Preparation, Characterization & Antimicrobial Activity of Active Packaging Film Incorporated with Licorice Root Extract

[1]Divya Yadav, [2]Divya Puri, [3]Mahak Sharma

[1] Student M.Sc,[2]Assistant Professor, [3]Associate Professor
[1]Department of Nutrition & Dietetics,
[1] Manav Rachna International Institute of Research & Studies, Faridabad, India

Abstract : The present study aimed at developing a packaging film incorporated with licorice root extract. The innovation behind using licorice root extract was to explore its antimicrobial property in food packaging sector, rather than its conventional usage in the pharmaceuticals and cosmetic industries The active component of licorice root named glycorrizic acid has antimicrobial properties and Hence, can be used as an active ingredient in making antimicrobial active packaging film. Two film samples i.e. test sample and control sample were prepared. The test film sample was made using Tween 80, PEG, food grade pectin, water and licorice root extract and the control sample was not incorporated with licorice root extract and only prepared with tween80, PEG, food grade pectin and water. The films were standardized using different formulations. Both the films were subjected to physical and mechanical tests. Both the films were analyzed for zone of inhibition for Rhizopus stolonifer. The data on obtained by performing different tests showed that there was significant difference at p≤0.05 in tear strength, tensile strength, thickness, bursting strength and zone of inhibition for Rhizopus stolonifer of the sample A and sample B.

Index terms : antimicrobial packaging , active packaging, licorice root extracts ,glycorizic acid , glabiridine.

Introduction

Active Packaging is very popular recently and will gradually revolutionize the packaging industry. Active packaging are of different types named as, Bio-Chemical active films, Antimicrobial packaging, Oxygen, CO_2, Gas-scavenging, Moisture control, Anti-Oxidation, Temperature Controlled Packaging, Active labels and indicators and Nano-technology enabled packaging (Karleigh Huff).Active packaging is accurately defined as "packaging in which subsidiary constituents have been deliberately
included in or on either the packaging material or the package headspace toenhance the performance of the package system (Robertson, 2006).This phrase emphasizes the importance of deliberately including a substancewith the intention of enhancing the food product.Active packag ing is an extension of the protection function of apackage and is commonly used to protect against oxygen and moisture.

Active packaging systems are developed with the goal of extending shelf life for foods & increasing the period of time that the food is high quality .

Active packaging technologies include some physical, chemical,or biological action which changes interactions betwen a package, product, a nd/or headspace of the package in order to get a desired outcome (Yam et al.,2005).The most common active systems scavenge oxygen from the package or the product and may even be activated by an outside source such as UV light (Gander, 2007). The active packaging relates to all the packaging systems that actively interact with the packaged food or with the food surface, to extend the shelf life and to improve the quality of the products, preserving their sensorial characteristics and safety along the storage period. In this definition are included several complex systems with different functions. In particular, a first classifications of active systems can be made, according to their functionality, in absorbers or scavenger systems (Lopez Rubio, 2004; Labuza, 1989; Vermeiren, 1999; Brody, 2001; Anon, 1995; Zagory, 1995), releasing or emitter systems (Lopez-Rubio, 2004; Vermeiren, 1999; Ahvenainen, 2003) and active systems based on the energy transport (Xia, 2008; Benim, 2007).

Antimicrobial Packaging

Antimicrobial packaging is a rapidly emerging technology. The need to package foods in a versatile manner for transportation and storage, along with the increasing consumer demand for fresh, convenient, and safe food products presages a bright future for AM Packaging. However, more information is required on the chemical, microbiological and physiological effects of these systems on the packaged food especially on the issues of nutritional quality and human safety (Floros et al., 1997). So far, research on AM packaging has focused primarily on the development of various methods and model systems, whereas little attention has been paid to its preservation efficacy in actual foods (Han 2000). Research is essential to identify the types of food that can benefit most from AM packaging materials. It is likely that future research into a combination of naturally-derived AM agents, biopreservatives and biodegradable packaging materials will highlight a range of the merits of AM packaging in terms of food safety, shelf-life and environmental friendliness (Nicholson 1998; Rodrigues and Han 2000; Coma et al., 2001). The reported effectiveness of natural plant extracts suggests that further research is needed in order to evaluate their antimicrobial activity and potential side effects in packaged foods. An additional challenge is in the area of odor/flavor transfer by natural plant extracts to packaged food products. Thus, research is needed to determine whether natural plant extracts could act as both an antimicrobial agent and as an odor/flavor enhancer. Moreover, in order to secure safe food, amendments to regulations might require toxicological and other testing of compounds prior to their approval for use (Vermeiren et al., 2002)

