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# Comparative evaluation of quality attributes and shelf life of minimally processed guava vis-a`-vis whole guava (*Psidium guajava*) fruits during storage

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ABSTRACT

Minimally processed guava (*Psidium guajava* L.) fruits cv. Lalit were compared with whole guava fruits for different quality attributes and shelf life during storage at 10 and 15°C. Whole and minimally processed guavas were analyzed for parameters, like total soluble solids, titratable acidity, ascorbic acid, total polyphenols, lycopene content, polygalacturonase activity and microbial load over a period of 15 days (d). Minimally processed fruits stored at 10°C showed relatively higher concentrations of ascorbic acid, polyphenols and lycopene, compared to whole guava fruits. Sensory evaluation results revealed that minimally processed fruits scored better than the whole guava fruits throughout the storage period, irrespective of the storage conditions. Bacterial load was low in both minimally processed and whole guava fruits stored at 10°C, compared to fruits stored at 15°C. Hence, it can be concluded from this study that minimally processed guava retained quality attributes and bioactive compounds, compared to whole guava fruits during storage at 10°C. Thus, minimally processed guava fruits can serve as suitable alternative to whole guava fruits for consumers' convenience as well as acceptance.

**Keywords:** Bioactive compounds, Microbial load, Minimally processed guava, Quality parameters, Sensory attributes, Shelf life

The market for minimally processed fruits and vegetables has significantly increased in recent years. In USA, the consumption of minimally processed fruits and vegetables accounts for about 48% of the total fresh fruit and vegetable market, because of the presence of high levels of vitamins, fiber, minerals and antioxidants in them (Patrignani *et al.* 2015). In addition, minimally processed fruits and vegetables regarded as ready-to-use or ready-to-consume, provide consumers with a safe and convenient product. Minimally processed fruits also help the consumers to have an idea about the internal defects, such as presence/absence of larvae inside the fruits, which are generally not visible in the whole fruits. Studies have shown that minimally processed fruits, such as pineapple, mango, cantaloupe, watermelon, strawberry and kiwifruit retained their nutritional properties during storage (Gil *et al.* 2006). Vitamin C, β-carotene and vitamin E were retained during storage of whole and fresh cut mango at 5°C (Robles-Sanchez *et al.* 2009). Minimally processed oranges retained their health related attributes, such as vitamin C, carotenoids and flavanones in comparison to whole fruit and hand peeled fruits during their storage at 4ºC for 12 d (Plaza *et al.* 2011). Fresh-cut peaches treated with a solution of ascorbic acid and citric acid and subjected to a combination of high pressure processing (HPP) and vacuum packaging (VP) showed retention of color and texture, lower content of ethanol and polyphenol oxidase activity (Denoya *et al.* 2015).

There is only a scanty literature available on different aspects of minimally processed guavas (*Psidium guajava* L.) and the available published literature does not comprehensively cover different facets of minimally processed guava fruits affecting their shelf life. The present study was therefore, undertaken to evaluate the changes in different quality attributes, bioactive compounds and microbial population dynamics in minimally processed guavas in comparison to whole fruits during their storage at 10 and 15°C. Commercial chiller temperatures in India are generally maintained at 8-10°C for storage of most fruits, vegetables including minimally processed ones. We also wanted to investigate if a comparable shelf life could be obtained during storage of whole and minimally processed

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guava fruits at 15°C which would eventually help in reducing energy costs for refrigeration as relatively higher running costs shall have to be incurred to maintain temperatures at 10°C than 15°C for long term storage.

