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Evaluation of pulsed electric field and conventional thermal processing on shelf life study of kokum juice

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Abstract
Kokum fruit juice is one of the refreshing summer drinks which have lot of health benefits. The high temperature during thermal processing of kokum fruit juice causes the changes in product flavors. The thermal processing also results in degradation of color, anthocyanin and ascorbic acid content present in fruit juice. In light of the increasing interest in and understanding of the advantages of alternative non-thermal methods, including pulsed electric field (PEF), the current work proposes the use of PEF to reduce the amount of bioactive chemicals lost. The study involves thermal (96 °C for 8 min), PEF (24 µs, 120 Hz and 38 kV/cm) and combination processing [PEF + Thermal (96 °C for 5 min)], of kokum fruit juice. The study also examines how storage affects the quality characteristics of Kokum juice that has undergone combination processing, thermal processing, and pulsed electric field (PEF) processing. The retention of quality and sensory attributes was higher in the PEF treated sample then that of thermal processed and combination processed sample. When contrasted with control and thermally processed samples, it was shown that the PEF and combination processed samples were extremely stable.

Keywords: Kokum juice, pulsed electric field (PEF), pasteurization, anthocyanin

Introduction

One of the significant indigenous tree spice crops, kokum (*Garcinia indica* Choisy), is grown in Goa, Coorg, and the Western Ghats of India (Devi *et al.*, 2013) [4]. According to Baliga *et al.* (2011) [2], this particular plant is commonly referred to by various names such as the wild mangosteen, kokum, goa butter tree, and kokum butter tree. The trees only produce fruit once a year, from March to May, during the warm months. Fruit is green when unripe and turns scarlet to dark purple as it ripens (Pasha & Ramachandran, 2014) [21].

The kokum fruit showcases a deep purple color, which stands out as one of its unique attributes. Additionally, when the fruit reaches full ripeness, it may also display a scarlet hue with subtle golden undertones. It has three to eight large seeds buried in a red acid pulp in a white pulpy material that resembles an orange segment (Krishnamurthy *et al.*, 1982) [13]. According to Krishnamurthy *et al.* (1982) [13], the fruit rind includes roughly 86% moisture, 1.92% protein, 14.28% crude fiber, 2.57% ash, 2.85% tannin, 5.71% pectin, 1% starch, 10% crude fat, and 22.80% acid (hydroxy citric acid). Phytochemical research has revealed that kokum rind contains the highest amount of anthocyanin, with 2.49 g/100 g of kokum fruit, in comparison to all other natural sources (Baliga *et al*., 2011) [2].The fruit ripens in April and May, and it blooms in November through February.

According to Swami *et al.* (2014) [29], garcinia contains a wealth of active substances, such as hydroxycitric acid, garcinol, xanthochymol, and isoxanthochymol. In addition to its medicinal properties for treating various ailments such as sores, tumors, dermatitis, heart issues, diarrhea, dysentery, and ear infections, this substance has also been found to aid in digestion. Kokum juice is utilized as a home medicine for liver and digestive issues (Krishnamurthy *et al.*, 1982; Mishra *et al.*, 2006) [13, 18].

It demonstrates a variety of phytochemical qualities, including effects that are anti-ulcerogenic, cardioprotective, anticancer, chemo preventive, free radical scavenging, and anti-obesity. Garcinia, which has therapeutic benefits, is also used to treat cardiac issues, piles, diarrhea, and tumor pain. Even Garcinia-based fat-burning pills and capsules are offered in the market (HM, R. 2015) [10]. Anthocyanin, a natural colorant used in the food and pharmaceutical industries, may be found in kokum. According to Leopoldini *et al.* (2010) [16], anthocyanins exhibit inhibitory actions against oxidative enzymes, stop the oxidation of ascorbic acid, scavenge free radicals, and subordinate the risk of cancer and heart disease.

Consumers today wish for food products that are just like the fresh, thus any processing should maintain the food's unique physicochemical characteristics and provide durability without compromising its essential qualities. Significant microbial inactivation as a result of thermal processing, and other undesirable effects, including enzyme inactivation, color change, taste changes, and vitamin loss, have been documented in numerous investigations. According to Kumar *et al.* (2006) [14], thermal treatments primarily influence nutritional (vitamins) and sensory (color, scent, texture, and flavor) qualities. As a result, PEF technology has become an important tool in the food industry, allowing for the production of high-quality, fresh-tasting food products that meet the demands of consumers while also reducing energy consumption (Shamsi and Sherkat, 2009) [27].

The consumption of juice made from fruits and vegetables has been scientifically demonstrated to be an essential element of a healthy diet. The population is getting older, there are more health issues, and people are more aware of the value of juices, all of which contribute to the rise in fruit and vegetable juice consumption. The economic impact of yeast-induced juice spoilage can be significant (Querol and Fleet, 2006) [22]. Juices that are not intended for immediate consumption should undergo a preservation process to ensure the ultimate quality and safety of the end product (Tran and Farid, 2004) [32].

