

## Retention of micro-nutritional components of lemon zest through edible coatings supplemented with lipid fractions

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### Abstract

The present work was carried out to study the effect of guar gum-based coating supplemented with lipid fractions (LFs) to minimise the post-harvest losses of lemon fruits. In addition to prolongation of storage life, the effects of these edible coatings on changes of bioactive compounds in lemon zest were also evaluated. Observations were recorded on bioactive compound contents at different time intervals. The antimicrobial coatings were also examined for antibacterial potential using two different assays against foodborne pathogens. Methanolic and ethanolic lipid fractions (LF) of fennel seeds supplemented coatings extended shelf life for 24 weeks and ethanolic LF supplemented coatings showed lowest rates of increase in micro-nutrient components. However, methanolic LF coatings exhibited higher inhibitory effect against *Escherichia coli* ATCC 8739 when assayed *in vitro*. Results positively support the use of these coatings to slow down biochemical reactions occurring in lemon zest.

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### Keywords

Lemon peels

Edible coatings

Lipid fractions

Shelf life

### Introduction

The utilisation of vegetables and fruits are known to provide myriad of health benefits. The markets which cater for the demand of fresh and ready-to-eat food commodities is dramatically increasing due to marked changes in the lifestyle of consumers (Soliva-Fortuny and Martín-Belloso, 2003). Organic products are gaining more importance. Globally, citrus fruits occupy an important position among tropical and sub-tropical fruits. Due to their versatile uses and a rich source of many nutraceutical-based compounds such as prenyl oxycoumarins which include bergamottin, auraptene, heraclenin, imperatorin and oxypeucedanin, more attention is being paid to cultivate lemons than any other citrus fruits. Lemons also have pharmaceutical importance due to higher contents of antiscorbutic vitamin C, phenols, flavonoids, pectins, molasses and limonene (Burdurlu *et al.*, 2006). The total phenolic contents of lemon pulp and zest are considerably higher than that of grapefruits and oranges. Lemon peels are commonly used in baking and commercially available in dried form. The peels are generally divided into epicarp/flavedo (i.e., coloured peripheral portion) and mesocarp/albedo (i.e., white soft middle portion)

(Rafiq *et al.*, 2018). Lemons are thermolabile fruits and suffer a chilling injury when stored below 10°C. The decrease in moisture content from the fruit's surface contributes to major postharvest losses. The main fungal disease that deteriorates the fruit quality are green mildew caused by *Penicillium digitatum*, blue mildew caused by *P. italicum*, scald skin and tail rot that contributes to major fruit losses (Eskandari *et al.*, 2014). Currently, researches have been conducted to exploit natural products for the control of decay and enhance the storage life of such perishable food commodities (Hammer *et al.*, 1999). Coatings supplemented with antimicrobial compounds are the widely exploited area to extend the shelf life of fresh produce. Lipid-based edible coatings are effective to prevent loss of water from the fruit's surface and add a glossy appearance to the epidermis. The addition of active components such as extracts could further enhance the potential of these coatings (Baldwin *et al.*, 1995).

The main objective of the present work was therefore to evaluate the effectiveness of guar gum edible coating supplemented with lipid fractions to extend the shelf life of lemons during cold storage, and to evaluate the delay in phytochemical changes in the lemon zest. To the best of our knowledge, there is no study conducted on such observations thus far.

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## Materials and methods

### Chemicals

All chemicals used in the present work were of analytical grade. DPPH (2, 2- diphenyl-1-picryl-hydrazyl) from Sigma Aldrich (D9132), metaphosphoric acid (Uni-Chem CAS # 37267-86-0), sodium hydroxide (Merck R: 35 S: 26-37/39-45), trichloroacetic acid (Riedel-de-Haën 76-03-09), L(+)-ascorbic acid (DJ 1099-4125), gallic acid monohydrate (Merck Index 14,4345), quercetin dihydrate (Daejung CAR 7417.1), sodium carbonate (DJ-7541-4105), Folin-Ciocalteu reagent (JUN 96703-8130), DCPIP(2,6-dichlorophenolindophenol) (ALF A10107), dimethyl sulfoxide (DJ-3047-4105), iron (II) sulphate heptahydrate (DJ 5017-4405), sodium nitrate (DJ 7599-4405), sodium nitrite (DJ 7601-4105), aluminium chloride hexahydrate (DJ-1032-4405), n-hexane (Daejung CAS # 11-0-54-3) and Guar gum were purchased from Parchem (CAS # 9000-30-0).

### Fruit material

Green, unripe lemon (*Citrus limon* L.) fruits were handpicked from indoor garden. The fruits were washed with tap water to remove any adhering dust particles and then air-dried under ambient air flow.