## MATERIALS AND METHODS

Mature and firm guava fruits of pink flesh cv. Lalit were procured from the fields of ICAR-Central Institute for Subtropical Horticulture (CISH), Lucknow, India. Infected, damaged or defective fruits were discarded and only healthy fruits were used for this study. Firmness of freshly harvested fruits was measured using a penetrometer (Mc Cormick fruit tester 0-12.5 scale) with 0.8 mm dia probe and the firmness was expressed as kg/cm. Fruits with firmness ranging between 3-9 kg/cm were selected for further experimentation. Although, firmness variability is apparently higher, such variations in freshly harvested raw fruits have been previously reported in literature (Robles-Sanchez *et al.* 2009). Fruits were dipped in 5% hydrogen peroxide solution for 5 min, followed by air drying at room temperature (Har and Perera 2013). Subsequently, the fruits were divided into two batches. First batch of guavas were cut into four wedges using a sterile knife and vacuum packed in 25µ thick low-density polyethylene (LDPE) pouches by removing air under a pressure of 760 mm Hg for 20s followed by sealing of packages. Second batch of whole guava fruits were vacuum packed in a similar manner as was the first batch. The packets containing whole and minimally processed guava were stored at 10 and 15°C for 15d for analysis of biochemical, enzymatic, sensory and microbial parameters during storage at regular intervals of 5d.

Samples were analyzed for total soluble solids, titratable acidity, reducing sugars, ascorbic acid, lycopene, total polyphenols and polygalacturonase activity. Samples were homogenized in a blender and used for evaluation of different quality parameters. Total soluble solids (TSS) was determined in the homogenized samples by placing a drop of juice on the screen of hand held refractometer (0-30%) and the observations were recorded, adjusted with the correction factor at 20ºC and expressed as ºBrix. Titratable acidity was measured by titrating the sample aliquot against 0.1 N NaOH using phenolphthalein as indicator and expressed as citric acid (%). Reducing sugars were estimated by Folin and Wu's method as described in AOAC (1975), while ascorbic acid was estimated by titrimetric method using 2,6-dichlorophenolindophenol dye solution (Ranganna 2000). Lycopene was estimated by extracting samples with acetone repeatedly until the sample turned colorless. The extractant was transferred to separating funnel containing 10-15 ml petroleum ether, water and sodium chloride. Extract containing lycopene was made up to the required volume with petroleum ether and measured at 503 nm in spectrophotometer using petroleum ether as blank (Ranganna 2000). Total polyphenols in the homogenized samples were analyzed by Folin-Ciocalteu's method and expressed as TAE g/kg (Ranganna 2000). Polygalacturonase activity was analyzed using D- galacturonic acid as a standard (Miller 1959). Briefly, 0.8 ml of 1% (w/v) polygalacturonic acid was dissolved in 50 mM phosphate buffer (pH 5.0) and 0.2 ml of the enzyme solution was incubated at 30°C for 30 min. Polygalacturonase activity was quantified by measuring the amount of D- galacturonic acid released in the medium. One unit of enzyme corresponded to the amount of enzyme needed to release one µmole of galacturonic acid per min under the standard assay conditions. All the colorimetric observations were recorded with UV-Vis spectrophotometer.

Twenty five gram sample from each package was collected immediately after opening the package and suspended in 225 ml sterile distilled water and subjected to homogenization in a pre-sterilized blender. Samples were aseptically drawn from the blender and the aliquots were subjected to serial dilutions using 9 ml sterile water blanks. One ml aliquot was spread plated on the sterilized media petriplates. Inoculated media plates were incubated at 37 $^{0}$C for enumeration of yeasts, molds and bacteria (common genera) including coliforms. Media used for quantification of bacteria, yeasts and molds were Nutrient medium, glucose yeast extract (GYE) and Potato Dextrose Agar (PDA), respectively. Presence of coliforms in the samples was detected using violet red bile agar (VRBA). All the samples were analyzed in triplicate and the results were expressed as CFU/g.

Sensory evaluation was done by a group of 20 semi-trained panelists who were well acquainted with the fresh fruit quality. Sensory data were collected for parameters, like color, flavor, texture and overall acceptability on a 9-point hedonic scale (Amerine *et al.* 1965). The final scores obtained from the panelists were tabulated and the score for overall acceptability was determined through analysis of the results.

