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Evaluation of the Physicochemical Properties and Antimicrobial Activities of Bioactive Biodegradable Films

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Abstract

The use of edible films to release antimicrobial constituents in food packaging is a form of active packaging. Different extractions of *Myrtus communis*, *Urtica urens*, *Ziziphus spina-christi*, and *Zygophyllum coccineum* were tested for their antimicrobial activity against the food pathogenic microorganisms *Escherichia coli*, *Salmonella typhimurium* and *Aspergillus niger* by using agar diffusion assay method. Soy-starch and gelatin edible films were prepared and incorporated with *Myrtus communis* and *Ziziphus spina-christi* essential oils separately and as a mixture in different concentrations. The films were characterized for their antimicrobial activity by using agar diffusion assay method and their physico-chemical properties. The films were studied on different food applications (orange, apple, lemon, tomato, pizza dough, chicken salami, meat salami, artificial cheese, mayonnaise, yoghurt and skimmed cheese). The results showed that, the films extended the shelf-life of the food products depending on the effective chemical compounds of the essential oils α-pinene and limonene.

Key Words: *M. communis, Z. spina-christi, essential* oils, α –pinene, limonene, edible films.

1. Introduction

Researches on the microbial spoilage of food has become important for food safety and keeping qualities. In food industry, all the steps of food production usually occur under sterilized condition. But at the final step where the food packaged, it usually exposes to post process surface contamination, which leading to the reduction of shelf life.

At the same way, the using of extracts from aromatic plants particularly the essential oils as antimicrobial agents are in an increasing interest (Shahidi Bonjar *et al.*, 2003), because there were considered as a rich source of biologically active compounds. They have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Burt 2004, Kordali *et al.*, 2005) and have a wide range of possible applications ranging from the food industry to pharmaceuticals (Burt 2004, Holley and Patel, 2005).

There are many techniques that have been used for maintaining the quality of food products and in the recent years the edible films and coatings have received considerable amount of attention as antimicrobial packaging materials. The main advantage is that they can be consumed with the packaged products and even if the films are not consumed they could still help in the reduction of environmental pollution (Bourtoom, 2008).

Various antimicrobial edible films can serve as a carrier for antimicrobial compounds to reduce, inhibit or retard the growth of the food pathogenic microorganisms in packed foods and packaging material (Coma *et al.*, 2001, Rodrigues and Han, 2000). The film can helps preventing brown coloration, moisture loss during storage, reducing the rate of rancidity causing lipid oxidation and also restrict of the volatile flavor loss (Pérez-Pérez *et al.*, 2006).

In general; edible films have been made from several polysaccharides, lipids, and proteins (Cagri *et al.*, 2004). Several attempts have been made in developing active packaging systems in which antimicrobial agents are incorporated into the polymeric material and are slowly released on the food surface (Devlieghere *et al.*, 2004) by diffusion through partitions (Han, 2000). Finally, there is a need to explain the advantages of using edible films as an antimicrobial food packaging materials to the consumers in order to help the industry to replace the synthetic packaging materials with the environmental friendliness biomaterials (Sonti, 2003). This study aims at

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incorporating the naturally-derived essential oils with the edible films to use them as antimicrobial food packaging materials to extend the food shelf-life.

2. Materials and Method

2.1. Plant Materials.

Both leaves and fruits of *Myrtus communis* and *Ziziphus spina-christi* were collected from Cairo arboretum; *Zygophyllum coccineum* was collected from Sinai (ras-sydr); and *Urtica urens* was collected from El – menofeya, while its seeds were purchased from Local herbal shop in Cairo. All the specimens were identified in the Herbaria of Botany Cairo University (CAI) and Ain-Shams University (CAIA).

A food grade soy protein (prolia) with a protein content of 55% was donated by "AWA" for food additives. Gelatin powder (animal gelatin) was purchased from a local herbal shop, bloom = (180-200) g. Corn starch was purchased from a local shop. Glycerol was obtained from Technogene Company, Dokki, Giza, Egypt. Ethanol was purchased from Sigma-Aldrich, Egypt. 1N sodium hydroxide purchased from El-gomhorea Company, Cairo, Egypt.

2.2. Organisms and Preparation of Cultures

The food deterioration microorganisms *Aspergillus niger, Escherichia coli* and *Salmonella typhimurium* were used as test microorganisms.

Both *Aspergillus niger* and *Escherichia coli* were obtained from Department of Microbiology, Faculty of Science, Ain-shams University. The fungal and bacterial species were received on PDA medium and nutrient agar medium respectively. They were maintained on agar slants of both Czapek's Dox and nutrient media, respectively.

Salmonella typhimurium kindly provided from the Veterinary Serum & Vaccine Research Institute. The bacterial species was received on nutrient agar medium, and then cultured on *Salmonella – Shigella* agar slants.

Czapek's Dox agar and Nutrient agar media prepared according to the "Hand book of microbiological media" (Atlas, 1979). SS agar was obtained from Oxoid LTD Company, Basinoctoke, Hampshire, England.

2.3. Preparation of Plant Extracts

Thirty six extracts of *Myrtus communis*, *Urtica urens*, *Ziziphus spina-christi* and *Zygophyllum coccineum* were used in this study. All the plants extractions (whether of air-dried or fresh parts or expressed Juice) prepared according to (Abo-Zaid, 2000), then stored at 4°C until use them in the experiments (not more than a week).

2.4. Extraction of Essential Oils

Myrtus communis and *Ziziphus spina-christi* were used for extraction of essential oils. According to (Abo-Zaid, 2000), 100 g of the air-dried leaves of both plants were crushed, then hydrodistilled for 3 hours. The yields were on average 0.5 % (v/w dried weight) and 0.01 % respectively. The essential oils were stored in dark vials at 4 °C. (The trials of the essential oils extraction from both *Urtica urens* and *Zygophyllum coccineum* through hydrodistillation method were failed).

