**Pasteurization Of Pomegranate Juice Using Microwave Energy**

**Abstract**

The pomegranate (*Punica granatum* L.) is an ancient fruit classified under the family Lythraceae, known for its therapeutic and functional properties. India stands first in world pomegranate production with 743.04 MT per annum and contributes 34% of the world production. Pomegranate juice makes an attractive, delicious drink also rich in micronutrients like physicochemical properties. For instance, a glass of pomegranate juice contains about 40% of the recommended daily allowance (RDA) of Vitamin C. The recent research revealed that, under heat processed pomegranate juice were showing more quality loss, instead we found thermal processing methods effective in protecting the physicochemical properties in pomegranate juice. However, the establishment of non-thermal processing is complex, and the cost of production is also high. On the other hand microwave treatment (MW) is continually being explored as alternatives to conventional thermal processing. The aim of this work was conducted to assess the effect of microwave exposure time and power level on the reduction of microbial load. Microwave oven is typically employing a radio wave frequency of around 2450MHz microwave. The fresh pomegranate juice was pasteurized with different Power levels and Times namely 420, 560, 700W and 30, 60, 90sec. In contrast, the impact on physicochemical properties and decrease in microbial load were found. A treated sample is potential nutritional content is observed, It produce there is no changes in pH, TSS, TA, Brix-Acid ratio and total sugar (5.5, 18.0, 0.38, 52.6 and 67). 700W/30sec may be a better method of processing pomegranate juice and microbial load decreased by up to 96% under the ideal condition. Therefor the Beverage processing industry when applying this technique, it leads to final processed juice with better nutritional qualities and enhance microbial load.

Key words: Pomegranate, fresh juice preservation, microwave energy, physicochemical properties, reduction of microbial load.

**1. Introduction**

The pomegranate (*Punica granatum* L.) is an ancient, mystical, and highly distinctive fruit, classified under the family Lythraceae. It is native from Iran (Persia) to the Himalayas in northern India but has been cultivated ancient time over the entire Mediterranean region. Now the pomegranate is available in most part of world including India, Southeast Asia, East Indies, and tropical Africa. Current global production of pomegranate reported was 350,000 tons (Citrogold, 2011). Pomegranate fruits are globular, have tough skin or rind, and generally have red, yellow, or yellow-red membranes and albedo. The fruit has several seeds, each of which is girdled with translucent, red, pink, or whitish, juicy, sweet- acid, orangish pulp (Ozgen et al., 2008). The total weight of the fruit is approximately 250–300g (AI-said et al., 2008).

Pomegranate fruit had high Physico-chemical properties of the extract from Bhagwa variety pomegranate fruit such as peel, juice content and seeds have been agreed by (F.a. al-said et al., 2004). Pomegranates contain high level of pH, Total Soluble Solids (TSS), Titrate acidity (TA). Pomegranate fruit contributes positively to mortal nutrition because it is a good source of vitamins (such as C, A, B, and niacin), minerals, and beneficial fiber (Simson and Straus, 2010).

The consumption of pomegranate has grown tremendously for health beneﬁts and functional properties due to high nutritional value. Pomegranate has potential therapeutic properties and functional properties like wide-ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and protection from ultraviolet (UV) radiation (Yahia, e.m. 2017). In India pomegranate fruits are sold as whole fruits as well as fresh fruit juice. But the consumers are reluctant to open and eat intact fruits, which has encouraged the development of methods to blow out airlines and pack these beautiful jewels as a minimally processed fresh product and ready to serve (RTS) pomegranate juices (Kong et al., 2020).

Nowadays many commercial pomegranate juice products (Tropicana®, Natura® etc.) are available in the refrigerated produce section of supermarkets and which has displayed considerable sales growth. Market demand for pomegranate products thereof showed a considerable increase, especially for pomegranate juice (Holland et al. 2009).

Therefore, the challenges to preserve vital nutrients suggest the application of rapid thermal treatment technologies for the preservation of pomegranate juice. Among these technologies, microwave heating has the potential to produce high-quality foods that display characteristics of fresh products, are microbiologically safe and have an extended shelf life (Juan carlos troiano et al., 2021).

