

Original Research Article

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Effect of Pasteurization Methods on Enzyme Activities, Microbial and Sensory Evaluations in Ready to Serve Watermelon Juice (*Citrullus lanatus*)

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ABSTRACT

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A comparative evaluation of the use of thermal, microwave and irradiation treatments for pasteurization of ready to serve watermelon juice was undertaken to study their relative impact on residual enzyme (polyphenol oxidase and peroxidase) activities microbial population, and sensorial changes during its refrigerated storage for a period of three months. Pasteurization using thermal and microwave treatments could effectively control the microbial load within the acceptable limit ($<1 \log \text{cfu mL}^{-1}$) over the entire three months of storage. Irradiation treatment of 0.5kGy resulted in the lowest residual polyphenol oxidase (RA_{PPO}) activity followed by the microwave (1.56W/ml) and thermal treatments. However, maximum reduction in peroxidase activity (RA_{POD}) was achieved using thermal and microwave treatments. At the end of the three months storage, watermelon juice pasteurized using microwave energy was sensorially found to be the most acceptable product.

Introduction

Cultivation of watermelon [*Citrullus lanatus* (Thunb.) var. *lanatus*] has significantly increased beyond the traditionally confined riverbeds of Yamuna, Ganges and Narmada in North, and Kaveri, Krishna and Godavari in the South because of the increased demand of fresh watermelon and its juice both in the domestic as well as international markets (Aguilo-Aguayo *et al.*, 2010). However,

traditional thermal treatments used for the processing of watermelon juice have failed to arrest the detrimental changes in its colour and viscosity mainly because of the catalysis by the intrinsic enzymes like polyphenol oxidase and peroxidase (Rodrigo *et al.*, 2006).

Use of microwave for the pasteurization of milk has been extensively reported (Chiu *et al.*, 1984; Villamiel *et al.*, 1996; Al-Hilphy and Ali, 2013; Rasooly *et al.*, 2014). Ability

of microwaves in reducing thermal exposure time, pathogenic microbial load while simultaneously retaining the quality and acceptability of the treated produce has invoked tremendous interest for its application in the beverage industry (Nikdel *et al.*, 1992; Tajchakavit *et al.*, 1997; Canumir *et al.*, 2002; Igual *et al.*, 2010). Recently this technology has been found effective in preserving the liquid egg white (Lotfian *et al.*, 2014)

Irradiation is one among the non-thermal processing method involves the exposure of food to ionizing energy for the purpose of shelf life extension. Irradiation doses of upto 10 kGy or more have been approved to be safe for application into food products by World Health Organization (WHO, 1999). Earlier reports suggest the application of irradiation for inactivation of enzymes and microbes (Eissa *et al.*, 2014; Alighourchi *et al.*, 2014)

Review suggesting non-availability of a standard thermal treatment for the pasteurization of watermelon juice on one hand and the availability of alternative non-thermal technologies which could be effectively used for pasteurization led to this study to understand the effect of these available alternative techniques like microwave application and irradiation vis-à-vis traditional thermal processing in reducing the change in colour, enzyme activities and microbial load, at the same time achieving better sensorial quality.

Materials and Methods

Sample preparation

Fresh watermelon fruits (var. Sugar baby) of uniform size were procured from the local market and stored under room temperature till further use. Fruits were cut, flesh separated,

seeds separated from the pulp and juice extracted from the de-seeded pulp. The extracted juice was subsequently clarified using a double-layered muslin cloth, tested for physicochemical parameters (titratable acidity- AOAC, 2002; Total Soluble Solids (TSS); organic acids-HPLC method; ascorbic acid- AOAC, 2002 and lycopene- with a slight modification to the method described by Ranganna, 2007) ingredients (sugar; acid regulator E-296; preservative E-211; and stabilizer E-440) added and thoroughly mixed and bottled.

Pasteurization Treatments:

The bottles were pasteurized as follows:

Irradiation treatment of 0.5 kGy was given using Co-60 irradiator (12000Ci, with 5000cc capacity, from BARC, Mumbai) available at ICAR-IARI.

Microwave treatment (1.56 W/ml) was given using a domestic microwave oven (model: WP700L17-3, Padmini make, India).

Thermal pasteurization (72°C for 20 s) was given using a precision water bath.

