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Egg yolk protein as a novel wall material used together with gum Arabic to encapsulate polyphenols extracted from *Phoenix dactylifera L* pits

Chiraz Ben Sassi[1], Ismael Marcet[2], Manuel Rendueles[2*], Mario Díaz[2], Sami Fattouch[1]

[1] *Echochemistry Laboratory, Department of Biological and Chemical Engineering, National Institute of Applied Sciences and Technology (INSAT), University of Carthage;* [2] *Department of Chemical and Environmental Engineering, University of Oviedo, C/ Julián Clavería s/n, E-33071 Oviedo, Spain*

Corresponding author:

Manuel Rendueles, E-mail : , Postal address: University of Oviedo, C/ Julián Clavería s/n, E-33071 Oviedo, Spain.

Abstract

In this study, previously delipidated egg yolk protein (EYP) was tested as a novel wall material, together with gum Arabic (GA), for encapsulating *Phoenix dactylifera L* (date) pit polyphenols. For this purpose, polyphenols were encapsulated by freeze-drying using different ratios of GA and EYP. Moisture content, color, microstructure of the powders, thermo-gravimetric analyses (TGA), encapsulation efficiency, antioxidant activity and an *in vitro* gastrointestinal simulation were carried out. An increase in the proportion of EYP produced a slight increase in the moisture content of the microparticles and higher ΔE values when the polyphenols were incorporated, but their morphology remained similar independently of the concentration of EYP tested. Furthermore, the microparticles containing a higher amount of EYP showed better thermal stability, higher encapsulation efficiency and better antioxidant properties. Finally, the *in vitro* gastrointestinal simulation study showed that a higher concentration of EYP than GA resulted in an improvement in the resistance of the microparticles to gastric and intestinal fluids. Overall, the EYP showed certain promising properties when it was used to encapsulate polyphenols.

Keywords: antioxidant compounds; date palm pits; delipidated protein; microparticles; freeze-drying.

1. Introduction

Phenolic compounds have usually been extracted from natural sources, and date palm pit extract represents an interesting polyphenol source that has shown antioxidant, anti-inflammatory, anti-bacterial and anti-viral properties (Bagheri, Madadlou, Yarmand & Mousavi, 2013; Sadeghi, Madadlou, & Yarmand, 2014). Since date pit extract has strong antioxidant activity, it may be used in the development and preservation of new functional foods. Nevertheless, the incorporation of polyphenols into food has some limitations, since they can affect the organoleptic characteristics (Jöbstl, O'Connell, Fairclough & Williamson,

2004). In addition, the polyphenols' high vulnerability to gastrointestinal fluids and to some environmental factors such as oxygen, light, moisture and temperature has to be considered, so they usually need to be protected with the aim of preserving their bioactive properties (Ballesteros, Ramirez, Orrego, Teixeira, & Mussatto, 2017; Munin & Edwards-Lévy, 2011).

The capacity of encapsulation technology to overcome all these problems has been assessed many times (Munin & Edwards-Lévy, 2011), and its use implies that, instead of using free compounds, the polyphenols are entrapped within a carrier material, improving their bioavailability and maintaining their stability until delivery (Soykut et al., 2019; Fang & Bhandari, 2010). Moreover, encapsulation can mask the astringent taste of polyphenols allowing their use at higher concentrations for greater health benefits (Soykut et al., 2019; Ballesteros Ramirez, Orrego, Teixeira & Mussatto, 2017).

However, despite the several biological benefits of date pit extract, little work has been done to investigate the possibility of encapsulating its bioactive compounds for better use. Bagheri, Madadlou, Yarmand & Mousavi, (2013) have used the desolvation method to encapsulate the date pit extract in whey protein particles, while other authors have studied the microemulsification-cold gelation of whey proteins method for nanoencapsulating date pit extract (Sadeghi, Madadlou, & Yarmand, 2014). Another study has confirmed the feasibility of encapsulating bioactive compounds from date pits by using the microemulsification-particulation method (Jivan, Yarmand, & Madadlou 2014). To the best of the authors' knowledge, no further studies have investigated the encapsulation techniques on date pit phenolic compounds.

Regarding encapsulation techniques, freeze-drying can preserve almost all the functional properties of the bioactive compound thanks to the low temperature of the lyophilization (Soykut et al., 2019; Ceballos, Giraldo, & Orrego, 2012), but this technique has some

drawbacks, such as a high economic cost compared with other drying procedures and the lack of control of the size of the microparticles produced (Ozkan, Franco, De Marco, Xiao & Capanoglu, 2019).

In addition, the encapsulation efficiency also depends on the coating material. In fact, many studies have elucidated the possible interactions between polyphenols and food matrices. Therefore, besides the encapsulation technique, the selection of the coating material is also important to achieve the successful incorporation of the bioactive compounds into the biopolymers (Anbinder, Deladino, Navarro, Amalvy, & Martino, 2011; Gouin, 2004).

With this in mind, gum Arabic, an edible biopolymer mainly composed of carbohydrates, possesses interesting emulsifying and rheological properties, and has been widely used by many researchers to encapsulate bioactive compounds (Barak, Mudgil & Taneja, 2020). However, according to the literature, some studies have concluded that the use of carbohydrate-protein mixtures can lead to an improvement in the encapsulation results (Yadav, Bajaj, Surajit & Mann , 2019; Shao, Feng, Sun & Ritzoulis, 2019).

In this regard, eggs may offer a possible source of protein. Egg yolk can be easily separated into two fractions, the plasma fraction, with a high lipid content (78%) and with noticeable emulsifying and gelling properties (Kiosseoglou & Paraskevopoulou, 2005; Le Denmat, Anton, & Beaumal, 2000), and the granular fraction, which mostly contains proteins and has poorer functional properties than those of the plasma fraction. As the food industry is not making sufficient use of the granular fraction, and with the aim of expanding its range of applications, this fraction has been used previously to prepare edible films (Marcet, Álvarez, Paredes, Rendueles, & Díaz, 2018; Marcet, Sáez, Rendueles, & Díaz, 2017), but the use of these proteins to encapsulate bioactive compounds in the form of microparticles has not been assessed yet.

