horticulturae

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Effects of Functional Edible Coatings and Storage on Bioactive Compounds, Antioxidant Properties and Sugars in Barhi Dates

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Abstract: Barhi dates at the Khalal stage were preserved using functional coatings developed using chitosan (CH) and olive-cake (OCE) and orange-peel (OPE) extracts in different ratios. The amounts of total flavonoids, total tannins, and total carotenoids, and antioxidant properties were evaluated. The coated and uncoated samples were also quantified for individual bioactive constituents including flavonoids and phenolic acids using liquid chromatography-mass spectrometry (LCMS). Significant ($p \leq 0.05$) improvements in the functional properties and phytochemical content were observed in coated fruits after the application of coatings (OCE+CH and OPE+CH) and during storage at 4 °C. The major phytochemicals detected were vanillic, syringic, ferulic, cinnamic, p-coumaric and protocatechuic acids, and quercetin-3-glucoside and rutin. The highest vanillic acid (536.78 mg/kg), syringic acid (157.39 mg/kg) and ferulic acid (96.42 mg/kg) were detected in 2% OPE+CH-coated dates. Application of functional CH coatings containing OCE and OPE was also found effective in preventing the conversion of sucrose to glucose and fructose through slowing down invertase activity. The application of OPE+CH and OCE+CH coatings can be potentially used to enhance the functional properties and slow down the ripening process in Barhi dates at an early stage (Khalal) of maturity.

Keywords: bioactive compounds; antioxidant properties; Barhi dates; Khalal stage; sugars; invertase; edible coating; olive cake; orange peel

Citation: Ghafoor, K.; Al-Juhaimi, F.Y.; Babiker, E.E.; Sarker, M.Z.I.; Ahmed, I.A.M. Effects of Functional Edible Coatings and Storage on Bioactive Compounds, Antioxidant Properties and Sugars in Barhi Dates. *Horticulturae* **2022**, *8*, 1185. https://doi.org/10.3390/horticulturae8121185

Academic Editors: Goran Fruk and Rajko Vidrih

Received: 23 November 2022
Accepted: 8 December 2022
Published: 12 December 2022

1. Introduction

The processing of agricultural produce results in a variety of by-products that may cause environmental and waste disposal issues. However, it has been reported that these by-products may serve as valuable sources of phytochemicals and natural antioxidants [1]. Synthetic antioxidants are often associated with certain undesirable effects on human health. Therefore, it is often considered that natural antioxidants can not only fulfill the desired objectives while added to foods as preservatives, colorants, and additives, but can also provide additional health benefits [2]. These phytochemicals also include phenolic and flavonoid compounds as these have been reported to carry antioxidant properties [3]. During olive-oil production, olive cake is produced as waste, which is considered a valuable source of natural antioxidants [4]. Fresh olive cake is reported to contain 4226.23 mg gallic acid equivalent/100 g dry mass (d.m.) of phenolic compounds, 931.15 µmol trolox equivalent/g d.m of oxygen radical absorbance capacity, and 47.45 µmol TE/g d.m. of free radical (DPPH) activity; these values are reported to be higher than some other food by-products [2]. Citrus fruits are increasingly produced around the globe and processed into various food products including juices, jams and others, which results in the production of around 44% peels among citrus by-products. These by-products may be disposed of or utilized as cattle feed and mixed with dried pulps [5,6]. The citrus peel is also considered a promising source of natural antioxidants, including phenolic acids, polymethoxylated

flavones, and glycosylated flavanones, which have been reported by researchers around the world [5–7].

Fruits and vegetables are cherished and consumed when fresh. Their consumption is associated with the provision of various essential and non-essential nutrients for different needs in the human body, such as for increasing immunity against different diseases [8]. The date (*Phoenix dactylifera* L.) fruit is one of the important fruits extensively cultivated in Middle Eastern and North African countries. It is considered a good source of energy and various nutrients [9]. Different varieties of date fruit are popular and among them Barhi is an important date variety. Barhi is particularly cherished and consumed when unripe. This unripe stage is short-lived and called Khalal [10]. The Barhi date at the Khalal stage ripens quickly due to the activity of different enzymes and the conversion of sucrose to simpler sugars. These changes result in the end of the Khalal stage and the onset of the Rutab stage [9]. Hence, there is a need to preserve these dates to extend and preserve the Khalal dates. Different techniques such as cryogenic freezing and the use of modified atmospheric packaging have been reported for preservation of dates at this stage [9,10]. Fresh fruits and vegetables can be preserved using various techniques that not only aim at minimizing post-harvest losses, but also at improving the quality of fresh produce. One such technique is the use of edible coatings, in which water-soluble hydrocolloids with gum-like properties are frequently used. Examples of such edible gums include chitosan and guar, xanthan, Arabic, gellan gums, etc. [11]. The edible coating acts as a barrier to transpiration and respiration processes by closing the stomata on the surface of fresh fruits and vegetables. The use of various types of edible gums, which sometimes can also be mixed with extracts from plant materials, has been reported [11]. Plant extracts can be rich sources of phytochemicals, which act as antimicrobial agents in addition to being natural antioxidants. Previous studies have used tulsi extract (0–5 mL/100 mL) along with Arabic gum and sodium caseinate for preserving guava [12], pomegranate-peel extract, locust bean, and chitosan for coating oranges [13], and ginseng extract with guar gum for coating sweet cherry [14]. Hence, the objective of carrying out this study was to use water extracts from olive cake and olive peel in the formulation of edible chitosan coatings with additional functional properties and enhanced bioactive compounds in Barhi dates stored under refrigeration. Different bioactive compounds (phenolic acids and flavonoids) were analyzed chromatographically in coated and uncoated Barhi fruits. Certain biological properties were evaluated, and the conversion of sugars (sucrose to fructose and glucose) and the role of invertase were studied in both coated and uncoated stored fruits.

2. Materials and Methods

2.1. Materials

Fresh Barhi dates were collected from the Qassim region of Saudi Arabia between August and October 2021. Dates were ensured to be at the Khalal stage of maturity after consultation with experts from King Saud University's College of Food and Agricultural Science in Riyadh, Saudi Arabia. The olive cake was prepared using fresh olives and subjected to a cold-pressing technique. The olive cake was dried (moisture 11.32 ± 1.43%) and ground to make a powder form. Orange peel was obtained from fresh oranges cultivated at a local farm in Riyadh, Saudi Arabia, followed by drying and grinding (moisture 7.67 ± 1.85%). Samples of olive-cake or orange-peel powders (50 g) were mixed with distilled water (200 mL) and heated at 70 °C for 30 min. After cooling, the extracts were filtered and the final volume of the extract was brought to 200 mL using distilled water [12,13].

2.2. Coating of Barhi Dates with Chitosan and Phytochemical-Rich Solutions

Chitosan (CH) powder was dissolved to prepare a stock solution (2% *w*/*v*) using 1% acetic acid and 1% glycerol as a plasticizer. The olive-cake extract (OCE) and olive-peel extract (OPE) as prepared in the previous section were then mixed with chitosan solution in 1 and 2.0% ratios followed by 5 min homogenization in a blender (Acapulco 30564,

Palson Co., Kunshan, China). Different solutions of coating materials were prepared and applied to fresh Barhi dates as reported previously [15]. Each batch of treated and untreated Barhi date was packaged in a polyethylene plastic container with 5–6 holes in the lid. The samples were stored at 4 °C for different periods (0, 7, 14, 21, and 28 days).