Licorice *Glycyrrhiza glabra*, family Leguminoseae, is a plant which grows in Egypt and other countries of the world. Its roots possess some nutritive value and medicinal properties. *Glycyrrhiza glabra Linn* , a commonly used herb in ayurvedic medicine. Studies indicate that *Glycyrrhiza glabra Linn* possesses antibacterial, antioxidant, antimalerial, antispasmodic, anti-inflammatory and anti hyper glycemic properties. Various other effects like antiulcer, antiviral, antihapatotoxic, antifungal and herpes simplex have also been studies . They are widely used as a cold beverage, in preparing some pharmaceutical preparations such as haematinic pills and to disguise the bitter taste

of other remedies (Fenwick et al., 1990). *Glycyrrhiza glabra Linn* is one of the most widely used herb from the ancient medical history of Ayurveda, both as a medicine and also as a flavoring herb. It is a very sweet, moist, soothing herb that detoxifies and protects the liver and is also a powerful anti-inflammatory, being used in conditions as varied as arthritis and mouth ulcers . *Glycyrrhiza glabra*, also known as licorice and sweetwood, is native to the Mediterranean and certain areas of Asia. Historically, the dried rhizome and root of this plant were employed medicinally by the Egyptian, Chinese, Greek, Indian, and Roman civilizations as an expectorant and carminative. In modern medicine, licorice extracts are often used as a flavoring agent to mask bitter taste in preparations, and as an expectorant in cough and cold preparations. Licorice extracts have been used for more than 60 years in Japan to treat chronic hepatitis, and also have therapeutic benefit against other viruses, including human immunodeiciency virus (HIV), cytomegalovirus (CMV), and Herpes simplex. Deglycyrrhizinated licorice (DGL) preparations are useful in treating various types
of ulcers, while topical licorice preparations have been used to sooth and heal skin eruptions, such as psoriasis and herpetic lesions.

METHODOLOGY

The study was carried out in the following six phases:

1. Procurement of Raw materials
2. Film Preparation
3. Physical Test of Packaging Films
4. Mechanical Test of Packaging Films
5. Microbiological Test of Packaging Films
6. Statistical Analysis

Procurement of Raw Materials

This phase consists of procurement of raw materials from the market. The Licorice roots and food grade pectin were collected from local market of Faridabad, India. The chemicals named Polyethylene Glycol (PEG) and Tween 80 were collected from Liberty chemicals shop situated in Delhi.

Film Preparation

Formation of Licorice Extract This phase foremostly consists of the licorice root extract formation followed by film formation using the same extract. Licorice roots were collected and dried in shade at room temperature. After complete drying, these roots were powdered in an electric blender. 10g of licorice root powder was then mixed with 300 ml water and heated at 60^0C under constant stirring for 4 hours. After cooling down, the solution was filtered using a strainer. Finally the licorice root extract was stored in refrigerator at 4^0C film prepararion.
Film Formation The film was formed using combination of following ingredients

2.5g Pectin + 2ml Tween 80 + 8g PEG 400 + 78.5ml water + 9ml licorice root extract (9.8%)
The above ingredients were mixed properly and stirred for half an hour using a magnetic stirrer. The solution was then poured over a ceramic tile and kept for drying at room temperature for 24 hours. The dried films were cut, peeled from the casting surface and stored wrapped in butter paper within air-tight sealed plastic bags.

Physical Test of Packaging Films

Flame Test
The sample was held at the edge of a flame. If no flame is produced quickly, hold the sample in the flame for about 10 seconds. If the material burns, note the color of the flame, the nature of the smoke, the presence of soot in the air and whether, while burning, the sample drips. Next, extinguish the flame and cautiously smell the fumes. To identify the odor, samples of known plastic samples for comparison can be most helpful. Finally, check your observations against the known characteristics of each plastic. The test was repeated thrice for each sample [ASTM 1980].

Biodegradability Test
Film samples were buried in soil, or performing a full-scale composting process with the biodegradable plastic, represent the ideal practical environmental conditions

Mechanical Test

Tensile Strength
The tensile strength of a paper is defined as the force applied parallel to the plane of the specimen of specified width and length under specified condition of loading. The test indicates the durability and serviceability of papers in many packaging operations such as wrapping, bagging, printing etc. Plastic films are normally tested at higher speeds because of higher extensibility. The stress strain curve helps in locating the yield point and knowing the yield strength.
Tensile strength was measured as per the method mentioned in IS:1060:Part:1, Standard
Method . Ten samples of 2.54cm x 12cm were cut from each film. Initial grip separation and crosshead speed were set at 50 mm and 50mm/min respectively. Tensile strength was calculated by dividing the maximum force by the initial specimen cross-sectional area and the percentage elongation at the break was calculated as follows:

$$\textbf{Tensile strength} = F_{max} / A$$

Where, F_{max} was the force applied and A was the area on which the force applied.