### Preparation of edible coating formulations

The edible coatings were prepared by using 1.5 g guar gum (w/v), 2 mL glycerol (v/v) and 200 µL (0.2%) of various lipid fractions (LFs) and making the final volume of 100 mL following the method of Naeem *et al.* (2018). Guar gum was added to distilled water at 20°C with subsequent addition of glycerol. Undiluted LFs were added to edible coatings, and the coating formulation were named after the type of LF added to the coating. The lipid fractions supplemented were ethanolic and methanolic LF of fennel seeds (EF and MF), ethanolic and methanolic LF of coriander seeds (EC and MC), ethanolic and methanolic LF of nigella seeds (EN and MN), and ethanolic and methanolic LF of bay leaf (EB and MB). The resulting mixture was kept on stirring for 2 h at room temperature, and left to stabilise for 10 min. Two controls were also run in this experiment i.e., untreated (uncoated and labelled as UN) and treated control (Guar gum + glycerol, and labelled as GG). The dried lemon fruits were dipped in coating solution three times with consecutive interval of 10 s. The coated lemons were hung tied with the help of thread to drip off excess of coating material overnight. On the next day, lemons were labelled with respective edible coating formulations. The lemons were then

stored in zip-lock bags in controlled temperature (10°C) in LEC Medical Pharmacy refrigerator until analysed.

### Pre-treatment of lemon zest

From the collected lemon fruits, lemon juice was collected for another study, and zest was collected for the present work. The zest was dried in hot air oven (Lab tech LCT-1075C, Daihan) at 45°C for 1 w. The dried peels were grinded using Waring Professional spice grinder (PG – 23096) and fine powder was stored in refrigerator in paper envelopes and in zip-lock bags until analysed.

### Preparation of lemon zest extract

To assess the effect of edible coatings on the phytochemical contents of lemon zest, extracts were prepared following the method of Maksimović *et al.* (2005) and Han *et al.* (2008). Briefly, 1 g powdered lemon zest was extracted with 50 mL 70% aqueous acetone under sonication for 20 min in sonicator (Soniclean PTY.LTD) at room temperature. After filtration, the extracts were used for biochemical analysis.

### Micro-nutrient component assays

#### Antioxidant activity by DPPH assay

Antioxidant abilities of extracts obtained from different coated lemons were subjected to DPPH assay following the method of Han *et al.* (2008). Briefly, 200 µL lemon zest extract were mixed with 2.7 mL DPPH (2, 2- diphenyl-1-picryl-hydrazyl). The concentration of DPPH solution used was 0.06 mM that was prepared in analytical grade methanol. After incubation for 30 min, the absorbance of the resultant mixture was measured at 515 nm using UV-Vis spectrophotometer (JascoV-670 UV-VIS-NIR Spectrophotometer Tokyo, Japan). The results were expressed as percent radical scavenging abilities using the formula:

$$\text{DPPH scavenging activity (\%)} = 1 - \frac{(S - SB)}{(C - CB)} \times 100$$

Eq.1

where, S = sample (lemon zest extract), SB = absorbance of blank sample (2 mL methanol + 0.2 mL lemon zest extract), C = absorbance of the control (2 mL DPPH + 0.2 mL methanol) and, CB = absorbance of the blank control (analytical grade methanol).

#### Antioxidant activity by ascorbic acid content

The content of ascorbic acid was evaluated

following the method of Barros *et al.* (2007). Briefly, 100  $\mu$ L lemon zest extract were added to 10 mL 1% metaphosphoric acid and left at room temperature for 45 min. Next, 1 mL of this homogenate was transferred to test tube containing 9 mL DCPIP dye (2, 6-dichlorophenolindophenol). After 30 min, the absorbance of the mixture was measured at 515 nm. The results were calibrated against the standard curve of L-ascorbic acid.

#### *Polyphenolic content*

##### *Total phenolic content*

The method of extraction of total phenolic content as mentioned by Waterhouse (2002) was performed on lemon zest extract. Briefly, 20  $\mu$ L lemon zest extract were mixed with distilled water (1.58 mL) and Folin-Ciocalteu reagent (0.1 mL). After leaving the mixture for 8 min, 0.3 mL sodium carbonate (25%) was added to the test tube. The tubes were further incubated at an ambient temperature in the dark for 2 h. The absorbance of the resultant mixture was measured at 765 nm. The results were expressed as mg/L gallic acid equivalents. Micro Folin-Ciocalteu method can specifically detect if extremely low levels of phenolic content are present in the samples. Standard curve of gallic acid was used to calibrate the experiment.

##### *Total flavonoid content*

The total flavonoid content of lemon zest extract was determined following the aluminium chloride colorimetric method (Hajlaoui *et al.*, 2009). Briefly, 250  $\mu$ L lemon zest extract were added to 1.25 mL distilled water and 75  $\mu$ L NaNO<sub>2</sub> (5%). After incubation for 6 min, 150  $\mu$ L AlCl<sub>3</sub>·6H<sub>2</sub>O (10%) and 0.5 mL NaOH (1 M) was added. The volume of the resultant mixture was made up to 2.5 mL with distilled water. The absorbance of the mixture was measured at 510 nm. Quercetin standard curve was used to calculate the flavonoid content in the samples.

##### *Total flavonol content*

Total flavonol content was determined following the method of Formagio *et al.* (2014). Briefly, 2 mL lemon zest extract were mixed with 3 mL (50 g/L) sodium acetate and 2 mL AlCl<sub>3</sub> (2%)/ethanol. The resulting mixture was vortexed and incubated at 20°C for 120 min. The absorbance after incubation period was measured at 440 nm using UV-Vis spectrophotometer (JascoV-670 UV-VIS-NIR Spectrophotometer Tokyo, Japan).