2.3. Total Flavonoids Determination

The total flavonoids were determined following a colorimetric method [16]. A 5 g coated or uncoated Barhi-fruit-flesh sample was extracted using 96% ethanol (120 mL) and distilled water (80 mL) at 70 °C for 3 h. After filtration, the extract samples were stored at 48 °C until used for the analysis of bioactive compounds and antioxidant properties. A sample of 1 mL of Barhi-fruit extract was mixed with 5% sodium nitrite (3 mL) and 10% aluminum chloride (3 mL) solutions followed by 5 min incubation at room temperature. Afterwards, 2 mL of 1M sodium hydroxide was mixed with the reaction mixture, and deionized water was used to bring the volume of this mixture to 10 mL. Finally, the spectrophotometric absorbance values were measured at 510 nm. A standard curve was prepared using varying concentrations of quercetin. The results for total flavonoids were described as mg of quercetin equivalent (QE)/100 g of date flesh.

2.4. Total Carotenoids Determination

The evaluation of total carotenoids in date extracts was based on a report by Ranjith et al. [17]. A sample of date extract (1 mL from the flavonoids method) was mixed with 500 µL of 5% NaCl and the mixture was centrifuged at 3000× *g* for 10 min. Afterward, the supernatant from the reaction mixture was diluted using n-hexane followed by absorbance measurement at 460 nm. Different solutions of varying concentrations of β-carotene (standard) were prepared. Afterwards, the same steps as for the date extract samples were performed to record the absorbance values to prepare a standard curve. The expression of the total carotenoids was as mg of β-carotene equivalent (BCE) per 100 g of date flesh.

2.5. Tannins Determination

The evaluation of date tannins was based on a modified vanillin–HCl in methanol method described by Alsawmahi et al. [9]. The Barhi fruit samples were dried and ground to a powder form. A 1 g sample of powdered date was extracted with 20 mL of 1% HCl in methanol. This extraction was carried out at 38 °C for 20 min. After extraction, the mixture was centrifuged at 2000× *g* for 4 min. A 1 mL sample of the supernatant was taken, and 5 mL of vanillin solution (0.5%) was mixed with it. The vanillin solution was prepared by mixing it with 12% HCl and methanol for 20 min at 38 °C. The blank used was 4% HCl in methanol and the spectrophotometric absorbance values were taken at 500 nm. The standard compounds used were (±)-catechin at different concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL). The quantification of tannins was carried out in terms of % catechin equivalents.

2.6. Ferric Reducing Antioxidant Power (FRAP)

Benzie and Szeto's [18] method was applied for the estimation of FRAP of extracts (flavonoids method) from Barhi-date flesh. A reagent consisting of 300 mM of acetate buffer at pH 3.6, 20 mM solution of ferric chloride hexahydrate, and 10 mM solution of TPTZ in 40 mM HCl was used for the FRAP assay. An aliquot of 3 mL of this reagent was mixed with fruit extract by maintaining the final concentration as 0.02–1 mg of extract in 1 mL of reagent. This mixture was then incubated at room temperature for 30 min followed by measurement of spectrophotometric absorbance at 593 nm. The standard curve was prepared using trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and the analytical results were given as milli mole trolox equivalent (TE)/100 g.

2.7. ABTS Cation Radical Scavenging Activity

This method involved the use of ABTS (2, 2- azinobis, 3- ethylbenzothiazoline, 6- sulfonic acid diammonium salt) to generate ABTS cations for measuring the anti-cation activity of date-flesh extracts. The method [19] involved the room-temperature reaction (10 h), in the dark, of 7 mM of ABTS with 2.45 mM of potassium persulfate. Ethanol was used for dilution of the reaction mixture before measurement of spectrophotometric absorbance (achieving a value of 0.700 ± 0.01) at 734 nm. Finally, extract samples were reacted with 3 mL of ABTS reagent while incubating at 23 °C for 6 min. Afterward, the measurement of absorbance value was conducted for the sample and standard (trolox) solution. The results for ABTS activity were presented as μmol trolox equivalent (TE)/100 g after comparison of sample results with those of the standard.

2.8. Flavonoids and Phenolic Acids Quantification

The extract from Barhi-date samples was prepared using the Soxhlet method and n-hexane as solvent. The dates were extracted for 10–12 h at 70 °C. The extract was cooled and then centrifuged (4000× *g*, 5 min, 5 °C). The supernatant was separated and the remaining residues were extracted again using the Soxhlet method. The separated supernatant parts were collected and combined followed by filtration using a 0.22 μm filter. The extracts were stored at −20 °C until analysis [20,21]. The phenolic compounds were quantified by using an HPLC system (Nexra X2; Shimadzu, Kyoto, Japan) equipped with a mass spectrophotometer (Sciex, Framingham, MA, USA). A C18 reverse phase column (XCB, Agilent Technologies, Santa Clara, CA, USA) was used for the separation of phenolic and flavonoid compounds. The mobile phases used in this study were A, 2% acetic acid in water and B, 100% acetonitrile. The injection volume was 1 μL and the column temperature was set as ambient. The elution profile was set according to the previously reported method [22]. The data obtained were processed using software (Analyst 1.6.3, Sciex, MA, USA).

2.9. Determination of Sucrose, Fructose and Glucose

The date sugars were evaluated in coated and uncoated Barhi-date fruits according to Lane–Eynon method [23]. In this method, a 5 g sample of ground Barhi-date sample was mixed with 100 mL of distilled water, followed by 10 min of boiling. The mixture was then treated with 2 mL of neutral lead followed by the dilution of sample to 200 mL. The mixture was then filtered. Afterward, 1 g of dry potassium oxalate was added to the reaction mixture for lead precipitation, which was then filtered out. The clarified mixture (25 mL) was again filtered using a 0.45-lm microfilter before analysis using an HPLC system (Shimadzu LC-10 AD, Shimadzu, Kyoto, Japan). The system consisted of a column (250 × 4.6-mm) in 5-lm Supelcosil LC-NH2 (Supelco, Bellefonte, PA, USA) column packing. A refractive index detector (RID-6A, Shimadzu Kyoto, Japan) was connected to this system and 20% water and 80% acetonitrile were used as the mobile phase. The flow rate of the mobile phase was set at 2.5 mL/min.

