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NUTRITIONAL, MICROBIAL AND SENSORY EVALUATION OF JAM STORED UNDER AMBIENT CONDITION USING PECTIN ISOLATED FROM POMELO (*Citrus maxima)* PEELS

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ABSTRACT

Fruit peels are agro wastes that may be considered as raw materials, however despite of their pollution and hazardous aspects, wastes from fruit processing industries have a great potential for regeneration into products of significant value as raw materials for other industries, while considering the fruit processing industries citrus is one of the major processed fruits, there is a substantial prospective to utilize citrus peels to isolate value-added ingredients as pectin. This study focused to use pomelo peels, as a source of pectin and its utilization in the fruit jam production with different blends of pectin and sugar levels and assess its nutritional, sensory qualities and stability during ambient storage condition. Quality parameters of jam formulations were assessed using standard AOAC methods. Organoleptic attributes were assessed by 30 semi-trained panelists using a seven-point hedonic scale. The foremost preferred jam formulations were stored and analyzed in ambient temperature at 70-75% RH for 12 weeks period. Quality parameters during storage of watermelon jams revealed the increasing trend in total soluble solids, titratable acidity and moisture content. Moreover, the declining trend in pH, and vitamin C content. Based on the quality parameters, sensory analysis and microbiological studies, the watermelon jam with 4.2 g (14% dry basis) extracted pomelo pectin and 65 g (65ºBrix) sugar, could be stored in ambient temperature for 12 weeks period of time with none significant change in quality characteristics.

Keywords: Agro waste, Citrus, Organoleptic, Pectin, Peels, Quality parameters

INTRODUCTION

Fruits are widely used as a magnificent source of phytochemicals and micro nutrients. Most of the fruits are seasonal and perishable, this needs some processing to preserve the nature of the fruits to maintain and preserve quality attributes and provide various products such as candies, jellies, jam and juices throughout the year, particularly in the off-seasons. Jams were produced as an attempt to conserve the fruit consumption (Baker *et al*., 2005). Jam is an intermediate moisture fruit-based product developed from fresh fruits by cooking fruit pulp with sugar, gelling agent, citric acid and other additives. According to the fruit products order specification, jam is made by cooking the pulp with the required amount of sucrose to a thick consistency that holds the fruit tissues in place (55% of sugar should be used for each 45% of fruit pulp) and the finished jams must have a brix value of at least 68.5°Brix (SLS 265:2011). Jam has substantial nutritional value with the specified level of vitamin C content, phenolic compounds, antioxidant and other nutrients. The crucial constituents within the preparation of jam are pectin, sucrose and acids in the appropriate proportion for accepted gel formation.

Gelling agents are food additives that provides the food with texture by the development of a gel substance. Typical gelling agents include alginic acid, sodium alginate, locust bean gum, starches, pectin, gelatin and agar. Since, 200 years pectin has been used in the jelly manufacturing industry (Willats *et al*., 2006), but only citrus albedo and apple pomace, sources have been commercially acceptable, as raw materials (Iglesias and Lozano, 2004).

Citrus fruits, peel as an adequate source of pectin, peel consists of two parts the epicarp and mesocarp. The part that is called epicarp is the outermost coloured surface of the peel, while the part that is called mesocarp is the soft white middle layer of the peel (Davies and Albrigo, 1994). Pectin is being isolated through several methods. Usually, commercial pectin is extracted during a several physico-chemical processes characterized by an extraction and precipitation step respectively, using hot dilute mineral acid and recovery through an overnight alcohol precipitation (Mollea *et al*., 2008). Pectin is commercially available as white to brown powder, made from citrus peels, that is used as gelling agent in foods such as jams, jellies, marmalades and ketchups (Kale and Adsule, 1995).

Watermelon (*Citrullus lanatus*) is an important cucurbitaceous crop, contains 92% water, with small amounts of protein, fat, vitamins and minerals. The vital nutritional components of the fruits are carbohydrates, especially sucrose, fibre, vitamin A and lycopene content and excellent source the Vitamin C. Sugar boosts the network formation and enhances gelling in jams, jellies and preserves through attraction of water and yanking it away from the pectin. Sugar is a dehydrating agent helps to prevent the growth of mainly molds and bacteria. Sugar provides jam its long-term keeping qualities. Citric acid helps the pectin to set. The optimum low pH is between 2.8 and 3.3 for the formation of gel (Apsara and Puspalatha, 2002). Jams and jellies are sugar containing food preserves that are possible to acquire microbial spoilage immediately after preparation. The molds and bacteria are notable spoilage sources of fruit products and their presence in the finished products beyond the permissible level are considered unfit for consumption (Vidhya and Narain, 2011).