All the experiments were conducted in triplicate and MS Excel was used for calculation of mean and SD values. A completely randomized blocking experimental design was used for this study and data were analyzed using WASP statistical software (Web Agri Stat Package, Version 2.0, ICAR Research Complex for Goa, Ela, Goa, India.) (Jayade *et al.* 2015, Bhuvaneswari *et al.* 2015).

## RESULTS AND DISCUSSION

*Effect of storage temperature on physico-chemical and bio-chemical properties:* Changes in the quality attributes of minimally processed and whole guava fruits during storage at different temperatures are presented in Table 1. Changes in TSS may be attributed to either ethylene synthesis during storage (Shah and Nath 2008) or to the utilization of sugars via glycolysis for maintenance of respiration over longer storage periods (Padmanabhan *et al*. 2014). Similar trend on increase and decrease in TSS has been previously reported for minimally processed guava and litchi fruits (Shah and Nath 2008, Mariano *et al.* 2011). Acidity in fruits and vegetables is an important quality attribute affecting the flavor and acceptability. Significant increase in titratable acidity, both in whole as well as minimally processed guava fruits from an initial value of 0.44% was seen during storage

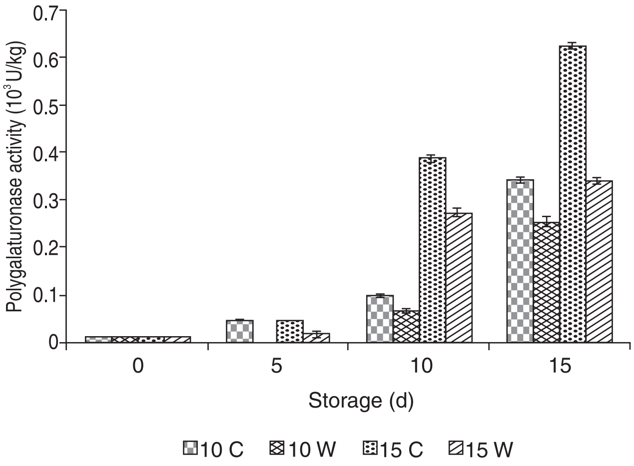


Fig 1 Changes in polygalacturonase activity in minimally processed fruit (C) compared with whole guava (W) fruit at 10 and 15 °C. Bars represent standard errors, n=3 , 10 C- Minimally processed guava fruits stored at 10 °C; 10 W- Whole guava fruits stored at 10 °C; 15 C- Minimally processed guava fruits stored at 15 °C; 15 W- Whole guava fruits stored at 15 °C

at 10 and 15°C (Table 1). Similar trend for increase in acidity was reported in kiwifruit and cantaloupe stored up to 9d at 5°C by Gil *et al*. (2006). Slight difference was observed in reducing sugars concentration in minimally processed and whole guava fruits during storage, irrespective of the storage temperatures. Increase in reducing sugars during storage at 10°C and a decrease at 15°C was seen at the end of the storage period for minimally processed guavas. Whole guava fruits showed a slight fall and increase in reducing sugars during storage at 10°C and 15°C, respectively at the end of the storage period (Table 1). Water loss leads to a loss of turgor and crispness, which is rapid in fresh-cut products due to the absence of cuticle and sub-epidermal layers and the exposure of internal tissues. However, rate of water loss