2.10. Determination of Invertase Activity

A previously reported method [24,25] was used to monitor invertase activity in coated and uncoated Barhi dates during storage at 4 °C. Date flesh (15 g) was cut into small pieces followed by 2 min blending with 100 mL of solution A, consisting of 4% NaCl solution and 1 g polyvinylpyrrolidone. The reaction mixture was then subjected to centrifugation at 20,000× *g* for 30 min at 4 °C. The supernatant was decanted and retained. The residue that remained after centrifugation was extracted again using solution A. The supernatants were then combined and 30 mL was dialyzed against several water changes at 2 °C. The process was carried out to ensure the absence of any detectable sugars. This dialysate was described as soluble invertase obtained from the supernatant extract. Insoluble invertase was obtained after washing the residue with water several times. The mixture for enzyme assay consisted of 1 mL of 0.5 M acetate buffer (pH 4.5), 1 mL of 1.5 M sucrose, 1 mL of enzyme extract and 2 mL of distilled water. The incubation of this mixture was carried out

at 30 °C for 20 min. A 1 mL sample from this mixture was taken after each 5 min during a 20 min incubation process. The 1 mL sample (every 5 min) was reacted with the reagent 3,5-dinitrosalicylic acid and the substrate. The measurement of the spectrophotometric absorbance was carried out at 550 nm. The standard used was a 0.01–0.1 µmol solution of glucose. The amount of reducing sugar released was calculated from the calibration graph. The activity of invertase was expressed in units (U). One unit was defined as the amount of enzyme needed to hydrolyze 0.5 µmol of sucrose per min under the above conditions.

2.11. Experimental Design and Statistical Analyses

The experiments were based on a random block design and a total of six treatments (control, CH, 1% OCE+CH, 2% OCE+CH, 1%OPE+CH, and 2%OPE+CH) were used in triplicate. The phytochemical contents, biological properties, sugar, and enzyme activity were all measured in triplicate during storage times of 0, 14, and 28 days. The statistical analyses included the application of analysis of variance (ANOVA) and Duncan's multiple range tests (DMRT) using SAS software (SAS Institute, Inc., Cary, NC, USA). The data from triplicate measurements of the listed attributes were presented as means and standard deviation (SD). The statistical significance was fixed at a probability (p) value of $\leq$0.05.

3. Results and Discussion

3.1. Bioactive Contents and Biological Properties of Barhi Dates

The total flavonoid (TFC), total tannin (TTC) and total carotenoid (TCC) content and biological properties as determined using ferric acid reducing power (FRAP) and ABTS cation radical scavenging assays are represented in Table 1. Significant ($p \leq 0.05$) differences were observed in the amount of total bioactive compounds (TFC, TTC and TCC) of coated and uncoated Barhi-date fruit. Hence, both treatment and storage time showed effects on the amount of such bioactive contents. Among the different treatments, TFC was found to be in the range of 96.38 mgQE/100 g in uncoated to 156.32 mgQE/100 g in Barhi dates coated with chitosan (CH) containing 2% olive-cake extract (OCE) before starting storage (0 days). There was a decreasing trend in all types of bioactive compounds during refrigeration storage of both coated and uncoated Barhi dates. However, the coated samples still had higher TFC even after 28 days of storage, where the highest (134.58 mgQE/100 g) TFC was observed in dates coated with CH containing 2% orange-peel extract (OPE). The higher TFC in coated Barhi dates at the start and end of storage can be attributed to the presence of these bioactive compounds in OPE and OCE. The protective effects of edible coatings on the loss of these bioactive compounds during storage of Barhi dates, could also be caused by another factor. Like TFC, the TTC was also significantly ($p \leq 0.05$) higher in coated dates than in uncoated ones. The TFC ranged from 1.89 mgCE/100 g in uncoated dates to 2.68 mgCE/100g in 2%OPE+CH-coated dates at the onset of the storage studies. The tannin content was still observed to be high (2.37 mgCE/100 g) in 2% OPE+CH-coated dates after 28 days of refrigerated storage. The TCC content was also significantly ($p \leq 0.05$) affected by both the coating treatment and the storage time.

The TCC content at 0 days ranged between 1.47 mgBCE/100 g in CH-coated and 2.98 mgBCE/100 g in 2% OPE+CH-coated dates. The use of OCE did not improve the TCC in Barhi dates. Significant ($p \leq 0.05$) reductions in TCC in stored samples were observed in uncoated dates; however, in the coated dates TCC was preserved during refrigeration storage until the 28th day. Table 1 also presents the data about biological properties (FRAP and ABTS) on the coated and uncoated dates, and it can be observed that these were consistent with the results of bioactive compounds. The highest FRAP value (5.24 mmolTE/100 g) was observed in 2% OPE+CH-coated dates at 0 days of storage, whereas the lowest one (2.18 mmolTE/100 g) was observed in the uncoated date sample after 28 days of storage. The highest ABTS scavenging activity (692.67 µmolTE/100 g) was observed in 2% OPE+CH-coated dates, whereas the lowest activity (395.24 mmolTE/100 g) was observed in the uncoated date sample after 28 days of storage. The total phenolic content (TPC) in the same samples was previously reported [15] to be in the range of

7.18 mgGAE/g in uncoated dates to 13.10 mgGAE/g in 2% OPE+CH in coated dates. The DPPH inhibition activities were also correlated with the TPC of the samples. In addition to the data on TPC and DPPH inhibition, various other quality attributes such as pH, acidity, moisture, water activity, soluble solids, color, moisture and microbial changes during storage of the same samples were also previously reported [15].

Table 1. Changes in total flavonoids, tannins and total carotenoids in fresh Barhi dates coated with chitosan (CH) and/or olive-cake (OCE) or orange-peel extracts (OPE) during cold storage (4 °C).