The widespread use of thermal pasteurization to destroy microorganisms in food has recently sparked attention in the progress of alternative methods in response to consumer preferences for particular fruits juices that have a unique combination of fresh-like functional properties, are processed with fewer organoleptic and nutritional changes than in the past, and require fewer additives than before (Gould, 2001) [8]. Fruit and vegetable juice processing research often aims to produce fresh-like products with sensory, nutritional, and functional qualities that are as unaltered as feasible. In order to produce safe, fresh-like nutritious, and healthful foods, pulsed electric field processing has a lot of potential for usage in the juice business as a substitute for thermal processing.

The response surface methodology (RSM) is a group of statistical and mathematical methods for developing empirical models. A response (output variable) that is impacted by a number of independent variables (input variables) is to be optimized through proper experiment design. An experiment is a series of tests or trials that involve changing the input variables to identify the factors that contribute to the variations in the output response. According to Vining *et al.* (2005) [33], response surface methods (RSM) are very effective for optimizing juice mixes and processing variables.

The objective of this study is to explore the potential of PEF treatment and combination processing in the development of kokum fruit juice. The aim is to investigate the effects of these techniques on the quality and characteristics of the juice. By utilizing PEF treatment and combination processing, the study aims to enhance the overall sensory attributes, nutritional value, and shelf life of the kokum fruit juice. The findings of this research will contribute to the development of an improved and innovative method for producing high-quality kokum fruit juice.

Materials and Methods

Sample preparation

Fresh Kokum fruits were collected from the agricultural fields in Putter local of Mangalore district, India. Visual observations were used to classify and grade immature and overripe fruits, which were then disposed of. After removing any undesired parts, such as leaves and stem material, the fruits were cleaned by thoroughly rinsing them in tap water to eliminate any dirt or foreign objects. Subsequently, the fruits underwent another round of cleaning in sterile water. Once the cleaning process was completed and the surface moisture was eliminated, the fruits were sliced in half. Careful attention was given to removing the fruit rinds from the seeds.

Extraction of kokum pulp

After destalking and washing of fruits, the seeds were detached from the fruit rinds. Then the fruit rinds were homogenized in the electric mixer to get the fine pulp. The obtained pulp was filtered through the four folds of muslin cloth and the clear fine pulp was used to prepare the ready to drink (RTD).

Preparation of Kokum ready to drink (RTD)

The acidity and T.S.S of the raw juice is observed, and then required quantity sugar was added to raise its °Brix to 20°Brix i.e. for preparation of 100 ml of juice, 20 ml of pulp, 27 gm of sugar and 80 ml of water was added. Before being processed, the juice was thereafter placed in a sterile stainless-steel jar after being filtered through a double layer of muslin fabric.

Flowchart

Selection of kokum fruits
↓
Washing of fruits
↓
Preparation of fruits (Removal of stalks and cutting them into two halves)
↓
Separation of seeds from rinds Extraction of pulp from fruit rinds
↓
Addition of sugar to raise its brix to 20°brix
↓
After preparation as per the formula, then it is kept in a sterile stainless steel jar before being processed
↓
Processing of the juice
↓
Filling the product in pre-sterilized pouches
↓
Labelling
↓
Storage of the product at cool and dry place

Fig 1: Flow chart for preparation and processing of Kokum ready to drink (RTD)

Processing of kokum fruit juice

Pulsed electric fields processing

A pilot scale continuous PEF system (Model: ELCRACK® HVP 5, DIL, German Institute of Food Technologies, Quackenbruck, Germany) was used to administer PEF treatments. The electrode gap used for the PEF treatments was 7mm. The system had a highest voltage of 80 kV, a maximum frequency of 1 kHz, and an adjustable pulse width of 4 to 32 s. Co-linear treatment chamber and a chilled chilling module (-50 °C) made up the system. The shape, polarity, width, difference in potential, electric current created across the electrodes, and pulse frequency of the transmitted electric pulses were all monitored using a digital oscilloscope (Model: Siemens Digital Touch Screen Oscilloscope, Made in Denmark). Temperatures were assessed utilizing two thermocouples manufactured by Testo AG, located in Lenzkirch, Germany. The input and exit locations of the unit were identified by affixing a pipe wrap-style probe to the surface of the stainless steel tubes. It should be noted that 35 °C does not represent the highest temperature ever documented.

A peristaltic pump (Type SK 20F-80L14TF $T_10/1$-S Getriebebau NORD GmbH & Co KG, Germany) was used to push the kokum fruit juice through the system at a flow rate of 40 ltr/hr. The processing units cleaning protocol (CIP) of PEF has been established and consists of a thorough flushing with water for 15 minutes prior to a disinfection cycle with a 0.5% (w/v) Sodium Hydroxide solution for 15 minutes, followed by a 5 minute water rinsing and a 5% (v/v) Hypochlorite-based solution for 20 minutes, before a final flushing with water.

Water samples from the final rinse were examined for any lingering pollution following the cleaning process. Juice was processed at frequencies between 85 and 100 Hz using pulse widths of 19–24 s, a constant electric field intensity of 38.1 kV/cm, and bipolar mode for all treatments in order to optimize PEF processing characteristics. 200 milliliters of processed samples from each group were placed into sterile prefabricated multilayer laminated pouches made of polyethylene terephthalate measuring 12 millimeters, aluminum foil measuring 9 millimeters, nylon measuring 15 millimeters, and cast measuring 80 millimeters. Utilizing a semi-automatic filling machine (Model No. SDA25X30, Sevana, Cochin, India) and a continuous band sealing machine (Model No. CS-3781 Sepack®, Sevana, Cochin, India), 200 ml of polypropylene (total thickness 116 µm) with dimensions of 15 x 20 cm was filled in an aseptic filling chamber with laminar air flow class 10.