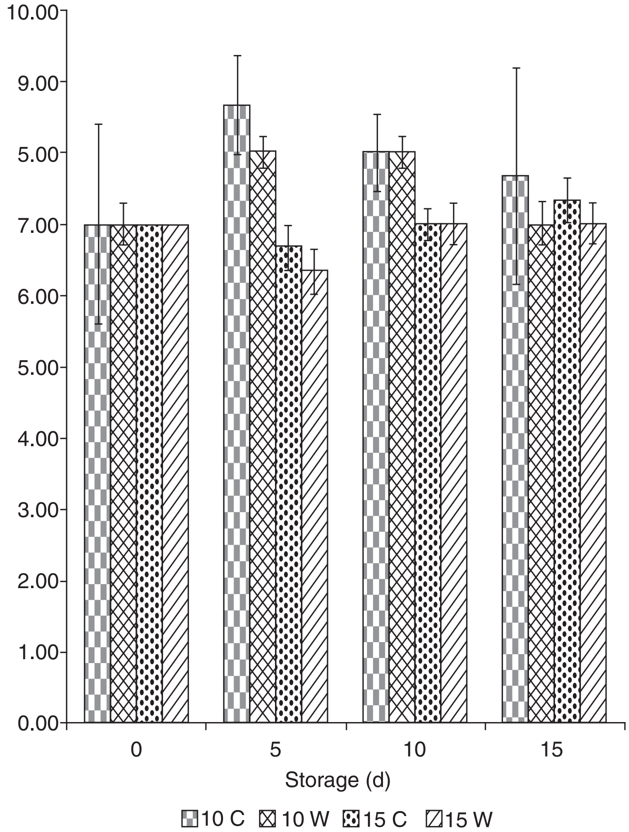


Fig 2 Sensory score for minimally processed (C) and whole guava (W) fruits during storage at 10 and 15 °C. Bars represent standard errors, n=7. 10 C- Minimally processed guava fruits stored at 10 °C; 10 W- Whole guava fruits stored at 10 °C; 15 C- Minimally processed guava fruits stored at 15 °C; 15 W- Whole guava fruits stored at 15 °C

Table 1 Changes in total soluble solids (TSS), titratable acidity (TA) and reducing sugars in vacuum packed minimally processed and whole guava fruits at different temperatures

| Parameter | Storage | | | | | |
|---|---|---|---|---|---|---|
| | Storage temperature | Sample | 0 | 5 | 10 | 15 |
| TSS (ºB) | 10 ºC | Fresh-cut | 12.46 $^a$ | 12.66$^a$ | 11.73$^b$ | 12.26$^a$ |
| | | whole | 12.46 $^a$ | 12.13$^b$ | 12.00$^a$ | 11.73$^b$ |
| | 15 ºC | Fresh-cut | 12.46 $^a$ | 11.80$^b$ | 11.53$^b$ | 11.46$^b$ |
| | | whole | 12.46 $^a$ | 12.06$^a$ | 12.26$^a$ | 12.20$^a$ |
| TA (% citric acid) | 10 ºC | Fresh-cut | 0.44 $^a$ | 0.79 $^a$ | 0.55$^b$ | 0.69$^a$ |
| | | whole | 0.44 $^a$ | 0.78 $^a$ | 0.75$^a$ | 0.62$^b$ |
| | 15 ºC | Fresh-cut | 0.44 $^a$ | 0.73 $^a$ | 0.66$^b$ | 0.69$^b$ |
| | | whole | 0.44 $^a$ | 0.67 $^a$ | 0.76$^a$ | 0.74$^a$ |
| Reducing sugars (%) | 10 ºC | Fresh-cut | 1.50 $^a$ | 1.67$^a$ | 1.63$^a$ | 1.59$^a$ |
| | | whole | 5.50 $^a$ | 5.17$^b$ | 5.35$^b$ | 5.38$^b$ |
| | 15 ºC | Fresh-cut | 1.50 $^a$ | 1.24$^a$ | 1.30$^b$ | 1.42$^b$ |
| | | whole | 5.50 $^a$ | 5.04$^b$ | 5.47$^a$ | 5.59$^a$ |

Values presented are mean of three replications, n-3. Values with the same superscript at each storage period and storage temperature (presented in rows) do not differ significantly.

also depends on the storage temperature. This could be a probable reason for higher reducing sugar concentration at the end of the storage for minimally processed guava fruits stored at 10°C.