The existence of molds in food may reduce food quality and also contamination of food with mycotoxins, which may cause allergic reactions and respiratory problems (Calado *et al*., 2014). Consumer acceptability testing in the form of well-planned sensory analysis, is an important aspect of any finished product's shelf-life evaluation, as a result the present study objectives were to assess the quality parameters, microbial safety and consumer acceptability of jam stored at ambient condition.

METHODOLOGY

Isolation of Pectin from Pomelo Peels

The pomelo peels were collected from juice processing centers in Batticalo, and the mesocarp the soft white middle layer of the peels was carefully removed and sun dried for 4 days, till their moisture content was negligible. The dried mesocarps were ground and fine particles were sieved with a mesh size of 60 and stored in polyester metalized bags in air tight glass containers at room temperature for further use. The peel pectin extraction procedure was followed by the method given by Methacanon *et al*. (2014). Comparing to other acids in extraction procedure the best solvent for extracting pectin is Hydrochloric acid (Kalapathy & Proctor, 2001). Dried pomelo peel powder of 30 g was transferred into a 1000ml beaker containing 300 ml of 0.01N HCl. The mixture was then boiled in an 80°C water bath for 1 hr, to recover the extract, the hot acid extract was filtered through a filter funnel equipped with a two-layer of muslin cloth and squeezed, a ratio of 1:2 (1 part extractant and 2 parts ethanol) 95% ethanol was added and allowed to precipitate overnight at 4°C, Whatman No. 1 was used to filter the precipitated gelatinous pectin which was then washed with ethanol twice to remove impurities and then it was weighed after 3 hrs of drying in an air dry oven at 40°C. The dried pectin was crushed into a fine powder and stored at ambient temperature in air tight containers for further use.

Studies on Development of Watermelon Jam Using Pectin from Pomelo peels

A number of preliminary experiments were conducted to find out the appropriate amount of watermelon pulp, sugar, pectin, citric acid and to develop the recipe for the jam formulation at different combinations of sugar and pectin levels.

Development of Jam Formulations

The weight of watermelon pulp, citric acid and sugar was weighed by using an electric balance (METTER PJ-300). Initially 500 g of watermelon pulp, sugar (g/100 g) and pectin (g/100 g) were added in the ratio of 80:0, 75:1.8, 70:2.3, 65:2.8 and 60:3.3 respectively into the stainless-steel pan then stirred thoroughly and kept for 15 minutes with occasional heating until temperature reaches 105°C. Meanwhile, 2 g of citric acid was added and cooked by stirring continuously, until it reaches end point as, total soluble solid 68ºBrix (SLS 265:2011). Sheet or flake test was done to determine the end point, then strawberry essence was added at the rate of 5ml (5 ml/100 g) and mixed thoroughly. At last, the jam was removed from the fire and cooled for 5 minutes. To maintain the inner pressure (10-12 kPa) the plastic cups were sterilized by spraying water at 68-70°C to dry. Then freshly prepared jam was filled into plastic cups and sealed with lids.

Experimental Plan

The experiment consists of five treatments namely, T1- 80 g sugar and no pectin added; T2- 75 g sugar and 1.8 g pectin isolated from peels; T3- 70 g sugar and 2.3 g pectin isolated from peels; T4- 65 g sugar and 2.8 g pectin isolated from peels; T5- 60 g sugar and 3.3 g pectin isolated from peels.

The formulations such as T1, T3, T4, T5 with high sensory scores in organoleptic evaluation were chosen for storage studies and stored at room temperature for a period of 12 weeks. The nutritional quality parameters such as moisture, titratable acidity, pH, ascorbic acid and total soluble solids were assessed at 2 week intervals. After 12 weeks of storage, the findings of sensory qualities were determined.

Quality Parameters

Pectin quality characteristics such as colour, solubility in both cold and hot water and in alkali, pectin yield, ash content, moisture content and degree of esterification were analyzed as described by Ranganna (2005). Nutritional qualities of the

freshly prepared watermelon jam were analyzed using recommended standard methods (AOAC, 2002). The Digital pH meter was used to determine the pH (HI HANNA Model 97150). The titratable acidity of the jam samples was measured by titrating them with standard alkaline and expressing the results as a percentage of citric acid. Hand refractrometer (Model ATOGA-S-29E) was used to measure the Total Soluble Solids (TSS). Titrimetrically, the ascorbic acid level was determined using the 2, 6 dichlorophenol indophenol dye method and moisture content was determined by the gravimetric method. During analysis each parameter were triplicated.