Thermal processing of kokum fruit juice

200 ml of ready-to-drink (RTD) kokum fruit juice were placed in sterile (thermally) prefabricated multilayer laminated pouches made of 12 mm Polyethylene terephthalate, 9 mm Aluminum foil, 15 mm Nylon, and 80 mm Cast. Using an impulse sealing machine (Model: HP Impulse Sealer, M/s Sunray Industries Mysore, India), 200 ml of polypropylene (total thickness: 116 µm) measuring 15 x 20 cm was hermetically sealed under sterile conditions. An air pressure of 4.5 kg/cm^2, a dwell time of 10 seconds, and a sealing temperature of 200 ºC were used to seal the pouches. The sealed and packed pouches were put through in-pack pasteurization in a steam jacketed kettle using a steel basket with the appropriate closure. A reference thermocouple was also installed in the steam jacketed kettle to maintain and monitor the temperature, and copper constantan thermocouples were fixed at the geometrical center of the pouch to measure heat penetration of the product. A data logger (Model: CTF 9004, M/s. Ellab, Denmark) was linked to thermocouple outputs. At regular intervals of 60 seconds, the thermo electromotive force was used to gauge the temperature inside the juice and steam jacketed kettle. Samples were taken as soon as the treatment period was over and placed in ice water for two to three minutes to cool. The thermal pasteurization was performed for 5 min (T_2) with standard product temperature (96 °C) and cooled immediately with cool water and then stored at 5 °C.

Combination processing of kokum fruit juice

The RTD kokum juice underwent a series of processes, including thermal processing and PEF. The kokum juice was initially treated with PEF, using the following parameters: a 40 ltr/hr flow rate, a 120 Hz frequency, a 24 µs pulse width, and an electric field strength of 38 kV/cm. The PEF processed sample was then packed and thermally treated using the in-pack pasteurization method for five minutes at 95 °C (T_4). After treatment, the sample was promptly cooled and kept for further research on storage.

Post-processing

The samples that underwent processing were segregated into aliquots as per the requirement for analysis. These aliquots were then assigned labels, with T_1 being the control, T_2 being subjected to pasteurization for 8 minutes, T_3 being subjected to PEF, and T_4 being subjected to a combination of PEF and pasteurization for 5 minutes.

Physico-chemical analysis of kokum fruit juice pH

At 23 °C, the pH was measured using a 700 Digital pH meter (Eutech Instruments, Made in Singapore). The pH buffers of 4.0, 7.0, and 10.2 were used to normalize the pH meter.

Acidity (%)

Acidity was determined through the titration method, as described by Ranganna (1986) [23]. A total of 10 ml of kokum fruit juice was taken and diluted to 100 ml with distilled water. The mixture was then filtered using Whatman paper. Next, 20 ml of the filtered material was transferred into a conical flask, and 2-3 drops of phenolphthalein indicator were added. The solution was titrated with 0.1 N NaOH until the end point, which is indicated by a pale pink color. The acidity was calculated using the following equation.

$$\%\ \text{Total acidity} = \frac{\text{titrate value} \times \text{normality of alkali} \times \text{volume made up} \times \text{equivalent wt of acid} \times 100}{\text{Volume of sample taken for estimation} \times \text{weight or volume of sample} \times 1000}$$

Reducing Sugar

Reducing sugar was estimated by Fehling's method (Ranganna, 1986) [23]. The process includes 25 ml of juice sample was taken in a 250 ml volumetric flask and 2 ml of lead acetate solution is added to precipitate protein. 2 ml of potassium oxalate solution added drop by drop through sides of the flask till white precipitate ceases and make up the volume with distilled water. Shake and filter through whatman no.1 paper. Pipette 5 ml of Fehling A and Fehling B solution in a conical flask and added two drops of methylene blue indicator and titrate against filtered sample with constant heat. Titration was continued until the brick red color was reached. Sugar reduction was estimated using the following formula.

$$\text{Reducing sugar}\ \% = \frac{\text{Felhing factor} \times 250 \times 100}{\text{Titrate value} \times \text{weight of sample} \times 1000}$$

Total sugar

Non reducing sugar was determined as per the Ranganna, 1986 [23]. In this method, part one solution of reducing sugar was used. 25 ml of the filtered solution was taken in 100 ml volumetric flask and 10 ml of concentrated HCL is added to it and kept overnight for hydrolysis. Then add a drop phenolphthalein indicator followed by 5% alkali i.e NAOH and make up volume. Pipette 5 ml of Fehling A and Fehling B solution in a conical flask and titrate against hydrolysed solution. Total sugar was calculated by using following equation

$$\text{Total sugar \%} = \frac{\text{Felhing factor} \times 100 \times 250 \times 100}{\text{Titrate value} \times 25 \times \text{weight of sample} \times 1000}$$

