المجلة العراقية
لبحوث السوق وحماية المستهلك

المجلد (7) العدد (1)
لسنة 2015

# Extending the storage life of some fruits by using Pullulan produced from locally isolate *Aureobasidium pullulans.*

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تأريخ استلام البحث: 2015/1/12 تأريخ قبول النشر: 2015/3/26

## Abstract

**Six isolates of *A. pullulans* were collected from many sources including *Hibiscus sabdariffa* (Roselle), old Roofs of houses and bathroom surface that referred as Ap ros1, Ap or2, 3, 4 and Ap bs5, 6 respectively, all these isolates were identified based on morphological characteristics and nutritional physiology profiles, all were able to utilize various carbon and nitrogen sources such as glucose, xylose, sucrose, maltose, ammonium sulfate, ammonium nitrate and ammonium chloride, also they showed positive test for starch and amylase, while α-cellulose, ethanol, and methanol were could not be assimilated and could not grow in 0.05% cycloheximide, the other tests referred to different results among isolates, the Ap ros1 isolate from *Hibiscus sabdariffa* (Roselle) was selected for production of pullulan.**

**Four bacterial strains of two gram (+) and two gram (-) were chosen for the tests. The effect of pullulan coating on bacterial growth was observed in all tested strains, it was 63, 65, 70 and 75% for *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimuram* respectively.**

**The weight loss of uncoated peach was 3.2, 4.8, 6.1, 7, 7.2, 8.3 and 9.1%, while in coated peach with 10% pullulan were 3.1, 4.5, 5.8, 6.2, 6.8, 7.9 and 8.9%, and in coated peach with 20% pullulan were 2.9, 3.8, 4.1, 4.6, 5.8, 6.4 and 7.6%, when these samples are stored at 3, 6, 9, 12, 15, 18 and 21 days respectively that stored at 25°C, and when uncoated and coated peach with 10 and 20% pullulan stored at 4°C . The weight loss in uncoated peach was 0.01, 0.06, 0.16, 0.23, 0.35, 0.46, 0.6, 0.68, 0.73 and 0.75%, while**

**in coated peach with 10% pullulan were 0.01, 0.05, 0.12, 0.16, 0.2, 0.3, 0.5, 0.58, 0.64 and 0.71%, and in coated peach with 20% pullulan were 0.015, 0.02, 0.04, 0.08, 0.1, 0.12, 0.18, 0.3, 0.46 and 0.5%, when these samples are stored at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days respectively.**

**The weight loss of uncoated pear was 1.4, 2, 2.6, 4.4, 5.5, 6.4 and 7.2%, while in coated peach with 10% pullulan were 1.2, 1.6, 2, 2.4, 4.3, 5.2 and 6.6%, and in coated peach with 20% pullulan were 0.8, 1, 1.4, 1.6, 3, 4.6 and 5.4%, when these samples are stored at 3, 6, 9, 12, 15, 18 and 21 days respectively that stored at 25°C, and when uncoated and coated pear with 10 and 20% pullulan that stored at 4°C. The weight loss is 0.02, 0.07, 0.14, 0.16, 0.3, 0.33, 0.44, 0.45, 0.52 and 0.54%, while in coated peach with 10% pullulan were 0.02, 0.06, 0.11, 0.16, 0.21, 0.3, 0.36, 0.38, 0.44 and 0.46%, and in coated peach with 20% pullulan were 0.01, 0.05, 0.08, 0.09, 0.1, 0.16, 0.19, 0.24, 0.26 and 0.28%, when these samples are stored at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days respectively.**

**kay words: storage life, fruits, Pullulan, *Aureobasidium pullulans.***

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# إطالة مدة حفظ بعض الفواكه باستعمال سكر البوليولان Pullulan المنتج من عزلة محلية للفطر *Aureobasidium pullulans*.

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جامعة بغداد

## الخلاصة

جمعت ستة عزلات من فطر *Aureobasidium pullulans* من مصادر عدة شملت كلا من ازهار الكجرات (Roselle) Hibiscus sabdariffa وسقوف البيوت القديمة واسطح الحمامات واعطيت الرموز Ap ros1 و Ap or2, 3, 4 و Ap bs5, 6 على التوالي، واخضعت جميع العزلات الى التشخيص بالاعتماد على الصفات المظهرية والمزرعية، ولوحظ امتلاك جميع العزلات قابلية استهلاك مصادر الكاربون والنتروجين مثل الكلوكوز والزايلوز والسكروز والمالتوز وكبريتات ونترات وكلوريد الامونيوم، كذلك اظهرت نتيجة موجبة لفحص النشأ والاماليز، وعدم تمثيل السليلوز والايثانول والميثانول ولم تستطيع النمو بوجود 0.05% من cycloheximide، واظهرت الفحوصات الاخرى نتائج متباينة بين العزلات، وتم اختيار العزلة Ap ros1 المعزولة من ازهار الكجرات *Hibiscus sabdariffa* (Roselle) لانتاج سكر البوليولان.