For preparation of different concentrations of the essential oils mixture; equal volumes of the essential oils of both *Myrtus communis* and *Ziziphus spina-christi* were mixed in a dark vial and kept at room temperature for 24 hours, then stored at 4 °C (shake well before it using). 0.1, 0.2, 0.3, 0.4 and 0.5 ml of the mixture then used.

2.5. Edible Films Preparation

Two different edible films were prepared, one of them from soy-starch and the other from gelatin.

2.5.1. Control Films

Film-forming solution of soy-starch prepared by using a modified method to that described by (Ghorpade *et al*., 1995). While, film-forming solution of gelatin prepared according to the method that described by (de Carvalho and Grosso, 2006).

Each film-forming solution were spread in a rectangular area of (11.5 ×32.5 cm).

2.5.2. Films Enriched with the Essential Oils

Films-forming solutions incorporated with the essential oils prepared by using a modified method to that described by (Maizura *et al.*, 2008) and by the same steps of preparation of control films.

Seven films sheets prepared for both of soy-starch and gelatin. A sheet containing 0.5 ml *Myrtus communis* essential oil, a sheet containing 0.5 ml *Ziziphus spina-christi* essential oil. And the other five sheets each one contained different concentration of the essential oils mixture (0.1, 0.2, 0.3, 0.4 and 0.5 ml).

2.6. Antimicrobial Activity

Screening of Antibacterial and antifungal activity for plants extractions, the essential oils and the edible films carried out by agar disc diffusion method.

2.6.1. For plant extractions

0.2 ml of suspension of tested microorganisms spread on the solid media surfaces. Sterilized filter paper discs (5 mm in diameter) saturated with plant extractions, left to dry at room temperature for an hour. Then placed on the cultures surfaces which previously inoculated. Control cultures contained only on sterile filter paper discs.

2.6.2. For Essential Oils

Sterilized filter paper discs (5 mm in diameter) were saturated with the essential oils, left to dry at room temperature for 30 minutes. Then placed on cultures surfaces which previously seeded with 0.2 ml of the inoculum. Control cultures contained only on sterile filter paper discs.

2.6.3. For Edible Films

Each film sheet cut into discs (13 mm in diameter), then placed on cultures surfaces which previously seeded with 0.2 ml of the inoculum.

All the plates incubated at 30° C for 48 h for bacteria and 6 days for fungus. Diameters of inhibition zones measured in millimeters. All the tests performed in triplicates, and the results analyzed statistically using ANOVA test (Schott, 1997).

2.7. Physicochemical Properties of Films

Color. Films Color of soy-starch and gelatin examined by using Kodak camera (dimensions of photo 862 × 962) to show the color of each film.

*Thickness***.** The thickness value represented by the mean of five measurements taken along the strips made on each film which used for testing tensile strength and percentage elongation at break. The films thickness measured automatically by a micrometer connected to the Universal Testing Instrument (Zwick \ Z010). The test was carried out at the National Institute for Standards.

*Solubility***.** Different pH solutions (1-14 pH) were prepared by using the distilled water and solutions of HCl-KCl Buffer (1-2.2), Citrate-Phosphate Buffer (2.6-7) and Glycine-NaOH Buffer (7.4-14). Small pieces of dried films samples (3 × 4cm) were placed in Petri dishes containing 60 ml of adjusted pH solution. The Test dishes were covered and incubated at room temperature for 48 hrs.

Water vapor permeability (WVP). Carried out by using a modified method to that described by (Kunte *et al.*, 1997). Fan was provided for the air circulation inside the desiccator cabinets at the first for 4 hours only after that, the test completed without it. All the tests were performed in triplicates.

Tensile strength (TS) and percent elongation (%E). According to (Kunte *et al.*, 1997), tensile testing was performed with the Universal Testing Instrument (Zwick \ Z010) on (50 ×4 cm) dample shape film strips. Initial grip separation set at 25 mm, while cross-head speed set at 50 mm/sec, the used lot cells (100 N). The test was carried out at the National Institute for Standards.

Scanning electron microscopy (SEM). Carried out for both films of soy-starch and gelatin by using SEM Model Philips XL 30 attached with EDX unit with accelerating voltage (15 K.V.). Samples were coated with gold and the films surfaces pictures taken by SEM at magnification of (500x).

Qualitative analyses by (GC-MS)

Glass jars were tightly sealed with film specimens (8 ×8 cm) of soy-starch film and gelatin film respectively. Film specimens cut from films sheets prepared in rectangular area of (11.5 ×32.5 cm) and enriched with 0.5ml of the essential oils mixture. All the samples were left at room temperature (27°C±2°C) for 7 days.

The air inside the jars examined by Gas Chromatography-Mass Spectrometer (from Agilent Technologies; 6890N, network GC system and 5975 inert XL Mass Selective Detector). It was carried out at the National Institute for Standards. The test performed in duplicate.

2.8. Applications on Foodstuffs

*Films Preparation***.** The films-forming solutions for soy-starch and gelatin were prepared by the same method which shown before in the preparation of edible films that enriched with the essential oils. For each film-forming solution, 0.5ml from the essential oils mixture was added then spread in rectangular area of (11.5 ×32.5 cm) on foil sheet.

Preparation of food samples. Different selected food products were covered with the edible films, each one with film area of (8 ×8 cm). Fruits (orange - apple - lemon), Vegetables (tomato), Bakery products (pizza dough), Meats products (chicken salami- meat salami) and Dairy products (artificial cheese) were directly attached with the films. Where, Ready meal (mayonnaise) and Dairy products (yoghurt-skimmed cheese) were packaged in glass jars and tightly sealed with films.

For control specimens, foil sheets free from films were used. The results were recorded daily during 100 days for odor changes and physical observation.

