Improvement Yogurt Texture: Innovative Use of Pineapple Juice Ultrasound to Manage Bromelain for Optimal Protein Breakdown

BY

Lamiaa I. El-Nawasany

Hanaa S. Sakr

Lamiaa A. Kadoum

Amira S. El-Rhmany

Dairy Chemistry Department, Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Giza

Doi: 10.21608/asajs.2025.459141

استلام البحث : ٣ / ٧ /٢٠٢٥

قبول النشــر : ٦ / ٩ / ٢٠٢٥

El-Nawasany, Lamiaa I. & Sakr, Hanaa & Kadoum, S. Lamiaa A. & El-Rhmany, Amira S. (2025). Improvement Yogurt Texture: Innovative Use of Pineapple Juice Ultrasound to Manage Bromelain for Optimal Protein Breakdown. ***Arab Journal of Agricultural Sciences***, Arab Institute for Education, Science and Literature, Egypt, 8(28),161- 200.

http://asajs.journals.ekb.eg

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Abstract:

Pineapple is a healthy tropical fruit that is high in vitamins, minerals, and antioxidants that fight oxidative stress, reducing the rate of long-term diseases like heart disease and cancer. The presence of bromelain, which is a pineapple proteolytic enzyme, presents an issue in mixing pineapple juice with protein-containing dairy foods. This is due to the fact that the enzyme breaks down protein, which is responsible for off-taste and sensory complaints. To solve this issue, a test was carried out via the application of applying an ultrasound treatment to pineapple juice. This was done via sonication of juice using high-frequency sound waves, 20 kHz for 20 minutes, and followed by blending with milk to obtain yogurt. The outcome revealed that sonication and mild heat treatment of milk at 70°C strengthened the chemical contents, i.e., phenolic compounds, flavonoids, DPPH antioxidant activity, and Ferric reducing power. That didn't decrease proteolysis and SN, i.e., the activity of the enzyme bromelain and its role in protein destruction was present. But when combining sound and heat treatment at 90°C, significantly enhanced rheological, bacterial, and sensory characteristics of yogurt were observed while simultaneously blocking proteolysis, but the negative effect on phenolic compounds, flavonoids, DPPH antioxidant activity, and Ferric reducing power.

Keywords: stirred yogurt, pineapple juice, bromelain, ultrasound

المستخلص:

تتناول الدراسة استخدام عصير الأناناس في تصنيع الزبادي، نظرًا لغناه بالفيتامينات والمعادن ومضادات الأكسدة التي تساهم في تقليل خطر الأمراض المزمنة. إلا أن وجود إنزيم البروميلين في الأناناس، والذي يكسر البروتينات، يسبب

مشاكل في الطعم والقبول الحسي عند خلطه مع منتجات الألبان. لحل هذه المشكلة، تم تطبيق معالجة بالموجات فوق الصوتية لعصير الأناناس (٢٠ كيلوهرتز لمدة ٢٠ دقيقة)، ثم خلطه بالحليب لصنع الزبادي. أظهرت النتائج أن المعالجة الصوتية مع التسخين المعتدل للحليب عند ٧٠°C حسّنت المحتوى الكيميائي للزبادي مثل المركبات الفينولية والفلافونويدات والنشاط المضاد للأكسدة، لكنها لم تقلل من نشاط البروميلين وتكسير البروتين. أما عند استخدام الموجات فوق الصوتية مع تسخين الحليب عند ٩٠°C، فقد تحسّنت الخصائص الريولوجية والبكتيرية والحسية للزبادي بشكل ملحوظ، وتم تثبيط نشاط البروميلين، لكن ذلك أثر سلبًا على مضادات الأكسدة والمركبات الحيوية.

1. Introduction

Stirred yogurt, or Swiss-style yogurt, is bulk fermented instead of being cup fermented. Thick consistency is formed by hard yogurt fermenting in the cup, but silky smooth consistency is formed through stirring and cooling the yogurt after fermentation (Hersh, 2021). Stirred yogurt contains two categories: plain and flavored. Honey or fruit puree can be added after fermentation. Stirred yogurt is also extremely versatile; it can be used as a base for other foods and smoothies, or consumed on its own with other ingredients added in like fruit or granola (Khadka, 2018). Stirred yogurt is a healthy food choice because it has several health benefits (Shah, 2017). Protein, as needed for the repair, formation of muscle, and normal bodily function, is present in sufficient amounts in stirred yogurt. Its content of calcium also provides good teeth and bones. Probiotics, in which stirred yogurt is present abundantly, are also responsible for good intestinal flora and the production of better digestion and higher immunity (Aryana and Olson, 2017). In addition, the yogurt is an excellent source of indispensable vitamins and minerals that help enhance overall health, including potassium, riboflavin, and vitamin B12.

Stirred yogurt consumption on a daily basis is beneficial to the cardiovascular system as it lowers the risk of cardiovascular disease by making sure there is proper blood pressure and cholesterol level. Li et al. (2021) and Hadjimbei et al. (2022) attest that stirred yogurt is also good to the gut as its probiotics lower the degree of symptom severity of such conditions as irritable bowel disease and inflammatory bowel disease.

Vitamin C, manganese, and vitamin B6 are some of the vitamins and minerals found in high amounts in pineapple, a fruit typically found in the tropics. Kumar et al. (2023) write that the nutrients play a leading role in sustaining bone structure, energy metabolism, and immune function. Flavonoids and phenolics found in high amounts in pineapple guard the body against inflammation and oxidative stress (Lalhruaitluangi and Mandal, 2024).

The immune system is also supported by the vitamin C content in pineapple, which helps the body fight infections and diseases (Sanchez and Andreas, 2020). The water content in pineapple guarantees detoxification and keeps the body hydrated (Horak, 2012). It also has the enzyme bromelain that facilitates nutrient absorption in the body through protein breakdown. Individuals suffering from inflammatory diseases such as arthritis can also derive immense benefits by consuming bromelain as part of their diet since bromelain is also reported to have anti-inflammatory properties (Kansakar et al., 2024).

The activity of the bromelain enzyme is the most significant of several things to keep in mind when adding pineapple to yogurt during processing (Roda and Lambri, 2019). The pineapple's protein-dissolving enzyme, bromelain, can soften the yogurt and make it more watery in consistency. The pineapple's acidity will also disrupt the balance of pH in yogurt, interfering with fermentation and resulting in sour flavor (Cichoke, 2002; Cao et al., 2022; Farahat and El-Batawy, 2013).

ISSN: 2537-0804 eISSN: 2537- 0855

Additionally, the addition of pineapple would result in an overmicrobial load that would inhibit yogurt cultures and upset the microbial balance, thereby shortening the product shelf life. As much as pineapple adds flavor, the majority consider grated unappetizing due to the fibrous characteristic that results in an unappetizing gritty texture. Syneresis is explained due to the higher water content in pineapple by Tarrega et al. (2016) and Zhou (2018), thus liquid separates from the yogurt since the general structure and appearance of the final product also alter.