اختبرت كفاءة البوليولان المنتج في دراسة تثبيط 4 انواع من العزلات البكتيرية توزعت بواقع عزلتين موجبة لصبغة كرام وعزلتين سالبة لصبغة كرام، واظهرت النتائج ان تأثير التغطية بالسكر في نمو البكتريا كان موجبا لجميع العزلات المستعملة، اذ بلغت نسبة التثبيط 63 و 65 و 70 و 75% لكل من بكتريا *Bacillus subtilis* و *Staphylococcus aureus* و *Escherichia coli* و *Salmonella typhimuram* على التوالي.

بينت نتائج فقدان الوزن، ان الخوخ الغير مغطى بمحلول البوليولان بلغت نسبة الفقد فيه 3.2 و4.8 و6.1 و7 و7.2 و8.3 و9.1%، بينما يلاحظ ان الخوخ المغطى

بالبوليولان ذي تركيز مقداره 10% فقد 3.1 و4.5 و5.8 و6.2 و6.8 و7.9 و8.9%، في حين ادت تغطية الخوخ بالبوليولان ذي تركيز مقداره 20% الى فقدان 2.9 و3.8 و4.1 و4.6 و5.8 و6.4 و7.6% عند خزن هذه النماذج لمدة 3 و6 و9 و12 و15 و18 و21 يوم على التوالي بدرجة حرارة 25م، وعند خزن الخوخ الغير مغطى والمغطى بالبوليولان بتركيز 10 و20% بدرجة حرارة 4م، لوحظ ان الوزن المفقود للخوخ غير المغطى بلغ 0.01 و0.06 و0.16 و0.23 و0.35 و0.46 و0.6 و0.68 و0.73 و0.75%، بينما اظهرت معاملة الخوخ بالبوليولان ذي تركيز 10% فقدان للوزن بلغ 0.01 و0.05 و0.12 و0.16 و0.2 و0.3 و0.5 و0.58 و0.64 و0.71%، في حين ادت تغطية الخوخ بالبوليولان ذي تركيز مقداره 20% الى فقدان وزن مقداره 0.015 و0.02 و0.04 و0.08 و0.1 و0.12 و0.18 و0.3 و0.46 و0.5% عند الخزن لمدة 1 و2 و3 و4 و5 و6 و7 و8 و9 و10 يوم على التوالي.

لوحظ ان ان الوزن المفقود للكمثرى الغير مغطاة بمحلول البوليولان كان 1.4 و2 و2.6 و4.4 و5.5 و6.4 و7.2%، بينما ادى تغطية الكمثرى بالبوليولان ذي تركيز مقداره 10% الى فقدان 1.2 و1.6 و2 و2.4 و4.3 و5.2 و6.6%، في حين لوحظ ان استعمال البوليولان ذي تركيز مقداره 20% ادى الى فقدان 0.8 و1 و1.4 و1.6 و3 و4.6 و5.4% عند الخزن لمدة 3 و6 و9 و12 و15 و18 و21 يوم على التوالي بدرجة حرارة 25م، وعند خزن الكمثرى غير المغطاة والمغطاة بمحلول البوليولان ذي تركيز مقداره 10 و20% بدرجة حرارة 4م، ظهر بان الوزن المفقود للكمثرى غير المغطاة كان 0.02 و0.07 و0.14 و0.16 و0.3 و0.33 و0.44 و0.45 و0.52 و0.54%، بينما فقدت الكمثرى المعاملة بالبوليولان ذي تركيز مقداره 10% وزنا نسبته 0.02 و0.06 و0.11 و0.16 و0.21 و0.3 و0.36 و0.38 و0.44 و0.46%، بينما لوحظ ان استعمال البوليولان ذي تركيز مقداره 20% الى فقدان وزن نسبته 0.01 و0.05 و0.08 و0.09 و0.1 و0.16 و0.19 و0.24 و0.26 و0.28% عند الخزن لمدة 1 و2 و3 و4 و5 و6 و7 و8 و9 و10 يوم على التوالي.

الكلمات المفتاحية: اطالة مدة الحفظ، الفواكه، سكر البوليولان، فطر Aureobasidium pullulans.

# Introduction

Pullulan $(C_6H_{10}O_5)$ $H_2O$ is a neutral, water- soluble polysaccharide produced from starch by the fungus *Aureobasidium pullulans*, by means of a process of fermentation. Chemically, this polymer consists of α-1,6 linked maltotriose residues, which in turn are composed of three glucose molecules connected to each other by α- 1,4 glycosidic bonds (2; 18).

