Food Science and Engineering
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UNIVERSAL WISER PUBLISHER

Research Article

Evaluation of the Antioxidant and Antimicrobial Properties of Pumpkin Pulp During Storage Through the Ultrasonication Process

Zunaira Arshad, Nabeel Ashraf, Ahsan Ali*, Ali Iqbal, Madiha Rafique, Maryam Gulzar, Adan Ahmad, Syed Ali Hassan

National Institute of Food Science and Technology, University of Agriculture, 38000, Faisalabad, Pakistan
E-mail: ashanali0789@gmail.com

Received: 6 September 2024; **Revised:** 6 December 2024; **Accepted:** 19 December 2024

Abstract: Pumpkin (*Cucurbita maxima*) is a seasonal fruit and it contains large amounts of bioactive compounds, including phenolic compounds, carotenoids, and anthocyanins, which lower the risk of cancers and prevent osteoporosis and hypertension. However, conventional pasteurization reduces the nutrient content of fruits and results in a loss of organoleptic properties. Food processing industries are exploring alternatives to thermal treatment (TT) due to the increasing demand for safe, high-quality, and minimally processed pumpkin pulp. The outcomes of thermal treatment and ultrasonication (US) on the overall quality of pumpkin pulp were examined in this study. Six treatments were used in the preparation of pumpkin pulp: T_0 (control), T_1 (TT at 90 °C for 2 min), and T_2 to T_5 (US of 37 kHz frequency for 5-20 min). Statistically analyzed data showed a significant increase in total phenolic contents (TPC) and total flavonoid contents (TFC) in US-treated samples (216 to 222 mg GAE/100 g) and (7.53 to 12.9 mg CE/100 g) respectively. In contrast, a significant decrease in microorganisms was found in all US-treated samples. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays increased significantly in US-treated samples (29.20 to 33.71%), (0.92 mg TE/g to 1.36 mg TE/g), and (4.52 mg TE/g to 5.15 mg TE/g) respectively. Nevertheless, a significant rise in ascorbic acid levels was observed in the T_5 sample (15.48 mg/100 g). All US-treated samples showed significantly fewer losses in bioactive compounds and effectively decreased microbial load during storage as compared to thermal treatment. This research indicated that the US T_5 showed the best results and demonstrated an ability to enhance the overall quality of pumpkin pulp, which can be effectively utilized for processing in industries.

Keywords**:** antioxidant, pumpkin, ultrasonication, antimicrobial

1. Introduction

The consumption of fruits and vegetables promotes health, energy, and quality of life. Fruit and vegetable intake decreases health-related diseases including cancer, cardiovascular diseases, and diabetes. Bioactive compounds including polyphenols, carotenoids, tocopherols, anthocyanin, vitamins, minerals, and fiber present in fruits have beneficial effects on health [1]. Recently, the market for food items has been growing that offer functional activity and enhance the well-being of consumers [2]. In Pakistan, Pumpkin (*Cucurbita maxima*), is commonly called halwa

DOI: https://doi.org/10.37256/fse.6120255657

Kadu and belongs to the Cucurbitaceae family. Pumpkin has been grown all over the world, and utilized in various ways based on local traditions and regions [3]. The prevalence of heart disease, gastrointestinal problems, and cancer require the incorporation of plant-based products into the human diet that possess enhanced bioactive properties and pumpkin is a good example of such product [4]. Pumpkin demand continues to rise mostly because it is lower in energy and contains significant levels of dietary fiber, polysaccharides, pectin, carotenoids, and flavonoid pigments that are becoming increasingly significant because of their antioxidant activity [5] minerals (potassium, calcium, magnesium, sodium, iron), vitamins (A, C, B_1, B_2, B_9), and other health-promoting substances [6]. The most significant advantage of the pumpkin is their low caloric content, which is primarily due to their high-water content which is approximately 80% of the total portion of plants [7].

Fruit and vegetable juices have high sugar content which leads to microbial growth rendering them at risk of quick degradation. Conventional heat treatment is a commonly used method for retarding the growth of microorganisms and extending the stability of juices up to a certain point in time. Conventional pasteurization of fruit and vegetable samples reduces their nutrient contents due to their high temperature. It has been observed that heat treatment has dramatically lowered the ascorbic acid concentration was 36 percent, total phenols concentration was 22 percent, flavonoids level was 25 percent, and antioxidant capacity was 18 percent in strawberry and blueberry juice [8]. Scientists are looking for substitutes for heat treatment to preserve food because heat treatment affects the sensory, physicochemical, and nutritional properties of food. The food industry and food researchers have investigated new substitutes technique that can retard microorganism's growth and certain enzymes found in various kinds of food, without destroying the nutritional and sensory components of foods [9].

It has been demonstrated that ultrasonic, either by itself or in combination with other preservation methods such as the use of antimicrobials and moderate temperatures (50 °C), is a promising substitute technology for food processing [10]. Ultrasonication (US) is a vital non-thermal method for accomplishing sustainable green chemistry. It utilizes sound waves of varying frequencies to achieve specific goals [11]. The two principles that solidify ultrasonication are the acoustic-cavitation effect (compression-collapse) and the piston effect (compression-decompression). Cavitation is an effect that releases energy when microbubbles are created in the medium during the process of compression and collapse. This leads to the creation of high-pressure (50-100 MPa) and High-temperature (550 °C) zones [12]. Ordonez-Santos et al. [13] found that gooseberry juice was processed in ultrasonic cleaner at 42 kHz and time 40, 20, and 10 min. The total content of carotenoid, phenolic, and retinol in the juice sample increased significantly after US treatment. The objectives of this research are to investigate the antioxidant and antimicrobial properties of pumpkin pulp and its optimum shelf life study through the ultrasonication process.

2. Materials and methods

This research was conducted in the Beverage laboratory of the National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan. The purpose of this study was to determine the antioxidant and antimicrobial effect of pumpkin pulp through the ultrasonication process during storage.

2.1 *Procurement of raw material*

Pumpkins (*Cucurbita maxima*) were brought from the local market of Faisalabad, Pakistan. Good quality pumpkins were brought to the lab where further processing of pumpkins was conducted. The sources of all the chemicals, reagents, and standards used in this investigation were Sigma-Aldrich (St. Louis, MO, USA).

