Journal

J. Biol. Chem. Environ. Sci., 2018, Vol. 13(4): 177-202
http://biochemv.sci.eg

EVALUATION OF JOJOBA WAX AS ANTIMICROBIAL AND ANTIOXIDANT TO APPLIED IN EDIBLE COATING FOR MAINTAINING QUALITY ATTRIBUTES OF FRESH-CUT EGGPLANT SLICES

Ammar M.S. [1] and Rabab H. salem[2]

[1]Food Science and Technology Department, faculty of Agriculture, Al- Azhar University, Cairo, Egypt.
[2]Food Science and Technology Department, Faculty of Home Economic, Al- Azhar University, Tanta, Egypt.

ABSTRACT

This research was aimed to evaluating jojoba wax as antimicrobial and antioxidant agent and study its application in sodium alginate edible coating for maintaining quality attributes of fresh-cut eggplant (*Solanum melongena*) slices during refrigerated storage at 4^{o}C. The results indicated that jojoba wax had high content of total monounsaturated fatty acid, the main fatty acid identified was gadoleic acid (20:1) (70.7%) followed by erucic acid (22:1) (14.2%) while oleic acid (18: 1) content was 8.7 %.The results indicated that DPPH scavenging capacity of jojoba wax is lower than that of ascorbic acid since the IC_{50} values were 99.18 and 14.20 µg/ml for jojoba wax and ascorbic acid; respectively. The results revealed that jojoba wax exhibited better inhibition activities against gram-positive than gram-negative bacteria. The minimum inhibitory concentration (MIC) of jojoba wax against tested bacteria ranged from 198 to 219 ul/ml. The antifungal activity of jojoba wax ranged between 4950 and 8750 AU/ml against *Fusarium oxysporum and Aspergillus parasiticus*; respectively. The coated fresh-cut eggplant slices were had higher lightness than that of control sample. Fresh-cut eggplant slices coated / uncoated had gradual increment in firmness values with

increased the storage time. The addition of jojoba wax to the edible coating result in lowering the microbial growth.

Keywords: Antimicrobial, antioxidant, eggplant, firmness, fresh- cut, jojoba wax, minimal processing,

INTRODUCTION

Consumers are exigent for foodstuffs that require minimal process, for example, fresh-cut vegetables because of busy lifestyles, an increase in health appreciation and increased financial capacity of the consumer **(Siew *et al.*, 1999)**. Fresh-cut vegetables are ready-to-use minimally processed foods **(Cantwell and Suslow, 2002)**, which highly nutritious but highly shabby because removing the peel or altering the size that lead to leakage of nutrients, quickened enzymatic reactions, rapid microbial growth, color change, texture change and weight losses, resulting in quality deterioration **(Sonti, 2003; Soliva and Martin, 2003 and Lafortune *et al.*, 2005)**. Many techniques have been used to overcome these problems and extend the shelf life of fresh produce from which applying of edible coating to the surface of products by dipping, spraying or brushing to create a modified atmosphere and act as a replacement of natural protective peel to provide a barrier to moisture, oxygen and solute movement **(McHugh and Senesi, 2000 and Suyatma *et al.*, 2005)**, control microbial growth, preserve the color and texture, which effectively extend the shelf life of the product **(Terzopoulou et al., 2003; Sonti, 2003; Lin and Zhao, 2007)**. Edible coatings may be composed of polysaccharides such as starch, cellulose and alginate or proteins such as collagen, zein, soybean, gluten proteins, and milk proteins or fats such as beeswax and fatty acids derivatives **(Casariego *et al.*, 2008)**. Polysaccharides edible coating are excellent oxygen, aroma, and oil barriers and provide strength and structural safety **(Krochta, 2001 and Falguera *et al.*, 2011)**. Alginates are structural polysaccharides extracted from brown algae comprising (1-4) linked units of β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) at different proportions and different distributions in the chain **(Hambleton *et al.*, 2011)**. Most applications of alginate is based on its gel-forming ability through cations binding (**Galus and Kadzinska, 2015)**. Sodium alginate-based edible films can be used to limit dehydration of meat, fish fruits and vegetables **(Pop *et al.*, 2015)**. Edible coatings can be

incorporated with antimicrobial and antioxidant agents to reduce enzymatic browning, microbial growth and pathogen growth on food surfaces which enhance the quality and extend the shelf life of coated foods **(Cutter, 2006; Vargas *et al.*, 2008 and Rojas-Grau *et al.*, 2009)**. Jojoba (*Simmondsia chinensis*) is an important oilseed crop contains about 50 % of liquid wax called jojoba oil which differs radically from other vegetable oils since it, contains no glycerides or glycerol, it is composed of fatty acids connected directly to fatty alcohols forming esters (straight chain alcohols and straight chain acids each have one double bond) and contains natural antioxidants (alpha-, gamma-, and delta-tocopherols), which occur in concentrations of about 50 ppm (**Léon *et al.*, 2004 ; El-Mallah and El-Shami, 2009)**. Raw jojoba wax has few impurities so it requires little or no refining **(Wisniak, 1994). Pooja *et al.* (2016)** reported that jojoba wax can be used as natural preservative in food against some bacterial species which cause food-borne diseases and food spoilage such as *E. coli*, *Pseudomonas aeruginosa*, *Kbebsiella pnuemoniae* and *Staphylococcus aureus*. Eggplant (*Solanum melongena*) fruit is a popular vegetable crop grown in the subtropics and tropics characterized with low calorie content, high content of pro-vitamin A, thiamine, riboflavin, niacin, ascorbic acid and polyphenols. Due to these aspects, the eggplant exhibits a high antioxidant potential and consequently help to prevent chronic and degenerative illnesses **(Sarker *et al.*, 2006; Nisha *et al.*, 2009; San José *et al.*, 2013 and Zaro *et al.*, 2014).** Eggplant is highly perishable due to the exposure of their internal cellular tissues caused by peeling and cutting which increase metabolism, respiratory rate, ethylene production, water loss, softening and microbial spoilage **(Manca *et al.*, 2017).** This research is aimed to studying the effect of addition jojoba wax to edible coating formulation on preserving quality and shelf-life of fresh-cut eggplant slices.