#### *Pigment content*

$\beta$ -carotene, lycopene, chlorophyll 'a' and chlorophyll 'b' contents were determined following the method of Bhumsaidon and Chamchong (2016). Briefly, 1 mL lemon zest extract was mixed with 10 mL of 4:6 (acetone: n-hexane). At 453 nm, 505 nm, 663 nm and 645 nm, the absorbances of the mixture were measured for different carotenoid and chlorophyll pigments. These pigments were calculated using the following formula:

$$\beta\text{-Carotene (mg/100 mL)} = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453} \quad (\text{Eq. 2})$$

$$\text{Lycopene (mg/100 mL)} = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453} \quad (\text{Eq. 3})$$

$$\text{Chlorophyll 'a' (mg/100 mL)} = 0.999A_{663} - 0.0989A_{645} \quad (\text{Eq. 4})$$

$$\text{Chlorophyll 'b' (mg/100 mL)} = -0.328A_{663} + 1.77A_{645} \quad (\text{Eq. 5})$$

#### *Total proanthocyanidin content*

The levels of proanthocyanidin in lemon zest extracts were determined by acid hydrolysis at 95°C as described by Majhenič *et al.* (2007). Briefly, 2 mL lemon zest extract was mixed with 20 mL ferrous sulphate solution (77 mg of FeSO<sub>4</sub>·7H<sub>2</sub>O was added to 500 mL of HCl:n-butanol in the ratio of 2:3). This mixture was incubated at 95°C for 15 min in a water bath. After cooling, absorbance of the mixtures was taken at 540 nm using UV-Vis spectrophotometer (JascoV-670 UV-VIS-NIR Spectrophotometer Tokyo, Japan). The results were expressed as the amount of proanthocyanidin in zest extract (mg PAC/g zest extract).

#### *Antibacterial activity of edible coating*

##### *Drop agar diffusion assay*

The edible coatings used to preserve lemons were evaluated for antibacterial potential. Five foodborne pathogens namely *Escherichia coli* ATCC 8739, *Vibrio parahaemolyticus* ATCC 17802, *Listeria monocytogenes* ATCC 13932, *Bacillus cereus* ATCC 11778 and *Vibrio alginolyticus* ATCC 17749 were selected as test microorganisms. The method of Lopes-Lutz *et al.* (2008) was used to assess the degree of inhibition these coatings provided against the selected pathogens. Zone of inhibition was observed in triplicates around the antibacterial coatings and tabulated in mm.

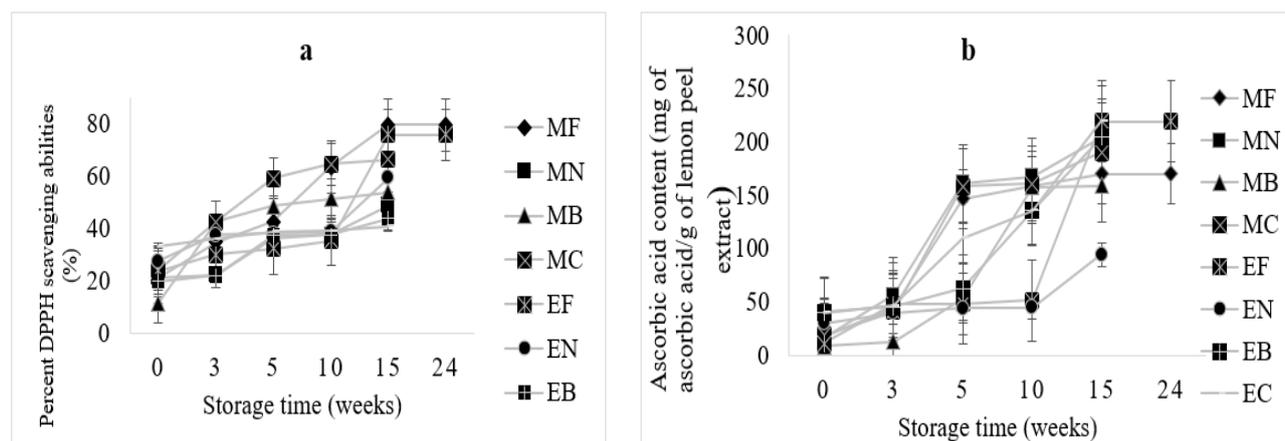


Figure 1. Effect of different edible coatings on antioxidant content in terms of (a) percent DPPH scavenging abilities, and (b) ascorbic acid content of lemon (peels) during storage (10°C, 80-85% relative humidity). The vertical bars represent the standard error of means of three replicates ( $n = 3$ ). MF; MN; MB; MC = guar gum coating added with methanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EF, EN, EB, EC = guar gum coating added with ethanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

#### Disk diffusion assay

Sterile paper discs impregnated with edible coatings were positioned on the surface of solidified Müeller Hinton agar for the detection of zone of inhibition (Oriani *et al.*, 2014). Triplicate results were expressed as zone of inhibition (mm) and tabulated.

