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THE EFFECT OF ADDING LOCAL ORANGE PEEL POWDER TO MICROBIAL INHIBITION AND OXIDATIVE REACTION WITHIN EDIBLE FILM COMPONENT

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Abstract

The objective was to increase the shelf life of cupcake by coating it with edible filming forms contain local orange peel powder and agar with different ratios and storage at refrigeration (6-8) ℃. The ability of three solution of edible films (6% gelatin without orange peel powder, 6% gelatin with 0.1% orange peel powder, 6% gelatin with 0.5% orange peel powder, 6% gelatin with 1% orange peel powder), to extend shelf -life of cupcake was examined during refrigeration storage. This coating was moisture saving, results of sensory and appearance evaluations of the filmed cupcake after storage were that the three samples have shown incorporeal prevalence in saving the sensorial properties with an acceptance degree of 6.75/7 in comparison with the standard sample that failed its sensorial properties after storage for 7 days, which gained low sensory acceptability. New edible coating composition was found to increase storage age and decrease microbial growth. Moreover, moisture was held to keep cakes undried. Membranes have shown high blocking towards water vapor and oxygen. There is significantly ($P \leq 0.05$) reducing in bacteria and there no growth of any mold or yeast during storage time in the three filmed cupcake samples. Peroxide value of samples at beginning of the experiment was 2.79 ml.eq./kg fat, after 1 week in refrigerating storage there is increasing in control sample up to 10.35 (ml.eq./kg fat) while the three samples of (0.1, 0.5, 1.0) % orange peel powder that showed a little increase in peroxide value reached 3.60, 3.75, 4.80 (ml.eq./kg fat) respectively, which indicated the antioxidant activity of orange peel powder.

Key words: Edible Film Component, Microbialinhibition, Oxidative Activity, Orange Peel Powder

Introduction

Orange is the most commercial fruit of the genus *Citrus* of the family *Rutaceae* which have leathery and oily rinds and edible juicy inner flesh with delicious taste. The fruit I rich in Vitamins A, B and C and a good source of beta-carotene, folate, pectin and minerals such as potassium, iron, selenium, iodine, phosphorus, manganese, sodium, chlorine and zinc. Furthermore, orange peel contains more than 170 different phytonutrients and more than 60 flavonoids combinations which highly evaluated as anti-cancer, anti-oxidants and anti-microbial agents (Al-Ani, 1989; Obidi *et al*., 2015 and Saonere Suryawanshi, 2011).

Several materials had been employed in film foaming such as Gelatin (A.O.A.C., 2008), Agar (E406) (Barreto *et al*., 2003). Generally, edible films processing employed polysaccharides or protein at 10-16%.

Many plasticizers should also utilize to import reliable flexibility, elasticity and breakage resistance during usage and storage (Williams and Phillips, 2000). The most common plasticizers used in film preparation include glycerol, polyethylene, sorbitol, sucrose and water (Stanley, 1995; Myllärinen *et al.,* 2002; Jagannath *et al.,* 2006; Cerqueira *et al.,* 2009; Bourtoom *et al.,* 2006 and Veiga-Santos *et al.,* 2007). A new generation of edible films were designed to increase their functional properties as incorporating natural antimicrobial and antioxidants agents, enzymes and other components such as probiotics, minerals and vitamins (Bifani *et al.,* 2007; Vargas *et al.,* 2008). Films with antimicrobial and antioxidants properties are characterized with slowing microbial penetration via films and enhancing nutritional values of coated foods (Hao Tian *et al.*, 2015 and Kierman, 2008).

The aim of present investigation was to assess incorporating orange peel in edible films manufacture. The prepared mixtures were applied to cupcake by dipping and brushing. Evaluation of the antimicrobial and antioxidant capacity of the manufactured product during storage at (6±2) °C.

Materials and Methods

Preparation of Orange Peel Powder

Orange peel was collected from different orange juice shops in Baghdad during 2017. Sun dried in clean environments. Grinded with electrical grinder to a mesh size of 60. Stored in sealed plastic bags at (6±2) °C.

