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Evaluation the Activity of Edible Coating for Maintenance the Shelf life of Raisins and Prunes

Youssef, M. A. and EL Kady, A. A.

Food Engineering and Packaging Dept., Food Tech. Res. Institutes, Agric, Res. Center, Giza, Egypt.

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ABSTRACT

This Research was carried out to extend the quality and shelf – life of raisin produced from seedless Thompson variety and prune produced from Hollwood plum variety. During storage period, these products turns sticky and hard due to exudates syrup and moisture loss .To overcome this problem the application of edible coating suggests being of proper assistance. In the research, four different edible coating materials including starch, pectin, soy protein and lipid were investigated. The coated raisins and prunes were kept under proper conditions for 6 months at 25 ± 1 ˚c. During this period of storage, moisture content and loss percentage in weight, microbial examination and sensory properties were evaluated. Moreover the thickness of coats were evaluated by the scanning electron microscopy. The data indicated that performance of lipid layer was better than other coats. This layer kept about 76.5 and 72.5 % of moisture content after 6 months of storage in raisin and prune samples respectively. Total mesophylic aerobic bacteria counts of raisins and prunes were decreased significantly ($p<0.05$). However, mould and yeast counts were increased during storage period.

Also, samples belong to lipid layers received higher color, flavor, taste, and texture scores when compared with the other treatment samples and were the most preferred in terms of general acceptance scores, on the other hand, the lowest acceptance scores were obtained for starch and pectin samples. Therefore the samples coated with lipid had better color, flavor, taste and texture scores at the end of storage (6 months at 25 ± 1 ˚c).

The scanning electron microscopy evaluation of coated samples showed that the pectin and lipid films was the most even among all of the other coating materials during 6 months of storage.

Key words: Raisin, Prune, edible coating, starch, pectin, soy protein, lipid layers

Introduction

Edible casing are thin layers of edible materials applied on food products that play an important role in their preservation, distribution and marketing (Jridi *et al*., 2013). Among the natural polymers able to form edible casing, polysaccharide (starch, pectin) protein (gelatin) lipid (wax) and composed films. Edible coatings can be applied by dipping products in coating materials and then allowing excess coating to drain as it dried and solidifies (Baldwin *et al.,* 2011).

Studies considering alternative systems for food protection that utilize biopolymers have increased significantly in the last years because these materials are entirely biodegradable and often edible and have few adverse environmental effects (Fadini et al., 2013). In this sense, edible coatings are receiving much attention as an efficient way to protect fresh and/or processed fruits from degradation during storage. They can used to control the permeability to water, oxygen and carbon dioxide, as well as lipid permeability in a food system (Espinel villacres *et al*., 2014). Also can offer several advantages to the fresh fruit and vegetable industry such as improvement in the retention of color, acids, sugar and flavor components, the maintenance of quality during shipping and storage, the reduction of storage disorders and improved consumer appeal (Lopez de lacey *et at*., 2013).

Starch, cellulose, gelatin, whey, chitosan, zein, carnauba, bees wax and fatty acids are the most commonly used compounds form edible coatings (Aquino *et al.,* 2015; Shit and Shah., 2014).

Polysaccharides and proteins show excellent mechanical and structural properties, but they have a poor barrier capacity against moisture transfer. This problem is not found in lipids due to their hydrophobic properties, especially those with high melting points such as beeswax and carnauba wax (Morillon *et al.,* 2002).

To overcome the poor mechanical strength of lipid compounds, they can be used in combination with hydrophilic materials by means of the formation of an emulsion or through lamination with an hydrocolloid film lipid layer. The efficiency was achieved by the formation of a homogeneous and continuous lipid layer inside the hydrocolloid Matrix (Karbowiak *et al*., 2007). In this way, it has been found that fatty acids can form stable layers in sodium caseinate or hydroxy propyl methyl cellulose matrices, whose properties depend on their chain length : the lower the chain length, the greater the layers (Jimenez *et al*., 2010).

Rasins and prunes are the important products for the dried fruit industry with worldwide production in excess of 970.000 metric / tons (IPA.,2014). They are widely consumed for its nutritional value which have a lot of sugars, high dietary fibre content, antioxidants and its effectiveness in enhancement of digestive function.

Corresponding Author: Youssef, M.M., Food Engineering and packaging Dept., Food tech. Res. Institutes, Agric., Res. Center, Giza, Egypt.