Microbial Analysis examination was conducted using Potato Dextro Agar (PDA) method and the total plate count was observed as the procedure mentioned below.

The peeled potato was cut into small pieces and added in 250 ml of distilled water and boiled. Weighed agar was boiled with 250 ml of distilled water until agar dissolve and placed in a 1000 ml of a flask. Then the needed amount of potato extraction and sucrose were added into the flask and stirred thoroughly. Cotton wool was used to plug the media, wrapped by using aluminum foil and media was sterilized by using an autoclave at 121ºC, 15psi for 20 minutes then allowed to cool. Petri dishes, forceps and needles were sterilized using a hot air oven at 180ºC for one hour and later allowed to cool. All the glasswares were sterilized with 70% of alcohol, then the media was poured into petri dishes, until media gets solidify kept in laminar flow. Different jam samples from T1 to T5 were placed in separate agar plates. Then petri dishes were covered and labeled accordingly. The dilutions were produced up to 10^{-4}, with the aid of laminar airflow. Incubation was done for 48 hours at 37°C, with results expressed in CFU/g. The total plate count of formulations was evaluated during a period of 1,2 and 3 months.

Sensory Evaluation

Sensory Evaluation was assessed using 30 semi-trained panelists. The sensory attributes which include colour, taste, texture, aroma and overall acceptability were assessed. The test was conducted in two session's morning 9.00 - 11.00 am and evening session 2.00 – 4.00 pm. A structured questionnarie was used for the sensory evaluation. To examine the degree of liking (7) and disliking (1) for the preference of the jam formulation, seven-point of hedonic scale was used following the storage period.

Statistical analysis

The trail was conducted in a Complete Randomized Design with triplicates of each formulation. The physicochemical data's parameters were subjected to analysis of variance (ANOVA) at a significance level of 5%. Duncan's Multiple Range Test (DMRT) was used to separate the means for storage studies. The Kruskal-Walli's test was used to assess data relating to sensory evaluation. Statistical Analysis System software statistical package was used for both nutritional and organoleptic analysis.

RESULTS AND DISCUSSION

Quantitative and Qualitative analysis of Pectin

The extracted pomelo pectin characterized in terms of pectin colour, solubility in water, and alkali (NaOH), pectin yield, ash content, moisture content, and degree of esterification, as these properties determines the applicability of pectin for a variety of applications.

Pectin content extracted from pomelo peel using HCl was 14% (dry basis) that is comparable acceptable with other commercial sources such as sugar beet and apple pomace (Ismail *et al*., 2012). Higher yield was obtained by using HCl at 80ºC, 60 min, this value remained in close accord with the findings provided by (Devi *et al*., 2014), extracted pectin from pomelo peels by two distinct acids (HCl and nitric) and at three different temperatures (60, 70 and 80ºC), time (30, 45 and 60 min).

Moisture content of pomelo pectin was below 11%. These values were lower than the pectin extracted from lemon pomace (Ismail *et al*., 2012). Stability and functionality of pectin is determined by the moisture content, the rate of hydration of pectin in gelation is affected by the moisture content.

The ash content indicates the inorganic impurities in the pectin. It was found to be 6.6% of ash content of pectin extracted using HCl which is 16.4% for commercial pectin. Higher ash content interferes in gelation (Devi *et al*., 2014). Therefore, it is important to have lower ash content is more favorable for gel formation. Degree of esterification of pomelo pectin was 67.64%, as a result it's classified as high methoxy pectin.

Nutritional Analysis of watermelon pulp primarily to develop the standard recipes of watermelon jam. Table 1 shows the Nutritional Analysis of watermelon pulp (*Citrullus lanatus*).

Table 1: Chemical Composition of Watermelon Pulp

Chemical composition	Watermelon pulp
Titratable Acidity (as % citric acid)	0.22±0.12
Total soluble Solids (°Brix)	14.56±0.01
pH	6.52±0.01
Vitamin C content (mg/100ml)	6.81±0.01
Total sugar (%)	6.25±0.01

The values are means of triplicates ± standard error

Quality parameters of Jam Formulations during Storage

Titratable Acidity

Titratable acidity is a crucial quality indicator refers to any acid that can lose protons in an acid-base reaction. If the acid level is too low, the product may be bland and unappealing (Rahman and Moshiur, 2017). Acidity is the direct proportional indicator of a product's ability to maintain its quality against microorganism growth. Figure 1 shows the changes in titratable acidity of the watermelon jam during storage.