Estimation of Anthocyanin content

The anthocyanin content was measured using spectrophotometric analysis, as described by Ranganna (1986) [23]. To extract the anthocyanin, a mixture of 10 ml of kokum fruit juice and 10 ml of ethanolic HCL (85:15) was prepared and refrigerated overnight at 4 oC. The next day, the mixture was filtered after being diluted with ethanolic HCL. The absorbance of the filtered solution was then measured at 535 nm, relative to a blank solution. The concentration of anthocyanin in the dried kokum rind was determined by reporting its absorbance. The calculation of anthocyanin concentration was based on a specific equation.

$$\text{Total optical density} = \frac{\text{OD} \times \text{Vol. made up} \times 100}{\text{Weight of sample}}$$

$$\text{Total Anthocyanin (mg/100 g)} = \frac{\text{Total OD} \times 100}{98.2}$$

CIE colour value (L* a* b*)

CIE (L* a* b*) values were determined using a computer and a Hunter Lab Scan Spectrophotometric colorimeter. (USA-made Hunter Lab Colour Flex EZ 45/0° color spectrophotometer). The results were expressed in accordance with the CIE LAB system, using illuminant D65 and a viewing angle of 10°. The samples were put in an optical glass tray with the colorimeter's white plate as the background (CFEZ0503 X = 79.05, Y = 84.00, Z = 87.76). This context was utilized to help standardize the measurements. The measurements were taken with a diaphragm of 30 mm. L (whiteness or brightness/darkness), a (redness/greenness), and b (yellowness/blueness) were used to represent the color values. Using Eqs. (1 and 2), total color difference (E*) and Browning Index (BI*), which measure the extent of color change after treatment, were determined (Tiwari *et al.*, 2009) [30].

$$\Delta E = \sqrt{(\mathrm{L}-\mathrm{L_0})^2 + (\mathrm{a}-\mathrm{a_o})^2 + (\mathrm{b}-\mathrm{b_o})^2} \qquad (1)$$

Browning Index = [100 (X-0.31)] 10.72 (2)

Ascorbic acid

Ranganna (1986) [23] technique was used to determine the ascorbic acid concentration of kokum fruit juice. In a pestle and mortar, a 10 ml sample of kokum fruit juice was combined with 20% Meta phosphoric acid and decanted into a 100 ml volumetric flask. The extraction was performed three times with a few cc of Meta phosphoric acid each time, and the volume was made up with distilled water. A standard 2, 6-dichlorophenol indophenol dye was titrated against 10 mL of vitamin C extract. The ascorbic acid content of each sample was calculated using the equation below.

$$\text{Ascorbic acid (mg/100 ml)} = \frac{\text{T.V} \times \text{dye factor} \times \text{volume made} \times 100}{\text{Volume taken for titration} \times \text{sample weight}}$$

Total soluble solids (°Brix)

The total soluble solids (°Brix) were measured using the RF.5580 Euromex Brix hand Refractometer. The measurements were conducted at a temperature of 25.0±2 °C. Following each analysis, the refractometer prism was thoroughly cleaned using distilled water.

Microbial analysis

Microbial analysis was performed according to (Rivas *et al.*, 2006) [24]. For the microbiological counts, materials were serially diluted and plated in total count agar (PCA) for total plate (aerobic) counts and acidified potato dextrose agar (PDA) for mold and yeast counts. For total plate counts, molds, and yeast, plates were incubated at 30°C for 48 hours and 5 days, respectively. Coliforms were cultured on Violet Red Bile Agar.

Sensory evaluation

Sensory quality was assessed using a 9-point Hedonic scale (Ranganna, 1986) [23]. Twenty semi-trained panelists were chosen to evaluate sensory taste and odor. The 100 ml samples (treated and processed kokum juice) were shown. Between tasting each item, the panelists were told to rinse their mouths with water. The panelists scored the preferred samples for overall acceptability (OAA) in contrast to the control (untreated).

Statistical analysis

The data was analyzed using the least-squares method and response surfaces were created with the Design Expert 7.0.0 program. ANOVA was conducted to determine the significance of each variable and to ensure the adequacy of the model, with a significance level of α=0.05. Interaction effects were determined using LS means at a significance level of α=0.05. All experiments were performed in triplicate and statistical differences were evaluated using the Student-Newman-Keuls test with ANOVA at a significance level of $p<0.05$. Co Stat Version 6.204 (Coplot: 2003) was used for the analysis.

Results and Discussion

Thermal processing (Pasteurization) of RTD kokum juice

Pasteurization of kokum fruit juice was performed at a product temperature of 96 °C over a variable treatment time and labeled as T_2 (pasteurization for 8 minutes) and T_4 (combined treatment of pulsed electric field and pasteurization for 5 minutes).

Pulsed electric field processing of RTD kokum juice

Input and output processing parameters of pulsed electric field are given in following table 1.

Table 1: PEF parameters

Parameters	Units	values	Parameters	Units	Values
In-put parameters			Out-put parameters		
Output	%	60	Flow rate	ltr/hr	38.2
Pulse width	µs	24	Pulse field strength	kv/cm	38.0
Frequency	Hz	120	Energy	KJ/ltr	219.3
Flow rate	ltr/hr	40	Frequency	Hz	108
Electrode gap	Mm	7	Load resistance	ohm Ω	113

Physico chemical analysis of RTD kokum fruit juice

Kokum juice was subjected to different processing parameters. After processing, juice is studied for various physico chemical characteristics such as pH, acidity, reducing sugar, total sugar, anthocyanin, ascorbic acid and color values. The evaluation and presentation of the impact of PEF processing on the physico-chemical properties of RTD kokum juice can be found in Table 2.