3. Results and Discussion

Four plants have reported in previous studies as medicinal plants were chosen; *Myrtus communis* (Montvale, 2000 and Tsybula and Kazarinova, 1996), *Ziziphus spina-christi* (Glombitza *et al.*, 1994 and Waggas, 2007), *Zygophyllum coccineum* (Batanouny *et al.*, 1999) and *Urtica urens* (Wichtl, 2002 and Randall, 2003) to be safe in using them as food additives. The antibacterial and antifungal activity for all plants extractions gave interesting results. Whereas Myrtus communis and Ziziphus spina-christi were the best of them (Table 1).

Table 1. Antimicrobial activities of plants extractions of Myrtus communis, Urtica urens, Ziziphus spina-christi, and Zygophyllum coccineum against the foodborne pathogens, E.coli, S. typhimurium and Aspergillus niger

plants species	Used part	Extraction solvent	inhibition zones diameter(mm)		
			E. coli	*S. typhimurium*	*A. niger*
M. communis	Dry Leaves	Distilled Water	10	12	9
		Alcohol	9	-	10
		Water + Alcohol	-	11	14
	Fresh Leaves	Distilled Water	9	9	-
		Alcohol	9	10	-
		Water + Alcohol	9	12	12
	Dry Fruit	Distilled Water	11	15	-
U. urens	Fresh Leaves	Distilled Water	-	-	-
		Alcohol	11	9	-
	fresh (Leaves+ Flowers+ Stems)	Distilled Water	6	-	-
		Alcohol	-	7	-
	Fresh Root	Distilled Water	9	-	-
		Alcohol	6	-	6
	Dry Leaves	Distilled Water	-	-	-
		Alcohol	-	-	7
	Dry (Leaves+ Flowers+ Stems)	Distilled Water	-	-	7
		Alcohol	8	-	7
	Dry Root	Distilled Water	-	-	8
		Alcohol	11	6	10
	Dry Seeds	Distilled Water	-	-	6
		Alcohol	7	-	6

	Expressed Juice	Fresh Intact Plant	-	-	10
Z. spina-christi	Dry Leaves	Distilled Water	-	10	-
		Alcohol	-	-	6
		Water + Alcohol	-	9	7
	Fresh Leaves	Distilled Water	-	7	7
		Alcohol	-	10	7
		Water + Alcohol	-	9	7
	Dry Fruit	Distilled Water	9	-	7
	Fresh Fruit	Distilled Water	8	10	-
	Seed Embryo	Distilled Water	-	-	-
Z. coccineum	Fresh Leaves	Distilled Water	-	-	11
		Alcohol	7	-	-
	Fresh Leaves and Stems	Distilled Water	9	-	-
		Alcohol	8	8	-
	Expressed Juice	Fresh Intact Plant	9	8	-

Each value is the mean of three replicates.
Control Cultures containing only sterile filter paper discs & did not show any inhibition zones. Filter paper discs diameter of 5 mm.
The results are not significant ($p > 0.05$) according to ANOVA test.

The mode of action was attributed to the disturbance of the cytoplasmic membrane, disrupting of the proton motive force, electrolyte flow and active transport and coagulation of bacterial cell contents (Burt, 2004). Whereas, the essential oils components in plants extractions may have an inhibitory effect on the mycelial growth of fungi (Özcan *et al*., 2005).

The extraction of the essential oils from *Urtica urens* and *Zygophyllum coccineum* through hydrodistillation method was failed. Therefore, only the essential oils of *Myrtus communis* and *Ziziphus spina-christi* were used. The method of extraction (Lemberkovics *et al.*, 2003) and the origin of the samples (Tuberoso *et al.*, 2006) have effect on the composition of essential oils in aromatic plants.

According to (Tuberoso *et al*., 2006 and Montvale, 2000), the essential oil yields of *Myrtus communis* were on average 0.5 for the dried leaves and 0.02 for berries. Therefore, the dried leaves were used for the extraction of the essential oil. Although *Myrtus communis* fragrance clearly appeared in its extracts; but the antimicrobial activity for its essential oil against all the tested microorganisms appeared more effective than the extracts (Figure1).

The essential oil of Ziziphus spina-christi showed more potent antimicrobial activity against all the tested microorganisms comparing with all the plant extractions (Figure1).

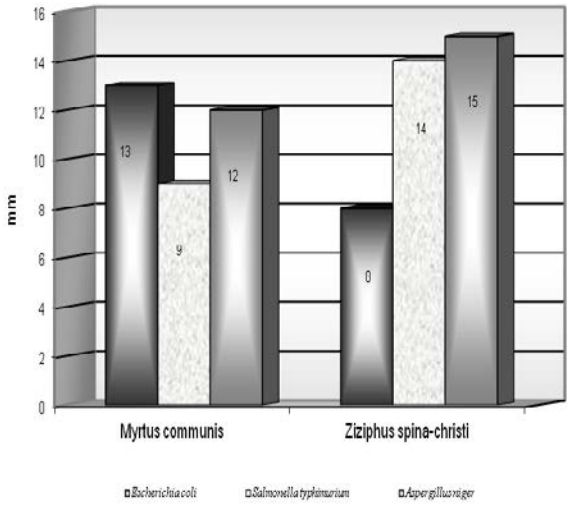

Figure 1. The antimicrobial activity of *M. communis* and *Z. spina-christi* essential oils against the foodborne pathogens *E. coli*, *S. typhimurium* and *A. niger* .
Each value represents the mean of three replicates. Filter paper discs diameter of 5 mm.
Control cultures contained only on sterile filter paper discs & did not show any inhibition zone.
The results are not significant ($p > 0.05$) according to ANOVA test.

This can be attributed to the absence of sugars that present in its extractions and include lactose, glucose, galactose, arabinose, xylose and rhamnose (Dweck, 2005). These sugars can be considered as a source of nutrient for the microorganisms.