Production of Laboratory Cupcake

Cupcake was prepared form the following ingredients (one and half cup flour, three quarters cup sugar, two teaspoons of baking powder, half teaspoon of salt, half cup of milk, half cup oil, two eggs, one teaspoon orange peel powder). The dry ingredients were added to the wet ingredients then well-mixed until it became creamy and smooth. After that, the mixture was poured in certain cups and baked at 180° C for 15 minutes (Jeyanthi Rebecca *et al*., 2016)

Preparation of Coating Solutions and Application

The coating solutions were prepared from Glycerol and Agar at 25% and 1.5% w/w respectively. Four coating solutions were prepared as orange-peel powder was added separately at (0, 0.1, 0.5, 1.0) % for the treatments M1, M2, M3 and M4 respectively. The coating mixtures were heated separately to boiling to ensure Agar melting. The cupcakes were coated separately by each coating mixtures via two methods mainly dipping and brushing. The recommended methods were employed (Armisén and Galatas, 2000; Bourtoom *et al*., 2006; Bifani *et al*., 2007; Veiga-Santos *et al*., 2007; Vargas *et al*., 2008 and Cerqueira *et al*., 2009).

Cupcake Coating with Membranes Solutions Using Brush

Polishing operations were performed on cupcake with filming solutions (M1, M2, M3, M4) that contains orange peel powder (0, 0.1, 0.5,1) % (wt./wt.) by two methods:

Dipping Method

Filming solutions were prepared according to the illustrated concentrations of orange peel powder with an addition of 1.5% Agar and 25% glycerol (wt./wt.) to each solution then cupcakes were sinking by a method of quick sinking in each hot solution after its preparation directly then left until the film get solidified.

Brush Filming Method

The solutions were prepared according to the method explained above then cupcakes were polished with the solutions using a suitable brush then left until films get solidified.

Sensory and Appearance Evaluations of Cupcake

Sensory assessment was made according to (Levent and Bilgicli, 2013). The evaluation was conducted by 20 people with experience and specialization in the Department of Food Science, Faculty of Agriculture, University of Baghdad. The evaluation included both males and females, Randomized and evaluated on the basis of appearance, texture, palatability, flavor, general acceptance and product values by giving grades 1-9, given a maximum of 9 and a minimum grade of 1.

Sensory evaluation was undertaken to assess the sensory characteristics of cupcake group one of replications which represents at zero time and the second group after being refrigeration for 7 days at (6±2) °C in comparison with the control replication and a sample form was used in sensory evaluation of cupcake.

Sensory evaluation was performed by two experts at the food science Department/ Collage of Agriculture/ University of Baghdad to assess the sensory characteristics according to the evaluation form that was certified from food and nutrition department at the American University of Kansas in 1975 (Department of Foods and Nutrition, 1975), included the sensory characteristics of appearance, texture, flexibility, flavor and overall acceptability.

Moisture Loss Percentage Test of Cupcake

Moisture loss percentage was estimated according to a certified method (Dhillon *et al*., 2004) as cupcakes were weighted at the zero time for treatments (M1, M2, M3, M4) after refrigeration at (6±2) °C for . Moisture loss was calculated according to the following:

$$\text{Moisture loss}(\%) = \frac{\text{cupcake weight before storage} - \text{cupcake weight after storage}}{\text{cupcake weight}}$$

Membrane Permeability of Water Vapor and Oxygen

Membrane permeability for water vapor and oxygen was conducted at ministry of science and technology, Baghdad, Iraq. The film solutions for different treatments (M1, M2, M3, M4) were poured into dishes of a known diameter to obtain films with suitable thickness for analysis

Microbiological Examination

The sterilized media were prepared containing 10 ml of agricultural medium of Nutrient Agar and Potato Dextrose Agar according to a certified method (Al-Saeedy, 1983) then a tinge of cake peel and core was taken by a vaccination nail loop with an average of 3

tinges to each cake separately. After that, they were cultured by the simple striking method and the dishes were stored at 37 °C for 1-2 days. The total count of bacteria cultured by using Nutrient Agar medium while the yeast and mold count were vaccinated with the dishes containing the agricultural medium of Potato Dextrose Agar and stored under 25 °C for 5-6 and finally the total count was measured.

