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Investigating the Impact of Wheat Bran and Heat-Resistant α -Amylase Enzyme Microcapsules on the Physicochemical Characteristics of Batter and Staling of Cake

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ABSTRACT

Staling represents a prevalent challenge within the bakery products sector, significantly contributing to the increased levels of waste in this industry. The objective of the present study was to assess the impact of microencapsulated heat-resistant α -amylase enzyme and wheat bran on the quality attributes of batter, as well as on the staling process of oil cake. Initially, α -amylase microcapsules were engineered utilizing varying ratios of chitosan and whey protein concentrate. The formulation comprising a ratio of 75:25 was identified as exhibiting the highest enzymatic efficiency, and was subsequently selected for further analysis. The microstructure was analyzed utilizing a scanning electron microscope (SEM). The microcapsule exhibited an almost spherical morphology characterized by irregular surfaces and depressions, along with observable accumulation. The present study investigates the influence of varying concentrations of wheat bran (ranging from 5% to 15%) and the inclusion of a micro-enzyme (at a concentration of 1%) on the properties of batter as well as the staling process of cakes. The findings indicate that the incorporation of wheat bran resulted in a statistically significant increase in both the viscosity and density of the cake batter ($p < 0.05$). However, the batters that included a combination of bran and micro-enzyme exhibited lower viscosity and density compared to those containing wheat bran alone. The incorporation of wheat bran, either as a standalone ingredient or in conjunction with enzyme microcapsules, resulted in a noticeable increase in the darkening of the cake color. Furthermore, this modification led to enhanced values of the a^* and b^* color indices when compared to the control group. Over time, there was a notable reduction in the moisture content of the cakes, accompanied by a corresponding increase in their hardness. The control sample exhibited the most pronounced intensity of these changes. The findings of this research indicate that the incorporation of wheat bran and α -amylase enzyme microcapsules facilitates the production of a functional cake characterized by a reduced staling rate.

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1. Introduction

Grain-based products represent a substantial component of the human diet, offering a diverse array of macronutrients and micronutrients. These foods are integral sources of protein, carbohydrates, dietary fiber, B vitamins, E vitamins, and essential minerals, including zinc and magnesium. However, during processing steps such as cooking and grinding, which are utilized to improve the palatability and preparation of these products, there is a concomitant decrease in certain bioactive compounds, resulting in a diminished nutritional value (1). In

contemporary society, there has been a notable increase in the demand for products that offer additional health benefits. Consequently, various industries are actively pursuing the fortification of food items with diverse bioactive compounds (2). Wheat bran is a highly nutritious material characterized by its significant content of dietary fiber and phytochemical compounds, including phenolic compounds. The consumption of wheat bran is associated with a myriad of health and physiological benefits, including a protective effect against breast and colorectal cancers, obesity, cardiovascular diseases, and various digestive disorders in humans (3). Wheat bran

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constitutes the outermost layer of the wheat kernel, which is separated as a by-product during the milling process. A small percentage of this bran is utilized for food applications (4). Numerous studies have demonstrated that wheat bran can enhance the nutritional value of products in the baking industry and facilitate the production of beneficial items (5).

Staleness represents a significant challenge within the bakery products industry. During the staleness process, various alterations transpire within the starch structure of the products. These alterations encompass the retrogradation of amylopectin and the rearrangement of polymers within the amorphous regions, in addition to the redistribution of water between the crystalline and amorphous regions (6). Enzymes represent a crucial class of additives within the baking industry, as they play a significant role in the standardization of flour, the improvement of the rheological properties of dough, and the enhancement of the texture of baked goods. Alpha-amylase enzymes represent a class of widely utilized enzymes that play a crucial role in the standardization and enhancement of flour. Moreover, they have been shown to extend the shelf life of baked goods by delaying the onset of staleness. This enzyme exerts its antibacterial properties by inhibiting the formation of the amylopectin network and diminishing its structural integrity, in addition to enhancing water stabilization (7). Commercial alpha-amylases are predominantly derived from microorganisms, although they can also be obtained from animal and plant sources. Microorganisms represent the most significant portion of the overall production of these enzymes. This phenomenon can be attributed to the rapid growth rates exhibited by microorganisms, which facilitate an expedited synthesis of enzymes (8). Enzymes hold a significant position within the food industry due to their catalytic properties. However, their functionality is markedly influenced by variations in environmental conditions, including temperature, ionic strength, and pH levels, rendering them susceptible to deactivation. Consequently, the implementation of efficient methodologies, such as the microcoating process, is essential for enhancing the stability of these materials and augmenting their resistance to degradation (9). In the process of microencapsulation, the central active material—potentially in the form of a liquid, gas, or solid—is enveloped by one or more wall materials (10). A diverse range of materials is utilized as wall constituents for capsules within the food industry. The agents selected for this purpose should possess low viscosity, be cost-effective, readily accessible, exhibit hydrophilic characteristics, and demonstrate biodegradability. Furthermore, it is essential that the formulation effectively encapsulates the primary active ingredient and provides protection against detrimental environmental conditions (11). Chitosan is recognized as one of the most prevalent and extensively utilized biopolymer materials for the microencapsulation of bioactive food compounds and additives. This biopolymer is characterized by its non-toxic nature, safety, hydrophilic properties, and significant mechanical strength, enabling it to effectively form a robust membrane. Furthermore, owing to its capacity to enhance stability in the presence of diverse chemical substances, it has been proposed as an advantageous wall material for the