Therefore, and given that the encapsulation of date pit extracts is a topic that has barely been studied, the aim of the present work is to test the performance of date pit polyphenol microparticles prepared by freeze-drying using gum Arabic (GA) and egg yolk protein (EYP) as a novel wall material. The microstructure, thermal properties, encapsulation efficiency, and antioxidant properties of these microparticles were tested. Furthermore, the *in vitro* gastrointestinal simulation of the encapsulated polyphenols was also assessed.

2. Material and methods

2.1 Material

Date palm seeds of the Deglet Nour variety were purchased from Tunisian local farm VACPA. The pits were first soaked in water, washed to get rid of any adhering date flesh, then air-dried and roasted at 200 °C for twenty minutes before being ground with a heavy grinder in order to obtain a fine powder which passed through 0.5 mm screens. The powder was then kept at 4 °C for further analyses. Hen eggs were purchased in a local market. Folin Ciocalteu reagent (ref. F9252), sodium carbonate (ref. 1613757), gallic acid (ref. G7384) ABTS (ref. A1888), Trolox (ref. 238813), imidazole (ref. I5513), pepsin from porcine gastric mucosa (ref. P7000), gum Arabic (ref. G9752) and pancreatin from porcine pancreas (ref. P7545) were acquired from Sigma-Aldrich (USA).

2.2 Preparation of date palm pit extract and delipidated EYP

The extraction of polyphenols from date pits was performed using an ultrasound device Sonopuls HD 2070 system (Bandelin, Germany); for that purpose, 4g of date pit powder was dissolved in 40 ml of distilled water and the pH was adjusted to 10.0 (which favored the liberation of the highest amount of antioxidant phenolic compounds according to preliminary tests) with NaOH (1.0 M) and HCl (1.0 M). On the grounds of preliminary test results, the amplitude of sonication was fixed at 40% (100% amplitude equivalent to 212 μm), the

98 temperature at 40 °C and the processing time at 20 min. At the end of the extraction, the pH
99 of the solutions was adjusted to 7.0 and then they were filtered using a vacuum pump and
100 Whatman no 1 paper, the permeate being recovered. The delipidated EYP was prepared
101 according to Marcet, Sáez, Rendueles, & Díaz (2017). Briefly, egg yolk was manually
102 separated from the albumen, and the vitelline membrane was discarded using tweezers. The
103 egg yolk was diluted with water, centrifuged at 10000g, and the granular fraction was
104 recovered in the sediment. Granules were lyophilized and delipidated with ethanol.

105 **2.3 Microencapsulation by freeze drying**

106 Date pit extract was encapsulated using EYP and GA as wall materials. The extraction of
107 antioxidant phenolic compounds from date pits was performed as described above. Then, the
108 extract was freeze dried using a lyophilization system before being encapsulated within the
109 wall materials. 20% (w/w) of coating wall material was mixed with distilled water. The
110 amount of extract was fixed at 15% (w/w) of the wall material. The mixture was homogenized
111 using an Ultra-turrax homogenizer (SilentCrusher M, Heidolph, Germany) at 6000 rpm for 5
112 minutes until a homogeneous dispersion was obtained. Five matrices were evaluated with
113 different GA:EYP proportions: 1:0; 0:1; 1:1; 1:3; 3:1. Furthermore, a blank without date pit
114 extract was prepared for each matrix. For freeze-drying, the samples were frozen at -80 °C for
115 12 hours and then placed into a lyophilization system for 48 hours. After freeze-drying, the
116 samples were crushed using a mortar and pestle, then sieved using a 250 μm sieve.

2.4 Analysis of microcapsules

2.4.1 Moisture content

Samples were placed in an HR73 Halogen Moisture Analyzer (Mettler Toledo, Germany) at 105 °C until they reached a constant weight, and the moisture content was calculated in terms of the weight loss (AOAC, 2000).

2.4.2 Colorimetric analysis

The microparticle color properties were measured using the L*, a*, b* system with an UltraScan VIS spectrophotometer (HunterLab, USA). Microparticles were measured on the surface of the white standard plate, which has L*, a*, b* values of 97.12, -0.14 and 0.13 respectively. The colors of the microparticles with date pit extract were compared to the same microparticles without extract and the ΔE parameter was calculated from:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

2.4.3 Encapsulation Efficiency (EE)

The encapsulation efficiency (EE) is the percentage of polyphenols that were successfully entrapped in the core of the wall materials. The total phenolic content (TPC) of the microparticles is already known and corresponds to the total phenolic content of date pit extract used in the encapsulation, whereas the surface phenolic content (SPC) was determined according to Sáez-Orviz, Camilleri, Marcet, Rendueles & Díaz (2019) with some modifications. 5 mg of microparticles were dispersed with 1 ml of distilled water. The mixture was then shaken gently for 1 minute and centrifuged at 10000g (Centrifuge 5415D, Eppendorf, Germany) for 5 minutes. In order to quantify the SPC, 50 µL of each sample was mixed with 50 µl of distilled water and 400 µL of the Folin Ciocalteu reagent (10% v/v). The mixture was then incubated in the dark at room temperature for ten minutes before adding 500

140 µL of sodium carbonate (7.5 % w/v), after which the mixture was stored again in a dark
141 chamber at room temperature for 30 minutes. Absorbency was determined at 765 nm in a
142 spectrophotometer. Results of polyphenol concentration were expressed as mg of gallic acid
143 equivalents per g of dried sample (GAE/g).

144 The Encapsulation Efficiency (%EE) was then calculated according to the following equation.

145 $$\%EE = \frac{TPC - SPC}{TPC} * 100$$

146 **2.4.4 ABTS assay**

147 Free radical scavenging activity of samples was determined by ABTS radical cation
148 decolorization assay. 25 mg of encapsulated powder was dissolved in 5 mL of 0.05 mol/L
149 NaOH. Then, 50 µL of diluted samples was mixed with 950 µL of $ABTS^{*+}$. After 10 minutes
150 of incubation in the dark at room temperature, the absorbance was measured at 734 nm. The
151 results were reported as mg Trolox equivalents (TE) /g dried sample.