Pineapple contains an enzyme called bromelain, which possesses many health benefits. Being anti-inflammatory, it is an excellent remedy for inflammation and swelling, and thus can be used for the treatment of sinusitis and osteoporosis. Bromelain also digests protein, speeding up digestion, calming gas and bloat (Jancic and Gorgieva, 2021). By limiting inflammation and speeding up tissue repair, it cures wounds and burns quicker as well (Kansakar et al., 2024).
Because of its anticoagulant effect, bromelain benefits cardiovascular health and inhibits blood clotting. In certain research, it is also stated that bromelain can increase chemotherapy by inhibiting the growth of certain cancer cells. In patients with sinusitis, it also inhibits inflammation and nasal stuffiness. Besides, through relief of joint pain and enhancement of joint function, bromelain is utilized for the treatment of osteoarthritis (De Decker et al., 2022; Noor et al., 2022).

The enzyme bromelain in pineapple is able to hydrolyze proteins. It is able to make the main protein of milk, casein, hydrolyze in yogurt and give it an undesirable, thinner consistency. Its function, in that it cleaves proteins into amino acids and short peptides, can also cause milk proteins to coagulate and lead to the milk having a lumpy or gritty texture.

ISSN: 2537-0804 **eISSN: 2537- 0855**

Besides, during the hydrolysis of proteins by bromelain, bitter amino acids may be liberated, which would be a negative attribute in the taste of the yogurt (Beniwal and Das, 2023 ; Ismail et al., 2019 ; Anjaly, 2021 ; Rathnakumar et al., 2023).

Sound waves with a frequency higher than the human hearing range typically more than 20 kHz are referred to as ultrasound. It has numerous applications, from industrial to medical diagnostics and treatment. Ultrasound is commonly used in the food sector for the extraction of bioactive components from plant materials, including polyphenols and antioxidants (Ensminger and Bond, 2024; Yusoff et al., 2022).

This is done through disruption of the cell wall, which increases penetration and extraction by solvents. Ultrasound also allows the creation of stable emulsions and complete dispersion of ingredients, improving the texture and flavor of milk products. Ultrasound can also disrupt microorganisms, decreasing the microbial count of food products and their shelf life. Ultrasound increases mixing and blending of the ingredients, a most important feature in guaranteeing product uniformity in the manufacture of soft drinks (Liu et al., 2022; Carrillo-Lopez et al., 2021; El-Nawasany et al., 2023).

The objective of this research was to strengthen whipped yogurt by adding pineapple juice with the aim of enhancing its flavor, nutritional value, and functional properties. The objectives of this research were to take advantage of the high nutrient content of pineapple juice and analyze the effect of heat and sonication on the added juice. The research also sought to establish how the pineapple bromelain enzyme breaks down the protein in yogurt and its effect on the properties of the end product.

2. Materials

Skimmed milk powder (USA) was used to make the stirred yoghurt; according to the seller, it included 34% protein, 51%

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lactose, 1.2% fat, 8.2% ash, and 4% moisture. We purchased pineapples (*Ananas comosus*) at Tanta's local market. *Streptococcus thermophilus* and *Lactobacillus delbrueeckii* sub ssp. bulgaricus (DVS) were present in the yoghurt culture used for production, which was purchased from Hansen Copenhagen Laboratories in Denmark. Misr Food Additives Company (Mifad) in Egypt provided the gelatin, while Emtenan Food Company in Egypt provided the stevia sugar.

2.1. Preparation of pineapple juice

Before being physically peeled with a sharp kitchen knife, the pineapples were first sorted and cleaned with tap water. After that, the pulp was thinly sliced and mixed in a pineapple juicer set to its highest speed (1200 rpm) for two minutes. The resulting pineapple juice was separated into two equal portions, one of which was ultrasonically treated for 20 minutes at 20 kHz while the other was placed in the refrigerator. Throughout the treatment, the temperature was closely watched to make sure it didn't rise over 60°C. The mixing procedure was carried out using a 4710 Series ultrasonic homogenizer (Parmer Instrument Co., Chicago, Illinois 60,648, USA). The support was submerged 2.5 cm from the surface.

2.2. Making stirred yoghurt

12 % skim milk powder (SMP), 0.5% gelatin as stabilizer, and 0.5% stevia as sweetener were used to make stirred yogurt. Milk was pasteurized at 90°C for 10 minutes. therapy C, the control treatment, was made by not adding addition of juice Fig.1. Conversely, 20% pineapple juice was added to the water in the other treatments (T1, T2, T3, and T4). T1 was pre-treated with unsonicated juice for 10 seconds at 70°C.; T2 (sonicated juice) of 10 seconds at 70°C, T3 (raw juice) for 10 seconds at 90°C, and T4 (raw juice) for 10 seconds at 90°C, were employed to use on the milk. They were inoculated using a primary stock of 0.2%, and the treatments were then plated into sterile 100 ml

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capacity plastic cups and incubated for four hours under a temperature of 42°C. The cups were stored at 4 C overnight and tested for 14 days. The treatments were done in triplicate. By 10 clockwise and counterclockwise rotation of the cups using a glass rod, the cups were rotated slowly to disassemble the gel for observation.

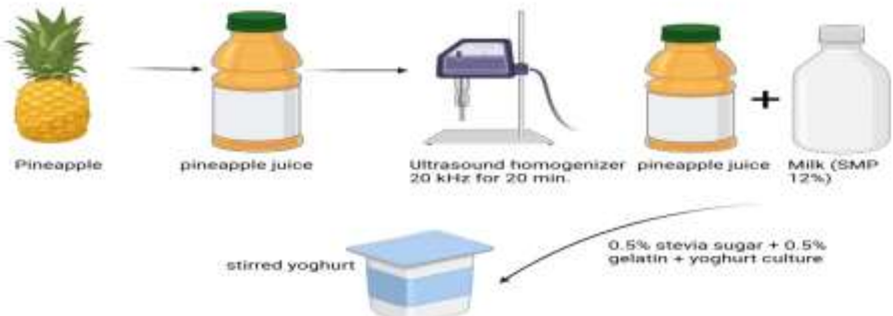

Fig 1 A basic schematic illustration of how pineapple juice is added to churned yogurt

2.3. Physicochemical composition

Total solids (TS), ash, fat, protein (TP), and soluble nitrogen (SN) (Kjeldahl) contents were analyzed as per the procedures describe d in AOAC (2010). The pH was measured at 25°C with a digital pH meter (Toptronic Milano, Italy). The volume of 0.1 N NaOH used for titration was calculated as a percentage of lactic acid, and titratable acidity (% lactic acid) was approximated by titrating with 0.1 M NaOH to the phenolphthalein endpoint. The sample preparation was done according to the method described by Costa et al. (2013) to estimate the total content of phenols (TPC) and flavonoids (TFC) in juice. The same method as El-Nawasany et al. (2023) was used for yogurt.

2.4. DPPH and Ferric reducing power (FRAP)

With a few minor modifications, the extraction procedure was carried out in accordance with the approach outlined by Xu et al. (2008). To clarify the pineapple juice, equal amounts of methanol (80%) were added. After 30 minutes of stirring at 250 rpm in a shaking incubator (SI-100R, Korea), the mixture was let to cool to room temperature. Centrifugation was then applied to the combination, and the supernatant was collected and tested for antioxidant activity.The ferric reducing power (FRAP) and free radical scavenging activity (DPPH) were evaluated using the approach outlined by El-Nawasany et al. (2023).

