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Bioactive Gelatin-based Date By-Product for Packaging Applications: Physico-Chemical and Biological Characterization

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Abstract: Biodegradable films from gelatin (Gn) with various date by-product (DBP) concentrations (1, 2, 3 and 4 wt %) were prepared. Elaborated films were examined in terms of physical properties (thickness, density, water solubility, water content, degree of swelling, color), and antimicrobial properties (*Escherichia coli* and *Staphylcoccus aureus*). Adding the highest concentration of DBP (4%), resulted an increase in the WHC of film as compared with control film. Moreover, the incorporation of 1% DBP reduced the moisture level of Gn based composite films as compared with the control film. Furthermore, Film with 4% of DBP had the lowest solubility which reached 39.39%. Incorporation of DBP from 1 to 4% showed decrease of L- and a-values. The active Gn-DBP 1% showed less lightness as compared to Gn-DBP 3%. The incorporation of DBP into film-forming solutions led to increased opaqueness for all gelatin-based composite films. The calculated opacity value was inversely proportional to transparency. Moreover, the Active Gn-DBP 1% and 2% film presented effective antibacterial activity against bacteria such *as Staphylococcus aureus* and *Escherichia coli.* The results showed an enhancement in the biodegradability of Gn-DBP films in moist soil. The results reveal the benefits of date by-products incorporated into gelatin based films as a potential material for active food packaging.
Keywords: Gelatin; Date by-product; Active packaging; Biodegradable film.

1. Introduction

In the recent years, active food packaging films have gained interests due to their biodegradability, non toxicity and their good interactions with food. These bio- films are also suitable alternatives for commercial synthetic films as these natural films enhance food quality and inhibits intrinsic food contamination. Nowadays, a wide variety of biopolymers derived from agriculture by-products have emerged as a potential source to produce eco-friendly food packaging materials [1]. This approach provides a new application to these natural polymers resulting in the formulation of economic and effective active food packaging films. Among natural polymers, gelatin is an excellent choice for packaging films as it is biodegradable and has good film forming properties. Gelatin is a water soluble protein derived by the hydrolysis of collagen present in the connective tissues able to form amorphous three-dimensional structures stabilized mainly by non-covalent interactions. However, gelatin films suffer from some limitations such as oxygen and water impediment ability resulting in moderate functional properties. The incorporation of natural compounds in biopolymer synthesis had been suggested to improve the functional property of the packaging [2, 3]. Recently, several studies have been performed to propose the use of natural compounds obtained from different sources, including plants, animals and by-products generated during fruit and vegetable industrial processing [4, 5, 6].

In Tunisia, date palm production is 195,000 tons per year (FAOSTAT, 2015). However, annual production of date generates a large volume of by-products which accounts for up to 30% of the total production which may lead to a serious environmental problem. Previous studies have shown that date contains natural antimicrobial compounds. The valorization of discarded second-grade dates for the production of low-cost value-added products remains a promising area. Hence, the present study was undertaken to develop Active gelatin-based date by-product packaging food film. The work aims to evaluate the physico-chemical characteristics and antibacterial activities.

2. Material and methods

2.1 Film Gelatin- date by-product preparation

A gelatin solution of 4% W/V was prepared and magnetically stirred for 15 min at 60°C for complete dissolution. After cooling, different concentrations of date by-product powder were added to the mixture (1, 2, 3 and 4 wt %) and the final solution is stirred again for 10 min. The glycerol was included as a plasticizer for films. Then, the hot film-forming solution is poured into petri dishes (25 ml), and was left at room temperature for at least 3 days to obtain the films. The dry films were removed from the petri dishes and stored. Two types of films have been prepared: the control films without date by-product and gelatin-based date by-product films with different concentrations (1, 2, 3 and 4 wt %).

2.2 Water holding capacity

For determining the percent water holding capacity (WHC), film sections of 2 x2 cm of size were weighed. Then the films were immersed for 1h, 2h and 3h in distilled water and were removed. After that, the final weight was measured.

$$\mathrm{WHC}(\%) = \frac{(\mathrm{w}\,i - \mathrm{w}\,f)}{wi} \times 100$$

where w*i* and w*f* were initial and final weight of film sections.

2.3 Film thickness

The film thickness measured by Mitutoyo 0-25 mm Micrometer with a resolution of 0.01 mm by measuring 5 random spots from each film.

2.4 Film moisture

The moisture content (MC) of the prepared films was determined by taking the weight (W1) of small pieces of films (2 cm x 2cm) which were placed in a Humid Room maintained enclosure. These sections of the films were then dried in a hot air oven at 105 °C for 24 h. The weight recorded after drying (W2) was taken as the dry weight of the sample. The percentage of MC was calculated using following equation:

$$\mathrm{MC}(\%) = \frac{(\mathrm{w}\,1 - \mathrm{w}\,2)}{w1} \times 100$$

where W1 and W2 were the original and final weights of film samples, respectively.