In general microwave oven pasteurization is a technique that increases the shelf life of processed juice by preventing or slowing the growth of microbial. Whereby fresh pomegranate juice generally pasteurized with different power levels and times 420, 560, 700W and 30, 60, 90sec. Tested at 420- 700W, during temperature increased from the microwave oven treatment starting 18 °C to 76 °C. Therefore, aim of the present study was to evaluate and compare the physic-chemical properties and microbial load in pasteurized pomegranate juice as well as focus on the most recent development, the current level of research in microwave food processing and potential prospects.

**2. Material and methods**

**2.1 Fresh Pomegranates**

Fresh pomegranate fruits of cultivar Bhagwa were procured from the locally available market and stored at 5º C till extraction of juice. The variety gives dark red to pink color with TSS content of 14.2-14 .5°Brix. Juice yield was also found to be maximum (20-45%) for this variety during the preliminary studies.

**2.2. Packaging materials**

A polypropylene (PP) bottle of 200ml capacity with 50 mm outer diameter and 170 mm height, this PP bottle we have received from Innotek industries Pvt. Ltd., Bengaluru. PP bottle used as a packaging material for pomegranate juice. These bottles are specially designed to withstand the high temperature and pressure during the sterilization under retorts using steam.

**2.3. Preparation of pomegranate juice**

Fresh pomegranate fruits were washed in cold tap water. The washed fruits were manually cut and the outer leathery skin which encloses the sacs/arils was separated. Then the juice was extracted from the arils by using a fruit juice extractor without crushing the seeds. The extracted juice was collected in a clean container. The juice obtained was clarified using muslin cloth. After filtration pomegranate juice 180mL was poured into a pre-sterilized polypropylene (PP) container. Filled juice is stored for 4º C until used for further experiments. All the utensils/ juice contacting surfaces involved in extraction of juice were cleaned thoroughly and sanitized with hot water before using to minimize the cross contamination. General process involved pomegranate juice processing.

2.4. Microwave treatment

The pomegranate juice in PP bottles was treated in a domestic microwave oven (IFB30SC2, IFB Industries Ltd., India), in which the samples were always positioned at an identical location inside the cavity with the same initial temperature and volume for all the treatments. Microwave heat treatments were scheduled and displayed on Table-2.1 (defined as zero dwell time) based on preliminary studies to obtain the desired final temperature (60-80°C). Juice samples were drawn before and after heat treatments, to get the initial and final number of survival microorganisms.



Fig. 2.1 Microwave treated Pomegranate Juice

Table-2.1. Factorial design for evaluation of microbial reduction in pomegranate juice under microwave heating

|  |  |  |
| --- | --- | --- |
| Run | MW Powers (W) | Heating Time (sec) |
| 1. | 420 | 90 |
| 2. | 560 | 60 |
| 3. | 700 | 30 |

2.5. Analysis of Microbial parameters

TPC was enumerated by employing Serial Dilution Agar Plating Method. Serial dilutions of the sample were prepared upto 10-5 by adding 1 ml water sample to 9 ml sterile physiological saline (0.8%). An amount consisting of 1 ml from each dilution was transferred aseptically onto duplicate sterile Petri dishes and approximately 18-20 ml of molten plate count agar (45°C) (Himedia, Mumbai, India) was added. The sample and agar were mixed thoroughly by rotating the plates several times. The plates were allowed to set and inverted, then one plate was incubated at 37°C and another at 20-220C for 24-48 hours. Colony counts were made from plates with less than 300 but more than 30 colonies and results expressed as actual colony counts multiplied by the dilution factor. Colony counts were expressed as colony forming units (cfu/ml) of the sample.

2.6. Analysis of chemical parameters

2.6.1. pH

The pH was measured at an ambient temperature (28±2°C) using digital pH meter (pHTutor, Eutech Instruments Pvt. Ltd., Singapore). The pH meter was calibrated with commercial buffer solutions at pH 9.1 and 4.0 before measurement. About 10 ml sample was inserted with a pH electrode and pH was recorded after stabilization.