Thickness Test

Thickness of a material is the perpendicular distance between the two outer surfaces of the material. Many physical properties of packaging materials are dependent upon the thickness e.g. Water Vapor Transmission Rate (WVTR) and Gas transmission Rate (GTR) of a film is inversely proportional to thickness and decrease with increase in thickness. Properties like tensile strength, sealability and seal strength, moisture, gas and light barrier properties are directly related to thickness. Dial gauge, micrometer, screw gauge, vernier calipers are used for the measurement of thickness. For paper boards, thickness is reported in points or in mm (1 point = 1/1000 of an inch); for papers it is in mm or inches. For films, thickness is reported in micron, mils or in gauge. In case of laminates the thickness of the constituent plies are more important as they influence the barrier properties.

A piece of sample was cut without any regularities of size 10cm ×10cm . The specimen was placed between two points of the micrometer, one of which was lifted gently and the film was inserted and the corresponding reading was noted. The thickness can be expressed in any unit such as micron, inches, mil etc.

(0.001" = 25 micron = 100 gauge = 1 mil)

Microbiological Test

Initial screening of potential antibacterial and antifungal compounds from plants may be performed with pure substances (Afolayan and Meyer 1997; Batista et al, 1994) or crude extracts (Vijaya et al., 1995). The methods used for the two types of organisms are similar. The two most commonly used screens to determine antimicrobial susceptibility are the broth dilution assay and the disc or agar well diffusion assay. In some cases, the inoculated plates or tubes were exposed to UV light to screen for the presence of light-sensitizing photochemicals (Vijaya et al, 1995).

Agar Well Diffusion Method

Agar well diffusion method is used to determine the antimicrobial activity. Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old broth culture of *Rhizopus stolonifer*. Wells (10 mm diameter) were made in each of the plates using sterile cork borer. Licorice root extract was prepared at
concentrations of 6%, 7%, 8% and 9% respectively . About 100µl of these concentrations of extract were added into the wells using a sterile syringe. The plates were further allowed to diffuse at room temperature for 2 hrs.

Control plate comprising inoculum without licorice root extract was prepared. The plates were incubated at 28^0C for 48 hours. The diameter of the inhibition zone (mm) was measured. Triplicates were maintained and the experiment was repeated thrice. For each replicate the reading was taken in three different fixed directions and the average value was recorded.

Statistical Analysis

Statistics is the science of the collection, organization and interpretation of data. It refers to a collection of methods used to process large amounts of data and report overall trends. Statistical analysis is particularly useful when dealing with noisy data and it provides ways to objectively report on how unusual an event is based on historical data. On the basis of information obtained from different tests conducted on film, analysis was done by calculating mean for all the value.

Difference among the mean values of the various test conducted on the films were calculated by using mean calculator. The independent-t test was performed to compare the means of different results of the films. Statistical analysis was performed using the SPSS software (*version 20*) and the significance was defined at $p < 0.05$ (two-tailed).

RESULTS AND DISCUSSION

Physical Tests The physical analysis of the active packaging film (APF) comprised of the following tests:

Flame Test The flammability test is used to determine the relative rate of burning of self-supporting plastics. This test is mainly used for quality control, production control and material comparisons. For quality control, production control and material comparisons. It cannot be used as a criterion for fire hazard. The flame was of orange colour with slow speed of drip with good scratch able properties for films enriched with licorice root extract. For the control sample with no extract, the flame was of orange colour with moderate drip speed with good scratch able properties. The odour was same like paraffin for both the samples and average time taken for a film of 15 sec. The parameters were checked in accordance with (Nummer L, Trombetta et al., 2012). The
residue of films after burning was black in colour.