2.2 *Preparation and selection of raw material*

2.2.1 *Grading and sorting*

Mature and fully ripe pumpkin was brought from Faisalabad. Sorting and grading of pumpkins were done manually based on different quality parameters like color, size, shape, and maturity. Defective and undesirable fruits were removed during sorting [14].

2.2.2 *Preparation of pumpkin pulp*

Pumpkins were cleaned using water to clear the soil, and peeling was done, after being chopped into portions, the pumpkin was put in the blender to make pulp [14].

2.3 *Thermal treatment (TT)*

The pulp was put in a 250 mL glass beaker and subjected to heat treatment using a water bath at the following conditions at 90 °C for 2 min. To avoid any light-induced interference, samples were treated in complete darkness [15].

2.4 *Ultrasonication (US) treatment*

Laboratory-scale ultrasound equipment (Elma E 60 H, Germany) with a frequency of 37 kHz was used to treat pumpkin pulp. Firstly, pumpkin pulp was added into a 250 mL beaker and placed carefully in the middle of the ultrasonic bath at the same water level at 30 °C. The pulp was treated with a constant US frequency of 37 kHz for 5-20 min and a power level of 600 W. All treated samples were performed in complete darkness to avoid any light-induced interference [16].

2.5 *Treatment plan*

Pumpkin Pulp (T_1 sample) was thermally treated in a water bath at 90 °C for 2 min. Samples T_2-T_5 were subjected to ultrasound treatments at a temperature of 30 °C for 5-20 min using a 37 kHz frequency. The T_0 sample served as a control group (Table 1) [17].

Table 1. Individual effects of ultrasonication and thermal treatment on pumpkin pulp were investigated

Treatments	Conventional pasteurization	Ultrasonication frequency (kHz)	Time (min)
T_0	-	-	-
T_1	90 °C	-	2
T_2	-	37 kHz	5
T_3	-	37 kHz	10
T_4	-	37 kHz	15
T_5	-	37 KHz	20

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

2.6 *Phytochemical analysis of pumpkin pulp*

2.6.1 *Determination of total phenolic contents (TPC)*

TPC in pumpkin pulp was analyzed through a spectrophotometer (U2020, IRMECO, Germany) by using Folin-Ciocalteu reagent. A 10% (w/v) Folin-Ciocalteu reagent solution was added to the pumpkin pulp. The mixture was thoroughly mixed, and after that, 2 mL of 20% Na_2CO_3 was added. A spectrophotometer was used to determine the TPC at 760 nm wavelength. The Gallic acid was used as a standard to calculate TPC [18]. All of the samples' total phenolic contents were determined using the formula:

$$C = c\frac{V}{m} \tag{1}$$

C = TPC (mg GAE/g);
c = Gallic acid concentration (mg/mL);
V = Extract volume (mL);
m = Extract mass (g).

2.6.2 *Determination of total flavonoid contents (TFC)*

TFC has been determined using the procedure described by Manzoor et al. [19]. The 0.25 ml sample of pumpkin pulp was mixed with 1.25 ml of water. Subsequently, a duration of reaction of approximately 6 minutes was allowed following the addition of 50 µl of $NaNO_2$ (5%), followed by the addition of 100 µl (10%) of $AlCl_3$. After 6 minutes, 0.5 ml of 1 M NaOH was added, and after that volume reached 2.5 ml by distilled water. A spectrophotometer (U2020, IRMECO, Germany) that was used for the measurement of absorbance of TFC in pumpkin pulp was carried out at 415 nm wavelength. The catechin was used as a standard to calculate the TFC in pumpkin pulp.

$$\text{TFC (mg/g)} = C \times V / g \tag{2}$$

C = concentration of the catechin equivalent from the standard curve (µg/mL);
V = Extract volume (mL);
g = Extract weight (g).

2.6.3 *Determination of DPPH-free radical scavenging activity*

The DPPH of pumpkin pulp was determined following the protocol by Aadil et al. [20]. A 2 mL DPPH solution was added to a 2 mL pumpkin pulp. After 30 minutes the absorbance was measured using a spectrophotometer at 517 nm wavelength. In the same way, the control (ethanol) was prepared. A reduction in absorbance has been linked to the proton-donating activity. The DPPH radical scavenging value was measured by a decrease in absorbance of the pumpkin pulp by the equation:

$$\text{DPPH (\%)} = 100 \times (A_0 - A_1 / A_0) \tag{3}$$

A_1 is the absorbance of the pumpkin pulp and A_0 is the control.

2.6.4 *ABTS radical scavenging assay*

The ABTS assay was performed using the Rice-Evans and Miller [21] Methodology. After adding extract into ABTS•+ reagent, the absorbance was measured at 734 nm. The regression formula was used to determine the antiradical capability against ABTS (mg TE/g DM).

2.6.5 *Ferric reducing antioxidant power (FRAP)*

The FRAP test is used to measure extracts' reducing capacity up to standards described by Pollini et al. [22]. After adding the sample to the FRAP reagent, the absorbance was measured at 593 nm. The regression formula was used to determine the reducing capacity in mg TE/g DM.

2.7 *Estimation of ascorbic acid*

Vitamin C contents of pumpkin pulp were determined by using acid-base titration following the protocol by Ayoub et al. [23]. About 50-100 mg of pulp was weighed and then homogenized in a 500 mL solution of meta-phosphoric acid formed by 15 g of meta-phosphoric acid mixed in 40 mL of acetic acid and volumeter with the distilled water. The homogenized material was filtered and diluted to achieve a final concentration of 10-100 mg ascorbic acid/100 ml. In 100 mL of meta-phosphoric acid-acetic acid solution, 50 mg of L-ascorbic acid was weighed to form the standard solution. Each sample was taken three times using the standard, and the results were titrated using the solution of dye until the rose-pink color was sustained for 10 seconds. The ascorbic acid of the pumpkin pulp was calculated using mg ascorbic acid/100 ml.

$$\text{Vitamin C (mg/100 mL)} = \frac{\text{Volume of sample used} \times \text{Titer} \times \text{Dye factor}}{\text{Sample reading} \times \text{Aliquot of extract taken}} \times 100 \quad (4)$$

2.8 *Microbial analysis of pumpkin pulp*

To evaluate the safety of the pulp, microbiological analyses of TPC, *E. coli* Yeast, and mold were performed. The microbiological analysis of pumpkin pulp was carried out following the pour plate method and counts were estimated in (CFU/mL) [24].

