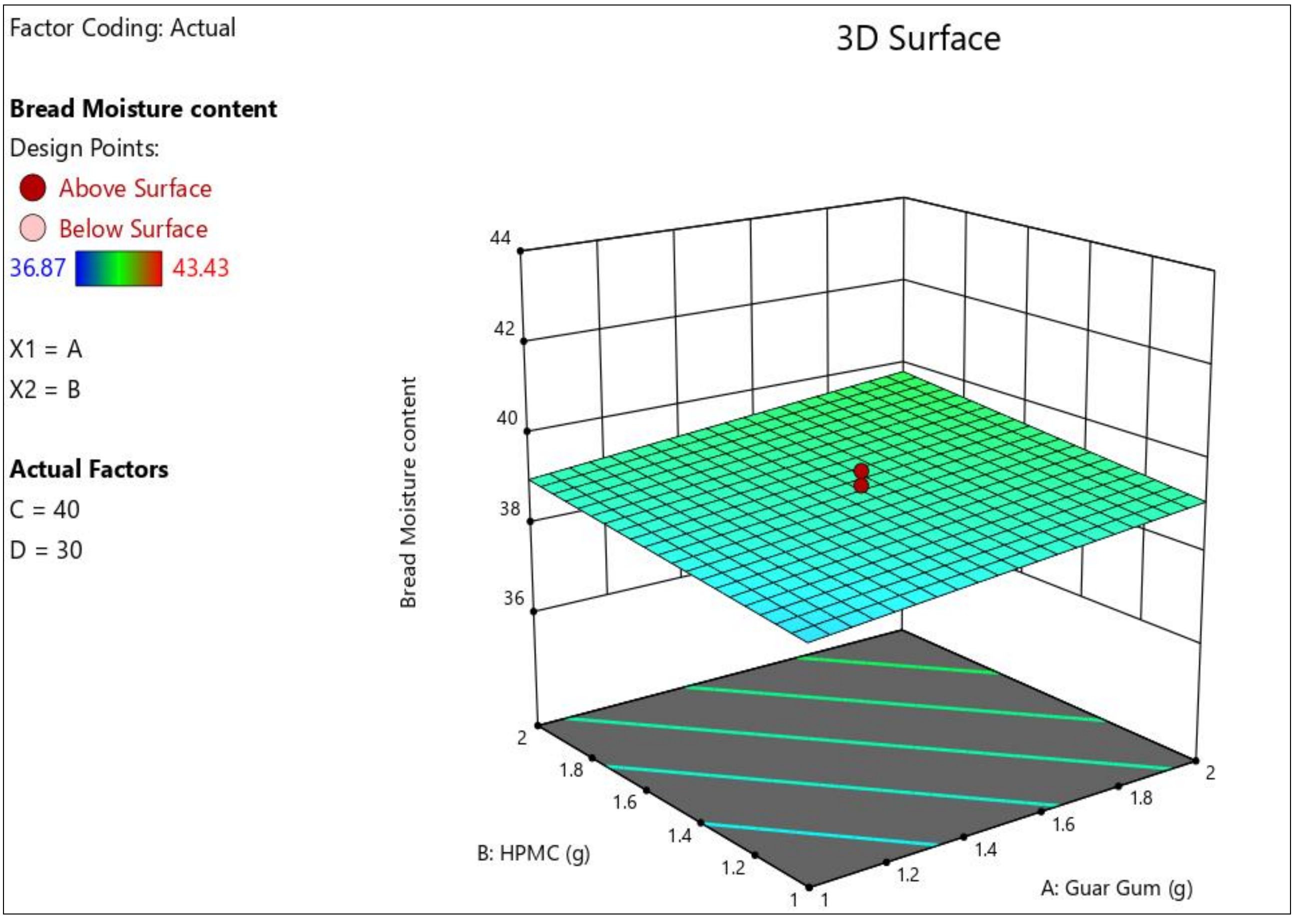

Fig 1: Moisture Content

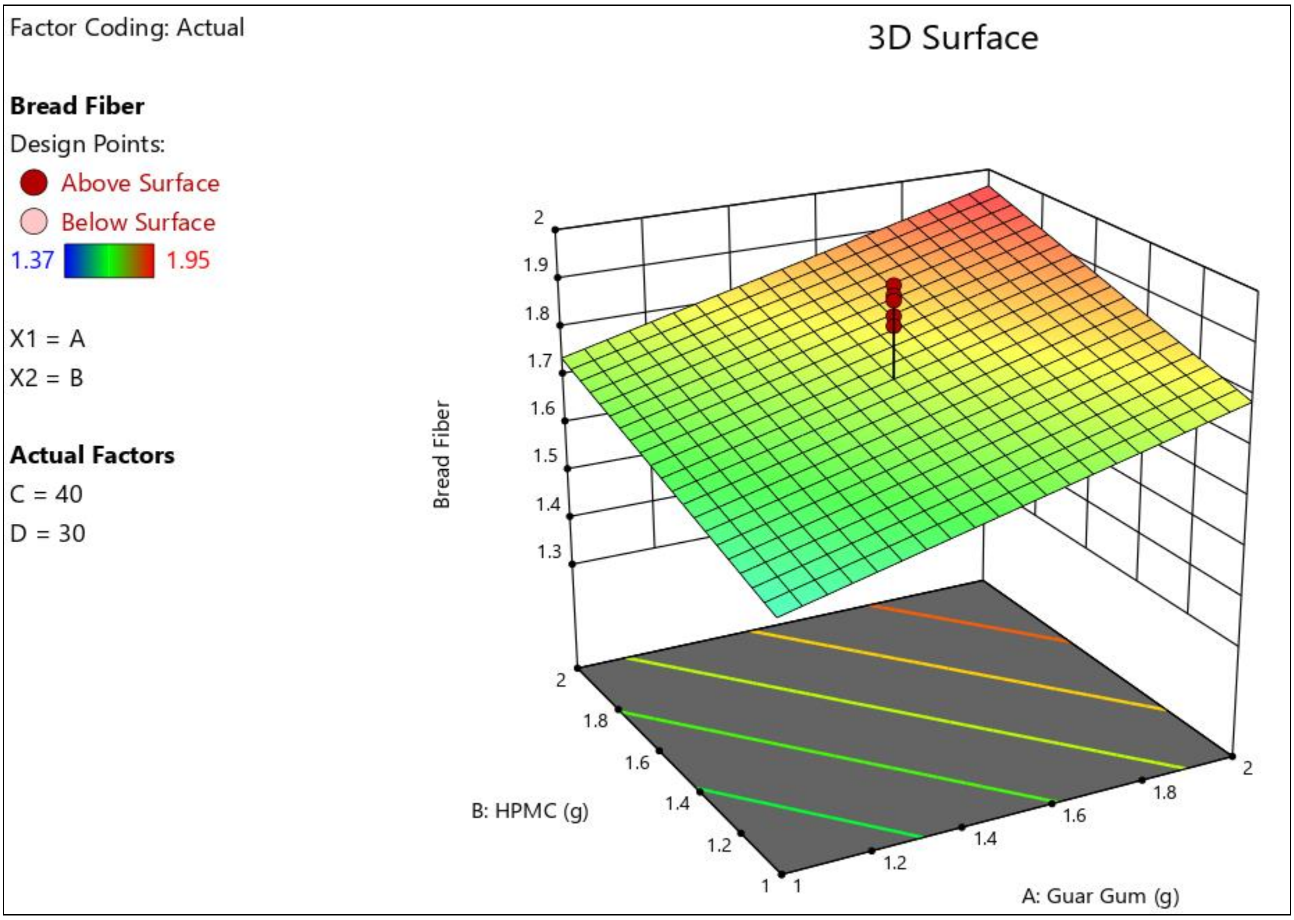

Fig 2: Fiber Content

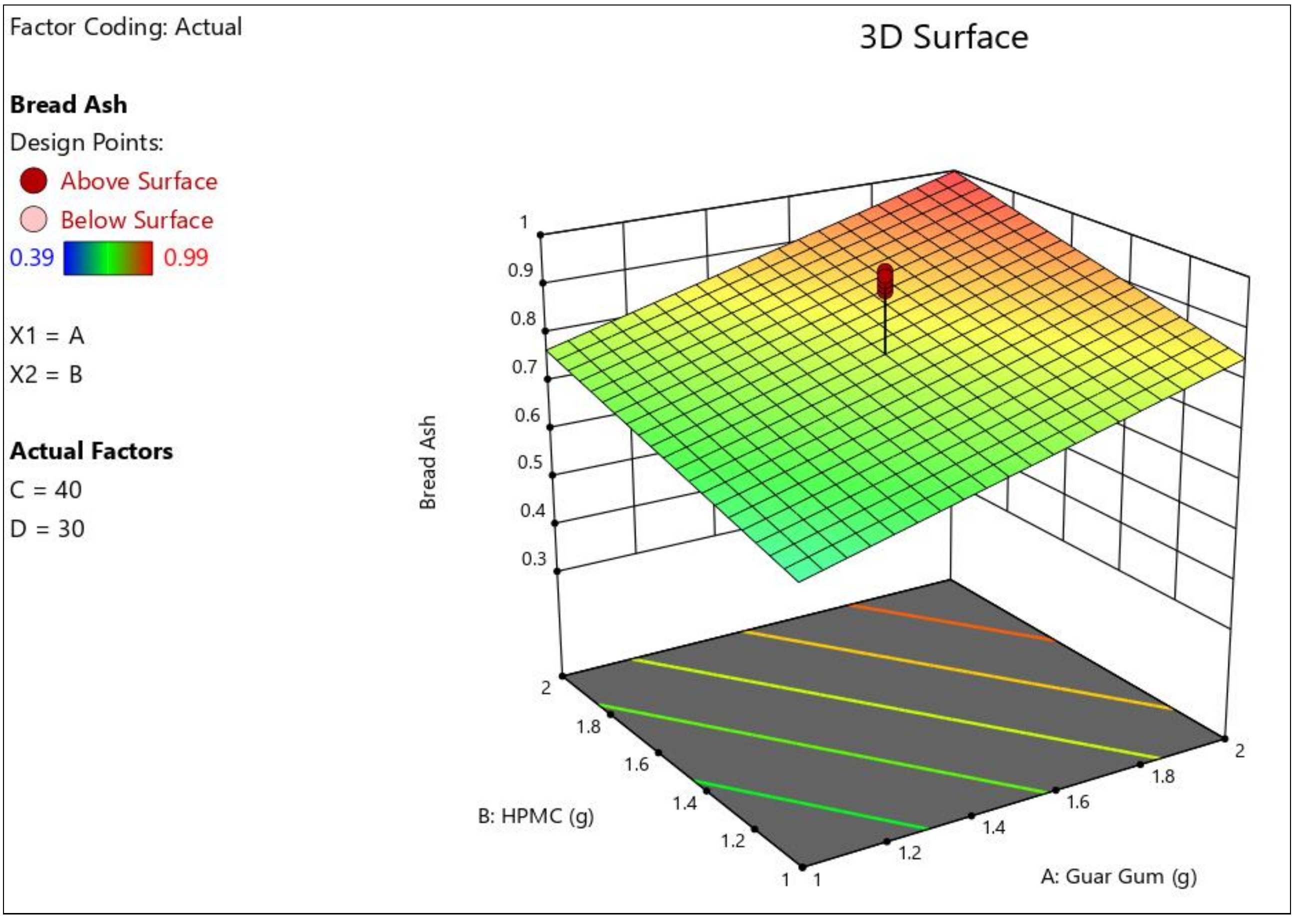

Fig 3: Ash Content

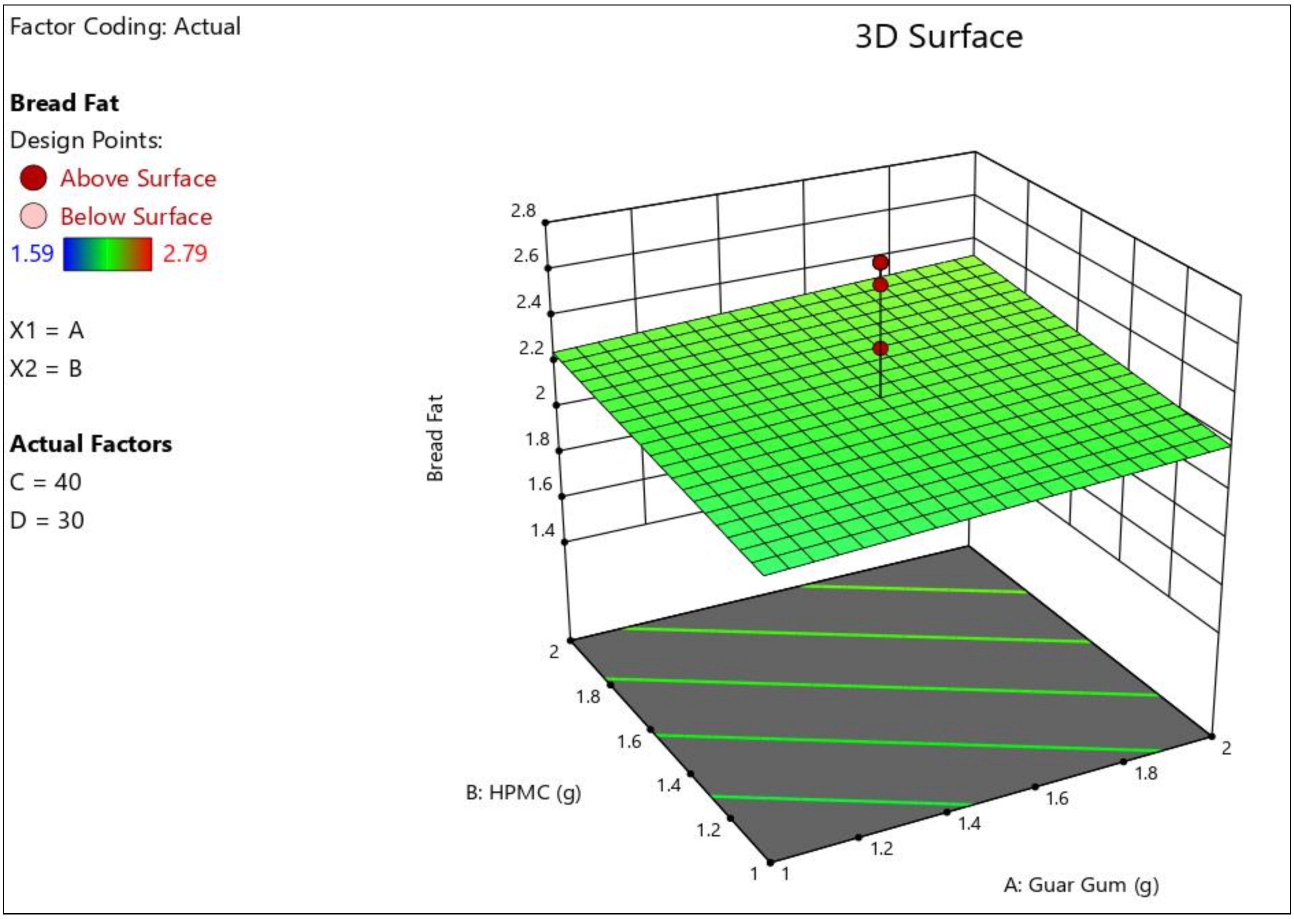

Fig 4: Fat Content

