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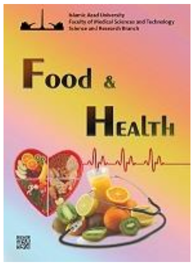

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The effect of pasteurization by ultrasound and thermal method on the color and microbial properties of grape juice

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A B S T R A C T

One of the most widely used methods for sanitizing and reducing the microbial load of fruit juices is thermal pasteurization, which damages bioactive pigments such as anthocyanins. This study was conducted to evaluate the possibility of pasteurization of grape juice by ultrasound (at powers 10, 105, and 200 W, temperatures 0, 30, and 60 °C, and times 2, 6, and 10 min) and to compare the microbial load and color properties by thermal pasteurization (pasteurization at 90 °C for 30 sec). According to the results, the number of color components (a*, b*, and L*) and the total count of microorganisms in pasteurized grape juice decreased significantly ($p \leq 0.05$) as the power, temperature, and time increased. Optimal conditions for simultaneous pasteurization by ultrasound to achieve the minimum microbial load and the maximum number of color components with 90.196% desirability were predicted at a power of 113 watts, a temperature of 60 °C, and a time of 8.850 min. The microbial load of pasteurized grape juice by ultrasound was 0 (CFU/ml), and the number of color components was L*= 18.8021, a*= 37.8406, and b*=27.2104. The results indicated that the pigments of grape juice could be preserved and the same safety in terms of microbial load reduction could be achieved through pasteurization by ultrasound.

1. Introduction

Grape, scientifically known as vinifera, is a genus of trees in the Vitaceae family. There are about 11 genera and more than 600 species in this family. Red grapes extremely affect memory enhancement and even control of Alzheimer's disease (1). Grapes are rich in phytonutrients, especially phenols and polyphenols. According to the Iranian National Standard No. 1634, grape juice is a non-fermented but fermentable product that can be obtained physically from healthy grapes, diluted concentrated grape juice, or grape puree with drinking water to reach the desired concentration with or without edible parts of the fruit such as fruit flesh, berries, fruit pieces, fruit puree, and permitted food additives and is stored and packaged in white or red colors by physical methods (2). Grape juice has a pH between 2.6 to 3.8 (3). All age groups from children to adults consume juices that can be beneficial to human health due to their valuable nutritional properties (4). Processing methods are of great importance in determining the quality, nutritional properties, safety, and shelf life of food products. Among the common methods, thermal methods can be mentioned that increase the shelf life of food products by reducing the population of microbes and enzymes. Despite their beneficial effects, these methods reduce the nutritional and sensory quality of products (5, 6). The most widely used method for pasteurizing juices is the thermal method. This method strongly affects the inactivation of microorganisms, but the application of high pasteurization temperatures reduces nutrients, antioxidants, color compounds, and, consequently, the quality properties of the juice. So, researchers try to find suitable alternatives to the thermal process (5, 7). Various methods have been proposed to eliminate the negative effects of thermal pasteurization, some of which are non-thermal methods such as ultrasound, high hydrostatic pressure, electric field, and hybrid methods (8, 9). However, unprocessed fruit juices can also be contaminated with microbes. The growth of