MATERIALS AND METHODS

Materials:

Plant material: Fresh Eggplant (*S. melongena*) of **dark** purple colour big size variety, at commercial maturity stage was obtained from Obour market, Cairo Governorate, Egypt.

Jojoba seeds: Jojoba seeds (*Simmondsia chinensis*) used in this study was obtained from Oil Crops Research Dept., Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

Coating materials: Food grade Sodium alginate **was obtained** from Morgan Industrial Chemical Co**mpany.**

Analytical chemicals and microbiological medium: Analytical chemicals and microbiological medium were purchased from EL-Gamhouria Trading Chemicals and Drugs Company.

Microorganism: ***Bacillus cereus*** **(ATCC11778),** ***E.coli*** **(ATCC11229),** ***Salmonella typhi*** **(ATCC14028),** ***Shegalla sonni*** **(ATCC29930),** ***Staphylococcus aureus*** **(ATCC33591),** ***Enterococcus faecalis*** **(ATCC47077),** ***Pesudomonas aeruginosa*** **(ATCC15692) and** ***Listeria monocytogenes*** **(ATCC19111**) *Aspergillus niger* (ITEM 3856), *Aspergillus parasiticus* (ITEM 11), *Aspergillus carbonarius* (ITEM5010), *Aspergillus flavus* (ITEM 698). *Aspergillus oryzae* (ITEMB5), *Fusarium oxysporum* (ITEM 12591), *Alternaria alternata* (ITEM) were brought from Mycobank at Institute of Food Science and Agriculture (ISPA), CNR, Bari, Italy.

Methods:

Extraction of jojoba wax:

After cleaning, jojoba seeds were manually extracted by cold hydraulic press at Fat and Oil Research Dept., Food Technology Research Institute, Agricultural Research Center, Giza, Egypt. Pressing technique was used for extracting the liquid wax as described by **(Tobares *et al.*, 2003).** The oil collected from the press was then filtered to remove any solid impurities and stored at 4 ± 1^{0}C.

Preparation of edible coatings:

Sodium alginate coating solution, were formulated as described by **Rojas-Graü *et al*., (2007)** as follows two grams of sodium alginate was dissolved in 100 ml distilled water and heated at 70°C until the solution became clear. Jojoba oil (1, 3, 5 ml/100 ml coating solution) was mixed with the coating solution by using a blender (model Braun KM 32).

Preparation of fresh-cut coated eggplant slices:

Fresh eggplant fruits with similar size, shape, color and lack of defects were selected, washed with tap water to remove surface impurities. Then, peeled and sliced into 1 cm thickness slices with a

sharp sterilized knife. The prepared eggplant slices were divided into five batches, the first batch used as control (uncoated), while the second batch coated with sodium alginate only (SA), where the other three batches coated with sodium alginate containing jojoba wax with different levels; sodium alginate/1% jojoba wax (SA/JW1); sodium alginate/3 %jojoba wax (SA/JW3) and sodium alginate/5 % jojoba wax (SA/JW5) by dipping for 10 minutes. Then, removed and placed on a wire tray to drain for 10 minutes. Eggplant slices for each treatment were packed in polypropylene plastic bags, then stored at 4 ± 1^{0}C and analyzed at 0, 3 and 6 days.

Analytical Methods:

1-Chemical characteristics of jojoba wax:

Iodine value, peroxide value and acid value of jojoba wax were determined according to the method of **AOCS (1997)**, while saponification value and % unsaponifiable matter: were determined according to the method of **AOAC (2000)**.

2-Evaluation of fatty acids profile of jojoba wax:

Fatty acids of methyl esters (FAME) of jojoba wax was measured according to **Alves and Bessa (2009)** at Food Technology Department, National Research Centre, Dokki, Giza, Egypt. Diluted FAME separated on an HP 5890 series II (Hewlett-Packard, Palo Alto, USA) equipped with an Innowax capillary column (30 m_0.20 mm_0.20 mm). The flow rate of carrier gas (hydrogen) 1.5mL/min. The column temperature was isotherm at 210°C. Detector and injector temperatures set at 240°C. Fatty acids identified by comparison of the retention times with authentic standards.