There are a significant interest in the industry of raisins and prunes to improve the industrial efficiency with the rising consumer demand for high quality products.

During storage period, the product turns sticky and hard due to exudates syrup and moisture loss, therefore the application of edible coating suggests being of proper assistance which enhance the boundary layer resistance resulting enhanced shelf–life of product and might impact in appropriate flavor and odors in the product (Ayoubi *et al*., 2015).

Also, edible coating can protect these products against pathogenic and spoilage microorganisms such as Aspergillus and Penicilium species which produced a mycotoxin (bradley *et al*., 2015)

Concerns that raisins and prunes may be contaminated by fumonisins stem from the persistent occurrence of Aspergillus Niger spores on raisins and prunes and the recent discovery of fumonisin production by A. niger on grapes or plums , which leads to the widespread occurrence of fumonisin. The presence of fumonisins in dried fruits suggested that they are produced mainly during the drying process concomitant (Du, Olsen, *et al*., 2008).

An emerging risk that exists from dried fruit in Central Europe. Recently, massive and frequent infestation of dried fruit imported from the Mediterranean region by the mite, Carpoglpyhus lactis L., has been found in 180 samples taken from supermarkets, 13% were contaminated; the contamination levels ranged from 180-660 mites/g of dried fruit. The contamination was found in dried apricots, figs, prunes and raisins, which mites were able to enter every dried fruit packing material tested including polypropylene and Al Foil. This indicated that mites may move from package to package in supermarkets. (Hubert *et al*., 2011)

The other emerging risk that exists from dried fruit, the mycotoxin produced by several species of Aspergillus and Pemicillium (Ochratoxin A) in contaminated raisins, prunes, figs, and dates, which collected from small and large supermarkets in seven areas of the United States between 2012 to 2014. Also the xerophilic mould, xeromyces bisporus as a spoilage organism isolated from packaged dried prunes.

Of the 665 samples analyzed: Ochratoxin A was detected in 48 raisin samples and 14 prune samples.

Also Ochratoxin A contamination levels exceeded the European Union regulatory limit in four raisin and two prune samples and these results suggest that these products are more frequently contaminated with Aspergillus and Penicillum than the other dried fruits (Palumbo *et al*., 2015)

Recent research should focus on the edible counting posses better antimicrobial potential for dried fruit applications such as effective essential oils, thyme and oregano essential oils which could be ascribed to the presence of phenolic compounds , particularly thymol and carnauba (Bradley *et al*., 2015). These oils have been evaluated by a number of researchers, however, limited data exist on the application of antimicrobial edible coatings incorporated with essential oils in real food systems (Du, Olsen *et al*., 2008)

Thus, the objectives of the present study were (1) to evaluate four edible casing materials for application on the surface of raisins and prunes in order to provide protection and brightness and enhance the boundary layer resistance resulting enhanced the shelf-life of the product (2) to determine effectiveness of these casing on micro – biological characteristics of raisins and prunes during storage.

Materials and Methods

Materials:

High methoxy pectin, corn starch and isolated soy protein were obtained from Sigma-Aldrich Co., St. Louis, Mo, USA, Polyvinyl alcohol, glycerol, Citric acid, Sodium hydroxide, Carnauba wax, Glyceryl monoestearate and Thyme oil were obtained from Braskem (Brazil).

Preparation of starch solution:

5 g of corn starch were mixed with distilled water (100 ml), polyvinyl alcohol (1.25 % w/v) was added as crosslink and 2 ml. glycerol (40% w/v) also added as a plasticizer and 10% citric acid w/w at room temperature (25˚) for 5 min. This suspension was transferred to a water bath at 90˚C for 30 min, and agitated by magnetic stirrer (500 rpm) followed by cooling at 40 ˚C before uses. (Zehra *et al*., 2010).

Preparation of pectin solution:

5 g of high methoxy pectin were mixed with distilled water (100 ml), polyvinyl alcohol (1.25 % w/v) and 2.5 % glycerol w/v at room temperature (25˚) for 5 min, then the suspension was transferred to a water bath at 90˚C for 30 min, and agitated by magnetic stirrer (500 rpm), followed by cooling at 40˚C (Zehsa *et al*., 2010).

Preparation of soy pectin solution:

Slowly dissolving 5% isolated soy protein (w/v) in distilled water while stirring, polyvinyl alcohol (1.25w/v) and glycerol (3.5 % w/v) was added and pH was adjusted to 10 with 0.1 NaoH. The solution was heated to 90 ˚C, for 30 min. followed by cooling at 40˚C and then filtering through four layers of cheese close by a vacuum pump (Zehra *et al*., 2010).