2.5. Minerals measurements

Using the Perkin Elmer Atomic Absorption Spectrophotometer (Model 2380, Japan), the AOAC (2003) technique was used to measure the mineral concentrations of Ca, Mg, Zn, Na, and Fe. The K concentration was also measured using the Flame Photometer (Model PE P7, England).

2.6. Determination of Proteolysis

To ascertain the extent of protein degradation, we used the OPA technique with modifications as outlined by Sah et al. (2015).

2.7. Apparent viscosity and water-holding capacity (WHC)

The apparent viscosity of yogurt samples was measured at 10 ± 2 °C using a Brookfield digital square viscometer (model DV-II, Canada) and reported in Pascals (Pa). Using the method described by Wu et al. (2009), the viscosity of yogurt was measured at a shear rate of 50 rpm. Using the procedures outlined by Costa et al. (2013), the viscosity of the juice samples was tested at 25 ± 1 °C with rotor zero at 25 rpm. Using the same methods as EL-Nawasany (2019), a 10 g sample of yogurt was centrifuged at 4500 rpm for 20 minutes at 10 °C in order to ascertain the water holding capacity (WHC).

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2.8. Microbiological analysis

The Oxoid Plate Agar Method, as described by Adegoke (2004), was the method used to determine the total bacterial counts (TBC counts). Using the techniques outlined by Adegoke (2004), the yeast and mold numbers were estimated.

2.9. Color measurements

A colorimeter (CR-410 Konica Minolta Chroma Meter, Japan) was used to measure the juice and yogurt's brightness (L*), red/green (a*), and yellow/blue (b*) values. Before beginning the analysis, the equipment was calibrated using a white calibration plate. The analysis was conducted once, in accordance with Cais-Sokolińska and Pikul's (2006) protocol.

A panel of 10 sensory specialists affiliated with the researchers from the Dairy Chemistry Department of the Animal Production Research Institute of the Agricultural Research Center evaluated the freshly made and preserved yogurt samples (Kailasabathy, 2006). To ensure the validity of the evaluation, the experts followed the guidelines, which included drinking water in between treatments and keeping any information on treatment differences confidential. This assessment took place over a period of 14 days.

2.10. Statistical analysis

All of the parameters were measured three times, and the mean values and standard deviation were reported. The statistical software program SPSS (version 20 for Windows, SPSS INC., IBM, New York) was used to conduct the analysis of variance and Duncan's test (Duncan, 1955).

3. Results and discussion

3.1. Chemical composition and pH of juice

The effect of ultrasonic waves on the chemical content and pH value alteration of pineapple juice is presented in Table 1. From the results, sonicated juice had a greater solid content than non-sonicated juice with values of 13.83 and 13.59 (%), respectively. Besides this, sonicated juice had higher TP, TPC, and TFC with 0.72 (%), 34.59 (mg GAE/g), and 17.79 (mg QUE/g), respectively, than non-sonicated juice. pH value of sonicated juice was 4.66, whereas that of non-sonicated juice was 4.81 with a highly significant difference.

Because each type of juice has unique qualities that affect how it reacts to waves, the effects of ultrasound therapy vary depending on the type of juice. Furthermore, different frequencies and intensities have different effects on the quantity and quality of juice (Lepaus et al., 2023). Pre-treatment parameters including fruit ripeness and treatment time and temperature also have an impact on the stability of nutrients and bioactive chemicals in juice (Dadan et al., 2021). Ultrasound treatment can enhance juice output, boost bioactive ingredient extraction, and better maintain nutritional stability as compared to conventional heat treatments (Lepaus et al., 2023).

Cavitation bubbles produced by ultrasound have the potential to break down cell walls, allowing more acids and other substances to enter the juice. Additionally, ultrasonic mechanical agitation can enhance the effectiveness of extracting acid from fruit material (Khan et al., 2022).

According to Roobab et al. (2023), ultrasonic treatment improves the extraction of phenolic and flavonoid components in juice by dissolving cell walls and releasing their bound compounds through cavitation bubbles produced by ultrasound. Additionally, the disintegration of cell structures is further facilitated by the localized heating generated by ultrasound, which increases the release of these chemicals. According to Lepaus et al. (2023), ultrasonic treatment produces greater quantities of phenolic and flavonoid components in juice than traditional extraction techniques.

ISSN: 2537-0804 **eISSN: 2537- 0855**

Table 1: The impact of ultrasonic waves on the chemical composition and pH changes in pineapple juice

Analysis	Pineapple juice (*)	
	Pre-sonication	Post-sonication
T.S (%)	13.59± 0.12[b]	13.83±0.08[a]
T.P (%)	0.63±0.11[b]	0.72±0.15[a]
TPC (mg GAE/g)	33.27±0.29[b]	34.59±0.14[a]
TFC (mg QUE/g)	17.04±0.15[b]	17.79±0.26[a]
pH	4.81±0.04[a]	4.66±0.08[b]

(*) The numbers obtained are the mean values of three specimens. The presented data showcases the average value along with the standard deviation of three duplicates for each specimen.

The distinct letters (a-b) within the columns indicate significant variation ($p \leq 0.05$) between treatments only for the same duration.

3.2. Color measurements and viscosity for pineapple juice

Table 2 shows the viscosity and color of pineapple juice before and after sonication. Viscosity of pineapple juice (Pascal sec) was 18.35 prior to sonication and 12.21 after sonication, and this was statistically different ($P<0.05$). Corresponding values were 92.37 and 88.54 for lightness (L*) values, -3.68 and -3.75 for negative and positive green and red (a*) values, and 14.03 and 14.35 for negative and positive blue and yellow (b*) values. The acoustic flow, which is generated by the motion of sound waves in a liquid, can influence the viscosity of the juice. Continuous flows and microflows are created by sound waves when they move through the juice and alter the mechanical properties of the juice, i.e., viscosity (Sulaiman and Silva, 2023).

ISSN: 2537-0804 eISSN: 2537- 0855

In addition, the mechanical oscillations of the sound waves can impart shear stress to the juice particles, which may modify their structure or disintegrate larger particles, thus changing the fluid's viscosity (Salehi, 2020).

Using Costa et al. (2013), sonication lowered the juice viscosity; the sonicated juice was 12.21 (Pascal second) compared to 18.35 for the unsonicated juice. Similarly, sonicated juice color was also altered, and L*a*b* values were 88.54, -3.75, and 14.35.

Table 2: Color measurements and viscosity of pineapple juice pre-sonication versus post-sonication

Analysis	pineapple juice	
	pre-sonication	post-sonication
Viscosity (Pascal second)	18.35±0.21[a]	12.21±0.16[b]
L*	92.34±0.15[a]	88.54±0.23[b]
a*	-3.68±0.09[b]	-3.75±0.13[a]
b*	14.03±0.12[b]	14.35±0.15[a]

*See legend to Table 1 for details.

The values L* refers to lightness, while a* indicates a negative value for green and a positive value for red. Likewise, the value b* indicates a negative value for blue and a positive value for yellow.