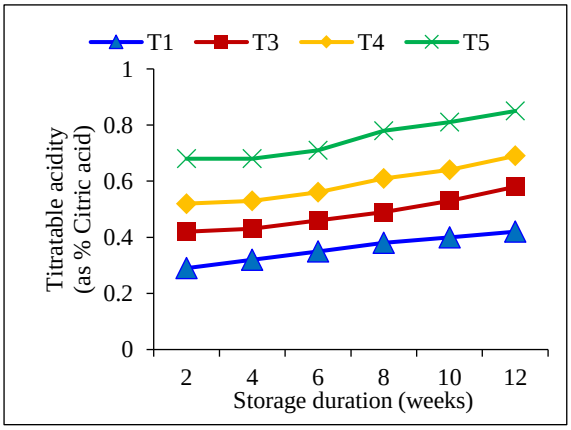

Figure 1: Changes in Titratable Acidity in Watermelon Jam during Storage

The values are means of triplicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

The overall result showed that, the acidity of watermelon jam significantly ($p<0.05$) showed an increment throughout the storage period at a highest value of 0.89% was observed in jam with 3.3g pectin after 12 weeks. The lowest value of 0.27% was showed in jam with no pectin added after two weeks. This might result from formation of carboxylic acids from alcohol which was produced from sugar and this might be due to the addition of acid into the watermelon jam.

pH

The result indicates that the pH of watermelon jam decreased with increasing duration of storage in all the formulations. There was a significant ($p<0.05$) reduction in pH during the storage period at ambient temperature (30°C). It was observed that the maximum pH (3.94) in the jam with no pectin added formulation in the 2ndweek and the least pH (3.31) was observed in T5 (3.3g pectin) at the end of storage. The pH of preserved products plays a role as a preservative (Ahmed *et al*., 2016). The result pertaining to the response of storage duration and different treatments of watermelon jam on pH are present in Figure 2.

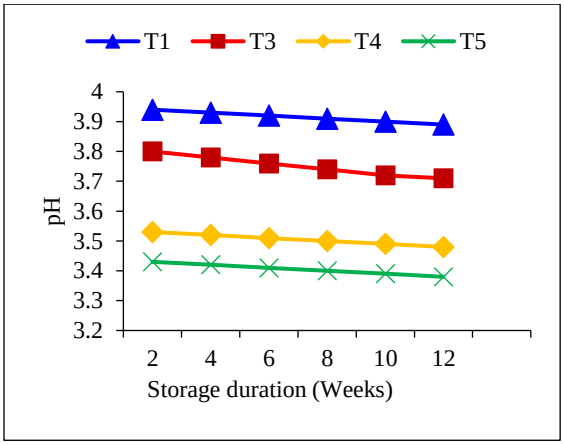

Figure 2: Changes in pH of Watermelon Jam during Storage

The values are means of replicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

It's possible the reduction in pH might be due to the formation of acids from added sugar during the storage. The findings are consistent with those of others who evaluated the pH which indicates declining tendencies over time intervals. Similar reduction in pH has been reported by Hussain *et al*. (2010), in watermelon lemon jam during storage

Ascorbic acid Content

The ascorbic acid content of the watermelon jam showed a reduction significantly ($p<0.05$) with the storage period (Figure: 3). According to DMRT, the vitamin C content had a significant ($p<0.05$) reducing trend to the storage in all formulations. Ascorbic acid is sensitive to oxygen, heat and light, it is easily oxidized, in the presence of oxygen by both enzymatic and non-enzymatic catalyst from ascorbic acid to dehydroascorbic acid.

The findings are consistent with those of Eskin (1979) and Land (1962) who found that decrease in ascorbic acid content of sample could be attributed to both non oxidative and oxidative changes during storage. The highest mean value 5.29mg/100ml for ascorbic acid was observed in T5 (Jam with 3.3g pectin) in the 2nd week and the least mean value 4.21mg/100ml was observed in T3 (Jam with 2.3g of pectin) in 12th weeks.