3-Determination of antioxidant and antimicrobial activity:

Preparation of extract from jojoba wax:

Jojoba wax treated with methanol and hexane in order to extract the bioactive phytochemicals **(Gutfinger, 1981)**. About 10 g of oil dissolved in 10 mL of hexane. The hexane layer sequentially extracted with 20 mL of 60% methanol (three times). The methanolic extracts pooled and evaporated in the rotary evaporator (Heidolph, 4010, Germany) to remove the solvent. The residue then dissolved in methanol (3mL) and stored at -20C until to determine the antioxidant and antimicrobial activity.

Radical-scavenging (DPPH):

The free radical scavenging activity was determined spectrophotometrically by the 1, 1-diphenyl 2-picrylhyorazyl (DPPH) assay **(Sanchez-Moreno, 2002)**. Gradual concentrations of jojoba wax extract and ascorbic acid as standard (between 25 and -100 μg/ml) were prepared and added to 1.95 ml of DPPH (0.025 mg/ml) prepared in methanol. After thirty minutes, the absorbance was measured at 515 nm using a spectrophotometer (BioTek, Synergy HT, USA). The capability of scavenging the DPPH radical was calculated by using the following equation:

$$\% \text{ scavenging} = [(A_0 - As)/A_0] \times 100$$

Where,

A_0 = the control absorbance (DPPH radical plus methanol without extract)

As = the sample absorbance

Radical-scavenging (ABTS):

The ABTS radical cation was prepared by adding 7 mM stock solution of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and 2.45 mM potassium per-sulfate together in 10 mL distilled water , stand overnight in the dark at room temperature **(Arnao *et al.*, 2001)**. Several concentrations of jojoba wax extract and ascorbic acid as standard (25–100 μg/ml) were prepared and added to 1 ml ABTS solution. The solutions were allowed to stand for 8 min. A control and blank was also performed simultaneously. The absorbance was read at 734 nm using a spectrophotometer (BioTek, Synergy H, USA).

Radical-scavenging (Nitric oxide):

The scavenging activity determined according to the method of **Garrat (1964).** The reaction mixture (3 ml) containing sodium nitroprusside (10mM, 2 ml), phosphate buffer saline (0.5 ml) and several concentrations of jojoba wax extract and ascorbic acid as standard (25–100 μg/ml) were incubated at 25 °C for two hours. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthyl-ethylene di-amine dihydrochloride (1%) was added, mixed and allowed to stand for 30 min. The absorbance of these solutions was measured at 540 nm using

a spectrophotometer (BioTek, Synergy HT, USA) against the corresponding blank.

4-Antibacterial activity of jojoba wax extract:

Agar diffusion test:

The bacterial inoculum uniformly spread using a sterile glass rod on a sterile petri dish contain PDA medium. Fifty microliters of jojoba wax extract added to each of the 5 wells (5 mm diameter holes cut in the agar gel, 10 mm apart from one another). The systems incubated for 24 hr at 37±1°C, under aerobic conditions. After incubation, confluent bacterial growth observed. Inhibition of the bacterial growth measured in millimeter (mm) **(Marston, 2011)**.

Disk diffusion test:

Agar plates prepared and the test microorganisms inoculated by the spread plate method **(CLSI, 2004)**. Whatman filter paper No. 1 approximately 6 mm in diameter (which are placed in a petri dish and sterilized in a hot air oven), soaked in 15 µL of jojoba wax extract and placed in the previously prepared agar plates. Each disc pressed down to ensure complete contact with the agar surface and distributed evenly so that they are no closer than 24 mm from each other, center to center. The agar plates then incubated at 37°C. After 16-18 h of incubation, each plate examined. The resulting zones of inhibition uniformly circular with a confluent lawn of growth.

Determination of minimal inhibition concentration (MIC):

The Minimum Inhibitory Concentration (MIC) determined by a microdilution method using serial concentration according to the **(Wiegand *et al*., 2008)**. The microorganism suspension of 50 µL added to the broth dilutions. These were incubated for 18 h at 37°C. The MIC of jojoba wax extract taken as the lowest concentration that did not give any visible bacterial growth.

Determination of fungal growth:

Plates were prepared by adding the mould (10^6 spores per 1.5 to 20% of Yeast Extract Sucrose (YES) medium). About 10µL of jojoba wax was spotted onto the sensitive mould plates. Flasks containing mycelia were filtered through Whatman filter paper no. 1 and then were washed with distilled water. The mycelia were placed on pre-weighed petri plates and were allowed to dry. The flasks containing dry mycelia were weighed **(Difco-Manual, 1984)**.

Determination of antifungal activity evaluation:

Antifungal activity evaluation of jojoba wax extract assessed by spot-on-the lawn assay **(Hoover and Harlander, 1993)**. Antifungal activity was expressed as arbitrary units (AU/mL) according to **Delgado *et al.* (2005)**.

AU/ml = (the highest dilution exhibiting inhibition zone X 1000) / Volume (µL).

5-Physical quality of coated fresh-cut eggplant:

Color: Color of samples was measured according to described by (**Amin *et al.*, 2014)** using a color meter (Konica Minolta, CR- 300, Germany). Color was measured using the CIE L*, a*, b* scale. The L* represents color lightness (0 = black and 100 = white). The a* scale indicates in the maximum the red (+a*) and in the minimum the green color (-a*) while the b* axis ranged from yellow (+b*) to blue (−b*). To determine darkening during storage, the color of each fruit was evaluated just after cutting and again after the different storage periods.