152 **2.4.5 Scanning electron microscopy (SEM)**

153 The morphology of the microparticles was analyzed using a scanning electron microscope
154 (SEM) (JSM-6610LV, JEOL, USA). A small amount of each powder was attached to a double-
155 sided adhesive tape fixed to stubs and coated with 3-5 mA palladium under vacuum in order to
156 be examined.

157 **2.4.6 Thermogravimetric analyses (TGA)**

158 Thermo-gravimetric analyses (TGA) were carried out using an SDTA851e TGA analyzer
159 (Mettler-Toledo, Switzerland) from 25 to 650 °C under a nitrogen atmosphere. The heating
160 rate was 10 °C/min. The first derivatives of the weight loss curve thermograms were
161 calculated (DTG curves).

2.4.7 *In vitro* gastric and intestinal release of the microencapsulate under simulated conditions

An *in vitro* gastrointestinal simulation was studied to determine the release of apparent phenolic content by the different encapsulated samples. The simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to the guidelines of the U.S. Pharmacopeia (2012). For the gastric digestion, 150 mg of sample was mixed in test tubes with 10 mL of SGF. Then 32 mg of pepsin (porcine stomach mucosa) was added to the mixture and a pH of 1.2 was maintained. After that, the test tubes were incubated in an orbital shaker at 37 °C for 2 h at 80 rpm before being centrifuged for 15 min at 10000g. Finally, the pH of supernatants was adjusted to 7.0 using 0.2 mol/L sodium hydroxide. The intestinal digestion was prepared in the same way. 150 mg of the samples was mixed with 10 mL of SIF. 100 mg of pancreatin was added to the mixture and a pH of 6.8 was maintained. Then, the test tubes were incubated at 36.6 °C for 2 h without shaking. The samples were centrifuged for 15 min at 10000g and the pH of the obtained supernatants was adjusted to 1.2 using 3 mol/L hydrochloric acid. After 15 min, the solution was neutralized (pH 7.0) using 0.2 M sodium hydroxide. Finally, the samples obtained with both SGF and SIF were evaluated for apparent phenolic content by the Folin–Ciocalteu method described above.

2.5 Statistical analysis

Analysis of variance (ANOVA) was applied. Least significant differences (LSD) were calculated by Fisher's test to determine significant differences between the tested samples. These analyses were performed using the SPSS software (Statistical Package for the Social Sciences) Version 18.0.

3. Results and Discussion

3.1 Moisture Content

Table 1 summarizes the moisture content of the freeze-dried samples. They ranged between 7.73 and 10.83%, the difference being significant ($p < 0.05$), and could be considered similar to values obtained by other authors. Jasen-Alves et al., (2019), using pea protein to encapsulate propolis extract, reported a maximum moisture content of 6.71% ± 0.80 for their microparticles. Da Silva et al., (2013) prepared microparticles by spray-drying using gum Arabic and OSA starch, and they found moisture values ranging from 4.9 to 12.6%.

In the case of these EYP-GA microparticles, when samples with the same microparticle wall composition were compared, a decrease in the moisture content was observed when the extract was incorporated. This could be because proteins and carbohydrates are interacting with water and polyphenols via hydrogen bonds, so the incorporation of polyphenols leaves fewer free groups available to bond water (Franks & Hatley, 1991; Meza, Verdini & Rubiolo, 2010). Furthermore, the moisture content rose significantly when EYP was incorporated into the samples in increasing proportions, which suggests that the protein possesses a higher capacity to bind water than that shown by the gum Arabic.

3.2 Colorimetric analysis

Table 1 shows the results for the color parameters. As is shown in this Table, the addition of the date pit extract significantly changed the color of the samples ($p < 0.05$), and the microparticles became darker, showing lower L* values, and more reddish, with higher a* values, than the control microparticles without phenolic compounds. Taking into consideration the appearance of the raw date pit extract, this change in the color of the microparticles was foreseeable (Figure 1). The addition of the phenolic compounds also

affected the b* parameter, but in this case, the changes in the degree of the yellowish/blueish character of the microparticles produced by the addition of the date pit extract were less noticeable. The differences between the microparticles with and without date pit extract can be evidenced by the parameter ΔE: the higher this parameter is, the higher the difference between samples is. In this case, the addition of date pit extract produced a major impact on the color of every sample tested, since the value for ΔE ranged between 30.8 and 40.0. Delving into these data, the EYP sample was affected the most by the addition of date pit extract, but it has to be considered that the degree of modification of the physicochemical properties of proteins, such as their UV-VIS spectrum, by the interactions between polyphenols and proteins depends on the unique structural complexity of each protein and the chemical properties of the polyphenols involved (Sęczyk, Świeca, Kapusta & Gawlik-Dziki, 2019).

3.3 Encapsulation Efficiency

According to Mahdavi, Jafari, Assadpoor & Dehnad (2016), a successful encapsulation method relies on achieving high retention of the core materials and minimum amounts of the core materials on the surface of powder particles. The results obtained (Table 2), indicated that the type of wall material had a significant effect ($p<0.05$) on the microencapsulation efficiency. The encapsulation efficiency ranged from 44.06 ± 4.28 % to 99.75 ± 0.01%. Clearly, the lowest encapsulation efficiency value was found when only GA was used as a coating material and, in fact, the encapsulation efficiency values decreased with the increase of GA concentration (Akdeniz, Sumnu & Sahin, 2017). Furthermore, the interactions between the wall material and the bioactive compound contributes to increasing the encapsulation efficiency (Jyothi et al., 2010). In this case, this enhancing effect of the EYP on the encapsulation efficiency may be produced due to the formation of stronger interactions between the proteins and the polyphenols than those produced between the GA and the

polyphenols. These interactions for both proteins and carbohydrates with the polyphenols are mainly hydrophobic and non-covalent hydrogen bonding, but their number and intensity is highly dependent on the protein and carbohydrate composition (Jakobek, 2015).

3.4 ABTS assay

This assay was performed to test if the enca.psulation process affects the antioxidant capacity of the bioactive compounds contained in the microparticles in any way. The 0.05 mol/L NaOH solution was selected, since in this solution the date pit extract, the gum Arabic and the egg yolk protein were dissolved completely, and therefore the microparticles are expected to fully release their antioxidant load.