3.3. Minerals measurements

Zn, Na, k, Mg, Fe, and Ca were found in pineapple juice in the following amounts, according to the analysis in Fig. (2): 0.15, 1.14, 109.82, 12.69, 0.33, and 13.23 (Per/100g), respectively.

Pineapple is a nutrient-rich fruit that contains vital elements including manganese, copper, magnesium, iron, and folic acid (vitamin B9), according to De Ancos et al. (2017).

ISSN: 2537-0804 eISSN: 2537- 0855

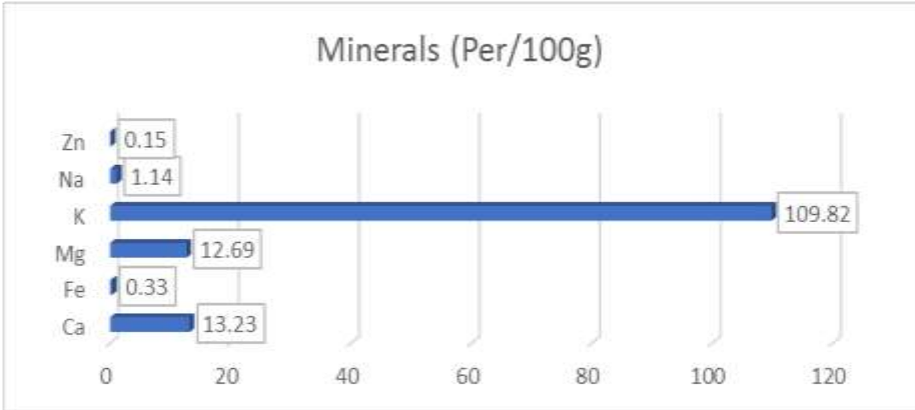

Fig. 2 Minerals measurements (per/100g) of pineapple juice pre-sonication

3.4. Fermentation time of yoghurt

The findings shown in Fig. 3 indicate that the added pineapple juice affected the stirred yogurt samples' fermentation time. As a result, the yogurt's pH dropped and its acidity levels rose. On the other hand, the juice's fermentation time was not significantly impacted by sonication or heat treatment. The control treatment's pH was 4.62 at 180 minutes, whereas the pH values of the other treatments ranged from 4.58 to 4.59.

Citric acid, the main acid component in pineapple juice, gives it its acidic flavor. According to Khalid et al. (2016), pineapple juice also includes malic acid and ascorbic acid. Although it is not an acid, an enzyme in pineapple juice, as proposed by Umair et al. (2022), can alter the impression of acidity by dissolving proteins and adding a stronger sensation.

ISSN: 2537-0804 eISSN: 2537- 0855

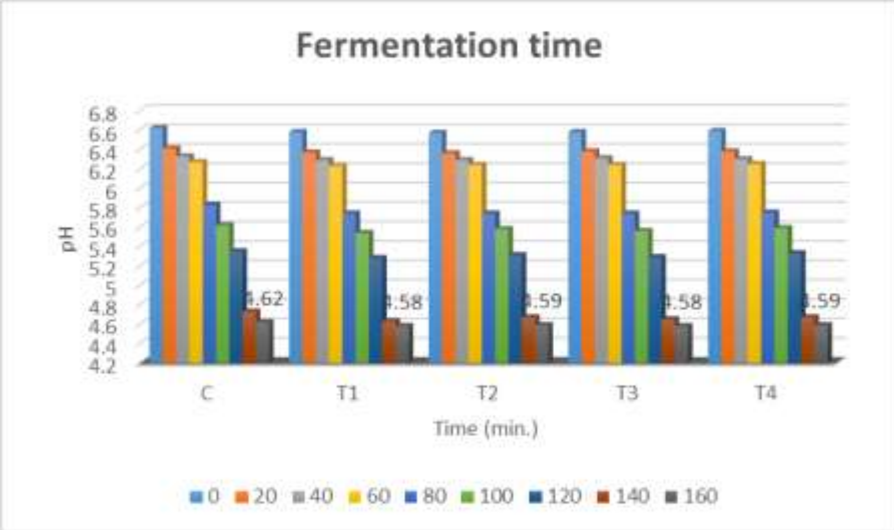

Fig. 3 Fermentation time of stirred yogurt enriched with pineapple juice

Treatment C served as the control, T1 mixed unsonicated juice with milk at 70°C for 10 sec, T2 added unsonicated juice to milk at 70°C for 10 sec, T3 added unsonicated juice to milk at 90°C for 10 sec, and T4 mixed unsonicated juice with milk at 90°C for 10 sec.

3.5. Chemical composition of stirred yogurt

The control group had the lowest total solids (TS) on fresh stirred yogurt (12.09%), whereas the T1, T2, T3, and T4 groups had TS of 14.52, 14.60, 14.70, and 14.76%, respectively. The corresponding values were 0.62, 0.72, 0.71, 0.72, and 0.72; 3.09, 3.21, 3.24, 3.13, 3.17; and 0.6, 0.49, 0.42, 0.46, and 0.38 for ash, TP, and SN. On C, T1, T2, T3, and T4, and it was 11.54, 22.98, 20.56, 22.87, and 20.20 on acetaldehyde, respectively (Table 3). More solids and proteins are released into the juice due to the cavitation bubbles created by the mechanical impact of ultrasonic waves, which may cause this effect. Treatment T1, which included a heat treatment at 70°C without sonication, had

ISSN: 2537-0804 eISSN: 2537- 0855

the greatest acetaldehyde level (22.98). Additionally, SN decreased due to sonication's effect on the proteolytic enzyme bromelain; fresh yogurt treated with treatment T4, which involved heat treatment at 90°C and juice sonication, had the lowest value (0.38%). Nevertheless, neither heat treatment nor sonication of the pineapple juice employed affected the ash levels.

Adding pineapple juice to yogurt significantly increased the TS, TP amount, and ash levels during storage. The results found that TS on controls on fresh was 12.09 and after 7 and 15 days of storage was 12.35 and 12.81%, respectively, and 3.09 on fresh and 3.17 and 3.21% after 7 and 14 days of storage for TP. The inclusion of natural sugars, protein, and minerals in pineapple juice may be the cause of this effect. This observation aligns with Meena et al. (2022). Additionally, the acetaldehyde assay showed alterations with the addition of juice, suggesting a rise in the acetaldehyde concentration. According to Hu et al. (2024), this phenomena could be explained by the citric acid in pineapple juice, which aids in the fermentation process' production of acetaldehyde.

Mechanical agitation combined with ultrasonic waves increases the effectiveness of solids and protein extraction from pineapple pulp (Khan et al., 2022). However, Badgujar et al. (2016) discovered that sonication decreased acetaldehyde levels, most likely due to the impact of sonication on enzyme activity.

The heat treatment of pineapple juice changed the chemical composition of yogurt. When the juice was heated to 90°C, the solids in treatments T3 and T4 increased. Water evaporating due to the high heat treatment may have caused this (Kuldilock, 2002). However, the total protein concentration decreased after the heat treatment because high temperatures break down proteins. Additionally, after being heated to 90°C, the juice's SN dropped, which may be explained by how temperature influences the bromelain enzyme's activity. According to Terefe et al. (2014), bromelain is a heat-sensitive enzyme that can cause structural deformation of the enzyme by oxidizing sulfhydryl groups in cysteine proteins.