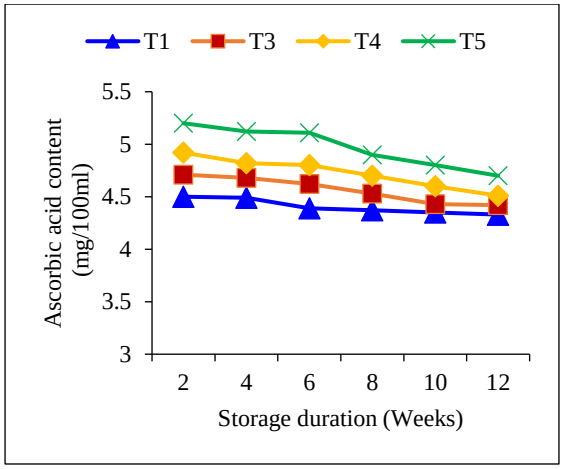

Figure 3: Changes in Ascorbic acid Content of Watermelon Jam during Storage

The values are means of replicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

Moisture Content

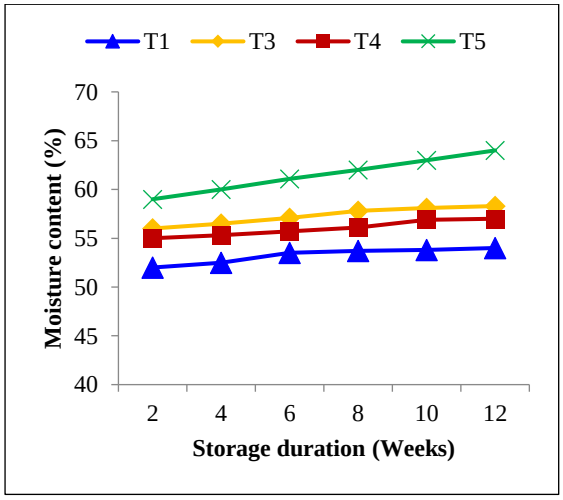

Figure 4: Changes in Moisture Content of Watermelon Jam during Storage

The values are means of replicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

The moisture content of less than 10% is responsible for the state of non-deterioration in dehydrated food (Ranganna, 2005). In all jam formulations, moisture content increased significantly ($p<0.05$) throughout the storage period extending a maximum value of 66.4% was observed in jam with 60g sugar at the end of 12 weeks storage period shown in Figure 4. The minimum moisture content observed in T5 (Jam with 80g sugar) value is 53.9%.

The moisture content gradually showed an increment during storage may be due to the hydrolysis of sugar into alcohol, carbon dioxide and water. Our findings are closely in accordance with the results attributed by Yousif and Alghamdi (1999) in date jelly.

Total Soluble Solids (TSS)

The results observed during the storage period of watermelon jam formulations are shown in Figure 5. According to DMRT, there was a significantly ($p<0.05$) increment in Total Soluble Solids among treatments. This could be due to the degradation of polysaccharides into simple sugars during storage, as well as the conversion of the insoluble fraction into a soluble fraction.

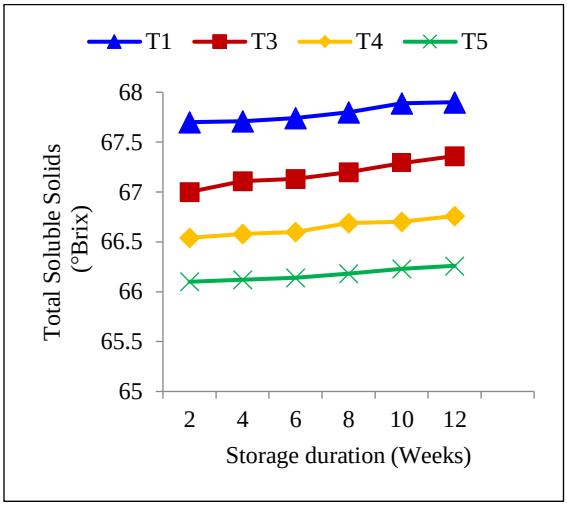

Figure 5: Changes in Total Soluble Solids (°Brix) of Watermelon Jam during Storage

The values are means of replicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

These findings were in agreement reported by Khan *et al*. (2012) with the increase in TSS of pear apple jam during storage, similar findings were in agreement reported by Shakir *et al*. (2007) with the increase in TSS of watermelon and lemon jam during storage. The formulation which had 80g of sugar showed a highest value (68.17°Brix) for TSS in the 12th week of storage period. The lowest mean value of (66.10°Brix) was observed in 2nd week of the storage period which had lowest of sugar content in the formulation.