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these microorganisms reduces the nutritional contents of the juice and creates an unpleasant taste and smell (10, 11). Ultrasound refers to a mechanical wave whose frequency is too high for the human ear to respond to, or in other words more than 20 kHz. High-frequency ultrasonic waves are used in industrial processing generally based on the proper use of a series of mechanisms activated by ultrasonic energy such as heat, agitation, propagation, joint surface instability, friction, mechanical rupture, chemical effects, etc. (12). Because of their purity and efficiency, these mechanisms can be used to produce or increase the range of specific processes, such as those concerning the food and pharmaceutical industries. Accordingly, many studies have been conducted on the effect of ultrasound on food (13, 14). Some of the benefits of using ultrasound in food processing include improved product quality, no heat utilization, increased efficiency, reduced energy consumption, high efficiency, industrial usability, easy installation, low energy cost, low maintenance cost, and simple operation (6). As a result, ultrasound can be used quickly (less than a second) and non-destructively during the food process to determine the properties and quality of food (15). Numerous studies on the use of ultrasound in fruit processing have been conducted recently and have reported this technology as an effective method to reduce the microbial load and maintain the nutritional and physical properties of these food products. For example, one can mention the study of the quality properties of pear juice pasteurized by ultrasound (16), the effect of the ultrasound process on the structural, physical, and lycopene content of guava fruit (17), the effect the ultrasound process on the color of raspberry juice (18), and the effect of the ultrasound process on the microstructural properties, pectin, carbohydrates, and rheological properties of kiwi juice (19). Due to the low background and the existence of different conditions of pasteurization (temperature, power, and time) by ultrasound, researchers and experts in the food industry have focused on finding other storage methods that have a minimal effect on the color and appearance of the food in addition to reducing the microbial load. This study was performed to determine the optimal conditions for the pasteurization of red grape juice by ultrasound under different conditions (temperature, power, and time) and to evaluate and compare the color properties and microbial load (total count of microorganisms) of red grape juice pasteurized by ultrasound under optimal conditions and thermal pasteurization (90 ° C for 30 seconds).

2. Materials and methods

2.1. Materials and chemicals

Red grapes were purchased from a local fruit shop in Tehran to provide samples. Chemicals such as phenolphthalein reagent and sodium hydroxide (Merck-Germany), buffers 4 and 7 (Carlo Erba-Italy), and plate count agar (Merck-Germany) were prepared.

2.2. Samples preparation

After preparing red grapes and dewatering them, the samples of red grape juice were pasteurized using an ultrasonic probe device (AMMM model, Switzerland) under different conditions of temperature (0, 30, and 60 °C), time (2, 6, and 10 min), and power (10, 105 and 200 W) according to Table 1. The optimal sample with the lowest microbial load (total count of microorganisms) and the best color properties was then compared with the control sample which was thermally pasteurized (90 °C for 30 sec).

Table 1. Red grape juice treatments were pasteurized by ultrasound different conditions.

Treatment	Power (w)	Temperature (°C)	Time (min)
1	105	30	6
2	10	60	6
3	10	30	2
4	200	0	6
5	10	0	6
6	105	60	10
7	105	60	2
8	105	30	6
9	200	30	10
10	105	30	6
11	105	0	10
12	200	60	6
13	10	30	10
14	105	0	2
15	200	30	2

2.3. Physicochemical properties

The physicochemical properties of grape samples including pH, acidity, and Brix were measured according to the Iranian National Standard No. 2685 (20).

2.4. Color analysis

The color of the samples was measured using a Hunter Lab (made in Iran). In this system, the exact position of the 3D color was described using the indices L*, a*, and b*. To this end, samples were placed in the cell and the number corresponding to the color parameters was read (21).

2.5. Microbial analysis

The total counting of fruit microorganisms was performed following the Iranian National Standard No. 1-5272 (Food Chain Microbiology-a comprehensive method for counting microorganisms) in plate count agar and incubation in aerobic conditions at 37 °C for 72 h, and the total count of microorganisms was expressed in CFU/ml (22).

2.6. Statistical analysis

Independent variables of power (10, 105, and 200 W), temperature (0, 30, and 60 °C), and time (2, 6, and 10 min) were considered for the pasteurization of red grape juice samples by ultrasound under different conditions. The treatments were designed and analyzed using the response surface methodology (Box-Behnken design) in Minitab 16. Duncan's one-way analysis of variance in Minitab 16 was used to compare the color and microbial components of the optimal sample with the control sample (thermal pasteurization).