Chemical Analysis of Fat Portion Fatty Extraction of Cupcake

Fatty extraction of cupcake was extracted according to (Bligh and Dyer 1959). cupcake samples were dried in order to get they ready to chemical tests by using electric over free of air under 60 °C then they were smashed, solvent difthylether and a petroleum ether were added to them and left sank until extraction. After that, fatty material was filtered and extracted by using a rotating evaporator then the fatty material was stored in tightly closed dark packages to perform the required tests.

Peroxide Value Estimation

About 5 gm of fat portion (as described above) was weighed and placed in a flask containing then 30 ml of acetic acid and chloroform mixture (30 ml and 20 ml respectively) was added to it and 0.5 ml of Potassium iodide KI saturated solution simultaneously prepared then the flasks were closed directly, shaken for 1 min and 50 ml of Distilled water was added to end the reaction. Moreover, it was titrated with Sodium thiosulfate solution 0.01 N standard by using starch index until blue color disappeared. The peroxide value was determined by the following equation:

$$\text{Peroxide number per kg fat} = \frac{\text{consumed thiosulfate} \times \text{standard} \times 1000}{\text{sample weight}}$$

Statistical Analysis

SAS, (2012) was used to determine the significant differences among the averages of least significant difference using randomized complete design (RCD) and compared the significant difference (LSD ≤ 0.05).

Results and Discussion

Cake Coating

The results of filming with the three solutions containing orange peel powder (0, 0.1, 0.5, 1) % (wt./wt.) using dipping method, showed that cake has lost its shape due to high moisture absorption in addition to non-storage resistance as cake pieces exposure to quick decomposition according to high water activity aw which encouraged microbials growth and spread. Whereas brush filming method has shown better results as a fine layer of film was formed and suitable quantity without absorption and inside permeability which reflected positively on shape and stability of cake during storage.

Sofia *et al*. (2015) noted that the archival properties of edible membranes can be affected by the polymer composition, the concentration of the plasticizer, the solvent type and other factors based on membrane melting, permeability and propagation properties, Edible coatings can withstand additives such as antimicrobial agents, antioxidants, flavorings and colors to improve food quality, provide safety conditions and ensure the safety of the food surface by controlling the release of active substances from the membrane matrix and coating to the food surface. Food (Oxidation of fat, melard reactions, enzymatic fermentation) as well as microbial growth occurs at high levels of water activity and moisture content in food

Moisture Loss Test

Most studies need to use commercial biomaterials to obtain accurate information for commercial application in the packaging of fresh foods and products using an edible polymer coating that adds nutritive value as well as longevity (Zeinab *et al*., 2015).

The (Table I) below shows the results of moisture loss of the second group which represents the control and three replications (0, 0.1, 0.5, 1) % after refrigerated at (6 ±2) ° C.

Table 1 : Moisture Loss Percentage of Control and Three Replications of Cupcake After Being Refrigerated

Replications	Cake weight (gm)		Moisture Loss (gm)	Moisture Loss Percentage (%)
	Fresh (0 day)	7 Days of Storage		
M1	32.4	30.5	1.9	5.8
M2	68.6	67	1.6	2.3
M3	53	53	No loss	0
M4	106	106	No loss	0
Chi-Square (χ^2)				1.392 NS

Notes: Each of these four replications is the average of 10 samples. NS: Non-Significant.

Control replication has exposed to high moisture loss after storage which was 5.8% despite what happened to the three cupcake replications that kept its moisture content during the storage period without a loss except a rare moisture loss in M1 which reached 2.3%. Therefore, this shows the clear effect of adding dried orange peel powder on decreasing the moisture loss during storage period.