protection and stabilization of enzymes (9). Enzymes demonstrate a high affinity for binding to the amino groups present in chitosan, with this interaction occurring via the formation of covalent bonds (8). Whey protein isolate, a derivative of dairy processing, represents one of the byproducts generated within the cheese manufacturing sector. This protein isolate exhibits not only substantial nutritional value but also advantageous physicochemical and functional characteristics. Whey protein isolate has the capacity to establish intermolecular interactions with various biopolymers, thereby functioning as an effective carrier that enhances the stability of active compounds. (12). The aim of this study was to examine the impact of microencapsulated heat-resistant α -amylase enzyme, along with the incorporation of wheat bran, on the quality and sensory attributes of dough and oil cake.

2. Materials and methods

2.1. Materials

Heat-resistant α -amylase enzyme, chitosan, Tween 80, and whey powder were procured from Merck, Germany. The culture media utilized in this study were procured from Qlab, Canada. The ingredients utilized in the formulation of the cake, specifically flour, wheat bran, eggs, sugar, salt, baking powder, and oil, were procured from local retail establishments within Tehran. All chemical reagents utilized in the experimental procedures were procured from Merck, Germany.

2.2. Preparation of heat-resistant alpha-amylase enzyme microcapsules

A chitosan solution and whey protein concentrate were prepared in a 75:25 ratio using deionized water as the solvent. The mixing and preparation of solutions were conducted within a one-hour timeframe, utilizing a magnetic stirrer. The process involved a fixed wall setup and a concentration ratio of 20% weight-to-weight. Subsequently, the prepared solutions were stored at refrigeration temperature for a duration of 24 hours to facilitate maximal water absorption. The pH of all solutions was meticulously adjusted to a value of 5.5 through the addition of hydrochloric acid. To mitigate the proliferation of microorganisms and inhibit microbial activity throughout the storage period, a concentration of 200 ppm of free sodium was incorporated into the samples. Tween 80 emulsifier and the enzyme were incrementally introduced to the mixture in a dropwise manner. The solution was homogenized using a magnetic stirrer for a duration of two minutes. Subsequently, the emulsion was promptly cooled using a cold phosphate buffer solution maintained at a temperature of 0-2°C while being subjected to mechanical stirring until the formation of spherical solid particles occurred. In conclusion, the resultant Korean solids were collected and filtered utilizing Whatman No. Three filter papers were utilized and subsequently rinsed with distilled water to eliminate any residual surfactants and enzymes. The

air-drying procedure was conducted at ambient temperature for a duration of 24 hours, resulting in the formation of discrete and free-flowing spherical solid aggregates. The synthesized microcapsules were stored at a temperature of 4°C (13). The efficacy of α -amylase enzyme microcapsules was assessed through the quantification of both free and encapsulated amylolytic activities. The activity of α -amylase was quantified using a spectrophotometer at a wavelength of 540 nm to assess the concentration of maltose generated. A unit of α -amylase is quantitatively defined as the quantity of enzyme that catalyzes the production of 1 micromole of maltose per minute. The encapsulation efficiency was ascertained using the following equation (13):

$$Y = 141.56x - 15.123(R^2 = 0.9904)$$

In order to evaluate the dimensions and morphological characteristics of the optimal microcapsule powder exhibiting the highest microencapsulation efficiency, a scanning electron microscope (SEM) was employed, operating at an accelerating voltage of 25 kV. For the purposes of this test, the powdered sample was deposited onto copper grids and subsequently subjected to a gold coating.