Each of the above treatment conditions have been finalized by conducting preliminary experimentation involving different experimental variables. Untreated juice was used as control. Each of the treatments was studied with five replications using a full factorial design. Pasteurized juice samples were stored under refrigerated conditions (4±2°C) for three months. Samples were analysed for enzyme activity at 10 days intervals. Microbial and sensory evaluation was carried out at 30 days intervals.

Enzyme activity

5 ml of juice sample was added to 5ml 0.1 M phosphate buffer (pH 7) containing 5 g of

polyvinyl pyrrolidone using magnetic stirrer for 15 min. The homogenate centrifuged (Sigma, 3-18K) at 2,500 rpm for 20 min., supernatant filtered through Whatman No.42 filter paper and the filtrate collected for enzyme analysis. PPO activity was determined using spectrophotometric method based on an initial rate of increase in absorbance at 410 nm (Soliva *et al.*, 2001). Phosphate buffer solution pH 7 (0.1 M, 1.95 ml), 1 mL of 0.1 M catechol as a substrate and 50 µl of the enzyme extract were pipetted into a test tube and mixed thoroughly. Then the mixture was rapidly transferred to a 1-cm path length cuvette. The absorbance at 410 nm was recorded continuously at 25°C for 5 min using ultraviolet-visible spectrophotometer (JASCO-V- 690).

Peroxidase (POD) activity was assayed spectrophotometrically at 470 nm using guaiacol as a phenolic substrate along with hydrogen peroxide (Diaz *et al.*, 2001). The reaction mixture contained 0.15 ml of 4% (v/v) guaiacol, 0.15 ml of 1% (v/v) H₂O₂, and 2.66 ml of 0.1 M phosphate buffer pH 7 and 40 µl of the enzyme extract. The blank sample contained the same mixture solution without the enzyme extract enzyme extract were pipetted into a test tube and mixed thoroughly.

Then the mixture was rapidly transferred to a 1-cm path length cuvette. The absorbance at 470 nm was recorded continuously at 25°C for 5 min.

The residual activities of PPO and POD were calculated according to the following equation,

$$\text{Residual activity} = \frac{\text{Enzyme activity in treated (Pasteurized) juice sample}}{\text{Enzyme activity in control (Unpasteurized) juice sample}} \times 100$$

Microbial evaluation

The juice samples were analysed for the population of total aerobic bacteria, yeast and mould and total coliforms using Standard Plate Count Agar (SPCA), Martin Rose Bengal Agar and Eosin Methylene Blue Agar (EMB) media respectively. Serial dilution technique with spread plating was used for enumeration of microbial population in the pasteurized juice and incubated at 30°C for 24 to 48 h for both fungi and bacteria and at 37°C for total coliforms. Results were expressed in terms of colony forming unit (cfu) per ml of juice sample.

Sensory evaluation

The sensory evaluation was conducted primarily to assess the flavour along with other characters like appearance, colour, taste, and overall acceptability of different treatments. A sensory panel of 10-untrained members was used to evaluate various sensory attributes using a 9-point hedonic scale (1=dislike extremely, 9=like extremely).

Analysis of results

Results were analysed for statistical significance by factorial analysis of Variance (ANOVA) and means were compared using Tukey's Studentized Range (HSD) Test using SAS-9.3 Software. The analysis of sensory evaluation was done by the non-parametric method Friedman mean rank for the comparison of the score assigned by the sensory panel. Chi-square analysis was done and treatments and storage period was compared using asymptotic significance (Asymp. Sig) level ($p < 0.05$).

Results and Discussion

The extracted watermelon juice was analyzed for pH, titratable acidity, organic acid, total

soluble solids, ascorbic acid and lycopene and the corresponding values were 5.64 ± 0.2 , $0.08 \pm 0.01\%$ (as malic acid), 3.38 mg citric acid /ml juice, 0.25 mg citric acid/ml juice, $8.9 \pm 0.46^\circ\text{B}$, 8.00 ± 0.17 mg ascorbic acid/100ml juice, and $5.7 \pm 0.13\text{mg}$ lycopene/100g juice respectively. The ready to serve juice prepared out of extracted watermelon juice had 4.4 ± 0.3 pH, $0.3 \pm 0.02\%$ titratable acidity (as malic acid), $12.2 \pm 0.2^\circ\text{B}$ total soluble solids, 0.2% pectin and 0.1% sodium benzoate.