3. Results and discussion

3.1. Physicochemical properties

The pH, acidity, and Brix of grape juice samples pasteurized by ultrasound under different conditions and pasteurized by the thermal method were 3.95, 0.369% malic acid, and 13, respectively, which were not significantly different. According to the results, the energy produced by ultrasound waves did not cause any change in the molecular structure of high molecular weight compounds in the acidity, pH, and Brix of the product. The results of the study were consistent with the results of previous studies. According to these studies, ultrasound processing had no significant effect on the acidity and Brix of grape juice (23) and barberry juice (24), and the pH of strawberry juice (25) and carrot juice (26). Ghourchi et al. (27) studied pomegranate juice ultrasound at different amplitudes (50, 75, and 100%), different times (0, 3, 6, and 9 min), and 25 °C and found that different powers and times of ultrasound application had no significant effect on pH, acidity, and Brix. This was consistent with the results of the present study. In their study, Saeeduddin et al. (16) investigated the quality properties of pear juice pasteurized by ultrasound (temperatures of 25, 45, and 65 °C, time 10 min and power of 750 W) and industrial methods (temperatures of 65 and 95 °C in 10 and 2 min). They found that the pH, acidity, and Brix of the samples were not significantly different.

3.2. Results of color components (a *, b *, and L *) and total count of red grape juice pasteurized by ultrasound

The color parameter L* is equivalent to the brightness and is between 0 (black) to 100 (total internal reflection (TIR)). The values of the color parameter a* are infinite with positive values equivalent to red and negative values equivalent to green. However, positive values of the color parameter b* are equivalent to yellow, and negative values are equivalent to blue (28). Tables 2 and 3 show the results of ANOVA and the predicted model of color components (a*, b*, and L*) and the total count of samples of pasteurized grape juice under different conditions, respectively. As can be seen in Table 2, the linear effects of power, temperature, and time, the square effects of power, and the interactions of power×temperature and power×time under different conditions on L* variations were significant ($p \leq 0.05$). Besides, the square effects of temperature and time and the interactions of temperature×time on the L* content of grape juice pasteurized by ultrasound were not significant ($p \geq 0.05$). The linear effects of power, temperature, and time and the square effects of time on a* content of grape juice pasteurized by ultrasound under different conditions were significant ($p \leq 0.05$). Furthermore, the square effects of temperature and power and the interactions of power×temperature, power×time, and temperature×time on a* content of grape juice pasteurized by ultrasound were not significant ($p > 0.05$). The linear effects of power, temperature, and time and the square effects of power on the b* content of grape juice pasteurized by ultrasound under different conditions were significant ($p \leq 0.05$). The square effects of temperature and time and the interactions of power×temperature, power×time, and temperature×time on the b* content of grape juice pasteurized by ultrasound were not significant ($p > 0.05$). Table 2 suggests that the linear effects of power, temperature, and time, the square effects of power, and the interactions of power×temperature on the total count of microorganisms in grape juice pasteurized by ultrasound under different conditions were significant ($p \leq 0.05$). The square effects of temperature and time and the effects of power×time and temperature×time on the total count of microorganisms of grape juice pasteurized by ultrasound were not significant ($p > 0.05$). According to the results, there was no significant difference was observed between the results of a*, b*, and L* and the total count of pasteurized grape juice samples under different tested and predicted conditions. The results also suggested that the number of a*, b*, and L* in pasteurized grape juice decreased significantly ($p \leq 0.05$) with increasing power from10 to 200 W, temperature from 0 to 60 °C, and time from 2 to 10 min.

Table 2. Results of ANOVA of response surface model of color components and total count of microorganisms of grape juice samples paste by ultrasound under different conditions (temperature, time, and power).