2.3. Preparation of Oil Cake Treatments

The composition of the oil cake was as follows: 100 grams of wheat flour, 24 grams of non-fat dry milk, 280 grams of sugar, 18 grams of egg white powder, 11.5 grams of baking powder, 250 milliliters of water, and 100 grams of oil. In the formulation of the cakes, wheat bran was incorporated as a partial substitute for flour, with replacement levels set at 5%, 10%, and 15%. An alpha-amylase enzyme microcapsule was incorporated into the formulation at a concentration of 1% by weight. To prepare the cake treatments, the oil and sugar were combined using a mixer for a duration of two minutes. Following this initial mixing process, water was subsequently incorporated into the mixture, and the components were stirred for an additional two minutes. The dry constituents of the formulation, which included flour, wheat bran, egg white powder, nonfat dry milk, and baking powder, were subjected to a sieving process. Following this step, the sieved dry ingredients were thoroughly mixed and subsequently incorporated into the aforementioned mixture, with a stirring duration of three minutes. The cake batter was subsequently introduced into the mold, and the baking procedure was conducted at a temperature of 170°C for a duration of 35 minutes. The baked cakes were allowed to cool and subsequently placed in plastic envelopes for storage at room temperature, maintained at $23 \pm 2^\circ\text{C}$, for a duration of 28 days.

2.4. Measuring the viscosity and firmness of cake dough

The viscosity of cake dough was assessed utilizing a Brookfield rotary viscometer manufactured in the United States. The viscosity and torque of each sample were assessed after a brief duration of one minute, utilizing varying rotational speeds within the range of 10 to 200 revolutions per minute

(rpm). In this experiment, the rheological parameters were assessed at a shear rate of 100 s^{-1} utilizing spindle number S0.6, conducted at an ambient temperature of 25 degrees Celsius (14). To evaluate the density of the cake dough, the volume of a 30-gram sample was determined immediately following its preparation, utilizing a graduated cylinder. The density of the dough was subsequently calculated by dividing the mass of the sample by its corresponding volume (15).

2.5. Examine the color of the cake batter

Dough color analysis was conducted by quantifying three color indices: L^* , a^* , and b^* , utilizing Image J software for the assessment. A sample image was obtained using a scanner set to a resolution of 300 pixels. Subsequently, the LAB color space was activated within the Plugins section to facilitate the calculation of the specified indices. The overall color change (ΔE) was computed using the equation presented below:

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$

2.6. Measurement of cake moisture content

The moisture content of the cakes was determined by subjecting them to heating at 105°C until a constant weight was attained (17).

2.7. Measuring the hardness of cake texture

To evaluate the staleness of the cake samples through the assessment of texture hardness over the storage period, cubic samples measuring $20 \times 20 \times 20\text{ mm}$ were extracted from the central portion of the cake. These samples were subsequently analyzed employing a Brookfield texture measuring device manufactured in Germany, utilizing an aluminum probe for the measurement. The samples were subjected to compression at a rate of 1 mm/s until achieving a maximum compression of 40% (18).

2.8. Statistical analysis of data

The experimental data were analyzed using one-way ANOVA to assess statistically significant differences among the mean values at a 95% confidence level, specifically in instances where a significant overall treatment effect was identified. Subsequently, Duncan's multi-range follow-up test was employed to ascertain these differences. The statistical analyses were conducted utilizing SPSS software, version 26, to derive the results.

3. Results and Discussion

3.1. Microencapsulation Efficiency of α -Amylase Enzyme Microcapsules

The investigation into the microencapsulation efficiency of enzyme microcapsules synthesized with varying ratios of chitosan and whey protein, as illustrated in Figure 1, indicated

that the microencapsulation efficiency of the microcapsules produced in this study ranged from 51.51% to 61.51%. The optimal efficiency was achieved with a chitosan coating to whey protein ratio of 25:75, yielding a weight percentage of 65.51%. Estevinho *et al.* (2014) appeared that microencapsulated yeasts with solvent chitosan: whey protein (1:29) had a microencapsulation proficiency of 66%. Horincar *et al.* (2019) detailed a microencapsulation proficiency of 59.07% for flavonoids in chitosan-whey protein coating.