The wall materials used in encapsulation in this study have an antioxidant activity themselves, as is shown in Figure 2, and this is in agreement with the literature (Montenegro, Boiero, Valle & Borsarelli, 2012; Sakanaka, Tachibana, Ishihara, & Juneja, 2004). Several mechanisms have been suggested for the GA antioxidant activity. In fact, GA has the capacity to scavenge free radicals, to quench reactive excited states, and to chelate metal ions (Montenegro, Boiero, Valle & Borsarelli, 2012). In the case of EYP, its antioxidant activity is mainly related to the strong metal chelating property of phosvitin, which represents 16% of the granular fraction of the EYP (Mecham & Olcott, 1949). Egg yolk phosvitin has a specific composition of amino acids that are highly phosphorylated by the addition of a covalently bound phosphate group, and therefore has great potential as an antioxidant agent. It is an iron-carrier in egg yolk and has the capacity to chelate various cations (Lu & Baker, 1986). Therefore, owing to the particular primary structure of egg yolk phosvitin, EYP has better antioxidant activity than GA. Thus, EYP can be used for functional food development, preventing the oxidation of lipids in food by chelating various metals (Marcet, Sáez, Rendueles & Díaz, 2017).

When the date pit extract was added, the antioxidant activity of the microparticles was significantly improved ($p < 0.05$). For instance, the antioxidant activity of free EYP-Extract was 101.35 ± 3.66 mg TE/g encapsulated powder, while the antioxidant activity of the encapsulated date pit powder using EYP alone as a coating material was significantly higher (123.30 ± 1.43 mg TE/g encapsulated powder). In addition, taking into consideration that the amount of date pit extract was fixed at 15% (w/w) of the wall material, then per gram of microparticles there is 150 mg of date pit powder, which showed an antioxidant activity of 42.86 ± 3.2 mg TE when this assay was performed without EYP or GA. Therefore, when the date pit extract is incorporated into the microparticles, an increase of 42.86 ± 3.2 mg TE is expected with respect to the antioxidant activity shown by the wall material alone. However, the antioxidant activity of the loaded microparticles shown in Figure 2 remained lower compared to the theoretical antioxidant activities expected, in particular when the wall material composition was high in EYP. It has been suggested that the use of proteins may increase the hardness of the lyophilized cake, it being therefore more difficult to crush it into microparticles, which could lead to higher degradation of some polyphenols because of the oxidation reactions produced during the grinding of the samples (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). The reason may also be associated with the possible formation of strong bounds between the EYP and the phenolic compounds, and although the microparticles were totally solubilized before this assay was performed, a fraction of the proteins could be forming complexes with a fraction of the phenol compounds, which could decrease the antioxidant activity. This kind of interaction could hinder, to some extent, the bioavailability of active compounds (Jakobek, 2015; Betz & Kulozik, 2011). Overall, in spite of this hindering effect of the EYP on the antioxidant properties of the date pit extract, and as can be seen in Figure 2, the higher the proportion of EYP was, the higher also was the

antioxidant activity of the microparticles. This is explained by the higher antioxidant activity of EYP compared with that of the GA, as mentioned above.

3.5 Microparticle morphology

Micrographs of the samples are shown in Fig 3. All freeze-dried encapsulated powders contained irregularly shaped particles and flaky structures. These are characteristic of microparticles produced by lyophilization (Ballesteros, Orrego, Teixeira & Mussatto, 2017). The images obtained agree with other researchers' work on the encapsulation of a variety of bioactive compounds (Laine, Kylli, Heinonen, & Jouppila, 2008; Gonçalves da Rosa et al., 2014). During the drying process, sublimation of ice occurred, leading to a porous structure which provides more rigidity. Thus, lyophilization is considered as the best way to encapsulate bioactive compounds (Aguilera & Stanley, 1999).

3.6 TGA Thermal Stability

The thermal stability of the samples was investigated using thermogravimetric analysis (TGA). According to Fig 5, samples showed similar behavior, with three different stages of weight loss during heating. The first stage, observed at temperatures up to 200 °C, is due to the loss of adsorbed and bound water present in the samples (Hijo et al., 2015). The second stage was from 200 to 400 °C with an average weight loss of 51.17%. This phase is associated with the decomposition of EYP and GA, which explains the important mass loss. The third stage was observed between 400 and 600°C and has an average recorded weight loss of 9.92%. Hence the overheating of samples has led to the decomposition of the wall materials. Based on the onset temperature, at which the weight loss started to occur, the microcapsules containing higher amount of EYP have better thermal stability. In fact, the onset temperature increased from 228 °C to 280.5 °C as the amount of EYP in the capsules increased, which can be attributed to the heat resistance of the granular EYP (Fuertes et al., 2017). The TGA

304 reveals, as well, that the encapsulated samples have better thermal stability compared to the
305 free date pit extract, which was to be expected. In fact, the degradation of the date pit extract
306 started at a lower temperature (140 °C) than that of any encapsulated sample. Therefore, EYP
307 and GA have successfully improved the thermal stability when they were used as coating
308 material for date pit extract.

309 **3.7 *In vitro* gastric and intestinal release of the microencapsulate under simulated**
310 **conditions**

