Use of Gamma Polyglutamic Acid produced from the local strain *Bacillus cereus* ARM24 in dietary systems

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Abstract:

This study aimed to evaluate the functional role of adding gamma-polyglutamic acid (γ-PGA), as a natural improver in laboratory bread and yogurt production. The results showed that adding the polymer at a concentration of 0.8%, improved the rheological properties of the dough, by increasing development time and stability, and strengthening the gluten network. This resulted in a significant increase in the specific volume of the bread, reaching 3.45 cm^3 gm, and improving its texture and flavor thanks to the peptides formed. The results also demonstrated the effectiveness of γ-PGA in delaying staling, by increasing the pulp and skin's moisture retention capacity, and preventing water migration during 5-day storage. In the case of yogurt, the polymer showed excellent biocompatibility with lactic acid bacteria starters, without affecting their numbers, while also acting as a stabilizer, to reduce whey seepage and increase water-holding capacity by strengthening the casein network. Furthermore, it regulated acidity and pH during the 21-day cooling period, which improved the sensory evaluation scores for appearance, texture, and overall product acceptability.

Keywords: Gamma, Polyglutamic Acid, *Bacillus cereus* ARM24, dietary systems.

1-Introduction

Gamma-polyglutamic acid is an environmentally friendly material, non-toxic and not derived from chemical (petroleum) substances, biodegradable, soluble in water, edible, and does not induce an immune response [24]. It is a homopolymer biopolymer, consists of 1000–15000 D and L units of glutamic acid. These units are linked together by an amide bond between the alpha-amino groups of glutamic acid and gamma-carboxyl groups, located at the end of the side chain of glutamic acid [17].

Gamma-polyglutamic acid is a naturally occurring substance, produced by many Gram-positive microorganisms, secreted extracellularly as an extracellular polymer, this acid is primarily produced by Bacillus spp. [23,6,5]

Gamma-polyglutamic acid (GPA) is receiving significant attention in the field of biomedical applications. It is used in pharmaceuticals, medical adhesives, vaccines, nanotechnology for cancer treatment, tissue engineering, and the absorption and removal of heavy metals. This polymer can also be used in wastewater treatment and as a dye adsorbent, it is incorporated into some food products [24,5], as a thickener, emulsifier, or antifreeze. It also improves calcium absorption in the intestines. In addition, it is a key ingredient in many Eastern foods, such as natto, sago, and kinema [19,6]. It is also used to improve the physicochemical and structural properties of some fish products when added in different concentrations [22], and as a stabilizer in yogurt production [26]. In addition to its importance as a biofilm that

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relying on local microbial sources to support sustainable food industries. The study aimed to utilize γ-PGA, by applying it in baked goods as an anti-drip agent, and in yogurt production as a stabilizer and texture improver.

Two control samples were used: one without the addition of γ-PGA, and the other with the addition of gelatin as a stabilizer at a concentration of 0.2%. The milk was then heated to 90°C for 10 minutes and cooled to 45°C. The starter culture, consisting of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* subsp., was then added at a concentration of 3%, after activation using skim milk containing 10^9 colony-forming units/ml (CFU/ml) as lactic acid bacteria, it was thoroughly mixed and then distributed into 125 ml plastic containers, which were tightly sealed, then, it was incubated for 6 hours at 40°C, until the pH reached 4.6. Afterward, the containers were removed and stored in the refrigerator. Tests were conducted at 1, 7, 14, and 21 days of storage [9].

2-2-2 Bacteriological and Physical Tests of Yogurt

1- Lactic acid bacteria count

A series of decimal dilutions were prepared for each sample, and 1 ml of each dilution was cultured on MRS agar supplemented with 0.5% calcium carbonate (CaCO3). The plates were incubated at 37°C for 48 hours, and the number of colonies surrounded by transparent halos was counted [10].

weighed and mixed with 10 mL of deionized water. It was placed in a

glass flask, and a few drops of phenolphthalein indicator (1%) were added. The solution was titrated against 0.1 N sodium hydroxide (NaOH) until it

plays a role in extending the shelf life of some food and agricultural products [22].

The importance of this research stems from the need for natural and safe food additives, as well as the importance of

2- Materials and Methods

2-1 Production of γ-PGA from *Bacillus cereus* ARM24

γ-PGA was obtained from the local strain of Bacillus cereus ARM24, this strain was isolated from agricultural soil, after performing morphological, microscopic, and biochemical tests according to [16], a screening process was performed based on γ-PGA production, was done using a spectroscopic method. The most efficient isolate was then selected, and its identification was confirmed by 16S rRNA assay. It was registered in the GenBank under the code PV018397. The γ-PGA polymer was characterized using several techniques, such as FTIR, TLC, NMR, and HPLC, by comparing it to a standard γ-PGA prepared by Sigma-Aldrich (Germany).