2.5 Water solubility

The film solubility (TS) was determined as described previously [7] by trimming the samples into small strips. Square pieces (2 cm x 2 cm) of each film were dried at 100 °C for 24 h to a constant weight. Each dried sample was immersed in 100 ml of distilled water for 24 h. Film samples were then removed from the solution and dried again at the same conditions. Final dry weights were recorded and solubility calculated as:

$$\mathrm{TS}(\%) = \frac{(\mathrm{w}\,1 - \mathrm{w}\,2)}{w1} \times 100$$

where W1 and W2 where the first and second drying weights of film samples, respectively.

2.6 Opacity

The opacity of gelatin films was performed using a spectrophotometer (Cary 300 Bio, UV-Vis Spectrophotometer, Varian Instruments, CA, USA), set at a wavelength from 200 to 800 nm. The opacities of films were determined by the following equation as described previously [8]:

$$\boldsymbol{Opacity}(\%) = \frac{\boldsymbol{A}}{\boldsymbol{x}}$$

where A was the absorbance of film at 600 nm and x was film thickness (mm).

2.7. Color measurement

Portable Konica Minolta colorimeters CR 300 was used to measure the surface color of the films. The color

space: *L**, *a** and *b** was measured, where *L**, is a measure of lightness black- white, the coordinate *a** represents the relative amounts of red- green and the *b** coordinate expresses the relative amounts of yellow- blue. Both *a** and *b** coordinate values vary approximately in the range from -100 to 100. The lightness *L**, increases in the range from 0 to 100 [9].

2.8. Biodegradation experiments

The biodegradability test of all samples in soil was carried out. Soil was poured into a plastic tray. The samples (2 cm x 2cm) were weighed and then buried in the soil to a depth of 5 cm. Water was sprayed once a day to sustain the moisture. At various time intervals (5, 10, 15, 20, 25 and 30 days), samples were carefully taken out, washed with distilled water and dried at 50 °C for 24 h and then weighed.

2.9. Antimicrobial activity test

The antimicrobial activity of the films against *Staphylococcus aureus* (ATCC25923) and *Escherichia coli* (ATCC25922) was determined according to the method described by Bahram et al. (2014) with minor modifications. Bacteria was cultured in the Luria Bertani medium (LB) at 37°C for 18h, and the final bacterial concentration was diluted to reach value of 0.1- 0.2 at 600 nm. After that, the film at different concentrations were added to the prepared bacterial suspensions and incubated at 37°C with 80 rpm/min for 8 and 24h. at the predetermined times, the absorbance value of all tested samples was red at 600 nm. The bacterial inhibition percentage was determined by the following equation:

$$Bacterialinhibition(\%) = \frac{(Ic - Is)}{Ic} \times 100$$

where, Ic is the absorbance value of the control bacterial solution and Is is the absorbance value of the bacterial suspension containing different films at each times.

3. Results

3. 1 Water holding capacity

The water holding capacity (WHC) of different films at different times was listed in Table 1. Active Gn-DBP 2% tends to hold less water content (362.01%). Adding the highest concentration of DBP (4%) resulted in an increase in the WHC of film. The incorporation of 1% DBP decreased the WHC that is considered as ideal for packaging food. These characteristics aid in stopping the intensification of microbes.

Table 1. Water solubility (WS), Water holding capacity (WHC) and Moisture content (MC) of gelatin films incorporated with different concentrations of DBP

Film samples	WS (%)	MC (%)	WHC (%)
Control	68.12±0.4	1.3±0.8	407.5±2.8
GN-DBP 1%	63.8±0.05	1.2±0.4	365.47±1.2
GN-DBP 2%	49.4±0.2	1.15±0.5	362.01±0.8
GN-DBP 3%	49.18±0.5	1.1±0.9	393.2±1
GN-DBP 4%	39.39±0.2	1.1±1.5	391.25±0.5

3. 2 Film moisture

Film moisture (MC) of all samples is shown in Table 1. Moisture content of the DBP films was reduced due to the DBP incorporation. As shown in Table 1, decrease in moisture content of the DBP films is due to the addition of the hydrophobic components of DBP. Furthermore, the incorporation of 1% DBP reduced the moisture level as compared with the control film. Packaging films should possess lower moisture content in order to inhibit moisture transfer between food and external environment.

3. 3 Film solubility

The water solubility (WS) of gelatin films incorporated with various concentrations of DBP is shown in Table 1. Water solubility is an important parameter for food storage. All films with DBP have reduced solubility in comparison to control film (68.12%). Film with 4% of DBP had the lowest solubility which was 39.39%. The decrease in value is attributed to high protein-polyphenols interaction in which it formed stronger film network structure. Control films had the higher solubility rate due to the OH group presence in gelatin which affects the gelatin film by its hydrophilic nature.