311 Polyphenols have several health benefits that depend on their bioavailability. The release of
312 polyphenols from the food matrix is influenced by different factors, such as the polyphenol
313 structure, interaction of polyphenols with food components and gastrointestinal digestion and
314 intestinal absorption (Archivio, Filesi, Varì, Scazzocchio & Masella, 2010). Therefore,
315 bioaccessibility is an important element in determining the bioavailability of phenolic
316 compounds (Ozdal et al., 2016). Table 2 shows the results of the *in vitro* simulation of
317 gastrointestinal digestion of encapsulated powders. Clearly, the use of GA alone as a coating
318 material allowed an important release of polyphenols from encapsulates after gastrointestinal
319 digestion. After the gastric and the intestinal digestion, 62.64 % and 69.59 % of phenolic
320 content were released, respectively, despite the fact that carbohydrates are frequently
321 recommended for the role of carriers of polyphenols through the gastrointestinal tract
322 (Jakobek, 2015). This unexpected result is probably not related to enzyme action but to the
323 high solubility of GA in water compared to the other gums (Montenegro, Boiero, Valle &
324 Borsarelli, 2012; Işık et al., 2014). However, the release of polyphenols was significantly
325 lower in the other samples and decreases with the increase in the proportion of EYP in the
326 encapsulated powders, from 40.80 to 11.75% in the gastric digestion, and from 51.32 to 3.01
327 % in the intestinal digestion, as the amount of EYP was progressively increased in the
328 composition of the microparticles. These results are in agreement with other studies, which

found that polyphenols-protein interactions limit the oxidative damage undergone by active compounds during their transit through the gastrointestinal tract (Jakobek, 2015). In fact, only a small amount of polyphenols are absorbed in the small intestine (Faria, Fernandes, Norberto, Mateus & Calhau, 2014). When they are safely delivered to the colon, they will positively interact with the gut microbiota which enhance their bioavailability and provide more health benefits.

4. Conclusion

In the present study, for the first time, date pit polyphenols were encapsulated using different ratios of EYP and GA in order to improve their bioavailability and their bioaccessibility. With respect to the TGA results and in particular, the onset temperature, the thermal stability of the encapsulated samples was improved in comparison with the date pit extract without encapsulation. It was found that the addition of EYP raised the thermal stability of the microparticles more than did GA. Furthermore, encapsulation efficiency was better when EYP made up a higher proportion of the wall material. Additionally, the antioxidant activities of the microparticles increased when EYP was used. In fact, EYP has antioxidant properties which further enhanced the antioxidant activity of the microparticles. After in-vitro digestion, the release of phenolic content in the gastric digestion was seen to be higher than in the intestinal digestion, but the incorporation of EYP limited the loss of polyphenols in the gastrointestinal tract.

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Table

Table 1: Moisture Content and color parameters (L*, a*, b* and ΔE) of the date pits aqueous extract microencapsulated with gum Arabic (GA) and Egg Yolk Protein (EYP)

Samples	Moisture content (%, w/w)	L	A	b	ΔE
GA	9.16 ± 0.02^{e}	89.28 ± 0.06^{a}	0.44 ± 0.00^{f}	8.89 ± 0.00^{g}	0.0
GA + Extract	7.73 ± 0.03^{i}	60.16 ± 0.03^{e}	10.47 ± 0.00^{a}	13.89 ± 0.0^{a}	30.8
EYP	10.83 ± 0.07^{a}	86.69 ± 0.18^{c}	0.41 ± 0.04^{f}	12.68 ± 0.10^{b}	0.0
EYP + Extract	9.15 ± 0.02^{e}	47.55 ± 0.13^{a}	8.79 ± 0.05^{e}	6.10 ± 0.04^{i}	40.0
GA:EYP (1:1)	9.62 ± 0.01^{c}	88.15 ± 0.02^{b}	0.02 ± 0.01^{h}	9.62 ± 0.0^{f}	0.0
GA:EYP (1:1) + Extract	8.02 ± 0.06^{g}	54.44 ± 0.07^{c}	9.22 ± 0.03^{d}	9.81 ± 0.04^{e}	35.0
GA:EYP (1:3)	10.59 ± 0.01^{b}	86.45 ± 0.04^{d}	$-0,06 \pm 0,00^{i}$	$10,04 \pm 0,00^{d}$	0.0
GA:EYP (1:3) + Extract	9.09 ± 0.01^{f}	50.83 ± 0.10^{g}	9.67 ± 0.02^{b}	8.61 ± 0.04^{h}	37.0
GA:EYP (3:1)	9.21 ± 0.00^{d}	89.37 ± 0.06^{a}	0.17 ± 0.01^{g}	9.89 ± 0.00^{e}	0.0
GA:EYP (3:1) + Extract	7.96 ± 0.10^{h}	56.32 ± 0.09^{f}	9.49 ± 0.035^{c}	11.48 ± 0.06^{c}	34.3

Data were shown in mean ± standard deviation. Different superscript letters in the same column indicated significant differences ($p < 0.05$)

Table 2 : SPC, Encapsulation Efficiency, Solubility and in vitro gastrointestinal release of apparent phenolic content in simulated gastric fluid

Samples	Encapsulation Efficiency (%)	% of TPC released (SGF)	% of TPC released (SIF)
GA + Extract	44.06± 4.28^{d}	62.64 ± 4.02^{a}	69.59 ± 7.91^{a}
EYP + Extract	99.75 ± 0.01^{a}	11.76 ± 7.36^{d}	3.01 ± 3.63^{d}
GA:EYP (1:1) + Extract	69.93± 0.13^{c}	31.2 ±5.27^{bc}	13.56 ± 2.70^{c}
GA:EYP (1:3) + Extract	91.55 ± 2.93^{b}	20.58 ±1.47^{cd}	6.62 ± 0.59^{d}
GA:EYP (3:1) + Extract	43.04 ± 0.54^{d}	40.8 ± 3.10^{b}	51.32 ± 2.37^{b}

Data were shown in mean ± standard deviation. Different superscript letters in the same column indicated significant differences ($p < 0.05$)

Figures caption

Figure 1 : Photos of the microencapsulates prepared by freeze drying: a=EYP+Extract; a'=EYP Extract free; b=GA+Extract; b'=GA Extract free; c= GA:EYP (1:1) + Extract; c'= GA:EYP (1:1) Extract free; d=GA:EYP(3:1) + Extract; d'=GA:EYP (3:1); e= GA:EYP(1:3) + Extract; e'=GA:EYP (1:3)

Figure 2: Antioxidant activity of the freeze-dried samples and the date pits extract. Data were shown in mean ± standard deviation. Different superscript letters in the same column indicated significant differences ($p < 0.05$)

Figure 3: SEM images of the surface morphology of the microencapsulates by freeze drying. a=GA+Extract; a'=GA Extract free; b=EYP+Extract; b'=EYP Extract free; c= GA:EYP (1:1) + Extract; c'= GA:EYP (1:1) Extract free; d=GA:EYP(1:3) + Extract; d'=GA:EYP (1:3) Extract free; e=GA:EYP (3:1) + Extract; e'=GA:EYP (3:1) Extract free