Preparation of lipid emulsion:

Five percent of carnauba wax were immersed in glyceryl monoestearate with a total solids content of 8 % with 150 ppm essential oil thyme (Thymus vulgaris) to produce the carnauba – based emulsion formula. (Ayoubi *et al*., 2015)

Methods:

In this study, raisin produced from Seedless Thompson variety and prune produced from Hollwood plum variety. Immediately after the drying process at EVAGRO company (Cairo – Alexandria Desert Road) samples were taken and these stages of coating are followed in Food Tech. Res .Institute Laboratory:

1- Preparation of coating solution.
2- Dipping of samples in solutions for 2-3 min.
3- Shaking of samples for 5 min. for removing the excessive solution.
4- Drying of samples in 10-15 C for 4 hours.
5- Packaging in polyethylene pouches and storage of packs in 20-25 C for 5 months in dark room.

Moisture content, microbial examination, sensory properties (texture, color, flavor, taste and overall acceptability) and statistical analysis were determined during storage in the first day and 45, 90,135 and 180 days after starting the storage, moreover, the thickness of coats could be evaluated by the scanning electron microscopy.

Moisture content:

The moisture content of raisin and prune samples were determined using a standard AOAC method (AOAC, 2000) by vacuum drying. In this method 5-10 g of ground sample was spread in an aluminium moisture dish and dried in a vacuum oven at 70 °C for 6 h under reduced pressure (<100 mm Hg). This was repeated at least three times to obtain a representative average.

Coats evaluation:

The samples should be selected, cleaned, Fixed, dehydrated and covered with conductor before the thickness of coats were evaluated by electronic microscopy to chose the best and more even coats on raisins and prunes during the storage period of 6 months (Salvatori *et al*., 1998).

Microbiological analysis:

At each sampling interval, a package from each treatment was aseptically opened. 10 g raisins or prunes fruits were homogenized for 150 sec in sterile stomacher bags containing 90 ml peptone water (1% W/v peptone) Serial dilutions were then made. Total mesophylic aerobic bacteria counts (TMAP) of samples were enumerated using Plate Count Agar (VM 218663; Merck, Basingstoke, Hampshire, England) and plates were incubated at 30 ˚C for 48-72 h.

Yeasts and moulds (YM) were enumerated using Potato Dextrose Ager (CM 0139, Oxoid). The pH was adjusted to 3.5 by adding 10 % (v/v) sterile lactic acid solution into media. Plates were incubated at 20 °C for 5 days and then yeasts and moulds colonies were counted.

Sensory Properties:

The sensory panel consisted of eight experienced personnel of the department. The panelists were familiarized with the raisin and prune samples daring the storage and were asked to evaluate the samples for color, taste, texture and general acceptance. The analysis was performed in the Food Engineering Research Laboratory, under white fluorescent lights according to the specifications of the International Standards Organization, ISO DP 6658. Samples were given scores out of five points, where one represents the most disliked and five represents the most liked. (Macfie and Bratchell .1989).

Statistical analysis:

Findings obtained from the replications were evaluated by the GLM procedure of the SAS Statistical Analyses Program according to the Completely Randomized Design (SAS, 2001). Duncan Analyses was applied on the results found statistically significant ($p < 0.05$) (Santé and Fernandez 2000).

Results and Discussion

Moisture content:

The Results of moisture content % and losing % during storage periods have been shown in Table (1). A thin layer of oil coating can limit moisture loss better than the other experimental layers.

As can be seen, the initial water contents of raisin and prune samples were 16.5 % and 24% respectively and the loss % in moisture content of control samples, which did not have any coating reached 52.7 % and 56.6

% after the storage period for 180 days at 25 °c. While in the case of samples with oil coating, the loss % reached to 23.6 % for raisins and 27.5% for prunes after the same period only. Therefore, it realized that the amount of wax determined the time of reaching to this moisture so that these samples had the most moisture content at the end of storage.