Source of variation	L*		a*		b*		Total count of microorganisms (CFU/ml)	
	P-value	F-value	P-value	F-value	P-value	F-value	P-value	F-value
Regression constant	0.001*	33.93	0.000*	37.01	0.000*	150.62	0.000*	88.95
Linear effects	0.000*	91.46	0.000*	106.42	0.000*	377.77	0.000*	236.01
Power (a)	0.000*	186.13	0.000*	222.83	0.000*	1043.65	0.000*	580.84
Temperature (b)	0.000*	78.80	0.000*	89.21	0.000*	76.47	0.000*	116.53
Time (c)	0.028*	9.46	0.043*	7.23	0.015*	13.20	0.022*	10.65
Square effects	0.069*	4.54	0.094	3.77	0.000*	74.06	0.001*	28.12
Power × Power (a^2)	0.034*	8.36	0.188	2.32	0.000*	219.79	0.000*	83.87
Temperature×Temperature (b^2)	0.153	2.83	0.434	0.72	0.828	0.05	0.957	0.00
Time × Time (C^2)	0.295	1.37	0.039*	7.68	0.880	0.03	0.559	0.39
Interactions	0.044*	5.78	0.530	0.83	0.985	0.05	0.154	2.73
Power × Temperature (a × b)	0.034*	8.45	0.192	2.27	0.972	0.00	0.036*	8.07
Power × Time (a × c)	0.035*	8.21	0.675	0.20	0.729	0.13	1.000	0.00
Temperature × Time (b × c)	0.447	0.68	0.851	0.04	0.944	0.01	0.737	0.13

Table 3. Predicted line equation of color components and total count of microorganisms of grape juice samples pasteurized by ultrasound different conditions (temperature, time, and power).

Source	Model	R^2 (%)	R^2-adj (%)
L*	$Y = 20.2600 - 1.1700a - 0.7613b - 0.2638c + 0.3650a^2 - 0.2125b^2 - 0.1475c^2 - 0.3525ab - 0.3475ac - 0.1000bc$	98.39	95.49
a*	$Y = 38.8567 - 1.0688a - 0.6763b - 0.1925c + 0.1604a^2 - 0.0896b^2 - 0.2921c^2 + 0.1525ab - 0.0450ac - 0.0200bc$	98.52	95.86
b*	$Y = 27.8800 - 1.5563a - 0.4213b - 0.1750c + 1.0512a^2 + 0.0163b^2 - 0.0112c^2 + 0.0025ab + 0.0250ac - 0.0050bc$	98.52	95.86
Total count (CFU/ml)	$Y = 7.33 - 12a - 5.375b - 1.625c + 6.7083a^2 - 0.0417b^2 + 0.4583c^2 + 2ab - 0.25bc$	99.38	98.26

The highest numbers of a*, b*, and L* (40.970, 30.860, and 21.880, respectively) in grape juice samples pasteurized by ultrasound were observed at 10 W, 0 °C, and 6 min, and the lowest numbers of a*, b* and L* (37.190, 27. 040, and 18.240) were observed at 200 W, 60 °C, and 6 min, respectively. Since most of the color of grape juice is composed of anthocyanins, the color reduction in the juice pasteurized by ultrasound can be attributed to the reduction in anthocyanin content. Anthocyanins are highly unstable and can be degraded during processing and lose their bioactive properties under conditions such as pH, temperature, light, oxygen, and the presence of enzymes and metal ions (29). According to researchers, anthocyanins (flavonoid compounds) are easily degraded by heat. This changes the color acceptability of juices (30). Some researchers say that the opening of the pyrylium loop and the formation of chalcone are the most important reasons for the destruction of anthocyanins. After the formation of chalcones, these compounds are destroyed and converted into other compounds (31). Wang et al investigated the effect of ultrasound on the color properties of kiwifruit juice, arguing that increasing the ultrasound time affects the color properties (19). In another study, the effect of the ultrasound on the color of raspberry juice was investigated by Wang et al (18). The results showed that ultrasound for 16 minutes caused the color change in the samples. This was in line with the results of the present study. In a study by Campoli et al. (17), the effect of ultrasound on the structural and physical properties and lycopene content of guava was investigated. The results suggested that ultrasound disrupted the cells of guava juice and did not change the color of the juice.