#### Statistical analysis

Analysis of variance was employed to compute significant differences between the means, and Duncan's test at  $p < 0.05$  was used to separate means using Statistical Package for Social Science (SPSS software version 24, SPSS Inc., USA).

### Results and discussion

The aesthetic appearance of lemon peel was observed as an indicator for the estimation of the shelf life of lemons at low temperature. Green unripe lemons were collected, stored at low temperature and their maturity was delayed by the application of edible coatings. The edible coating that comprised of different lipid fractions that were extracted by two different solvent systems, were employed in this guar gum-based edible coatings. The lemons treated with different coating formulations exhibited different post-harvest storage life. The longest duration shelf life i.e. 24 weeks was observed for lemons that were coated with MF and EF edible coatings. The novelty of the present work is that these edible coating applications not only prolonged the shelf life of coated lemon fruits but also retarded the growth of contaminating pathogens. In MF and EF coated lemons, the fruits turned yellow and were in fully

ripened state at the end of shelf life study period. The untreated (uncoated; UN) control had storage life of 25 days while treated control (GG) decayed after 11 days. Two different parts of lemons were used to quantify the phytochemical contents namely lemon juice (reported in a different study) and lemon zest. The basic purpose of the present work was to report the effects on peel's phytochemical contents when the fruit was coated and subjected to storage. The micro-nutrient component assays were performed to give a detailed picture of the biochemical changes occurring in the peel.

#### Antioxidant capacity in terms of DPPH scavenging abilities

Antioxidant capacity of lemon zest extract was determined by the relative capacity of antioxidants present in extracts to scavenge high lipophilic DPPH (2,2-diphenyl-1-picrylhydrazyl) molecule. Antioxidant activity tends to increase as the fruit reaches maturity and declines when the fruit approaches senescence as observed by Shamloo *et al.* (2013) (Figure 1a). As the lemon fruits were collected in green, unripe stage, the antioxidant levels increased during the storage period. The rate of this antioxidant increase was significantly slowed down using these edible coatings. Lowest rate of antioxidant content increase was observed for EC (19.44%) coated lemon. Both the controls also had a significant increase in antioxidant activity (65% in GG and 20.82% in UN) but this rate of increase was observed during shorter duration of time, elucidating their failure to decelerate the antioxidant activities.

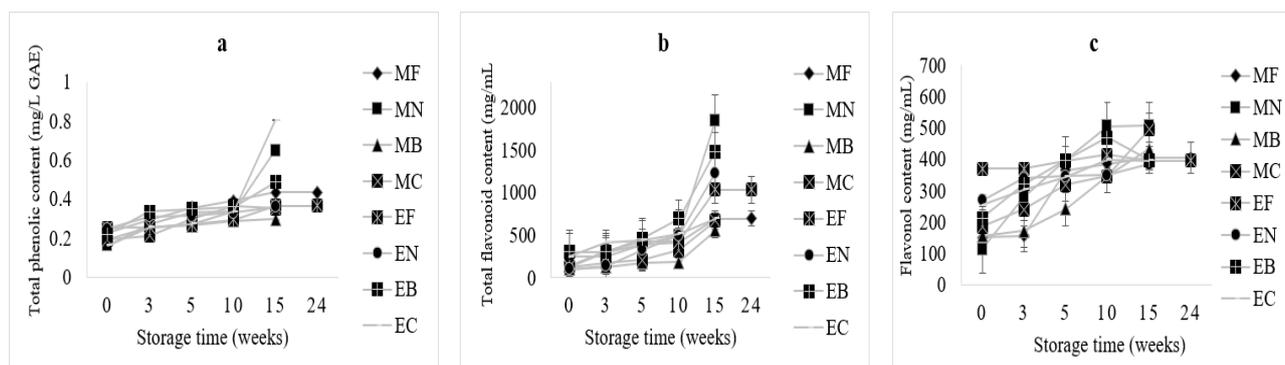


Figure 2. Effect of different edible coatings on polyphenols in terms of (a) total phenolic content, (b) total flavonoid and (c) total flavonol content of lemon (peels) during storage (10°C, 80-85% relative humidity). The vertical bars represent the standard error of means of three replicates ( $n = 3$ ). MF; MN; MB; MC = guar gum coating added with methanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EF, EN, EB, EC = guar gum coating added with ethanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

#### Antioxidant content in terms of ascorbic acid content

The ascorbic acid content of lemon zest extract showed an elevated amount during storage days (Figure 1b). This increase was sufficiently higher for both controls during shorter duration. The lowest rise in ascorbic acid was observed for EN coated lemon fruits. The rate of increase in ascorbic in GG was 34% and 67% in UN. Storage temperature and time are the major factors involved in the retention of vitamin C in fruits. Increase in time of storage causes the vitamin C losses in fruits. Different fruits show different retention capacities of vitamin C, and it is entirely dependent on the processing methods and packaging techniques (Uckiah *et al.*, 2009). The ascorbic acid content in fruits increase during ripening process and once the fruit has approached maturity it begins to decline during the senescence (Rahman *et al.*, 2016). The factors that accelerate the vitamin C losses during storage of fruits are higher temperatures, low relative humidity, chilling injuries and physical damages. In addition to these factors, enzymes also cause the degradation of vitamin C. Ascorbic acid is catalysed to dehydroascorbic acid by the activity of enzyme ascorbate oxidase, thus leading to enzymatic degradation (Lee and Kader, 2000).