2-2 Use of γ-PGA in Yogurt Production

2-2-1 Production of γ-PGA-Enriched Yogurt

Yogurt was produced by adding γ-PGA, derived from a local isolate, at concentrations of 0.04, 0.06, 0.08, and 0.1 gm 100 ml of skim milk, supplied by the Omani company Al-Mudhesh and recovered at a rate of 13% (w/v).

2- Measurement of Titratable Acidity

The percentage of titratable acidity in the yogurt samples was estimated as described in [8]. 10 gm of yogurt was

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following equation:

turned pink. The total acidity was then calculated based on lactic acid using the

$$Total\ acidity\ (\%) = \frac{\text{Ml No. of NaOH released} \times \text{Standard base} \times \text{Acid equivalent weight} \times 100}{\text{Sample weight}}$$

* The equivalent weight of lactic acid is 0.09.

2-3 Use of γ-PGA in Lab Loaf Production

Prepare wheat flour from the General Company for Grain Processing, Al-Mithaq Mill, Basra Branch, consisting of a blend of 15% Australian wheat, 15% American wheat, and 70% local wheat blend with a 70% extraction rate (first-grade fraction). Store the flour in nylon bags and keep refrigerated at 4°C until use.

2-3-1 Determination of the Rheological Properties of Wheat Flour Added to γ-PGA

Rheological tests were conducted on flour added to γ-PGA at the following rates: 0.2, 0.4, 0.6, and 0.8 gm per 100 gm of flour (w/w). The tests were performed at the Quality Control Laboratory of the General Company for Grain Processing in Baghdad, as follows:

1- Farenograph test

The test was performed according to the method described in AACC. (2000), and the following readings were taken:

Absorbance: This is the amount of water, in milliliters, required, to bring the center of the curve's apex to the 500 Brabender line. Absorbance is expressed as a percentage of the flour's weight based on 14% moisture content. It is calculated using the following equation:

3- **pH measurement**

The pH of the curd was measured using a pH meter, and the device was calibrated using buffer solutions [12].

3- Water Holding Capacity (WHC) Measurement

The water holding capacity of the curd samples was measured as described in [26].10 gm of curd were weighed and centrifuged at 3000 rpm for 20 minutes. The filtrate was then weighed. The water holding capacity was estimated using the following equation:

$$WHC = \frac{W2}{w1}$$

W1: Sample weight.

W2: Filter weight.

4- Susceptibility to Syneresis

The susceptibility of whey to syneresis was estimated as described in [3]. 10 gm of the curd sample were placed on filter paper (Whatman No. 4) at a temperature of 4°C. The volume of whey that drained after 2 hours was measured using a graduated cylinder.

2-2-3 Sensory Evaluation of Yogurt

The sensory evaluation of yogurt was conducted, by ten reviewers, including professors and graduate students from the Department of Food Science, College of Agriculture, University of Basrah, according to [13].

$$Absorption = \frac{(X - Y + 300)}{3}$$

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Dough stability time: This is the time, in minutes, for the peak of the curve to remain above the Brabender 500 line.

Dough elasticity: This is measured in Brabender units and is the point at which the curve drops below the Brabender 500 line 12 minutes after the curve reaches its maximum height.

Farinograph number: This is a single number that provides a general overview of the dough's properties, including its development time, stability, and elasticity. It typically reflects the strength of the flour.

conditions for another 10 minutes. Shape the dough and place it in pre-greased molds. Grease the molds lightly with butter for the final proofing for 60 minutes under the same conditions. Bake the dough pieces in a preheated electric oven at 180°C for 45 minutes.

2-3-3 Study of the qualitative characteristics of laboratory-made bread

The bread pieces were weighed after cooling, and their volume was measured using the displacement method with millet grains, as described in AACC. (2000), to calculate the specific volume shown in the following equation:

$$Specific\ volume\ \left(\frac{cm3}{gm}\right) = \frac{volume\ (\text{cm3})}{\text{weight (gm)}}$$

2-3-4 Sensory evaluation

The sensory evaluation of the lab-made bread was conducted by ten judges, including professors and graduate students from the Department of Food Science, College of Agriculture, University of Basrah, to assess its external and internal characteristics according to the data included in the sensory evaluation form prepared by the American Institute of Baking, according to [7].

Where:

X: The amount of water in milliliters required to make the center of the curve's apex lie on the 500 Brabender line.