Residual PPO

Poly phenol Oxidase (PPO) residual activity detected in the juice samples immediately after the thermal, microwave and irradiation was 70.77%, 31.36% and 34.17% respectively. The effect of pasteurization on residual PPO activity is shown in Figure 1. Watermelon juice having the most delicate flavour compounds showed an irregular trend in residual PPO irrespective of the method of pasteurization. It was clear from the data that thermal pasteurization could not inactivate the PPO activity in watermelon juice. Here, a steady increase in the residual activity was observed till 80th day. Even a maximum activity upto 93.98% was seen from the 70th day's observation. Liu *et al.*, (2012) observed PPO residual activity of 49.1 % immediately after thermal pasteurization at 95°C for 1 min. The PPO activity in strawberry puree from two cultivars was highly resistant to thermal inactivation even at temperature as high as 100°C Terefe *et al.*, (2010). But, surprisingly Igual *et al.*, (2010) obtained 94% PPO inactivation through conventional pasteurization than microwave pasteurization (88%) in grape fruit juice. Earlier reports suggest that non thermal pasteurization techniques like irradiation, Pulsed Electric Field (PEF) and High Pressure carbon dioxide (HPCD) could be completely inactivate PPO activity (Ertugay *et al.*, 2013; Liu *et al.*,

2012). But none of the methods could completely inactivate the enzyme activity.

It was seen from the present study that irradiation at 0.5kGy was the most effective method to reduce the PPO activity. Even the mean value for the entire three months storage period was 32%. Till 70th day it could maintain the lowest activity of 20.24% but, there was a sudden increase in the activity after 80 days of storage. Still it showed only 32.3% activity at the end of storage (90th day).

Hanotel *et al.*, (1995) observed 30% inhibition of polyphenol oxidase and peroxidase activities after irradiation in cut witloof chicory. However, the efficacies of inhibition to PPO activity depend on the irradiation dose. Eissa *et al.*, (2014) observed that inhibition to PPO activity increased as the irradiation dose from 1kGy to 5kGy with 91%. But, concerning the nutritional and biochemical quality of fruit juice the irradiation dose was limited to 0.5kGy in the present study. The result of this study also revealed that microwave pasteurization is also effective in reducing the residual PPO activity. The initial residual activity of microwave pasteurized juice samples were 31.36% which was later on increased to 87.92% on the 70th day then fallen down to 62.28% on the 90th day. The report of Math *et al.*, (2014) strongly supports this finding.

Residual POD

The graphical representation of the result of pasteurization methods on residual peroxidase (POD) in watermelon juice during 90 days of storage at $4 \pm 2^\circ\text{C}$ is presented in Figure 2. One interesting finding was the effectiveness of thermal was not immediate. The observed residual activity immediately after the three sets of pasteurization was 52%, 12% and 4% for thermal, microwave and irradiation respectively.

Fig.1 Effect of pasteurization methods on residual polyphenol oxidase (RA_{PPO}) activity during storage (%) at 4±2 °C