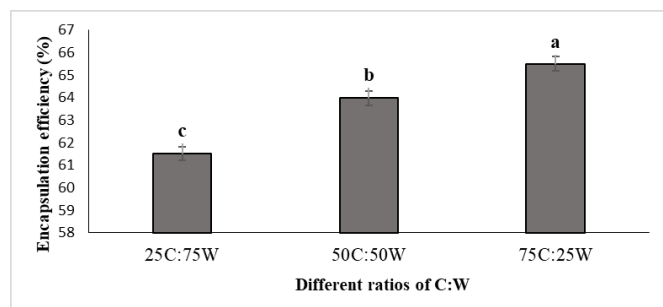


Fig.1. Encapsulation efficiency of α -amylase microcapsules prepared with different ratios of chitosan-whey. Bars represent mean ($n=3$) \pm SD. Different letters on the bars indicate significant difference at 5% level of probability among samples. C: chitosan; W: whey.

3.2. Morphology of α -amylase Enzyme Microcapsules

The morphology of the ideal microcapsule with the most noteworthy microencapsulation productivity (chitosan coating: whey (75:25)) is appeared by the SEM magnifying instrument, looking at the shape and the coming about picture in Figure 2. These microcapsules have sporadic shapes of diverse sizes and take after broken glass or a flake-like structure, which are characteristic of the powders gotten by the freeze-drying handle (21). The folds and spaces on the surface of microcapsules can too be credited to surface dissipation (22). Ponders have appeared that the coating of whey, in conjunction with carbohydrate polymers such as chitosan and maltodextrin, makes a more wrinkled divider, showing that microcapsules have lower penetrability to gases, increased security, and way better maintenance of dynamic substances. This is often since the combination of carbohydrates and proteins within the divider structure upgrades the warm resistance of proteins and decreases protein denaturation, in this manner expanding the effectiveness of the capsules and protecting their physical structure more successfully (23). Moreover, the SEM picture appeared that the agglomerated microcapsules had breaks on the surface, but no tearing was watched, which affirms their great basic astuteness. The nonappearance of the α -amylase chemical on the free surface demonstrates great embodiment execution. Analysts have basically detailed that the embodiment of bioactive compounds defined with chitosan as the divider fabric employing a drying strategy comes about in a circular, uniform, and flaky framework. (24, 25). Tavares et al. (2019) appeared an unpredictable and flake-like surface within the SEM comes about amid the encapsulation of garlic extricate within the chitosan: whey coating. Agglomerated

microcapsules with basic judgment and without the nearness of yeast on the free surface were moreover watched amid the microencapsulation handle of baker's yeast utilizing the combined coating of chitosan and cheese whey.

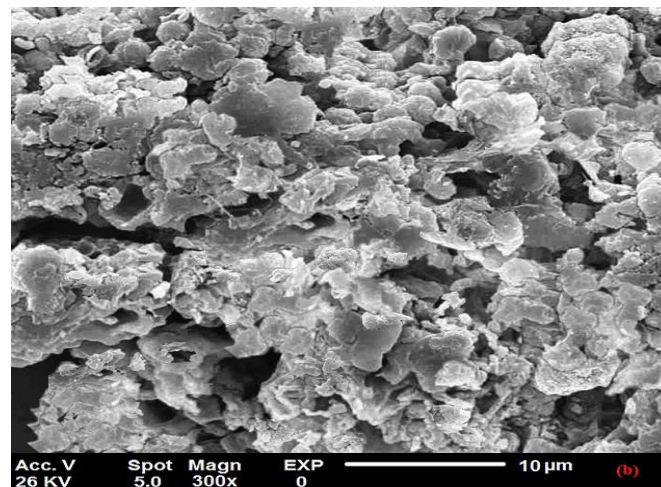


Fig.2. SEM image of α -amylase microcapsules prepared with 75% chitosan-25% whey.

