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The Effect of Using Alpha Amylase and Monoglyceride Enzymes on Pan Bread Quality

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ABSTRACT

The increasing consumption of pan bread, there is a pressing need to extend its shelf life while preserving its high quality. To achieve this, specific enzymes have been utilized to enhance both longevity and technological properties, ensuring the product remains viable in the market for a longer duration. This study was investigated the effects of alpha-amylase and monoglyceride enzymes in varying concentrations. The experiments include 9 samples (control, T_1, T_2, T_3, T_4, T_5, T_6, T_7 and T_8) respectively for studying the chemical composition, Rheological properties, sensory evaluation, physical properties, texture profile analysis of pan bread and staling evaluation were studied. The technological evaluation showed that the most effective formulations were T_3 (wheat flour (ext. 72%) + 0.03% α-amylase and T_7 (100% wheat flour (ext. 72%) + 1.5% monoglycerides) respectively. Also, showed that these formulations exhibited superior performance in sensory evaluation, rheological properties, and texture analysis. Additionally, they retained quality throughout storage periods of 2, 4, and 6 days. It can be concluded that alpha-amylase and monoglyceride enzymes in mold bread production can improve its shelf life and overall quality.

Keywords: Pan bread, alpha amylase, monoglyceride, rheological properties, staling and storage.

1. Introduction

Flour and bread are the main and strategically important food product of the world. The food security of a country depends on the state of the grain economy and the broad availability of the population. (Seibel, 2006) Food additives play a crucial role in ensuring food safety and reliability, helping to minimize potential issues during production (Al Shorman, 1999). One major challenge in bread preservation is staling, a complex process involving several physical and chemical changes that occur during storage, excluding microbial spoilage. These changes negatively impact consumer acceptance and result in substantial financial losses for both manufacturers and buyers. The primary physicochemical alterations associated with staling include starch retrogradation, starch-gluten interactions, and moisture redistribution among components (Gray and Bemiller, 2003).

Jiang *et al.* (2005) stated that one of the most notable effects of bread staling is the gradual increase in crumb firmness. As awareness of the adverse effects of synthetic additives grows particularly in developed nations there is a rising demand for natural, bioorganic, and chemical free food products.

Enzymes have become a preferred alternative to synthetic additives, as they do not remain active in the final product. Alpha-amylase is particularly significant in starch-based industries due to its ability to break down carbohydrates (Gupta *et al.,* 2003). Research suggests that incorporating amylases into baked goods enhances consumer preference and purchasing decisions. Consequently, amylases have been widely used in bread production as anti-staling agents and dough standardizers (Goesaert *et al.,* 2007).

Goesaert *et al.* (2005) also emphasized that α-amylases are considered highly safe for this purpose. Their application increases the availability of fermentable sugars, promoting yeast fermentation and enhancing the Maillard reaction, which contributes to better flavor and improved crust color.

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Additionally, monoglycerides constitute approximately one-third of the emulsifiers used in the baking industry and are widely recognized as anti-staling agents (Stauffer, 2000). These compounds help maintain bread softness after baking and throughout its shelf life, preventing rapid firming of the crumb (Gaupp and Adams, 2015).

The aim of study was using monoglycerides and alpha-amylase develop nutritious bread without the use of chemical additives. By enhancing its shelf life and maintaining superior freshness, the bread remains available to consumers for an extended period utilized.

2. Materials and Methods

2.1. Materials

Wheat flour (72% extraction) was sourced from South Cairo Mills Company, Giza, Egypt.Alpha-amylase enzyme was supplied by Industrial Zone in 6th of October City, while monoglyceride was obtained from Egypt Oil and Soap Company.Salt, sugar, dry yeast, and corn oil were purchased from the local market in Giza, Egypt.

2.2. Methods

2.2.1. Pan Bread manufacturing

The basic bread formula consisted of 100 g of wheat flour, 1 g of salt, 5 g of sugar, 2.0 g of active dry yeast, and 3 g of corn oil, with water added based on farinograph absorption, along with enzymes. Enzyme concentrations (0.01–0.04% α-amylase, 0.5–2.0% monoglycerides) were selected based on industry standards (Stauffer, 2000) and prior studies demonstrating efficacy in reducing staling (Goesaert *et al.,* 2005; Gaupp and Adams, 2015). The wheat flour, yeast, and water were mixed for 5 minutes, followed by the addition of all ingredients, which were then mixed for an additional 10 minutes until the dough reached the desired consistency. The dough was then divided into 100 g portions, hand-rounded, and allowed to rest for 5 minutes. Afterward, it was panned and left to ferment for 1 hour at 30 ±5°C with a relative humidity of 85%. Finally, the fermented dough was baked at 180°C for 20 minutes, following a modified method by Frutos *et al.* (2008).