Treatment	Storage Period (Days)		
	0	14	28
	Total flavonoids (mg QE/100 g)		
Uncoated	96.38 ± 2.54 ^{ep}	86.72 ± 3.24 ^{fq}	80.36 ± 3.42 ^{fr}
CH	96.82 ± 1.86 ^{ep}	91.58 ± 4.25 ^{eq}	87.25 ± 4.18 ^{er}
1% OCE+CH	136.62 ± 4.31 ^{dp}	112.45 ± 5.15 ^{dq}	110.36 ± 3.69 ^{dq}
2% OCE+CH	156.32 ± 4.39 ^{ap}	138.45 ± 4.63 ^{aq}	118.67 ± 4.18 ^{cr}
1%OPE+CH	139.62 ± 6.24 ^{cp}	129.45 ± 3.74 ^{cq}	126.38 ± 5.18 ^{br}
2%OPE+CH	145.64 ± 5.17 ^{bp}	136.47 ± 3.98 ^{bq}	134.58 ± 5.47 ^{aq}
	Total tannins (mg CE/100 g)		
Uncoated	1.89 ± 0.54 ^{bp}	1.72 ± 0.39 ^{bp}	1.66 ± 0.32 ^{bp}
CH	1.91 ± 0.36 ^{bp}	1.85 ± 0.27 ^{bp}	1.72 ± 0.24 ^{bp}
1% OCE+CH	2.32 ± 0.42 ^{ap}	2.14 ± 0.46 ^{ap}	2.08 ± 0.52 ^{abp}
2% OCE+CH	2.63 ± 0.23 ^{ap}	2.35 ± 0.38 ^{ap}	1.97 ± 0.39 ^{abp}
1%OPE+CH	2.45 ± 0.75 ^{ap}	2.28 ± 0.43 ^{ap}	2.17 ± 0.61 ^{ap}
2%OPE+CH	2.68 ± 0.39 ^{ap}	2.49 ± 0.51 ^{ap}	2.37 ± 0.57 ^{ap}
	Total carotenoids (mg BCE/100 g)		
Uncoated	1.58 ± 0.23 ^{bp}	0.93 ± 0.11 ^{bq}	0.72 ± 0.17 ^{cr}
CH	1.47 ± 0.19 ^{bp}	1.24 ± 0.17 ^{bp}	1.05 ± 0.12 ^{bq}
1% OCE+CH	1.59 ± 0.16 ^{bp}	1.34 ± 0.21 ^{bp}	0.95 ± 0.21 ^{bcq}
2% OCE+CH	1.52 ± 0.27 ^{bp}	1.38 ± 0.16 ^{bp}	1.27 ± 0.22 ^{bp}
1%OPE+CH	2.36 ± 0.32 ^{ap}	2.11 ± 0.15 ^{bp}	1.87 ± 0.15 ^{bq}
2%OPE+CH	2.98 ± 0.33 ^{ap}	2.62 ± 0.32 ^{bp}	2.38 ± 0.26 ^{aq}
	FRAP (mmol TE/100 g)		
Uncoated	3.65 ± 0.72 ^{cp}	2.45 ± 0.42 ^{cq}	2.18 ± 0.14 ^{cq}
CH	3.78 ± 0.35 ^{cp}	3.52 ± 0.27 ^{bp}	3.05 ± 0.25 ^{bq}
1% OCE+CH	4.36 ± 1.24 ^{bp}	3.91 ± 0.19 ^{bp}	3.75 ± 0.21 ^{bp}
2% OCE+CH	4.89 ± 1.22 ^{bp}	4.57 ± 0.34 ^{ap}	4.09 ± 0.52 ^{aq}
1%OPE+CH	4.65 ± 0.72 ^{bp}	4.25 ± 0.46 ^{ap}	4.17 ± 0.51 ^{ap}
2%OPE+CH	5.24 ± 0.82 ^{ap}	4.73 ± 0.38 ^{ap}	4.28 ± 0.63 ^{ap}
	ABTS (µmol TE/100 g)		
Uncoated	569.38 ± 20.42 ^{ep}	487.25 ± 33.65 ^{eq}	395.24 ± 18.37 ^{fr}
CH	621.45 ± 31.25 ^{dp}	574.19 ± 27.62 ^{dq}	462.75 ± 17.29 ^{er}
1% OCE+CH	668.14 ± 24.36 ^{cp}	627.17 ± 35.62 ^{cq}	487.65 ± 24.32 ^{dr}
2% OCE+CH	669.42 ± 18.47 ^{cp}	631.52 ± 29.86 ^{bq}	514.23 ± 15.48 ^{cr}
1%OPE+CH	689.51 ± 33.69 ^{bp}	654.25 ± 35.12 ^{aq}	582.45 ± 24.36 ^{br}
2%OPE+CH	692.67 ± 41.28 ^{ap}	654.28 ± 25.45 ^{aq}	627.36 ± 35.67 ^{ar}

Values are means of triplicate samples (±SD). Means not sharing a common superscript(s) a, b, c, d, e or f in a column for each storage period or p, q, or r in a row for each treatment are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range test.

In the present study, significant improvements in TFC, TCC, TTC, FRAP and ABTS values can be attributed to the phytochemical-rich extracts from orange peel and olive cake. The total phenolic and total flavonoid content of water extracts from orange peel have been reported as 49.04 and 29.86 mg/g of peel, respectively [6]. The same water extracts were also reported to contain antioxidant enzymes, polyphenolic compounds and antioxidant properties. Moreover, the health effects of orange-peel extracts are related to their effects in reducing the formation of reactive oxygen species, formation of thiobarbituric acid reaction substances (TBARS), increment in mitochondria membrane potential and reduced

caspase-3 activation. The orange-peel water extract demonstrated a strong cytoprotective effect against oxidative stress, that can be attributed to the presence of the above reported phytochemicals in orange peel [6]. In another study [26], the total flavonoid and total phenolic content in orange peel were reported to be 40.25 mg/g and 66.36 mgGAE/g of peel, respectively. The TBARS assay also revealed that flavonoids were responsible for the inhibition of lipoprotein oxidation resulting from cupric (Cu^{2+}) or peroxynitrite ($ONOO^{-}$) radicals. The phenolic and flavonoid-rich extracts of orange peel have been reported to have 1.7 mg/mL of antiradical activity against DPPH radicals, 6.09 mmol/TEAC g ABTS scavenging ability and a 71.99 mg/GAE 100 g FRAP value [27]. In another study, orange peel was reported to contain 5.55, 5.73 and 9.98 mg/100 g of flavonoids, tannins and total phenolics, respectively [28]. Murador et al. [29] developed an ultrasonic-assisted ionic liquid method for the extraction of carotenoids from orange peel. A total carotenoid content of 32.08 µg/g was detected in the extract. It has been reported that olive fruit also contains a wide range of phenolic compounds including phenolic alcoholic, secoiridoid derivatives, phenolic acids and flavonoids. However, most of the bioactive compounds (98%) were wasted with the olive cake, whereas only 2% were present in olive oil [4]. Furthermore, the antioxidant activities of olive cake as determined using oxygen radical absorbance capacity or ORAC and DPPH free radical inhibition assays were 659.11–931.15 µmolTE/g d.m and 60–95%, respectively [2]. The total tannin content of olive cake has been reported to be in the range of 9.8 to 13.8 g/kg [30]. The total phenolic content and DPPH radical scavenging activity in uncoated Barhi dates were found to be 7.18 mg GAE/g and 43.78%, respectively. All coated samples (OPE and OCE) contained significantly ($p < 0.05$) higher amounts of phenolics and DPPH scavenging activity. The highest values of 13.10 mg GAE/g phenolics and 78.25% DPPH scavenging were detected in Barhi coated with 2% OPE and 2% OCE, respectively [15]. It is generally considered that refrigeration storage of fruits and vegetables affects their phenolic, phytochemical, and antioxidant content, and their activity. Based on this fact, a study [8] involving 19 fruits and vegetables stored at 4 °C reported a loss of phenolic compounds, such as ellagic, gallic, sinapic, vanillic, chlorogenic, and sinapic acids, with simultaneous decreases in TPC, TFC, TCC and total anthocyanins. These commodities also showed significant reductions in antioxidant activity as observed by DPPH, ABTS and FRAP assays during refrigeration storage. Five different date cultivars were studied for changes in phenolic, tannin and vitamin-C content. These amounts decreased during maturation and ripening processes. Furthermore, it was also observed that the antioxidants in these dates decreased with the progress of the ripening process and hence a direct correlation between bioactive compounds and their activity was reported in Saudi dates [31].