Pullulan characterized by nontoxic for human and animal, low caloric and totally biodegradable, have good adhesive characteristics that enables it to be applied for coating of food products, colorless, odorless, no taste, can be very thin and have a big barrier characteristics in relation between oxygen and carbon dioxide, which had effect upon growth inhibition of most of the tested microorganisms, responsible for decay of food, so it can be an useful element to ensure microbiological safety of food(3).

This polysaccharide is of economic importance with increased application in food, pharmaceutical, agricultural and chemical industries(22). Pullulan produces a high viscosity solution at a relatively low concentration and can be used for oxygen impermeable films and fibers, thickening or extending agents, or adhesive or encapsulating agents, despite being a α-D-glucan, pullulan is resistant to α-D-amylolysis and may be used in low-calorie food formulation(24). The use of pullulan in biomedical field is increasing contemporarily due to its non toxic, non immunogenic, biocompatible and inert nature. Pullulan as a carrier for drug delivery(11), tissue engineering and grafting(19), liver targeting of drug loaded pullulan(20), anti cancer drug(21), as drug delivery systems, particularly in the form of micro gels and nano gels(25), gene delivery(17), bio imaging by nanoparticles shell(8). Pullulan is also being used for the production of biodegradable plastics in Japan and U.S.A., because it resembles polyethylene with properties like tensile strength and ability to form thin transparent oxygen impermeable films(9).

Pullulan produced by the fungus *Aureobasidium pullulans*, by means of a process of fermentation. *Aureobasidium pullulans* (De Bary) G. Around is a ubiquitous, polymorphic and oligotrophe black yeast like micro fungus that occurs frequently in wide range of tropical and temperate environment with fluctuating moisture content in

phyllosphere, and also isolated from damp indoor surfaces, food and feed substances, it has also been found in the osmotically stressed environments like hyper saline water in salterns and the rocks(10).

Increasing consumer demand for extended plant material storage duration has mobilized the food industry to search for methods to maintain raw material quality and protection of a wide group of food products(15). All fruits and vegetables are harvested when they are completely ripe, the main problem with them it continues to ripen while they are being stored because it causes irreversible wilt and weight loss which results to shortened shelf-life and loss of economic and commercial value, so applying edible coatings that defined as thin layers of biomaterials, which form a barrier around products in the form of tightly adhering coating, and may concurrently be consumed together with the protected food, this edible coatings manufacture from natural materials like polysaccharides, proteins and lipids might be one of the effective methods to prolong shelf-life stability at storage in the room temperature for these types of foods by slows down gases exchange, controls respiration process, limits mass loss and R.H. transmission and decrease in microorganisms growth on the surface (4; 12). So this study was aimed to produce Pullulan from locally isolated *Aureobasidium pullulans* and used to extending the storage life of some fruits.

## Materials and methods

**Fungal isolation:**

Many strains of *A. pullulans* were isolated from *Hibiscus sabdariffa* (Roselle) by using medium containing: glucose 2%, ammonium sulphate 0.06%, dipotassium hydrogen orthophosphate 0.5%, sodium chloride 0.1%, magnesium sulphate 0.04% and yeast extract 0.04% with pH 5.0 and incubated at 42°C (9). Also *A. pullulans* strains were isolate from some old Roofs of houses and bathrooms surfaces at different areas in Baghdad using sterile cotton swabs that aseptically smeared onto corn meal agar (CMA) and half-strength malt extract agar (MEA) plates, all *Aureobasidium* like colonies were selected and subcultures on potato dextrose agar (PDA) and yeast malt agar (YMA) to pure cultures (16). The yeasts were then identified based on morphological observation and nutritional physiology test (6; 1).

**Production of pullulan:**

Pullulan was produced by transferring cells from agar slants to 50ml of medium containing (g\l): 0.6 $(NH_4)_2SO_4$; 5 $K_2H\ PO_4$; 1 NaCl; 0.2 $MgSO_4.7H_2O$ and 2.5 yeast extract, the pH of medium was adjusted to 6.0 before sterilization, glucose 2% was autoclaved separately for 15min at 121°C and added to the medium under aseptic conditions, in 250ml erlenmeyer flasks and incubated for 48h at 30°C and 200rpm(13).

**Isolation and purification of extracellular polysaccharide:**

The culture medium after fermentation was heated at 100°C in water bath for 15min, cooled to room temperature and centrifuged at 12,000rpm at 4°C for 10min to remove cells and other precipitates, then 3ml of the supernatant were transferred into a test tube and added to it 6ml of cold ethanol (99%) and mixed thoroughly and held at 4°C for 12h to precipitate the extracellular polysaccharide.

After removal of the residual ethanol, the precipitate was dissolved in 3ml of deionized water at 80°C and the solution was dialyzed against deionized water for 48h to remove small molecules in the solution, the exopolysaccharide was precipitated again by using 6ml of the cold ethanol and the residual ethanol was removed, the precipitate was then dried at 80°C to a constant weight, pullulan weight was measured using sensitive balance and expressed in (g/l) (23).