3.3. Dough Viscosity

Batter arrangement is an vital step within the handling of flour items. The arrangement of a ceaseless arrange of wheat gluten gives both thickness and flexibility to batters. It is recognized that mixture quality is specifically controlled by the structure of the gluten arrange. Moreover, consistency plays a part in holding gasses that cause the batter to swell, which, in turn, is essential for the porosity of the ultimate item (27). The consistency values of glues with diverse details are appeared in Table 1. Concurring to the comes about, the least thickness was related with the control test (cP 6.2300), and the expansion of wheat bran, either alone or in combination with microenzyme, essentially expanded the consistency of the mixture compared to the control test. This increment was subordinate on the rate of bran; as the bran rate expanded, a more noteworthy increment in thickness was watched. This increment in consistency may be related to the higher water-holding capacity of wheat bran (28). In this respect, Lebesi *et al.* (2009) too appeared that the expansion of dietary strands to the mixture of cupcakes expanded their consistency and credited this to the assimilation of water by the filaments. In any case, the comes about of the display ponder appeared that the thickness of batters containing a combination of bran and microenzyme was lower than that of batters containing bran alone. This can be since the amylase protein assaults starch atoms and breaks them down into decreasing sugars. This disturbance within the starch structure can decrease the consistency of the mixture and, as a result, lead to a diminish within the consistency and gas-holding capacity of the batter. The microencapsulation prepare in microcapsules can for the most part diminish the consistency decrease of mixture caused by the α -amylase protein due to the slower discharge of the protein from the microcapsule structure (31). Zhang *et al.*

(2018) moreover gotten comparable comes about whereas examining the impacts of glucose oxidase chemical microcapsules on the properties of wheat mixture. Too, Yang *et al.* (2020), whereas examining the expansion of cyclodextrin glucosyl transferase chemical, appeared that when the chemical was included to the mixture, amylase hydrolyzed parts of the inner chain of starch, coming about in remaining amylopectin, little atomic starch clusters, and maltooligosaccharides, which diminish the consistency of the batter.

Table.1. The viscosity and density cake batter treatments.

Treatments	Viscosity (cP)	Density (g/cm ³)
Control	2300.6 ± 18.0 ^g	0.91 ± 0.06 ^e
WB5	3159.3 ± 8.6 ^d	1.09 ± 0.01 ^d
WB5+EM	2728.3 ± 17.5 ^f	0.92 ± 0.05 ^e
WB10	3263.3 ± 15.9 ^c	1.15 ± 0.00 ^c
WB10+EM	2846.0 ± 36.0 ^e	0.98 ± 0.01 ^e
WB15	4464.0 ± 60.8 ^a	1.50 ± 0.08 ^a
WB15+EM	3396.0 ± 27.2 ^b	1.22 ± 0.00 ^b

Values represent mean ± SD. Different letters indicate significant difference among treatments at 5% probability level. WB: Wheat bran; EM: Enzyme microcapsules.

3.4. Density of cake dough

The batter thickness test measures the alter within the thickness of a batter test, conducted beneath conditions similar to those within the maturation chamber. The least thickness demonstrates the greatest capacity of the mixture to extend (33). Batter thickness speaks to air circulation amid blending, and in common, lower thickness compares to more air circulation and, in this way, a better volume of the ultimate item. Consistency encompasses a noteworthy impact on batter thickness and cake volume since it decides the capacity of the mixture to hold gas amid blending and heating. Be that as it may, as it were an ideal thickness can lead to great cake quality, so controlling mixture consistency is pivotal (34). Agreeing to the displayed comes about (Table 1), distinctive medicines had a critical impact on mixture thickness. Including wheat bran alone or in combination with microenzyme essentially expanded mixture thickness; in any case, batters containing wheat bran alone had a better thickness than those containing the bran and microenzyme combination. The increment in thickness was subordinate on the rate of bran included to the batter detailing, with density increasing from 1.09 cm³/g within the control treatment to 1.50 cm³/g within the treatment containing 15% wheat bran. In the event that mixtures are less thick, they are likely to contain more air volume, which influences the ultimate volume of each cake. This volume is additionally impacted by the sum of gas misfortune amid handling, the potential collapse of the structure after cooking, and starch gelatinization (35). This behavior was too detailed within the inquire about conducted

by Sudha *et al.* (2007) and Majzoobi *et al.* (2016) after consolidating apple pomace flour and grain fiber into wheat flour mixture. Also, Majzoobi *et al.* (2012) appeared that including bran to wipe cake player essentially expanded its thickness. They detailed that the mixture thickness expanded due to bran's solid water-binding properties. In expansion, mixture containing more fiber can trap less discuss amid blending, coming about in higher thickness. The diminish within the thickness of batters containing wheat bran after microenzyme consolidation can be ascribed to the decreased soundness of the starch structure, which is basically dependable for the extension of the batter (37). Amylases can for the most part hydrolyze non-starch polysaccharides, subsequently diminishing the clear thickness of cake tests. Concurring to the comes about, Shahryari *et al.* (2019) appeared that the expansion of α-amylase diminished the thickness of cake hiter.