Figure 4: TGA (solid lines) and DTG (dash lines) of the different freeze-dried powders and of the date pits extract

Figure

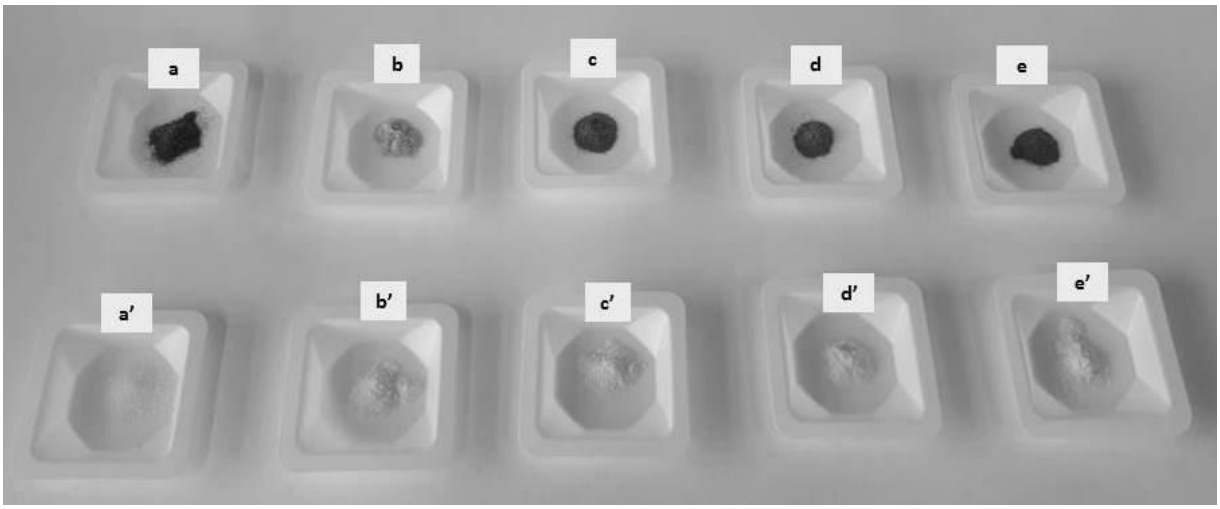


Fig.1

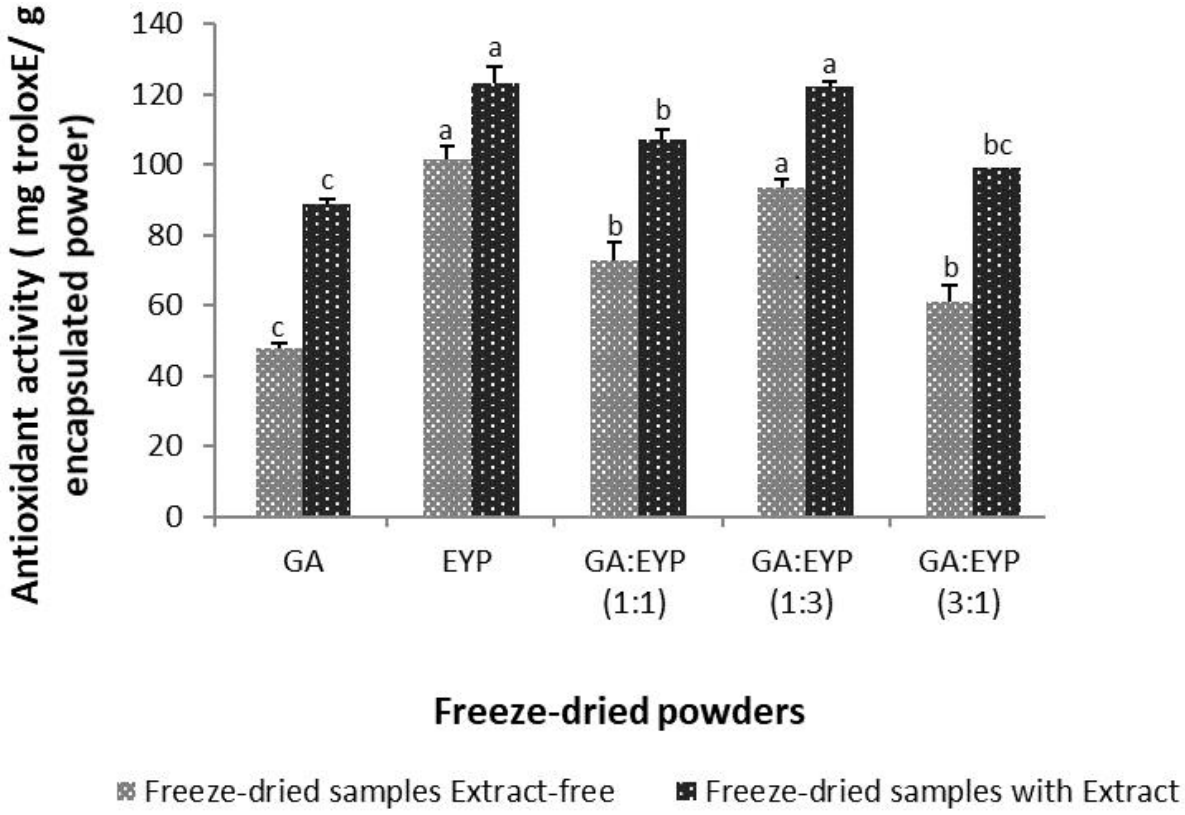

Antioxidant activity (mg troloxE/ g encapsulated powder)
140
120
100
80
60
40
20
0
c
c
a
a
b
b
a
a
b
bc
GA
EYP
GA:EYP (1:1)
GA:EYP (1:3)
GA:EYP (3:1)
Freeze-dried powders
Freeze-dried samples Extract-free
Freeze-dried samples with Extract