Y: The weight of the flour, estimated in grams, equivalent to 300 grams of flour at 14% moisture content.

Dough development time: This is the time, in minutes, from the moment water is added until the curve reaches its maximum height.

2-3-2 Loaf Preparation

The one-stage straight dough method described in [1] was used to prepare the loaf. Table (1) shows the ingredients of the dough.

Table (1) Ingredients of the Laboratory Loaf Bread Mix.

Contents	Weight (gm)
Wheat flour	100
γ-PGA	0.2, 0.4, 0.6, 0.8
Water	According to Farinograph readings
Sugar	6
Hydrogenated fat	1
Yeast	3
Salt	1.5

Mix the dry ingredients together thoroughly. Prepare the yeast mixture by adding water at 30°C. Knead the ingredients well until the desired consistency is achieved and form the dough into a ball. Place the dough in a proofing container for the first proofing for 45 minutes at 30°C and 80-85% relative humidity. After the first proofing, manually release the gases by punching for 10 seconds, then leave the dough for a second proof under the same

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The method described in [1] in section (20-56) was followed to estimate the swelling power, and it was calculated using the following equation:

$$Swelling\ power = \frac{C - B}{B} \times 100$$

Where:

C: Weight of sample after impregnation

B: Weight of sample before impregnation

graduated cylinder, and adding 75 mL of deionized water. The contents of the cylinder were then thoroughly mixed for 15 minutes. Afterward, the mixture was left to stand for one hour to allow all the pulp to settle. It was then manually filtered, and the volume of pulp sediment was calculated in milliliters.

2-3-7 Statistical Analysis

The results were analyzed using Analysis of Variance (ANOVA). A Completely Randomized Design (CRD) was employed. Significant differences between means were compared using the RLSD test at a probability level of 0.05, using the SPSS version 23 statistical software.

2-3-5 Loaf Storage

Laboratory loaf made from wheat flour and containing varying concentrations of γ-PGA was placed in sealed nylon bags and stored at 4°C for periods of 1, 3, and 5 days to monitor for any freezing that might occur during these periods.

2-3-6 Swelling Tests

2-3-6-1 Swelling Power Test

2-3-6-2 Percentage of moisture in the pulp and crust

The percentage of moisture in both the pulp and crust of the laboratory bread was estimated according to the method described in [2].

2-3-6-3 pH Measurement

The method described in [2] was followed to determine the pH of bread produced and stored for different time periods. This was done by placing 1 gram of pulp in 10 ml of deionized water. The mixture was then filtered through filter paper, and the pH of the filtrate was measured.

2-3-6-4 Volume of Sediment

The volume of sediment was estimated using the method described by [18], by weighing 10 g of pulp in a 100 mL

3- Results and Discussion

3-1 Use of γ-PGA in Yogurt Production

3-1-1 Total Lactic Acid Bacteria Count

Table (2) shows the total lactic acid bacteria count in yogurt enriched with γ-PGA. Statistical analysis revealed no significant differences at the probability level ($P \leq 0.05$) (R.L.S.D = 0.159) with increasing γ-PGA concentration across all storage periods. The control sample (1) on day 1 showed the highest logarithm of bacteria count at 10.60 colony-forming units (CFU/ml). The lowest logarithm of bacteria count was recorded at the highest polymer concentration of 0.1%, at 10.47 CFU/ml. This is because γ-PGA is acidic due to the presence of free carboxyl groups (COOH-). The highest value was recorded during the final storage period (21). The control sample (1) had a value of 10.46. The lowest value was for the highest polymer concentration, reaching 10.33 colony-forming units/ml. These results are consistent with what was confirmed in [13], which stated that γ-PGA

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showed no significant effect in any of the treatments at a probability level (P≤0.05) on the first day. Acidity increased during the remaining storage periods, and all treatments showed significant differences. Concentrations of γ-PGA (0.08% and 0.1%) were less effective than the other concentrations and control samples for all storage periods. This is consistent with [13] indicated regarding the lack of effect of low γ-PGA concentrations on acidity development during refrigerated storage. These results also agree with the findings of [26], which showed that adding γ-PGA improves yogurt stability and reduces the sharp increase in acidity during refrigeration. This is attributed to the enhancement of γ-PGA casein network and restriction of the metabolic activity of lactic acid bacteria.

Table (3) Percentage of total titration acidity of γ-PGA-fortified yogurt during storage periods.