3. 4 Film thickness and Opacity

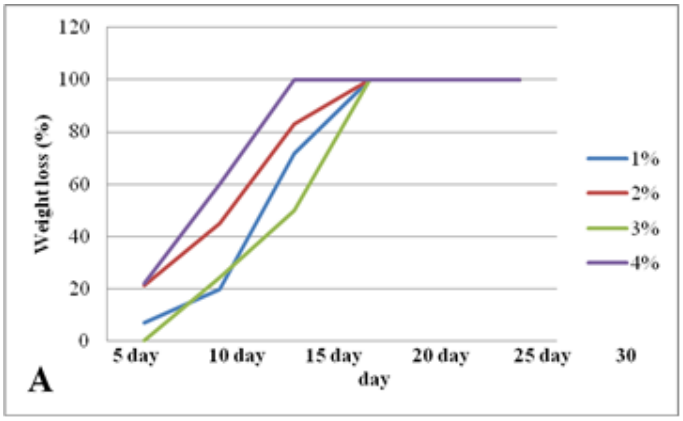
The effects of DBP addition on film thickness and opacity are listed in Table 2. The incorporation of different percent of date into the films did not prove a significant difference in thickness**.** The incorporation of DBP resulted in alteration in the transparency of the developed films. The calculated opacity value was inversely proportional to transparency. A significantly higher value (4.66) was observed for the Gn-DBP 4%.

Table 2. Opacity measurement of films.Values given are mean ± standard deviation.

DO (nm)	Films with different concentration of DBP			
	Gn-DBP 1%	Gn-DBP 2%	Gn-DBP 3%	Gn-DBP 4%
200	4.4±0.01	4.52±0.01	4.56±0.02	4.66±0.01
280	2.35±0.03	2.71±0.02	3.45±0.02	3.93±0.01
350	0.57±0.01	0.75±0.02	0.98±0.02	1.2±0.04
400	0.26±0.02	0.3±0.01	0.38±0.02	0.5±0.02
500	0.15±0.03	0.16±0.01	0.18±0.01	0.23±0.02
600	0.1±0.01	0 .10±0.02	0.11±0.03	0.15±0.03
800	0.08±0.03	0.1±0.03	0.11±0.04	0.12±0.04

3. 5 Biodegradability

The biodegradation results were shown in Fig 1. On the 5th day a few changes in the appearance of films were seen (Fig1.B). During these days, the films were swollen remarkably and changed their colors to brown, after which they began to lose their outline shapes. On the 10th day, the biodegradability rate of composite biofilms was accelerated (Fig.1.A). The time was dependent to the DBP incorporation percent to the gelatin film. The high rate of degradation was recorded in samples containing 4% of DBP. On the 20th day, the biodegradation was complete for all the films.



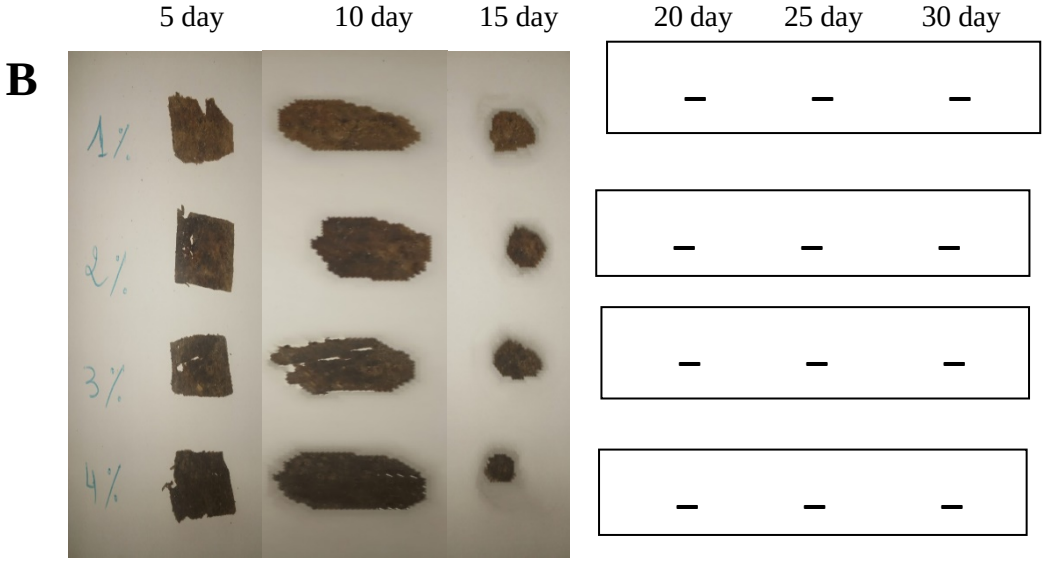


Figure 1. A Weight losses of the films.B Appearances of the gelatin films after the specified days of soil biodegradation.