Table 1: Flame Test Results of Packaging Films

SAMPLE	ODOUR	DRIP	DRIPPING SPEED	COLOUR OF FLAME	TIME
A	Paraffin	Yes	Moderate	Orange	15 secs
B	Paraffin	Yes	Slow	Orange	15.7secs

* Values are represented as Mean ±SD of triplicate determination, Sample-A: Control (i.e. packaging film without licorice root extract) and Sample-B: Test Sample (i.e. packaging film with licorice root extract)

Biodegradability Test

The films were buried in soil for about a week to check the biodegradability of films. The term biodegradable plastics normally refer to an attack by microorganisms on no water soluble polymer-based materials (plastics). This implies that the biodegradation of plastics is usually a heterogeneous process. Because of the lack of water solubility and the size of the polymer molecules, microorganisms are unable to transport the polymeric material directly into the cells where most biochemical processes take place; rather, they must first excrete extracellular enzymes which depolymerise the polymers outside the cells (Joachim et al.,2014). The films were completely degraded and no residue was reported.

Table 2: Biodegradability Test Results of Packaging Films

PARAMETER	SAMPLE-A	SAMPLE-B
Biodegradability Test	Positive	Positive

* Values are represented as Mean ±SD of triplicate determination, Sample-A: Control (i.e. packaging film without licorice root extract) and Sample-B: Test Sample (i.e. packaging film with licorice root extract)

Mechanical Tests

The Mechanical test analysis of active packaging film (APF) comprised of the following tests:

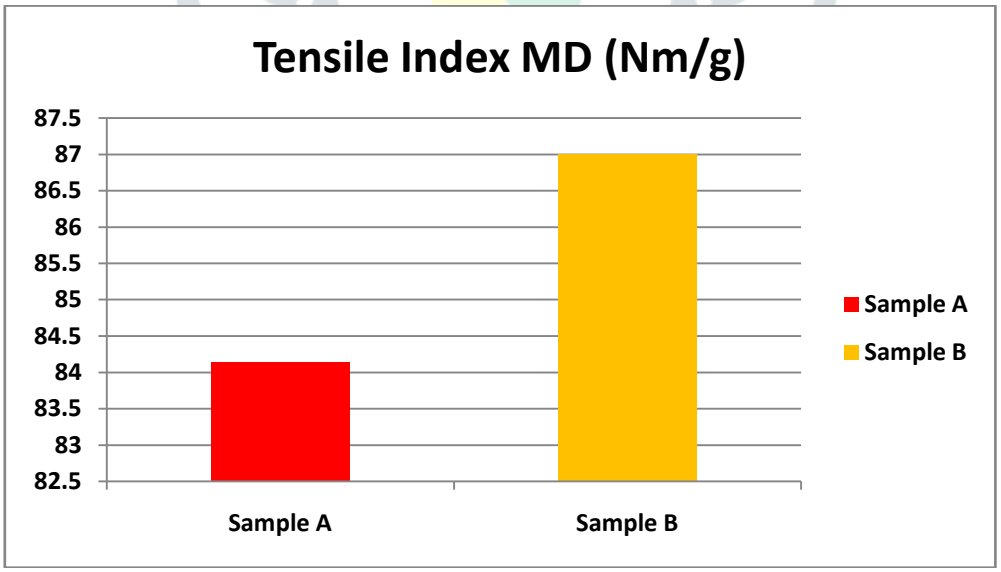
Tensile Strength As food packaging function to protect food during handling, transportation and marketing require high TS (Sousa et al., 2010b; Pitak and Rakshit, 2011). Tensile strength (TS) is the key indicators of films strength [Ghasemloua et al., 2014]. It expresses the maximum stress developed in a film specimen during tensile testing. The incorporation of licorice root extract in pectin based films caused a significant increase in tensile strength of the film as compared to the control one which was not incorporated with licorice root extract. The tensile strength was calculated in two dimensions i.e. Machine Dimensions (MD) and Cross Dimensions (CD). Tensile strength of Sample B i.e. Test sample was greater than sample A i.e. control sample. The results are depicted the given Table.

Table 3: Tensile Index Test Results of Packaging Films

PARAMETER	SAMPLE A	SAMPLE B	p value
Tensile index CD (Nm/g)	38.56±0.09	41.94±0.06	.00001
Tensile index MD (Nm/g)	84.15±0.17	87±0.079	.00001