3.3. Results of color components (a, b*, and L*) and total count of red grape juice pasteurized by ultrasound*

Table 4 shows the comparison of the number of tested and predicted color components (a*, b*, and L*) and the total count of grape juice samples pasteurized by ultrasound under different conditions (power, temperature, and time). According to the results, there was no significant difference was observed between the results of a*, b*, and L* and the total count of pasteurized grape juice samples under different tested and predicted conditions. The results also suggested that the number of a*, b*, and L* in pasteurized grape juice decreased significantly ($p \leq 0.05$) with increasing power from 10 to 200 W, temperature from 0 to 60 °C, and time from 2 to 10 min. The highest numbers of a*, b*, and L* (40.970, 30.860, and 21.880, respectively) in grape juice samples pasteurized by ultrasound were observed at 10 W, 0 °C, and 6 min, and the lowest numbers of a*, b* and L* (37.190, 27. 040, and 18.240) were observed at 200 W, 60 °C, and 6 min, respectively. Since most of the color of grape juice is composed of anthocyanins, the color reduction in the juice pasteurized by ultrasound can be attributed to the reduction in anthocyanin content.

Table 4. Comparison of the number of tested and predicted color components (a*, b* and L *) and the total count of microorga in grape juice pasteurized by ultrasound under different conditions (power, temperature and time).

Treatment	b * tested	b * predicted	a * tested	a * predicted	L * tested	L * predicted	Total count tested (CFU/ml)	Total count predicted (CFU/ml)
1	27.960	27.880	38.840	38.857	20.250	20.260	7.000	7.333
2	30.160	30.080	39.330	39.167	21.380	21.174	19.000	18.625
3	30.740	30.676	39.830	39.941	21.610	21.564	29.000	28.125
4	27.730	27.810	38.220	38.382	20.150	20.356	5.000	5.375
5	30.860	30.928	40.970	40.825	21.880	21.991	32.000	33.375
6	27.280	27.284	37.620	37.586	18.710	18.775	Not Detected	0.500
7	27.500	27.644	37.960	38.011	19.250	19.503	3.000	4.250
8	27.830	27.880	38.850	38.857	20.220	20.260	7.000	7.333
9	27.150	27.214	37.530	37.419	18.650	18.696	Not Detected	0.875
10	27.850	27.880	38.880	38.857	20.310	20.260	8.000	7.333
11	28.280	28.136	39.030	38.979	20.750	20.497	13.000	11.750
12	27.040	26.973	37.190	37.335	18.240	18.129	Not Detected	0.375
13	30.200	30.276	39.450	39.646	21.590	21.731	25.000	24.875
14	28.480	28.476	39.290	39.324	20.890	20.825	15.000	14.500
15	27.590	27.514	38.090	37.894	20.060	19.919	4.000	4.125