3.6 Color

Color parameters of different films are presented in Table 3. Incorporation of date by-product from 1 to 4% showed decrease of L- and a-values. The active Gn-DBP 1% showed less lightness as compared to Gn-DBP 3%. The higher b-values of films after the addition of DBP indicated that the films were close to yellowish color. Changes of films color are most likely contributed to natural pigments of phenolic compounds present within the DBP extract.

Table 3. Color value for active gelatin-based DBP films. Where *L**, is a measure of lightness black- white, the coordinate *a** represents the relative amounts of red- green and the *b** coordinate expresses the relative amounts of yellow- blue.

	L*	a*	b*
Control	2.22± 4	2.47±3	19.92±4
Gn-DBP 1%	76.09±5	4.68±2	25.28±1
Gn-DBP 2%	71.13±3	3.5±1	24.99±1
Gn-DBP 3%	58.03±2	4.3±4	23.29±2
Gn-DBP 4%	75.27±2	5.32±	29.8±2

3.7 Antimicrobial activity of Active gelatin-based DBP films

Table 4 illustrates the percentage of bacterial inhibition of all samples after 24h. Films containing DBP at different percentages effectively inhibit the growth of microbial strains during the tested incubation time. As shown in Table 4, the antibacterial activity of the films increased with the increase of the content of DBP. In all samples, the film incorporated with 3% of DBP showed the highest bacterial inhibition of 70 % and 67.13% after 24h incubation in *S.aureus* and *E.coli* suspensions, respectively. This could be due to the presence of various phenolic compounds present in DBP extracts, which could combine with the bacterial cells wall and inactivate their function. In contrast, Control films did not show any antimicrobial activity against tested microorganisms.

Table 4. The antibacterial activity evaluation of different samples against *E. coli* and *S. aureus*.

	Bacterial inhibition (%)	
	E. coli	***S. aureus***
Control films	0	0
Gn-DBP 1%	20	30
Gn-DBP 2%	35	42
Gn-DBP 3%	50	68
Gn-DBP 4%	67.13	70

4. Discussion

The protein-based films become one of the key points in the research of food packaging materials. In general, the protein-based films have moderate mechanical properties and good oxygen barrier properties, but they are sensitive to water. Some physical or chemical post-treatment methods can be applied to make the properties of protein-based films improved for specific applications [10, 11]. Herein the DBP was used to elaborate active packaging formulations as substitutions for synthetic additives. When compared to other films, the Gn-DBP 1 percent film has the lowest percentage of Water Holding Capacity. The gelatin offered great protection against the evaporation of water on the surface of the food and the oxidation of other nutrients [12]. Furthermore, proteins can prevent moisture and taste loss, manage exchange, and transport active compounds due to their inherent qualities. In comparison to other films, the Gn-DBP 1 percent film had the lowest MC content. Similar results were observed in gelatin films with rosmarinic acid [13]. On the other hand, the solubility of edible film is a critical metric in food storage packaging [14]. The addition of a small amount of DBP reduced solubility, according to our findings. Hydrophilic and hydrophobic components influence solubility. The treatment with 1 and 2 Wt percent DBP is the most appropriate concentration for obtaining the characteristics of water solubility. As a result, this film proved to be the most appropriate for food packing. Our results are in agreement with previous studies who reported that gelatin film incorporated with natural extracts showed a significantly lower solubility [15-16]. Biodegradation, on the other hand, is described as the degradation of an organic material produced by biological activity (biotic degradation), which is mostly induced by bacteria via enzymatic action. Outdoor soil burial tests are used to assess substances for biodegradation speed under real-world situations. Many writers conducted outdoor soil burial experiments for varied lengths of time [17,18,19]. Previous reports showed that a film incorporated with natural extract similarly obtained degraded in approximately two weeks [20]. DBP's inclusion into gelatin films offered

possible antibacterial action, which inhibited bacterial growth in food. As a result, the addition of DBP to Gelatin film has improved the antibacterial properties of the film, which is critical for active food packaging.

5. Conclusion

The current study demonstrated the conversion of date by-products biomass into a primary antibacterial ingredient for active food packaging films. Incorporation of date by-product into gelatin film improved different properties such as Water Holding Capacity, Film Moisture and Film solubility. In contrast, the results revealed that addition of date by-product decreased the film transparency. The Active gelatin-based DBP films also showed a potent antimicrobial activity against food borne pathogenic bacteria such as *S. aureus* and *E.coli*. In conclusion, gelatin films enriched with date by-products are expected to be useful as a novel antibacterial and biodegradable food packaging material.

6. References

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