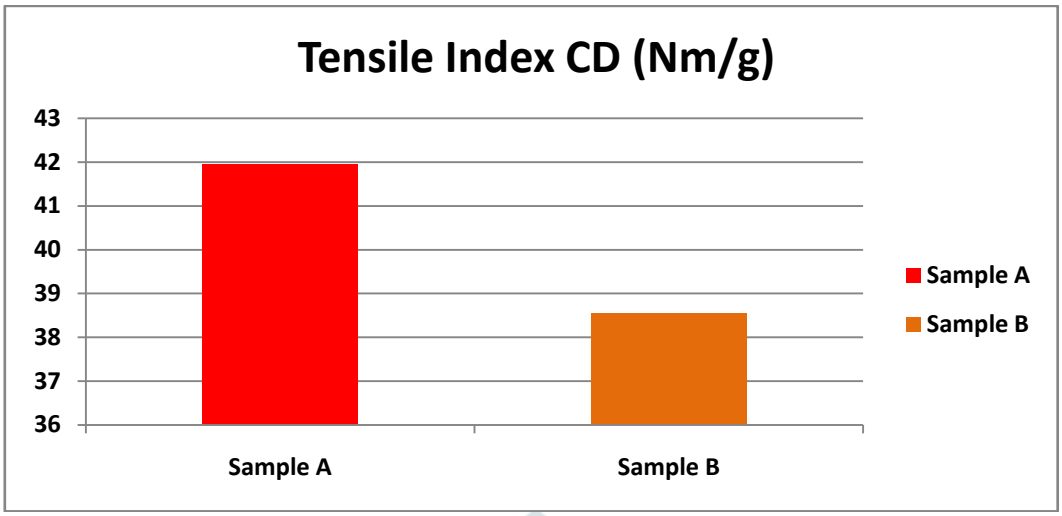
* Values are represented as Mean ±SD of triplicate determination , Sample-A: Control film (i.e. packaging film without licorice root extract) and Sample-B: Test Sample (i.e. packaging film with licorice root extract) *Significance value p≤0.05 for independent t-test administered

Graphical Representation of Tensile index (MD) of Packaging Films



*Sample-A: Control (i.e. packaging film without licorice root extract) and Sample-B: Test Sample (i.e. packaging film with licorice root extract)

Graphical Representation of Tensile index (CD) of Packaging Films



*Sample-A: Control (i.e. packaging film without licorice root extract) and Sample-B: Test Sample (i.e. packaging film with licorice root extract

Thickness Test

The films thickness is an important characteristic in determining the feasibility of films as packaging materials for food products since the thickness of the films affects other characteristics of the film, such as tensile strength, elongation, and water vapour permeability (Hugh et al., 1994; Galus and Lenart, 2013). The films thickness is dependent on both film composition and processing parameters (Garcia et al., 2009).

The thickness of control and test samples was reported to be 77.283±0.125µm and 79.22±0.0763 µm respectively. The test sample (i.e. active packaging film with licorice root extract) was found to be thicker in comparison to the control sample (i.e. packaging film without licorice root extract). It might be deduced that the test packaging film had a better tensile strength and water vapor permeability as compared to the control film.

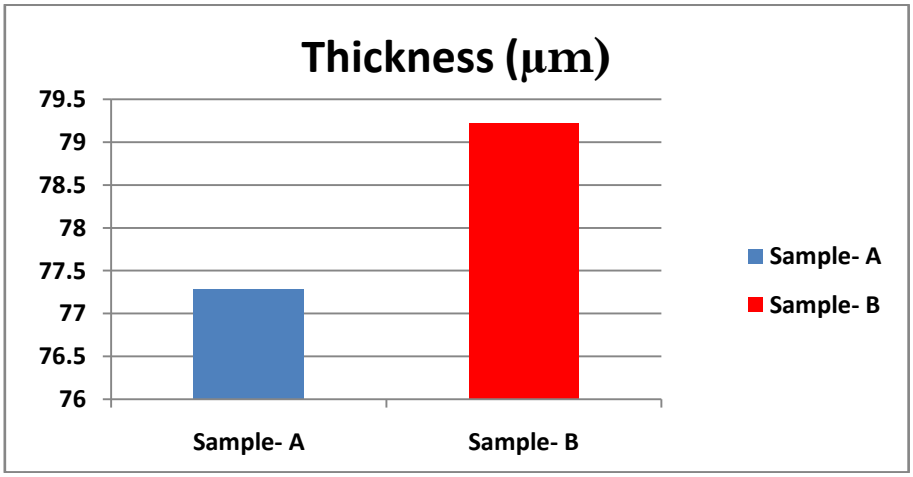
Table 4: Thickness Test Results of Packaging Films

PARAMETER	SAMPLE- A	SAMPLE- B	p- value
Thickness (µm)	77.28±0.12	79.22±0.07	0 .00001

* Values are represented as Mean ±SD of triplicate determination, Sample-A: Control (i.e. packaging film without licorice root extract) and Sample-B: Test Sample (i.e. packaging film with licorice root extract)
*Significance value p≤0.05 for independent t-test administered

Since the p -value for thickness is less than 0.05 (two-tailed), therefore it can be deduced that the thickness for both the packaging films (i.e. Sample-A and Sample-B) were significantly different from each other. The thickness is a contribution of the materials used for the production of packaging film.