3.2. Chromatographic Evaluation of Bioactive Compounds in Barhi Dates

A liquid chromatography-mass spectrometry (LCMS) technique was used for quantification of various types of polyphenols in coated and uncoated date fruit. A total of 19 compounds were quantified in freshly treated and stored (14 days) date samples. the analytical results are presented in Table 2. The detected compounds included caffeic, syringic, vanillic, chlorogenic, cinnamic, p-coumaric, ferulic, protocatechuic, and gallic acids, and catechin, apigenin, kaempferol, luteolin, naringenin, epicatechin, procyanidin B2, quercetin-3-glucoside, quercetin, and rutin. The vanillic, syringic, ferulic, cinnamic, p-coumaric and protocatechuic acids and quercetin-3-glucoside and rutin were in particularly higher quantities. Quantities of certain compounds were significantly ($p \leq 0.05$) reduced after storage up to 14 days. In the case of certain other compounds, the differences due to storage were insignificant. Significant ($p \leq 0.05$) differences were observed in the amounts of all the phenolic compounds concerning coating treatments. Increasing the OPE and OCE concentration in coating material improved the amount of polyphenols in Barhi date fruit samples. The amounts of apigenin, catechin, chlogenic, gallic, luteolin, naringenin, procyanidin and quercetin were <1.0 mg/kg in uncoated, fresh Barhi dates. Significant improvements were observed when either OPE and OCE were added to the coating ma-

terial (chitosan solution). The storage period invariably affected their amounts during two weeks of storage. Cinnamic-acid levels were found to be 18.54 mg/kg in uncoated dates and decreased to 16.35 mg/kg after two weeks. However, the 2% OPE+CH-coated dates contained 20.63 mg/kg of cinnamic acid in freshly coated and 19.67 mg/kg in the same dates stored for 2 weeks, indicating that OPE might have contained this polyphenolic compound and also that the coating material might have prevented its loss during storage. The ferulic-acid content of uncoated dates was 78.46 mg/kg whereas, those coated with 2% OPE+CH showed a significantly ($p \leq 0.05$) high value (96.42 mg/kg) that remained higher (87.65 mg/kg) after 14 days of storage in comparison to the uncoated dates, either fresh or stored for 14 days. Vanillic acid was found to be the polyphenol with the highest quantity among all the tested samples. The uncoated fresh dates contained 432.54 mg/kg of it, which decreased to 382.61 mg/kg after 14 days. The vanillic acid content of 2% OPE+CH-coated dates was 536.78 mg/kg in freshly coated dates; however, it decreased to 507.19 mg/kg after 14 days of storage. The amount of certain polyphenols was also higher in OCE+CH-coated dates such as p-coumaric acid, which was 28.67 mg/kg in 2% OCE+CH-coated dates and significantly higher than other samples.

Table 2. Polyphenolic Compound (Phenolic Acids and Flavonoids) Amounts (mg/kg) in Barhi dates coated with chitosan (CH) and/or olive-cake extract (OCE) or orange-peel extract (OPE) during refrigerated storage.