Firmness measurement: Firmness was measured as described by **(Chen and Opara, 2013)**, in order to obtain the maximum penetration force expressed in Newton (N) using a digital pressure tester provided with cone head (force-Goug, FGV100XY, Shimpo instruments).

6- Microbiological quality of fresh-cut eggplant coated:

Determination of total aerobic bacterial count: Total aerobic bacterial counts was determined using plate count agar medium **(Difco-Manual, 1984)**. The plates of bacteria medium were inverted and incubated at 37°C for 48 hrs. Following the appropriate length of incubation, the colonies were counted by using 3 replicates. The data was expressed as (log /g).

Determination of yeast and mould count: Yeast and mould count was determined using potatoes dextrose agar medium **(Difco-Manual, 1984)**. The plates were incubated at 20 – 25ºC for 5 days. If excessive growth develops, colonies counted first after 3 days and then again after 5 days, and reported as yeast and mould count per gram.

7- Statistical Analysis:

Results were analyzed using SPSS (version16.0 software Inc. Chicago, USA) **(SPSS, 1998)**. Treatment means were compared using least signification differences (LSD) at 0.05 levels of probability. Values are expressed as a mean ± SD; n=3.

RESULTS AND DISCUSSION

Chemical characteristics of jojoba Wax:

Table 1 shows the chemical characteristics of jojoba wax. The Iodine value is the amount of iodine (in grams) necessary to saturate 100g of oil sample and is a measure of the amount of unsaturation in fats and oil as well as an indicator of their susceptibility to oxidation **(Knothe, 2006).** The data pointed that iodine value was (80.5 g I_2/100 g) for jojoba wax, which reflect its high unsaturated fatty acids content. Which is supported by fatty acid composition **(Table 2).**

Fresh oils contain trivial peroxides due to the self- protection via the presence of assured natural antioxidant **(Fahimdanesh and Bahrami, 2013)**. From the results it could be noticed that peroxide value (meqO_2/kg) of jojoba wax 0.95meqO_2/ kg, which indicate that it can be stored for a long period without deterioration. Also, **Table 1** shows the acid value of jojoba wax which indicates that the free fatty acid content reach to 0.97 mg KOH/g which in agree with the findings of **Sandha and Swami (2008)**.

The chain length and the molecular weight of fatty acids could be supposed by estimating the saponification value. The data show that saponification value for jojoba wax was 86 mg/g KOH. The saponification value indicates the polar nature of jojoba oil, which can impart rust protection, antifoaming, and oily characteristics. Moreover, the saponification and iodine values of jojoba wax are relatively low compared with other vegetable oils, such as castor, soybean and rapeseed, suggesting better stability **(Sivasankaran *et al.*, 1998**).

Table 1: Quality characteristics of jojoba wax under study

Characteristic	Value
Iodine Value (mg I_2/g)	80.50±0.10
Peroxide Value (meqO_2/kg)	0.95±0.01
Acid Value (mg KOH/g)	0.97±0.01
Saponification Value (mg/g KOH)	86±0.50
Unsaponification Matter (%)	48.50±0.44

All data were mean of triplicate determinations.

Fatty acids profile of jojoba wax:

Table 2 shows fatty acid composition (%) of jojoba wax. The results indicate that saturated fatty acids were found to be compose mainly of Palmitic acid (2.20%) followed by Arachidic Acid (1.10%). Jojoba wax had high content of total monounsaturated fatty acid, the main fatty acid identified was gadoleic acid (20:1) (70.70%) followed by erucic acid (22:1) (14.2%) while oleic acid (18: 1) content was 8.70 %. These results are in agreement with those reported by **Al-Qizwini *et al*. (2014)** who found that the highest fatty acid identified of Jojoba wax was (20:1). Also these results were similar to that obtained by **El-Mallah and El-Shami (2009).**

Table 2: Fatty acids composition (%) of jojoba wax under study

Synonym	Fatty Acid	%
C16:0	Plamitic Acid	2.20
C16:1	Plamitoleic Acid	0.70
C18:0	Stearic Acis	0.40
C18:1	Oleic Acid	8.70
C20:0	Arachidic Acid	1.10
C20:1	Gadoleic Acid	70.70
C22:0	Behenic Acid	0.50
C22:1	Erucic Acid	14.20
C24:0	Lignoceric acid	0.30
C24:1	Nervonic Acid	1.20

Radical-Scavenging of jojoba wax:

DPPH is relatively stable free radical can be reduced primarily by more reactive reducing components such as phenolic substances **(Stratil *et al.*, 2007)**. The results of DPPH scavenging assay (Inhibition %) of jojoba wax extract are presented in **Table (3).** It is clear that as the concentration of antioxidant increased, the scavenging effect also increased. The results reveal that jojoba wax extract showed lower scavenging assay than that of ascorbic acid at all concentrations. The wax extract was able to reduce the stable radical DPPH to 1,1-diphenyl-2-picrylhydrazine with an IC_{50} value of 99.18 µg/ml. These result are in agreement with that of **Suhas *et al.*, (2016)** who reported that the percentage of inhibition is increased with increasing the concentration of jojoba wax since 200 µg showed 29.59% inhibition.

Table 3: DPPH scavenging assay (Inhibition %) of jojoba wax extract versus ascorbic acid.