Fig.2

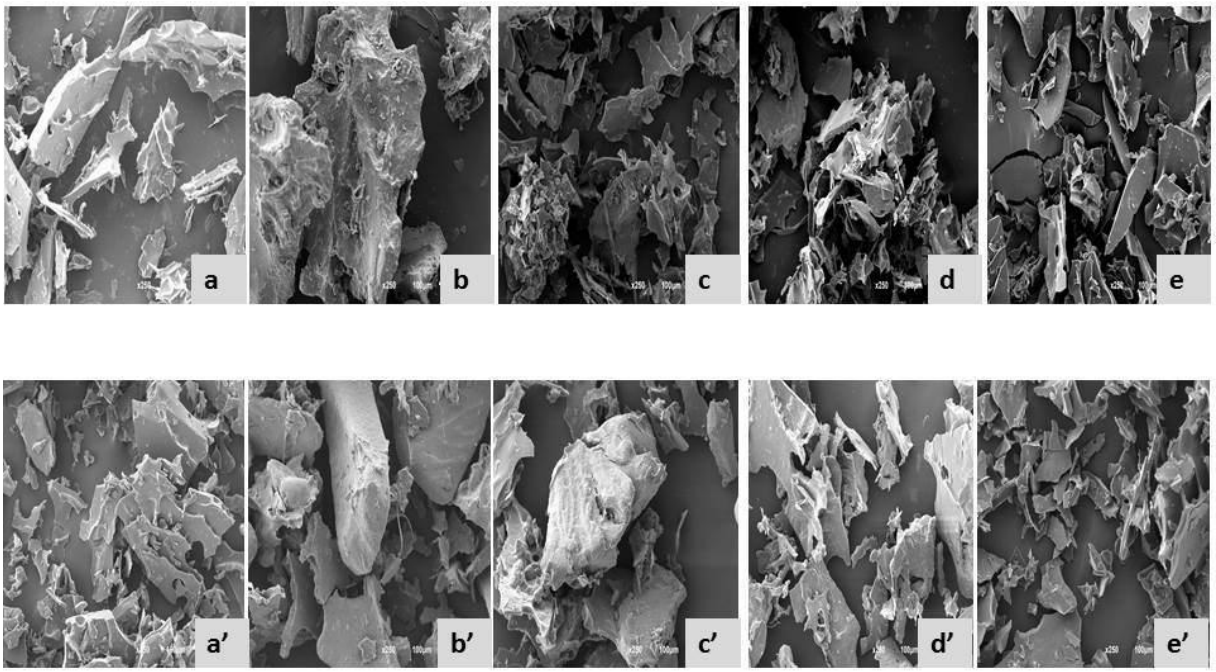
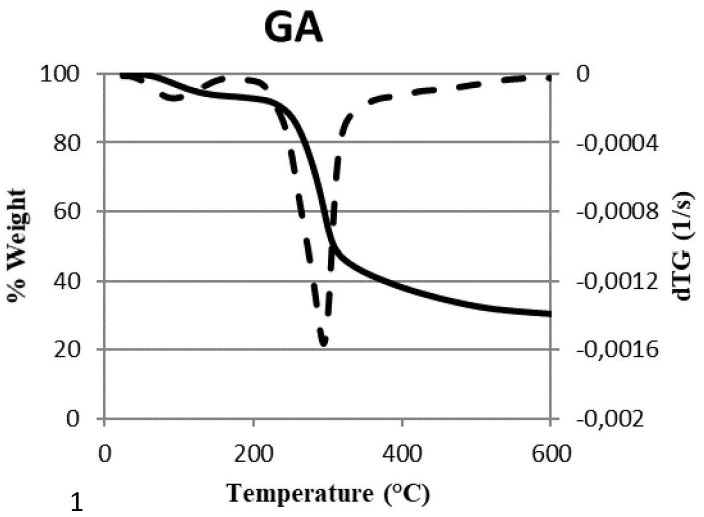
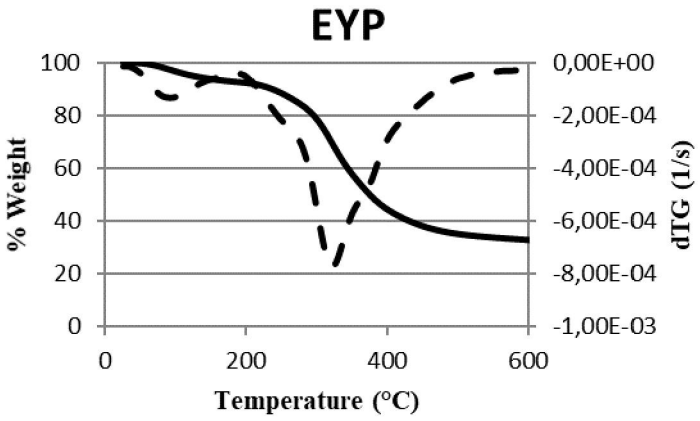
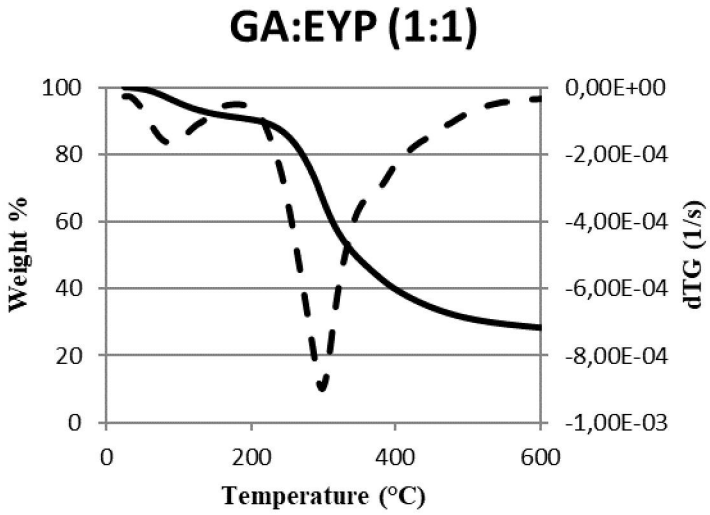