Table 3: Chemical composition of stirred yogurt enriched with pineapple juice

Item	Chemical composition				
	T.S %	**Ash %**	**T.P %**	**S.N %**	**Acetaldehyde**
			Fresh		
C	12.09±0.03^{e}	0.62±0.01^{b}	3.09±0.02^{d}	0.36±0.01^{e}	11.54±0.02^{d}
T1	14.52±0.03^{d}	0.72±0.01^{a}	3.21±0.01^{a}	0.49±0.01^{c}	22.98±0.14^{a}
T2	14.60±0.03^{c}	0.71±0.01^{a}	3.24±0.01^{a}	0.42±0.01^{c}	20.56±0.06^{b}
T3	14.70±0.04^{b}	0.72±0.02^{a}	3.13±0.03^{c}	0.46±0.01^{b}	22.87±0.08^{a}
T4	14.76±0.02^{a}	0.72±0.02^{a}	3.17±0.02^{b}	0.38±0.01^{d}	20.20±0.08^{c}
			7 Days		
C	12.35±0.03^{d}	0.64±0.02^{b}	3.17±0.02^{e}	0.46±0.02^{e}	10.05±0.04^{e}
T1	14.80±0.04^{c}	0.73±0.01^{a}	3.28±0.01^{c}	0.59±0.02^{a}	21.34±0.04^{a}
T2	14.89±0.03^{b}	0.73±0.01^{a}	3.31±0.01^{b}	0.53±0.02^{c}	19.81±0.05^{c}
T3	15.00±0.04^{a}	0.74±0.02^{a}	3.23±0.02^{d}	0.56±0.01^{b}	21.08±0.05^{b}
T4	15.05±0.03^{a}	0.74±0.01^{a}	3.28±0.01^{c}	0.49±0.01^{d}	19.08±0.02^{d}
			14 Days		
C	12.81±0.0	0.65±0.0	3.21±0.0	0.52±0.0	8.54±0.03^{e}

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	4^{d}	1^{b}	3^{e}	2^{e}	
T1	15.٣4±0.0	0.75±0.0	3.42±0.0	0.68±0.0	$19.81±0.03^{a}$
	2^{c}	1^{a}	1^{b}	1^{a}	
T2	15.٤4±0.0	0.74±0.0	3.48±0.0	0.63±0.0	$18.32±0.04^{c}$
T3	4^{b}	2^{a}	2^{a}	1^{c}	$19.54±0.05^{b}$
T4	15.40±0.0	0.74±0.0	3.3±0.02	0.66±0.0	$17.93±0.04^{d}$
	3^{a}	1^{a}	d	2^{b}	
	15.48±0.0	0.74±0.0	3.38±0.0	0.58±0.0	
	3^{a}	1^{a}	1^{c}	2^{d}	

Treatment C served as the control, T1 mixed unsonicated juice with milk at 70°C for 10 sec, T2 added unsonicated juice to milk at 70°C for 10 sec, T3 added unsonicated juice to milk at 90°C for 10 sec, and T4 mixed unsonicated juice with milk at 90°C for 10 sec.

The distinct letters (a-d) within the columns indicate significant variation ($p \leq 0.05$) between treatments only for the same duration.

3.6. Degree of protein hydrolysis of stirred yogurt

According to the results in Fig. (4), the degree of protein hydrolysis showed how much heat treatment and sonication affected the process. The T4 treatment showed the best results, with degrees of protein hydrolysis of 6.91, 6.65, and 6.31% at roughly 0, 7, and 14 days of storage.

Lactic acid bacteria produce proteolytic enzymes during the yogurt-making process, breaking milk protein peptide bonds to produce free lactic acid and peptides (Kieliszek et al., 2021). The presence of the enzyme bromelain, which breaks down proteins, might cause issues when utilizing pineapple juice to make yogurt. Since different enzymes react differently to ultrasound therapy, the effect of ultrasound treatment on enzyme activity depends on several variables, including frequency, intensity, exposure length, temperature during treatment, and the type of enzyme involved (Nadar and Rathod, 2017).

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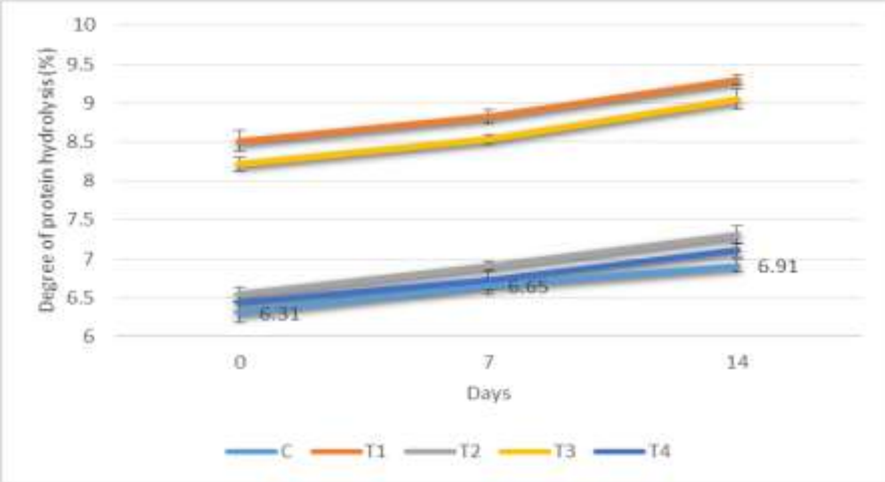

Fig. 4 Degree of protein hydrolysis of stirred yogurt enriched with pineapple juice

*See legend to Fig 3 for details.

3.7. Acidity and pH of stirred yogurt

The pH results correlated with acidity changes with the most acidic treatment (T2) recording the lowest pH values of 4.54, 4.49, and 4.41, and the pH values of 1.33, 1.52, and 1.74 at 0 and 14 days, respectively, in the same order (Fig. 5).

As seen in Fig. 5, adding pineapple juice to yogurt raised its acidity compared to the control, consistent with research by Gangwar et al. (2016). This is explained by the fact that the natural acids in pineapple juice, such as citric and ascorbic acids, increased the acidity of yogurt (Antoniolli et al., 2012). Yogurt's increased acidity was also influenced by the bromelain enzyme found in pineapple and its possible impact on protein interactions. It's possible that the juice's sonication altered the structure of the proteins, raising the juice's acidity and eventually affecting the acidity of the yogurt (Kaur and Gill, 2019). By potentially affecting the bromelain enzyme, heat treatment of the juice at 90°C had the opposite effect, decreasing the acidity of the yogurt (Bala et al., 2012).