Table 1: The ingredients of produced pan bread.

Ingredients (g)	Con.	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Wheat flour (72 %)	100	100	100	100	100	100	100	100	100
α-amylase	-	0.01	0.02	0.03	0.04	-	-	-	-
Monoglycerides	-	-	-	-	-	0.5	1.0	1.5	2.0
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sugar	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Corn oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Dry yeast	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Water %	As farinograph								

Control = 100% Wheat flour 72 ext. % (WF), T_1= 100 % (WF) 72 ext. % + 0.01 % α-amylase, T_2= 100 % (WF) 72 ext. % + 0.02% α-amylase, T_3= 100 % (WF) 72 ext. % + 0.03% α-amylase, T_4 = 100 % (WF) 72 ext. % + 0.04% α-amylase, T_5= 100 % (WF) 72 ext. % + 0.5 % Monoglycerides, T_6= 100 % (WF) 72 ext. % + 1.0 % Monoglycerides, T_7= 100 % (WF) 72 ext. %+ 1.5 % Monoglycerides and T_8 = 100 % (WF) 72 ext. % + 2.0% Monoglycerides.

2.2.2. Chemical Composition

Chemical Composition of Wheat Flour (72%) the chemical composition of wheat flour (72% extraction) was analyzed following the official methods of (AOAC 2019). The total carbohydrate content was calculated by difference, as described by Fraser and Holmes (1959), using the formula:

$$\text{Carbohydrate \% (dry basis)} = 100 - (\text{Protein \%} + \text{Fat \%} + \text{Ash \%} + \text{Fiber \%}).$$

2.2.3. Rheological properties

The farinograph and extensograph were used to evaluate the rheological properties of the flour samples at the Department of Bread and Dough, Food Technology Research Institute, Giza, Egypt, following the method outlined by (AACC 2016).

2.2.4. Sensory Evaluation

The sensory attributes of pan bread were assessed by ten panelists from the Food Technology Research Institute, Agricultural Research Center. The evaluation followed the AACC (2016) method, using a 100-point scoring system based on the following criteria: textures (15), general appearance (15), taste (15), odor (15), sponge (10), crust color (10), crumb color (10), distribution of crumb (10), and overall acceptability were calculated (100).

2.2.5. Physical Properties

The physical characteristics of pan bread were measured according to AACC (2016). These included: height, weight (w), volume (v), and specific volume v/w (cm^3/g) of the produced bread.

2.2.6. The texture profile analysis (TPA)

A Brookfield CT3 Texture Analyzer (Stable Micro Systems, USA) was used to assess the texture profile of pan bread. The analysis measured: Hardness (N), Cohesiveness, Gumminess(n) and Resilience. Samples were compressed twice to 40% of their original height, using the following settings: using settings as text-TPA, probe-36 mm cylindrical, Pre-text speed -2 mm/s, post- text speed -2mm. All experiments were conducted under ambient conditions.

2.2.7. Pan Bread Staling

Bread staling was assessed using the Alkaline Water Retention Capacity (AWRC) method, as described by (Kitterman and Rubenthaler 1971). The percentage of absorbed alkaline solution in a 5 g baked sample was calculated using the formula:

$$AWRC= (W2 - W1) / WS,$$

where W1 is the weight of the empty tube, W2 is the weight of the tube with the sample after centrifuge, and WS is the weight of the sample.

2.3. Statistical Analysis

Analysis of Variance (ANOVA) was performed on the organoleptic evaluation, staling, and biological experiments of different pan bread samples. The data were analyzed using a completely randomized design (CRD) with Microsoft Excel 2010. The Least Significant Difference (LSD) was calculated at a 0.05 significance level, following the method described by Levine *et al*. (1999).

3. Results and Discussion

3.1. Chemical composition of wheat flour (72% extraction)

Table 2 presents the chemical composition of 72% extraction wheat flour. The results indicate that its components are as follows: 85.01 % total carbohydrates, 13.0% moisture, 11.98% protein, 0.71% fat, 0.50% ash, and 1.80% fiber. These findings closely align with those reported by Abd El-Salam *et al.* (2018).

Table 2: Chemical composition of wheat flour (72% extraction).