Table 1: Effect of different coatings on moisture content and weight loss % of raisins and prunes during storage.

sample	Treatment	Storage time / days									
		Zero		45		90		135		180	
		m.c %	loss	m.c %	loss	m.c %	loss	m.c %	loss	m.c %	loss
Raisins	Control	16.5	---	13.6	17.6	11.0	33.3	8.3	49.7	7.8	52.7
	Starch	16.5	---	14.0	15.1	12.0	27.2	10.5	36.3	9.0	45.5
	Pectin	16.5	---	14.4	12.7	12.5	24.2	10.6	35.7	9.2	44.2
	Soy protein	16.5	---	14.7	10.9	13.0	21.2	11.5	30.3	9.7	41.2
	Lipid	16.5	---	15.5	9.1	14.2	13.9	13.3	19.4	12.6	23.6
Prunes	Control	24.0	---	19.5	18.7	15.3	35.4	11.4	52.5	10.4	56.6
	Starch	24.0	---	20.2	15.8	16.6	30.8	13.0	45.8	12.4	48.3
	Pectin	24.0	---	20.5	14.6	16.8	30.0	13.3	44.5	12.6	47.5
	Soy protein	24.0	---	21.3	11.3	17.5	27.0	14.6	39.2	13.6	43.3
	Lipid	24.0	---	22.2	7.5	20.4	15.5	18.0	25.0	17.4	27.5

M.C %: Moisture content percentage.

Fig (1, 2) illustrates the effects of different coatings on moisture content in raisins and prunes. Different coatings did not have any significant effect on moisture content with the exception of lipid compound layers. It is known that carbohydrate films are weak in moisture pressure and can not prevent moisture loss, they have a poor barrier capacity against moisture transfer, therefore it reduces constantly during storage time (Morillon *et al*., 2002).

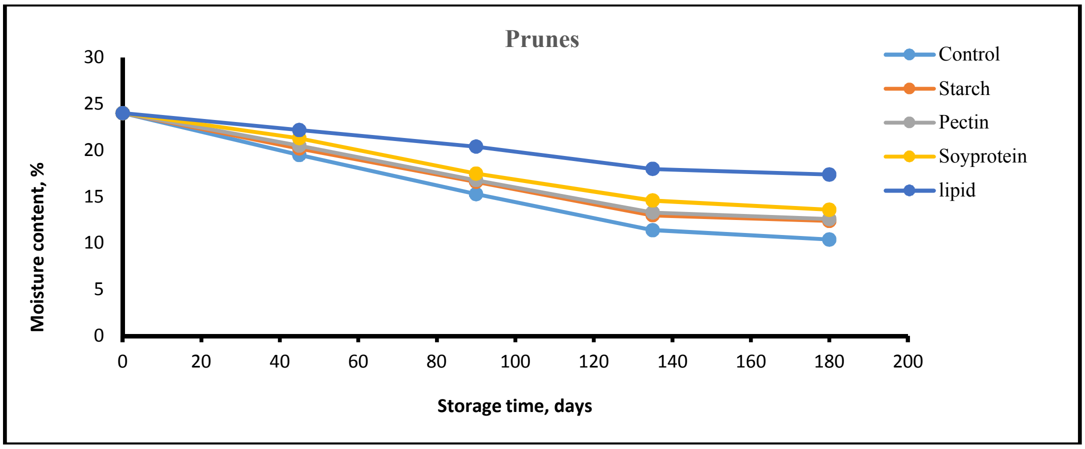

Fig. 1: Moisture content of prunes with different coatings during storage time.

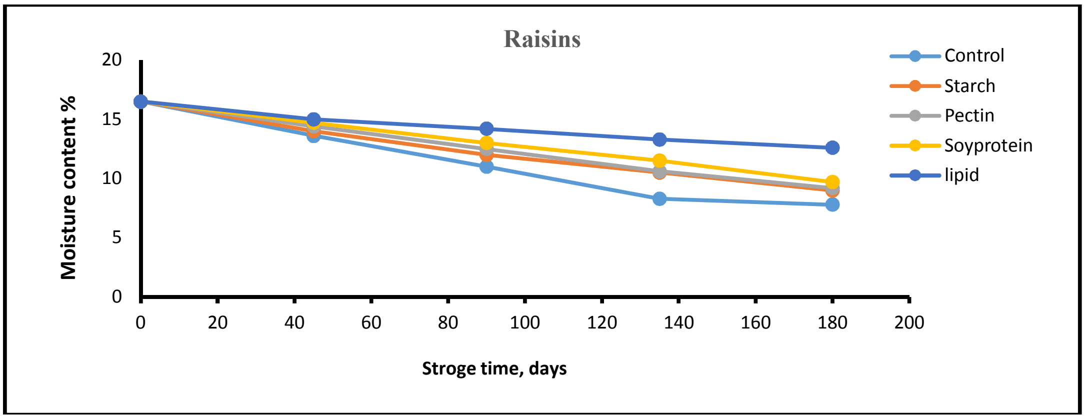

Fig. 2: Moistures content of raisins with different coatings during storage time.