The results of these experiments emphasize the work of Gill and Holley (2006) which concluded that, "at bactericidal concentrations of the essential oils" the bacterial-cytoplasmic membrane disrupted by increasing its non-specific permeability because the essential oils components may be possess ATPase inhibiting activity. Also there were concluded that, the other secondary effects at sublethal concentrations cannot be discounted and can be expected as a consequence of membrane interactions. By the same way and according to Özcan *et al.* (2005), the essential oils has inhibitory effect on the mycelial growth of fungi. Whereas Curini *et al.* (2003) found that, the essential oil of *Myrtus communis* causing morphological alterations of fungal hyphae.

So it is obvious that, the plants extractions and essential oils have remarkable lethal effects on the tested microorganisms *Escherichia coli*, *Salmonella typhimurium* and *Aspergillus niger* by inhibiting their survival remarkably.

The results of the antimicrobial activity of edible films which incorporated with the essential oils of *Myrtus communis* and *Ziziphus spina-christi* whether individually (Table 4**)** or as a mixture (Table 5) demonstrated wide variation in activities against the tested microorganisms.

Table 4. Antimicrobial activity of the films incorporated with *M. communis* and *Z. spina-christi* Essential Oils (separately) on *E. coli*, *S. typhimurium* and *A. niger* by the disc diffusion method.

Film	Essential Oil	Diameter of inhibition Zones (mm)		
		E. coli	*S. typhimurium*	*A. niger*
Soy-Starch	Control	20	20	21
	M. communis	21	22	22
	Z. spina-christi	20	21	25
Gelatin	Control	18	–	20
	M. communis	21	25	32
	Z. spina-christi	18	22	33

Each value represents the mean of three replicates. Films discs diameter of 13 mm.
Each prepared film sheet (11.5 ×32.5 cm) containing 0.5 ml of an essential oil.
Control films prepared without adding any of Essential oils. The results are significant ($p > 0.01$) according to ANOVA test.

Table 5. Antimicrobial activity of the films incorporated with different concentrations of the essential oils mixture on *E. coli*, *S. typhimurium* and *A. niger* by the disc diffusion method.

Film	essential oils mixture Conc. (ml)	Diameter of inhibition Zones (mm)		
		E. coli	*S. typhimurium*	*A. niger*
Soy-Starch	0.1	28	20	30
	0.2	28	23	32
	0.3	30	20	31
	0.4	25	22	30
	0.5	26	20	25
Gelatin	0.1	20	20	44
	0.2	20	18	31
	0.3	20	20	33
	0.4	27	25	30
	0.5	27	21	30

Each value represents the mean of three replicates.
Films discs diameter of 13 mm.
Each prepared film sheet (11.5 × 32.5 cm) containing different concentration of essential oil mixture.
The results of both soy-starch and gelatin are highly significant ($p < 0.01$) according to ANOVA test.

The films materials come from biological tissues; soy (protein) and starch (carbohydrate) from plants, and gelatin (protein) from animal tissue. Therefore, may be some of the chemicals residues which come from their origin attached to the powders (Pérez-Pérez *et al.*, 2006). This may be explaining the antimicrobial activity of the control films.

There is no single antimicrobial agent can cover all the requirements for food preservation. Therefore, the antimicrobial activity of the essential oils mixture of *Myrtus communis* and *Ziziphus spina-christi* were examined. And according to the presented results, the minimum inhibitory concentration not detectable and it cannot be unified to all the microorganisms (Table 5). This is because of the potential differences of the effect of the essential oils on their cell wall (Gill and Holley, 2006).

Differences between both films were appeared through the physicochemical tests.

Color is an important property because it could affect consumer acceptance of such films for both edible and nonedible packaging applications. Differences observed clearly between both films (Figure 2).

Figure 2. Photo of gelatin and soy -starch films.

This is attributed to the used powders color of both soy protein (yellow) and gelatin (white). The Soy protein consists of two major protein fractions referred to as the 7S (conglycinin) and 11S (glycinin). Each fraction has a quaternary (subunit) structure (Kinsella *et al.*, 1985) and make up to 37 and 31%, respectively of the total extractable proteins (Gennadios *et al.*, 1994). Both 7S and 11S contain cysteine residues (Sulfur-containing amino acid) (Kinsella *et al.*, 1985). For that soy powder has the yellow color which became faint with adding of starch. While gelatin contain on high content of the amino acids glycine, proline and hydroxyproline which are free of sulfur.

The films thicknesses were measured automatically by a micrometer connected to the Universal Testing Instrument (Zwick \ Z010). There were 0.1272 mm and 0.1704 mm for soy-starch and gelatin, respectively

Although plasticizers are hydrophilic substances causing an increase in the films solubility (Gennadios *et al.*, 1994). It must be added to the film-forming solutions. Because it decreasing the accumulation of intermolecular forces along polymer chains. Thereby "softening" film structure, decreasing tensile strength and increasing elongation (Mellan, 1961). Whereas, films made without plasticizer were extremely brittle and shattered (Brandenburg *et al.*, 1993).

Polymers which contain groups that can associate through hydrogen or ionic bonding causing the susceptibility of films to absorb moisture (Salame, 1986).

Therefore, both gelatin and soy-starch films are susceptible to moisture absorption. All the film pieces of gelatin were completely dissolved in all the pH solutions. While, not all soy-starch film pieces dissolved. The film pieces that immersed in the pH values 1, 9 and 13 were remained stable; this result confirmed by Gennadios *et al.* (1993). So, the soy-starch film can withstand the highly acidic and highly alkaline solutions.

Also, the edible films that produced from polysaccharides and proteins were showed limited resistance to moisture transmission. This is due to the inherent hydrophilicity of the film-forming substances and to the considerable amount of hydrophilic plasticizers that incorporated into the films to ensure formation of free-standing films (Guilbert *et al.*, 1996 and Callegarin *et al.*, 1997**)**. Therefore, both films were showing limited water vapor permeability.