Raw materials	Moisture	Protein	Fiber	Fat	Ash	Carbohydrates
WF (72%) ext.	13.00	11.98	1.80	0.71	0.50	85.01

3.2. Sensory evaluation of pan bread

Pan bread was evaluated by ten certified sensory panelists. The incorporation of monoglycerides and α-amylase into 72% extraction wheat flour significantly enhanced all sensory attributes. The addition of these enzymes in varying proportions improved key quality parameters, making it a crucial factor in assessing pan bread quality. Data in Table 3 indicate that there were significant differences ($p \leq 0.05$) among all enzyme ratios tested. While both 0.03% α-amylase (T_3) and 1.5% monoglycerides (T_7) significantly outperformed the control ($p \leq 0.05$), their near-identical sensory scores (95.0 and 95.95) suggest comparable consumer acceptability. Practical selection should consider secondary factors: T_3 may be favored for cost efficiency if α-amylase is cheaper. T_7 could be prioritized if its superior anti-staling performance (e.g., slower crumb hardening) aligns with extended shelf-life goals."Therefore, all subsequent experiments were conducted using these predefined enzyme ratios.

Table 3: Sensory evaluation of pan bread with enzymes addition.

Samples	Control	α-amilase				Monoglycerides			
	-	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Texture (15)	13.00^{d} ±0.22	13.50^{c} ±0.82	14.00^{b} ±0.91	14.30^{a} ±0.10	14.10^{a} ±0.23	13.70^{c} ±0.5	14.10^{b} ±0.60	14.50^{a} ±0.22	14.45^{a} ±0.33
General appearance (15)	13.00 ^{d}±0.21	13.50^{c} ±0.71	14.00^{b} ±0.84	14.50^{a} ±0.08	14.40^{a} ±0.01	14.00^{c} ±0.72	14.20^{b} ±0.91	14.50^{a} ±0.44	14.50^{a} ±0.32
Taste (15)	13.50^{d} ±0.75	13.00^{c} ±0.66	14.10^{b} ±0.99	14.50^{a} ±0.10	14.60^{a} ±0.05	14.00^{c} ±0.50	14.20^{b} ±0.78	14.50^{a} ±0.51	14.60^{a} ±0.94
Odor (15)	13.50^{d} ±0.80	13.00^{c} ±0.63	14.00^{b} ±1.20	14.50^{a} ±0.90	14.40^{a} ±0.47	14.00^{a} ±0.22	14.10^{a} ±0.32	14.50^{a} ±0.54	14.45^{a} ±0.47
Sponge (10)	7.50^{d} ±1.30	8.50^{c} ±1.22	8.50^{b} ±1.15	9.10^{a} ±1.00	9.20^{a} ±1.00	9.10^{b} ±0.9	9.30^{a} ±0.74	9.55^{a} ±0.51	9.60^{a} ±0.21
Crust color (10)	7.70^{d} ±0.85	8.50^{c} ±0.55	9.00^{b} ±0.89	9.50^{a} ±0.53	9.40^{a} ±0.33	7.50^{a} ±0.95	8.70^{a} ±1.58	9.50^{a} ±1.95	9.40^{a} ±1.00
Crumb color (10)	7.50^{d} ±0.87	8.00^{c} ±0.99	8.50^{b} ±0.84	9.10^{a} ±0.99	9.10^{a} ±0.89	8.00^{a} ±1.33	9.00^{a} ±1.20	9.50^{a} ±1.01	9.45^{a} ±0.87
Distribution of crumb (10)	7.80^{d} ±1.04	8.50^{c} ±1.00	9.00^{b} ±0.85	9.50^{a} ±0.56	9.43^{a} ±0.57	8.50^{c} ±0.91	9.00^{b} ±0.84	9.50^{a} ±0.50	9.44^{a} ±0.71
Overall acceptability (100)	83.5^{d} 2±1.40	86.5^{c} ±2.08	91.0^{b} ±2.02	95.0^{a} ±0.50	94.63^{a} ±0.01	88.6^{c} ±1.90	92.7^{b} ±2.28	95.95^{a} ±0.03	95.89^{a} ±0.04.

Control = 100% Wheat flour 72 ext. % (WF), T_1= 100 % (WF) 72 ext. % + 0.01 % α-amylase , T_2= 100 % (WF) 72 ext. % + 0.02% α-amylase, T_3= 100 % (WF) 72 ext. % + 0.03% α-amylase , T_4 = 100 % (WF) 72 ext. % + 0.04% α-amylase, T_5= 100 % (WF) 72 ext. % + 0.5 % Monoglycerides, T_6= 100 % (WF) 72 ext. % + 1.0 % Monoglycerides, T_7= 100 % (WF) 72 ext. %+ 1.5 % Monoglycerides and T_8 = 100 % (WF) 72 ext. % + 2.0% Monoglycerides.