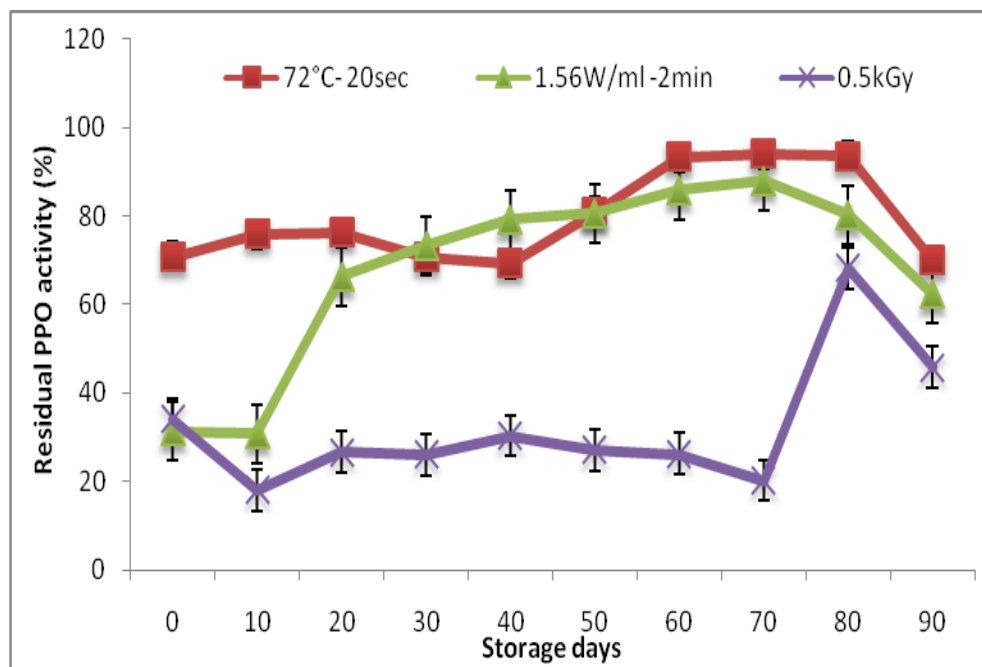


Fig.2 Effect of pasteurization methods on residual peroxidase (RA_{POD}) activity during storage (%) at 4±2 °C

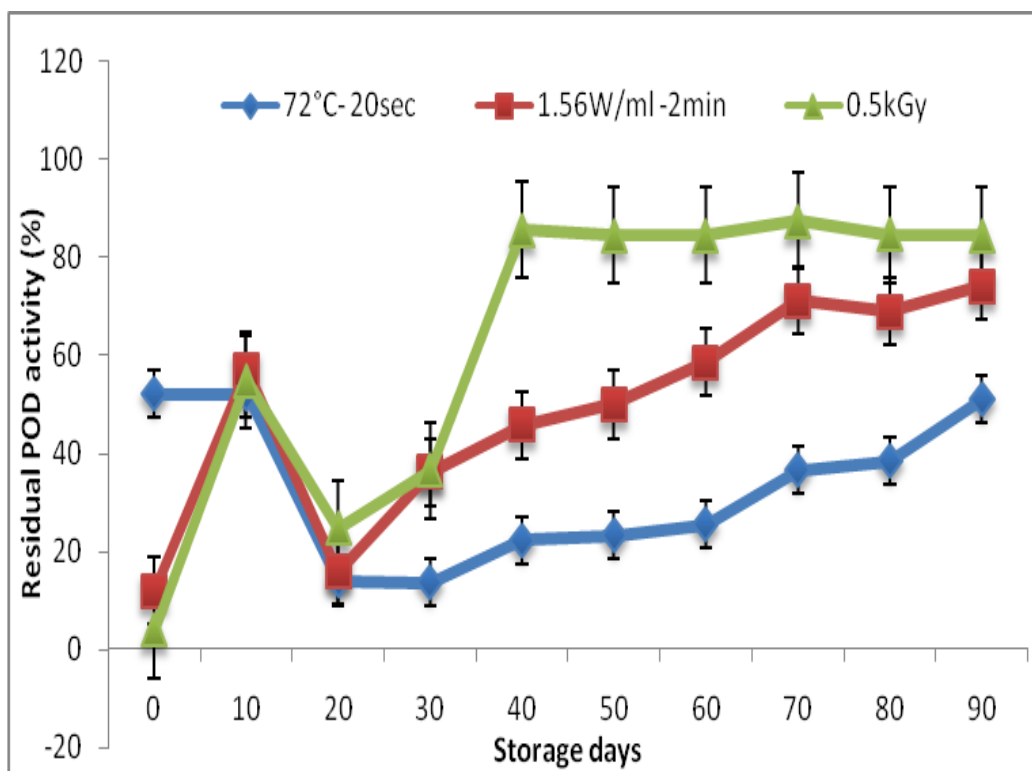


Fig.3 Effect of pasteurization methods on microbial load (log cfu/ml) during storage at 4±2 °C