Sensory and Appearance Evaluations of Cupcake

Table II shows the results of sensory evaluations of cupcake of the first replications group that represents the beginning of experiment and second group after one week of refrigeration by comparison with the control.

Table II : Cupcake Sensory and Appearance Evaluation Average Results

Storage period (days)	Replicates	Sensory Characteristics				
		Appearance	Texture	Flexibility	Flavour	General Acceptance
Fresh (0 day)	M_1	5.83± 0.07	6.00± 0.12	6.16± 0.10	5.83± 0.07	6.00± 0.08
	M_2	6.16± 0.14	5.50± 0.08	6.66± 0.15	6.00± 0.11	6.50± 0.12
	M_3	6.00± 0.09	5.50± 0.06	6.66± 0.12	5.83± 0.09	6.16± 0.12
	M_4	5.83± 0.09	5.83± 0.08	5.50± 0.09	5.50± 0.08	5.83± 0.10
LSD Value	---	1.09 NS	0.871 NS	1.363 NS	1.026 NS	1.117 NS
7 days of storage	M_1	6.50± 0.13	3.50± 0.06	2.50± 0.03	5.50± 0.07	3.50± 0.06
	M_2	6.50± 0.09	6.50± 0.12	7.50± 0.16	6.50± 0.13	6.75± 0.13
	M_3	6.50± 0.13	7.50± 0.26	6.50± 0.11	6.50± 0.10	6.75± 0.10
	M_4	6.50± 0.13	6.50± 0.11	6.50± 0.15	7.50± 0.24	6.75± 0.12
LSD Value	---	NS	2.09 *	1.86 *	1.95 *	2.16 *

Notes: The highest mark that can be given to each characteristic is 7
7= excellence, 6= very good, 5= good, 4= moderate, 3= acceptable, 2= poor, 1= very poor
Each of these four replications is the average of 10 samples -- * ($P<0.05$).

Results of the sensory evaluations of first replications group at the beginning of experiment includes no differences in sensory characteristics (appearance, texture, flexibility, flavor and general acceptance) within (0, 0.1, 0.5) % replications while 1.0% replication showed a light (significant degree $P\leq0.05$) decrease in flexibility that reached 5.50 in comparison with the other replications that showed higher degrees of flexibility with a degree of 6.66. it could be because of the increase in orange peel which contains pectin that kept the moisture content inside the cupcake.

Furthermore, the results of the second replications group after refrigeration presented that the three replications are better than the control in the sensory characteristics in (Table II) above. The general acceptance of the control was obviously reduced after refrigeration to become 3.5 in comparison with the three other replications that reached 6.75. the reason might be the moisture loss of the control during the storage what negatively affects the texture and flexibility as shown in moisture loss results.

Membrane Permeability Test of Water Vapor and Oxygen

Water Vapor Membrane Permeability

Membranes of Agar material are recognized in its ability of forming a coherent and flexible membrane with perfect rheological characteristics which keep water vapor and subsequently save the flexibility of the product by lesser vapor loss. It can be noticed that a gradual decrease in water vapor permeability throw the membrane with a concentration increase of orange peel extraction. Thus, it indicates the membrane cohesion increase with the increase of the concentration. Water vapor permeability are shown in (Table III).

Table III : Results of Water Vapor Permeability Test

Replicates	Water vapor permeability (g.mm/day.m^2kPa)
M_1(0% Orange peel)	0.389 ± 0.08
M_2(0.1% Orange peel)	0.13 ± 0.334
M_3(0.5% Orange peel)	0.18 ± 0.321
M_4(1% Orange peel)	0.10 ± 0.274
LSD Value	0.251 *

Notes: Each of these four replications is the average of 10 samples -* ($P<0.05$).