This problem is not found in lipids due to their hydrophobic properties, especially those with high melting points such as carnauba wax and beeswax. Somewhat, protein films can more limit moisture loss during storage than starch and pectin layers (Henry, 2012).

Microbiological analysis results:

Changes in microbiological attributes (TMAB and YM) of raisins and prunes are presented in Table (2). As can be seen , initial TMAB and YM at zero time of storage were 2.60 and 1.20 log cfu/g in raisin samples and 3.84 and 1.43 log cfu/g in prune samples respectively . During the storage period, it was observed that the TAMB and YM counts of all samples were decreased month by month.

At the end of the storage period of 6 months at 25 ± 1 ˚C, the logarithmic changes of TAMB counts of control, lipid . soy protein, pectin and starch treatments in raisin samples became 1.45, 1.80, 1.74, 1.60 and 1.54 log cfu /g, respectively, while in prune samples became 1.75 , 2.94 , 2.90 , 2.82 and 2.44 log cfu /g, respectively.

Due to polysaccharides have a poor barrier capacity against moisture transfer, starch and pectin samples having the lowest counts of TMAB, while in lipid samples, due to their hydrophobic properties, having the highest counts (Morillon *et al*., 2002). Soy protein layers having intermediate -counts.

Palumbo *et al*. (2015) stated that raisins and prunes more frequently contaminated with low levels of several species of Aspergillus, Penicillium and Rhizopus, therefore edible coating such as lipid emulsion posses better supplementary layers and antimicrobial potential for raisins and prunes application to prevent fungous contamination. This supports our findings, in our study the lowest YM count was found for the lipid samples at the end of storage which it reached 2.17 and 2.42 log cfu / g in raisin and prune samples respectively, while the highest YM count was found for the starch and pectin samples which it reached 2.34 and 2.31 log cfu/g in raisins and 3.10 and 3.00 log cfu/g in prunes (Table 2), and also soy protein layers having intermediate – counts.

Table 2: Changes in the total mesophlic aerobic bacteria (TMAB) and mould – yeast (TMY) counts of the raisin and prune samples during storage at 25 ± 1 ˚c (log cfu/g).

Kind of product	Storage time (days)	TMAB					TMY				
		Control	Lipid layer	Soy protein layer	Pectin layer	Starch layer	Control	Lipid layer	Soy protein layer	Pectin layer	Starch layer
Raisins	0	2.60	2.60	2.60	2.60	2.60	1.20	1.20	1.20	1.20	1.20
	45	2.39	2.52	2.50	2.30	2.20	1.69	1.53	1.66	1.86	1.92
	90	2.14	2.35	2.32	2.25	2.12	2.00	1.72	1.85	2.11	2.15
	135	1.86	2.10	2.06	2.15	2.00	2.15	1.90	2.04	2.24	2.27
	180	1.45	1.80	1.74	1.60	1.45	2.40	2.17	2.24	2.31	2.34
Prunes	0	3.84	3.84	3.84	3.84	3.84	1.43	1.43	1.43	1.43	1.43
	45	3.40	3.72	3.69	3.48	3.30	2.12	1.69	1.75	1.85	1.98
	90	3.19	3.33	3.28	3.27	3.11	2.73	1.73	1.93	2.47	2.51
	135	2.45	3.08	3.77	3.00	2.92	2.90	2.08	2.22	2.63	2.75
	180	1.75	2.94	2.90	2.82	2.44	3.28	2.42	2.61	3.00	3.10

Sensory analysis results:

Changes is sensory analyses scores of raisin and prune samples are presented in Table (3). Samples were evaluated in terms of color, flavor, taste, texture and general acceptance. As seen in Table (3) color, flavor and texture scores of samples were decreased significantly ($p<0.05$) in starch and pectin treatments, and there were no significant changes reported in taste scores.

Samples belong to lipid layers received higher color, flavor, taste and texture scores when compared with the other treatment samples and were the most preferred in terms of general acceptance scores. This evinced that the lipids can from stable layers on the surface of dry fruits and it has been proved, in emulsion – based films, that the smaller the particle size or lipid globules and the more homogeneously distributed, the lower water vapor permeability (Fakhourzi, *et al*., 2015).The storage period was found significant on the changes in appearance and color scores of the samples ($p < 0.01$).