3.5. Cake batter color

The color indices of different cake batter treatments are presented in Table 2. The addition of wheat bran resulted in a significant decrease in the brightness index and a significant increase in the redness and yellowness indices, as well as the ΔE index of the dough samples. The effect of bran color intensity in food depends on the type of food and the amount added. Wheat bran contains natural dark pigments and has a larger particle size than wheat flour, which can influence the brightness of the dough (40).

Table.2. Color indexes of cake batter treatments.

Treatments	L*	a*	b*	ΔE
Control	69.74 ± 0.35 ^a	9.38 ± 0.29 ^f	12.48 ± 0.04 ^g	-
WB5	68.00 ± 0.12 ^b	12.01 ± 0.29 ^e	16.81 ± 0.05 ^f	5.36 ± 0.29 ^c
WB5+EM	68.38 ± 0.46 ^b	12.34 ± 0.18 ^e	17.48 ± 0.15 ^e	5.97 ± 0.55 ^c
WB10	58.85 ± 0.15 ^c	18.27 ± 0.50 ^d	24.75 ± 0.13 ^d	18.66 ± 0.42 ^d
WB10+EM	59.06 ± 0.55 ^c	19.31 ± 0.12 ^c	25.83 ± 0.82 ^c	19.81 ± 0.63 ^c
WB15	51.34 ± 0.42 ^d	22.30 ± 0.31 ^b	31.02 ± 0.44 ^b	29.14 ± 0.48 ^b
WB15+EM	51.55 ± 0.49 ^d	23.34 ± 0.53 ^a	32.11 ± 0.32 ^a	30.18 ± 0.51 ^a

Values represent mean ± SD. Different letters indicate significant difference among treatments at 5% probability level. WB: Wheat bran; EM: Enzyme microcapsules

Majzoobi *et al.* (2013) and Onipe *et al.* (2017) too detailed comparative discoveries. Ndlala *et al.* (2019) also showed that the incorporation of wheat bran into cereal dough resulted in diminished brightness and enhanced levels of both redness and yellowness in the mixture. The findings of this study revealed that incorporating microenzyme did not significantly alter the brightness index of cakes made with wheat bran; however, it did enhance the a*, b*, and ΔE indices of the dough. Research presents varying perspectives on the incorporation of enzymes into dough. Schoenlechner *et al.* (2013) demonstrated that the

incorporation of transglutaminase and xylanase proteins did not significantly affect the color parameters of the test samples following enzyme application. Nevertheless, it was observed that the levels of redness and yellowness demonstrated a decrease subsequent to the addition of transglutaminase. Kim *et al.* (2020) indicated that the inclusion of amylase and xylase in the formulation leads to a decrease in brightness, yellowness, and redness in frozen dough when compared to its fresh counterpart. Alp *et al.* (2008) demonstrated that the incorporation of trans-glucominase resulted in a decrease in both brightness and yellowness indices, whereas the redness index exhibited an increase.

3.6. Moisture content of cake

Moisture content constitutes an essential quality parameter that plays a significant role in determining the shelf life of food products. Throughout the cooking process, water evaporates from the surface of the dough and subsequently condenses within its interior. The properties of the dough, along with the baking conditions, play a crucial role in influencing the reduction of moisture content in baked products (44). The variations in moisture content of the oil cake treatments throughout the 28-day storage period at ambient temperature are presented in Table 3. During the initial phase of the storage period, the incorporation of wheat bran into the cake formulation, with an increase in its concentration from 5% to 15%, resulted in a significant elevation of the moisture content. The incorporation of amylase microenzyme into the formulation of bran-enriched cakes resulted in a significant reduction in the moisture content of the cakes ($p > 0.05$). The increase in moisture content observed in cakes enriched with wheat bran can be attributed to the presence of dietary fibers, which possess a significant capacity for water absorption and retention. Over the 28-day storage period, a significant reduction in moisture content was observed across the various treatment groups ($p < 0.05$). Initially, moisture content ranged from 16.19% to 19.04% on day one, while by the conclusion of the storage period, it varied from 12.25%. The increased humidity observed in the fiber-containing samples, in comparison to the control group, can be attributed to the structural modifications and alterations in the characteristics of insoluble fibers, which facilitate their transformation into soluble fibers. This conversion enhances the fibers' capacity to absorb moisture, particularly due to the presence of hydroxyl groups that enable water retention (36). The moisture-retaining capacity of microenzymes can be ascribed to the hydrophilic characteristics of the microcapsule wall, which facilitates the retention of moisture (7).