Graphical Representation of Thickness of Packaging Films



*Sample-A: Control (i.e. packaging film without licorice root extract) and Sample-B: Test Sample (i.e. packaging film with licorice root extract

Microbiological Test

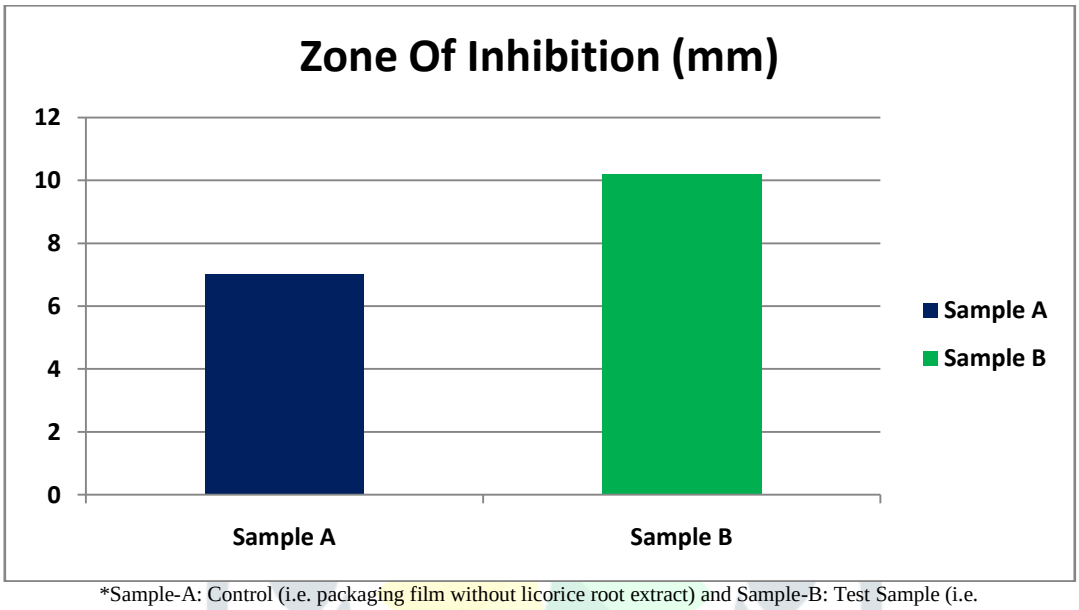
Antimicrobial Disc Plate Assay: Zones of inhibition generated by the licorice extract were used as a measure of their antimicrobial activity. Sterile cork borers were used to bore a single well into each agar plate. 100 µL of licorice root extraction solutions transferred into the 12 mm wells in the assay plates and incubated for 24 h at 37 °C and zones of inhibition measured with a ruler (n = 3). Sterile water was used as a control.

Table 5: Antimicrobial Disc Plate Assay Test Results of Packaging Films

PARAMETER	SAMPLE A	SAMPLE B	p value
Zone of Inhibition (mm)	7.01±0.012	10.21±0.018	.00001

* Values are represented as Mean ±SD of triplicate determination, Sample-A: Control (i.e. packaging film without licorice root extract) and Sample-B: Test Sample (i.e. packaging film with licorice root extract)
*Significance value p≤0.05 for independent t-test administered

Graphical Representation for Zone of Inhibition for Antimicrobial Activity



*Sample-A: Control (i.e. packaging film without licorice root extract) and Sample-B: Test Sample (i.e. packaging film with licorice root extract)