Antioxidant concentration (µg/ml)	Jojoba wax	Ascorbic acid
25	18.75±0.20	89.97±0.55
50	41.50±0.26	91.33±0.70
75	43.74±0.35	91.57±0.45
100	61.63±0.56	91.67±0.46
IC_{50}	99.18±0.66	14.20±0.30

IC_{50}=the value for 50% scavenging activity.

Table 4 shows ABTS scavenging activity (Inhibition %) of jojoba wax and ascorbic acid The results indicate that jojoba wax showed a significant effect in inhibiting ABTS, reaching up to 45.48 and 57.48% at a concentration of 75 and 100 µg/ml; respectively compared with 91.67 and 92.18 %; respectively for ascorbic acid. This indicates that the radical scavenging potential of the jojoba wax is lower than that of ascorbic acid. Similar results were reported by **Suhas *et al.*, (2016)** who reported that at level of 200 µg jojoba wax showed inhibition percent reached to 26%.

Table 4: ABTS scavenging assay (Inhibition %) of jojoba wax extract versus ascorbic acid.

Antioxidant concentration (µg/ml)	Jojoba wax	Ascorbic acid
25	38.41±0.65	90.21±0.54
50	43.81±0.55	91.44±0.42
75	45.48±0.35	91.67±0.50
100	57.48±0.53	92.18±0.22
IC50	104.90±0.40	14.40±0.35

IC50= the value for 50% scavenging activity.

The results of Nitric oxide scavenging assay (Inhibition %) are shown in **Table (5)** .The results revealed that, the scavenging activity was increased by increasing the concentration of antioxidants (jojoba wax and ascorbic acid). The ascorbic acid exhibited higher scavenging activity 92.62% (at concentration 100µg /ml) compared to 49.83 % for jojoba wax. The IC_{50} values of jojoba wax extract was 79.5 µg/ml comparing to 14.00 µg/ml for ascorbic acid which reflects its lower antioxidant activity as compared to ascorbic acid.

Table 5: Nitric oxide scavenging assay (Inhibition %) of jojoba wax extract versus ascorbic acid.

Antioxidant concentration (µg/ml)	Jojoba wax	Ascorbic acid
25	37.53±0.25	89.33±0.50
50	39.88±0.20	91.42±0.35
75	46.16±0.34	92.34±0.50
100	49.83±0.55	92.62±0.60
IC50	79.50±0.35	14.00±0.20

IC50= the value for 50% scavenging activity.

Antibacterial activity of jojoba wax:

Table 6 illustrates the inhibition zone diameter (mm) of jojoba wax against some microorganisms by both of desk diffusion and well diffusion assay. Jojoba wax was recorded 17 and 15 mm inhibition zone diameter by desk diffusion assay method while in well diffusion assay method, jojoba wax had inhibition diameter zone of 16 and 14 mm against *Bacillus cereus* and *Salmonella typhi*; respectively. Jojoba

wax exhibited better inhibition activities against gram-positive than gram-negative bacteria. *Bacillus cereus* and *Salmonella typhi* are most susceptible and *Escherichia coli* and *Staphylococcus aureus* are least susceptible to jojoba wax. This result was similar to these obtained by **Pooja *et al.*, (2016)**, The antimicrobial activity of Jojoba oil against *Staphylococcus aureus* showed zone of inhibition 17mm by agar well diffusion method. While with *Pseudomonas aeruginosa* and *Klebsiella pnuemoniae*, the zone of inhibition was 15mm and 13mm; respectively.

Table 6: Zone inhibition diameter (mm) of jojoba wax extract against some microorganisms.

Bacteria Strain	Inhibition zone diameter (mm) of jojoba wax extract	
	Desk diffusion assay	Well diffusion assay
Bacillus cereus	17±0.25	16±0.28
Staphylococcus aureus	13±0.15	14±0.20
Salmonella typhi	15±0.22	14±0.26
Escherichia coli	14±0.30	13±0.42

Minimum inhibitory concentration (MIC) of jojoba wax extract:

Table 7 shows the minimum inhibitory concentration (MIC) of jojoba wax against tested bacteria. The results show that MIC of jojoba wax against tested bacterial strains ranged from 198 to 219 *ul*/ml. The lowest MIC values observed for *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Enterococcus faecalis* were 198 *ul*/ml, 200 *ul*/ml, 202 *ul*/ml and 205 *ul*/ml; respectively. On the other hand, higher MIC values of jojoba wax noticed against Escherichia *coli* (217 *ul*/ml), *Shegalla sonni* (218 *ul*/ml) and *Pesudomonas aeruginosa* (219 *ul*/ml). The susceptibility of tested pathogenic bacteria was exhibit the following decreasing order: *Pesudomonas aeruginosa* > *Shegalla sonni* > *Escherichia coli* > *Enterococcus faecalis*> *Staphylococcus aureus.* It was interesting to note that both Gram negative and Gram positive bacteria were sensitive to jojoba wax but Gram positive bacteria were more sensitive than Gram negative bacteria.