**Hydrolysis of the purified extracellular polysaccharide and assay of reducing sugar:**

The purified pullulan was dissolved in 3ml deionized water at 80°C in water bath, the dissolved substrate was hydrolyzed by incubating the mixture of 0.5ml of the substrate, 0.4ml of 0.2M $Na_2HPO_4$ / 0.1M citric acid buffer (pH 5.0) and 0.1ml pullulanase for 2h at 40°C (9). The total sugar was determined according to the phenol sulfuric acid method (7) using sucrose as the standard for the confirmation of pullulan.

**Antibacterial Activity Test:**

**Bacterial strains:**

Four bacterial isolates: *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Salmonella typhimurium* were obtained from biological Lab. of market research and consumer protection

center, University of Baghdad, Iraq. All isolates were maintained in their appropriate nutrient agar slants at 4°C throughout the study and used as stock cultures

**Methods of carrying out of tests strains' culture under pullulan coating:**

One ml. of inoculums of microorganisms ($10^5$ CFU/ml) was carried over on Petri plates and a nutrient agar medium was poured, after that, 1ml of 10% water solution of pullulan was carried over on surface of medium, for quicker drying of the pullulan coating the plates were placed for about 1h in a laminar chamber with a constant flow of sterile air, in parallel control plates were prepared (without pullulan coating), after the coating has got set, the plates with the pullulan coating and without it were incubated at the temperature of 37°C for 24-48 h (depending on the strain).

The grown up colonies of bacteria were counted, the test was carried out in triplicate, on the base of the obtained results, the results of inhibition degree of pullulan was calculated by dividing the number of colonies grown on pullulan coating over number of colonies grown on control plates, thus obtained results were used for calculation of inhibition degree of tested bacteria growth by the pullulan coating (3).

**Sample preparation:**

Dried pullulan was dissolved in a distilled water obtain 10 and 20% solutions, the mixture was then heated up to 80°C in a water bath and continuously stirred to dissolve, the solution sterilized in 117°C for 10 min and stored in cooling refrigeration (4).

**Weight loss:**

Peaches and pears were washed, dried up, weighted and then covered with 10 and 20% pullulan solutions by using a sterile little brush, as soon as the pullulan solution dried, the fruits were weighed again and stored at 4°C for 10 day and 25°C for 21 day, the percentage of weight loss was calculated in relation to the initial weight of the fruits, which were weighed for the whole period of storage (15).

# Results and discussion

**Fungal isolation:**

Six isolates of *A. pullulans* were collected from three sources including *Hibiscus sabdariffa* (Roselle), old roofs of houses and bathroom surface that refer it Ap ros1, Ap or 2, 3, 4 and Ap bs5, 6 respectively (Table, 1), all these isolates were identified based on morphological characteristics and nutritional physiology profiles, all they were able to utilize various carbon and nitrogen sources such as glucose, xylose, sucrose, maltose, ammonium sulfate, ammonium nitrate and ammonium chloride, also they showed positive test for starch and amylase, while α-cellulose, ethanol, and methanol were showed negative test, and all isolates could not be grow in 0.05% cycloheximide, the other tests refer to different results between isolates, and with return to literature characteristics key (6; 1), the Ap ros1 isolate from *Hibiscus sabdariffa* (Roselle) was selected to production of pullulan.

**Table (1):** Growth reactions and other tests of *Aureobasidium ullulans*.

| *Test | **Isolate | | | | | |
|---|---|---|---|---|---|---|
| | Ap ros1 | Ap or2 | Ap or3 | Ap or4 | Ap bs5 | Ap bs6 |
| D-glucose | + | + | + | + | + | + |
| D-xylose | + | + | + | + | + | + |
| sucrose | + | + | + | + | + | + |
| maltose | + | + | + | + | + | + |
| inulin | w | - | w | w | w | w |
| D-glucosamine | w | w | w | - | w | - |
| Starch (soluble) | + | + | + | + | + | + |
| Glycerol | + | + | w | + | w | + |
| methanol | - | - | - | - | - | - |
| ethanol | – | – | – | – | – | – |
| 10% MgC12 | + | + | - | w | - | w |
| 10% NaCl | + | w | - | + | - | - |
| 0.05% cycloheximide | - | - | - | - | - | - |
| acid production | w | + | + | w | + | - |
| urease test | + | + | w | + | w | + |
| amylase | + | + | + | + | + | + |
| cellulase | - | - | - | - | - | - |
| Ammonium sulfate | + | + | + | + | + | + |
| Ammonium nitrate | + | + | + | + | + | + |
| Ammonium chloride | + | + | + | + | + | + |

***Growth was determined by OD600 measurement after 7-day cultivation:**
**+: growth (OD >0.05).**
**- : no growth (OD <0.02).**
**w: weak growth (0.05>OD >0.02).**
**** Isolate:**
**Ap ros1: *Hibiscus sabdariffa* (Roselle).**
**Ap or2, 3, 4: old Roofs of houses.**
**Ap bs5, 6: bathroom surface.**

## Effect of pullulan coating on growth of selected bacterial strains:

Four bacterial strains, two gram positive and two gram negative were selected for the tests of pullulan inhibition, the obtained results of the bacterial strains explain inhibition degree of growth on substrate with pullulan coating (Figure, 1), the effect of pullulan coating on bacterial growth was observed in all tested strains, it was 63, 65, 70 and 75% for *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhimuram* respectively.