REFERENCES

[1] Ahvenainen R., Vermeiren L., Heirligs L., Delieghere F., Debevere J.. Novel food packaging techniques. Woodhead Publishing Limited: Cambridge England 2003.
[2] AirlessSpraySystems.TheEfficientChoiceforManyLiquidPaintingApplications; Nordson Corporation: Armhest, OH, USA, 2004. antimicrobial films based on chitosan matrix. Journal of Food Science.
[3] Andrade, R.; Skurtys, O.; Osorio, F. Atomizing spray systems for application of edible coatings. Comp. Rev. Food Sci. Food Safety 2012.
[4] Asad Abbas, Muhammad Zubair, Nasir Rasool, Komal Rizwan(2015) Antimicrobial Potential of Glycyrrhiza glabra. Journal of Drug Design and Medicinal Chemistry 201.
[5] ASTM. Standard test methods for flame properties of materials. Method D-635. In Annual Book of ASTM Standards. American Society for Testing and Materials. Conshohocken, PA. 1980.
[6] Baba M, Shigeta S. Antiviral activity of glycyrrhizin against Varicella-zoster virus in vitro.Antiviral Res 1987.
[7] Biodegradability of Polymers: Regulations and Methods for Testing Dr. Rolf-Joachim M¸ller Gesellschaft f¸r Biotechnologische Forschung mbH, Braunschweig, Germany.
[8] Bosquez-Molina, E.; Guerrero-Legarreta, I.; Vernon-Carter, E.J. Moisture barrier properties and morphology of mesquite gum–candelilla wax based edible emulsion coatings. Food Res. Int. 2003.
[9] Brody A.L., Strupin sky E.R., Kline L.R. Active Packaging for Food applications. Technomic Publishing Company, Inc. Pennsylvania, USA, 2001
[10] Cagri R, Avena Bustillos RJ, Krochta JM. Water vapor permeability of caseinate-based edible films as affected by pH, calcium cross linking and lipid content. Journal of Food Science. 2001 .
[11] Chiu, P.E.; Lai, L.S. Antimicrobial activities of tapioca starch/decolorized hsian-tsao leaf gum coatings containing green tea extract sin fruit-based salads, romaine hearts and pork slices. International journal of Food Microbiology
[12] Coma V, Sebti I, Pardon P, Deschamps A, Pichavant FH. 2001. Antimicrobial edible packaging based on cellulose ethers, fatty acids, and nisin incorporation to inhibit Listeria innocua and Staphylococcus aureus. J Food Prot
[13] Costa, C.; Conte, A.; Del Nobile, M.A. Effective preservation techniques to prolong the shelf life of ready-to-eat oysters. J. Sci. Food Agric. 2014.
[14] Dhanapal,A.;Sasikala,P.;Rajamani,L.;Kavitha,V.;Yazhini,G.;Banu,M.S.Ediblefilmsfrompolysaccharides. Food Sci. Qual. Manag. 2012.