Phytochemical	Storage (Days)	Treatment					
		Uncoated	CH	1% OCE + CH	2% OCE + CH	1% OPE + CH	2% OPE + CH
Apigenin	0	0.23 ± 0.0^{ar}	0.26 ± 0.01^{aq}	0.35 ± 0.02^{ap}	0.47 ± 0.06^{ap}	0.33 ± 0.04^{ap}	0.37 ± 0.07^{ap}
	14	0.18 ± 0.01^{br}	0.24 ± 0.01^{bq}	0.31 ± 0.05^{ap}	0.45 ± 0.08^{ap}	0.28 ± 0.06^{aq}	0.35 ± 0.09^{ap}
Caffeic acid	0	2.08 ± 0.59^{aq}	2.10 ± 0.36^{aq}	2.68 ± 0.43^{aq}	3.18 ± 1.05^{ap}	3.17 ± 0.62^{ap}	3.26 ± 1.13^{ap}
	14	1.23 ± 0.35^{bq}	1.69 ± 0.21^{aq}	2.14 ± 0.53^{ap}	2.75 ± 0.31^{ap}	2.85 ± 0.29^{ap}	2.71 ± 0.23^{ap}
Catechin	0	0.91 ± 0.15^{aq}	0.88 ± 0.13^{aq}	1.35 ± 0.41^{ap}	1.48 ± 0.23^{ap}	1.39 ± 0.17^{ap}	1.42 ± 0.23^{ap}
	14	0.82 ± 0.13^{aq}	0.83 ± 0.21^{aq}	1.24 ± 0.42^{ap}	1.37 ± 0.36^{ap}	1.08 ± 0.13^{aq}	1.13 ± 0.11^{apq}
Chlorogenic acid	0	0.38 ± 0.02^{aq}	0.39 ± 0.14^{apq}	0.45 ± 0.06^{ap}	0.46 ± 0.07^{ap}	0.42 ± 0.11^{ap}	0.44 ± 0.18^{ap}
	14	0.27 ± 0.05^{aq}	0.31 ± 0.03^{apq}	0.37 ± 0.08^{ap}	0.36 ± 0.10^{ap}	0.39 ± 0.09^{ap}	0.41 ± 0.15^{ap}
Cinnamic acid	0	18.54 ± 3.52^{aq}	18.62 ± 2.98^{aq}	18.62 ± 3.75^{aq}	19.34 ± 4.16^{aq}	19.06 ± 2.65^{apq}	20.63 ± 3.93^{ap}
	14	16.35 ± 3.43^{br}	17.62 ± 4.25^{bq}	17.36 ± 3.69^{bq}	18.16 ± 2.73^{bq}	18.13 ± 3.92^{bq}	19.67 ± 4.31^{bp}
p-Coumaric acid	0	21.43 ± 5.46^{bt}	24.36 ± 4.32^{as}	27.14 ± 2.98^{ar}	28.67 ± 4.19^{ap}	26.58 ± 4.37^{ar}	27.69 ± 3.92^{aq}
	14	22.01 ± 5.32^{aq}	21.87 ± 3.64^{bq}	22.48 ± 4.61^{bq}	25.12 ± 4.12^{bp}	24.38 ± 2.71^{bp}	25.19 ± 4.06^{bp}
Epicatechin	0	4.87 ± 1.31^{ap}	4.92 ± 1.58^{ap}	5.26 ± 2.32^{ap}	5.89 ± 1.67^{ap}	4.77 ± 2.07^{ap}	5.16 ± 1.87^{ap}
	14	4.39 ± 2.11^{ap}	4.56 ± 1.65^{ap}	4.75 ± 1.92^{ap}	4.87 ± 1.32^{bp}	4.52 ± 2.11^{ap}	4.67 ± 1.63^{ap}
Ferulic acid	0	78.46 ± 4.99^{au}	82.96 ± 7.24^{ar}	79.35 ± 6.33^{at}	80.35 ± 5.69^{as}	90.35 ± 5.36^{aq}	96.42 ± 10.09^{ap}
	14	73.87 ± 6.82^{bt}	76.38 ± 5.66^{bs}	78.62 ± 6.38^{ar}	79.36 ± 5.12^{ar}	84.32 ± 4.59^{bq}	87.65 ± 3.84^{bp}
Gallic acid	0	0.73 ± 0.15^{aq}	0.72 ± 0.31^{aq}	0.84 ± 0.26^{aq}	0.91 ± 0.33^{apq}	1.06 ± 0.29^{ap}	1.31 ± 0.35^{ap}
	14	0.72 ± 0.14^{aq}	0.71 ± 0.23^{aq}	0.75 ± 0.09^{aq}	0.85 ± 0.17^{apq}	1.05 ± 0.13^{ap}	1.07 ± 0.09^{ap}
Kaempferol	0	1.85 ± 0.32^{aq}	1.84 ± 0.33^{aq}	2.13 ± 0.56^{ap}	2.32 ± 0.49^{ap}	1.96 ± 0.63^{apq}	2.25 ± 0.68^{ap}
	14	1.79 ± 0.36^{ap}	1.81 ± 0.29^{ap}	2.07 ± 0.63^{ap}	2.06 ± 0.37^{ap}	1.80 ± 0.24^{ap}	1.99 ± 0.35^{ap}
Luteolin	0	0.87 ± 0.09^{ar}	0.91 ± 0.08^{aqr}	1.07 ± 0.19^{aqr}	1.63 ± 0.09^{apq}	2.42 ± 0.31^{ap}	2.53 ± 0.42^{ap}
	14	0.76 ± 0.16^{ar}	0.75 ± 0.22^{ar}	1.34 ± 0.35^{aq}	1.08 ± 0.18^{aqr}	2.36 ± 0.24^{ap}	2.18 ± 0.19^{ap}
Naringenin	0	0.78 ± 0.15^{ar}	0.83 ± 0.13^{ar}	1.13 ± 0.24^{aqr}	1.36 ± 0.36^{ap}	1.46 ± 0.33^{ap}	1.63 ± 0.27^{ap}
	14	0.63 ± 0.16^{aq}	0.72 ± 0.35^{aq}	0.83 ± 0.26^{apq}	1.08 ± 0.19^{ap}	1.12 ± 0.22^{ap}	1.36 ± 0.42^{ap}
Procyanidin B2	0	0.47 ± 0.06^{aq}	0.53 ± 0.06^{aq}	1.32 ± 0.16^{apq}	1.53 ± 0.23^{ap}	1.39 ± 0.17^{ap}	1.64 ± 0.36^{ap}
	14	0.52 ± 0.14^{aq}	0.39 ± 0.09^{aq}	0.86 ± 0.11^{apq}	1.04 ± 0.17^{ap}	1.17 ± 0.25^{ap}	1.29 ± 0.09^{ap}
Protocatechuic acid	0	54.79 ± 6.32^{ar}	55.62 ± 5.42^{ar}	57.36 ± 4.23^{aq}	60.38 ± 6.25^{ap}	59.69 ± 4.68^{ap}	60.92 ± 4.61^{ap}
	14	54.36 ± 5.78^{aq}	54.98 ± 3.62^{aq}	55.46 ± 4.21^{aq}	55.33 ± 3.98^{aq}	54.43 ± 4.76^{aq}	56.81 ± 5.72^{ap}
Quercetin-3-glucoside	0	42.16 ± 6.27^{aq}	43.65 ± 5.78^{apq}	44.36 ± 8.42^{apq}	45.19 ± 6.24^{apq}	46.92 ± 5.39^{ap}	50.74 ± 6.27^{ap}
	14	39.68 ± 2.57^{ap}	40.37 ± 3.95^{ap}	41.73 ± 4.27^{ap}	43.49 ± 4.18^{ap}	44.18 ± 3.95^{ap}	46.93 ± 5.74^{ap}
Quercetin	0	0.88 ± 0.21^{ar}	0.93 ± 0.17^{ar}	1.29 ± 0.17^{aq}	1.68 ± 0.33^{aq}	2.11 ± 0.39^{ap}	2.74 ± 0.33^{ap}
	14	0.71 ± 0.07^{ar}	0.79 ± 0.11^{ar}	1.24 ± 0.06^{aq}	1.13 ± 0.16^{aq}	1.76 ± 0.24^{aq}	2.57 ± 0.27^{ap}
Rutin	0	29.87 ± 3.98^{at}	29.71 ± 4.12^{at}	30.78 ± 2.91^{as}	33.63 ± 3.07^{ar}	35.64 ± 2.77^{aq}	37.61 ± 3.66^{ap}
	14	28.36 ± 5.21^{brs}	27.63 ± 4.36^{bs}	28.75 ± 6.74^{br}	31.72 ± 5.36^{bq}	32.47 ± 4.87^{bpq}	33.29 ± 4.08^{bp}
Syringic acid	0	128.74 ± 13.69^{as}	129.47 ± 7.25^{as}	127.69 ± 6.37^{at}	130.67 ± 4.96^{ar}	140.98 ± 7.32^{aq}	157.39 ± 4.87^{ap}
	14	127.86 ± 14.36^{br}	128.53 ± 13^{ar}	125.63 ± 6.27^{bs}	127.69 ± 10.69^{br}	137.29 ± 9.42^{bq}	138.47 ± 12.15^{bp}
Vanillic acid	0	432.54 ± 36.25^{as}	442.68 ± 34.63^{ar}	428.67 ± 23.56^{at}	419.37 ± 22.14^{au}	517.36 ± 15.92^{aq}	536.78 ± 16.25^{ap}
	14	382.61 ± 45.25^{bu}	399.45 ± 52.36^{bt}	412.87 ± 28.87^{bs}	408.24 ± 49.26^{br}	435.98 ± 50.45^{bq}	507.19 ± 38.23^{bp}

Data are means ± SD of three samples. Means not sharing a common superscript(s) a, or b in a column for each phenolic compound during storage or p, q, r, s, t or u in a row for each phenolic compound of treated samples are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range test.

According to a previous study [32], date fruit is a good source of polyphenols and four free polyphenols (namely, protocatechuic, vanillic, syringic, and ferulic acids) and nine other bound polyphenols (namely, gallic, protocatechuic, p-hydroxybenzoic, vanillic, caffeic, syringic, p-coumaric, ferulic, and o-coumaric acid). These polyphenols were quantified using a high-performance liquid chromatography method. In another study [33] various groups of phenolic compounds, such as benzoic acid and its derivatives, cinnamic acid and its derivatives, flavonoid glycosides and its esters, flavan-3-ols, proanthocyanidins and anthocyanins, have been reported in date fruit. Orange peel, which is an important