Treatments (%)	Storage periods (days)			
	1	7	14	21
0.04	0.76	0.90	1.06	1.25
0.06	0.76	0.88	1.02	1.22
0.08	0.74	0.85	0.93	1.09
0.1	0.74	0.83	0.89	1.02
Control Sample (1)	0.78	0.92	1.09	1.26
Control Sample (2)	0.77	0.90	1.04	1.24
$R.L.S.D_{0.05}$	TRT	SP	Interaction	
	0.079	0.056	N.S	

γ-PGA proved highly effective in slowing this decrease. 0.1% concentration treatment showed the best stability, reaching 4.11 at the end of the 21-day period, compared to the control sample 1, which had a pH of 3.51. This difference is very significant, as confirmed by the R.L.S.D. (Related has excellent biocompatibility with yogurt starters. Low levels of γ-PGA do not affect the number of lactic acid bacteria. However, the effect of γ-PGA during storage periods showed significant differences at a probability level (P ≤ 0.05) (R.L.S.D = 0.103) between the first and last storage days (21 days). The number of bacterial cells decreased gradually and continuously. This is due to the bacteria continuing to produce lactic acid, leading to a decrease in pH, which in turn causes bacterial cell death.

Table (2) Logarithm of the total number of lactic acid bacteria in γ-PGA-fortified yogurt during storage periods.

Treatments (%)	Storage periods (days)			
	1	7	14	21
0.04	10.58	10.55	10.50	10.44
0.06	10.54	10.52	10.48	10.40
0.08	10.50	10.47	10.43	10.39
0.1	10.47	10.44	10.39	10.33
Control Sample (1)	10.60	10.56	10.52	10.46
Control Sample (2)	10.58	10.55	10.50	10.44
$R.L.S.D_{0.05}$	TRT	SP	Interaction	
	0.159	0.103	N.S	

3-1-2 Acidity Test

Table (3) shows the acidity test results for yogurt fortified with γ-PGA. γ-PGA

3-1-3 pH

Table (4) shows a natural decrease in pH values when supplemented with γ-PGA, during the 21-day storage period. This is attributed to the continued activity of lactic acid bacteria during refrigeration.

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storage. Control sample 1 yielded a value of 0.31, and control sample 2 yielded a value of 0.37. This improvement is attributed to γ-PGA's high water-binding capacity and its electrostatic interaction with casein proteins. This leads to the formation of a denser, less porous protein network that effectively retains water. This mechanism aligns with the findings of [26] in his study, which confirmed that water carrying capacity increases with increasing γ-PGA content. Similarly, [13] study linked the decrease in whey separation with increased water carrying capacity in skimmed milk yogurt to the binding effect of γ-PGA. Water. Although water-holding capacity decreased significantly throughout the storage period up to day 21, γ-PGA samples maintained significantly higher water-holding capacity values than the control samples, indicating enhanced stability. The fact that the interaction between concentration and storage period was not significant suggests that the improvement provided by γ-PGA is relatively constant. Its rate of decline does not vary significantly over time, supporting its use as an effective long-term stabilizer under refrigerated conditions.

Table (5) Water holding capacity values of γ-PGA-enriched yogurt during storage periods.

Treatments (%)	Storage periods (days)			
	1	7	14	21
0.04	0.33	0.30	0.24	0.20
0.06	0.34	0.30	0.24	0.21
0.08	0.36	0.33	0.27	0.22
0.1	0.43	0.38	0.33	0.28
Control Sample (1)	0.31	0.29	0.22	0.18
Control Sample (2)	0.37	0.33	0.30	0.25
$R.L.S.D_{0.05}$	TRT	SP	N.S	
	0.054	0.039	0.169	

Interaction) value of 0.169. This effect is attributed to the role of γ-PGA as a stabilizer, strengthening the casein protein network, thus limiting the activity of lactic acid bacteria during refrigeration.

The results were consistent with [13] study, which found that adding γ-PGA at a concentration of up to 0.01%, did not affect the growth of lactic acid bacteria. This confirms that the role of γ-PGA is primarily to stabilize the product during storage, not to influence the initial fermentation process. [26] study also confirmed that the pH of yogurt fortified with 0.02% and 0.04%. γ-PGA was higher compared to the control sample and the other concentrations (0.06%, 0.08%).

Table (4) pH values of γ-PGA-enriched yogurt during storage periods.