Tensile strength (TS), elongation (E %) and the elastic modulus (E- Modulus), are measurements helps in the description of the mechanical properties for the films and the relation with their chemical structures (Ninnemann, 1968). Tensile strength is an important mechanical property, which expresses the maximum stress which developed on the film during the tensile testing (Briston, 1988). Whereas the elongation, is the ability of the film to stretch; it determined at the point where the film breaks under tensile testing. And It expresses as, the percentage of change of the original length of the specimen between the grips of the testing machine (Briston, 1988). While the elastic modulus, is the mathematical description of an object or substance's tendency to be deformed elastically (i.e., non-permanently) when a force is applied to it (Hartsuijker and Welleman, 2001). However, soy-starch film showed lower TS, lower E% and lower E- Modulus than gelatin film (Figure 3, Table2 and Figure 4, Table 3 respectively).

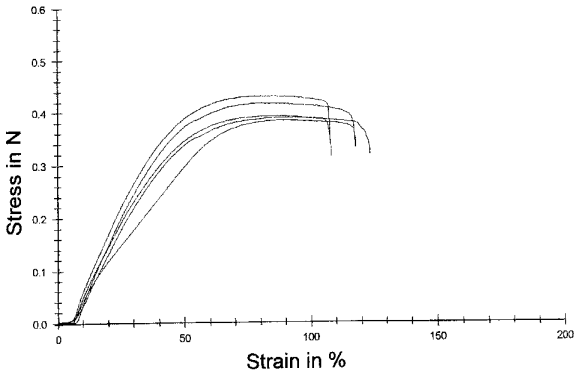

Figure 3. Tensile strength (TS), percent elongation (%E) and elastic modulus (E- Modulus) of Soy-starch film
Test standard: ASTM D638-02, Load cell: 100N.
Thickness of protein-carbohydrate film = 0.1272 mm

Table 2. Statistics of TS and %E of Soy-starch film.

n=5	E-Modulus MPa	RM MPa	ε F max %	RB MPa	ε Break %
X	1.94	0.87	81.78	0.71	110.79
S	0.44	0.08	6.38	0.07	6.81
V	22.68	9.59	7.80	9.34	6.15

X: the mean of five measurements. **S:** Standard deviation.
V: Poisson's ratio.

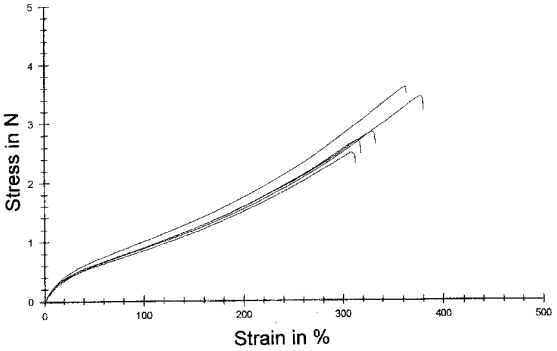

Figure 4. Tensile strength (TS), percent elongation (%E) and elastic modulus (E- Modulus) of gelatin film.
Test standard: ASTM D638-02, Load cell: 100N.
Thickness of protein film = 0.1704 mm

Table 3. Statistics of TS and %E of gelatin film.

n=5	E- Modulus MPa	RM MPa	ε F max %	RB MPa	ε Break %
X	4.08	5.33	338.49	4.98	341.49
S	0.75	0.81	30.31	0.88	29.33
V	18.28	15.23	8.95	17.76	8.59

X: the mean of five measurements. **S:** Standard deviation.
V: Poisson's ratio.

Those results can be explained as follow; in case of gelatin film. The amino acids chains rearranged with the help of glycerol (plasticizer) during drying the film (Mellan, 1961). This led to the formation of a uniform surface structure (Figure 6). Whereas in case of the soy-starch film, also glycerol helped in the rearrangement of amino acids chains for soy protein. But, although the addition of starch improved the mechanical properties of the film (comparing with a film was prepared free from starch). Addition of starch caused the formation of uneven surface (Figure 5) which affected the film tensile strength, elongation and elastic modulus.

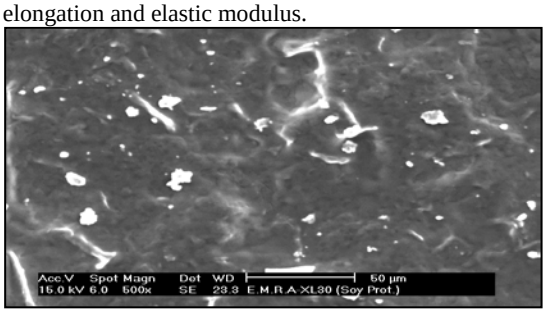

Figure 5. SEM of soy-starch film surface.

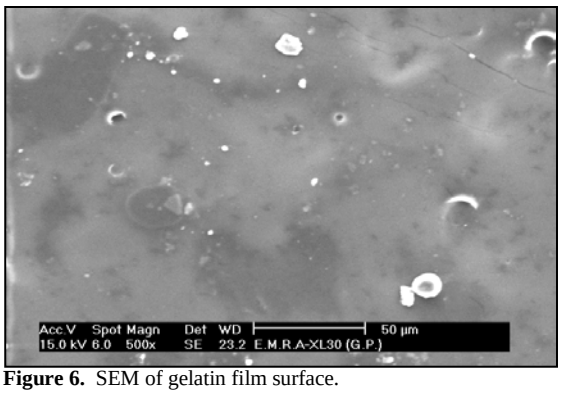

Figure 6. SEM of gelatin film surface.

The results that detected quantitatively by GC-MS showed the presence of α-pinene in soy-starch film, while α-pinene and limonene in gelatin (Figure7 and Figure 8, respectively**)**.