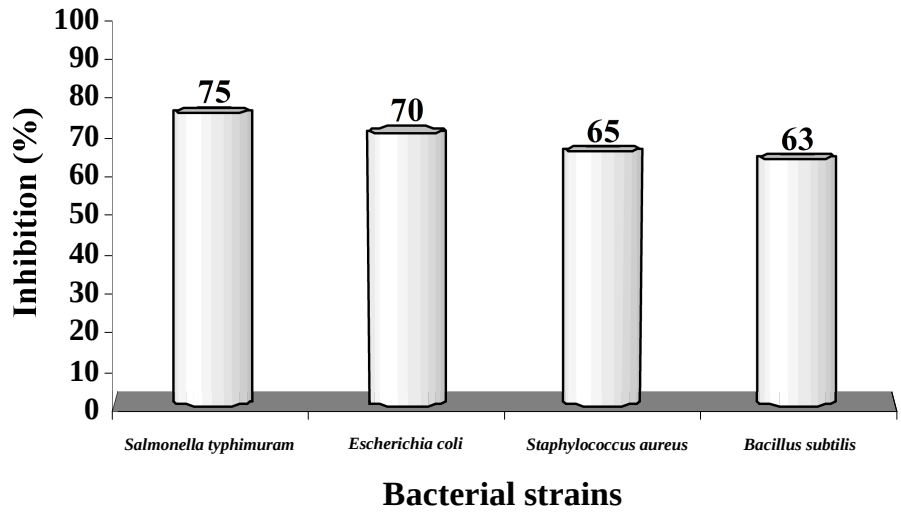


**Fig. (1):** The percentage of growth inhibition for chosen bacterial strains through pullulan coating.

Many research were referred the effect of pullulan on microorganisms, Chlebowska-Smigiel and Gniewosz(4) studied the effect of pullulan on moulds, yeasts and bacterial growth, they found that the inhibition of pullulan was 100% on bacterial species such us *Citrobacter freundii* ATCC 8090, *Lactobacillus plantarum* ATCC 4080, and *Pseudomonas fluorescens*, while the inhibition was 80% on *Lactobacillus brevis*. The inhibition of pullulan was 70% on *Escherichia coli* ATCC 25922 and *Tetracoccus* sp. and 60% on *Micro-coccus luteus* ATCC 9341 and *Bacillus subtilis* ATCC 6650.

They suggest that pullulan coating can be a useful application to ensure food safety and quality from microbiological deterioration, because it had important effect on relation between oxygen and carbon dioxide in microorganisms. Gniewosz, et al(12) was studied antimicrobial activity of pullulan film incorporated with meadowsweet flower extracts (*Filipendulae ulmariae flos*) on postharvest quality of apples.

They found that inhibition of pullulan on *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Salmonella enteritidis* ATCC 13076 and *Escherichia coli* ATCC 25922 was more than degree of mold species such as *Penicillium expansum* ATCC

7861, *Rhizopus arrhizus* ATCC 11145 and *Aspergillus niger* ATCC 9142, also they refer that gram-positive bacteria like *S. aureus* and *B. subtilis* were demonstrated higher resistance (MIC 0.6-6.0 mg/ml). Krasniewska, et al(15) was used pullulan coating that enriched with plant extracts from *Satureja hortensis* L. in different concentrations to maintain pepper and apple quality and safety and they obtained that the inhibition zone was significantly increased ($p < 0.05$) for all the tested strains as the concentrations of extracts in the pullulan film, in 5% addition of extract to the pullulan film inhibited the growth of gram-positive bacteria, while gram-negative bacteria were inhibited by 20% in the film, they explain that, the gram-negative bacteria are more resistant to antimicrobial compounds than gram-positive bacteria and this fact may be related to the structure of the cell wall of gram-negative bacteria which is characterized by the presence of an additional external lipopolysaccharide (LPS) membrane. Kanmani and Lim (14) were refered to antimicrobial activities for pullulan-mediated silver nanoparticles against some microorganisms that causes food-born and multidrug resistant pathogens, they found that all bacterial pathogens were highly inhibited in a dose-dependent manner, increases in the inhibition zones were observed with an increase in the amount of AgNPs. The bacterial pathogens like *Pseudomonas. aeruginosa* was more susceptible to AgNPs followed by *Klebsiella. pneumoniae* and *E. coli*, the food-born pathogen *Listeria. monocytogenes* was less susceptible to the AgNPs, overall, the gram negative bacterial pathogens were highly suppressed by the AgNPs compared to gram positive bacterial pathogens, that probably due to the thick cell wall of gram positive bacteria, which are generally composed of a three-dimensional thick peptidoglycan (PG~20-80nm) layer compared to that of gram negative bacteria (~7-8nm), the PG layer possesses linear polysaccharide chains cross linked by more short peptides and thus forms a complex structure which makes it difficult for AgNPs to penetrate gram positive bacteria.