Treatments (%)	Storage periods (days)			
	1	7	14	21
0.04	4.60	4.45	4.04	3.56
0.06	4.62	4.47	4.13	3.63
0.08	4.62	4.52	4.28	3.90
0.1	4.62	4.55	4.41	4.11
Control Sample (1)	4.61	4.44	4.01	3.51
Control Sample (2)	4.60	4.46	4.05	3.52
$R.L.S.D_{0.05}$	TRT	SP	Interaction	
	0.076	0.058	0.169	

3-1-4 Water Holding Capacity

Table (5) shows the water holding capacity values of γ-PGA-enriched yogurt during storage periods. The results demonstrate the effectiveness of this polymer as a stabilizer, as a concentration of 0.1% showed a significant difference compared to the control samples. It yielded the highest value of 0.43 on the first day of

3-1-6 Sensory evaluation

Table (7) presents the results of the panel test of yogurt enriched with γ-PGA. Clear differences exist between treatments in most of the studied sensory characteristics. The results showed that adding γ-PGA at increasing concentrations led to a noticeable improvement in appearance, texture, and overall acceptability. However, no significant difference was observed in taste. 0.08% concentration treatment showed the highest evaluation scores in all four characteristics (appearance 8.10, taste 7.55, texture 7.80, and overall acceptability 8.10). 0.1% concentration treatment was the most suitable in terms of improving sensory characteristics without negatively affecting taste. This improvement is attributed to γ-PGA's ability to enhance texture and increase the stability of the yogurt's colloidal structure. This is achieved through water retention and reducing syneresis (water separation), resulting in a more homogeneous appearance and smoother texture. Consumer-friendly. These characteristics also contributed to a higher overall acceptance rate compared to the control samples, which recorded lower values, particularly in the overall acceptance rate of control sample 1 (6.15).

Table (7) Sensory evaluation Form for Yogurt.

Treatments (%)	Panel test			
	Appearance (9)	Taste (9)	Texture (9)	General Acceptance (9)
0.04	7.20	6.90	6.95	7.30
0.06	7.50	7.30	7.30	7.50
0.08	8.10	7.55	7.80	8.10
0.1	8.10	7.40	8.15	7.90

3-1-5 Whey Permeability

Table (6) shows the whey permeability values of γ-PGA-enriched curd during storage periods. The results showed a gradual decrease in whey permeability with increasing γ-PGA concentration, reaching a maximum concentration of 0.1%. The lowest whey permeability value was 0.71 on the first day, showing significant differences compared to the control sample 2, which contained added gelatin as an artificial stabilizer. This confirms the ability of γ-PGA, as a stabilizer, to improve the curd's water retention capacity and strengthen the casein network. [13,26] indicated that γ-PGA forms a denser, less porous network due to its high water-binding properties, which reduces gel shrinkage and whey expulsion. Despite the significant increase in whey permeability during the storage periods (1-21 days), due to the rearrangement of the protein matrix, the treatments The γ-PGA-enhanced samples maintained significantly better relative stability than the control samples throughout the storage period.

Table (6) Whey permeability values (ml) for γ-PGA-enriched curd during storage periods.

Treatments (%)	Storage periods (days)			
	1	7	14	21
0.04	0.95	1.03	1.22	1.35
0.06	0.90	0.97	1.12	1.29
0.08	0.82	0.92	1.01	1.19
0.1	0.71	0.83	0.91	1.01
Control Sample (1)	1.10	1.21	1.30	1.41
Control Sample (2)	0.83	0.93	1.04	1.10
$R.L.S.D_{0.05}$	TRT	SP	Interaction	
	0.063	0.051	N.S	

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Sample (2)		0			Control Sample (1)	6.90	7.00	6.35	6.15
R.L.S.D$_{0.05}$	0.6193	N.S	0.852	0.747	Control	7.80	7.3	7.25	7.20

3-2 Use of gamma-polyglutamic acid in laboratory bread making

3-2-1-Effect of adding gamma-polyglutamic acid on the rheological properties of wheat flour

3-2-1-1 Percentage of water absorption

The results in Table (4-8) show no significant differences in water absorption between the control sample and the treatments containing γ-PGA. The addition of γ-PGA at the concentrations used did not affect the amount of water absorbed by the dough. Despite the hydrophilic nature of γ-PGA, the lack of significance here may be attributed to the polymer's role being to redistribute water within the gluten network, improving water retention rather than increasing its total quantity. These results are consistent with [4] regarding the role of γ-PGA in regulating the hydrophilicity within the dough without affecting the amount of water absorbed.

Table (8) Farinograph readings for wheat flour fortified with γ-PGA.