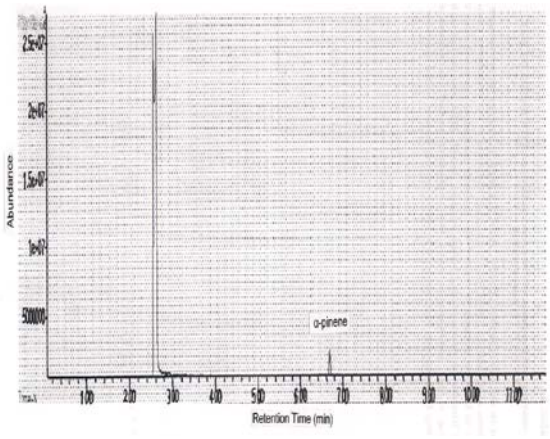

Figure 7. Chromatogram of GC-MS for soy-starch film.
Temp. : 22± 2°C **RH:** 43±5%
Area for α-pinene = -2.76 (Representing the amount in the area of film specimen of (8 ×8 cm)).

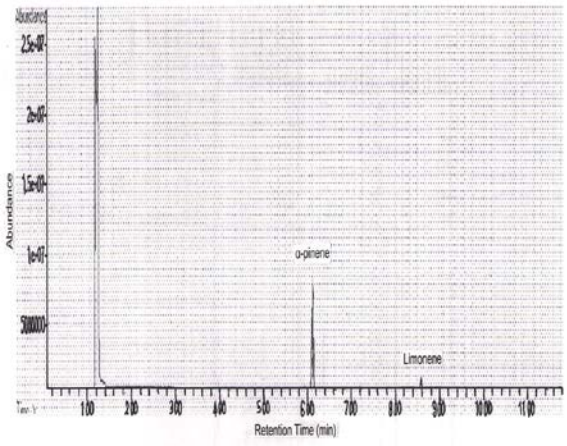

Figure 8. Chromatogram of GC-MS for gelatin film.

Temp. : 22± 2°C **RH:** 43±5%
Area for α-pinene = - 11.4 & **for limonene =** - 0.83
(Representing the amount for both of them in the area of film specimen of (8 ×8 cm))

This test proved that, the antimicrobial activity of the films which directly attached to the food products was due to the existence of the essential oils components in the films. Also, the antimicrobial activity of the films which covered the jars was due to the liberalized essential oils components from the films to the space above the surface of the packaged food (not packaged under vacuum condition).

α-pinene is an organic compound of the terpene class, one of two isomers of pinene (Figure 9).

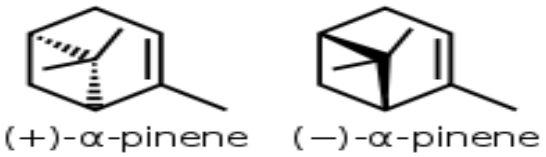

Figure 9. α-pinene structure

(1S, 5S)-2, 6, 6-Trimethyl bicycle [3.1.1] hept-2-ene ((−)-α-Pinene).

It is an alkane, contains a reactive four- membered ring and of melting point 64° C. It is found in the oils of many plant species (Simonsen, 1957). The four-membered ring makes it a reactive hydrocarbon (Richter, 1945); therefore, α-pinene easily attached to both soy-starch and gelatin amino acids.

Limonene is a hydrocarbon, classified as a cyclic terpene and is a chiral molecule in which biological sources produce one enantiomer (Simonsen, 1947) (Figure 10).

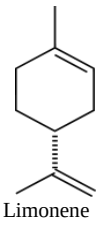

Figure 10. Limonene structure

1-methyl-4-prop-1-en-2-yl-cyclohexene (Racemic: DL-limonene).

It is a relatively stable terpene, which can be distilled without decomposition, although at elevated temperatures it cracks to form isoprene (Pakdela *et al.*, 2001). It is considered by some researchers to be a significant chemopreventive agent (Crowell, 1999).

Gelatin structure contains on high content of amino acids glycine, proline and hydroxyproline (Bourtoom, 2008). While, it contains on many of glycine (almost 1 in 3 residues arranged every third residue), proline and 4-hydroxyproline residues (Chaplin, 2009). So, the availability of free hydrogen bond in glycine amino acid in gelatin skeleton permit the binding of limonene through the second C=C double bond with gelatin. This explains the presence of limonene only in gelatin film.

The used food products showed different susceptibility towards both films of control and those enriched with the essential oils mixture (Figure 11).

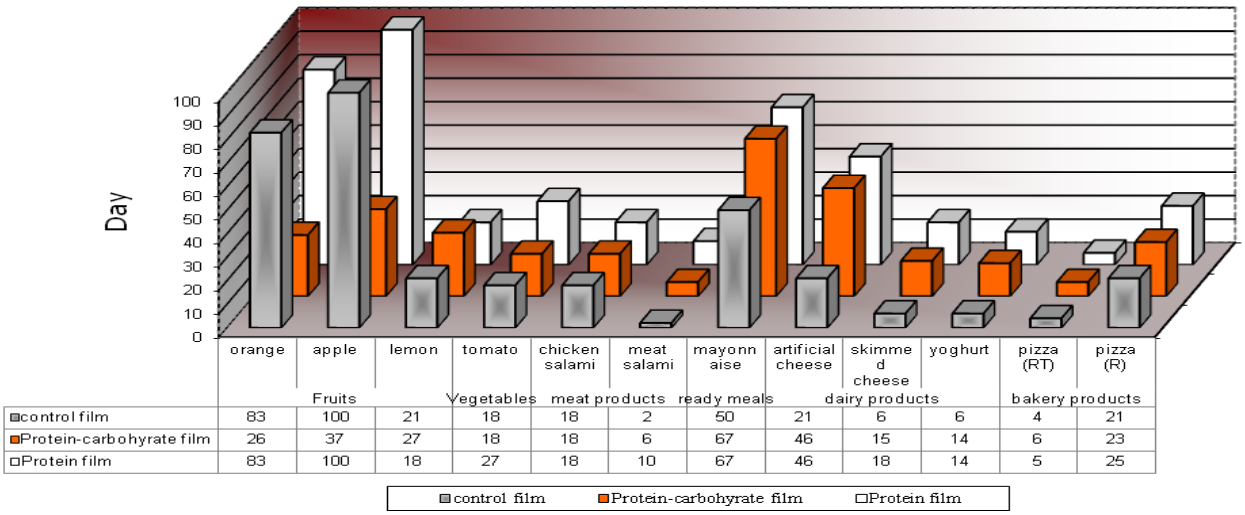

Figure 11. The effect of films enriched with essential oils mixture on the shelf-life of different food products.