2.9 *Sensory analysis*

Sensory analysis will be performed of the pumpkin pulp to check the overall acceptance, color, and mouthfeel [25]. For sensory analysis, Hedonic scale was used from 1-9. By using a hedonic scale, the panelists choose the rating level according to their preferences and how much they like it based on their emotional and preference statements. This scale assessed whether the person who tasted the product liked it or disliked it. Fresh, untreated pulp was used as a control. The untreated sample was compared to the thermal and ultrasonic treated samples and every treatment was different. All samples were coded using random three-letter (A, B, C, D) and sent to panelists (20 mL). The assessment team must be well known with assessment Performa. Provide a glass of water to the panelists and educate them to rinse their palate with water and drink water between the two samples.

2.10 *Storage study*

Pumpkin pulp was placed in the refrigerator for one month and evaluated its quality parameters after 0, 7, 14, 21, and 28 days [26].

2.11 *Statistical analysis*

The data were analyzed statistically by following the method of Montgomery [27]. The data were expressed as mean values ± standard deviation (SD). Factorial analysis under two-way analysis of variance (ANOVA) was conducted at a significance level of $p < 0.05$. Statistics 10 software was used to determine the significant differences between mean values.

3. Results and discussion

3.1 *Effect on total phenolic contents of pumpkin pulp*

The effect of US and thermal treatments on the TPC contents in pumpkin pulp is shown in Table 2. All of the examined treatments had a significant effect on the TPC of the pumpkin pulp. T_4 and T_5 showed the best result in which total phenolic contents increased with US increasing time. T_4 showed the highest phenolic content (224.2 mg GAE/100 g) as compared to T_5, T_3, and T_2 (222.2 mg GAE/100 g), (22.3 mg GAE/100 g) and (220.6 mg GAE/100 g) respectively. Thermal treatment showed significant decreasing trends in the total phenolic contents in pumpkin pulp as compared to the ultrasonicated treated sample. Our findings were similar to those of Nadeem et al. [28], who reported an increase in the TPC value of the US-treated carrot-grape blend juice. US processing can cause the release of phenolic chemicals by breaking down the cell walls of plant cells. This increase can be attributed to the abrupt changes in pressure or shear force caused by the implosion of bubbles, leading to cavitation and subsequent destruction of cells. A notable rise in phenolic content has also been reported in apple juice due to sonication processing [29]. Results showed that TPC significantly decreased with respect to storage time but ultrasonicated samples found less decreasing trends in addition to thermal as well control. Thermal treatment had higher losses of total phenolic contents at 28 days (209.8 mg GAE/100 g) respectively as compared US treated samples T_4 (217.5 mg GAE/100 g) and T_5 (216.8 mg GAE/100 g). High temperature leads to more losses of phenolic compounds [30]. Heat induce losses in phenolic compounds were observed in Barberry juice [31]. Goh et al. [32] found that conventional pasteurization affects the heat-sensitive phenolic compounds in pineapple juice by inactivating the pectinase enzyme. Similar trends were noted by Calderón-Martínez et al. [33], who noticed a decrease in TPC value during storage of Gulupa pulp.

Table 2. Mean values of TPC (mg GAE/100 g) of pumpkin pulp treated with ultrasonication and conventional pasteurization during storage of 28 days

Treatments	Storage days				
	0	7	14	21	28
T_0	219.7 ± 0.41^{f}	217.7 ± 0.44^{gh}	215.9 ± 0.70^{kl}	213.8 ± 0.30^{op}	211.3 ± 0.53^{r}
T_1	216.4 ± 0.42^{ijk}	214.7 ± 0.38^{mn}	213.1 ± 0.39^{p}	211.4 ± 0.51^{qr}	209.8 ± 0.43^{s}
T_2	220.6 ± 0.35^{de}	218.5 ± 0.46^{g}	216.2 ± 0.91^{jkl}	214.3 ± 0.28^{no}	212.2 ± 0.40^{q}
T_3	222.3 ± 0.54^{c}	220.0 ± 0.51^{ef}	217.7 ± 0.43^{gh}	215.4 ± 0.42^{lm}	213.1 ± 0.41^{p}
T_4	224.9 ± 0.93^{a}	223.6 ± 0.79^{b}	220.4 ± 0.60^{def}	219.8 ± 0.42^{ef}	217.2 ± 0.34^{hi}
T_5	222.2 ± 0.44^{c}	221.1 ± 0.43^{d}	219.9 ± 0.32^{ef}	218.1 ± 0.70^{g}	216.8 ± 0.40^{ij}

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

3.2 *Effect on total flavonoid contents of pumpkin pulp*

The ultrasound treatment and thermal treatment showed a significant effect on the TFC contents in pumpkin pulp as shown in Table 3. T_4 and T_5 showed the best result in which total flavonoid contents increased with US processing time. T_5 showed the highest flavonoid content (12.9 mg CE/100 g) as compared to T_4 (11.3 mg CE/100 g), T_3 (10.1 mg CE/100 g), and T_2 (9.46 mg CE/100 g) at 0 days. Thermal treatment showed si sgnificant decreasing trends in the total flavonoid contents (7.53 mg CE/100 g) in pumpkin pulp as compared to US-treated sample T_5 (12.9 mg CE/100 g) at 0 days. Chakraborty et al. [34] found phenolic was thermally degraded more quickly at higher temperatures, which led to lower concentration of bioactive compounds in the pineapple puree. US treatments significantly increase the extraction of flavaniod contents with increasing time. These findings were similar to Choo et al. [35], in which US-treated noni juices undergo an ultrasound treatment process that can result in the release of phenolic compounds. This release occurs owing to the disintegration of the cell walls of the plant cells. The increase in TPC and TFC in noni juice during sonication may attributed along with the release of previously bound phenolics and flavonoids [35]. Similar findings were reported by Saeeduddin et al. [36] in sonicated pear juice. On the 7th day, T_5 had a slightly increase TFC (13.1 mg CE/100 g) in comparison to 0 day (12.9 mg CE/100 g). While T_1 showed more decreasing trend (6.94 mg CE/100 g) in comparison to 0 day (7.53 mg CE/100 g) as showed in Table 3. US-treated samples had less losses of TFC at T_3 (5.99 mg CE/100 g), T_4 (6.26 mg CE/100 g), T_5 (9.98 mg CE/100 g) as compared to T_1 (3.91 mg CE/100 g) at 28 days. The results showed that US-treated samples had TFC value decrease less significantly as compared to thermal and control at 28 days as shown in Table 3. Similar results were reported by Walia et al. [37], who observed a decline in the total flavonoid contents (TFC) value over 30 days while studying the storage of fig juice. The process of breaking down phenolic compounds through oxidation may be the cause of a decline in total flavonoid content during sonication.