#### Polyphenolic content

##### Total phenolic content

Like antioxidant activity, total phenolic content also showed an increasing trend throughout the storage period of lemon fruits. This rate was lowest in lemon fruit coated with EF *i.e.*, 30.70% (Figure 2a). Phenolic compounds are well known to exhibit

health benefits. Fruit peels are rich sources of these compounds and contribute to health benefits and disease prevention by scavenging reactive oxygen species such as superoxide radical, hypochlorous acid, hydrogen peroxide and hydroxyl radicals (Rafiq *et al.*, 2018). The release of phenolic compounds results from plant defence mechanism during chilling injury (Artés-Hernández *et al.*, 2007). The rate of increase in total phenolic content was 62.6% in GG and 60.78% in UN during 11 and 25 days of storage.

##### Flavonoid and flavonol content

A gradual increase was observed in the contents of flavonoid and flavonol in lemon peels. Previous studies reported the same results for strawberries (Gil *et al.*, 1997), pears (Amiot *et al.*, 1995; Price *et al.*, 1997) and onion bulbs (Price *et al.*, 1997) during cold storage. The flavonoid content increased at a faster rate in both controls (UN and GG) as compared to treated samples *i.e.*, 86.37% in GG and 41.9% in UN. This rate was slowest in lemon peels which had the coating of MB (81.82%) for the period of 15 weeks. Similar results were observed for flavonol contents (Figure 2b and 2c). The lowest rate of flavonol content increase was observed in peels coated with EF *i.e.*, 16% for the period of 24 weeks. These rates were significantly different from both the controls (75.5% in GG and 73.73% in UN) as the flavonol contents were elevated drastically during shorter duration of storage life.

##### Pigments content

Carotenoids are fat-soluble pigments which contribute to the yellowish colour of food commodities. They prevent the oxidative damage in the cell and are a lipophilic radical scavenger.

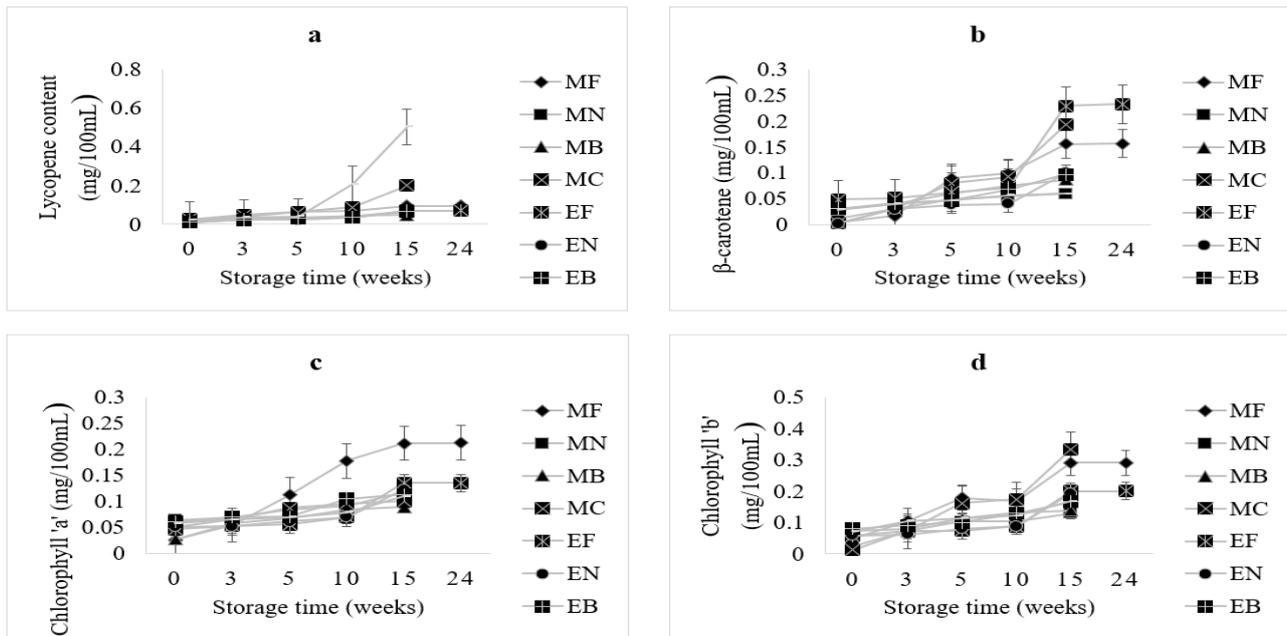


Figure 3. Effect of different edible coatings on pigment contents (a) lycopene (b)  $\beta$ -carotene (c) chlorophyll 'a' and (d) chlorophyll 'b' contents of lemon (peels) during storage ( $10^{\circ}\text{C}$ , 80-85% relative humidity). The vertical bars represent the standard error of means of three replicates ( $n = 3$ ). MF; MN; MB; MC = guar gum coating added with methanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EF, EN, EB, EC = guar gum coating added with ethanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