At the end of the storage period of 6 months, the lowest acceptance scores were obtained for starch and pectin samples while the higher acceptance scores were obtained for lipid samples which at the end of storage period, received four points out of five. The middle acceptance scores were obtained for soy protein samples scores.

These results were in agreement with those of Yeygel (2001). According to Yeygel (2001), the higher acceptance scores of dry fig and apricot samples were obtained for wax treatments at the end of 8 months storage at ambient temperature. Also Rosello *et al*. (1994) found the same sensorial results for raisins during storage.

Table 3: Changes in sensory scores of raisin and prune samples daring storage period.

Kind of product	Storage Time (Days)	Starch layer					Pectin layer					Soy protein layer					Lipid layer				
		C	F	Ta	Te	GA	C	F	Ta	Te	GA	C	F	Ta	Te	GA	C	F	Ta	Te	GA
Raisins	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	45	3	3	5	3	2	4	3	5	4	4	3	4	4	5	4	4	5	5	5	5
	90	2	3	4	2	2	2	2	5	4	3	2	3	4	4	3	4	5	5	5	5
	135	2	2	4	2	2	2	2	4	4	3	2	3	4	4	3	3	4	4	5	4
	180	2	2	4	1	1	1	1	4	4	2	1	3	3	4	3	3	4	4	4	4
Prunes	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	45	3	3	5	3	2	4	4	5	5	3	4	4	4	5	5	4	5	5	5	5
	90	2	2	5	2	2	3	3	4	4	3	3	4	4	4	4	3	4	5	5	4
	135	2	2	4	2	1	2	2	4	4	3	2	3	4	4	3	3	4	4	4	4
	180	2	2	4	1	1	1	1	4	4	1	1	3	3	3	3	3	4	4	4	4

C,Color; F, Flavor; Ta, Taste; Te, Tecture; GA, General acceptance.

The scanning electron microscopy evaluation of coated samples showed that the pectin (Fig :3,5) and lipid (Fig 4,6) films were the most even among all of the other coating materials daring 6 months of storage in either case of prune or raisin samples.

Fig. 3: The picture of prunes which was coated with pectin casing

Fig. 4: The picture of prunes which was coated with lipid casing

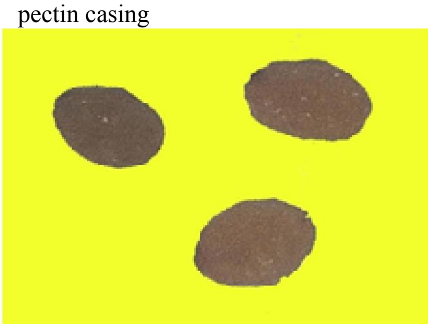

Fig. 5: The picture of raisins which was coated with pectin casing

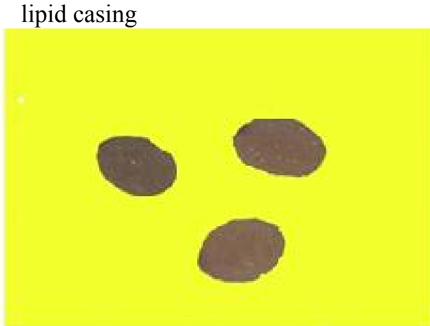

Fig. 6: The picture of raisins which was coated with lipid casing

Conclusion

Nowadays , trends in the use of edible coating is based on the barrier effect against gases flow , structural resistance to water loss (permeability to water vapor) and maintain its quality and sensory acceptability during the storage of the product.

In this connection, the examination showed that the best coating in raisins and prunes were lipid film, which the significant observation was that the samples were coated with lipid at this formula kept about 76.5 and 72.5 % of moisture content for raisins and prunes respectively after 6 months of storage at ambient temperature.

Its use was effective as natural treatment on sensory evaluate terms scores for consumer acceptance, and was the most even among all of the other coating materials.

As, result of this research , the count changes in TAMB counts were connection with moisture content in the samples during the storage period, the decreasing amount of water, TMAB counts decrease with the same patters, but in YM counts, changes were connection with the type of edible coating used.

The industry could apply optimized lipid film for commercial purposes. Thus, future research in this area should focus on application of lipid assistant layers on processed dry fruits such as raisins and prunes to extend the shelf-life of these products.

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