

Chemical and Technological Studies on Pink Grapefruit (*Citrus paradise* L.) Peels. 1-Effect of Storage Conditions on Gross Chemical Composition, Phytochemical Components and Oil Stability of Pink Grapefruit Peels

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Abstract: The storage temperature is one of the most important factors affecting on the fruit peels composition and quality after harvesting. The pink grapefruit (*Citrus paradise* L.) peels was subjected in current study to examine the effect of different storage conditions on phytochemical constituents, the antioxidant activity and constants of peels oil. The phytochemical constituents of the pink grapefruit peels such as lycopene, phenolics, flavonoids and vitamin C were assessed the level of their concentrations for examine their antioxidant activities and the effect of storage on those components. The antioxidant effects was determined from the changes in acid value, peroxide number, conjugated diene and triene contents and the delay of rancidity of pink grapefruit peels oil during storage conditions for 60 days in refrigerator temperature at $8 \pm 1^\circ\text{C}$ and for 40 days in room temperature at $16 \pm 4^\circ\text{C}$. Results of a proximate composition of pink grapefruit peels during storage conditions showed that significantly decreased ($P \leq 0.05$) in moisture, carbohydrate and energy values at most storage periods. On the other hand, protein, fat, fiber and ash contents were significantly increased ($P \leq 0.05$) in most different storage periods. The Antioxidants components such as: Lycopene, total phenolics and total flavonoids content; as well as, antioxidant activity of pink grapefruit peels were significantly increased ($P \leq 0.05$) during storage conditions, while, ascorbic acid levels were significantly ($P \leq 0.05$) decreased during storage periods at different storage conditions. The rates of increase in antioxidant activity of samples stored at room temperature were higher than in the refrigerator. The contents of peroxide number, conjugated diene and triene was significantly ($p \leq 0.05$) increased for most storage periods at room temperature ($16 \pm 4^\circ\text{C}$), while, non-significant differences ($p \leq 0.05$) were found during storage periods at refrigerator temperature ($8 \pm 1^\circ\text{C}$). On the other hand, acid value was significantly decreased ($p \leq 0.05$) after 20 days of storage at room temperature, while, non-significant differences ($p \leq 0.05$) were found during storage periods at refrigerator temperature. The storage at refrigerator temperature ($8 \pm 1^\circ\text{C}$) improved the shelf-life of pink grapefruit peels and to maintain health promoting compounds.

Key words: Grapefruit peels • Bioactive compounds • Antioxidant activity • Storage conditions • Peels oil properties

INTRODUCTION

Vegetables and some fruits such as citrus, cherries, grapes, pineapples, that have a respiration rate which at a given temperature, remains fairly constant, or even fall slightly, during the post-harvest period [1]. As the fruit matures on the tree, the pulp becomes juicy and sweet (or acid in lemons and limes) and remain so for a long time. The most important characteristic of freshly harvested

fruits and vegetables is that, they are still alive and respiring [2]. Transpiration is one of the major processes that affect commercial and physiological deterioration of fruits and vegetables, it induces wilting, shriveling and loss of firmness, crispness and succulence all components of freshness. The desiccation resulting from moisture loss reduces the commercial value of the product adversely affecting its appearance, texture, flavour and weight [3].

Citrus fruits are highly consumed worldwide as fresh produce, juice and most often the peel is discarded as waste which contains a wide variety of secondary components with substantial antioxidant activity in comparison with other parts of the fruit [4]. Therefore, a large amount of peel is produced every year. Citrus peel, the primary waste, is a good source of molasses, pectin and limonene and is usually dried, mixed with dried pulps and sold as cattle feed [5]. Citrus peels are subdivided into the epicarp or flavedo (coloured peripheral surface) and mesocarp or albedo (white soft middle layer). The grapefruits as agents able to lower blood pressure interfere with calcium channel blockers and the grapefruit juice having anti-genotoxic effects [6, 7].

Citrus (*Citrus* L. from *Rutaceae*) is one of the most popular world fruit crops, contains active phytochemicals that can protect health. In addition to this, it provides an ample supply of vitamin C, folic acid, potassium and pectin. The contribution of citrus species in deterrence of life threatening diseases have been assessed [8, 9] and it has been reported that citrus fruits, citrus fruit extracts and citrus flavonoids exhibit a wide range of promising biological properties due to their phenolic profile and antioxidant properties [10].

Lycopene is a phytonutrient and an antioxidant which is responsible for the characteristic deep red colour of fruits and their food products. Lycopene act as an antioxidant reduces oxidative stress. It may play a significant role in many health concerns, including cardiovascular disease, diabetes, cancer, osteoporosis, liver disease, cataracts and male infertility. Lycopene a carotenoid without pro-vitamin-A is present in many fruits and vegetables. Fruits and vegetables that are high in lycopene include autumn olive, gac, tomatoes, watermelon, pink grapefruit, pink guava, papaya, sea buckthorn, wolfberry and rosehip [11].

Major bioactive compounds known for health benefits are phytochemicals, especially phenolics in fruits and vegetables. Studies have reported that plant phenolics are not only present in edible parts of plant but their presence with multiple biological effects has also been reported in non-edible parts of the plants. Many researchers found out that the citrus plants contain a wide range of flavonoids constituents which is the secondary metabolites compound from plants. Although flavonoids glycosides can had a wide range of biological activities, the protective role of flavonoids in living systems was mostly due to their antioxidant potential, which is related to transfer of reactive oxygen species (ROS), chelation of

metal catalysts, activation of antioxidants enzymes and inhibition of certain type of oxidases and colon cancer [12, 13].

Citrus byproducts are promising sources of bioactive ingredients and of valuable technological and nutritional properties. These byproducts can be used as ingredients and food additives [14, 15] in food industry for their cheap valuable component [16]. Peels are generated as the primary citrus byproducts represent about 50-65% of fruit weight during processing. These byproducts discarded and considered as a huge load to the environment [17, 18, 19]. In Egypt and many Mediterranean countries, a major quantity of the citrus peels does not process. Some efforts were made to use these residues as livestock feed [20, 21].

Among antioxidant vitamins, vitamin C plays several roles in human health [22]. More than 90% of the vitamin C in human diets is supplied by fruits and vegetables, being citrus fruits the most important sources of vitamin C because of the large quantities consumed [23, 24]. Also, citrus peels are good source of phenolic compounds can be extracted and employed as natural antioxidants to prevent oxidation of some foods or may be utilized in designing functional foods [25, 26]. Citrus peels described as rich source of unique phenolic compounds to citrus, especially the characteristic flavanone glycosides (mainly naringin, hesperidin, narirutin and neo hesperidin). Huge amounts of flavanones and many polymethoxylated flavones which are very rare in other plants are contained in citrus peels [27, 28].

Citrus peel oils have been used widely in beverages, cosmetics, pharmaceuticals and perfumery industry, whereas seed oils are used in cooking and for treatment of leather and textile. The quality, freshness and uniqueness of citrus oils are major considerations pertaining to their value and applications. However, large amounts of volatile components, as well as unsaturated compounds, render the oils unstable and prone to change with time and storage conditions. Most of the qualitative changes of citrus peel oil during storage occur due to evaporation, oxidation, polymerization, rearrangement and cyclization of some labile constituents in the presence of heat, light, oxygen, moisture and catalysts resulting in the loss of volatile components and formation of off-flavor artifacts [29, 30].

In recent years, white and pink grapefruits (*Citrus paradise* Macf.) have attracted much attention because of their nutritional and antioxidant properties. High levels of bioactive flavanones glycosides, namely,

naringin and narirutin, have been reported in seed and peel residues released after grapefruit juice extraction, although further research is required to explore the composition of this fruit variety and its byproducts in more detail [31, 32, 33].

The aim of this study was to investigate the effect of different storage conditions; refrigerator temperature ($8 \pm 1^\circ\text{C}$) for 60 days, room temperature ($16 \pm 4^\circ\text{C}$) for 40 days on the phytochemical constituents, the antioxidant activity of the pink grapefruit peels and the chemical constants of oil extracted from peels during these conditions.

MATERIALS AND METHODS

Materials

Samples: Pink grapefruit (*Citrus paradise* L.) foster variety was brought from botanical farm - Faculty of Agriculture - Assiut University in January 2017. The fruits studied were botanically classified using the synthetic proposal of Mabberley [34]. Morphological characterization of the sample (8 fruits analyzed) was performed (Table 1) for botanical description weight, shape and other distinctive general characters, such as peel (flavedo and albedo) thickness and the smoothness of the surface, number of segments of the endocarp and seeds presence were described according to horticultural criteria defined by Hodgson [35].

Storage Samples: The fruits were gathered either directly from trees and shrubs and stored. For examination only healthy looking fruits were chosen (without mechanical damages and bacterial infection) and mature (Fig. 1).

The fruits were stored for 60 days at refrigerator temperature ($8 \pm 1^\circ\text{C}$) and for 40 days at room temperature ($16 \pm 4^\circ\text{C}$) (shelf life) (Fig. 2). Every 20 days (refrigerator); 10 days (shelf life) the fruit peels (outer yellow layer and inner white layer) were taken, cut into a cube size approximately $4 \times 4 \times 4 \text{ mm}^3$ and dried at 50°C in hot-air oven. The dried samples were milled then the whole yellow flour was analyzed.

Experimental Methods

Proximate Composition: Moisture, crude protein, crude oil, crude fiber and ash were determined as described in the AOAC methods [36]. Triplicate determinations were

carried out for each sample and the means were reported. The total carbohydrates content was determined by difference according to FAO [37] as follows: Carbohydrate % $\leq 100 - (\text{protein \%} + \text{ash \%} + \text{lipid \%} + \text{crude fiber \%})$. The caloric value was calculated using value of 4 k.cal/g for protein, carbohydrates and 9 k.cal/g for fat according to Livesy [38].

Lycopene and Ascorbic Acid: Lycopene content of samples extract was determined using a colorimetric method by Rao *et al.* [39]. Lycopene of samples was extracted with hexane, methanol and acetone (2:1:1) for 1h. Absorbance of the extract at 502 nm was measured using spectrophotometer against the blank extract solvent. Ascorbic acid was determined according to the method described by Sahlin *et al.* [40]. The results were expressed as mg/100 g dry weight (DW).

Total Phenolic, Flavonoid Contents and Antioxidant Activities Assay: The total phenolic compounds of samples was determined using modified Folin-Ciocalteu colorimetric method [41], while the aluminium chloride colorimetric assay was used for flavonoids determination, as described by Marinova *et al.* [42]. The hydrogen peroxide scavenging ability of samples was used to determine antioxidant activity assay according to the method of Ruch *et al.* [43].

Chemical Constants of Pink Grapefruit Peels Oil: The oil of pink grapefruit peels was extracted by n-hexane during storage conditions (at room temperature and refrigerator), the oil was used to determine the chemical constants; acid value, peroxide number, conjugated diene and triene. Free fatty acid (acid value) content was determined according to the method outlined in the IUPAC [44]. Peroxide value was determined according to the method described in A.O.C.S. [45]. Contents of conjugated dienes (CD) and conjugated trienes (CT) were calculated according to the method described by Fathi *et al.* [46] which is based on the measurement of solution absorbance (5 mg of oil sample dissolved in 10 mL cyclohexane) at 234 and 270 nm for CD and CT, respectively.

Statistical Analysis: The experimental data were subjected to an analysis of variance (ANOVA) for a completely randomized design using a statistical analysis system [47].

Table 1: Morphological characterization of pink grapefruit purchased in botanical farm

Sample	Weight (g)	Shape	Skin	Flavedo (mm)	Albedo (mm)	Segments number	Seed number
Pink grapefruit	325.56	Subglobose	Smooth dotted	2.00	4.00	12	48



Fig. 1: Pink grapefruit (Foster variety)



Fig. 2: Storage of pink grapefruit at room temperature and refrigerator

RESULTS AND DISCUSSION

Approximate Composition of Pink Grapefruit Peels

During Storage: The moisture contents of pink grapefruit peels stored at room temperature, refrigerator was shown in Fig. (3) and Fig. (4). It was clear that the moisture content of grapefruit peels decreased significantly ($P \leq 0.05$) during the storage periods at room temperature or refrigerator with the continuation of the storage period as it was 82.42% at the beginning of storage and decreased to 57.83% after 40 days of storage at room temperature ($16 \pm 4^\circ\text{C}$) while it decreased to 55.95% after 60 days of storage in the refrigerator ($8 \pm 1^\circ\text{C}$).

From the results in Fig. 3 and Fig. 4, it can be observed that the rate of moisture loss after 40 days was 29.83% at room temperature and 21.63% at refrigerator temperature after 40 days. There is a gradual decrease in the moisture content in the grapefruit peels as a result of the transpiration process occurs in the fruits, which leads to water loss, noting that the rate of transpiration at room

temperature is higher than that occur in the refrigerator due to temperature difference between the room and refrigerator. In postharvest study by Shimshon *et al.* [48] showed that water loss of citrus fruits during storage is a factor in the postharvest weight loss. It causes accelerated softening and loss of attractive appearance of fruits. The resultant water stress enhances senescence. Loss of weight involves mainly the peel, not the pulp of the fruits. As the peel is a major marketing feature, peel appearance is as important economically, if not more than the flavour of the pulp.

Approximate composition of pink grapefruit peels during storage conditions for 60 days in refrigerator ($8 \pm 1^\circ\text{C}$) and for 40 days at room temperature ($16 \pm 4^\circ\text{C}$) are presented in Table 2. From these data it could be noted that, protein, fat, fiber and ash contents were significantly increased ($P \leq 0.05$) in most different storage periods. The highest rates of protein, fat, fiber and ash contents were found (49.61%, 85.22%, 62.23% and 38.90% respectively) in samples stored in the refrigerator at end

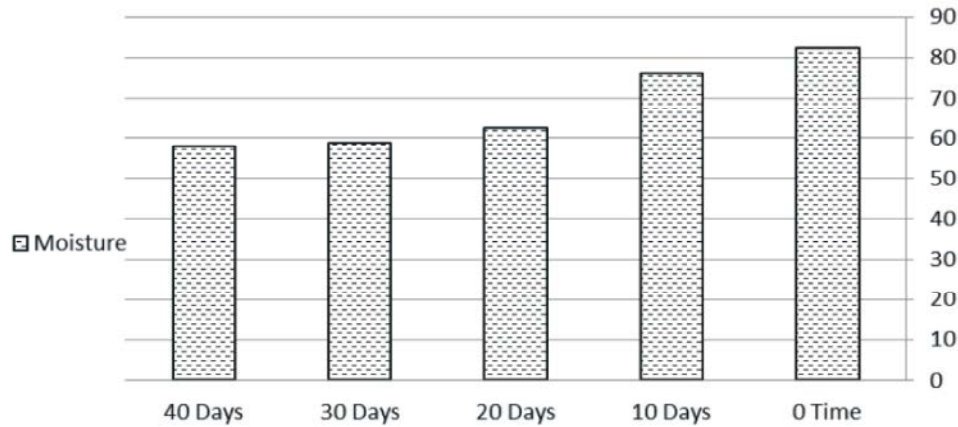


Fig. 3: Moisture content of pink grapefruit peels stored at room temperature (shelf life)

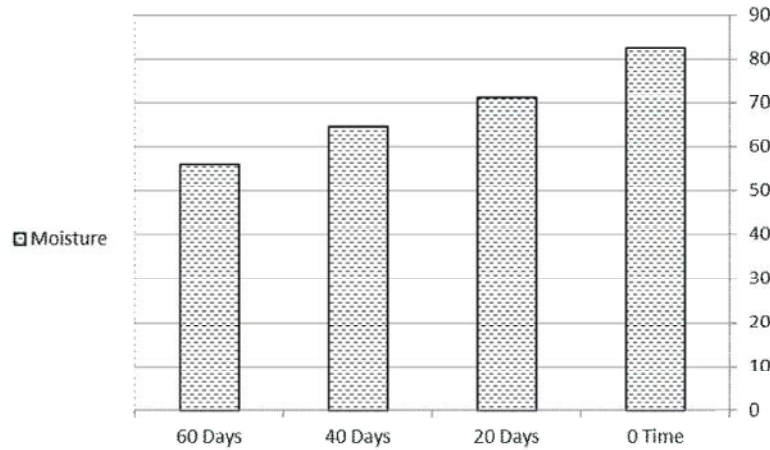


Fig. 4: Moisture content of pink grapefruit peels stored at (8 ± 1°C) (refrigerator)

Table 2: Proximate composition (g/100 g dry peel) of pink grapefruit peels during storage conditions (at room temperature and refrigerator)

Storage period (days)		Crude Protein	Crude fat	Crude Ash	Crude fiber	Total carbohydrates	The caloric value (Kcal/100g)
Shelf life	0	6.43	3.79	2.78	8.22	79.08	376.15
	10	6.50	4.14	3.29	9.61	76.46	369.10
	20	7.01	5.48	3.76	10.72	73.03	369.48
	30	7.29	6.23	3.90	11.21	71.37	370.71
	40	8.41	6.46	4.02	11.38	69.73	370.70
Refrigerator	0	6.43	3.79	2.78	8.22	79.08	376.15
	20	6.85	6.47	3.36	9.65	73.67	380.31
	40	8.31	6.75	3.69	10.44	70.81	377.23
	60	9.62	7.02	4.51	11.42	67.43	371.38
L.S.D 0.05	0.28	0.21	0.13	0.38	4.67	5.18	

storage time (Table 2), these increments may be due to the decreasing of moisture content during storage time in all samples. On the other side, non-significantly decreased ($P \leq 0.05$) in total carbohydrate in most storage periods, except for end of storage period was significantly decreased ($P \leq 0.05$) when compared with zero time (Table 2). The highest rate of low carbohydrate was found (14.73%) in samples stored in the refrigerator at end storage time. It is known that the peel of fruits is a living

system containing active enzymes that continue to work even during storage causing the respiration process which occurs in the peels [3]. The process of respiration due to analysis of carbohydrates by enzymes, producing carbon dioxide and heat energy leading to evaporation of water from the peels. So the moisture content, total carbohydrates was decreased during storage on room temperature or refrigerator and the oil, protein, ash, crude fiber was increased as a result of that decrement.

Table 3: Bioactive components and antioxidant activity of pink grapefruit peels during storage conditions (at room temperature and refrigerator)

Storage period (days)	Vitamin C (mg vit C/100g D.W.)	Lycopene (mg lycopene /100g D.W.)	Total phenolics (mg GAE/ 100 g D.W.)	Total flavonoids (mg catechin /100g D.W.)	Antioxidant activity (scavenging H ₂ O ₂ molecules /100g D.W.)
Shelf life					
0	52.26	42.93	1078.16	173.85	51.91
10	44.22	45.18	1152.96	192.58	52.10
20	32.16	46.09	1183.76	205.03	52.30
30	21.11	47.83	1210.51	218.75	52.43
40	18.09	49.85	1274.65	227.35	52.59
Refrigerator					
0	52.26	42.93	1078.16	173.85	51.91
20	35.18	49.12	1092.85	183.50	52.17
40	32.16	50.64	1154.29	196.73	52.38
60	24.12	51.18	1186.37	210.45	52.56
L.S.D 0.05	2.61	1.81	7.56	0.27	

Table 4: Acid value, peroxide number, conjugated diene and triene contents of pink grapefruit peels oil during storage conditions (at room temperature and refrigerator)

Storage period (days)	Acid value (mg of KOH/g of oil)	Peroxide value (meq O ₂ /kg oil)	Conjugated diene	Conjugated triene
Shelf life				
0	3.17	2.88	0.22	0.07
10	2.63	3.43	0.33	0.18
20	2.01	4.93	0.44	0.23
30	1.38	7.59	0.52	0.29
40	1.27	9.89	0.60	0.33
Refrigerator				
0	3.17	2.88	0.22	0.07
20	2.86	3.15	0.27	0.11
40	2.42	4.24	0.32	0.15
60	2.30	5.12	0.38	0.18
L.S.D 0.05	0.61	1.72	0.07	0.05

Table (2) also contains energy values; it is clear that from these values there is a decrease in their values with increased storage period at room temperature. Also, there is a relationship between the contents of oil, protein, carbohydrates and energy values, as a result of the difference in these contents whether it decrease or increase, this effects on the caloric values calculated from those contents. The caloric value was ranged from 376.15 Kcal/100g to 371.38 Kcal/100g; from 370.70 Kcal/100g to 376.15 Kcal/100g for grapefruit peels samples stored at refrigerator and room temperature, respectively.