Increased PG activity was seen in minimally processed fruits, compared to whole guava fruits (Fig 1). Storage of samples at 15°C showed higher PG activity in minimally processed fruits (Fig 1). Low PG activity at 10°C could be attributed to low ethylene production at lower temperatures, leading to a fall in the PG activity. Ethylene production increases in fruits due to wounding and mechanical injury, leading to an increase in the PG activity (Karakurt and Huber 2003, Toivonen and Brummel 2008). Similar results were observed when sliced papaya was stored at 4ºC (Paull and Chen 1997). Imsabai (2002) observed higher PG activity in durian fruits stored at 27°C, compared to fruits stored at 12°C. Lower PG activity could be positively correlated with maintenance of firmness in whole guava fruits in the present study.

*Effect of treatments and storage temperatures on bioactive compounds:* Ascorbic acid as vitamin C contributes to the antioxidant property of fruits. Increase in ascorbic acid content was observed in minimally processed fruits at 10°C, however in whole guava fruits, ascorbic acid slightly increased initially and later declined towards the end of the storage period. This may be because of the reversible oxidation of ascorbic acid to dehydro-acorbic acid, which resulted in decrease in ascorbic acid concentration. In case of minimally processed fruits, retention of ascorbic acid may be correlated to high polyphenol content which might have protected the ascorbic acid by polyphenol's ascorbate-sparing effect (Piga *et al.* 2003). Similar trend on increase and decrease in ascorbic acid levels was observed in fresh-cut and whole cantaloupe stored at 5°C by Gil *et al.* (2006).

Polyphenols from plants play an important role against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Pandey and Rizvi 2009). Total polyphenol content reduced by 24-30% at 10°C and 21-26% at 15°C for both minimally processed and whole guava fruits at the end of the storage period, but no significant difference was observed in polyphenol content between the treatments. It has been observed that phenolic content reduces when fruits and vegetables are exposed to oxygen, light and high temperatures. Polyphenoloxidase activity increases during cutting of fruits and vegetables, which in turn increases the oxidation process, resulting in loss of phenolics (Robles-Sanchez *et al.* 2009).

Lycopene is ranked as the most potent antioxidant among other carotenoids, such as α-tocopherol, α-carotene, β-cryptoxanthin, β-carotene and lutein (Heber and Lu 2002). Increased lycopene content was observed in minimally processed guava fruits stored at 10°C towards the end of the storage period. This may be due to slight variation in the maturity stage of fruits during harvesting. The process of induction and accumulation of bioactive compounds continue during refrigerated storage, depending on the temperature (Carmona *et al.* 2007). This effect could be more pronounced in minimally processed fruits which are subjected to mechanical injury and have large surface area. Apparently, minimally processed guava fruits showed higher lycopene content during storage at 10°C. Rivera-Lopez *et al.* (2005) presumed that the increase or decrease in lycopene content in fruits and vegetables could be due to increase in the respiration rate by 1-7 folds, because of processing operations. Increase in ethylene production, synthesis and/or loss of phytochemicals also takes place during processing (Rivera-Lopez *et al.* 2005). Minimally processed fruits showed increase in lycopene content during storage, whereas, reduction in lycopene by 41 and 23% was observed in whole guava fruits stored at 10 and 15°C, respectively. Significant loss in carotene has been reported in papaya cubes stored at 20°C compared to those stored at lower temperatures (Rivera-Lopez *et al.* 2005). This loss could be attributed to enzymatic degradation mediated by lipoxygenase and through secondary reactions or co-oxidation during fatty acid oxidation. Lycopene was either retained or increase in its concentration was observed at the end of the storage period for minimally processed guava fruits stored at 10°C. Similar observations were reported by Lana *et al.* (2005) for fresh-cut and intact tomatoes.