γ-PGA addition (gm)	Water absorbance (%)	Dough development time (%)	Stability (minutes)	Softening level after 10 minutes of starting	Softening level after 12 minutes from reaching the peak	FQN
0	57.4	4.7	5.3	81	124	62
0.2	57.4	5.5	6.5	50	96	78
0.4	57.2	6	7.2	41	93	86
0.6	56.5	5.5	8.1	36	78	95
0.8	56.3	6.3	8.6	30	78	101
R.L.S.D	N.S	0.64	0.72	7.67	12.92	10.99

dough development time indicates an improvement in dough strength and its need for longer mechanical stress, to achieve maximum cohesion, this is due to the formation of cross-links. This is due to the presence of γ-PGA between the gluten chains. These results are consistent with [11] and, [15] which confirmed that γ-PGA

3-2-1-2 Percentage of dough development time

Table (8) shows clear and significant differences between the control sample and the other concentrations. Except for the (4 and 8) gm concentrations, no significant differences were shown. This increase in

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significant difference between the control sample and all other concentrations except for concentrations (6 and 8) gm. This increase reflects an improvement in the qualitative characteristics of the dough. This number combines stability and development time. This result confirms that γ-PGA is an effective flour quality improver. It increases the market and manufacturing value of flour. This aligns with modern trends in the use of natural food additives that improve flour quality [11,20].

3-2-2 Baking Test and Sensory Properties of Laboratory Bread:

The results, shown in Table (9) and Figure (1), illustrate the physical and sensory properties of bread produced by fortifying wheat flour (70% extraction) with γ-PGA. Physically, although no significant differences were recorded at the probability level $P \leq 0.05$ in bread weight, the volume and specific volume showed a significant increase with increasing concentration. The specific volume was 2.84 cm^3 gm in the control sample, reaching a peak of 3.45 cm^3 gm at a concentration of 0.8%. This increase is attributed to γ-PGA's high ability to form a gel-like network within the dough, enhancing gluten elasticity and its ability to trap carbon dioxide gas [25]. This also reinforces the conclusions reached in [11] regarding γ-PGA's ability to protect the gluten network from deterioration during the baking process.

increases the flexibility and cohesion of the gluten network, thus requiring a longer time for the dough to fully develop.

3-2-1-3 Stability

Table (8) shows significant differences between the control sample and the other concentrations, while no significant difference was observed between the 6 and 8 gm concentrations. This increase demonstrates that the addition of γ-PGA gave the paste a higher resistance to mechanical stress for a longer period before the paste lost its elasticity. This result is consistent with [20] study which demonstrated that γ-PGA enhances paste stability by protecting the disulfide bonds in the gluten network, preventing rapid gluten dissociation, thus making the paste more stable and durable [4].

3-2-1-4 Dough Weakness Degree:

Table (8) shows a significant decrease in dough weakness degree for all concentrations. This decrease confirms the role of γ-PGA as a stabilizer that prevents the deterioration of the dough's structural integrity. These results support the findings of [15] that PGA reduces the loss of elasticity in the gluten network. It maintains the dough's structural integrity under conditions of continuous stress, thus improving flour quality and causing it to exhibit strong flour behavior.

3-2-1-5 Farinograph Number:

Table (8) shows the Farinograph Number (FQN) values, revealing a

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Table (9) Results of sensory evaluation of the external and internal characteristics of laboratory bread produced from 70% extraction flour with different concentrations of γ-PGA added.

Concentrate (%)			0	0.2	0.4	0.6	0.8	R.L.S.D
Weight (gm)			172.3	175.5	179.0	177.5	174.0	N.S
Volume (cm^3)			490	515	565	580	600	20.17
Specific volume (cm^3gm)			2.84	2.93	3.16	3.27	3.45	0.24
10	External properties	Volume mark	6	6	7	8	9	0.89
8		crust color	7	8	7	8	8	N.S
3		crust description	2	1	2	2	2	0.51
3		baking description	1	1	2	2	2	0.42
3		symmetry	1	1	1	3	3	0.20
3		Cut-off and spreading line	2	2	2	2	2	N.S
10	Internal properties	Granular description	7	7	8	9	9	0.54
10		pulp color	9	9	9	9	9	N.S
10		Pulp smell	8	8	8	8	8	N.S
15		Pulp taste	11	11	12	13	13	1.05
10		chewing	7	7	9	9	9	0.54
15		texture	9	11	12	13	15	0.59
100	Total		70	72	79	86	89	3.39

Regarding the internal properties of the laboratory bread, the results show an improvement in texture and chewiness. Texture scored 9 for the control sample and 15 for the 0.8% concentration. This change in the internal texture can be explained by the results of [4] study that used electron microscopy (SEM), to demonstrate that γ-PGA creates a denser and more homogeneous gluten network with fine and regular pores. The significant increase in chewiness also clearly indicates a decrease in pulp hardness. This is consistent with [15], who confirmed the effectiveness of γ-PGA in delaying bread retrogradation through its strong binding to water molecules and preventing their migration.