Table 3. Mean values of TFC (mg CE/100 g) of pumpkin pulp treated with ultrasonication and conventional pasteurization during storage of 28 days

Treatments	Storage days				
	0	7	14	21	28
T_0	8.51 ± 0.34^{hi}	7.49 ± 0.39^{ijk}	5.97 ± 0.76^{lmn}	4.01 ± 0.75^{o}	2.29 ± 0.51^{p}
T_1	7.53 ± 0.45^{ijk}	6.94 ± 0.84^{klm}	5.54 ± 0.46^{mn}	4.90 ± 0.90^{no}	3.91 ± 0.81^{o}
T_2	9.46 ± 0.42^{fgh}	8.97 ± 0.73^{gh}	7.63 ± 0.59^{ij}	6.37 ± 0.47^{klm}	5.54 ± 0.47^{mn}
T_3	10.1 ± 0.52^{ef}	9.35 ± 0.56^{fgh}	8.94 ± 0.79^{gh}	7.46 ± 0.47^{ijk}	5.99 ± 0.84^{lm}
T_4	11.3 ± 0.51^{cd}	10.9 ± 0.75^{cde}	9.88 ± 0.99^{efg}	8.44 ± 0.41^{hi}	6.26 ± 0.53^{lm}
T_5	12.9 ± 0.75^{ab}	13.1 ± 0.70^{a}	11.9 ± 0.80^{bc}	10.6 ± 0.38^{de}	9.98 ± 0.80^{efg}

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

3.3 *Effect on DPPH of pumpkin pulp*

The impact of ultrasound treatment and thermal treatment on the DPPH value in pumpkin pulp during the 28th day, of storage is shown in Table 4. US treatments found a significant impact on the DPPH of the pumpkin pulp. T_4 and T_5 showed the best result in which DPPH contents increased with increasing US time. T_5 showed the highest DPPH value (33.71%) as compared to T_4, T_3, and T_2 (32.67%), (31.95%) and (31.10%) at 0 days. The DPPH results showed increased levels in all US-treated samples. During ultrasound treatment, the results of pumpkin pulp samples exhibited a substantial rise ($p \leq 0.05$) from 30.85 to 33.71%. Aadil et al. [24] also found during the 28-day storage period, the grapefruit juices exhibited an ongoing upward trend in DPPH free radical scavenging activity following ultrasound treatment. This rise could be attributed to the enhancement in the overall phenolic contents of the fluids. Table 4 provides information on the mean value of DPPH and results showed that DPPH significantly decreased concerning storage time but ultrasonicated treated samples showed less decreasing trends as compared to thermal and control. Thermal treatment T_2 had higher losses of DPPH (22.69%) at 28 days as compared to US-treated samples T_3 (27.86%), T_4 (28.76%), and T_5 (29.43%). Similar trends were observed by Bhat and Goh [38], who observed a decline in the DPPH value over 30 days while studying the storage of strawberry juice. Similar trends were observed by Aadil et al. [24], who found that high concentrations of phenolic contents in vegetables and fruits are directly correlated with a considerable increase in antioxidant activity.

Table 4. Mean values of DPPH (%) of pumpkin pulp treated with ultrasonication and conventional pasteurization during storage of 28

Treatments	Storage days				
	0	7	14	21	28
T_0	30.85 ± 0.05^{ef}	29.35 ± 0.04^{ghi}	27.87 ± 0.03^{l}	24.76 ± 0.07^{n}	19.92 ± 0.03^{q}
T_1	29.20 ± 0.04^{hij}	28.89 ± 0.07^{ijk}	26.44 ± 0.04^{m}	26.61 ± 2.92^{o}	22.69 ± 0.04^{p}
T_2	31.10 ± 0.04^{e}	30.55 ± 0.05^{f}	29.3 ± 0.05^{ghij}	27.53 ± 0.05^{l}	25.44 ± 0.04^{m}
T_3	31.95 ± 0.05^{cde}	30.94 ± 0.04^{ef}	29.85 ± 0.08^{g}	28.55 ± 0.03^{k}	27.86 ± 0.06^{l}
T_4	32.67 ± 0.04^{b}	31.95 ± 0.04^{cd}	30.62 ± 0.05^{ef}	29.67 ± 0.06^{gh}	28.76 ± 0.06^{jk}
T_5	33.71 ± 0.06^{a}	33.42 ± 0.03^{a}	32.35 ± 0.04^{bc}	31.78 ± 0.08^{d}	29.43 ± 0.04^{gh}

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

3.4 *FRAP assay*

The impact of ultrasound treatment and thermal treatment on the FRAP value in pumpkin pulp during the 28th day, of storage is shown in Table 5. Ultrasonicated samples have a significant effect on FRAP of the pumpkin pulp. T_4 and T_5 showed the best result in which FRAP contents increased with increasing time at 37 kHz. T_5 showed the highest FRAP value (1.36 mg TE/g) as compared to T_4 (1.3 mg TE/g), T_3 (1.19 mg TE/g), and T_2 (0.98 mg TE/g) at 0 days. The results showed increased levels in all US-treated samples. Choo et al. [35] determined that the antioxidant potential of noni juice was significantly enhanced by 60 minutes of sonication as compared to the control group. The rise in bioactive substances like phenolic and organic acids may be the cause of an increase in antioxidant activity. Thermal treatment had higher losses of FRAP value (0.54 mg TE/g) at 28 days as compared to US-treated samples (1.11 mg TE/g) because thermal treatment leads to degradation of fruit pulp at high temperatures. Mgaya-Kilima et al. [39] also found that when a blend juice of guava, mango, papaya, and roselle was stored after pasteurization, its antioxidant activity was reduced.