3.3. Farinograph properties

The best sensory-acceptable pan bread samples were selected for further mold testing after completing the sensory evaluation experiment. The farinograph rheological test results was recorded in table 4 whish showed that water absorption decreased in T_3 (64.3%) and T_7 (63.5%) compared to the control sample (65.5%). The observed 1.2-2.0% reduction in water absorption with enzyme treatments aligns with established mechanisms of starch modification. α-Amylase (0.03%) hydrolyzes damaged starch, reducing its water-binding capacity by approximately 30% (Goesaert *et al*., 2005), while monoglycerides (1.5%) form starch-lipid complexes that limit water retention during storage (Purhagen *et al.*, 2011). This water redistribution improves dough handling without compromising final product quality. The dough development time increased in T_3 (1.5 min.) and T_7 (2.0 min.), while the control dough developed in 1.0 min. However, the arrival time remained constant at 1.0 min. across all samples. Additionally, the data in Table 4 indicate that enzyme addition enhanced dough stability, leading to a notable increase in stability time, while significantly reducing degree of softening (B.U). This effect is likely due to the functional role of enzymes in improving dough properties. These findings align with those reported by (Mohamed *et al.* 2019).

Table 4: Farinograph parameters of wheat flour substituted with addition of enzymes.

Sample	Water Absorption %	Arrival time (min)	Dough Development (min)	Stability time (min)	Degree of softening (B.U)
Control	65.5	1.0	1.5	4.5	80
T_3	64.3	1.0	2.0	8.5	70
T_7	63.5	1.0	1.0	9.00	60

Control = 100% Wheat flour 72 ext. % (WF)T_3= 100 % wheat flour ext.72 % (WF) + 0.03% α-amylase and T_7= 100 % wheat flour ext.72 % (WF) + 1.5 % Monoglycerides

3.4. Extensograph parameters.

The monoglycerides and impact α-amylase on the extensograph properties of dough were illustrates in table 5 of adding specific concentrations of. The results revealed that, compared to the control sample, the treated samples exhibited an increase in: Resistance of Extension (R) (B.U), Extensibility (E) (mm), and Energy (cm^2) dough extensibility (min) all increased. The enhanced dough qualities of bread dough could be explained by this dough softening effect. These findings aligned with the findings of Al-Refaie *et.al.* (2018).

Table 5: Extensograph parameters of wheat flour substituted with addition of enzymes.

Sample	Resistance of extension (R) (B.U)	Extensibility (E)(mm)	Proportional number (R/E)	Energy (cm^2)
Control	200	120	1.66	30
T_3	300	150	2.00	45
T_7	400	170	2.35	47

Control = 100% Wheat flour 72 ext. % (WF)T3= 100 % wheat flour ext.72 % (WF) + 0.03% α-amylase and T_7= 100 % wheat flour ext.72 % (WF) + 1.5 % Monoglycerides

3.5. Physical characteristics of pan bread

Bread containing enzymes exhibited higher specific volume, porosity, and aldehyde content compared to the enzyme-free control the data was recorded in Table 6. These findings align with data reported by previous researchers (Kim and Yoo, 2020; Baratto *et al.,* 2015).

According to Kostyuchenko *et al.* (2021), the addition of α-amylase enzyme significantly improves the physical properties of bread. Additionally, monoglycerides, which directly influence starch retrogradation, are widely used to increase loaf volume and enhance crumb softness (Purhagen *et al*., 2011).

Table 6: Physical properties of pan bread

Sample	Weight (g)	Volume (cm^3)	Specific volume (cm^3/g)
Control	117.5 ^{a} ±0.3	400 ^{b} ±3.5	3.40 ^{b}±1.9
T_3	117.0 ^{a} ±0.2	443 ^{a} ±1.5	3.78^a ±0.8
T7	116.80 ^{b} ±0.4	440 ^{a} ±2.0	3.77 ^{a}±0.9

Control = 100% Wheat flour 72 ext. % (WF)T_3= 100 % wheat flour ext.72 % (WF) + 0.03% α-amylase and T7= 100 % wheat flour ext.72 % (WF)+ 1.5 % Monoglycerides

3.6. Effect of texture measurement on pan bread storage for different time.

The results were showed in Table 7, indicated that the initial hardness values for bread containing α-amylase (0.3%) and monoglycerides (1.5%) were lower than those of the control sample. Over time, hardness increased across all samples, demonstrating the effectiveness of enzymes in maintaining bread freshness and slowing the firming of the crumb.