Table 2: Physico-chemical properties of RTD kokum juice

Parameters	T_1	T_2	T_3	T_4
pH	2.60	2.72	2.65	2.71
Acidity%	0.20	0.29	0.26	0.22
Reducing sugar%	2.96	10.6	3.2	3.46
Total sugar%	19.45	18.64	19.28	18.68
Vitamin c (mg/100 ml)	5.35	3.57	4.85	4.14
Antocyanin (mg/100 ml)	12.65	8.76	10.58	9.08
L a b values	L*=2.11	L*=2.45	L*=2.27	L*=2.6
	a*=8.17	a*=7.49	a*=8.62	a*=8.16
	b*=2.07	b*=2.05	b*=2.52	b*=2.19

T_1 = Control, T_2 = pasteurization for 5 min, T_3= PEF treated, T_4 = PEF + pasteurization for 5 min.

After PEF processing, the RTD kokum juice's total soluble solids and pH barely changed. Rivas *et al.* (2006) [24] published findings from a related investigation showing that the pH and °Brix of PEF-processed blended orange and carrot juice barely changed. Additionally, Yeom *et al.* (2000) [34] found no appreciable alterations in orange juice treated with PEF during storage. Additionally, Kumar *et al.* (2014) [15] found no appreciable differences in the pH, °Brix, or acidity of PEF-treated mango nectar.

One of the crucial indicators of RTD juice quality is color. Using a Hunter color equipment and CIE color lab values, the color degradation of PEF-treated RTD kokum juice was examined. The color values for redness (a*), yellowness (b*), and brightness (L*) of the PEF-treated juice were determined to be 8.62, 2.52, and 2.27, respectively. Juice that had undergone thermal processing had color values for redness (a*), yellowness (b*), and brightness (L*) of 8.17, 2.07, and 2.11, respectively. We can see from this number that juice that has undergone heat processing has more color deterioration. Colour is important quality factor of fruit juice for the consumer. El Syed *et al.,* (2020) [5] found that there is no changes in the colour of the PEF treated strawberry juice as compared to thermal processing. One of the main causes of the color change in PEF-treated kokum juice is the breakdown of anthocyanin. Juice from the kokum fruit contains a lot of anthocyanin. Fruit juice that had undergone thermal processing contained 8.76 mg and fruit juice that had undergone PEF processing contained 10.58 mg of anthocyanin. According to these findings, thermally processed kokum fruit juice loses more anthocyanin than PEF-treated juice. In comparison to control samples, the anthocyanin content of the PEF treated samples did not show any obvious signs of degradation, and neither did the color values (table 2). A similar study by Kumar *et al.* (2014) [15] found that the carotene content in PEF-treated mango nectar was retained at high levels.

Fresh RTD kokum juice's ascorbic acid level was 5.35 mg per 100 ml. In pasteurized kokum juice, the ascorbic acid level was 3.57, but it was 4.85 mg per 100 ml in PEF-treated samples (table 2). A vitamin called ascorbic acid is very readily broken down when exposed to heat, air, or light. Based on these results, the ascorbic acid content was significantly degraded (p 0.05) by PEF processing. Our findings are in line with those of authors Elez-Martinez and Martin- Belloso (2007) [6] and Odriozola-Serrano *et al*. (2008) [19] who assessed the Vitamin C losses in orange and strawberry juices, respectively, treated with pulsed electric fields. These results are same with the results obtained by El Syed *et al.*, (2020) [5], Schilling *et al.,* (2007) [26] who reported that PEF treatments of fruit juices did not affect the physiochemical properties like pH, acidity, soluble solids and colour of the fruit juices.

Shelf life evaluation study

Effect of pasteurization, PEF, and combination processing on pH during storage of RTD Kokum fruit juice

The pH of an unprocessed fresh kokum juice was found 2.60. The stability of bioactive compounds in fruit juice can be described by pH, which is one of the primary quality characteristics (Sanchez *et al*., 2006) [25]. Thermal pasteurized kokum juice had a higher pH than raw kokum juice (Fig 2). It could be related to thermal breakdown of the ascorbic acid in the juice, although the pH shift in PEF processed kokum juice is not as significant as in thermal processing. During the storage period of RTD kokum juice at a temperature of 5 °C, it was observed that there was no significant alteration in the pH of the T_1 sample (which was the unprocessed/control sample) from the beginning until the 15th day. T_2 (pasteurization for 8 minutes) samples had an initial pH of 2.72 on the 30th day, with no significant alterations identified, but on the 45th day, the pH was discovered to be much lower. The same procedure is used in T_4 (combination processing) and in PEF-treated sample T_3. In a study conducted by Rivas *et al.* (2006) [24], it was found that the pH of thermally treated juice, specifically a blend of orange and carrot juice, did not exhibit any variation during storage. This suggests that the thermal treatment process did not have a significant impact on the pH levels of the juice. Similarly, Yeom *et al.* (2000) [34] also reported no significant changes in the pH of heated orange juice over the course of storage. These findings indicate that the pH of thermally treated and heated juices remains stable during storage, highlighting the potential for these products to maintain their quality over time.