Table 5. Mean values of FRAP (mg TE/g) of pumpkin pulp treated with ultrasonication and conventional pasteurization

Treatments	Storage days				
	0	7	14	21	28
T_0	0.97 ± 0.03^{efg}	0.88 ± 0.04^{ghijk}	0.78 ± 0.04^{klmn}	0.67 ± 0.03^{o}	0.43 ± 0.05^{q}
T_1	0.81 ± 0.06^{ijklmn}	0.79 ± 0.02^{jklmn}	0.75 ± 0.04^{lmno}	0.71 ± 0.02^{no}	0.54 ± 0.04^{p}
T_2	0.98 ± 0.04^{efg}	0.94 ± 0.05^{fgh}	0.89 ± 0.01^{fghij}	0.82 ± 0.03^{ijklm}	0.67 ± 0.04^{o}
T_3	1.19 ± 0.28^{bc}	0.99 ± 0.06^{ef}	0.91 ± 0.04^{fghi}	0.88 ± 0.02^{ghijk}	0.72 ± 0.04^{efgh}
T_4	1.3 ± 0.09^{a}	1.28 ± 0.03^{ab}	1.1 ± 0.04^{cd}	1.07 ± 0.03^{de}	0.84 ± 0.04^{hijkl}
T_5	1.36 ± 0.07^{a}	1.33 ± 0.02^{a}	1.16 ± 0.04^{cd}	1.11 ± 0.04^{cd}	0.94 ± 0.03^{fgh}

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

3.5 *ABTS assay*

The impact of ultrasound treatment and thermal treatment on the ABTS value in pumpkin pulp during the 28th days, of storage is shown in Table 6. US treatments found a significant impact on the ABTS value of the pumpkin pulp. T_4 and T_5 showed the best result in which ABTS contents increased with increasing time at 37 kHz. T_5 showed the highest ABTS value (5.15 mg TE/g) as compared to T_4, T_3, and T_2 (4.98), (4.82 mg TE/g), and (4.77 mg TE/g) respectively. Thermal treatment showed significant decreasing trends of ABTS value in pumpkin pulp as compared to the ultrasonicated treated sample. The ABTS assay results showed increased levels in all US-treated samples. During ultrasound treatment, the results of pumpkin pulp samples exhibited a substantial rise ($p \leq 0.05$) from 4.77 to 5.15 with increasing time at 0 days. Similar findings were reported by macadamia skin [40] and lemon myrtle [41], which concluded that higher recovery of bioactive compounds was associated with longer radiation exposure. The antioxidant activity of pasteurized baobab juice showed a similar pattern with storage [42]. Results showed that ABTS value significantly decreased concerning storage time but ultrasonicated samples have less decreasing trends as compared to thermal and control. On the 7th day, T_5 had a slightly decreased ABTS (5.09 mg TE/g) in comparison to 0 days (5.15 mg TE/g) while T_1 showed more decreasing trend (4.47 mg TE/g) in comparison to 0 days (4.52 mg TE/g) as shown in Table 6. This study showed that the application of ultra-sonication had a significant effect on the ABTS value of pumpkin pulp during storage.

Table 6. Mean values of ABTS value (mg TE/g) of pumpkin pulp treated with ultrasonication and conventional pasteurization

Treatments	Storage days				
	0	7	14	21	28
T_0	4.52 ± 0.05^{jk}	4.47 ± 0.04^{jk}	4.41 ± 0.04^{mn}	4.36 ± 0.04^{n}	4.16 ± 0.03^{pq}
T_1	4.45 ± 0.04^{lm}	4.38 ± 0.03^{n}	4.29 ± 0.03^{o}	4.2 ± 0.03^{p}	4.13 ± 0.04^{q}
T_2	4.77 ± 0.04^{ef}	4.65 ± 0.04^{g}	4.53 ± 0.02^{jk}	4.47 ± 0.04^{klm}	4.29 ± 0.03^{o}
T_3	4.82 ± 0.07^{e}	4.74 ± 0.05^{f}	4.68 ± 0.03^{gh}	4.6 ± 0.02^{hi}	4.35 ± 0.03^{n}
T_4	4.98 ± 0.05^{c}	4.83 ± 0.04^{de}	4.76 ± 0.04^{f}	4.67 ± 0.05^{g}	4.54 ± 0.05^{ij}
T_5	5.15 ± 0.04^{a}	5.09 ± 0.03^{ab}	5.03 ± 0.02^{bc}	5.01 ± 0.03^{c}	4.89 ± 0.04^{d}

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

3.6 *Effect on vitamin C of pumpkin pulp*

US and thermal treatment on vitamin C concentration in pumpkin pulp is shown in Table 7. All of the examined treatments had a significant impact on the vitamin C of the pumpkin pulp. Ultrasonication treatments significantly increase the value of vitamin C with an increase in processing time. T_4 and T_5 showed the best result in which vitamin C content was increasing with an increasing time at 37 kHz. T_5 showed highest vitamin C content (15.48 mg/100 g) as compared to T_4, T_3 and T_2 (14.45 mg/100 g) and (14.27 mg/100 g) and (13.74 mg/100 g). The degradation of pulp is mostly caused by heat and oxygen. The improvement in vitamin C levels can be attributed to the elimination of trapped oxygen caused by cavitation. Our findings showed an increasing trend of vitamin C levels similar to vitamin C levels in sonicated grapefruit juice were reported by Aadil et al. [24]. Results showed that vitamin C significantly decreased concerning storage time but ultrasonicated treated samples showed less decreasing trends as compared to thermal and control. On the 7th day, T_5 had low vitamin C decreasing (14.31 mg/100 g) in comparison to 0 day (15.48 mg/100 g) While T_1 showed more decreasing trend (7.65 mg/100 g) in comparison to 0 day (9.81 mg/100 g) as showed in Table 7. Thermal treatment had higher losses of vitamin C at 28 days (3.07 mg/100 g) as compared to US-treated sample T_4 (10.42 mg/100 g) because vitamin C is heat sensitive, and thermal treatment leads to degradation of fruit pulp. These studies showed that the application of ultra-sonication had a significant effect on the vitamin C value of pumpkin pulp.