Pizza (RT): Pizza dough kept at room temperature. Pizza (R): Pizza dough kept in the refrigerator.

Although, the direct attachment of the films which enriched with the essential oils mixture have the limited benefits because the active substances either neutralized on the food surface or diffused rapidly from the surface into the food mass (Quintavalla and Vicini, 2002). Good results obtained by this way with Meat Salami, Artificial Cheese, and the refrigerated Pizza dough. Whereas, the essential oils components can be "in some cases" insufficient to inhibit the microbial growth or adsorbed rapidly on the food stuff surface. This supposition can be explaining the results obtained with Chicken Salami, and Pizza dough which kept at room temperature.

Theoretically, food products packaged in containers and sealed with the films enriched with the essential oils mixture can be the best process. That is because; the air which filled the space over the food stuff be saturated with essential oils components (evidenced by GC-MS test), adsorbed and diffused slower than that directly attached with the food surface.

However, the use of such packaging materials is not meant to be a substitute for good preservation practices, but it should enhance the safety of food as an additional hurdle for the growth of pathogenic microorganisms.

If the types of food products can be divided in general into solid, semi-solid and soft food stuffs; this work succeeded in designing a protection process to the solid and semi-solid one by using the simplest techniques and materials. According to the results "and after the purification and chlorophyll removal process", extractions of *Myrtus communis*, *Urtica urens*, *Ziziphus spina-christi* and *Zygophyllum coccineum* can be used as antibacterial food additives.

4. Conclusion

The microbicidial activities for the films of soy-starch and gelatin that enriched with 0.5ml of the essential oils mixture were attributed to the presence of α-pinene in soy-starch film, and α-pinene and limonene in gelatin film. From the physicochemical properties of films, those edible films were found to be suitable for packaging solid and semi-solid food products.

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References

Abo-Zaid EN. 2000. **Volatile Oils**, 1st ed. Dar-el-Arabeya. [In Arabic]

Atlas RM. 1979. **Hand Book of Microbiological Media**, 2nd ed. CRC Press.

Batanouny KH, Abou Tabl S, Shabana M and Soliman F. 1999. Wild medical plant in Egypt. An inventory to support conservation and sustainable Use. Academy of Scientific Research and Technology, Egypt. International Union for Conservation (IUCN), Switzerland. pp: 207.

Bourtoom T. 2008. Edible films and coatings: characteristics and properties. *Inter Food Res J*., **15** (3): 1-12.

Brandenburg AH, Weller CL and Testin RF. 1993. Edible films and coatings from soy protein. *J Food Sci*., **58(5)**:1086-1089.

Briston JH. 1988. **Plastics Films**, 3rd ed. Wiley: New York.

Burt SA. 2004. Essential oils: their antibacterial properties and potential applications in foods: a review. *Inter J. Food Microbiol*. **94**:223–253.

Cagri A, Ustunol Z and Ryser ET. 2004. Antimicrobial edible films and coatings. *J Food Prot*.,**67(4)**: 833-848.

Callegarin F, Gallo JAQ, Debeaufort F and Voilley A. 1997. Lipids and biopackaging. *J Am Oil Chem Soc*., **74**: 1183 – 1192.

Chaplin M. 2009. Gelatin. Licensed under a Creative Commons Attribution-Noncommercial – No Derivative Works 2.0 UK: England & Wales License.

Coma V, Sebti I, Pardon P, Deschamps A and Pichavant FH. 2001. Antimicrobial edible packaging based on cellulosic ethers, fatty acidsmand nisin incorporation to inhibit *Listeria innocua* and *Staphylococcus aureus*. *J Food Prot*.,**64(4):** 470 - 475.

Crowell PL. 1999. Prevention and therapy of caner by dietary monoterpenes. *J Nutr*., **129 (3**): 775S–778S.

Curini M, Bianchi A, Epifano F, Bruni R, Torta L and Zambonelli A. 2003. Composition and *in vitro* antifungal activity of essential oils *of Erigeron canadensis* and *Myrtus communis* from France. *Chem Natural Compounds*, **39(2):** 191-194.

De Carvalho RA and Grosso CRF. 2006. Properties of chemically modified gelatin films. *Braz J Chem Eng*., **23 (1**): 45 - 53.

Devlieghere F, Vermeiren L and Debevere J. 2004. New preservation technologies: Possibilities and limitations. *Inter Dairy J*.,**14**: 273-285.

Dweck AC. 2005. A review of *Ziziphus spina- christi*. *Personal Care Magazine*, **6**: 53-55.

Gennadios A, Mchugh TH, Weller CL and Krochta JM. 1994. Edible coatings and films based on proteins. In: Krockta JM., Baldwin EA and Nisperos-Carriedo MO, (eds). **Edible Coatings and Films to Improve Food Quality**. Technomic: Lancaster, PA. pp 201- 277.

Gennadios A, Brandenburg AH, Weller CL and Testin RF. 1993. Effect of pH on properties of wheat gluten and soy protein isolate films. *J Agric Food Chem*., **41**:1835-1839.

Ghorpade VM, Gennadios A, Hanna MA and Weller CL. 1995. Soy protein isolate / poly (ethylene oxide) films. *Cereal Chem*.,**72(6):** 559-563.

Gill AO and Holley RA. 2006. Disruption of *Escherichia coli*, *Listeria monocytogenes* and *Lactobacillus sakei* cellular membranes by plant oil aromatics .*Inter J Food Microbiol*.,**8**: 1 – 9.