[15] Falguera, V.; Quintero, J.P.; Jiménez, A.; Muñoz, J.A.; Ibarz, A. Edible films and coatings: Structures, active functions and trends in their use. Trends Food Sci. Technol. 2011.
[16] Fenwick GR, Lutomski J and Nieman C, Liquorice, Glycyrrhiza glabra Linn L.Composition, uses and analysis. J Food Chemistry, 1990.
[17] *Fenwick GR, Lutomski J and Nieman C, Liquorice, Glycyrrhiza glabra Linn L.Composition, uses and analysis. J Food Chemistry, 1990.*
[18] Fenwick, G.; Lutomski, J. and Nieman, C. (1990): Glycyrrhiza glabra L. (Liquorice): Composition, uses and analysis. Food Chem.
[19] Floros JD, Dock LL, Han JH. 1997. Active packaging technologies and applications. Food Cosmetic Drug Packaging.
[20] *Galdi M. R, Nicolais V, Di Maio L, Incarnato L. (2006) "Effect of oxygen scavenger fillers on mechanical and barrier properties of a PET monolayer film". Proceedings of Shelf Life International meeting SLIM 2006. Catania, 21-23 June 2006.*
[21] Galus, S., & Lenart, A. (2013). Development and characterisation of composite edible films based on sodium alginate and pectin . Journal of Food Engineering,115(4), 459-465.doi:10.1016/j.foodeng.2012.
[22] Gander, P. 2007. The smart money is on intelligent design. Food Manufacture Feb: xv-xvi
[23] Ghasemloua M, Weller CL, Hanna M, Froning GW. Mechanical and barrier properties of egg albumen films. *Journal of Food Science*. 2014
[24] Hamzah,H.M.;Osman,A.;Tan,C.P.;MohamadGhazali,F.Carrageenanasanalternativecoatingforpapaya (carica papaya l. Cv. Eksotika). Postharvest Biol. Technol. 2013.
[25] Han C.D., Baek D.M., Kim J.G. (1990) Effect of microdomain structure on the order-disorder transition temperature of polystyrene-block polyisoprene-block-polystyrene copolymers. Macromolecule.
[26] Han, J. H. and Floros, J. D. 1997. Casting antimicrobial packaging films and measuring their physical properties and antimicrobial activity. Journal Plastic Film Sheeting.
[27] Iino S, Tango T, Matsushima T, et al. Therapeutic effects of stronger neo-minophagen C at different doses on chronic hepatitis and liver cirrhosis. Hepatol Res 200.
[28] Jalal Bayati Zadeh, Zahra Moradi Kor , Masoud Karimi Goftar (2013) "Licorice (Glycyrrhiza glabra Linn) As a Valuable Medicinal Plant". International journal of Advanced Biological and Biomedical Research Volume 1, Issue 10, 2013.
[29] Judd D, Aalders B, Melis T (1989) The Silent Salesman. Singapore: Octogram Design.
[30] Karleigh Huff,Active and Intelligent Packaging: Innovations for the Future
Department of Food Science and Technology,Virginia Polytechnic Institute and State University (Virginia Tech) Blacksburg.
[31] Khan, M.I.; Nasef, M.M. Spreading behaviour of silicone oil and glycerol drops on coated papers. Leonardo J. Sci. 2009.
[32] Kumar, G.; Prabhu, K.N. Review of non-reactive and reactive wetting of liquids on surfaces. Adv. Colloid Interface Sci. 2007
[33] Labuza TP, Breene WM (1989) Applications of "active packaging" for improvement of shelf life and nutritional quality of fresh and extended shelf life of foods. Journal of Food Processing and Preservation 13.
[34] Lopez TS, Ranasighe DC, Patkai B, Farlane DM (2011) Taxonomy, technology and applications of smart objects. Information of Systems Frontiers 13
[35] Lopez-Rubio A., Almenar E., Hernandez-Muñoz P., Lagaròn J.M., Català R., Gavara R. (2004) Overview of Active polymer based tecnologies for food applications. Food Review International. 2004.
[36] Lu, F.; Ding, Y.; Ye, X.; Liu, D. Cinnamon and nisin in alginate–calcium coating maintain quality of fresh northern snakehead fish fillets. LWT—Food Sci. Technology 2010.
[37] Mannouch, S. Spray Gun Technique. Itw Devilbiss Industrial Training Centre.
[38] Mchugh, M. A., & Krukonis, V. J. (1994) . Special Applications, Supercritical Fluid Extraction,333-365.doi:10.1016/b978-0-08-0518176.50015-1.
[39] Méndez-Vilas, A. Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education; Microbiology Book Series 1; Formatex Research Center: Badajoz, Spain, 2013.
[40] Nakagawa K and Asami M, Effect of glycyrrhizin on hepatic lysosomal systems. Japanese J Pharmacology, 1981; 31: 849–851
[41] Nakamura H., Hoshino J. (1983) Techniques for the preservation of food by employment of an oxygen absorber. Technical information Mitsubishi Gas Chemical Co., Tokyo Ageless Division.
[42] Nasr, G.; Yule, A.; Bendig, L. IndustrialSpraysand Atomization: Design,Analysisand Applications; Lightning Source UK Ltd.: Milton Keynes, UK, 2002.
[43] Nicholson MD. 1998. The role of natural antimicrobials in food packaging biopreservation. J Plastic Film Sheeting 14(3):234-41.
[44] Nithya, V.; Murthy, P.S.; Halami, P.M. Development and application of active films for food packaging using antibacterial peptide of bacillus licheniformis me1. Journal Applications of Microbiology 2013.
[45] Sachdeva A , Vashist D, Rajni, Divya Puri , Antimicrobial activity of active packaging film to prevent bread spoilage International Journal of Food Science and Nutrition 2017.
[46] Šikalo, Š.; Marengo, M.; Tropea, C.; Ganic´, E.N. Analysis of impact of droplets on horizontal surfaces. Exp. Therm. Fluid Sci. 2002
[47] Silvestru, B.M.; Pâslaru, E.; Fras Zemljic, L.; Sdrobis, A.; Pricope, G.; Vasile, C. Chitosan coatings applied to polyethylene surface to obtain food-packaging materials. Cellul. Chem. Technol. 2014.
[48] Skurtys, O.; Acevedo, C.; Pedreschi, F.; Enronoe, J.; Osorio, F.; Aguiler, J.M. Food hydrocolloid edible films and coatings. In Food Hydrocolloids: Characteristics,Properties andStructures; Nova Science Publishers, Inc.: New York, NY, USA, 2010.
[49] Vermeiren L, Devlieghere F, Debvere J. 2002. Effectiveness of some recent antimicrobial packaging concepts. Food Add Contamin 19(Suppl.):163-71.
[50] Yam, K. L., Takhistov, P. T., and Miltz, J. 2005. Intelligent packaging: concepts and applications. Journal of Food Science. 70.