Table 7. Mean values of vitamin C (mg/100 g) of pumpkin pulp treated with ultrasonication and conventional pasteurization during storage of 28 days

Treatments	Storage days				
	0	7	14	21	28
T_0	14.25 ± 0.59^{a}	12.59 ± 0.83^{d}	10.66 ± 0.93^{hi}	8.94 ± 0.76^{o}	6.55 ± 0.56^{r}
T_1	9.81 ± 0.67^{m}	7.65 ± 0.99^{q}	6.13 ± 0.93^{s}	4.84 ± 0.86^{t}	3.07 ± 0.89^{u}
T_2	13.74 ± 0.94^{b}	13.30 ± 0.94^{c}	12.77 ± 0.98^{d}	11.51 ± 0.87^{f}	9.54 ± 0.98^{ij}
T_3	14.27 ± 0.98^{d}	13.72 ± 0.98^{e}	13.15 ± 0.81^{g}	11.58 ± 0.98^{jk}	10.58 ± 0.76^{n}
T_4	14.45 ± 0.97^{ef}	14.35 ± 0.97^{gh}	13.59 ± 0.91^{kl}	12.38 ± 0.80^{n}	10.42 ± 0.97^{op}
T_5	15.48 ± 0.93^{j}	14.31 ± 0.76^{l}	13.52 ± 0.98^{mn}	12.70 ± 0.98^{n}	10.21 ± 0.78^{p}

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

3.7 *Effect on total plate count of pumpkin pulp*

The effect on the microbes attributes in pumpkin pulp by ultrasound treatment and thermal treatment are shown in Table 8. All of the examined treatments showed a significant effect on the TPC of the pumpkin pulp. On day 0, ultrasonication effectively lowered all population levels to a point where they were undetectable (< 2 log). The ultrasonic treatments drastically reduced the growth rate of all microbial populations. Moreover, the reduction in the rate of development was more pronounced in the US for 20-min samples compared to 15-min, 10-min, and 5-min samples. This indicates that longer ultrasonic exposure times result in significantly greater decreases in the growth rate of the local microflora. The substantial decrease in the growth rate of microbes in ultrasonicated samples may be attributed to the physical phenomena of cavitation that happens during ultrasound treatments. Our findings were similar to the US-treated strawberry juices, in which the ultrasound treatment process causes cavitation as reported by Tomadoni et al. [43]. Results showed that microbes were significantly increased with respect to storage time but ultrasonicated samples have less increasing trends in addition to thermal and control. During the 28th day, ultrasonicated treatment had a microbial load of 5.39 CFU/ml as compared to control and thermal treatment which had 8.63 CFU/ml and 7.47 CFU/ml. A significant reduction in microbial load was observed in the US-treated sample. Microorganisms may degrade as a result of the cavitation process, which generates free radicals and increases the temperature [44].

Table 8. Mean values of total plate count (CFU/mL) of pumpkin pulp treated with ultrasonication and conventional pasteurization during storage of 28 days

Treatments	Storage days				
	0	7	14	21	28
T_0	4.30 ± 0.26^{l}	5.83 ± 0.46^{g}	6.77 ± 0.59^{de}	8.20 ± 0.90^{b}	8.63 ± 0.45^{a}
T_1	3.95 ± 0.65^{n}	4.88 ± 0.61^{j}	5.70 ± 0.45^{h}	6.93 ± 0.56^{c}	7.47 ± 0.43^{b}
T_2	3.20 ± 0.52^{p}	4.13 ± 0.58^{m}	5.82 ± 0.49^{g}	6.06 ± 0.61^{f}	7.32 ± 0.53^{b}
T_3	2.82 ± 0.64^{q}	3.93 ± 0.58^{n}	4.71 ± 0.27^{k}	5.74 ± 0.42^{gh}	6.81 ± 0.56^{cd}
T_4	2.43 ± 0.42^{r}	3.21 ± 0.34^{p}	4.40 ± 0.60^{l}	5.46 ± 0.42^{i}	6.25 ± 0.39^{e}
T_5	2.18 ± 0.40^{s}	2.88 ± 0.62^{q}	3.19 ± 0.40^{o}	4.51 ± 0.30^{j}	5.39 ± 0.45^{i}

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

3.8 *E. coli count*

The ultrasonication significantly affected the *E. coli* value of the pumpkin pulp during 28 days, as demonstrated in Table 9. There was a slight increase in Escherichia coli count over 28 days in relation to thermal and untreated samples. The colonies in US-treated samples significantly decreased as time increased. The T_0 untreated sample had the greatest *E. coli* count compared to the thermal (T_2) and US-treated samples (T_2, T_3, T_4, and T_5). After US treatment, the *E. coli* count in pumpkin Pulp was reduced to 3.25, 3.1, 2.7 and 2.2 CFU/mL as compared to thermal (T_1) 3.81 CFU/mL and untreated sample (T_0) 4.21 CFU/mL at 0 days, with significantly decrease in colonies in samples as time increased. The T_4 and T_5 samples showed the best results due to more exposure time which showed a significant decrease in *E. coli* count of 3.5 and 2.9 CFU/mL respectively after 28 days of storage study. The ultrasonic treatment may have caused the disintegration of microbial cell membranes; leading to a decrease in microbes in pumpkin pulp. These results supported by Zhang et al. [45] investigated how ultrasound affected the microbial properties of pumpkin juice. Longer exposure of ultrasonication times leads to a significant reduction in microbial loads. Tahi et al. [46] found the viability of Staphylococcus aureus cells in orange juice using sonication and showed that sonication at 30, 60, and 90 min reduced the number of colonies.