Narsaiah *et al.* (2019) demonstrated that the moisture content of bread samples enriched with garlic essence microencapsulated in alginate exhibited a significant increase. This enhancement in moisture retention can be ascribed to the hydrophilic properties inherent to alginate when utilized as a wall material. Nevertheless, the research conducted on free-form carbohydrase enzymes demonstrates that these enzymes do not influence the moisture content in products within the baking industry. Meng *et al.* (2020) exhibited that the

application of carbohydrate enzymes, specifically amylase and xylase, did not produce a statistically significant impact on the composition of rice cakes in comparison to the control sample throughout the duration of the storage period.

Table 3. Changes in moisture content and hardness values of oil cake treatments during the storage period

Treatments	Storage time (Day)	Moisture (%)	Hardness (N)
Control	0	16.55 ± 0.35 ^{A,de}	14.62 ± 0.13 ^{C,a}
	14	15.80 ± 0.08 ^{B,e}	19.43 ± 0.45 ^{B,a}
	28	12.25 ± 0.09 ^{C,e}	24.82 ± 0.45 ^{A,a}
WB5	0	16.97 ± 0.14 ^{A,d}	13.62 ± 0.21 ^{C,c}
	14	16.25 ± 0.22 ^{B,d}	18.59 ± 0.09 ^{B,c}
	28	13.74 ± 0.03 ^{C,d}	22.15 ± 0.36 ^{A,c}
WB5+EM	0	16.19 ± 0.18 ^{A,e}	13.98 ± 0.09 ^{C,b}
	14	15.72 ± 0.06 ^{B,e}	19.01 ± 0.24 ^{B,ab}
	28	12.43 ± 0.17 ^{C,e}	23.08 ± 0.02 ^{A,b}
WB10	0	18.63 ± 0.32 ^{A,ab}	12.75 ± 0.16 ^{C,e}
	14	17.46 ± 0.12 ^{B,b}	17.92 ± 0.09 ^{B,d}
	28	14.44 ± 0.06 ^{C,b}	21.60 ± 0.09 ^{A,d}
WB10+EM	0	17.60 ± 0.20 ^{A,c}	13.03 ± 0.09 ^{C,d}
	14	16.79 ± 0.05 ^{B,c}	18.80 ± 0.06 ^{B,b}
	28	14.06 ± 0.02 ^{C,c}	22.24 ± 0.25 ^{A,c}
WB15	0	19.04 ± 0.27 ^{A,a}	11.44 ± 0.17 ^{C,f}
	14	18.17 ± 0.15 ^{B,a}	16.89 ± 0.18 ^{B,e}
	28	14.77 ± 0.13 ^{C,a}	20.99 ± 0.34 ^{A,c}
WB15+EM	0	18.39 ± 0.16 ^{A,b}	11.45 ± 0.11 ^{C,f}
	14	17.57 ± 0.08 ^{B,b}	17.71 ± 0.03 ^{B,d}
	28	15.04 ± 0.33 ^{C,a}	21.28 ± 0.02 ^{A,c}

Values represent mean ± SD. Small and big different letters indicate significant difference among treatments and storage period at 5% probability level, respectively.

3.7. Moisture content of cake

Gomez *et al.*, (2007) demonstrated that the firmness of texture serves as a significant indicator of consumer acceptability in the baking industry, particularly regarding products such as bread and cakes. Consequently, a negative correlation exists between the hardness of the product's texture and consumer satisfaction, indicating that lower product hardness is associated with higher levels of consumer satisfaction. Firmness is quantified as the maximum force necessary to compress the cake to a predetermined degree at a specified rate of application.