Table 7: Minimum inhibitory concentration (MIC) of jojoba wax extract against tested bacterial

Bacteria	Strains	Jojoba wax extract (ul/ml)
Bacillus cereus	ATCC11778	198
Escherichia coli	ATCC11229	217
Salmonella typhi	ATCC 14028	211
Shegalla sonni	ATCC 29930	218
Staphylococcus aureus	ATCC 33591	202
Enterococcus faecalis	ATCC47077	205
Pesudomonas aeruginosa	ATCC 15692	219
Listeria monocytogenes	ATCC19111	200

Fungal growth:

Table 8 shows the fungal growth inhibition of jojoba wax extract against tested fungal (*A. favus, Penicillium, A. Paraciticus, F. graminearum*). The results indicate that fungal growth inhibition was directly proportional to the addition level jojoba wax extract since a significant decreasing in mycelial weight of the tested fungal strains was observed when comparing the mycelial weight of jojoba wax extract treated fungus with untreated ones (since the mycelial weight of untreated fungi was higher than that of treated ones with increasing ration ranged between 26.68 and 32.48 % after 8 days of incubation). *F. graminearum* was had the lowest value of mycelial weight followed by *A. Paraciticus* at all storage period. Also the data revealed that, after 8 days of incubation, *A. favus* control had the highest value of mycelial weight (14.32 g) compared to other fungal strains while *F. graminearum* plus jojoba wax was had the lowest value of mycelial weight (8.25 g). It is evident that, jojoba wax is effective in retardation of fungal growth.

Table 8: Fungal growth inhibition of jojoba wax extract against tested fungal.

Fungal Strains	Mycelial dry weight (g)			
	2 days	4 days	6 days	8 days
Aspergillus flavus / jojoba wax	1.71±0.13	4.37±0.10	7.61±0.25	10.88±0.11
Aspergillus flavus Control	3.65±0.15	6.91±0.09	10.88±0.12	14.32±0.28
Penicillium / jojoba wax	1.31±0.14	3.28±0.13	6.57±0.17	9.37±0.16
Penicillium Control	2.35±0.15	5.34±0.14	8.41±0.19	11.87±0.16
Aspergillus parasiticus / jojoba wax	0.97±0.10	2.27±0.13	5.43±0.11	9.82±0.18
Aspergillus parasiticus Control	2.61±0.08	5.22±0.11	8.92±0.18	12.77±0.12
F. graminearum / jojoba wax	0.51±0.14	1.27±0.17	4.72±0.09	8.25±0.19
F. graminearum Control	1.84±0.17	3.54±0. 22	7.22±0.12	10.93±0.21

Antifungal activity of jojoba wax:

Table 9 shows the antifungal activity (AU/ml) of jojoba wax against tested fungal strains. The results showed that, jojoba wax is effective against all tested fungal strains. Comparison of the sensitivity of tested fungal strains towards the jojoba wax showed that *Fusarium oxysporum* was the most resistant with antifungal activity with 4950 AU/ml, the lowest antifungal effect of jojoba wax). On the other hand, the strongest antifungal activity effect was exerted against *Aspergillus parasiticus* with antifungal activity value of 8750 AU/ml. These results suggest that the constituents of jojoba wax have definite potential against fungi.

Table 9: Antifungal activity (AU/mL) of jojoba wax extract against tested fungal.

Fungal	Strains	Activity (AU/mL)
Aspergillus niger	ITEM 3856	5600
Aspergillus parasiticus	ITEM 11	8750
Aspergillus carbonarius	ITEM5010	7200
Aspergillus flavus	ITEM 698	6700
Aspergillus oryzae	ITEMB5	6700
Fusarium oxysporum	ITEM 12591	4950
Alternaria Alternata	ITEM	5800

Quality characteristics of sodium alginate / jojoba wax coated eggplant slices during cold storage at 4 ^{o}C:

Color parameters of coated/uncoated fresh-cut eggplant: The lightness (L value) of fresh-cut vegetables has a major impact on the perceived appearance of the product. **Table 10** demonstrates color parameters (lightness, redness and yellowness) of sodium alginate with/without jojoba wax coated eggplant slices during cold storage at 4^{o}C. From analysis of the data given in **Table 10**, it could be observed that, uncoated eggplant slices had higher lightness (L*) (81.89) followed by fresh-cut eggplant coated with sodium alginate/1% jojoba wax (SA/JW1) (81.32) at zero time. All samples of fresh-cut eggplant had gradual decrement in lightness value with increased the storage time (increase in the darkness, decrease in the L* values). From the same table, it could be reported that, the lowest redness (a*) value (-1.12) was recorded for fresh-cut eggplant coated with sodium alginate/5% jojoba wax (SA/JW5). On the other hand, after 6 days of storage redness (a*) values in all samples ranged between 5.32 for uncoated eggplant slices and 9.13 for fresh-cut eggplant coated with sodium alginate/3% jojoba wax (SA/JW3).These data showed that values of yellowness (b*) were increased as the storage time increased. The data showed that no difference between all coated samples at the end of storage time, whereas the highest yellowness value was observed for control sample.

Table 10: Color parameters of sodium alginate / jojoba wax coated eggplant slices during cold storage at 4 ^{o} C.