Anthocyanins are highly unstable and can be degraded during processing and lose their bioactive properties under conditions such as pH, temperature, light, oxygen, and the presence of enzymes and metal ions (29). According to researchers, anthocyanins (flavonoid compounds) are easily degraded by heat. This changes the color acceptability of juices (30). Some researchers say that the opening of the pyrylium loop and the formation of chalcone are the most important reasons for the destruction of anthocyanins. After the formation of chalcones, these compounds are destroyed and converted into other compounds (31). Wang et al investigated the effect of ultrasound on the color properties of kiwifruit juice, arguing that increasing the ultrasound time affects the color properties (19). In another study, the effect of the ultrasound on the color of raspberry juice was investigated by Wang et al. (18). The results showed that ultrasound for 16 minutes caused the color change in the samples. This was in line with the results of the present study. In a study by Campoli et al. (17), the effect of ultrasound on the structural and physical properties and lycopene content of guava was investigated. The results suggested that ultrasound disrupted the cells of guava juice and did not change the color of the juice. The results of tested and predicted microbial load (total count of microorganisms) of grape juice pasteurized by ultrasound under different conditions (power, temperature, and time) are given in Table 4. The results indicated that power, temperature, and time significantly affected the microbial load (total count of microorganisms) of grape juice pasteurized by ultrasound ($p \leq 0.05$). Furthermore, the microbial load of pasteurized grape juice was significantly reduced ($p \leq 0.05$) with increasing power from 10 to 200 W, temperature from 0 to 60°C, and time from 2 to 10 min. The highest total count of microorganisms (32 CFU/ml) in grape juice samples pasteurized by ultrasound was observed at 10 W, 0 °C, and 6 min, and the lowest total count of microorganisms (Not Detected) in grape juice samples pasteurized by ultrasound was observed at 105 W, 60 °C, and 10 min; 200 W, 30 °C and 10 min; and 200 watts, 60 °C, and 6 min. This indicated the adequacy of ultrasonic pasteurization in grape juice samples. According to Iranian National Standard No. 3414, the total count of microorganisms should be less than 1 CFU/ml (32). Thermal ultrasound treatment increases the inactivation of microbes in the juice. The effectiveness of microbial destruction depends on the amplitude of the sound waves, the processing time, the treatment temperature, the volume of the processed juice, and the composition of the juice. Microorganisms do not respond to ultrasound treatment in the same way. Microbial degradation results from ultrasound treatment due to cell membrane thinning, localized heat generation, increased pressure, and the production of free radicals (33). Physical stresses caused by ultrasonic cavitation are the main mechanism responsible for inactivating microbes. The hydrophobic surfaces of the cell wall of microorganisms help to destroy the cavitation bubbles formed during the application of ultra-sonication, leading to severe damage to the cell wall (34). Cells are destroyed by various factors such as physical and chemical mechanisms that are created during cavitation, the formation of free radicals, and hydrogen peroxide, which causes the cell membrane of microbes to become thinner (35). The results of this study confirmed the results of other studies. In their study, Saeeduddin et al investigated the quality properties of pear juice pasteurized by ultrasound (temperatures of 25, 45, and 65 °C, time 10 min, and power of 750 W) and industrial methods (temperatures of 65 and 95 °C in 10 and 2 min). They argued that the microorganisms were completely inactivated in the industrial method at 95 °C. Pasteurization by ultrasound at 65 °C for 10 min resulted in the best results so that microbial activity was reduced (16). In various studies, the use of ultrasound has been reported as an effective method to reduce microbial load and increase the shelf life of juices such as blueberry juice (35), orange juice (36), carrot juice (37), and strawberry juice (38) so that the cavitation created by these waves increases the sensitivity of these microorganisms to heat, temperature, water activity, and osmotic pressure by destroying the cell membrane of bacteria.

3.4. Simultaneous optimization of color components and microbial load of grape juice by pasteurized ultrasound

Simultaneous optimization conditions of color components L*, a*, and b* and the total enumeration of microorganisms in grape juice pasteurized by ultrasound under different conditions can be seen in Fig. 1.

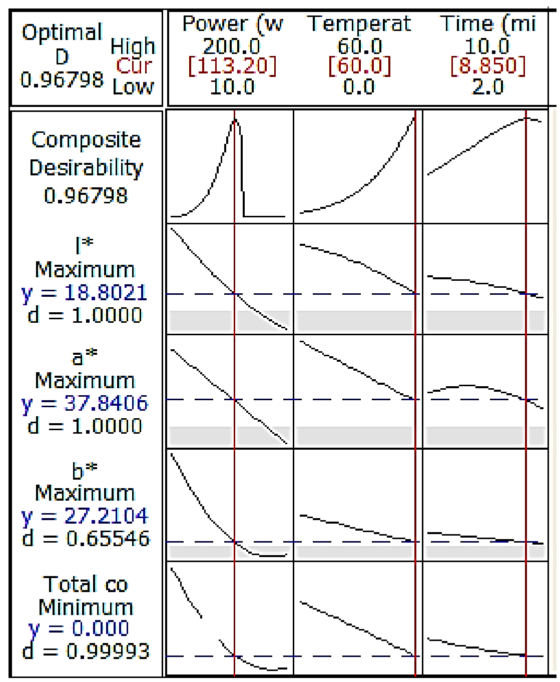

Fig. 1. Simultaneous optimization of color components and microbial load (total count of microorganisms).