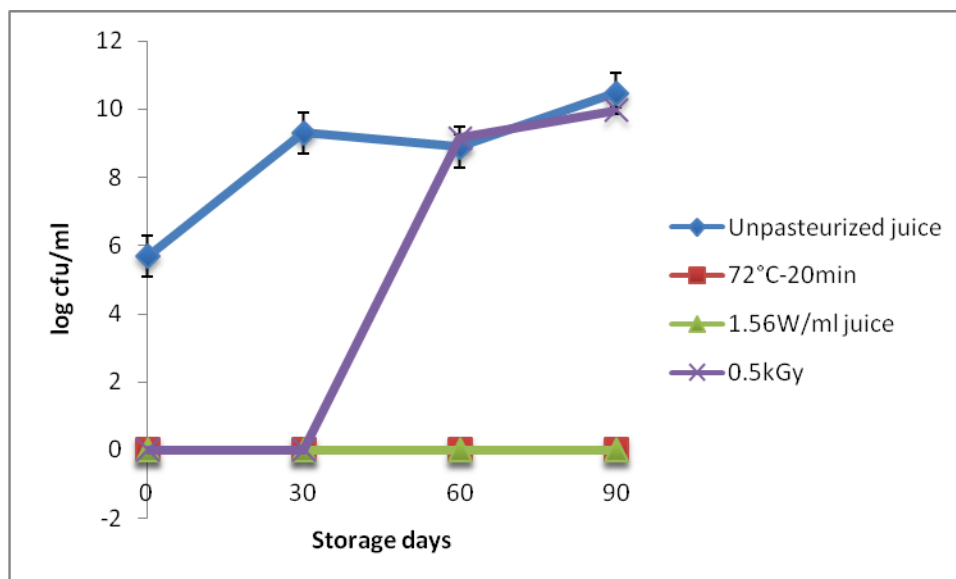
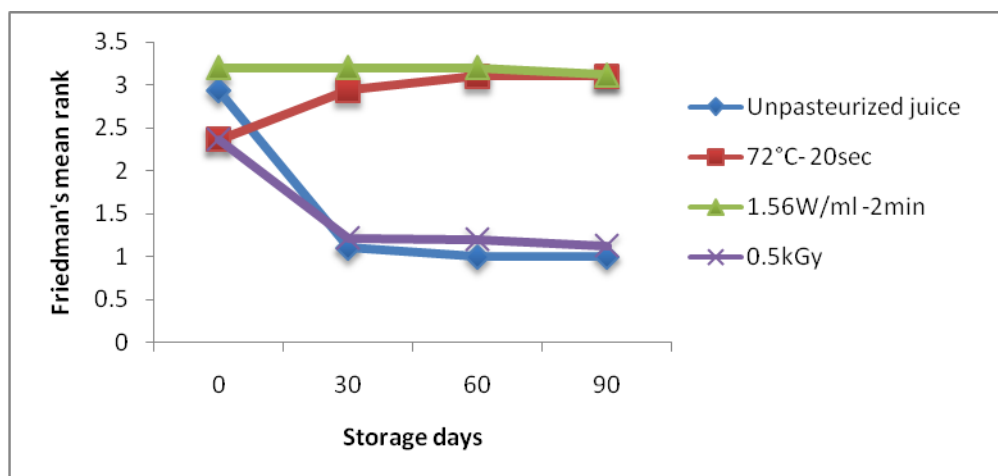


Fig.4 Effect pasteurization methods on sensory evaluation (overall acceptability) during storage at 4±2 °C



Initially irradiation showed maximum efficiency in inactivating the PPO. But, unfortunately the effect remained only for maximum 10 days. There was a sudden increase in the activity to 54.81% as per the 10th day's observation. Irradiated sample remained satisfactory for a period of 30th day. Peroxidases (POD) influence both colour and flavour of fruit juices. Activity of POD could be the reason behind the extreme off taste and flavour experienced during the sensory

evaluation in the irradiated juice sample after one month period. This enzyme might be altered fresh watermelon flavor leaving behind an undesirable flavour. A study on enzyme inactivation in cantaloupe juice with the use of gamma irradiation suggested that, it is not a perfect method for inactivation of the PPO and POD (Wang *et al.*, 2006). Contradictory to the result of PPO inactivation, the maximum efficiency in inactivating the POD was found in thermal

pasteurized juice. At the end of storage period the maximum residual activity was 50.98%, where it was 74.1 and 84.53% in microwave pasteurized and irradiated samples. Reports of Igual *et al.*, (2014) was in accordance with the effectiveness of thermal pasteurization as compared to microwave pasteurization in grape fruit juice.

One recent report (Marszałek *et al.*, 2015) clearly gave evidence that, only thermal pasteurization could able to inhibit the PPO and POD activities in strawberry puree in comparison with high pressure processing (HPP). However, in the present study thermal pasteurization was effective for reducing the only the activity of POD and not PPO.