Carotenoids such as  $\beta$ -carotene and lycopene were quantitatively determined in lemon peel extracts (Figure 3a and 3b). The slowest increase in carotenoid contents was observed in EF and EC for lycopene and  $\beta$ -carotene, respectively. Carotenoids are present in abundant levels in lemon peels which belong to provitamin A carotenoids ( $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin). Derivatives of  $\alpha$ -carotene are present in lemon zest. The carotenoid content varies with cultivar, maturity, season, climate and tissue type. In general, the contents of carotenoids increase with increasing intensity of yellow pigmentation of lemon fruits during the early phase of fruit growth. The intensity of colour correlates with the content of chlorophyll pigments and their respective proportions. In the present work, the chlorophylls 'a' and 'b' gradually decreased and carotenoids subsequently increased during the ripening process (Figure 3c and 3d). The level of chlorophyll 'b' decreased at a lesser rate as compared to chlorophyll 'a' (Win *et al.*, 2006). This breakdown of chlorophyll pigment is an important part of ripening process. Pheophorbide- $\alpha$ -oxygenase pathway (PaO) is involved in chlorophyll catabolic pathway. This pathway converts the pheide-a (green) chlorophyll into red chlorophyll catabolite (Hörtensteiner, 2006). The rate of increase in  $\beta$ -carotene, lycopene, chlorophylls 'a' and 'b' in GG was 18.4%, 19.75%, 5%, 12.7% while in UN was 20%, 84.3%, 25% and 61.5%, respectively.

#### Proanthocyanidin content

Proanthocyanidin are plant polyphenols involved in antioxidative reactions in the fruits. These classes of phenolic compounds contribute significantly to sensory quality and aesthetic attributes of fruits. In the present work, proanthocyanidin content did not significantly increase in the peel extracts obtained from different treatments of lemon fruits. The lowest rate of increase was observed for EN coated lemons (Figure 4). The rate of increase in proanthocyanidin content in GG and UN was 45%

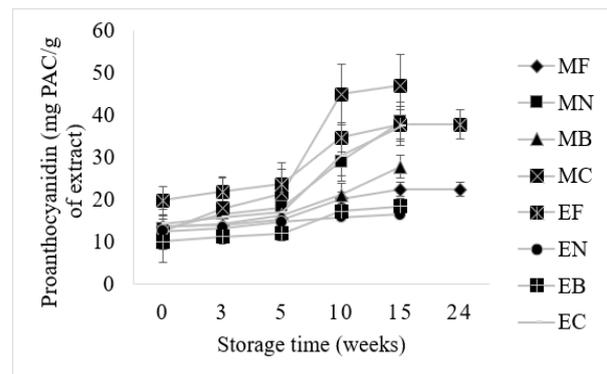


Figure 4. Effect of different edible coatings on proanthocyanidin content of lemon (peels) during storage ( $10^{\circ}\text{C}$ , 80-85% relative humidity). The vertical bars represent the standard error of means of three replicates ( $n = 3$ ). MF; MN; MB; MC = guar gum coating added with methanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EF, EN, EB, EC = guar gum coating added with ethanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively.

Table 1. Antibacterial activity of edible coatings by disk diffusion assay.

Coating formulation	Zone of inhibition (mm)				
	<i>Vibrio alginolyticus</i> ATCC 17749	<i>Vibrio parahaemolyticus</i> ATCC 17802	<i>Escherichia coli</i> ATCC 8739	<i>Bacillus cereus</i> ATCC 11778	<i>Listeria monocytogenes</i> ATCC 13932
MB	12.67 ± 0.20 <sup>ab</sup>	11.00 ± 0.10 <sup>a</sup>	15.33 ± 0.15 <sup>c</sup>	13 ± 0.20 <sup>a</sup>	16.67 ± 0.41 <sup>a</sup>
MC	11.67 ± 0.23 <sup>ab</sup>	13.33 ± 0.15 <sup>a</sup>	19 ± 0.1d	12.33 ± 0.15 <sup>a</sup>	14.26 ± 0.20 <sup>a</sup>
MF	13.67 ± 0.30 <sup>ab</sup>	11.67 ± 0.15 <sup>a</sup>	21 ± 0.1d	14.67 ± 0.15 <sup>a</sup>	16.67 ± 0.20 <sup>a</sup>
MN	14.33 ± 0.30 <sup>b</sup>	18.67 ± 0.15 <sup>b</sup>	25 ± 0.1e	25 ± 0.20 <sup>b</sup>	12.33 ± 0.20 <sup>a</sup>
EC	12.33 ± 0.57 <sup>ab</sup>	13.33 ± 0.15 <sup>a</sup>	9.66 ± 0.41 <sup>a</sup>	10.67 ± 0.49 <sup>a</sup>	13 ± 0.10 <sup>a</sup>
EN	16.33 ± 0.35 <sup>b</sup>	13.32 ± 0.15 <sup>a</sup>	14.33 ± 0.23 <sup>b,c</sup>	14.33 ± 0.23 <sup>a</sup>	15.33 ± 0.40 <sup>a</sup>
EB	9.00 ± 0.36 <sup>a</sup>	13.33 ± 0.55 <sup>a</sup>	10.67 ± 0.20 <sup>ab</sup>	10.67 ± 0.20 <sup>a</sup>	12.33 ± 0.05
EF	14.00 ± 0.1 <sup>b</sup>	16.67 ± 0.28 <sup>b</sup>	13.33 ± 0.15 <sup>ab,c</sup>	15.33 ± 0.25 <sup>a</sup>	13 ± 0.10 <sup>a</sup>
Control (GG + glycerol)	14 ± 0.1 <sup>b</sup>	N.D.	N.D.	N.D.	13 ± 0.10 <sup>a</sup>