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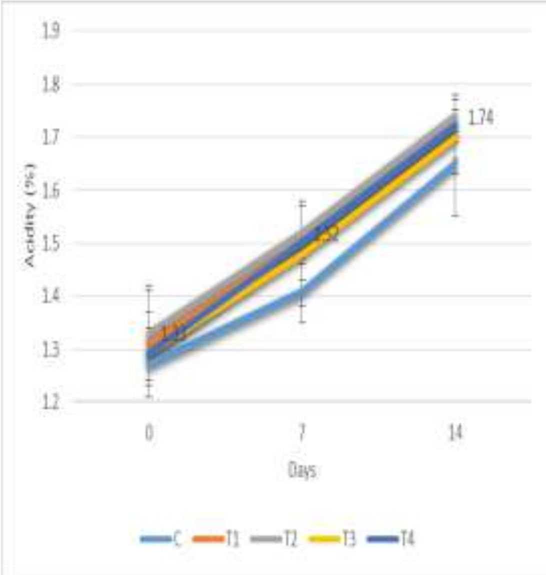

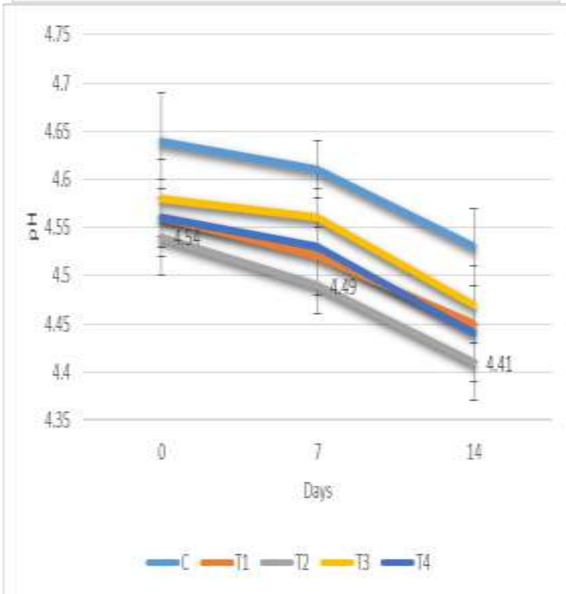

Fig. 5 Acidity and pH of stirred yogurt enriched with pineapple juice

*See legend to Fig 3 for details.

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3.8. DPPH and Ferric reducing power of stirred yogurt

The optimal treatment by T3 was given, where the juice was given parallel sonication and heating treatments at 70 °C. In treatment, the DPPH values were 22.68, 22.5, and 22.33 at 0, 7, and 14 days, respectively, while the corresponding Ferric reducing power values were 0.49, 0.48, and 0.44 (Fig. 6).

The result indicates the increase in DPPH and Ferric reducing power following pineapple juice application, which can be attributed to its antioxidant contents rich in vitamin C, bromelain, phenolic, and flavonoids (Khalid et al., 2016). Sonication accounted for a principal contribution to a rise in DPPH and ferric-reducing capability, perhaps owing to the appearance of cavitation bubbles by acoustic waves that bring about the extraction of antioxidant metabolites from cells of plants (Xu et al., 2022). Conversely, the action of heating treatment was such that higher temperatures led to declines in both DPPH and ferric-reducing capability, with an optimum value at 70 °C. This result is possibly due to the preservation of vitamin C and other phenolics, as vitamin C loses its effectiveness in temperatures over 70 °C and decomposition is enhanced at high temperatures (Santos and Silva, 2008).

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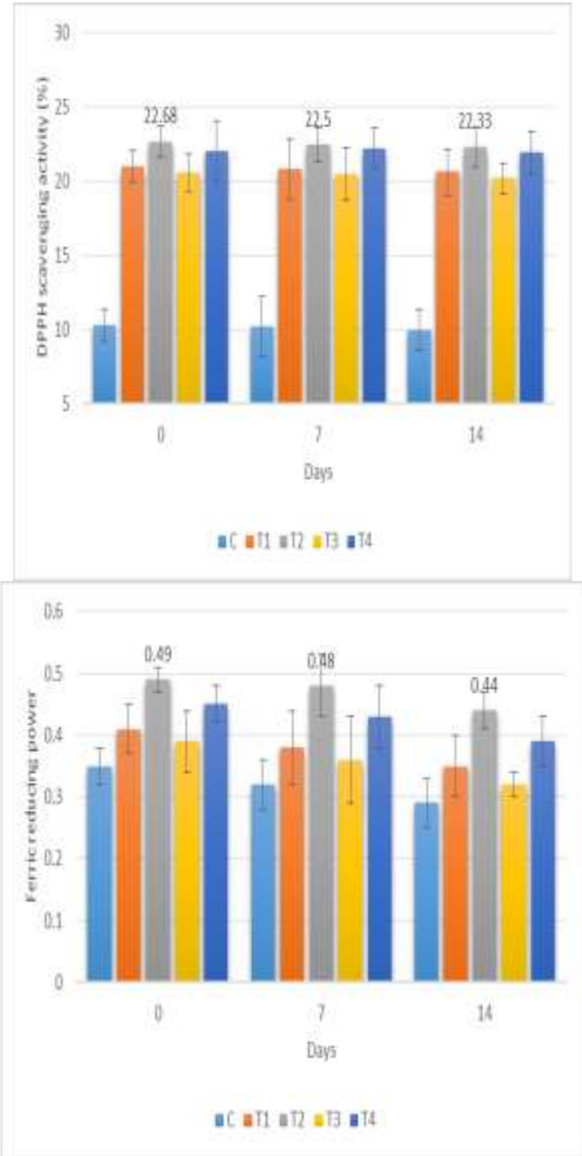

Fig. 6 DPPH scavenging activity and ferric reducing power of stirred yogurt enriched with pineapple juice

*See legend to Fig 3 for details.

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3.9. TPC and TFC content of stirred yogurt

As indicated from Fig7, the best treatment was the T2, in which sonication and heat treatment at 70°C were applied, with total phenolic content of 33.97 (mg GAE/g) and flavonoid content of 11.16 (mg QμE/g) after 14 days.

Pineapple juice has been reported to be abundant in phenolic and flavonoid chemicals by Khalid et al. (2016), which include caffeic acid, ferulic acid, quercetin, and mimosin as prominent constituents. Flavonoid and phenolic chemicals increased when pineapple juice was added during the yogurt-making process (Fig. 7). Interestingly, the control sample and other pineapple juice-added samples differed greatly, as Bakar et al. (2024) have also indicated. Additionally, sonication significantly influenced the treatment of juice; in treatments T2 and T4, sonicated juice increased the amount of phenolic and flavonoid compounds in yogurt samples. The chemicals become soluble after sonication, which makes it easy to detect them in the juice. Besides, it may play an important role in releasing flavonoid and phenolic chemicals from plant cells (Zinoviadou et al., 2015).

However, the flavonoid and phenolic content of the juice was differently a ffected by heat treatment at 90°C and 70°C; the 90°C treatment reduced the quantity of these compounds as a whole. This observation can be explained by the harmful effects that high temperature has on flavonoids and volatile phenolic chemicals, which may result in loss or damage. Besides, heat treatment has also been found to induce changes in chemical structures that reduce antioxidant activity, and influence the bromelain enzyme, leading to a reduction in concentration (Jutamongkon and Charoenrein, 2010).