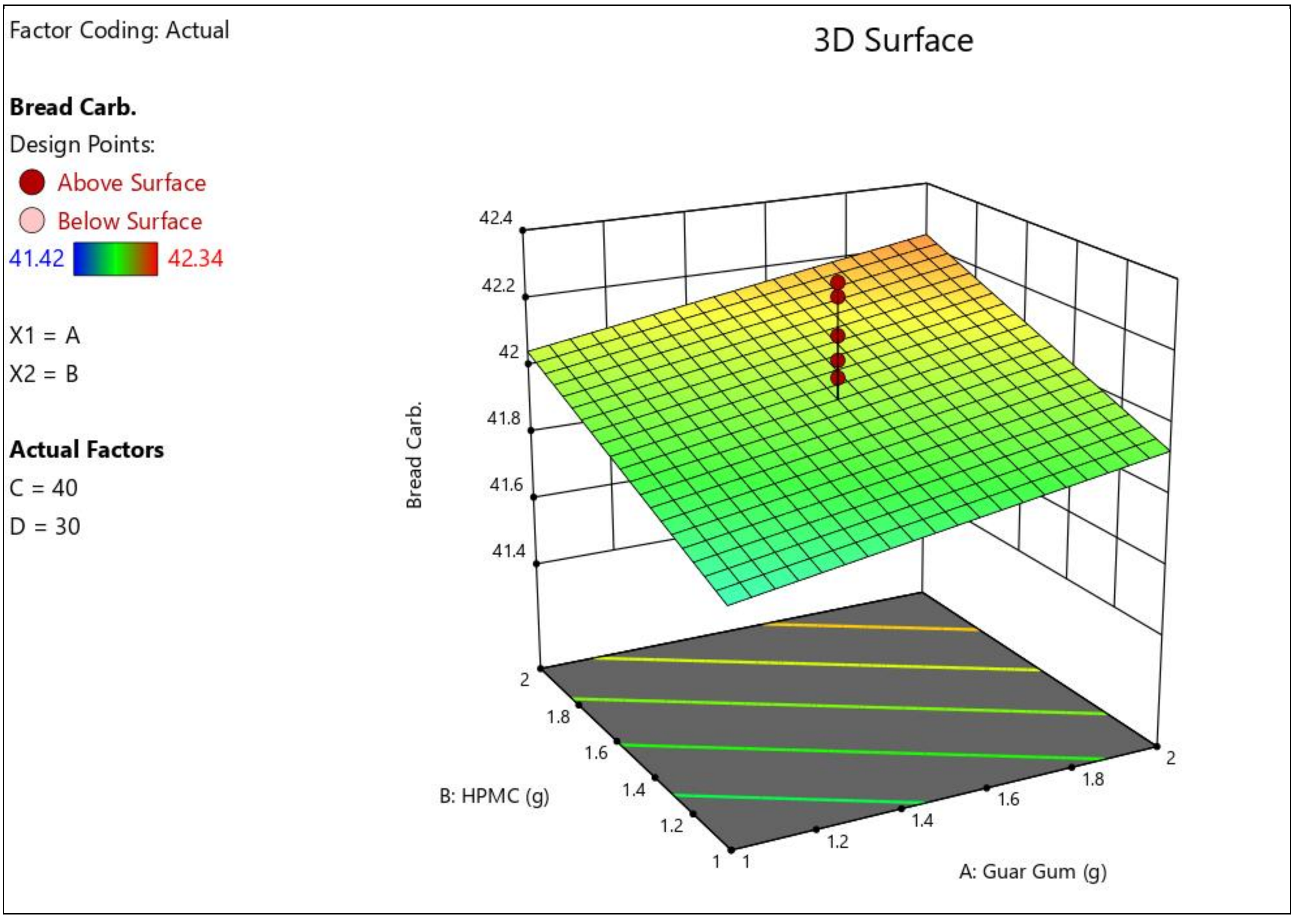

Fig 5: Carbohydrate Content

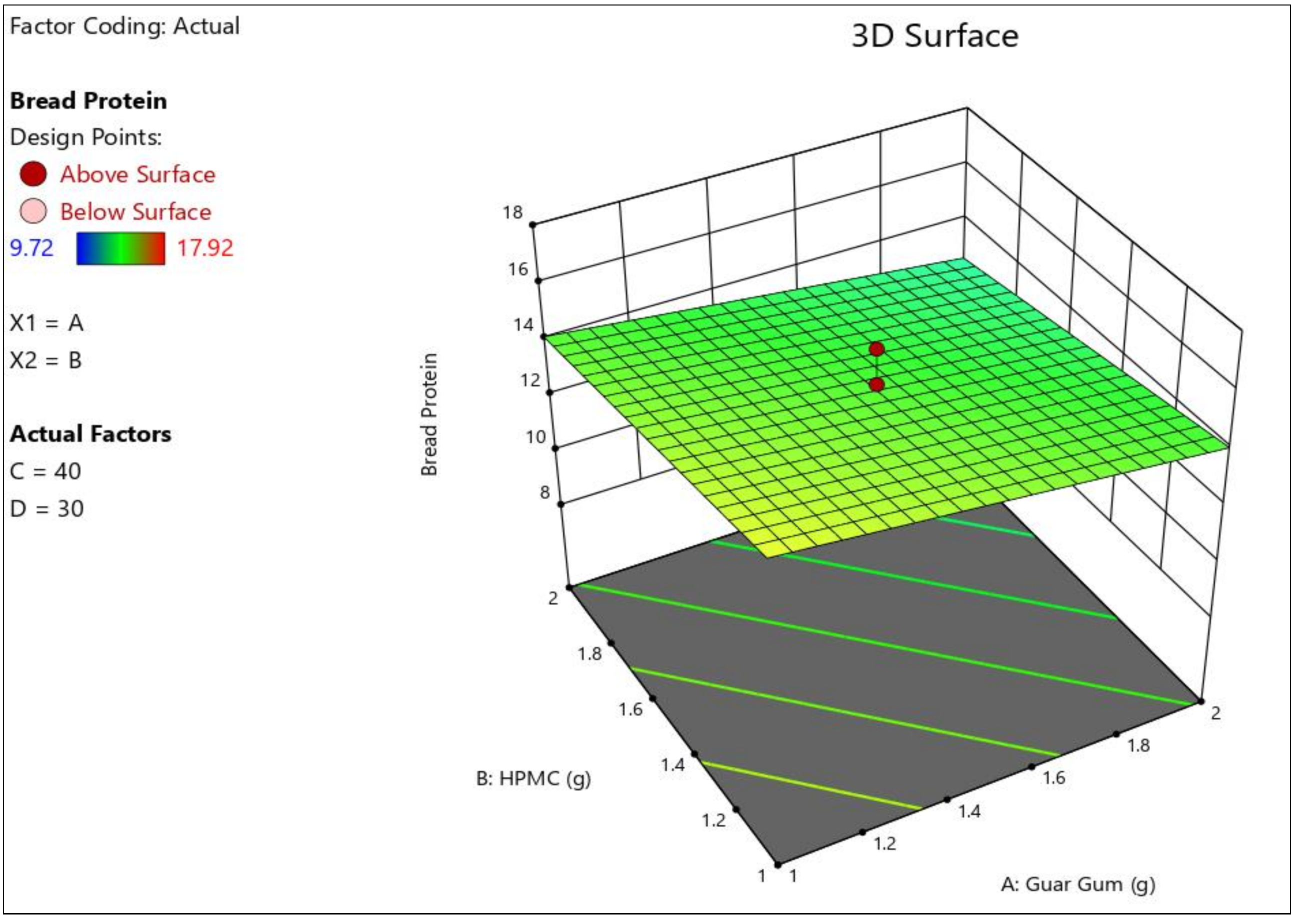

Fig 6: Protein Content

Conclusion

The present study emphasized the effect of hydrocolloids, frozen storage and thawing on the major nutritional content viz., proximate contents. 30 Different treatments were formulated using RSM software and based on extensive study and data analysis it can be concluded that proximate composition of bread, influenced by hydrocolloids, freezing storage duration and thawing temperature. Moisture significantly affected by Guar Gum and HPMC combinations which indicate moisture binding capacity of hydrocolloids. Fiber, ash, carbohydrate and protein content also fluctuated across experiments. Hydrocolloids notably impacted fiber, ash and carbohydrate but had minimal effect on fat content. Protein alterations due to freezing highlight the importance of proper freezing techniques to maintain gluten integrity. This study emphasizes the complex interplay of ingredients and freezing conditions in bread composition, aiding quality control. Further research in this area could explore additional variables to refine bread formulations and enhance product quality in diverse culinary contexts.

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