Storage period/days	Treatments*				
	Control	SA	SA/JW1	SA/JW3	SA/JW5
(L*) lightness					
0	81.89^{a}±0.45	80.17^{b}±0.34	81.32^{a}±0.20	80.52^{b}±0.41	79.02^{c}±0.33
3	42.47^{e}±0.30	49.12^{b}±0.19	52.34^{a}±0.17	45.43^{d}±0.11	46.78^{c}±0.16
6	35.41^{e}±0.13	49.57^{b}±0.21	47.06^{d}±0.17	48.42^{c}±0.23	50.83^{a}±0. 14
(a*) redness					
0	-2.11^{c}±0.11	-1.26^{ab}±0.14	-1.32^{ab}±0.17	-1.4^{b}±0.16	-1.12^{a}±0.12
3	2.71^{d}±0.13	10.69^{a}±0.16	9.30^{c}±0.15	10.44^{a}±0.18	9.76^{b}±0.14
6	5.32^{c}±0.22	9.41^{b}±018.	9.90^{a}±0.12	9.13^{b}±0.23	9.36^{b}±0.19
(b*) yellowness					
0	26.40^{b}±0.26	26.82^{b}±0.33	26.45^{b}±0.38	27.62^{a}±0.40	27.60^{a}±0.31
3	32.04^{a}±0.32	30.84^{b}±0.35	27.49^{d}±0.28	28.58^{c}±0.29	28.60^{c}±0.30
6	32.22^{a}±0.22	28.90^{bc}±0.20	29.19^{b}±0.26	28.63^{c}±0.23	28.69^{c}±0.29

*Control (uncoated fresh-cut eggplant slices); SA, coated with sodium alginate only; SA/JW1, coated with sodium alginate/1% jojoba wax; SA/JW3, coated with sodium alginate/3% jojoba wax and SA/JW5, coated with sodium alginate/5 % jojoba wax. Means (± SD) sharing similar superscripts in a row are statistically non-significant ($p<0.05$).

Firmness of coated/uncoated fresh-cut eggplant: Table 11 illustrates firmness values (Newton) of eggplant slices coated with sodium alginate with/without jojoba wax during cold storage at 4^{o}C. The data reveal that there are no significant difference in firmness between coated and uncoated fresh-cut eggplant slices which ranged between (28.30- 28.50 Newton) at zero time. The data show that an accumulative increasing could be seen in the average values of firmness for the control sample from zero time to 6 days at cold storage giving the values of (28.40, 30.70 and 35.60 Newton); respectively. From the obtained data it could be noticed that all samples of fresh-cut eggplant had gradual increment in firmness value with increased the storage time, this increase could be mainly attributed to loss of water which causing more compact texture which is very clear in control sample which had the highest firmness value at the end of storage time (35.60 Newton) as compared to coated samples (30.20 – 32.00 Newton), since edible coatings decrease the loss of water from freshly cut eggplant slices and maintain its soft texture.

Table 11: Firmness value (Newton) of sodium alginate / jojoba wax coated eggplant slices during cold storage at 4 ^{o} C.

Storage period/days	Treatments				
	Control	SA	SA/JW1	SA/JW3	SA/JW5
0	28.40^{a}±0.14	28.30^{a}±0.12	28.50^{a}±0.10	28.30^{a}±0.21	28.40^{a}±0.13
3	30.70^{b}±0.20	31.40^{a}±0.13	30.00^{c}±0.14	29.70^{d}±0.18	29.00^{e}±0.10
6	35.60^{a}±0.19	32.00^{b}±0.15	30.50^{d}±0.18	31.20^{c}±0.15	30.20^{d}±0.16

*Control (uncoated fresh-cut eggplant slices); SA, coated with sodium alginate only; SA/JW1, coated with sodium alginate/1% jojoba wax; SA/JW3, coated with sodium alginate/3% jojoba wax and SA/JW5, coated with sodium alginate/5 % jojoba wax. Means (± SD) sharing similar superscripts in a row are statistically non-significant ($p<0.05$).

Microbiological quality of coated/uncoated fresh-cut eggplant:

Microbial safety is regarded as an important factor in preservation of fresh-cut fruit and vegetables (**Bico *et al.,* 2009**). The presented data in **Table 12**, indicated that uncoated fresh-cut eggplant had higher total bacterial count (4.11 log /g) followed by fresh-cut eggplant coated with sodium alginate (3.95 log /g) at zero time. In this study, samples were stored at relative low temperature (4^{o}C), which could reduce microbial growth (**Carbonaro and Mattera, 2001**). As shown in the same Table, total bacterial counts (TBC), yeast and mould counts (YMC) of all treated samples were lower than those of control sample along the storage time period. Data in **Table 12**, reveals that, after 3days of storage, fresh-cut eggplant coated with sodium alginate / Jojoba oil 5 % (SA/JW5) had the lowest value of total bacterial count (3.70 log /g) followed by fresh-cut eggplant coated with sodium alginate/ Jojoba oil 1% (SA/JW1) and fresh-cut eggplant coated with sodium alginate/Jojoba oil 3% (SA/JW3) whose had the same bacterial count (4.08 log /g). These results showed that addition of jojoba wax to sodium alginate coating delayed the increase in bacterial population beside the low temperature applied during the cold storage. A similar effect was observed in reducing growth of yeast and mould population. Data of **Table 12** reveals that, mould and yeast count of tested samples were gradually increased as cold storage time increased. Mould and yeast count were 4.00 and 3.84 log /g in fresh-cut eggplant coated with sodium alginate (SA) and fresh-cut eggplant coated with sodium alginate/Jojoba oil 1% (SA/JW1); respectively. These results indicate that the incorporation of jojoba wax result in lowering the microbial growth of fresh-cut eggplant slices which is opposite proportion to the jojoba wax addition level since as the jojoba wax level increased the microbial growth decreased.