Martínez-Cervera *et al.*, (2011) demonstrated that the texture firmness of cake is influenced by several interrelated characteristics, including the dough density, overall volume, degree of porosity, and moisture content of the final product. The array of factors influencing the formation and nucleation of bubbles within the dough, as well as the release of gas during the baking process, significantly determines the textural firmness of the resultant cake. Staleness, defined as the hardening of confectionery textures during storage, constitutes a multifaceted phenomenon influenced by several interrelated

factors. These include the retrogradation of amylopectin, the rearrangement of polymers within the amorphous regions, the distribution of moisture between amorphous and crystalline domains, as well as the overall loss of moisture content. The findings from the analysis of texture hardness variations in various cake treatments throughout the storage period at room temperature (refer to Table 3) indicate that the incorporation of wheat bran, whether utilized independently or in conjunction with microenzyme, resulted in a noteworthy reduction in the texture hardness of the cakes. The results obtained in this study align with previous research on dough density. Existing literature indicates that the texture of cake is significantly influenced by the density of the dough, a phenomenon that can be attributed to the quantity of air retained within the dough matrix. The observed reduction in tissue hardness associated with the incorporation of wheat bran may be attributed to the enhanced water-holding capacity of bran fiber, as well as the structural configuration of the fibrous network (49). During the storage period, a notable increase in the firmness of the cake texture was observed ($p < 0.05$), which can be attributed to moisture migration from the interior to the surface of the product, as well as the subsequent evaporation of moisture from the surface. Nevertheless, the texture firmness of the control sample was found to be significantly greater than that observed in the other treatment groups. One of the significant parameters influencing the staleness of cake is the migration of water between the outer shell and the internal core during the storage process. The translocation of water from the core to the shell of the cake may elucidate certain aspects of the hardening phenomenon observed in the core tissue during storage (50). Liu et al., (2023) demonstrated that the incorporation of wheat bran into chiffon cake yielded comparable outcomes during the investigation. A notable reduction in tissue hardness was observed subsequent to the introduction of the amylase enzyme. Amylase catalyzes alterations in the structural conformation of amylopectin and amylose, thereby modifying the overall network architecture of starch as well as influencing the interactions with other biomolecules, including proteins and lipids. The influence of amylase on the reduction of tissue hardness can be attributed to its enzymatic action, which facilitates the hydrolysis of glycosidic bonds in starch, resulting in the formation of dextrins with lower molecular weight. This process induces a delay in the formation of the double helix structure of amylopectin, thereby inhibiting the aggregation of amylopectin molecules into a cohesive three-dimensional network. This attenuation ultimately results in a reduction of viscosity (53). Furthermore, low molecular weight dextrins generated through enzymatic hydrolysis may function as emollients and contribute to the mitigation of hardness (54). The application of amylases in the preparation of baked goods appears to mitigate texture hardening of these products through three primary mechanisms: (1) a reduction in the particle size distribution of incorporated starch, (2) a decrease in the rigidity of the starch gel network, and (3) a diminished interaction between starch and protein. It is well established that the antimicrobial properties of α -amylases can be attributed to their ability to hydrolyze amylopectin, resulting

in the formation of soluble branched-chain polymers with low molecular weight (55). The investigation into the effects of incorporating carbohydrases, specifically amylases and xylases, revealed a notable reduction in the texture of the rice cake samples. The observed decrease in hardness can be ascribed to the hydrophilic characteristics exhibited by the wall materials utilized in microcapsules and nanoemulsions (46). In the course of examining the impact of amylase incorporation on cake quality, it was observed that there was a deterioration in the texture of cakes that included amylase. (37).

4. Conclusion

The findings of this study indicate that the incorporation of wheat bran, along with an increase in its proportion within the cake formulation, resulted in a marked enhancement in both the viscosity and density of the dough when compared to the control sample. Nonetheless, cakes incorporating treatments enriched with bran containing α -amylase microenzyme demonstrated reduced viscosity and density compared to those composed solely of bran. The incorporation of wheat bran into the dough, along with an increase in its concentration, resulted in a notable reduction in brightness intensity, accompanied by an enhancement of the redness and yellowness parameters. Additionally, these modifications contributed to a significant alteration in the overall color composition of the doughs. The incorporation of the microenzyme resulted in a noticeable enhancement of the color of the cake dough, exhibiting intensified red and yellow hues. The findings of the staleness evaluation of cakes, assessed through the parameters of moisture content and textural firmness throughout the storage period, demonstrate a significant influence of wheat bran, both independently and in conjunction with α -amylase microenzyme. This combination exhibited enhanced moisture retention and a reduced rate of textural hardening in comparison to the control group. From this analysis, it can be concluded that the integration of wheat bran and heat-resistant α -amylase microenzyme serves as an effective method for preserving the quality of cakes and mitigating the rate of staleness.

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