Table 9. Mean values of *E. coli* count (CFU/mL) of pumpkin pulp treated with ultrasonication and conventional pasteurization during storage of 28 days

Treatments	Storage days				
	0	7	14	21	28
T_0	4.21 ± 0.03^{i}	4.4 ± 0.17^{h}	5.4 ± 0.05^{d}	6.53 ± 0.05^{b}	7.9 ± 0.05^{a}
T_1	3.85 ± 0.04^{l}	4.1 ± 0.05^{j}	4.51 ± 0.05^{g}	5.2 ± 0.06^{e}	6.2 ± 0.05^{c}
T_2	3.25 ± 0.04^{o}	3.48 ± 0.06^{n}	3.8 ± 0.06^{l}	4.2 ± 0.05^{i}	4.69 ± 0.07^{f}
T_3	3.1 ± 0.06^{pq}	3.2 ± 0.04^{o}	3.45 ± 0.04^{n}	3.7 ± 0.04^{m}	3.98 ± 0.02^{k}
T_4	2.71 ± 0.07^{s}	2.85 ± 0.04^{r}	3.04 ± 0.04^{q}	3.19 ± 0.05^{op}	3.5 ± 0.04^{n}
T_5	2.2 ± 0.06^{v}	2.31 ± 0.04^{u}	2.45 ± 0.03^{t}	2.68 ± 0.03^{s}	2.9 ± 0.06^{r}

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

3.9 *Yeast and mold*

US treatments have significantly decreased the yeast and mold count in comparison to thermal and control treatments. The yeast and mold were progressively significant decreased as the US time increased (5, 10, 15, and 20 min). At day 0 there is highest count was observed in untreated sample (T_0) 2.98 CFU/mL and thermal treated sample (T_1) 2.85 CFU/mL as shown in Table 10. While ultrasound treated samples have significantly decrease in yeast and mold count (T_2) 2.7 CFU/mL, (T_3) 2.5 CFU/mL, (T_4) 2.31 CFU/mL, and (T_5) 2.02 CFU/mL. The primary process by which ultrasonic inactivates bacteria is cavitation. Microbubbles are formed, expand, and collapse during the process, producing extreme heat and pressure. Additionally, other phenomena including agitation, pressure, shock waves, acoustic transmission, and the production of free radicals happen when ultrasound is applied [47, 48]. Yıkmış [49] applied ultrasonication on watermelon juices without heat influences at different processing durations (4-16 min), resulting in a satisfactory reduction of yeast and mold count. Starek et al. [50] studied the effect of ultrasound on microbes in fresh tomato juice. The juice was demonstrated to be microbiologically pure even after ten storage days when subjected to higher US field intensity to an ultrasonic field (40 W/cm^2) for ten minutes. Ultrasonication treatment accelerated the growth of bacteria. Many authors reported, that the degree and length of ultrasonication determine how ultrasound affects the growth of microbial cells [51].

Table 10. Mean values of yeast and mold count (CFU/mL) of pumpkin pulp treated with ultrasonication and conventional pasteurization during storage of 28 days

Treatments	Storage days				
	0	7	14	21	28
T_0	2.99 ± 0.05^{ghi}	3.88 ± 0.04^{d}	4.45 ± 0.06^{c}	4.92 ± 1.13^{b}	7.21 ± 0.04^{a}
T_1	2.85 ± 0.05^{hij}	3.27 ± 0.03^{efg}	3.52 ± 0.06^{def}	3.76 ± 0.06^{d}	3.66 ± 0.53^{d}
T_2	2.71 ± 0.03^{ijk}	2.95 ± 0.05^{ghi}	3.25 ± 0.05^{efg}	3.62 ± 0.03^{de}	3.85 ± 0.05^{d}
T_3	2.49 ± 0.07^{jklm}	2.65 ± 0.05^{ijk}	2.8 ± 0.06^{hij}	2.99 ± 0.05^{ghi}	3.15 ± 0.06^{fgh}
T_4	2.31 ± 0.06^{lmno}	2.4 ± 0.05^{klmn}	2.55 ± 0.05^{ijkl}	2.75 ± 0.07^{ijk}	2.94 ± 0.05^{ghi}
T_5	2.02 ± 0.04^{o}	2.1 ± 0.05^{no}	2.26 ± 0.05^{mno}	2.5 ± 0.06^{jklm}	2.8 ± 0.03^{hij}

T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

3.10 *Sensory evaluation of pumpkin pulp*

The sensory evaluation method was an adaptation of quantitative descriptive analysis (QDA) developed by Stone [52].