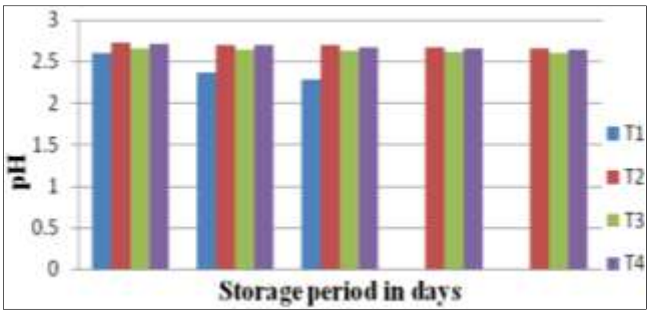

Fig 2: Effect of storage on pH

Effect of storage on total acidity of RTD Kokum fruit juice

The study investigated the impact of different treatments (PEF, thermal, and combination processing) and storage on the total titratable acidity of raw kokum fruit juice. The initial titratable acidity of the juice was measured at 0.19%. Regular measurements were taken throughout the storage period, and it was observed that there was no significant change in acidity during the initial stages of storage. However, as the storage period progressed, an increase in acidity was observed. These findings align with a study conducted by Bull *et al.* (2004) [3], which examined the thermally processed Valencia and Navel orange juice and reported no significant alterations in total titratable acidity over the storage duration.

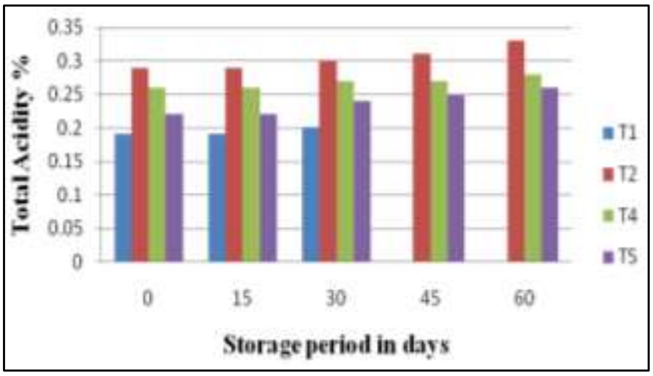

Fig 3: Effect of storage on total acidity

Effect of storage on ascorbic acid of RTD Kokum fruit juice

The ascorbic acid content of the fresh kokum juice was found 5.35 mg/100 ml (T_1). Ascorbic acid degradation in thermal pasteurized kokum juice was about 44% whereas in PEF treated juice it was 10%. There was a notable decline observed in the ascorbic acid content of the stored samples of T_2, T_3, and T_4 over a period of 60 days, as depicted in Figure 4. During storage, there was significant decrease in the ascorbic acid content but when compared to thermal processed sample degradation was less in PEF treated sample (Klopotek *et al.*, 2005; Odriozola-Serrano *et al.*, 2008) [12, 19]. The results are in consistent with El sayed *et al.*, (2020) [5] who has studied the effect of PEF and thermal pasteurization on strawberry juice. The level of vitamin C in decreased in thermal pasteurization as compared to PEF during storage.

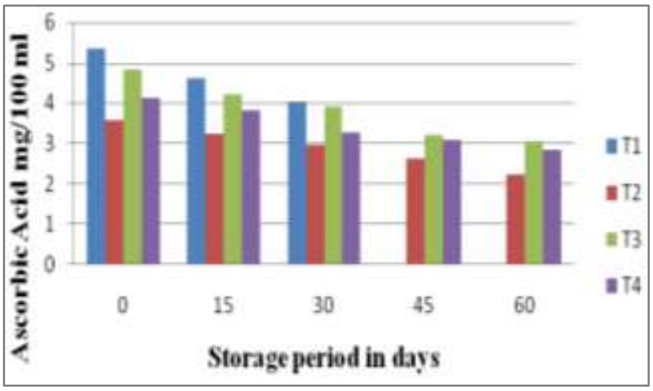

Fig 4: Effect of storage on ascorbic acid

Effect of storage on CIE color (L* a* and b*) of RTD Kokum fruit juice

Color is a crucial attribute that plays a significant role in determining the quality of fruit juices. The primary factor responsible for the reduction in kokum juice is thermal stress. The control juice sample (T_1) exhibited redness (a*), yellowness (b*), and brightness (L*) values of 8.17, 2.07, and 2.11, respectively. On the other hand, the PEF processed samples displayed redness (a*), yellowness (b*), and brightness (L*) values of 8.62, 2.52, and 2.27, respectively. However, during the storage at 5 °C, the processed T_2, T_3, and T_4 samples experienced a significant depletion ($p < 0.05$) in their redness (a*), yellowness (b*), and luminosity (L*) values. The study conducted by Ayhan *et al.* (2001) [1] revealed that PEF treatment resulted in a significant increase in luminosity (L*) and yellowness (b*) values, while decreasing the redness (a*) values of the juice. This suggests that the changes in particle size induced by PEF treatment could be responsible for the observed increase in luminosity and yellowness. Similarly, Kumar *et al.* (2014) [15] found that PEF-treated mango nectar exhibited better color preservation compared to thermally treated mango nectar.