**2.6.2. Total soluble solids**

The total soluble solids (TSS) content was determined as °Brix at an ambient temperature (28±2°C) by digital Refracto meter (RX-7000α, Atago India Instruments Pvt. Ltd., India). TSS of the sample was measured after resetting the readings to zero with distilled water.

**2.6.3. Titrate acidity**

Titrable acidity (TA) was estimated as suggested by Kong T *et. al.,* (2020). Where, 20 ml of sample was taken in a 250-ml beaker and 80 ml distilled water was added. This solution was then titrated against standardized 0.1 N NaOH with phenolphthalein indicator to detect the end point (pH = 8.2±0.1). The volume of NaOH was converted into citric acid equivalent by using the following equation,

TA (%WT/VOL) = N×V1×Eq.Wt

V2×1

**2.6.4. Brix-Acid ratio**

The taste and flavor are determined by the brix/acid ratio (TSS: TA) both during harvest and during post-harvest processing. Variations in fruit juice's TSS and TA concentrations affect the acid ratio, or Brix.

**2.6.5. Total sugar**

1.0g of sample was taken into boiling tubes and hydrolyzed by keeping them in boiling water bath for 30min to 1hour with 5ml 0f 2.5NHCL. it was cooled to room temperature and neutralized with solid sodium carbonate until the effervescence ceases and made up the volume to 100ml and centrifuge. 0.5ml and 1ml of the supernatant were collected and used for further analysis. The volume was make upto 1.0ml in all the tubes including the sample tubes by the addition of distilled water. 4ml of 0.2% Anthrone reagent was added to each test tube followed by heated in a boiling water bath for 10min. Test tubes were cooled at room temperature. Dark green color was appeared on heating the sample. The capital density (OD) value of the colored solution was then measured through 630nm wavelength in a colorimeter against blank. Standard graph was drawn by plotting the concentration of glucose on X axis and optical density on Y axis. From the graph the amount of carbohydrate present in the sample was calculated.

**3. Result and discussion**

**3.1. Analysis of Microbial parameters**

Table-1 shows the effect on the juice temperature exposed to different microwave energies. A progressive increase of the pomegranate juice temperature as the energy increased was observed, microwave temperature does not increase near the boiling point. Tested at 420-700W, during temperature increased from the microwave treatment starting at 18C or less to 76°C or more at 700 watts of electricity. While both 700W and 1050W reached high temperatures, only the treatment at 700 W achieved the desired level of microbial reduction.

Microbiological results presented in Table 3.1 showed reduced desired level of microbial load at 720 and 560 W power levels for 30 and 60 sec. Average reduction profiles were compared in pomegranate juice to 420W power level. These outcomes showed 1CFU/mL for pasteurized pomegranate juice. Were discovered to be present in processed juice and no microbes were detected in 700W/30s samples. This is due to reduction of microbial load up to 96% at 700W/30sec.

Complete the pasteurization for the 180 mL sample, Nevertheless, it is important to note that spores of some bacteria and yeast may have survived during pasteurization process. This process suggested that they were successfully reduced below detection level Table 3.1.

Table-3.1. Effect of microwave power levels on microbiological parameters of pomegranate juice.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| S  No | MW power (W) | Final temperature1 (°C) | Effective heating time2(s) | 10-2 (1CFU/ML) | 10-4(1CFU/ML) | 10-6(1CFU/ML) |
| 1. | 420 | 67 | 90 | 17 ±1.69 | 9±1.43 | 3±1.32 |
| 2. | 560 | 64 | 60 | 13±1.65 | 4±1.35 | 0±0.00 |
| 3. | 700 | 76 | 30 | 5±1.33 | 0±0.00 | 0±0.00 |
| 1Mean and standard deviation (n = 3) | | | | | | |