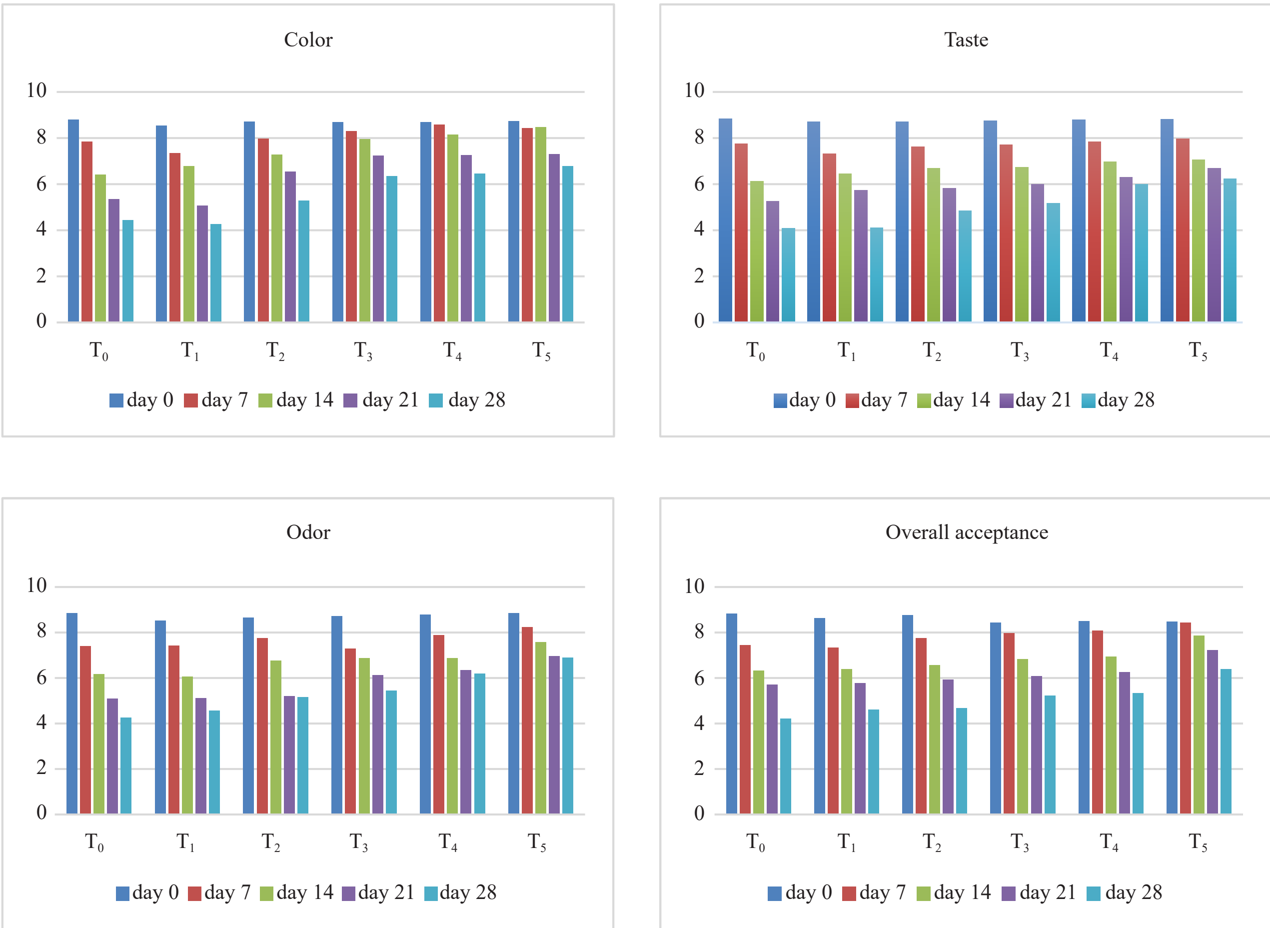

Figure 1. Sensory analysis of pumpkin pulp treated with ultrasonication and conventional pasteurization during storage of 28 days
T_0 = Control sample, T_1 = Thermal treatment at 90 °C (2 min), T_2 = Ultrasonication treatment at 37 kHz (5 min), T_3 = Ultrasonication treatment at 37 kHz (10 min), T_4 = Ultrasonication treatment at 37 kHz (15 min), T_5 = Ultrasonication treatment at 37 kHz (20 min)

Color: All of the examined treatments showed a significant effect on the color value of the pumpkin pulp as shown in Figure 1. T_5 showed the highest color value (8.73) as compared to T_4 (8.68), T_3 (8.68), and T_2 (8.72) at 0-day study. Thermal treatment showed a significant decrease in the color value in pumpkin pulp. Similar findings were reported by Yi et al. [53], who found that processing induced the breakdown of pigments which is responsible for the discoloration of apple juice. Ultrasound's beneficial effects are correlated to its effective removal of dissolved oxygen from the juice [54]. Color values were decreased significantly during storage. Similar findings were reported by Fikry et al. [39] in date based energy drink treated ultra-sonication. In comparison to the control, the US-40 sonicated samples maintained an acceptable evaluation over 28 days.

Taste: All of the US-treated samples showed a significant effect on the taste value of the pumpkin pulp as shown in Figure 1. T_5 showed the highest taste score (8.85) as compared to T_4 (8.82), T_3 (8.1), and T_2 (8.79). These findings were similar to US-treated fresh-cut quince as reported by Yildiz et al. [55]. The efficacy of the US treatment in minimizing the browning and decomposing of fresh fruits has been demonstrated. During storage, taste values were decreased significantly. A similar result was reported by Pratheepa et al. [56] in grape juice during storage. The changes were observed after 15 days in US-treated samples due to oxidation of phenolic compounds and some chemical reactions in juice.

Odor: T_5 showed the highest odor value (8.85) as compared to T_4 (8.79), T_3 (8.72), and T_2 (8.65) as shown in Figure 1. US-treated samples less decreased color values as compared to thermal treatment because high-temperature treatments accelerate the decomposition and condensation of the volatile compounds that contribute to the juice's aroma [57]. These findings were similar to US-treated red wine juice as reported by Ahmad et al. [58], in which the extraction of aromatic compounds is accomplished using 48 kHz ultrasonic waves. During storage aroma values decreased significantly and US-treated samples showed less decreasing trends of aroma value as compared to thermal and control.

Overall acceptance: All of the examined treatments showed a significant ($p > 0.05$) effect on the overall acceptance of the pumpkin pulp. During the 7th day, T_5 had a slightly less decreased acceptance value (8.42) in comparison to other treatments. Our findings were similar to those of US-treated date-based energy drinks, as reported by Fikry et al. [39], where ultrasonicated samples revealed increasing trends against control as well pasteurized samples. The sonicated samples were preferred for up to 21 days compared to untreated samples. The results revealed that overall acceptance values significantly decreased concerning storage time. Similar results were reported by Hassan [59] on-the-shelf stability of pulp leather during storage.

4. Conclusion

US is a novel technique that can enhance the nutritional and therapeutic properties of pumpkin pulp. The results showed that US processes are superior to thermal treatment from a quality perspective. The outcome of this research revealed that the T_5 treatment significantly enhanced the total flavonoid content (12.9 mg CE/100 g), total phenolic content (222 mg GAE/100 g), DPPH (33.71%), FRAP (1.36 mg TE/g) and ABTS (5.15 mg TE/g) of pumpkin pulp. The US improved the quality parameters of pumpkin pulp, which can also be used for industrial purposes. US could be the preferred technique due to its positive impact on the extraction of bioactive compounds from pumpkin pulp, while thermal treatment has limitations in terms of quality and results in losses in bioactive compounds. Limited research has assessed the influence of ultrasonication along with thermal treatments on bioactive compound retention and stability of pumpkin pulp. This is the first report on the development of processed pumpkin pulp, and it could be useful for developing novel value-added products that improve microbial stability and encourage the intake of less appealing fruits and vegetables. Our findings showed that ultrasonication can be applied in the fruit and vegetable processing industry to produce minimally treated pulp to meet consumer needs. Future research should concentrate on combining US with heat to attain more product stability for a longer period and study the US effect on the physicochemical parameters of pumpkins.

Acknowledgments

All the authors reviewed and approved the final draft carefully. We are thankful to the National Institute of Food Science and Technology for providing research facilities.

Authors contributions

Zunaira Arshad and Nabeel Ashraf: designed the study, wrote the original draft, performed the experiments, and examined the data. Zunaira Arshad wrote the original draft, Conceptualization, data collection, picture and table making, and draft writing. Ahsan Ali and Ali Iqbal: Supervision, review, and editing. Madiha Rafique and Maryam Gulzar: Data curation and draft review Adan Ahmad and Syed Ali Hassan: Draft review, Supervision, review, and editing.

Conflict of interest

The authors declare no competing financial interest.

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