Effect of storage on reducing and non reducing sugar of RTD Kokum fruit juice

Figures 5 and 6 depict the results for the reduction in sugar and overall sugar content. In the untreated (T_1) kokum juice, the reducing sugar was measured at 2.96% and the total sugar at 19.45%. The impact of processing on sugar content was minimal, with the PEF treated samples showing reducing sugar and total sugar levels of 3.2% and 19.12% respectively. Notably, significant changes in sugar content were observed in the PEF treated samples during the storage at 5 °C.

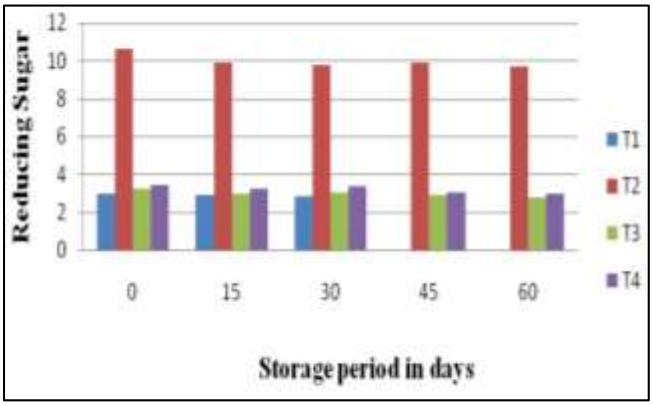

Fig 5: Effect of storage on reducing sugar content

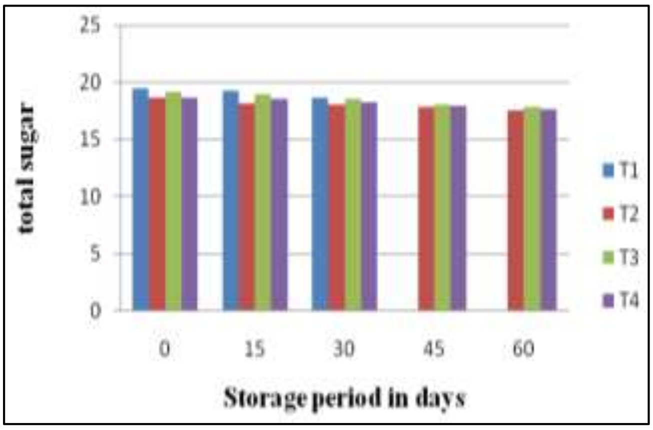

Fig 6: Effect of storage on non reducing sugar

Effect of storage on anthocyanin content of RTD Kokum fruit juice

Kokum fruit juice is rich source of anthocyanin. The anthocyanin content in unprocessed fresh sample of kokum juice was found 12.65 mg /100 ml, whereas in PEF treated it was 10.58 mg /100 ml. During storage, for some interval of time there was slight raise in the anthocyanin content (Fig 7). The anthocyanin dilapidation is more in thermal processing and at refrigerated condition the anthocyanin degradation was less compared to room temperature. Hellstrom *et al.,* (2013) [9] studied the stability of anthocyanin at storage at different temperature in fruit juices.

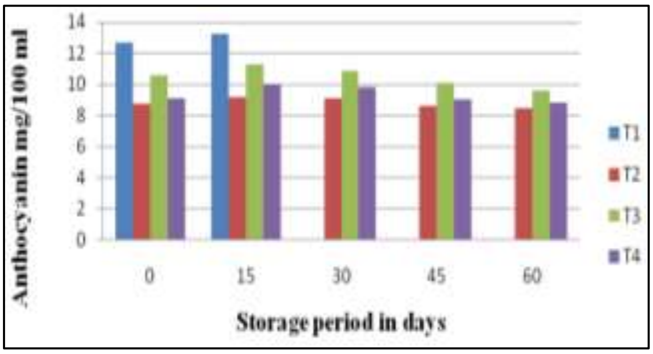

Fig 7: Effect of storage on anthocyanin

Effect of storage on microflora of RTD Kokum fruit juice

The impact of different processing methods (PEF, thermal, and their combinations) and storage conditions on the microflora was examined in this study. The control samples (T_1) initially had a microbial load of 9×10^2, 1×10^1 for total plate count, yeast, and mold, and were positive for coliforms. After processing, the T_2 sample showed no growth for total plate count and yeast and mold, but was positive for coliforms. On the other hand, the T_3 and T_4 samples had a total plate count of 2×10^1 and no growth, respectively, and were confirmed to have no yeast, mold, and coliforms. After storage, the T_2 sample still had no growth for total plate count and yeast and mold, but remained positive for coliforms. The T_3 and T_4 samples, however, showed no growth for yeast and mold and coliforms, with T_3 having a load of 5×10^2 and T_4 having a load of 4×10^2.

The bacteria are sensitive to storage temperature. Because microbial development is very quick at higher temperatures (27 °C), the counts were always greater in the samples maintained at that temperature. The shelf life of the juices was determined while keeping the natural microbial ecology in mind. The kokum juice possesses an important phenolic compound called as Garcinol. Other than this there are some compounds in kokum juice such as furfural and its derivatives, cyanidin- 3-glucose which is present as anthocyanin in the rind and caffeine. Of all these, furfural and cyanidin-3-glucose are potent antimicrobials. Kokum showed antibacterial activity against gram positive and gram negative organisms (Sutar *et al.*, 2012) [28]. There is significant relations between electric field strength as well as treatment time and microbial inactivation efficiency (Ghanshyam *et al.*, 2011; Huang and wang, 2009; Toepfl *et al.*, 2007) [7, 11, 31].