While cohesiveness and resilience decreased in all pan bread samples, this trend is consistent with the findings of (Gambaro *et al.* 2006 and Caballero *et al.* 2007).

According to Table 8, the AWRC (Alkaline Water Retention Capacity) values were highest at zero time across all samples but decreased over 2, 4, and 6 days of storage. In enzyme-enriched bread samples, AWRC values initially increased, whereas in the control sample, they showed a steady decline. This indicates that enzymes enhance moisture retention, delaying the staling process. Statistical analysis confirmed that the control sample exhibited the highest staling values, as shown in Table 8. These results align with the findings of (Al-Refaie *et al.* 2018), further supporting the positive role of enzymes in extending bread freshness.

3.7. Staling evaluation of pan bread during storage at 25±1°C

The crust of bread acquires a delicate, leathery texture due to moisture migration from the crumb were illustrated in Table 8. Research has shown that amylopectin recrystallization remains the primary factor in pan bread staling.

The addition of α-amylase has been proven to reduce amylopectin retrogradation and slow the firming rate of the crumb. This enzyme plays a crucial role in modifying the viscoelastic properties of dough by binding to the amylose fraction of wheat starch at the high temperatures required for baking (Caballero, 2007). By doing so, α-amylase slows starch retrogradation during cooling and storage (Gaupp and Adams, 2015).

To extend the shelf life of bread and prevent spoilage, specific enzymes were incorporated. As indicated in Table 8, the AWRC (Alkaline Water Retention Capacity) values were highest at zero time across all samples. In enzyme-enriched pan bread, AWRC values increased over time, whereas in the control sample, they declined. This demonstrates the moisture retention ability of enzymes, which helps delay the staling process.

Statistical analysis confirmed that the control sample had the highest staling values, reinforcing the effectiveness of enzyme addition. These findings align with those reported by Al-Refaie *et al*. (2018).

Table 7: Textural profile analysis of pan bread during storage at 25±1°C.

Characteristics	Storage time	Control	T_3	T_7
Hardness (n)	Zero time	9.95	8.50	8.42
	2 day	12.5	9.25	9.18
	4 day	20.60	12.00	11.89
	6 day	27.70	15.30	15.21
Cohesiveness	Zero time	0.45	0.50	0.51
	2 day	0.41	0.48	0.49
	4 day	0.35	0.44	0.45
	6 day	0.30	0.41	0.42
Gumminess(n)	Zero time	4.50	3.60	3.55
	2 day	6.50	3.75	3.70
	4 day	8.55	3.91	3.98
	6 day	9.60	4.20	4.28
Resilience	Zero time	0.23	0.25	0.26
	2 day	0.20	0.23	0.24
	4 day	0.19	0.20	0.22
	6 day	0.17	0.19	0.20

T_3= 100 % w Control = 100% Wheat flour 72 ext. % (WF) wheat flour ext.72 % (WF) + 0.3% α-amylase and T_7= 100 % wheat flour ext.72 % (WF) + 1.5 % Monoglycerides

Table 8: Alkaline water retention capacity (AWRC) of pan bread during storage.

Sample	(AWRC)% values for the storied samples			
	(AWRC)% Zero time	(AWRC)% 2 days	(AWRC)% 4 days	(AWRC) % 6 days
Control	280.30 ^{b} ±2.4	240.56 ^{b} ±1.9	200.000 ^{b} ±3.4	150.08 ^{b} ±3.4.
T_3	300.00 ^{a} ±1.2	290.56 ^{a} ±0.3	275.89 ^{a} ±2.0.	269.00 ^{a} ±2.8
T_7	302.10 ^{a} ±0.8	290.00 ^{a} ±0.4	274.000 ^{a} ±1.3	265.00 ^{a} ±2.5

Control = 100% Wheat flour 72 ext. % (WF)T_3= 100 % wheat flour ext.72 % (WF) + 0.03% α-amylase. and T_7= 100 % wheat flour ext.72 % (WF) + 1.5 % Monoglycerides

4. Conclusion

It can be concluded that from our study and the paper provides valuable insights into enzyme-assisted bread quality improvement. Alpha amylase and monoglycerides are important enzymes for improving the sensory qualities, physical attributes, rheological characteristics and staling values of pan bread made from 72% wheat flour.

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