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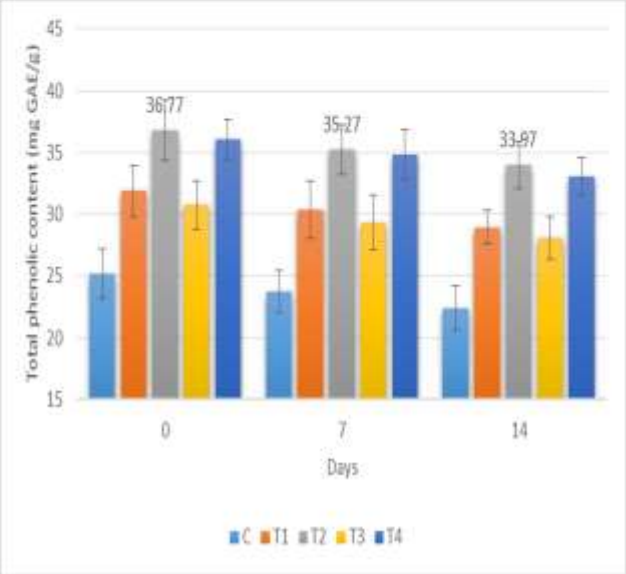

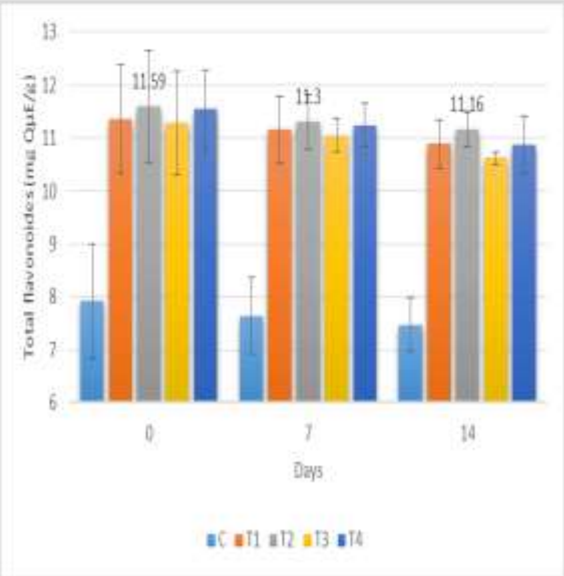

Fig. 7 TPC and TFC of stirred yogurt

*See legend to Fig 3 for details.

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3.10. Rheological properties of stirred yogurt

Results in Fig. (8) showed that adding sonicated juice enhanced the rheological characteristics, increasing viscosity and decreasing water holding. The optimal treatment in terms of viscosity was T4, which recorded 484.66 Pa at 14 days, even though heat treatment at 90°C was preferable to 70°C. On the other hand, T1 showed the lowest water holding, with values of 54.97% at 14 days, at a temperature of 70°C and no sonication.

Expert assessors have determined that rheological qualities are a powerful indication of sensory features (Heymann and Lawless, 2013). Because sonication breaks down plant fibers and fragments bigger molecules, it tends to reduce viscosity in juices. Additionally, it homogenizes the juice by uniformly dispersing the molecules (Terefe et al., 2016). In a similar trend, heat can improve molecular dispersion and hence increase homogeneity (Aghajanzadeh et al., 2023).

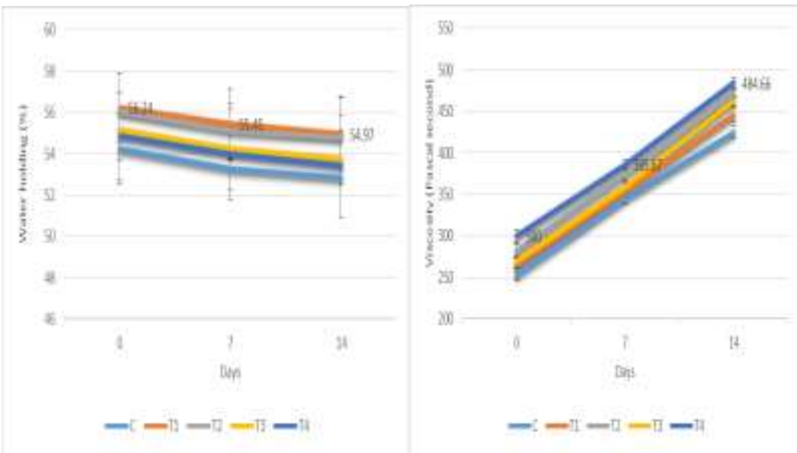

Fig. 8 Rheological properties of stirred yogurt

3.11. Microbiological analysis of stirred yogurt

Juice sonication and heat treatment had a discernible effect on the findings shown in Table (4), with treatment T4

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producing the greatest results when compared to the other treatments, recording 0.34 (log cfµ/qX10) at 14 days. This result emphasizes how effective the sonication procedure is, especially when combined with heat treatment at 90°C. The quality of the fermentation process in the yogurt may also be determined by looking at total bacterial counts (TBC). The study discovered that the development and activity of yogurt bacteria, which give yogurt its unique flavor, were unaffected by sonication or juice heat treatment.

The study of yeast and mold counts in yogurt is paramount, given that yeast and molds play a pivotal role in preserving food safety, ensuring its safe delivery to consumers, and influencing sensory properties and consumer acceptance (Lund et al., 2000).

Table 4 Microbiological analysis of enriched with pineapple juice

	C	T1	T2	T3	T4
			yeast and mold counts (log cfµ/q X10)		
0 Days	ND	ND	ND	ND	ND
7 Days	ND	0.67±0.04[a]	0.43±0.05[b]	ND	ND
14 Days	0.39±0.04[d]	0.89±0.04[a]	0.68±0.06[b]	0.52±0.02[c]	0.34±0.06[d]
			TBC counts (log cfµ/q X10)		
0 Days	8.13±0.2[a]	7.62±0.05[b]	7.41±0.05[c]	7.19±0.1[d]	7.03±0.12[d]
7 Days	9.62±0.08[a]	9.11±0.08[b]	8.9±0.07[c]	8.71±0.02[d]	8.63±0.05[d]
14 Days	11.03±0.09[a]	10.67±0.03[b]	9.49±0.06[c]	9.43±0.05[c]	9.29±0.05[d]

*See legend to Table 3 for details.

3.12. Color measurements of stirred yogurt

Table (5) indicates that the inclusion of pineapple juice generally causes significant color changes compared to the control sample. Nevertheless, the brightness improved with juice sonication, as the color brightened with sonication and when the temperature was raised to 90°C.

Color is a crucial visual characteristic in food products, and it is desirable to maintain minimal changes in color after processing (Pathare et al., 2013). Furthermore, color changes can signify food spoilage and the growth of spoilage microorganisms (in't Veld, 1996). Sonication enhances color clarity by eliminating large particles from the juice, rendering it more visually appealing (Bhattacharjee et al., 2017). In addition, sonication and high temperature led to reduced red color and increased blue color.