Effect of storage on Sensory of RTD Kokum fruit juice

Sensory analysis was done by 10 semi trained members using 9 point hedonic scale. The results were obtained from the panel members and average score was taken. Figure (8) depicts the hedonic scale rating of 9 for untreated and processed kokum juice. The sensory scores for color, appearance, consistency, flavor, and overall acceptability (OAA) of untreated (T_1) kokum juice were 8.8, 8.7, 8.8, 8.8, 8.5, and 8.7, respectively. T_2, T_3, and T_4 samples maintained at 5 °C were found to be considerably reduced during storage. When compared to the samples processed by thermal pasteurization (T_2), the PEF (T_3) and PEF + pasteurization 5 min (T_4) samples received good ratings. Min *et al.* (2003) [17] has reported that PEF processed orange juice possessed significantly higher attributes than thermally processed orange juice.

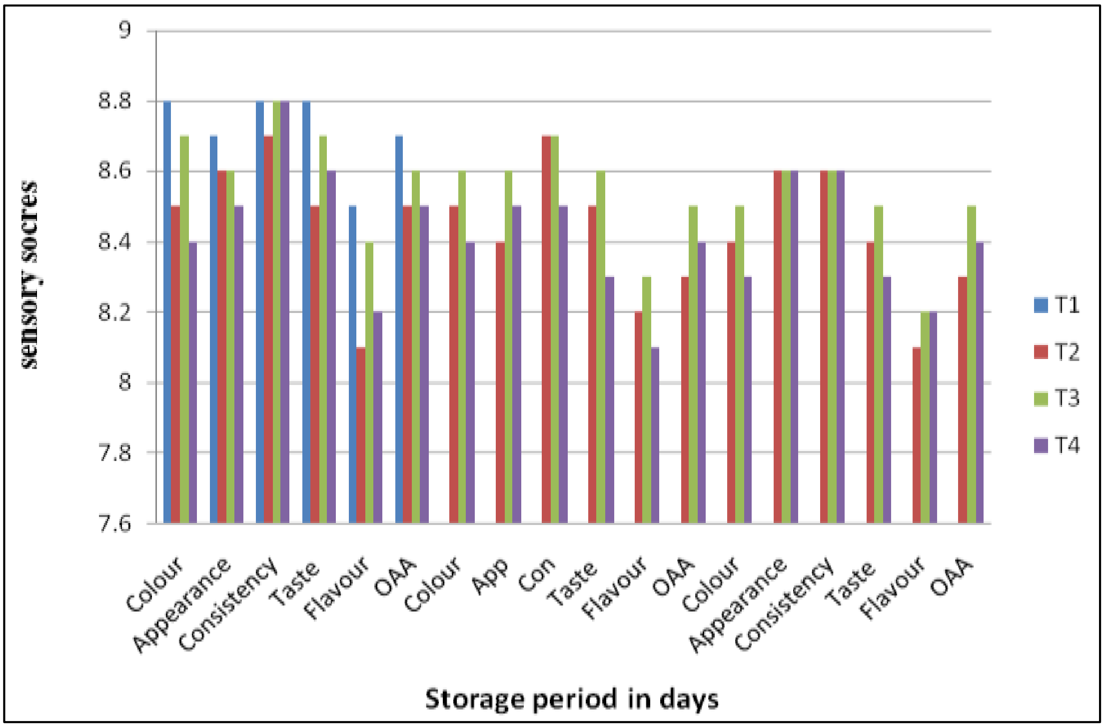

Fig 8: Effect of storage on sensory

Conclusion

Garcinia is a rich source of active compounds including Hydroxycitric acid, garcinol and anthocyanin, due to this it as lot of neutracetical properties such as antiobesity, reduce the risk of cancer and heart diseases, antiulcer, antioxidation and antiglycation activity. The shelf life of fresh fruit is less i.e. about one week hence lot of value added products can be prepared by kokum such as kokum amsul, kokum agal, kokum butter, kokum syrup etc. The RTD kokum juice was subjected to thermal, PEF and combination treatment. It was observed that the thermal treatment, as such or in combination, the degradation in color, anthocyanin and vitamin C is more when compared to PEF treated kokum juice. PEF processing had a statistically significant effect on TPC, anthocyanin and sensory score (OAA) of RTD kokum fruit juice. The study aimed to examine the impact of storage on the quality characteristics of kokum fruit juice, specifically in relation to thermal, PEF (pulsed electric field), and combination processing. The analysis revealed significant differences in color, vitamin C, and anthocyanin content between thermally processed samples and those treated with PEF. Furthermore, when compared to pasteurized samples, both PEF and combination treated samples exhibited high shelf stability. Notably, the retention of ascorbic acid and anthocyanin was found to be higher in the PEF exposed sample compared to the thermal and combination treatment samples. Consequently, the study concludes that PEF, along with its combination treatment, is an effective method for preserving the quality of kokum fruit juice, surpassing the stability achieved through thermal processing.

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