The results showed that the optimal conditions for achieving the maximum number of L* (18.8021), a* (37.8406), and b* (27.2104) and the minimum total count of microorganisms (Not Detected) in grape juice pasteurized by ultrasound with 96.798% desirability was predicted at 113 W, 60 ° C, and 8.850 min. In a study by Lieu et al. (39), the effect of ultrasound (35 kHz frequency and 360 W power) on grape juice yeasts was investigated. The results showed improved properties due to increased sugar, phenol, and color and reduced microbial load of grape juice. Gomez et al applied ultrasound to calcium-containing orange juice, stating that aerobic mesophilic bacteria were reduced by a logarithmic rate of 1.38. There was also a significant change in color indices immediately after processing and a lack of pH and acidity (40).

3.5. Comparison of color properties and the total count of optimal samples pasteurized by ultrasound and thermal pasteurization

Color components and total count of microorganisms of the sample pasteurized by ultrasound under optimal conditions (113 W, 60 ° C, and 8.850 min) and the control sample (thermally pasteurized at 90 °C for 30 sec) are compared in Table 5. The results showed that there was no significant difference between the total count of microorganisms in the sample pasteurized by the thermal method and the optimal sample pasteurized by ultrasound. The number of color components (a*, b*, and L*) in the sample pasteurized by thermal method (control) were significantly lower than in the sample pasteurized by ultrasound. According to Table 5, the color compounds of pasteurized grape juice are less damaged by ultrasound. So, ultrasonic pasteurization improves the quality, nutritional properties, and safety of the product. Ultrasound efficiency in inactivating microorganisms is influenced by physicochemical properties, food volume, type of microorganisms, processing temperature, and power and time of ultrasound application.

Table 5. Comparison of the sample pasteurized at 90 °C (control sample) and the sample pasteurized by ultrasound.

Test	Optimal sample pasteurized by ultrasound	Pasteurized sample at 90 °C for 30 sec
L*	18.85± 0.17 A	15.43 ± 0.24B
a*	37.90 ± 0.19A	35.13 ± 0.14 B
b*	27.13±0.29A	24.83±0.25B
Total count (CFU/ml)	Not Detected A	Not Detected A

In general, ultrasound combined with another complementary method can kill all microorganisms and reduce the negative effects of the conventional pasteurization (thermal) method (13). The mechanism of inactivation of microorganisms, which is mainly based on the physical and chemical properties of the product, also has different effects on the chemical properties of the product, among which the production of free radicals such as hydroxyl and hydrogen radicals by acoustic-chemical reactions can be noted.

4. Conclusion

The study was conducted to evaluate and compare the physicochemical, color, and microbial properties of red grape juice by pasteurized ultrasound and thermal method while increasing the power from 10 to 200 W, increasing the temperature from 0 to 60 °C, and increasing the time from 2 to 10 min. The results indicated that pH (3.95), acidity (0.369), and Brix (13) remained the same in all treatments. The results also showed that the number of color compounds (a*, b*, and L*) and the total count in pasteurized grape juice decreased significantly (p≤0.05) with increasing power from 10 to 200 W, temperature from 0 to 60 °C, and the time from 2 to 10 min. It was concluded that grape juice with desirable color properties could be prepared and safety in terms of microbial load reduction could be achieved by using ultrasonic pasteurization.

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