The effect of microwave pasteurized watermelon juice was intermediate in reducing both PPO and POD activities. It showed only 31.36% and 12% initial PPO and POD activities. Faster inactivation of PPO and peroxidase POD was observed in microwave pasteurized coconut water than the conventional thermal process (Matsui *et al.*, 2008). In comparison with conventional pasteurization, microwave heating was found effective in higher enzyme destruction the enzymes (Tajchakavit and Ramaswamy, 1997).

Microbial evaluation

The pH of fresh watermelon juice was 5.64 ± 0.2 and the prepared ready to serve juice after adding the acid regulator was 4.4 ± 0.3 . Spoilage in the juice mainly resulted from the growth of acid tolerant bacteria, yeasts or molds that are sensitive to pasteurization. In this study, counts of total aerobic bacterial (TAB) and yeast and mould (Y&M) were almost similar during the microbial enumeration. The initial mean populations of the TAB and Y&M in fresh watermelon juice and the prepared ready to serve juice were 5.69 ± 0.09 , 5.54 ± 0.01 and 5.68 ± 0.03 ,

5.53 ± 0.01 log cfu/ ml respectively. The test for total coliforms was found to be negative. The effect of pasteurization methods on inhibiting the microbial population is represented in Figure 3. Thermal and microwave pasteurization was effective as to extend the shelf life of watermelon juice by inhibiting the growth of bacteria, yeast and mould. The juice remained free of contamination for the entire three months storage period. Similar to the observations of Naresh *et al.*, (2015) in mango juice irradiation, the initial microbial load was significantly reduced by gamma irradiation at 0.5kGy. But later an increase in the population was observed. Aligurchi *et al.*, (2008) reported that the effect of irradiation in reducing the growth rate of bacteria and fungi of pomegranate juice was only for three days at 4°C. Result of the present study revealed that the irradiation could effectively control the microbial load in watermelon juice to the maximum extend of one month. Interestingly, thermal and microwave pasteurized juice were able to retain the quality of the juice over the entire storage period (<1 log cfu ml⁻¹). The obtained result was in agreement with Diaz and Aguayo (2013) who reported that the complete inactivation of microbial population in thermally pasteurized watermelon juice. Microwave treatment widely used in milk pasteurization because of its effectiveness in reducing thermal exposure to inactivate pathogenic while maintaining high quality. The internal components of the microbial cells disrupt due to the coupling of electromagnetic energy with critical molecules such as protein or DNA.

Even at higher microbial inoculums, mild microwave treatment could able to reduce more than 5-log (Geveke and Brunkhorst, 2004). Canumir *et al.*, (2002) observed a 2-4 log reduction of total coliforms in apple juice depending on the power level (270–900 W) and time (40, 50, 60 and 90s).

Sensory evaluation

The effect of pasteurization on sensory notes with respect to overall acceptability of the sensory parameters like appearance, colour, flavour and taste was evaluated. The trend in overall acceptability is shown in Figure 4. Panellists could not found any significant difference in appearance, flavour and taste immediately after different pasteurization. But it showed a clear difference in overall acceptability. Unlike other reports of fruit juice irradiation, the irradiated watermelon juice sample obtained the lowest score. Naresh *et al.*, (2015) did not find any significant changes in the sensory scores of irradiated mango juice at 1, 3 and 5 kGy. Degradation of colour was observed for all the treatments except the irradiated juice during the entire storage. In fact, the irradiated juice turned darker in colour. This could be directly linked to the improved lycopene content after irradiation. Regarding the flavour of the juice, immediately after pasteurization all the treatments except the unpasteurized juice showed similar flavour. Presence of off flavour was detected from irradiated juice sample on the 30th day of evaluation. The panellists preferred microwave pasteurized juice for the taste properties followed by thermal pasteurized juice.

The most acceptable overall quality characteristics in ready to serve watermelon juice were found in microwave pasteurized juice (1.56W/ml juice) with a mean Firedman's score of 3.5 followed by the thermal pasteurization (3.3). During the storage study of centrifuged watermelon juice Diaz and Aguayo (2013) observed a marked reduction in colour and flavour in during the 10th day itself. In that case, the result obtained from the present study concludes that microwave pasteurization is a reliable method in comparison with the conventional thermal

pasteurization in retaining especially the flavour and colour of the juice while reducing the microbial population.

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