Effective Heating time

Total plate count

Fig 3.1. Effect of microwave power levels on microbiological parameters

of pomegranate juice

**3.2. Analysis of physicochemical parameters (pH, TSS, TA, TS)**

According to the characteristic values for pomegranate juice presented in Table-3.2, no differences were observed pasteurized juice, even though pH, total soluble solids, total acidity and total sugar percentage tended to be increased. Pasteurized juice is crucial because it establishes the impact of the various heat treatments on juice quality. The Pomegranate juice's pH value indicates how acidic it tastes. pH of pasteurized juice respectively 5.4, 5.5 and 5.6. Every pH measurement was still within the acceptable limit for pomegranate juice. Juice's suitability as substrates or settings for specialized pathogenic microorganisms, as well as the degradation action of bacteria due to its high-water content as compared to its initial concentrated state, generally occurs prior to the pasteurization procedure. The bulk of the vegetative forms of bacteria were reduced from the samples via pasteurization techniques

The total soluble solids (TSS) of pasteurized juice were significantly higher, which is recorded at 20.0 and 22.2°Brix respectively. This result complied with the minimum Brix requirement as outlined by Codex general standard for fruit juices and nectars (CODEX STAN 247‐2005) (FAO/WHO Food Standards, 2005). The Thermal treatment has increased the TSS of juice which due to evaporation of water during treatment also hydrolysis of polysaccharides into sugars.

Organic acids content is an important figure for fruit juice as they influence the organoleptic properties (flavour, colour, and aroma), stability and microbiological control of the juice, whereby this is determined by titratable acidity (TA), which indicates that sample significantly alters the TA percentages 0.32% and 0.39%, respectively. These values of TA agreed as studied by Cam et al. (2009).

The total sugar (TS) of pomegranate juice to verify that there were no alterations of pasteurized juice. Pasteurized juice was containing higher, which is recorded at 67±0.49 respectively as compared to other treated samples (65±0.47). Next there was no discernible difference in the brix-acid ratio between all pasteurized juice. TA and TSS, resulting in a significant increase in brix/acid ratio at most storage temperatures for ‘Bhagwa’. Hence, it can be concluded that further studies on the microbial growth rate of treated samples during storage are necessary to provide insights into the requirements for filling, packaging, and storage parameters that could extend the shelf life of juice without the use of preservatives.

The power levels having no significant effect (p ≤ 0.05) on pH, titrable acidity (TA) and total soluble solids (TSS) of pomegranate juice during microwave pasteurization. The high values observed were, 65 g/100ml (Total sugar), 5.4 (pH), 0.38 (titrable acidity), 20.0 (Brix), respectively. Similar results were observed during the thermal processing of pomegranate juice by Alighourchi and Barzegar (2009).

Table 3.2. Effect of microwave power levels on chemical qualities of pomegranate juice

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| S.No | Power levels  (W) | Effective  heating time(s) | pH | TA  (g/100ml) | TSS  (°Brix) | TS  (g/100ml) | Brix-Acid ratio |
| 1 | 420 | 90 | 5.6±0.09 | 0.39± 0.01 | 16.0± 0.08 | 66±0.47 | 41.02±0.52 |
| 2 | 560 | 60 | 5.4±0.012 | 0.38±0.01 | 20.0 ±0.08 | 65±0.47 | 52.63±2.92 |
| 3 | 700 | 30 | 5.5±0.08 | 0.38±0.01 | 18.0 ±0.08 | 67±0.49 | 47.36±1.58 |
| # All values shown are mean ± standard errors (n=3) | | | | | | | |

Microwave power

Chemical Qualities

Fig 3.2 Effect of microwave power levels on chemical qualities of pomegranate juice

**Conclusion**

Currently, people are more health conscious; they want to eat food inclusive of all essential nutrients with lower preservative. Microwave treatment are useful for the food processing industry because of enhancing nutritive parameters and microbiological parameters. Microbial loads populations were lower on the sample at 700W for 30sec. At this condition, reduction generally over to 96% of microbes. 700Wm30se power levels having no significant effect (p ≤ 0.05) on pH, titrable acidity (TA) and total soluble solids (TSS) of pomegranate juice during the microwave pasteurization. The high values observed were ,67±0.49 g/100ml (Total sugar), 5.5±0.08 (pH), 0.38±0.01 (titrable acidity), 20.0±0.8 (Brix), respectively. The optimized condition of 700W for 30sec can be the better treatment condition for producing the pomegranate juice.

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