by-product of orange-fruit processing, is a good source of various polyphenols, which can be categorized as phenolic acids, stilbenes, and flavonoids. The predominant polyphenols were myricetin (2.10 mg/g DW), o-coumaric acid (1.13 mg/g DW), benzoic acid (0.81 mg/g DW), naringin (0.72 mg/g DW), benzoic acid (0.76 mg/g DW) and quercetin (0.36 mg/g DW) [34]. In addition, caffeic acid, gallic acid, p-coumaric acid, catechin, rutin, ferulic acid, p-coumaric acid, ellagic acid, vanillic acid and cholorogenic acid have also been reported in orange peel, making it a good source of these important antioxidants [34,35]. Different bioactive compounds in orange-peel extracts can play a significant role in the reduction of oxidative stress in living cells [6]. Similarly, the olive cake is also reported to be a good source of free polyphenols, such as protocatechuic acid, hydroxybenzoic acid, sinapic acid, p-coumaric acid, rutin and hesperidin, and certain bound polyphenols (syringic acid, sinapic acid, caffeic acid and protocatechuic acid) with appreciable antioxidant activity [36]. Hence, the presence of different polyphenols in the coated and uncoated Barhi dates can be attributed to either the bioactive compounds in dates or to those in orange-peel and olive-cake extracts. The coated fruit showed presence of higher phenolics due to their presence in the by-product extracts as well as the protective effect of coating material during storage. The edible coatings could represent a mono- and bilayer to protect various post-harvest losses in nutritional and quality attributes of fresh fruits and vegetables. The delay in various deleterious changes in fruits and vegetables can also be attributed to changes in the generation of reactive oxygen species (ROS), which improve non-enzymatic and enzymatic antioxidant systems. Different types of antioxidant enzymes and non-enzymatic antioxidants, such as anthocyanins, phenols, anthocyanins, and flavonoids, play a major role in this defensive mechanism in coated fruits and vegetables [37]. Bioactive compounds in fresh fruits are generally considered highly susceptible to biotic and abiotic stresses during post-harvest handling and storage. Hence, use of edible coatings has been applied to preserve the bioactive compounds and their antioxidant activity in various sensitive fruits including black berry and sweet cherry [38–40].

3.3. Content of Sucrose, Fructose and Glucose and Invertase Activity in Barhi Dates

The uncoated (control) and coated (using chitosan, OPE+chitosan and OCE+chitosan) using different coating solutions were evaluated bi-weekly for sugars (sucrose, fructose, and glucose) during storage at 4 °C for four weeks, and the changes in their concentrations (%) are presented in Table 3. There were non-significant differences in all sugar types among different samples on day 0 of the trial. However, there were significant ($p \leq 0.05$) reductions in sucrose and incremental reductions in fructose and glucose, as determined biweekly until 28 days of storage. The sucrose content of uncoated and CH-coated dates decreased rapidly in comparison to OPE- and OCE-coated dates. All types of OCE- and OPE-coated dates showed significantly ($p \leq 0.05$) higher sucrose content at 14 days and 28 days of storage. The dates coated using 1 and 2% OPE with CH demonstrated the highest sucrose content (>19%) after 28 days of storage while the uncoated dates showed only 12.47% of unconverted sucrose. There were simultaneous increases in both fructose and glucose content. The highest fructose content (39.45%) was observed in uncoated dates after 28 days of storage. The initial fructose content was 23.45% at the start of the storage study, demonstrating a 17% increase in fructose content. In the case of 1% OPE+CH-coated dates, only a 7% increase in fructose content (from 23.69 to 30.66%) was observed at 28 days of storage. In the case of glucose content (21.89% at 0 days, 32.58% at 14 days and 38.92% at 18 days), the increase was much quicker and larger in the case of uncoated dates. The coated samples showed glucose contents in the range of 29.67 to 33.68% after 28 days of refrigeration storage, demonstrating the significant effects ($p \leq 0.05$) of coating (particularly when OPE and OCE were added to the coating solution) on the prevention of sucrose conversion to both fructose and glucose.

Table 3. Changes in sucrose, fructose and glucose in fresh Barhi dates coated with chitosan (CH) and/or olive-cake (OCE) or orange-peel extracts (OPE) during cold storage at 4 °C for four weeks.

Treatment	Storage Period (Days)		
	0	14	28
	Sucrose (%)		
Uncoated	24.67 ± 1.26 ^{ap}	14.65 ± 0.59 ^{dq}	12.47 ± 1.19 ^{dr}
CH	24.71 ± 1.32 ^{ap}	18.66 ± 1.21 ^{cq}	12.79 ± 0.52 ^{dr}
1% OCE+CH	23.97 ± 0.69 ^{ap}	20.48 ± 0.62 ^{bq}	14.47 ± 0.63 ^{cr}
2% OCE+CH	23.74 ± 2.45 ^{ap}	21.26 ± 0.35 ^{aq}	18.52 ± 0.57 ^{br}
1%OPE+CH	24.53 ± 1.35 ^{ap}	22.17 ± 0.18 ^{aq}	19.45 ± 0.49 ^{ar}
2%OPE+CH	24.19 ± 1.39 ^{ap}	21.89 ± 0.31 ^{aq}	19.76 ± 0.28 ^{ar}
	Fructose (%)		
Uncoated	23.45 ± 1.55 ^{ar}	29.45 ± 0.79 ^{aq}	39.45 ± 3.47 ^{ap}
CH	23.78 ± 2.63 ^{ar}	27.47 ± 0.63 ^{bq}	35.68 ± 2.74 ^{bp}
1% OCE+CH	23.74 ± 1.42 ^{ar}	27.33 ± 0.72 ^{bq}	32.57 ± 2.45 ^{cp}
2% OCE+CH	24.05 ± 0.78 ^{ar}	27.18 ± 1.63 ^{bq}	31.45 ± 3.18 ^{dp}
1%OPE+CH	23.69 ± 0.69 ^{ar}	26.87 ± 1.75 ^{bq}	30.66 ± 2.83 ^{ep}
2%OPE+CH	23.53 ± 0.82 ^{ar}	25.87 ± 0.96 ^{cq}	30.78 ± 1.76 ^{ep}
	Glucose (%)		
Uncoated	21.89 ± 1.72 ^{ar}	32.58 ± 2.48 ^{aq}	38.92 ± 1.34 ^{aq}
CH	22.47 ± 2.54 ^{ar}	25.98 ± 1.65 ^{bq}	33.78 ± 2.53 ^{bq}
1% OCE+CH	21.63 ± 3.61 ^{ar}	25.27 ± 1.87 ^{bq}	29.67 ± 1.93 ^{eq}
2% OCE+CH	22.08 ± 1.12 ^{ar}	23.68 ± 2.67 ^{dq}	28.55 ± 2.72 ^{fq}
1%OPE+CH	22.31 ± 3.14 ^{ar}	24.37 ± 1.63 ^{cq}	31.62 ± 1.58 ^{cq}
2%OPE+CH	21.55 ± 0.73 ^{ar}	24.06 ± 0.69 ^{cq}	30.83 ± 2.07 ^{dq}

Values are means of triplicate samples (±SD). Means not sharing a common superscript(s) a, b, c, d or e in a column for each storage period or p, q, or r in a row for each treatment are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range test.

Date fruit is considered a high-sugar fruit. Its sugar or sweetness gradually increases as a result of ripening and enzyme activity. Once fully ripe, the date fruit may contain 60 to 70% weight of sugars, including simple sugars such as sucrose [41]. Barhi, which is a famous date variety and especially cherished in the Khalal (yellow) stage of maturity, may contain lower amounts of fructose and glucose at this stage [9]. Once fully ripe, the total sugars of Barhi may increase and become 11.17 g/100 g of fresh weight, of which the sucrose, glucose and fructose content can be 43.49, 33.96 and 22.54%, respectively [42]. One of the objectives of this study was to slow down the ripening process by reducing sucrose conversion. This could also preserve the characteristic astringent taste of Barhi dates at the Khalal stage. The other quality attributes, including sensory attributes, color and texture (also associated with ripening), were previously reported [15] and both the current and previous report [15] show consistencies.