**Weight loss:**

Results in (Figure, 2) showed the weight loss for uncoated and coated peach with 10 and 20% pullulan water solutions that storage at 25°C for 21 days.

The percentage of weight loss of uncoated peach was 3.2, 4.8, 6.1, 7, 7.2, 8.3 and 9.1%, Values of weight loss in coated peach with

10% pullulan were 3.1, 4.5, 5.8, 6.2, 6.8, 7.9 and 8.9%, while values in coated peach with 20% pullulan were 2.9, 3.8, 4.1, 4.6, 5.8, 6.4 and 7.6% when stored for 3, 6, 9, 12, 15, 18 and 21 days respectively.

(Figure 3) showed the results of weight loss for uncoated and coated peach storage at 4°C for 10 days.

The percentage values of weight loss for uncoated peach increased gradually between the first and tenth day of stored were 0.01, 0.06, 0.16, 0.23, 0.35, 0.46, 0.6, 0.68, 0.73 and 0.75%. Values of coated peach with 10% pullulan were 0.01, 0.05, 0.12, 0.16, 0.2, 0.3, 0.5, 0.58, 0.64 and 0.71%, while values in coated peach with 20% pullulan were 0.015, 0.02, 0.04, 0.08, 0.1, 0.12, 0.18, 0.3, 0.46 and 0.5%, when samples stored for 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days respectively.