Data are means of triplicates ( $n = 3$ ) ± SD. Data with different superscripts in the same column were significantly different at  $p < 0.05$ . MF; MN; MB; MC = guar gum coating added with methanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively; EF, EN, EB, EC = guar gum coating added with ethanolic essential oil of fennel, nigella seeds, bay leaf and coriander seeds, respectively. N.D. = no detection of antimicrobial activity.

and 89%, respectively. Proanthocyanidin content does not change significantly during ripening process of fruits and vegetables. Flavonoid compounds result in the bitter and astringent taste of many fruits. Specifically, in citrus fruits, bitter taste is the result of the presence of specific phytochemicals such as naringin, neohesperidin and limonin. Monomers of these proanthocyanidin compounds give bitter flavour as compared to astringent taste of the fruit. The increase in the size of the proanthocyanidin molecules astringent taste is relative to the bitterness. This capacity continues until the molecules become too big to remain soluble. The extremely bitter flavour of flavedo and albedo of lemon fruits are the indicators of the presence of proanthocyanidin molecules in that portions (Senter *et al.*, 1992).

These results indicated that not only edible coatings such as guar gum-based edible coatings could delay the rate of bioactive compounds, but this rate was further decelerated by the supplementation of lipid fractions which provided a hydrophobic barrier, thus slowing the rate of biochemical reactions. Comparing the results of both controls, it could be concluded that GG coated lemon decayed earlier but the rate of biochemical reactions was higher in UN as compared to GG, indicating the significance of a barrier in the form of a coating.

#### Antibacterial activity of edible coatings

The antibacterial activity of edible coating *in vitro* was analysed using disk diffusion assay and drop agar diffusion assay. The results were expressed in the form of respective zone of inhibition around the well/disk containing the antibacterial agent. The LFs of coriander seeds, nigella seeds, fennel seeds

and bay leaves are known to possess a wide spectrum of antibacterial potential against Gram-positive and Gram-negative bacteria. The antibacterial activities of these LFs as supplemented in the concentration of 0.2% in edible coatings were evaluated. The control having the composition of guar gum + glycerol also exhibited antibacterial activity against *Listeria monocytogenes* ATCC 13932 and *Vibrio alginolyticus* ATCC 17749 only when assayed through the disk diffusion assay. However, no zone of inhibition was observed against *Escherichia coli* ATCC 8739, *Bacillus cereus* ATCC 11778 and *Vibrio parahaemolyticus* ATCC 17802. The edible coating supplemented with methanolic and ethanolic LFs exhibited activities against all the tested food isolates. The highest zone of inhibition was observed for methanolic LF supplemented edible coating against *E. coli* ATCC 8739 having zone of inhibition of 25 mm (Table 1). The genus *Escherichia* is involved in the contamination of food products during processing or packaging. *Escherichia coli* are a Gram-negative bacterium with outer cellular membrane that aids in the virulence of this pathogen (Burt, 2004). Despite this pathogenic characteristic of *E. coli*, the lipid fraction could combat this bacterium even in the agar matrix. The drop agar diffusion method was also used to screen the antibacterial activity of the edible coatings. The results obtained exhibited that ethanolic LF supplemented edible coatings (EN/EF/EB/EC + glycerol + guar gum) failed to generate any zone of inhibition against all the tested pathogens. On the contrary, methanolic LFs supplemented edible coatings yielded zone of inhibition against *E. coli* ATCC 8739 which were 11, 13, 8 and 9 mm for MF, MN, MB and MC formulated coatings, respectively.

Control (GG + glycerol) failed to inhibit any test culture. From these results, it could be concluded that when methanolic LFs were tested for antibacterial potential either by disk diffusion method or by agar well diffusion method, they proved to be potent against Gram negative bacterium i.e., *E. coli* ATCC 8739. It could also be said that these edible coating formulations are more antibacterial in action against Gram-negative isolates as compared to the Gram-positive ones. These results justify that if these edible coatings are applied to the fruits during post-harvest storage they might not get contaminated by pathogenic bacteria. Another remarkable observation is that these LFs when added to the edible coatings possessed antibacterial properties even at very low concentrations i.e., 0.2%. This makes this coating formulation as cost-effective apart from being edible, GRAS (generally regarded as safe) and biodegradable.