*Sensory evaluation:* Minimally processed guava scored better in comparison to whole guava fruits stored at either of the storage temperatures throughout the storage period. However, minimally processed fruits stored at 10°C scored slightly higher than those stored at 15°C, which could be attributed to textural properties that were better maintained at 10°C. This indicates that the minimally processed fruits maintained their color, texture and flavor during storage (Fig 2). Robles-Sanchez *et al.* (2009) also reported higher sensory score for fresh-cut mango treated with a combination of ascorbic acid, citric acid, $CaCl_2$ and stored at 5°C for 5 d. It is evident from the results of this study that minimally processed guavas can be safely stored at 10°C up to 15 d without a significant change in the sensory attributes.

*Microbial population dynamics in minimally processed and whole guava fruits during storage:* Fresh-cut fruits and vegetables are more perishable than the whole fruits due to availability of more surface area for microbes to grow (Bhagwat 2006). Initial total bacterial population ranged from 2.95-3.26 log CFU/g (Fig 3). Significant increase in bacterial population was not seen until 10 d and the bacterial count even after 15 d was less than 5 log CFU/g (Fig 3). Higher bacterial count was observed in minimally processed fruits, compared to whole fruits. Bacterial load was higher in fruits stored at 15°C, largely due to the growth and proliferation of bacteria as most bacteria prefer temperatures in the range of 15-40°C. Higher bacterial count could also be due to processing operations involved in the preparation of minimally processed fruits which lead to the release of enzymes and other substrates, that act as medium for the growth of the microorganisms. In addition, cutting operations in fruits form leaky membranes that allow diffusion of small molecular weight compounds and water

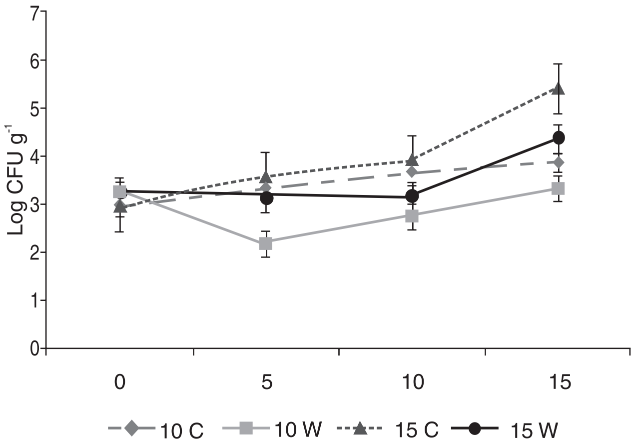


Fig 3 Change in the bacterial population of minimally processed (C) and whole guava (W) fruits during storage at 10 and 15 ºC. Bars represent standard errors, n=3.

into intercellular spaces and wound surfaces facilitating the growth of microorganisms (Siddiqui *et al.* 2011). We have not yet come across any universally accepted safe limits for population of spoilage organisms. However, some countries have established legal limits for microbial population in minimally processed fresh fruits. The Spanish legal limit (RD 3484/2000, 2001) for microbial populations on minimally processed fruit for safe consumption are 7, 5, and 3 log CFU/g for aerobic bacteria, yeasts and molds, respectively (Barth *et al.* 2009). Yeast and molds were well within the specified limits for samples stored at 10°C, yeast and molds count was relatively higher in minimally processed samples at 15°C towards the end of the storage period (data not shown). However, even at 15°C, the yeast and mold population in whole and minimally processed guava fruits was within the acceptable limits. Coliforms were not detected in either of the samples throughout the storage period.

Minimal processing of guava at 10°C led to a product with better physic-chemical attributes and nutritional quality than whole fresh fruits during storage for 15 d. Minimally processed guava stored at 10°C showed relatively higher concentration of bioactive compounds than the whole fruits during storage. Microbial population results indicated that minimally processed and whole fruits could be safely stored at 10°C for 15 d. It can be concluded from this study that minimally processed guava fruits retained quality parameters at par or even better than the whole guava fruits, thereby offering convenience, extended shelf life and more choice to the consumer. However, further studies are needed to evaluate the effect of vacuum packaging and other modes of packaging on quality parameters of minimally processed guava fruits for longer shelf life.

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