Table IV : Results of Oxygen Permeability Test

Replicates	Oxygen permeability (ml/m^2.day)
M_1(0% Orange peel)	9.91± 0.47
M_2(0.1% Orange peel)	9.85± 0.25
M_3(0.5% Orange peel)	9.65± 0.44
M_4(1% Orange peel)	9.21± 0.52
LSD Value	1.263 NS

Notes: Each of these four replications is the average of 10 samples - . NS: Non-Significant.

Oxygen Membrane Permeability

Agar material membranes works as barriers to oxygen, carbon dioxide, fats, flavors and odors between nutritious compositions and the surrounding atmosphere. It was noticed that cake painting with those films works to decrease oxygen permeability from and to cake pieces.

Table IV above refers to the decrease of oxygen permeability with the increase of orange peel extraction concentration. The reason behind that is the transverse overlap which occurs between filming solution ingredients that gives higher cohesion to the cover.

Microbial Tests

The (Table V) below shows the microbial tests results of cupcake to control and three replications (0.1, 0.5, 1.0) % at the experiment beginning and the end of refrigeration.

Table V: Microbial load of cupcake was a result of edible film containing orange peel powder

Storage period (days)	Replications	Total number	Total number or moulds and yeasts
Fresh (0 day)	Control	50 x 10^3	50 x 10^1
7 days of storage	Control	50 x 10^2	1 x 10^1
	0.1%	1 x 10^2	No Growth
	0.5%	10 x 10^2	No Growth
	1.0%	8 x 10^2	No Growth
LSD value			17.39 *

(P<0.05).

Table VI : Peroxide Value of Cupcake (ml equivalent/Kg fat)

Storage period (days)	Replications	Peroxide value (ml equivalent)
Fresh (0 day)	Control	2.79± 0.06
7 days of storage	Control	10.35 ± 0.52
	0.1%	3.60 ± 0.12
	0.5%	3.57 ± 0.09
	1.0%	4.80 ± 0.15
LSD value	--	2.584 *

* (P<0.05)

Total number of molds and yeasts of control replication were shown at the beginning of experiment as 50 x 103 and 50 x 101 respectively while the results after refrigeration were 50 x 102 and 1 x 101 respectively. This was because of high moisture loss of cake during storage period. However, the results of the three other replications showed no growth of molds and yeasts because of orange peel role in preventing the microbial growth as mentioned by (Obidi *et al*., 2013). Citrus fruits have some antimicrobial properties specially in its peel (Moosavy *et al*., 2017). Furthermore, the bacterial number of the three replications (0.1, 0.5, 1.0)% at the end of storage was decreased significantly in comparison with the control sample by full logarithmic round which reached 1 x 102, 10 x 102, 8 x 102 respectively.

Fatty Tests

The method of working membranes and coatings to prolong the food shelf life of the food includes control of the transmission of moisture between food and the environment and control the rate of breathing, as well as control the release of chemicals such as antimicrobial or anti-oxidants and is not implemented for basic materials such as fats and oils and support for the installation of food as well as increasing the stability of vehicles Flavor (Aday and Caner. 2010). (Table VI) explains the

chemical analysis results (peroxide value) of cupcake fat of the control replication at the experiment beginning which reached 2.79 mm equivalent while that number was increased significantly of the control at the end of refrigeration up to 10.35 in comparison with peroxide value of filmed cupcake if the three replications (0.1, 0.5, 1.0) % that showed a little increase in peroxide value to 3.6 – 3.57 – 4.80 ml equivalent/kg oil respectively.

That might be because of low permeability provided be the used filming solutions in cupcake filming regards oxygen. The results of testing those films showed a positive reflection on fatty material stability in addition to the role of aromatic oils in antioxidant orange peels. 0.5% replication has the highest fatty material stability during storage which showed less deterioration of fatty material with a peroxide value of 3.57 ml equivalent/kg oil, however, the other two concentrations (0.1 & 1)% caused also stability against oxidation because of essential oil from orange peel get more stability to coating polymers (Siddique *et al*., 2011 and Djenane, 2015).

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