Fig.3

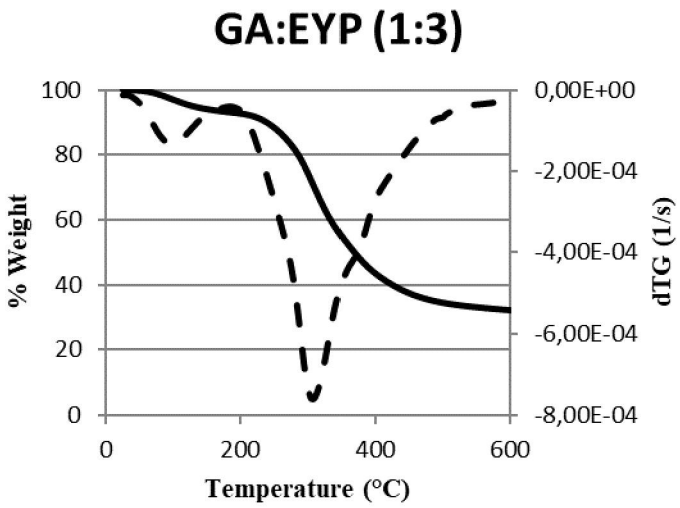

GA
100
80
60
40
20
0
% Weight
0
-0,0004
-0,0008
-0,0012
-0,0016
-0,002
dTG (1/s)
0
200
400
600
Temperature (°C)

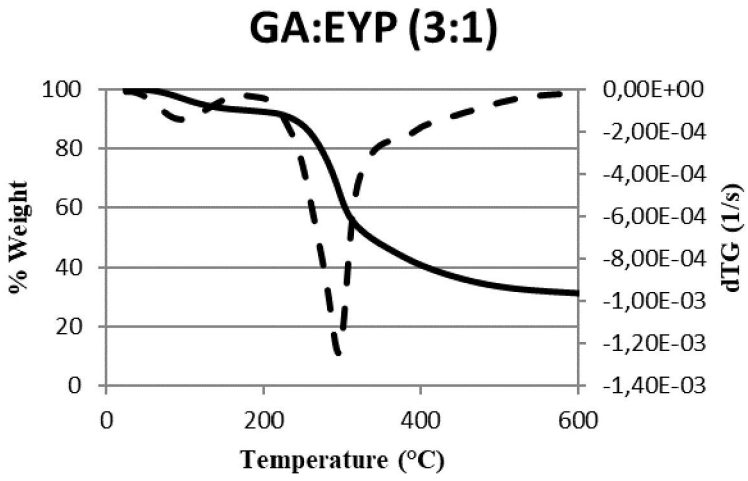
1


EYP
100
80
60
40
20
0
% Weight
0,00E+00
-2,00E-04
-4,00E-04
-6,00E-04
-8,00E-04
-1,00E-03
dTG (1/s)
0
200
400
600
Temperature (°C)


GA:EYP (1:1)
100
80
60
40
20
0
Weight %
0,00E+00
-2,00E-04
-4,00E-04
-6,00E-04
-8,00E-04
-1,00E-03
dTG (1/s)
0
200
400
600
Temperature (°C)

2


GA:EYP (1:3)
100
80
60
40
20
0
% Weight
0,00E+00
-2,00E-04
-4,00E-04
-6,00E-04
-8,00E-04
dTG (1/s)
0
200
400
600
Temperature (°C)


GA:EYP (3:1)
100
80
60
40
20
0
% Weight
0,00E+00
-2,00E-04
-4,00E-04
-6,00E-04
-8,00E-04
-1,00E-03
-1,20E-03
-1,40E-03
dTG (1/s)
0
200
400
600
Temperature (°C)

3

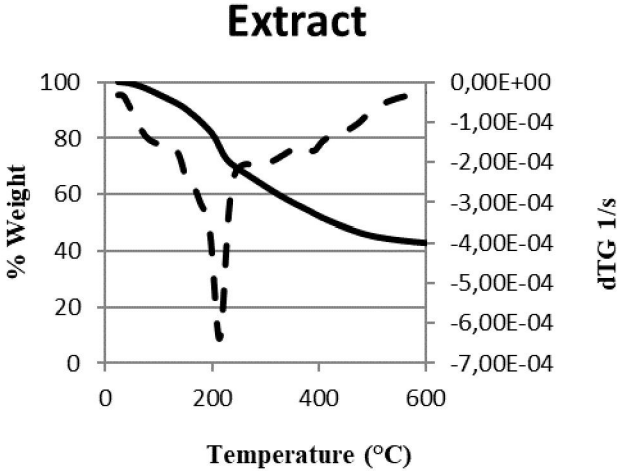

Extract
100
80
60
40
20
0
% Weight
0,00E+00
-1,00E-04
-2,00E-04
-3,00E-04
-4,00E-04
-5,00E-04
-6,00E-04
-7,00E-04
dTG 1/s
0
200
400
600
Temperature (°C)

Fig.4

***Credit Author Statement**

Credit author statement:

Chiraz Ben Sassi: Investigation and validation. **Ismael Marcet**: Methodology, writing. **Manuel Rendueles**: Conceptualization, writing – Review and editing. **Mario Díaz:** Resources, Supervision. **Sami Fattouch:** Resources, Supervision.

Conflict of Interest Statement

The manuscript entitled:

Egg Yolk Protein and Gum Arabic as encapsulation materials of polyphenols extracted from *Phoenix dactylifera L* pits for Food Applications

Chiraz Ben Sassi[1], Ismael Marcet[2], Manuel Rendueles[2*], Mario Díaz[2], Sami Fattouch[1]

[1] *Echochemistry Laboratory, Department of Biological and Chemical Engineering, National Institute of Applied Sciences and Technology (INSAT), University of Carthage;* [2] *Department of Chemical and Environmental Engineering, University of Oviedo, C/ Julián Clavería s/n, E-33071 Oviedo, Spain*

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with above work submitted to **LWT Food Science and Technology.**

Thank you and best regards.

Yours sincerely,

Manuel Rendueles, Professor

Department of Chemical and Environmental Engineering, University of Oviedo, C/ Julián Clavería s/n, E-33071 Oviedo, Spain

E-mail: