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Application of guar gum-based edible coatings supplemented with spice extracts to extend post-harvest shelf life of lemon (*Citrus limon*)

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RESEARCH ARTICLE

Abstract

Guar gum based edible coatings supplemented with condiments' extracts (methanolic and ethanolic extract of fennel, bay leaf, coriander and nigella seeds) were assayed for lemon for the maintenance of parameters that determine the quality during postharvest life. Rate of change in pH, titratable acidity and total soluble solids were studied during the shelf life of lemons. Results obtained from all the assays were communicated as mean ± standard deviation. One-way analysis of variance and t-test was employed on the studied factors at $P \leq 0.05$ to study the effect of guar gum containing extracts. The shelf life of ethanolic and methanolic extract of fennel coated lemons was significantly increased up to 24 weeks at 10 °C (85% relative humidity) while all other treatments had 15 weeks of storage stability. Treated lemons showed less changes in physicochemical attributes than both controls (untreated control; UN and treated control; GG). In addition to biochemical parameters, the storage parameters of lemon, such as antioxidant content, ascorbic acid, total phenolic content, carotenoid and chlorophyll pigments and total flavonoid content were significantly delayed in treated lemons as compared to controls. Bacterial load was also reduced in treated lemons. Coatings based on guar gum fortified with extracts could be an effective tool to delay ripening process and maintain quality of lemons during cold storage. As the constituents of these coatings are derived from food grade products, these coatings are therefore safe for human consumption and eco-friendly. These coatings not only protect the lemon from the contaminating microbes but also have bactericidal effects on the bacteria that were already present inside the lemon. Moreover, the active component of these coatings, i.e. extracts are derived from culinary spices that are easily available throughout the year.

Keywords: shelf life, bioactive compounds, green unripe lemons, micro-nutritional component analysis

1. Introduction

Lemon fruits (*Citrus limon*) are known to possess the highest contents of health-promoting properties, such as anti-inflammatory, antioxidant, prevents kidney stones, etc., among all other fruits and vegetables (Benavente-García *et al.*, 1997). Both the pulp and the peel of lemons are rich in ascorbic acid, polyphenols (e.g. narirutin, diosmin, phlorin) and carotenoids (e.g. Zeta carotene, eta-carotene and alpha-carotene) which contribute to the major antioxidant potential of these fruits (Marlett, 1992; Marlett and Vollendorf, 1994; Peleg *et al.*, 1991). The ascorbic acid content of lemons has been reported as 680 mg/l of juice (Artés-Hernández *et al.*, 2007) which depends on area of cultivation, maturity stage of harvest and postharvest duration. Lemon fruits are non-climacteric commodities with relatively lower rate of respiration and ethylene generation. They do not suffer major postharvest losses, such as loss of firmness or physical deformities and therefore, can have a storage life of 6-8 weeks at low temperatures (Kader, 2002). They are considered important agricultural commodities in many parts of the world and are occasionally grown in quantities higher than actual demand of the consumer. Although the fruit has a good storage life but due to improper postharvest storage conditions and microbial contamination, a substantial amount of produce gets wasted annually. Therefore, extension of its storage life may be helpful for its consumption during the off-season when the prices of these fruits are very high (Bisen *et al.*, 2012). The microbial contaminants of lemons include *Penicillium digitatum, Penicillium italicum*, *Geotrichum candidum*, *Phythophthora citrophthora* and *Phythophthora parasitica* (Taverner *et al.*, 2001). The post-harvest losses can be reduced by enhancing the storage life through reduction in rate of respiration and transpiration, bacterial and fungal contamination and preventing physical deformities at the surface of fruits (Bisen and Pandey, 2016). During the shelf life, the losses can be prevented to an extent by making use of growth regulators, wax emulsion, cold storage conditions, fungicides, silver nitrate, calcium treatment, oil coating, chemical application, irradiation, polyethylene packaging and edible coatings (Chahal and Bal, 2003). Although modern technologies are being used, the post-harvest losses are still high. Among the different treatments used to enhance the shelf life of fruits, the application of edible coatings is the most cost effective one (oil, wax, polysaccharide) (Bisen *et al.*, 2012; Chiabrando and Giacalone, 2014; Embuscado and Huber, 2009; Lin and Zhao, 2007). The present study deals with the incorporation of extracts in edible coatings to enhance the shelf life of lemons at low temperature. The spice extracts have been reported earlier to possess polyphenols, flavonoids, flavonols, antioxidants and were capable to yield antibacterial effects both *in vitro* and *in vivo* too (Pasha *et al.*, 2018). The resin of these edible coatings was Guar gum. Guar gum belongs to the class of galactomannans and are present in the endosperm of leguminous plants and function as the storehouse of polysaccharides (Gidley and Reid, 2006). The galactomannans have a wide range of applications; they can be employed as gelling agent and stabilisers of emulsions. They are also used in textile, biomedical, pharmaceutical, food products and in cosmetics (Srivastava and Kapoor, 2005; Vieira *et al.*, 2007). Guar gum is known to possess antibacterial properties which can be highly boosted by the addition of antimicrobial extracts. The proposed edible coating used glycerol as a plasticiser at 2% concentration. The purpose of glycerol is to alter the tensile strength of coatings by enhancing the mobility of polymers and by decreasing internal hydrogen bonding between polymer chains while improving the intermolecular spacing. Plasticisers, such as glycerol alter the water binding capacity and augments the permeability to oxygen (Sothornvit and Krochta, 2000) and also have an impact on the water vapor barrier attributes (Taylor *et al.*, 2016; Yang and Paulson, 2000).

Currently researches have been conducted to find a safer option for the preservation of food commodities that are not only safe for human consumption but also does not harm the environment. Therefore, the purpose of this study is to evaluate the use of guar gum coatings fortified with extracts to extend the shelf life of green, un-ripe lemons during low temperature storage (12±2 °C). The storage quality parameters and microbiological analysis were also performed on the treated and untreated fruits during the shelf life. To date, there is no study on the addition of selected extracts to guar gum coatings for the preservation of lemon fruits therefore, it is a novel research to explore such biodegradable and eco-friendly alternatives for the preservation of lemon fruits.

2. Materials and methods

Chemicals and raw material

All chemicals were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA). Green, un-ripe lemons (*Citrus limon* var. kagzi) were collected from trees growing in the indoor garden. The lemons were selected on the basis of uniformity in maturity stage, size, colour and free from any dysmorphic characteristics. The lemons were washed with tap water and dried under ambient air flow.

Preparation of coating solution

The coating solutions were prepared by dissolving 1.5 g guar gum, 2 ml glycerol and 0.2 ml of different extracts in 97.8 ml of distilled water (20 °C) thus making volume of coating solution equal to 100 ml. Eight different extracts were used for this study; ethanolic and methanolic extract of fennel (EFEO and MFEO), ethanolic and methanolic extract of nigella seeds (ENEO and MNEO), ethanolic and methanolic extract of bay leaf (EBEO and MBEO) and ethanolic and methanolic extract of coriander seeds (ECEO and MCEO). The extracts were prepared according to the method of Cheikh-Rouhou *et al.* (2007). This formulation of edible coating was selected after repeated trials. Food grade guar gum powder (1.5 g) was added to 100 ml of distilled water at 20 °C and thereby glycerol (2 ml) as a plasticiser was added and subjected to continuous stirring for 120 minutes and left undisturbed at room temperature for 10 minutes. The coating was then supplemented with 0.2 ml of different extracts (undiluted) and stirred well until homogenised. Two control lemon fruit sets were also run alongside, i.e. uncoated/untreated (UN) and treated control (guar gum and glycerol (GG) without addition of extracts).

Coating of lemon

For each set of coating formulation, five lemons were coated per batch. However, only three readings were selected for each tested parameter. The lemons were tied with white cotton thread and assorted according to different treatments. Subsequently, lemons were dipped in respective coating solution for 30 seconds and removed from the coating solution for 10 seconds. The process was repeated thrice; hung and coated with respective coating treatments. Coated lemons were left undisturbed to drip off excess coating solution for 18 hours at ambient temperature and air flow. Dried lemons were stored in zip-lock bag in a refrigerator (PG 102C-RM; LEC Medical, Prescot, UK) maintained at 12±2 °C.

Preparation of lemon juice samples

The biochemical and microbiological attributes of lemon fruits were analysed using lemon juice as a substrate in triplicate. Lemon juice was extracted with the help of a manual citrus juice press. The peels were collected and stored for another study. The juice was collected in a conical flask and diluted with distilled water in a ratio of 1:4.

Physicochemical analysis

To assess the biochemical changes occurring inside the lemon fruits during storage, physicochemical analysis was performed. Hand refractometer (0-32%) ATAGO N-1α (Tokyo, Japan) was used to measure total soluble solids (TSS) of lemon juice and expressed as ° Brix. The prism was cleaned with distilled water after taking every reading (Davis *et al.*, 2003). The pH values were assessed using digital pH meter (MP220 pH meter; Mettler Toledo, Columbus, OH, USA). 10 ml of lemon juice from coated fruits and controls were added to 50 ml beaker and pH was recorded. Titratable acidity was measured using 1 ml of lemon juice after dilution with 4 ml of distilled water (Tiwari *et al.*, 2008). After addition of 2-3 drops of phenolphthalein as an indicator, the conical flask was shaken, and titration was carried out with 0.1 N NaOH. All the readings were taken in triplicate and results were expressed as g of citric acid/100 ml of lemon juice using the following equation:

$$TA=\frac{v \times 0.067 \times 0.1 \times 100}{m} \quad (1)$$

Where, v is the volume of 0.1 N NaOH used and m is the mass of lemon juice.

Assessment of micro-nutritional quality

Antioxidant ability by DPPH assay

The antioxidant ability of lemon juice samples was examined through determination of the free radical capturing impact on DPPH radical (2,2-diphenyl-1-picrylhydrazyl) according to the method of Han *et al.* (2008). 200 µl of diluted lemon juice sample was added to 2.7 ml of methanolic DPPH solution (0.06 mM). The homogenate was agitated vigorously and left undisturbed in dark for 30 minutes. Absorbance of the samples was measured using the UV-Vis spectrophotometer (JascoV-670 UV-VIS-NIR Spectrophotometer, Tokyo, Japan) at 515 nm. Readings were expressed as percent scavenging abilities of each lemon juice sample and controls.

Ascorbic acid content

Ascorbic acid of extracted lemon juice samples was determined according to the method of Barros *et al.* (2007). Lemon juice samples (0.1 ml) was added to 10 ml of 1% meta phosphoric acid and incubated for 45 minutes at room temperature. 1 ml of this homogenate was mixed with 9 ml of DCPIP (2,6-Dichlorophenolindophenol) dye solution. Absorbance measurements of the samples were carried out using UV-Vis spectrophotometer (JascoV-670 UV-VIS-NIR Spectrophotometer) at 515 nm. Results were expressed as mg of ascorbic acid/ g of lemon juice.

Determination of chlorophyll 'a', chlorophyll 'b', β-carotene and lycopene contents

Carotenoids and chlorophyll pigments were determined according to the method of Bhumsaidon and Chamchong (2016). 1 ml of undiluted lemon juice sample was homogenised with 10 ml of 4:6 (acetone: n-hexane) for 60 seconds using ultra homogeniser and filtered through Whatman no. 2 filter paper (Whatman, Maidstone, UK). Absorbance measurement of the filtrate was carried out at 663, 645, 505 and 453 nm. All the measurements were repeated thrice.

Determination of total phenolic contents

For the total phenolic content (TPC), each sample was analysed in triplicate; 20 µl of the lemon juice sample was mixed with 100 µl of Folin-Ciocalteu reagent after the addition of 1.58 ml of distilled water. After incubation period of 1-8 minutes, 300 µl of 25% Na_2CO_3 was added following the micro-method described in Waterhouse (2002). After 2 hours of incubation in dark, the absorption of the homogenates was measured using JascoV-670 UV-VIS-NIR Spectrophotometer at 765 nm. Results were expressed as gallic acid equivalents (GAE mg/l) of lemon juice samples.

Determination of total flavonoid content

Total flavonoid content was measured according to the method of Hajlaoui *et al.* (2009). 250 µl of lemon juice sample was diluted with distilled water and subsequently mixed with 75 µl of 5% $NaNO_2$. After 6 minutes, 150 µl of 10% $AlCl_3.6H_2O$ was added to the test tube. After 5 minutes, 0.5 ml of 1M NaOH was mixed. Finally, the mixture was adjusted to 2.5 ml with distilled water. The absorption of the resulting mixture was measured at 510 nm using UV-Vis spectrophotometer (JascoV-670 UV-VIS-NIR Spectrophotometer). The results were expressed as mg/ml of quercetin equivalents. All the measurements were taken in triplicate.

Microbiological assessment

Effect of the application of edible coatings on the increase/decrease in bacterial counts in each lemon juice sample during the shelf life was determined. The endpoint for the shelf life was the appearance of any visible fungal growth on the peel of lemon fruits. Dilutions of the lemon juice i.e. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} were prepared in peptone water (Das *et al.*, 2013). 50 µl of this sample were spread on the surface of solidified tryptone soy agar. The plates were incubated at 35±2 °C for 18 hours. The results were reported as log cfu/ml for each lemon juice sample (Irkin *et al.*, 2015).

Statistical analysis

Analysis of variance was employed to compute significant differences between the means, and Duncan's test at $P \leq 0.05$ was used to separate means using SPSS software (version 24, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

Comparison of shelf life of lemons

Lemons have a fairly long storage life if collected at green immature stage as compared to the ones that are gathered at yellow maturity phase (Durner, 2013). These can be stored at low temperatures for up to 6-8 weeks without any loss in fruit's physiological or biochemical characteristics. Different coating treatments had different impact on the enhancement of storage life of lemons at 10 °C. The completion of storage interval was marked as soon as the peel of the fruit showed any signs of physical deformity, such as loss in firmness, formation of crevices on the surface or any sort of visible fungal contamination, i.e. growth of moulds. The untreated (uncoated) control had a storage life of 25 days, while treated control decayed merely after 11 days. The shelf life of all treatments varied significantly. The treated samples, i.e. MBEO, MCEO, MNEO, EBEO, ECEO and ENEO survived up to 15 weeks, while MFEO and EFEO had storage life of 24 weeks. Uckoo *et al.* (2015) reported the storage life of lemons for 4 weeks at 10 °C to retain phytochemicals in fruits. D'Aquino *et al.* (2017) showed that treatment with sodium hypochlorite and imazalil increased the shelf life up to 8 weeks when stored at 20 °C. Different researchers (Eskandari *et al.*, 2014; Ghosh *et al.*, 2015; Jawandha *et al.*, 2014) reported the use of edible coating to prolong the shelf life of lemons, but none of these edible coatings extended the shelf life beyond 2 months of storage. The same coating formulation was utilised for the preservation of tomato fruit (Naeem *et al.*, 2018a) and mango (Naeem *et al.*, 2018b) for 60 and 24 days, respectively, during cold storage.

Physicochemical analysis

The change in pH of lemon juice samples over the period during storage were presented as rate of change during the shelf life in Table 1. The rate of pH was reduced by the application of the proposed edible coatings. However, both the controls had a total rate of increase in pH (25% for GG and 34% for UN) but this rate was higher than those observed for treated samples within shorter duration of time. This showed that these coatings slowed down the rate of increase in pH by providing a semi-permeable barrier around the fruit's surface (Moraes *et al.*, 2012).

Total soluble solids of treated and both the controls were expressed as ° Brix in Figure 1. The comparison of the readings obtained at zero and last day of storage revealed significant differences in the values obtained on the days of assessment, i.e. 15 weeks for MBEO, MCEO, MNEO, EBEO, ECEO and ENEO and 24 weeks for MFEO and EFEO at $P \leq 0.05$. The TSS content of lemon juice for EBEO and MBEO coated lemons showed a small increment during storage as observed by Alikhani and Daraei Garmakhany (2012). The highest values of TSS (1.36 ° Brix) were observed for lemons coated with ENEO during 90 days of storage. The TSS values did not show significant increase for untreated and treated control samples during storage. The alterations in values of TSS is directly linked with hydrolytic changes in starch molecules during ripening process in postharvest storage (Kays, 1991). Release of oligosaccharins due to hemicellulose and pectins during storage can have an impact on fruit ripening (Côté and Hahn, 1994).

Table 1. Effect of edible coating on pH and titratable acidity of lemon (juice) during storage at 10 °C.[1]

Treatments	Shelf life (weeks)	Rate of change in pH	Rate of change in titratable acidity
MNEO	15	13.20 ± 0.22^{b}	22.53 ± 0.17^{a}
MCEO	15	15.91 ± 0.11^{d}	22.53 ± 0.17^{a}
EBEO	15	19.21 ± 0.17^{e}	60.26 ± 0.83^{b}
ENEO	15	13.29 ± 0.17^{b}	75.01 ± 0.03^{f}
MBEO	15	10.67 ± 0.17^{a}	67.14 ± 0.07^{d}
ECEO	15	13.96 ± 0.05^{c}	66.50 ± 0.02^{c}
EFEO	24	19.03 ± 0.05^{e}	72.25 ± 0.23^{e}
MFEO	24	22.53 ± 0.17^{f}	74.49 ± 0.13^{f}

[1] Values are mean ± standard deviation. Values in the same column with different superscripts are significantly different at $P \leq 0.05$. MFEO; MNEO; MBEO; MCEO, guar gum coating added with methanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EFEO, ENEO, EBEO, ECEO, guar gum coating added with ethanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

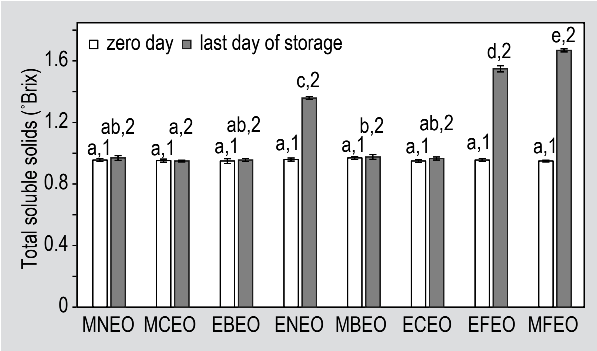

Figure 1. Effect of different edible coatings on total soluble solids of lemon (juice) during storage (10 °C, 80-85% relative humidity). The vertical bars with error bars represent the average ± standard error (n=3). Different lowercase letters over bars represent significant differences due to different treatments at *P*≤0.05 whereas numbers on bars indicate significant differences at zero and last day of storage, i.e. 15 weeks for MBEO, MCEO, MNEO, EBEO, ECEO and ENEO and 24 weeks for MFEO and EFEO at *P*≤0.05. MFEO; MNEO; MBEO; MCEO, guar gum coating added with methanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EFEO, ENEO, EBEO, ECEO, guar gum coating added with ethanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

Drop in titratable acidity values were observed in all the lemons stored at low temperature and are presented in Table 1. The highest rate was observed for MCEO and lowest for EBEO coated lemons. The controls also showed decrease in titratable acidity 79.16% and 66% for treated control and untreated control, respectively as shown by Nabifarkhani *et al.* (2015). The rate of decrease in titratable acidity in both control samples was observed during lesser duration of time as shown by Alikhani and Daraei Garmakhany (2012). On contrary, lower rates were observed for coated lemons over the long duration of time as observed by Alikhani and Daraei Garmakhany (2012). This significantly showed that edible coatings successfully prevented the decrease in titratable acidity by providing a semi-permeable film around the commodity. As organic acids are primary substrates for respiration, therefore, their reduction can be observed in fruits with high rates of respiration (El-Anany *et al.*, 2009). It is believed that edible coatings decelerate respiration rate and may therefore, prolong the consumption of organic acids (Yaman and Bayoındırlı, 2002).

Quality parameters

Antioxidant ability

The total antioxidant capacity in terms of radical scavenging abilities of DPPH was observed (Figure 2A). The lemon samples showed a gradual increase in antioxidant capacities over the period of time (Shamloo *et al.*, 2013). The lowest rate of antioxidant increase was observed for MFEO. Thus, MFEO coating significantly reduced the rate of antioxidant increase during ripening. GG had a rate of 56% and UN 65% increase in antioxidant abilities observed during 25 and 11 days respectively. As the fruit matures, the antioxidant levels increase in the fruits. This rise in antioxidant contents is mainly due to increase in lipophilic antioxidant compounds. The presence and quantity of phytochemicals depend on the plant genotype and environmental conditions such as availability of water and micronutrients in the soil under pre- and post-harvest conditions of lemons (Uckoo *et al.*, 2015). Some other factors such as content of ascorbic

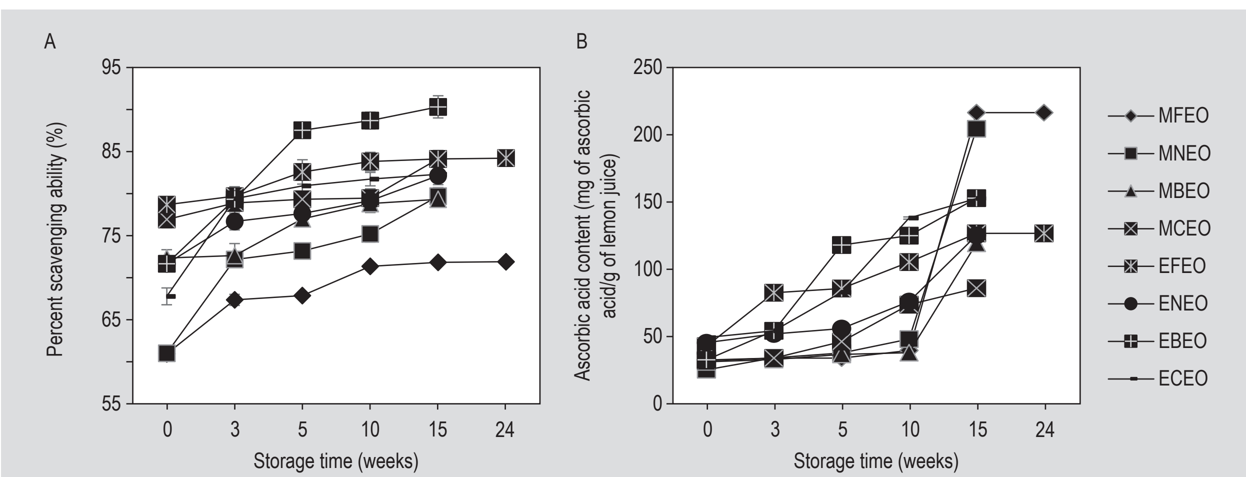

Figure 2. Effect of different edible coatings on (A) total antioxidant activity (DPPH) and (B) ascorbic acid content of lemon (juice) during storage (10 °C, 80-85% relative humidity). The vertical bars represent the standard error of means of three replicates. MFEO; MNEO; MBEO; MCEO, guar gum coating added with methanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EFEO, ENEO, EBEO, ECEO, guar gum coating added with ethanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

acid, β-carotene and Vitamin E greatly also influence the antioxidant potential (Ali *et al.*, 2013).

Ascorbic acid content

Figure 2B shows the ascorbic acid content of the treated samples, treated control and untreated control procured from lemons during storage at 10 °C. The ascorbic acid content of all the lemons increased as the time duration was increased. The rate of increase in ascorbic acid in MBEO, MCEO, MNEO, MFEO, EBEO, ECEO, EFEO and ENEO was 73.83, 84.9, 87, 84.79, 78.65, 67.7, 74.13 and 63.69%, respectively. However, the rate of increase in ascorbic acid in treated control (GG) and untreated control (UN) was found out to be 40 and 94.05%, respectively. These results proved that edible coatings greatly influence the biochemical reactions such as synthesis of organic acids (ascorbic acid) that was occurring inside the lemon fruits during the post-harvest life. This retention of vitamin C content might be due to decrease in respiration of fruits or halting of oxidation of vitamin C in the fruits. The untreated control showed considerable levels of vitamin C during storage life due to increased respiration rates. Vitamin C is highly influenced by oxidative degradation and mild oxidative reaction of ascorbic acid leading to the formation of dehydroascorbic acid (Hassan *et al.*, 2014). The antioxidant and ascorbic acid content showed similar trends in their rates as both the contents showed a linear trend of increase and this increase was observed after 10th week of storage period in most of the treatments.

Carotenoids and chlorophyll content

The carotenoids, such as β-carotene and lycopene, are generally increased during post-harvest storage (Figure 3 and 4). However, the inverse behaviour is observed for chlorophyll content (chlorophyll 'a' and 'b') when the fruits are picked in immature stage and subjected to modified atmosphere packaging. As chloroplast vacuoles are utilised to synthesise the carotenoid pigments during the fruit's maturity phases, the green colour is lost in matured fruits. The increase in lycopene content is totally dependent on the temperature and rate of respiration during storage (Javanmardi and Kubota, 2006). The lemon juice from treated samples showed a gradual increase in carotenoid content while a decrease in chlorophyll content was observed. The control samples also showed a similar variation, but these fluctuations were measured during shorter duration of time as compared to treated samples. The rate of increase in β-carotene, lycopene, chlorophyll 'a' and 'b' in GG was 43, 76, 77.9 and 79.88%, respectively, that in UN was 86%, 84, 80 and 84%, respectively.

Total phenolic content

TPC was estimated by Folin-Ciocalteu Micro method (Figure 5A). Generally, all the samples as well as both the controls showed an increase in the TPC. The lowest TPC was observed for EFEO. However, GG and UN had a rate of 82.13 and 85.45%, respectively. Edible coatings have a role in reducing the rate of increase in phenolic content during the shelf life but could not halt it as various biochemical processes are continuously occurring in the cellular compartments. A slight change in TPC was observed during the shelf life as observed by Alikhani-Koupaei (2014). The increase in TPC is directly linked with the enhancement

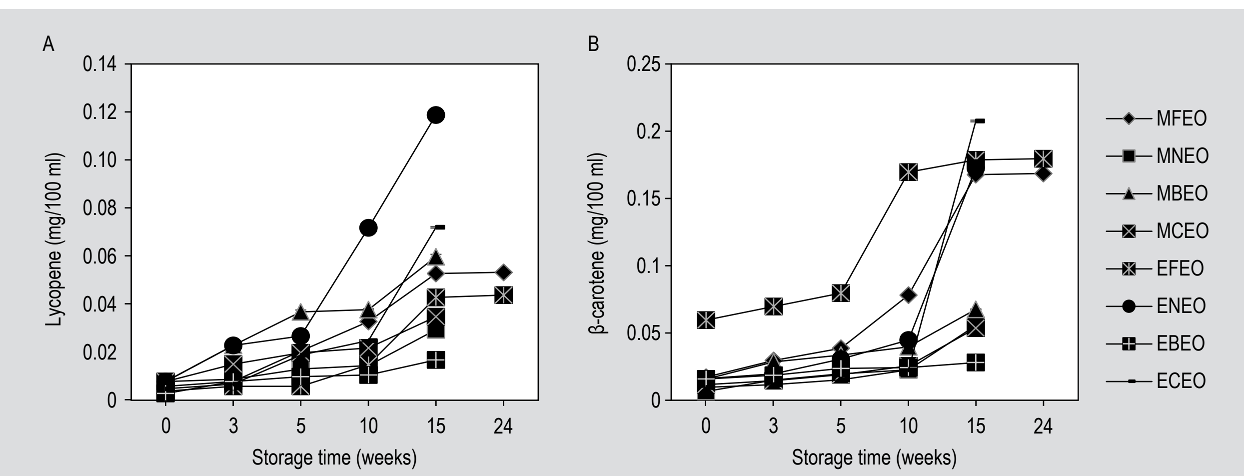

Figure 3. Effect of different edible coatings on (A) lycopene and (B) β-carotene content of lemon (juice) during storage (10 °C, 80-85% relative humidity). The vertical bars represent the standard error of means of three replicates. MFEO; MNEO; MBEO; MCEO, guar gum coating added with methanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EFEO, ENEO, EBEO, ECEO, guar gum coating added with ethanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

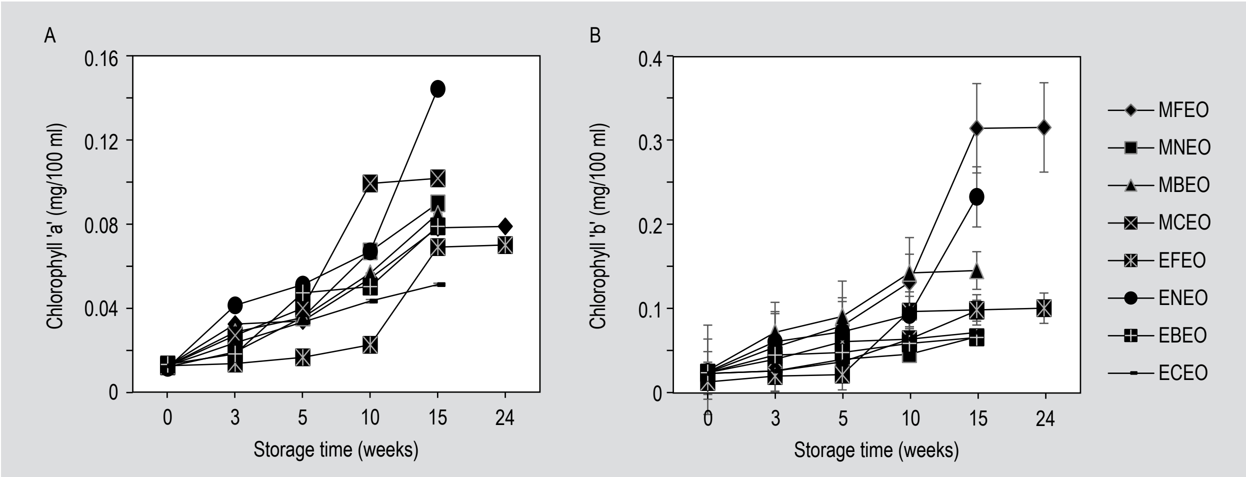

Figure 4. Effect of different edible coatings on (A) chlorophyll 'a' and (B) chlorophyll 'b' content of lemon (juice) during storage (10 °C, 80-85% relative humidity). The vertical bars represent the standard error of means of three replicates. MFEO; MNEO; MBEO; MCEO, guar gum coating added with methanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EFEO, ENEO, EBEO, ECEO, guar gum coating added with ethanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

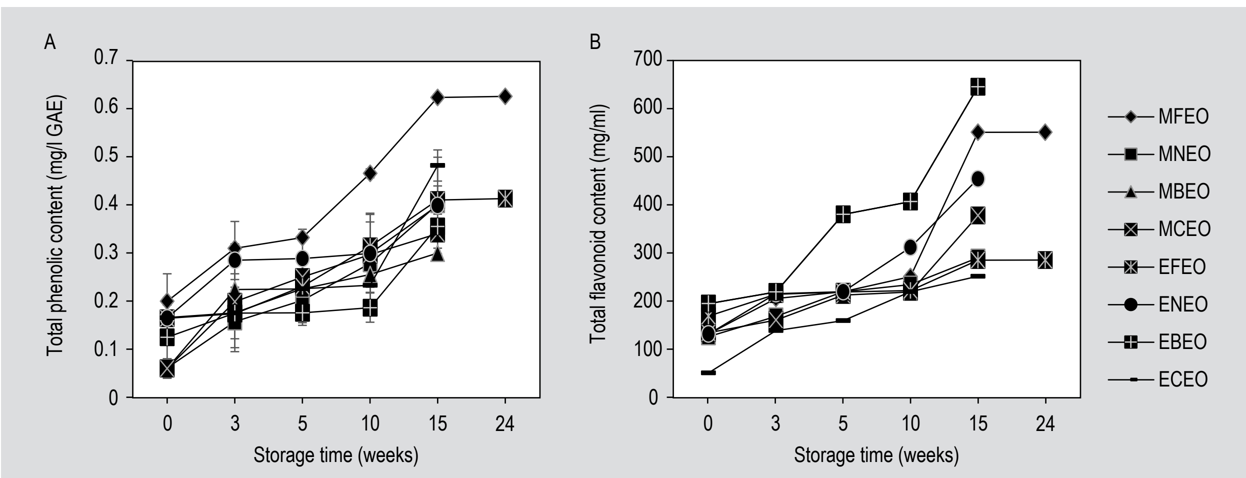

Figure 5. Effect of different edible coatings on (A) total phenolic and (B) total flavonoid content of lemon (juice) during storage (10 °C, 80-85% relative humidity). The vertical bars represent the standard error of means of three replicates. MFEO; MNEO; MBEO; MCEO, guar gum coating added with methanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EFEO, ENEO, EBEO, ECEO, guar gum coating added with ethanolic extract of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

of antioxidant potential of the fruit (Sharifani *et al.*, 2015). The phenolic compounds are synthesised at a higher rate during the ripening process, but the levels begin to decline as the fruit approaches senescence. Phenolic compounds play a vital role in the fruit quality and in phytochemical levels (Shamloo *et al.*, 2015). These compounds are also promoters of plant defence reactions, shielding the plant from reactive oxygen species, pests and insects (Uckoo *et al.*, 2015). The results of TPC and ascorbic acid content can be correlated as in both parameters, the highest values were observed for MFEO coated lemons.

Total flavonoid content

Lemons are rich in flavonoids. Narirutin, didymin, hesperidin are the major flavonoids present in lemon juice. Flavonoids are polyphenolic compounds that are released during plant defence mechanism with the major role played by hesperidin. Various researchers reported that didymin, narirutin and hesperidin also possess antimicrobial as well as antioxidant activities *in vivo* (Uckoo *et al.*, 2015). Lemon juice samples obtained from treated fruits and both controls showed a rise in flavonoid content during storage period, i.e. 38.4% in GG and 59.6% in UN. EFEO coated lemon had the slowest rate of rise in flavonoids (Figure 5B). The results are expressed as quercetin equivalents. Like phenolic compounds, flavonoid content is also dependent on stage of maturity of the fruit as their levels decreases as the fruit reaches senescence.

When comparing the effect of both the solvents (methanol and ethanol) on the rates of biochemical reactions, it could be concluded that in most studied parameters, ethanolic extracts incorporated coated fruits showed lower levels of bioactive compounds increase as compared to the methanolic extracts incorporated coated fruits.

Microbiological analysis

The edible coatings not only served as semi-permeable barrier for the retention of phytochemical compounds in fruits but also served as antibacterial barrier for the contaminating microorganisms during storage conditions. The antioxidant and antimicrobial compounds in the edible coatings, such as extracts which were used in this study, retain in edible coating. They diffuse into the fruit tissues whenever needed from the fruit's surface to inhibit the combating microbes consequently the inner tissues present aseptic environment (Shan *et al.*, 2013). These coatings not only decreased the bacterial counts during the storage period, but also delayed the growth of contaminating fungi in lemon fruits, the latter was also the endpoint of storage life in this study. The bacterial count of lemon juice samples on day zero of treatment was log 8 cfu/ml of lemon juice which significantly dropped for all the treated samples (Figure 6). However, both controls showed an increase in viable colonial counts. In nearly all treated samples, the colonial counts were reduced to half of its initial value with the lowest counts observed for MFEO coated lemons. T-test was used to compare the readings obtained at initial and final day of shelf life and the values were found to be statistically significant at $P \leq 0.05$. A drop in bacterial count was also observed by Alikhani-Koupaei *et al.* (2014) during the controlled storage. These results signify that MFEO coating was highly potent to inhibit further increase in bacterial counts.

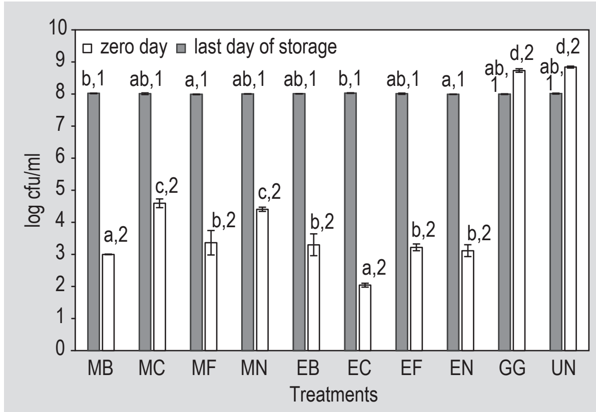

Figure 6. Effect of different edible coatings on microbial load of lemon (juice) during storage (10 °C, 80-85% relative humidity). MB, MC, MF and MN; microbial loads in lemon fruits having a treatment of guar gum coating added with methanolic extract of bay leaf, coriander seeds, fennel seeds and Nigella seeds, respectively. EB, EC, EF, EN, GG and UN; microbial loads in lemon fruits having a treatment of guar gum coating added with methanolic extract of bay leaf, coriander seeds, fennel seeds and Nigella seeds, treated control and untreated control, respectively. Final day of storage, i.e. 15 weeks for MB, MC, MN, EB, EC and EN and 24 weeks for MF and EF. Vertical bars with error bars indicate the average ± standard error (n=3), lowercase numbers indicate statistical significance at zero and last day of storage at *P*≤0.05.

4. Conclusions

The present study showed remarkable results in preservation of lemons during cold storage coated by MFEO and EFEO ($P \leq 0.05$). Both coatings successfully extended the shelf life of lemons up to 180 days without any loss in phytochemical components or in physicochemical attributes. Results also justify that coating lemons with guar gum complexed with extracts delayed the ripening process by slowing down the biochemical reactions in this fruit. This suggested that proposed edible coatings not only maintained micro-nutritional attributes but also enhanced the postharvest shelf life during cold storage.

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References

Ali, A., Maqbool, M., Alderson, P.G. and Zahid, N., 2013. Effect of gum arabic as an edible coating on antioxidant capacity of tomato (*Solanum lycopersicum* L.) fruit during storage. Postharvest Biology and Technology 76: 119-124.

Alikhani, M. and Daraei Garmakhany, A., 2012. Effect of microencapsulated essential oils on storage life and quality of strawberry (*Fragaria ananassa* cv. Camarosa). Quality Assurance and Safety of Crops and Foods 4: 106-112.

Alikhani-Koupaei, M., 2014. Liposomal and edible coating as control release delivery systems for essential oils: comparison of application on storage life of fresh-cut banana. Quality Assurance and Safety of Crops and Foods 7: 175-185.

Alikhani-Koupaei, M., Mazlumzadeh, M., Sharifani, M. and Adibian, M., 2014. Enhancing stability of essential oils by microencapsulation for preservation of button mushroom during postharvest. Food Science and Nutrition 2: 526-533.

Artés-Hernández, F., Rivera-Cabrera, F. and Kader, A.A., 2007. Quality retention and potential shelf-life of fresh-cut lemons as affected by cut type and temperature. Postharvest Biology and Technology 43: 245-254.

Barros, L., Ferreira, M.-J., Queiros, B., Ferreira, I.C. and Baptista, P., 2007. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chemistry 103: 413-419.

Benavente-García, O., Castillo, J., Marin, F.R., Ortuño, A. and Del Río, J.A., 1997. Uses and properties of citrus flavonoids. Journal of Agricultural and Food Chemistry 45: 4505-4515.

Bhumsaidon, A. and Chamchong, M., 2016. Variation of lycopene and beta-carotene contents after harvesting of gac fruit and its prediction. Agriculture and Natural Resources 50: 257-263.

Bisen, A. and Pandey, S., 2016. Effect of post harvest treatment on biochemical composition and organoleptic quality in Kagzi lime fruit during storage. Journal of Horticultural Science 3: 53-56.

Bisen, A., Pandey, S.K. and Patel, N., 2012. Effect of skin coatings on prolonging shelf life of kagzi lime fruits (*Citrus aurantifolia* Swingle). Journal of Food Science and Technology 49: 753-759.

Chahal, S. and Bal, J., 2003. Effect of post-harvest treatments and packaging on shelf life of Umran ber at cool temperature. Journal of Research 40: 363-370.

Cheikh-Rouhou, S., Besbes, S., Hentati, B., Blecker, C., Deroanne, C. and Attia, H., 2007. *Nigella sativa* L.: Chemical composition and physicochemical characteristics of lipid fraction. Food Chemistry 101: 673-681.

Chiabrando, V. and Giacalone, G., 2014. Effect of essential oils incorporated into an alginate-based edible coating on fresh-cut apple quality during storage. Quality Assurance and Safety of Crops and Foods 7: 251-259.

Côté, F. and Hahn, M.G., 1994. Oligosaccharins: structures and signal transduction. Signals and signal transduction pathways in plants. Springer, New York, NY, USA, pp. 143-175.

D'Aquino, S., Suming, D., Deng, Z., Gentile, A., Angioni, A., De Pau, L. and Palma, A., 2017. A sequential treatment with sodium hypochlorite and a reduced dose of imazalil heated at 50 °C effectively control decay of individually film-wrapped lemons stored at 20 °C. Postharvest Biology and Technology 124: 75-84.

Das, D.K., Dutta, H. and Mahanta, C.L., 2013. Development of a rice starch-based coating with antioxidant and microbe-barrier properties and study of its effect on tomatoes stored at room temperature. LWT – Food Science and Technology 50: 272-278.

Davis, A.R., Fish, W.W. and Perkins-Veazie, P., 2003. A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. Postharvest Biology and Technology 28: 425-430.

Durner, E.F., 2013. Principles of horticultural physiology. CABI, Wallingford, UK.

El-Anany, A., Hassan, G. and Ali, F.R., 2009. Effects of edible coatings on the shelf-life and quality of Anna apple (*Malus domestica* Borkh) during cold storage. Journal of Food Technology 7: 5-11.

Embuscado, M.E. and Huber, K.C., 2009. Edible films and coatings for food applications. Springer, New York, NY, USA.

Eskandari, A., Heidari, M., Daneshvar, M.H. and Taheri, S., 2014. Studying effects of edible coatings of Arabic gum and olive oil on the storage life and maintain quality of postharvest sweet lemon (*Citrus limetta*). International Journal of Agriculture and Crop Sciences 7: 207.

Ghosh, A., Dey, K. and Bhowmick, N., 2015. Effect of corn starch coating on storage life and quality of Assam lemon (*Citrus limon* Burn.). Journal Crop and Weed 11: 101-107.

Gidley, M.J. and Reid, J.G., 2006. Galactomannans and other cell wall storage polysaccharides in seeds. Food Polysaccharides and their Applications: 181-215.

Hajlaoui, H., Trabelsi, N., Noumi, E., Snoussi, M., Fallah, H., Ksouri, R. and Bakhrouf, A., 2009. Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. World Journal of Microbiology and Biotechnology 25: 2227-2238.

Han, J., Weng, X. and Bi, K., 2008. Antioxidants from a Chinese medicinal herb – *Lithospermum erythrorhizon*. Food Chemistry 106: 2-10.

Hassan, Z., Lesmayati, S., Qomariah, R. and Hasbianto, A., 2014. Effects of wax coating applications and storage temperatures on the quality of tangerine citrus (*Citrus reticulata*) var. Siam Banjar. International Food Research Journal 21(2): 641-648.

Irkin, R., Degirmencioglu, N. and Guldas, M., 2015. Effects of organic acids to prolong the shelf-life and improve the microbial quality of fresh cut broccoli florets. Quality Assurance and Safety of Crops and Foods 7: 737-745.

Javanmardi, J. and Kubota, C., 2006. Variation of lycopene, antioxidant activity, total soluble solids and weight loss of tomato during postharvest storage. Postharvest Biology and Technology 41: 151-155.

Jawandha, S., Singh, H., Arora, A. and Singh, J., 2014. Effect of modified atmosphere packaging on storage of Baramasi lemon (*Citrus limon* (L.) Burm). International Journal of Agriculture, Environment and Biotechnology 7: 635.

Kader, A.A., 2002. Postharvest technology of horticultural crops, 3311. University of California Agriculture and Natural Resources, St. Davis, CA, USA, 535 pp.

Kays, S.J., 1991. Postharvest physiology and handling of perishable plant products. Van Nostrand Reinhold Inc., New York, NY, USA.

Lin, D. and Zhao, Y., 2007. Innovations in the development and application of edible coatings for fresh and minimally processed fruits and vegetables. Comprehensive Reviews in Food Science and Food Safety 6: 60-75.

Marlett, J.A. and Vollendorf, N.W., 1994. Dietary fiber content and composition of different forms of fruits. Food Chemistry 51: 39-44.

Marlett, J.A., 1992. Content and composition of dietary fiber in 117 frequently consumed foods. Journal of the American Dietetic Association 92: 175-186.

Moraes, K.S.d., Fagundes, C., Melo, M.C., Andreani, P. and Monteiro, A.R., 2012. Conservation of Williams pear using edible coating with alginate and carrageenan. Food Science and Technology 32: 679-684.

Nabifarkhani, N., Sharifani, M., Daraei Garmakhany, A., Ganji Moghadam, E. and Shakeri, A., 2015. Effect of nano-composite and thyme oil (*Tymus Vulgaris* L) coating on fruit quality of sweet cherry (Takdaneh Cv) during storage period. Food Science and Nutrition 3(4): 349-354.

Naeem, A., Abbas, T., Ali, T.M. and Hasnain, A., 2018a. Effect of antioxidant and antibacterial properties of guar gum coating containing spice extracts and its application on tomatoes (*Solanum lycopersicum* L.). Journal of Food Measurement and Characterization 12(4): 2725-2734.

Naeem, A., Abbas, T., Ali, T.M. and Hasnain, A., 2018b. Effect of guar gum coatings containing essential oils on shelf life and nutritional quality of green-unripe mangoes during low temperature storage. International Journal of Biological Macromolecules 113: 403-410.

Pasha, A., Abbas, T., Ali, T.M. and Hasnain, A., 2018. Inactivation of food borne pathogens by lipid fractions of culinary condiments and their nutraceutical properties. Microbiology Research 9(1).

Peleg, H., Naim, M., Rouseff, R.L. and Zehavi, U., 1991. Distribution of bound and free phenolic acids in oranges (*Citrus sinensis*) and grapefruits (*Citrus paradisi*). Journal of the Science of Food and Agriculture 57: 417-426.

Shamloo, M., Sharifani, M., Garmakhany, A.D. and Seifi, E., 2013. Alternation of flavonoid compounds in Valencia orange fruit (*Citrus sinensis*) peel as a function of storage period and edible covers. Minerva Biotecnol 25: 191-197.

Shamloo, M., Sharifani, M., Garmakhany, A.D. and Seifi, E., 2015. Alternation of secondary metabolites and quality attributes in Valencia orange fruit (*Citrus sinensis*) as influenced by storage period and edible covers. Journal of Food Science and Technology 52(4): 1936-1947.

Shan, B., Cai, Y.-Z., Brooks, J. and Corke, H., 2013. Spice and herb extracts as natural preservatives in cheese. Handbook of cheese in health: production, nutrition and medical sciences. Wageningen Academic Publishers, Wageningen, the Netherlands, 280 pp.

Sharifani, M., Shamloo, M.M., Garmakhany, A.D. and Seifi, E., 2015. Quality attributes of Valencia orange fruit using different edible covers. Acta Horticulturae 1065: 1529-1532.

Sothornvit, R. and Krochta, J., 2000. Plasticizer effect on oxygen permeability of β-lactoglobulin films. Journal of Agricultural and Food Chemistry 48: 6298-6302.

Srivastava, M. and Kapoor, V., 2005. Seed galactomannans: an overview. Chemistry and Biodiversity 2: 295-317.

Taverner, P., Tugwell, B. and Wild, B., 2001. A guide to the common postharvest diseases and disorders of navel oranges and mandarins grown in inland Australia. South Australian Research and Development Institute (SARDI), Adelaide, Australia.

Taylor, J., Muller, M. and Minnaar, A., 2016. Improved storage and eat-ripe quality of avocados using a plant protein-based coating formulation. Quality Assurance and Safety of Crops and Foods 8: 207-214.

Tiwari, B., Muthukumarappan, K., O'donnell, C. and Cullen, P., 2008. Colour degradation and quality parameters of sonicated orange juice using response surface methodology. LWT – Food Science and Technology 41: 1876-1883.

Uckoo, R.M., Jayaprakasha, G.K. and Patil, B.S., 2015. Phytochemical analysis of organic and conventionally cultivated Meyer lemons (*Citrus meyeri* Tan.) during refrigerated storage. Journal of Food Composition and Analysis 42: 63-70.

Vieira, Í.G.P., Mendes, F.N.P., Gallão, M.I. and De Brito, E.S., 2007. NMR study of galactomannans from the seeds of mesquite tree (*Prosopis juliflora* (Sw) DC). Food Chemistry 101: 70-73.

Waterhouse, A.L., 2002. Determination of total phenolics. Current Protocols in Food Analytical Chemistry 6(1): 1.1.1-1.1.8.

Yaman, Ö. and Bayoındırlı, L., 2002. Effects of an edible coating and cold storage on shelf-life and quality of cherries. LWT – Food Science and Technology 35: 146-150.

Yang, L. and Paulson, A., 2000. Effects of lipids on mechanical and moisture barrier properties of edible gellan film. Food Research International 33: 571-578.