Bioactive Components and Antioxidant Activity of Pink Grapefruit Peels: Data presented in table 3 showed the bioactive components such as: ascorbic acid, lycopene, total phenolic compounds and total flavonoids content; as well as, antioxidant activity of pink grapefruit peels during storage conditions for 60 days in refrigerator temperature at $8 \pm 1^\circ\text{C}$ and for 40 days room temperature at $16 \pm 4^\circ\text{C}$.

Ascorbic acid levels were significantly ($P \leq 0.05$) decreased in pink grapefruit peels during storage period. The reduction rates of ascorbic acid at the end of the storage period of the storage at room temperature (65.38%) were higher than in the refrigerator (53.84%). On the other side, lycopene, total phenolic compounds and total flavonoids content were increased in pink grapefruit peels during storage period. The increment rates of lycopene, total phenolics, total flavonoids

content at the end of the storage period were recorded 16.12%; 19.21%, 18.22%; 10.04%, 30.77%; 21.05% for shelf life and refrigerator, respectively.

In general antioxidant activity gives an indication of the efficiency of the antioxidant compounds found in the treatments and their ability to act as antioxidants. Data in Table (2) revealed that antioxidant activity of pink grapefruit peels slightly increased and did not change significantly during the entire experimental period at deferent temperatures during storage period. The rates of increase in antioxidant activity at the end of the storage at room temperature (1.31%) were higher in peels which stored in refrigerator (1.25%). Although the rates of phenolic compounds, flavonoids and lycopene increased during periods of storage, the rate of increased, antioxidant activity of grapefruit peels was affected during this period, may be due to the rate of decreased in ascorbic acid content (Table 3). These results agree with the observation that fruits and vegetables show a gradual decrease in ascorbic acid content as the temperature or time at storage increases. The observed effect of temperature conditioning and/or storage time on ascorbic acid degradation could be explained due to direct oxidative destruction of ascorbinase activity, or by indirect degradation through polyphenol oxidase, cytochrome oxidase and peroxidase activity. Ascorbic acid is very susceptible to chemical and enzymatic oxidation during storage of fruits and vegetables. The decreasing in vitamin C content due mainly to oxidation of

ascorbic acid to dehydroascorbic acid, but ascorbic acid was probably protected by the ascorbate-sparing effect of the polyphenols that may be attributed to their higher redox potential as compared to ascorbic acid [49, 50, 51].

Acid Value, Peroxide Number, Conjugated Diene and Triene Contents of Pink Grapefruit Peels Oil: Acid value, peroxide number, conjugated diene and triene contents of pink grapefruit peels oil during storage conditions for 60 days in refrigerator temperature at $8 \pm 1^\circ\text{C}$ and for 40 days in room temperature at $16 \pm 4^\circ\text{C}$ are presented in Table (4).

It is important to noted that acid value was significantly decreased ($p \leq 0.05$) after 20 days of storage at room temperature ($16 \pm 4^\circ\text{C}$), while, non-significant differences ($p \leq 0.05$) were found during storage periods at refrigerator temperature ($8 \pm 1^\circ\text{C}$) Table (4).

On the other hand, peroxide number, conjugated diene and triene contents were increased linearly during storage periods at different storage conditions. Significantly increased ($p \leq 0.05$) in peroxide number, conjugated diene and triene contents of most storage periods at room temperature ($16 \pm 4^\circ\text{C}$), while, non-significant differences ($p \leq 0.05$) were found during storage periods at refrigerator temperature ($8 \pm 1^\circ\text{C}$). The increasing in peroxide number, conjugated diene and triene contents may be due to the oxidation of some fatty acids involved in the composition of oil and helped to increase the temperature during storage at room temperature. These results are in good agreement with those obtained by Hyang and Masayoshi [29].

CONCLUSION

A proximate composition, the phytochemical components as well as, antioxidant activity and stability of oil extracts of pink grapefruit peels, clearly affected by storage periods and different storage conditions. The storage at refrigerator temperature ($8 \pm 1^\circ\text{C}$) improved the shelf-life of pink grapefruit peels and to maintain health promoting compounds.

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