Table 5 Color change of stirred yogurt enriched with pineapple juice

Treatments	L*	a*	b*
		0 Days	
C	24.56±0.04	6.61±0.08	7.85±0.10
T1	10.16±0.1	-10.32±0.08	10.53±0.08
T2	12.36±0.06	-5.81±0.07	11.05±0.09
T3	15.41±0.06	-9.34±0.06	11.6±0.07
T4	18.31±0.07	-5.33±0.07	14.44±0.05
		7 Days	
C	23.83±0.06	6.42±0.06	7.45±0.06
T1	9.83±0.06	-10.22±0.06	10.27±0.05
T2	11.81±0.07	-5.64±0.06	10.72±0.12
T3	15.03±0.1	-9.17±0.06	11.39±0.09
T4	17.82±0.08	-5.08±0.06	14.21±0.05
		14 Days	
C	23.65±0.07	6.12±0.03	7.16±0.05
T1	8.64±0.05	-10.02±0.08	9.98±0.08
T2	11.64±0.04	-5.5±0.03	10.52±0.08
T3	14.91±0.08	-8.89±0.04	11.24±0.06
T4	17.63±0.06	-4.98±0.06	13.89±0.11

*See legend to Table 3 for details.

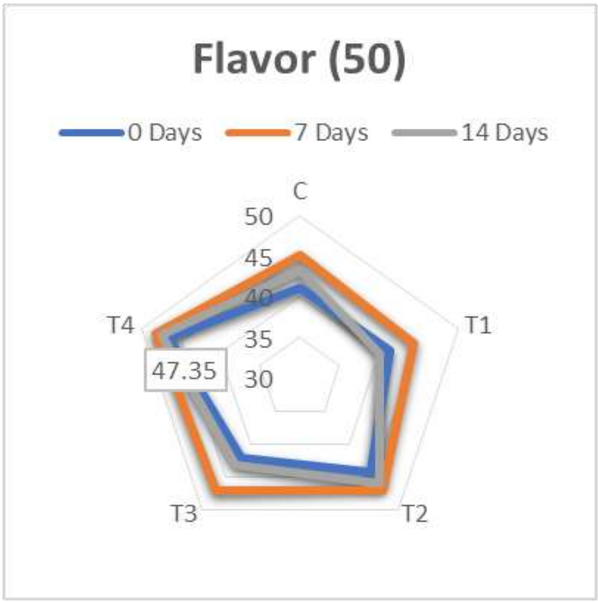
ISSN: 2537-0804 **eISSN: 2537- 0855**

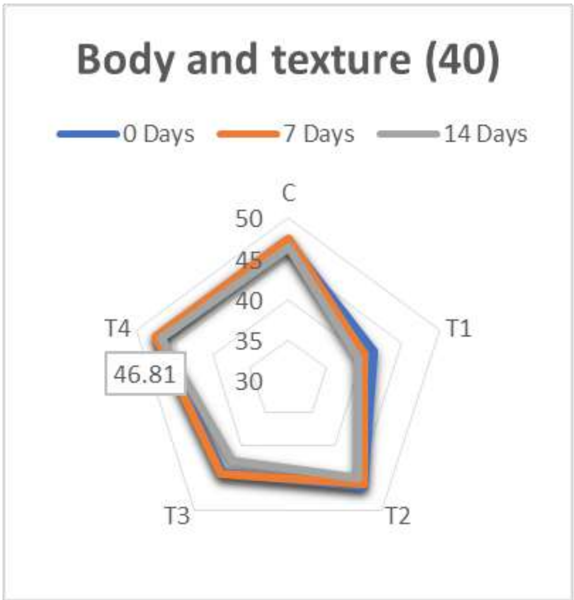
L* denotes lightness, where a higher value represents increased brightness, and a lower value indicates darker shades. Additionally, a* values signify hues ranging from green to red when positive, while b* values indicate colors from blue when negative to yellow when positive.

3.13. Sensory evaluation of stirred yogurt

Fig. 9 displays the findings of the sensory examination. With ratings of 47.35, 46.81, and 9.15 at 14 days, respectively, the judges from the Animal Production Research Institute's Dairy Chemistry Department mostly approved of treatment T4's flavor, body and texture, and appearance.

A new variety of meals and goods have entered the market as a result of the increased interest in eating healthily. It's crucial to remember that consumer approval of a new, healthful product is not always 100% certain. The sensory qualities of the meals must be overshadowed by its advantages, even though they could give consumers more value (Frewer et al., 2003). A product's ability to satisfy customers and if it fulfills their demands will determine its level of acceptability. As a result, it is essential to take customer feedback into account both at the beginning of the product development process and while evaluating the end product's acceptability (Bayarri, 2011).





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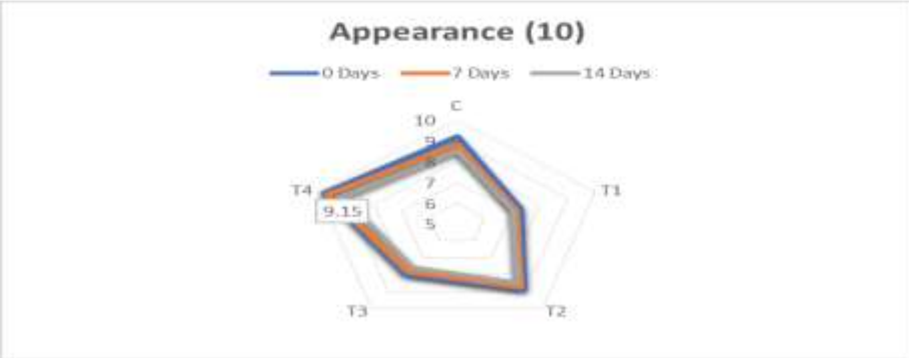

Fig.9 Sensory evaluation of stirred yogurt enriched with pineapple juice.

*See legend to Fig 3 for details.

4. Conclusion

This research aimed to investigate the possibility of reducing the effect of the enzyme bromelain on proteolysis in milk during its use in yogurt production. This was accomplished by the use of ultrasound combined with heat treatment of pineapple juice used to enrich yogurt, aiming at the development of a healthy and consumer-acceptable functional yogurt with a low level of proteolysis due to the activity of bromelain. The results indicated that the combination of ultrasound and heat treatment was most efficient at improving the quality of the resulting functional yogurt. Ultrasound significantly improved antioxidant activity and the level of bioactive compounds such as flavonoids and phenols. Besides, ultrasound resulted in a significant reduction in bacterial counts and juice clarity compared to heat treatment alone. Though heat treatment was more effective in inhibiting microorganisms, it also lowered the content of beneficial bioactive compounds significantly. Therefore, further research is needed to determine the optimum processing parameters i.e., time, power, frequency, and amplitude for the use of ultrasound in preservation. By combining ultrasound with

ISSN: 2537-0804 eISSN: 2537- 0855

moderate heat or other new technologies, i.e., high-voltage cold plasma treatment, high-pressure treatment, and antibacterial agents, food processing plants can create high-quality juices and dairy products with extended shelf life.

Acknowledgements

The authors thank the Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Giza, Egypt.

Funding

No fund

Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that they have no conflict of interest.

Authorship contribution

This work was done by all authors

Ethical Approval

None to be declared

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