#### *Antibacterial activity of edible coatings with respect to the compounds in lemon zest extract*

The main compounds of lemon zest extract that were measured in the present work were antioxidant content in terms of radical scavenging abilities, ascorbic acid content, total phenolic, flavonoid, flavonol, proanthocyanidin, carotenoid and chlorophyll contents. The lemon zest extract that was prepared from lemon peels had varying degree of micro-nutritional contents. The antioxidant content in terms of radical scavenging abilities of MB, MN, MF, MC, EB, EN, EF and EC were measured as 53.72%, 48.79%, 79.53%, 66.49%, 43.77%, 59.34%, 75.78% and 40.89%, respectively. The antioxidant content in terms of ascorbic acid content of MB, MN, MF, MC, EB, EN, EF and EC were measured as 158.8 mg/L, 204 mg/L, 170.1 mg/L, 190 mg/L, 204.52 mg/L, 94.07 mg/L, 219.42 mg/L and 219.62 mg/L, respectively. The total phenolic content of MB, MN, MF, MC, EB, EN, EF and EC were measured as 0.29 mg/L GAE, 0.65 mg/L GAE, 0.43 mg/L GAE, 0.35 mg/L GAE, 0.48 mg/L GAE, 0.36 mg/L GAE, 0.37 mg/L GAE and 0.80 mg/L GAE, respectively. The total flavonoid content of MB, MN, MF, MC, EB, EN, EF and EC were measured as 559 mg/mL, 1849 mg/mL, 698.6 mg/mL, 669.2 mg/mL, 1,483.6 mg/mL, 1,231 mg/mL, 1038.2 mg/mL and 693.3 mg/mL, respectively. The total flavonol content of MB, MN, MF, MC, EB, EN, EF and EC were measured as 432.4 mg/mL, 507.8 mg/mL, 405.2 mg/mL, 495.8 mg/mL, 398 mg/mL, 384 mg/mL, 414.4 mg/mL and 396 mg/mL, respectively. The proanthocyanidin content of MB, MN, MF, MC, EB, EN, EF and EC were measured as 27.72 mg PAC/g of

extract, 38.44 mg PAC/g of extract, 27.72 mg PAC/g of extract, 47.04 mg PAC/g of extract, 18.42 mg PAC/g of extract, 16.48 mg PAC/g of extract, 37.78 mg PAC/g of extract and 37.34 mg PAC/g of extract, respectively. The lycopene content of MB, MN, MF, MC, EB, EN, EF and EC were measured as 0.049 mg/100 mL, 0.06 mg/100 mL, 0.09 mg/100 mL, 0.2 mg/100 mL, 0.06 mg/100 mL, 0.06 mg/100 mL, 0.07 mg/100 mL and 0.2 mg/100 mL, respectively. The  $\beta$ -carotene content of MB, MN, MF, MC, EB, EN, EF and EC were measured as 0.08 mg/100 mL, 0.05 mg/100 mL, 0.15 mg/100 mL, 0.193 mg/100 mL, 0.09 mg/100 mL, 0.1 mg/100 mL, 0.23 mg/100 mL and 0.06 mg/100 mL, respectively. The chlorophyll 'a' content of MB, MN, MF, MC, EB, EN, EF and EC were measured as 0.08 mg/100 mL, 0.11 mg/100 mL, 0.21 mg/100 mL, 0.101 mg/100 mL, 0.11 mg/100 mL, 0.12 mg/100 mL, 0.13 mg/100 mL and 0.11 mg/100 mL, respectively. The chlorophyll 'b' content of MB, MN, MF, MC, EB, EN, EF and EC were measured as 0.13 mg/100 mL, 0.13 mg/100 mL, 0.29 mg/100 mL, 0.33 mg/100 mL, 0.16 mg/100 mL, 0.19 mg/100 mL, 0.2 mg/100 mL and 0.17 mg/100 mL, respectively. However, the antibacterial activities of MB, MN, MF, MC, EB, EN, EF and EC were observed against *E. coli* ATCC 8739 as 15.33 mm, 25 mm, 21 mm, 19 mm, 10.67 mm, 14.33 mm, 13.33 mm and 9.66 mm, respectively. From the data of micro nutritional component analysis and antimicrobial activities of respective edible coatings, it could be concluded that the methanolic LFs supplemented had higher levels of compounds as well as these compounds were active in antibacterial action. Further study could be directed towards the isolation of these compounds and to find out their biological activities *in vitro* and *in vivo* with respect to their applications in food preservation.

#### **Conclusion**

The present work demonstrated that the proposed edible coatings could be used to extend the shelf life of lemons during cold storage with remarkable results obtained for MF and EF coated lemons ( $p \leq 0.05$ ). These coatings also successfully delayed the variations in phytochemicals present in lemon zest up to 180 days. These coatings also exhibited considerably high levels of antimicrobial potentials against foodborne pathogens especially the Gram-negative strains *in vitro*. *In vivo*, these coatings successfully inhibited the growth of contaminating microorganisms including fungus. Therefore, the present work proposed the potential application of guar gum-based edible coatings containing lipid

fractions to enhance the safety of foods and food products by slowing down the rate of microbial contamination. Further studies are henceforth suggested to investigate the active components in these lipid fractions to be utilised in pure forms in the development of antimicrobial packaging systems.

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