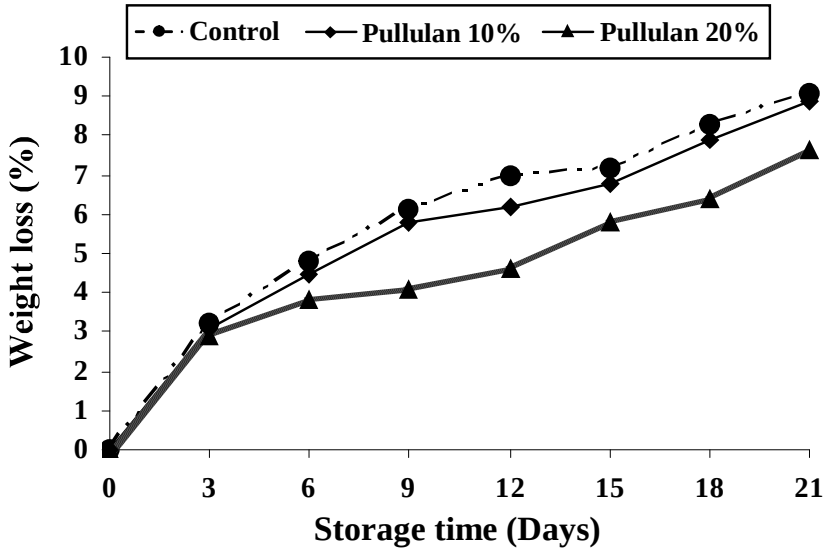


**Fig. (2):** Changes in the peaches weight loss stored at 25°C for 21 days for different treatments.

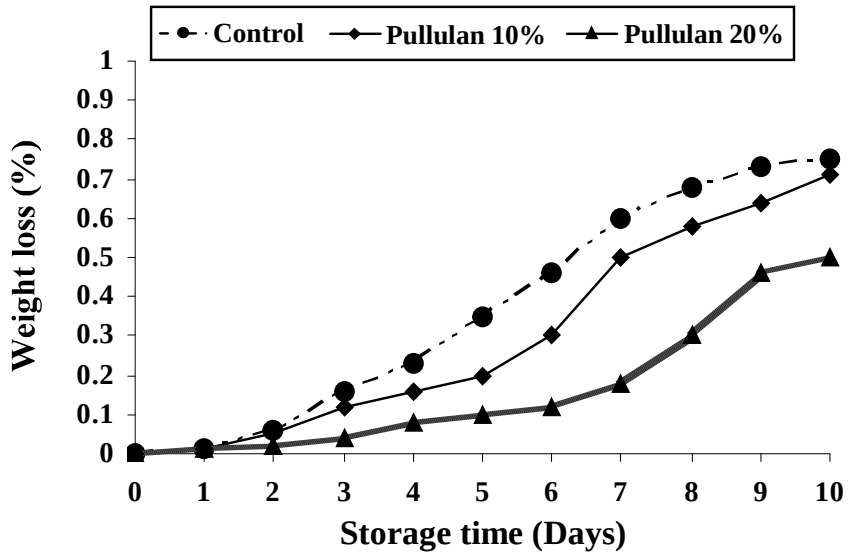


**Fig. (3):** Changes in the peaches weight loss stored at 4°C for 10 days for different treatments.

The changes in weight loss for uncoated and coated pear with 10 and 20% pullulan that stored at 25°C are presented in (Figure 4), the weight loss values of uncoated pear were 1.4, 2, 2.6, 4.4, 5.5, 6.4 and 7.2%, the values in coated peach with 10% pullulan were 1.2, 1.6, 2, 2.4, 4.3, 5.2 and 6.6%, while in coated peach with 20% pullulan were 0.8, 1, 1.4, 1.6, 3, 4.6 and 5.4%, when these samples stored at 3, 6, 9, 12, 15, 18 and 21 days respectively.

(Figure, 5) showed the uncoated and coated pears with 10 and 20% pullulan stored at 4°C for 10 days.

The weight loss values of uncoated pears were 0.02, 0.07, 0.14, 0.16, 0.3, 0.33, 0.44, 0.45, 0.52 and 0.54% for 10 days storage.

The values for coated pears with 10% pullulan were 0.02, 0.06, 0.11, 0.16, 0.21, 0.3, 0.36, 0.38, 0.44 and 0.46%, while coated pears with 20% pullulan were 0.01, 0.05, 0.08, 0.09, 0.1, 0.16, 0.19, 0.24, 0.26 and 0.28%, as there samples stored for 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days respectively.