Microbiological Test for Jam formulations

The total plate count for all the samples ranges from 8.45×10^4 - 32×10^4 cfu/g reflecting the significant ($p<0.05$) differences among samples The total plate counts do not exceed the standard ($\times10^6$ cfu/g)

(ICMSF, 2002). The reduction in the microbial levels may be due to the intense heat application involved in jam production as well as addition of sugar and citric acid while processing, sugar and acid reduces the pH and makes the environment unfavorable for microorganism growth.

Sensory Analysis of Watermelon Jam Following at the End of Storage

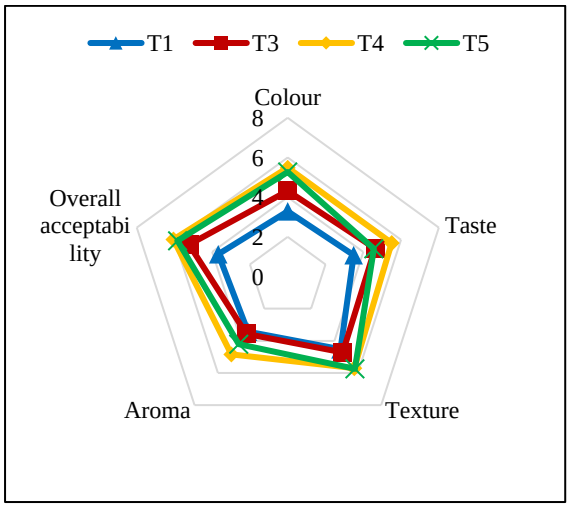

Figure: 7 Sensory Analysis of Watermelon Jam during Storage

The values are means of triplicates ± standard error

(T1- 80g sugar and no pectin added; T3- 70g sugar and 2.3g pectin extracted from lemon peels; T4- 65g sugar and 2.8g pectin extracted from lemon peels; T5- 60g pectin and 3.3g pectin extracted from lemon peels).

In this investigation, organoleptic scores reduced while increasing the storage period at room temperature 30±1°C. Sensory scores are given in Figure 7. The sensory scores with respect to colour of all jam formulations revealed a slight decrement after 12 weeks of storage (Figure 7). According to the mean value of sensory scores the highest mean value for colour was observed in formulation T4 (2.8 g pectin and 65 g sugar). These jam formulations had natural colour pigments. In storage it could be attributed to enzymatic or non-enzymatic browning (Maillard reactions). Figure 7 shows a general decrease in taste score of watermelon jam formulations. The watermelon formulations containing (2.8g pectin and 65g sugar) showed the highest mean value 4.72 for taste and formulation with (80 g sugar and no pectin added) had the least mean value score 3.49 for taste score, oxidation of ascorbic acid into dehydroascorbic acid and tannins to gallic acid, increase the acidity of the jam creates sourness.

Figure 7 shows a general decrease in texture score of watermelon jam formulations during storage. The watermelon formulations containing (3.3 g pectin and 60 g sugar) had the highest mean value score of 5.01 and formulation containing (80 g sugar and no pectin added) had the least mean value score of 4.52 for texure, the possible reason for the loss of texture score it is possible that this is due to pectic acids, which are then converted to sugars galacturonic acids. According to the results obtained for aroma shown in Figure 7, the mean scores for aroma revealed that there was significant decrement in the aroma of watermelon jam after the storage period of 12 weeks.

The reduction in aroma during storage could be due to the initial processing of jam cause losses of volatile aromatic compounds. Figure 7 shows a general decreasing trend in overall acceptability. The mean scores for overall acceptability revealed that there were significant reductions in overall acceptability of watermelon jam after a storage period of 12 weeks. A decrease in overall acceptability might be due to loss of appearance, flavor uniformity in food products during storage.

CONCLUSIONS

The current findings shown that pomelo peel is an adequate source of pectin, the natural biodegradable polymer. Pomelo pectin contains a large quantity of pectin, making it a viable commercial source for jam manufacturing. Jams with low gel strength can be improved by adding isolated pomelo peel pectin during processing to get the desired gel strength (Mollea et al., 2008). The watermelon jam prepared with 65 g (65 °Brix) sugar and 4.2 g (14% dry basis) pectin is the best formulation novel biomaterial for commercial preparation, and could be stored at ambient condition 30±1 °C and 70-75% RH for 12 weeks without any significant changes in the quality, sensory and microbial characteristics.

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ICMF, International Commission on Microbiological specification for foods

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