Regarding external characteristics, a significant improvement was observed in size, uniformity, and cut line at concentrations of 0.6 and 0.8%. This difference in the bread's external structure reflects the rheological stability that γ-PGA imparts to the dough. This aligns with the findings of [20] that γ-PGA enhances dough stability and its resistance to mechanical and thermal processing. Conversely, the crust color was not significantly affected. This confirms that γ-PGA does not negatively interfere with Maillard reactions, thus preserving the desired natural color of the bread.

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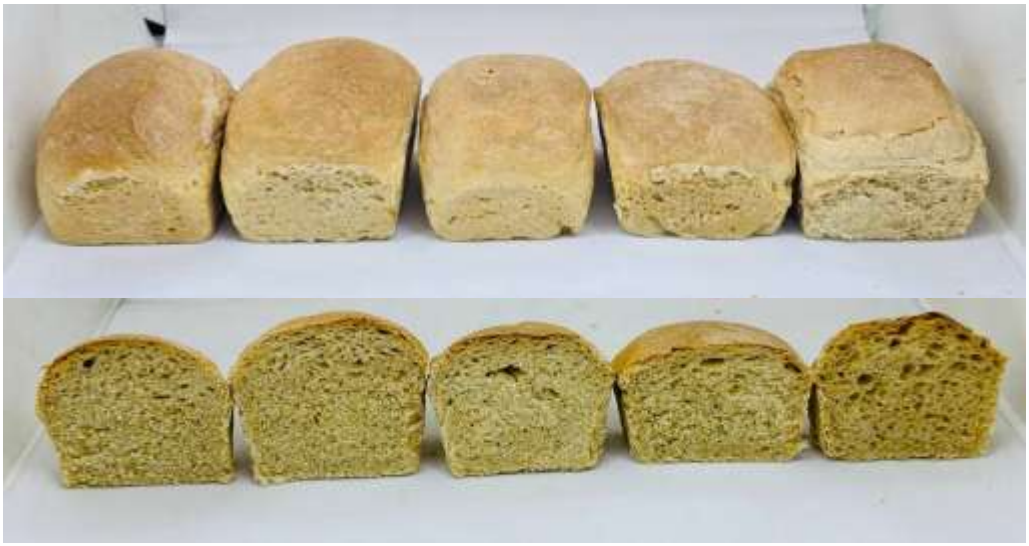

0.8 0.6 0.4 0.2 0

Figure (1) Effect of adding different concentrations of γ-PGA on the properties of laboratory bread.

flavor, a property that gives the taste a sense of depth, fullness, and continuity in the mouth by activating calcium receptors on the tongue. In addition, the polymer's hydrogel nature contributed to trapping the volatile aromatic compounds produced during fermentation and preventing their evaporation, leading to a concentration of the bread's natural flavor and making it more palatable and sensoryly superior compared to the control sample [14].

The results also showed no significant difference in aroma and pulp color values. However, there was a significant difference in taste between the control sample and the concentrations (0.6 and 0.8%). This change in taste of the laboratory bread is attributed to the functional role of γ-PGA, which acts as a biosynthetic source of gamma-glutamyl peptides, resulting from thermal hydrolysis during the baking process. These derived peptides act as enhancers of the kokumi

of the bread, demonstrates that a concentration of 0.8% γ-PGA represents the optimal ratio for producing high-quality bread.

The results also showed that the overall sensory evaluation score increased significantly from 70 to 89. This qualitative improvement in the rheological properties

laboratory bread, respectively. There were clear and significant differences between the concentrations and the control sample. 0.8% concentration resulted in the highest moisture content of 36.86% for the pulp after one day of storage, and 35.91% and 35.05% for the third and fifth days, respectively. This decrease in moisture distribution for the pulp was not significant with respect to storage periods. However,

3-2-3 Effect of adding gamma-polyglutamic acid on bread settling

3-2-3-1 Effect of adding gamma-polyglutamic acid on the moisture content of the pulp and crust of stored bread

Tables (10) and (11) show the moisture content results for the pulp and crust of the

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phenomenon was discussed by Saleh *et al.* (2014) in their study on the deterioration of local bread quality. They indicated that moisture loss is the primary factor causing staleness. The results are consistent with [25] and [4], which found that γ-PGA converts free water into bound water, thus preserving the softness of the pulp and preventing it from drying out. It also maintains the crust's texture by regulating vapor transport.