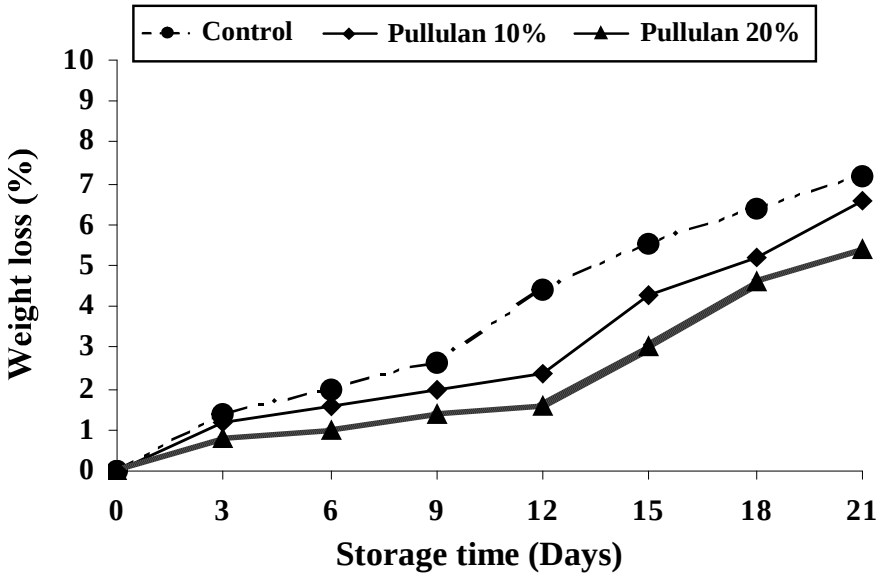


**Fig. (4):** Changes in the pears weight loss stored at 25°C for 21 days for different treatments.

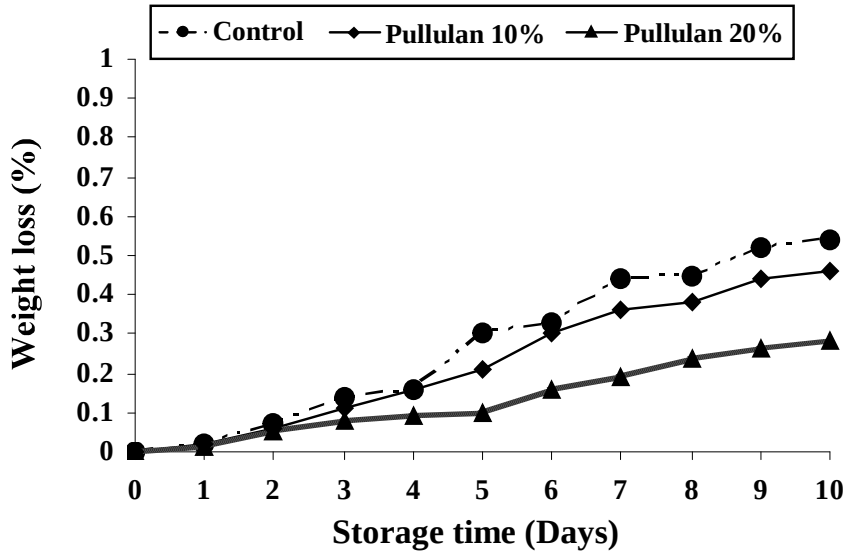


**Fig. (5):** Changes in the pears weight loss stored at 4°C for 10 days for different treatments.

The main problem in fresh fruits and vegetables are turnover on the markets is softening that caused by the natural process of maturation and water loss, this changes are considered undesirable by consumers (5). Weight loss in fresh fruits and vegetables caused a short shelf-life and loss of economic value, additionally, this parameter has a strong impact on the appearance, due to shrinkage, both temperature and storage time contributed to increase weight loss (15). Storage at 16°C reported higher weight loss than storage at 6°C, polysaccharide coatings exhibit high barrier properties toward oxygen, the rate of oxygen transfer through pullulan films is lower compared to polypropylene and polyethylene films, this enables the formation of pullulan coatings to reduce the rate of fruit and vegetable respiration (3). So many researches discuss this important parameter. Chlebowska-Smigiel, et al (4) used Pullulan coatings to prolong apples shelf-life stability by coating apples with 15 and 20% pullulan stored at 4°C and 22°C for 39 days, they noticed that the highest mass loss was with 15% pullulan coat samples during the first two days of storage and then between $18^{th}$ and $22^{nd}$ day at 4°C, the coated with 20% pullulan also the highest mass loss was observed in the first two days and then considerable fall between $13^{th}$ and $18^{th}$ day of storage, also they found on the following days, apples mass losses were tended to decrease independently from the pullulan and the values of changed was from 0.49-0.58% after the first day of storage up to 0.11-0.13% after the very last day, and for the comparative apples (without pullulan coating) the mass loss was evidently highest than for these coated and was 0.9% after first day of storage and 0.2% after the last day, and observed the mass losses for the apples covered with the 15 and 20% pullulan were less than for the control apples (uncoated) when stored at room temperature of 22°C during 39 day, the highest mass losses for the control apples were observed during the first 11 days of storage (and they were 1.35-0.74%), at the same time the mass losses of the apples covered with the protecting pullulan coatings were only 0.82-0.51%, the coatings produced from either 15 or 20% pullulan had better physical properties at 4°C than at 22°C. In lower temperature it was thin, shiny and smooth, at 22°C starting from the $5^{th}$ day of storage, became wrinkled and the fruit surface started to go off. Krasniewska, eta al (15) use pullulan coating that enriched with plant extracts (SH) from *Satureja hortensis* L. to maintain pepper and apple quality and safety, they noticed that weight loss increased significantly ($p<0.05$)

during the storage of at 6 and 16°C for uncoated peppers compared to peppers coated with pullulan and pullulan (SH) extract, while water loss was observed significantly lower ($p<0.05$) in coated samples, and throughout storage at 6°C, the uncoated peppers and peppers coated with pullulan and with pullulan (SH) extract lost respectively 12.01, 10.47 and 8.87% of their initial weight at 28 days of storage, the weight loss in uncoated peppers stored at 16°C was the highest 12.66% at 14 day, while peppers coated with pullulan and pullulan (SH) extract reached lower values of 10.26 and 9.75%, respectively, and a similar behavior was observed for apples stored at 2°C and 16°C, weight loss for coated apples was significantly lower ($p<0.05$) than that of the controls, apples stored at 2°C showed losses of about 2.84% for uncoated samples, whereas for apples coated with pullulan and with pullulan (SH) extract the weight loss was 1.93 and 1.61%, respectively, reductions in weight loss in apples stored at16°C were 4.13, 2.93 and 2.86% respectively, for uncoated apples, those coated with pullulan and with pullulan (SH) extract. Gniewosz, et al (12) used pullulan film incorporated with meadowsweet flower extracts (*Filipendulae ulmariae flos*) on postharvest quality of apples, they refer that the highest weight losses were noted in the samples stored at 24°C, during the first 8 days, the losses in sample weight did not different significantly statistically, which was probably caused by rapid water evaporation from the surface (uncoated samples) and from coatings (coated samples), at the end of the storage period, weight losses about 0.5% lower were noted in the case of samples coated with pullulan coatings with meadowsweet flower extracts (EMFs) compared to uncoated ones, the samples stored in refrigerated conditions demonstrated lower weight losses, the addition of (EMFs) to pullulan coatings contributed to a decrease in weight loss of up to 2.5% when used pullulan coatings with ethanol meadowsweet flower extracts (eEMF) and 2.3% with pullulan coatings with water-ethanol mixture meadowsweet flower extracts (weEMF).

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