Glombitza KW, Mahran GH, Mirhom YW, Michel KG and Motawi TK. 1994. Hypoglycemic and antihyperglycemic effects of *Ziziphus spina- christi* in rats. *Planta Med*., **60**: 244-247.

Guilbert S, Gontard N and Gorris LGM. 1996. Prolongation of the shelf- life of perishable food products using biodegradable films and coatings. *Lebensmittel-Wissenschaft und-Technologie*. **29(1)**:10-17.

Han JH. 2000. Antimicrobial food packaging. *Food Technol*., **54 (3)**: 56-65.

Hartsuijker C and Welleman JW. 2001. **Engineering Mechanics**, Vol. 2. Springer.

Holley RA and Patel D. 2005. Improvement of shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiol*., **22:** 273–292.

Kinsella JE, Damodaran S and German B. 1985. Physiochemical and functional properties of isolated proteins with emphasis on soy proteins. In: Altschul AM and Wilcke HL, (Eds), **New Protein Foods.** Orlando, FL: Academic Press. pp.107-179.

Kordali S, Kotan R, Mavi A, Cakir A, Ala A and Yildirim A. 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium, A. dracunculus, Artemisia santonicum,* and *Artemisia spicigera* essential oils. *J Agric Food Chem.*, **53**: 9452–58.

Kunte LA, Gennadios A, Cuppett SL, Hanna MA and Weller CL. 1997. Cast films from soy protein isolates and fractions. *Cereal Chem.*, **74(2):**115–118.

Lemberkovics E, Kéry A, Kakasy E and Simái B. 2003. Effect of extraction method on the composition of essential oils. ISHS Acta Horticulture 597. International Conference on Medicinal and Aromati Plants (Part II).

Maizura M, Fazilah A, Norziah MH and Karim AA. 2008. Antibacterial activity of modified *Sago starch-alginate* based edible film incorporated with lemongrass (*Cymbopogon citratus*) oil. *Inter Food Res J*., **15(2):** 233-236.

Mellan I. 1961. **The Behavior of Plasticizers**. Pergamoon press, New York.

Montvale NJ. 2000. **PDR for Herbal Medicine**, 2nd Edition. Medical Economics Company.

Ninnemann KW. 1968. Measurements of physical properties of flexible films. In: Sweeting OJ,(Ed), **Science and Technology of Polymer Films.** Interscience, London, England. pp. 546-649.

Özcan M, Kasik G and Öztürk C. 2005. Inhibitory effect of essential oils of myrtle, laurel, pickling herb and thyme and savory on the mycelial growth of *Agaricus campestris* (Lange) Sing. *J Essential Oil Bearing Plants*, **8(2):**120-125.

Pakdela H, Panteaa D and Roy C. 2001. Production of dl-limonene by vacuum pyrolysis of used tires. *J Analytical and Applied Pyrolysi.*, **57(1):** 91–107.

Pérez-Pérez C, Regalado-González C, Rodríguez-Rodríguez CA, Barbosa-Rodríguez JR and Villaseñor-Ortega F. 2006. Incorporation of antimicrobial agents in food packaging films and coatings. In: Guevara-González RG and Torres-Pacheco L, (Eds.), **Advances in Agricultural and Food Biotechnology**. pp193-216.

Quintavalla S and Vicini L. 2002. Antimicrobial food packaging in meat industry. *Meat Sci*., **62 (3):** 373 – 380.

Randall C. 2003. Historical and modern uses of Urtica. In: Kavalali GM, (Ed.). *Urtica*. **Therapeutic and Nutritional Aspects of Stinging Nettles.** Taylor & Francis, London, New York. pp. 12-24.

Richter GH. 1945. **Textbook of Organic Chemistry**, 2nd Edition. John Wiley & Sons, New York. pp. 663-666.

Rodrigues ET and Han JH. 2000. Antimicrobial whey protein films against spoilage and pathogenic bacteria. Proceedings of the IFT Annual Meeting. Dallas, June 10-14. Chicago, Ill.: Institute of Food Technologists. pp191.

Salame M. 1986. Barrier polymers. In: Bakker M, (Ed.), **The Wiley Encyclopedia of Packaging Technology**. New York: Johh Wiley and Sons. pp.48-54.

Schott JR. 1997. **Matrix Analysis for Statistics**. New York: John Wiley and Sons.

Shahidi Bonjar GH, Nik AK, Heydari MR, Ghasemzadeh MH, Farrokhi PR, Moein MR, Mansouri S and Foroumadi A. 2003. Anti-Pseudomona and Anti-Bacilli activity of some medicinal plants of Iran. *DARU*. **11(4):** 157-163.

Simonsen JL. 1957. **The Terpenes**, 2nd Edition. (Vol. 2). Cambridge University Press.

Simonsen JL. 1947. **The Terpenes**, 2nd Edition. (Vol. I). Cambridge University Press.

Sonti, S. 2003. Consumer perception and application of edible coatings on fresh-cut fruits and vegetables (MSc thesis). Faculty of Agricultural and Mechanical College: Louisiana State University.

Tsybula NV and Kazarinova NV. 1996. Sanative effect of volatile compounds produced by intact common Myrtle *Myrtus communis* L. in interiors. *Bulletin Exper Biol Med*., **121 (5):** 597-600.

Tuberoso CIG, Barra A, Angioni A, Sarritzu E, and Pirisi FM. 2006. Chemical composition of volatiles in Sardinian Myrtle (*Myrtus communis* L.) alcoholic extracts and essential oils. *J Agric Food Chem.*, **54**: 1420-1426.

Waggas AM. 2007. Acute effect of Sidr leaves extract on some neurotransmitter contents in different brain areas of male albino rats. *Saudi J Biol Sci*., **14 (1):** 93-101.

Wichtl M (Ed.). 2002. **Teedrogen und Phytopharmaka. Ein Handbuch für die Praxis auf wissenschaftlicher Grundlage**, 4. Auflage. Wissenschaftliche Verlagsges, M.B.H., Stuttgart. pp. 617-619.