The ripening process in Barhi dates and other fruits is closely associated with the activity of different enzymes such as polyphenoloxidase, methylesterase, polygalacturonase, invertase, etc., that affect, texture, color and composition of the fruit [9,43]. The invertase enzyme catalyzes the reaction involving the breakdown of sucrose to glucose and fructose [44,45]. This enzyme plays its role by cleaving the O-C (fructose) bond and alpha-1,2-glycosidic bond of sucrose [45]. Due to its different industrial applications, it may also be recovered from date fruit [46]. In the case of fruit, invertase plays a role in fruit maturation and ripening, and eventually degradation and spoilage [44]. The activity of invertase was evaluated for both coated and uncoated dates in the current study and results are presented in Figure 1. The enzyme activity was around 9.0 U/g at 0 days for both coated and uncoated dates; however, it increased significantly after 14 days and 28 days of storage. The lowest invertase activity (10.66 ± 3.09 U/g) after 14 days was observed in 2% OPE+CH-coated dates. After 28 days of storage the lowest activity (17.99 ± 2.42 U/g) was detected in 2% OCE+CH-coated dates. The uncoated dates demonstrated higher enzyme activities of 18.12 ± 3.67 and 26.32 ± 4.23 U/g after 14 and 28 days of storage, respectively.

The data showed that invertase activity was significantly reduced by the use of edible functional coatings during refrigeration storage of Barhi dates. The slowing down of invertase activity can reduce the ripening and subsequently spoilage of fruit. Enzyme activity generally increases after harvest and during storage of fruit, increasing the conversion of sucrose to glucose and fructose. Due to these reasons, Alhamdan et al. [10] observed that even when Barhi dates at the Khalal stage of maturity were stored at −20 °C, the glucose and fructose content demonstrated substantial increases during the first three months of storage and there was a simultaneous decrease in sucrose content (81% of the original value at the start of storage). These changes in sugar composition were attributed to increased enzyme activity during this period. The invertase-enzyme activity may be slower in fruit when stored at freezing temperatures in comparison to refrigeration or room temperature storage as the activity is dependent on temperature in addition to pH, moisture and other factors [47]. In the current study, the functional coatings provided a barrier to the transfer of moisture, reduced the microbial activity and controlled other environmental factors [15] that may have resulted in lower invertase activity and reduced conversion of sucrose to glucose and fructose.

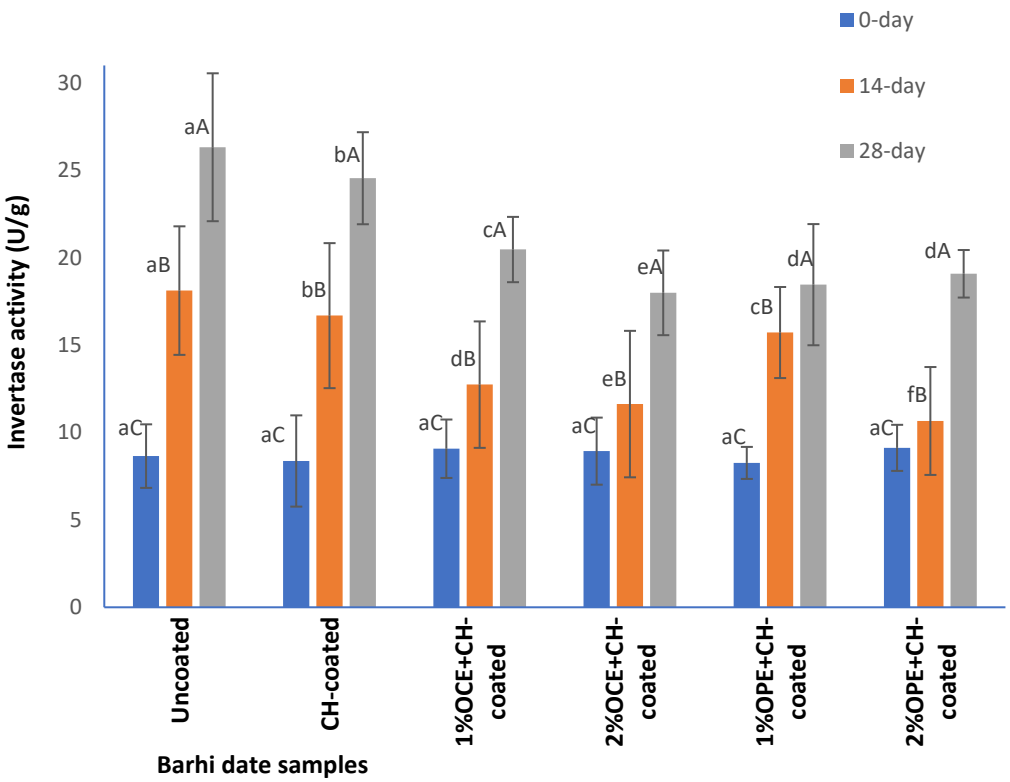

Figure 1. Invertase activity of Barhi dates coated with chitosan (CH) and/or olive-cake (OCE) or orange-peel extracts (OPE) during cold storage (4 °C) for 28 days. Values are means of triplicate samples (±SD) and represented by standard error bars. Same colored bars (same storage period) in different treatments not sharing a common lower-case superscript(s) a, b, c, d, e or f and different upper case letters A, B, or C within same treatment are significantly different at $p \leq 0.05$ as assessed by Duncan's multiple range test.

4. Conclusions

Barhi-date fruit is a good source of certain natural antioxidants such as flavonoids, phenolic, tannins and carotenoids with appreciable antioxidant properties. These functional properties of Barhi dates can be significantly improved by the use of edible coatings made using phytochemicals from agricultural by-products such as olive cake and orange peel. Furthermore, different phytochemicals were chromatographically quantified in Barhi dates, and vanillic acid, syringic acid, rutin, quercetin-3-glucoside, protocatechuic acid, ferulic

acid, p-coumaric acid and epicatechin were detected as major polyphenols. The use of edible coatings containing phytochemical-enriched extracts not only enhanced the amount of various bioactive compounds and their functional properties, but it also preserved them and their antioxidant potential during refrigerated storage for four weeks. The protective effects of coating can be attributed to the creation of a barrier between the fruit and the environment, thereby controlling moisture and pH, as well as the antioxidant potential of fruit against reactive oxygen species. Because of these factors, the use of functional coatings resulted in lower invertase activity in coated Barhi dates, resulting in less sugar conversion over four weeks. More research could be carried out to determine the effects of the edible functional coatings used here on the activity of other fruit-quality-deteriorating enzymes. Hence, the application of edible functional coatings can be effectively used to preserve Barhi dates and has potential for future application in the date industry.

Author Contributions: Conceptualization, methodology, resources, supervision, project administration, funding acquisition, writing—review and editing, K.G.; validation, F.Y.A.-J.; investigation, writing—original draft preparation, visualization, E.E.B.; software, formal analyses, data curation, M.Z.I.S.; data curation, I.A.M.A. All authors have read and agreed to the published version of the manuscript.

Funding: This project was funded by the National Plan for Science, Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, award number (2-17-04-001-0031).

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: The authors have no conflict of interest.

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