3-2-3-2 Effect of Adding Gamma Polyglutamic Acid on the Absorption Strength and Settlement Volume of Stored Bread Pulp

The results in Tables (12) and (13) indicate a significant improvement in the absorption strength and settling volume values of the laboratory bread fortified with γ-PGA compared to the control sample. 0.8% concentration recorded the highest values. This increase is attributed to the hydrophilic properties of γ-PGA, which enhance the ability of the pulp's molecular components (starch and gluten) to absorb water and swell, thus delaying the shriveling process. A decrease in absorption strength is a key indicator of bread quality deterioration and hardening, resulting from a reduction in the starch's water-retention capacity [18]. The addition of γ-PGA successfully delayed this phenomenon by forming strong hydrogen bonds that prevented the bonding of amylose chains. Furthermore, the increased sediment size reflects the stability of the dough's colloidal system and its ability to retain high internal moisture. This is consistent with the conclusions of [25] and [4] regarding the role of γ-PGA in improving the structure and gluten hydration, which ensures that bread retains high elasticity and a soft texture for longer storage periods.

the results for the crust did not show any significant differences with respect to storage periods, but the differences were significant with respect to the concentration. 0.8% concentration resulted in the highest moisture content, reaching 25.08%, while the control sample yielded 14.09%. This increase in moisture content is attributed to the high ability of γ-PGA to bind to water within the gluten network, which reduces moisture migration from the pulp to the crust during storage. This

Table (10) Effect of adding γ-PGA on the moisture content (%) of laboratory bread pulp.

Concentrate (gm)	Storage periods (day)		
	1	2	3
0	21.00	20.02	19.12
0.2	23.01	22.20	21.30
0.4	30.02	28.96	28.01
0.6	33.75	32.23	31.31
0.8	36.86	35.91	35.05
$R.L.S.D_{0.05}$	Concentrate		day
	2.79		N.S

Table (11) Effect of adding γ-PGA on the moisture content (%) of the crust of laboratory bread.

Concentrate (gm)	Storage periods (day)		
	1	2	3
0	14.09	13.02	12.08
0.2	16.86	16.00	15.04
0.4	20.34	19.44	18.51
0.6	22.21	21.31	20.80
0.8	25.08	24.11	23.10
$R.L.S.D_{0.05}$	Concentrate		day
	2.21		N.S

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is likely due to the acidic nature of γ-PGA, which contains free carboxyl groups (COOH) along its polymer chain, leading to a slight increase in overall acidity [21]. This decrease in pH plays an important role in improving the quality of baked goods in several ways. On the one hand, it contributes to increased yeast and flour enzyme activity and improves gluten elasticity. On the other hand, the slightly acidic environment acts as a natural preservative, reducing microbial contamination (Saleh *et al*., 2014).

Table (12) Effect of adding γ-PGA on pulp absorption strength (%) of laboratory bread.

Concentrate (gm)	Storage periods (day)		
	1	2	3
0	26.13	23.25	20.41
0.2	28.33	26.56	22.79
0.4	33.09	23.29	27.01
0.6	35.98	33.82	31.28
0.8	39.06	36.78	34.72
$R.L.S.D_{0.05}$	Concentrate		day
	2.56		2.06

Table (13) Effect of adding γ-PGA on the precipitate volume (ml) of laboratory bread.

Concentrate (gm)	Storage periods (day)		
	1	2	3
0	42	32	21
0.2	55	45	33
0.4	58	47	35
0.6	60	51	39
0.8	71	63	55
$R.L.S.D_{0.05}$	Concentrate		day
	4.32		3.35

Table (14) Effect of adding γ-PGA on the pH of laboratory bread.

Concentrate (gm)	Storage periods (day)		
	1	2	3
0	6.01	5.92	5.87
0.2	5.97	5.90	5.84
0.4	5.97	5.88	5.84
0.6	5.95	5.79	5.76
0.8	5.91	5.74	5.70
$R.L.S.D_{0.05}$	Concentrate		day
	N.S		0.10

3-2-3-3 Effect of Adding Gamma Polyglutamic Acid on the pH of Stored Bread

Table (14) shows a slight but significant decrease in the pH of bread with increasing storage periods of γ-PGA. However, with respect to concentrations, the pH decreased gradually and no significant difference was observed. This

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4- Conclusions

The study concludes that the addition of γ-PGA acts as a functional enhancer and effective biostabilizer in both baked goods and dairy products. In bread making, the polymer successfully strengthened the gluten network, improved the rheological properties of the dough, and increased its specific volume. It also effectively delayed stelling by enhancing the product's moisture retention capacity. In yogurt making, γ-PGA demonstrated high biocompatibility with bacterial starters. It effectively contributed to improving protein structure, increasing water-holding capacity, and reducing whey leaching. Furthermore, it played a role in regulating acidity and pH during refrigerated storage. Overall, this resulted in improved texture, appearance, and overall sensory acceptability of the final products.

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