Table 12: Microbiological Load (log /g) of sodium alginate incorporated with jojoba wax coated eggplant slices during cold storage at 4 ^{o} C.

Storage period/days	Treatments				
	Control	SA	SA/JW1	SA/JW3	SA/JW5
Total bacterial count (log /g)					
0	$4.11^{a}\pm0.21$	$3.95^{ab}\pm0.18$	$3.77^{b}\pm0.14$	$3.63^{b}\pm0.15$	$3.30^{c}\pm0.20$
3	$4.81^{a}\pm0.19$	$4.17^{b}\pm0.13$	$4.08^{b}\pm0.12$	$4.08^{b}\pm0.19$	$3.70^{c}\pm0.16$
6	$5.06^{a}\pm0.14$	$4.50^{b}\pm0.18$	$4.41^{c}\pm0.16$	$4.14^{c}\pm0.15$	$4.00^{c}\pm0.18$
Mould and yeast count (log/g)					
0	$3.74^{a}\pm0.12$	$3.71^{a}\pm0.13$	$3.53^{ab}\pm0.10$	$3.36^{bc}\pm0.14$	$3.20^{c}\pm0.19$
3	$3.84^{a}\pm0.16$	$3.78^{a}\pm0.11$	$3.66^{ab}\pm0.13$	$3.50^{b}\pm0.10$	$3.41^{b}\pm0.18$
6	$4.04^{a}\pm0.10$	$4.00^{a}\pm0.15$	$3.84^{ab}\pm0.16$	$3.70^{b}\pm0.12$	$3.47^{c}\pm0.13$

*Control (uncoated fresh-cut eggplant slices); SA, coated with sodium alginate only; SA/JW1, coated with sodium alginate/1% jojoba wax; SA/JW3, coated with sodium alginate/3% jojoba wax and SA/JW5, coated with sodium alginate/5 % jojoba wax. Means (± SD) sharing similar superscripts in a row are statistically non-significant ($p<0.05$).

CONCLUSION

Finally it could be concluded that the shelf life of fresh-cut eggplant slices could be extended with the incorporation of jojoba wax in the sodium alginate edible coating applied to fresh-cut eggplant slices because of its antioxidant and antimicrobial characteristics which maintain the quality characteristics (color, firmness and microbiological) of coated Fresh-cut eggplant slices.

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تقييم شمع الجوجوبا كمضاد للميكروبات ومضاد للأكسدة لتطبيقة في مواد التغطية الغذائية لحفظ صفات الجودة لشرائح الباذنجان حديثة التقطيع

محمد صابر عمار [1] و رباب حسن سالم [2]

قسم علوم وتكنولوجيا الأغذية – كلية الزراعة – جامعة الأزهر –ا لقاهرة –

جمهورية مصر العربية

قسم علوم وتكنولوجيا الأغذية – كلية الاقتصاد المنزلي – جامعة الأزهر – طنطا – جمهورية مصر العربية

استهدف هذا البحث تقييم شمع الجوجوبا (زيت الجوجوبا) كمادة مضادة للميكروبات ومضادة للأكسدة ودراسة تأثير إضافته إلي مادة التغطية الغذائية الجينات الصوديوم لحفظ صفات الجودة لشرائح الباذنجان حديثة التقطيع الطازجة خلال التخزين المبرد علي درجة حرارة 4 ^{5}م. وقد أظهرت النتائج أن محتوي شمع الجوجوبا من الأحماض الدهنية أحادية عدم التشبع كما يلي حمض الجادوليك (20 : 1) هو الحمض السائد بنسبة 70.7 ٪ يليه حمض الأيروسيك (22 : 1) 14.2٪ وكان محتواه من حمض الأوليك (18 : 1) 8.7٪ . وقد أظهرت النتائج أيضاً أن قدرة شمع الجوجوبا كمضاد للأكسدة كانت أقل قدرة حمض الأسكوربيك حيث كان التركيز المطلوب لقيمة التثبيط النصفي IC_{50} 99.18 و 14.20 ميكروجرام /مل من شمع الجوجوبا وحمض الأسكوربيك علي التوالي. وقد بينت النتائج أن شمع الجوجوبا أظهر نشاط تثبيطي أفضل ضد البكتيريا الموجبة عن البكتيريا السالبة لجرام. تراوح أقل تركيز مثبط لشمع الجوجوبا ضد اليكتريا المختبرة بين 198 -219 ميكروجرام/مل. تراوح النشاط المضاد للفطريات لشمع الجوجوبا بين 4950 و 8750 وحدة نشاط/ مل ضد فطر *Fusarium oxysporum* و فطر *Aspergillus parasiticus* علي التوالي. كانت قيم اللون للعينات المغطاة أعلي منها للعينة المرجعية. وكذلك أظهرت النتائج انخفاض تدريجي لقيم صلابة عينات شرائح الباذنجان حديثة التقطيع بزيادة وقت التخزين. وبينت لنتائج أن إضاقة شمع الجوجوبا إلي مادة التغطيية أدي إلي خفض النمو الميكروبي.