Microencapsulation of phenolic compounds from olive pomace using spray drying: a study of operative parameters

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Abbreviations

ARP: Antiradical Power, CAE: Caffeic Acid Equivalents, DM: Dry Matter, DP: Dried Powder, $DPPH^{\cdot}$: 2,2-diphenyl-1-picrylhydrazyl, DTA: Differential Thermal Analysis, DTG: Differential Thermogravimetric analysis, HPTE: High Pressure and Temperature Extraction, IT: Inlet Temperature, IT130: Inlet Temperature 130 °C, IT160: Inlet Temperature 160 °C, MD: Maltodextrins, MD10: Maltodextrins 100 g/L, MD50: Maltodextrins 500 g/L, ME: Microencapsulation Efficiency, OT: Outlet Temperature, OP: olive Pomace; OPE: Olive Pomace Extract, SC: Swelling Capacity, SEM: Scanning Electron Microscopy, sp: surface polyphenols, SP: Surface polyphenol Percentage, TG: Thermogravimetric analysis, TP: Total Polyphenols, WAI: Water Absorption Index, WSI: Water Solubility Index.

Abstract

In this work, polyphenols from olive pomace were extracted with a high pressure-high temperature agitated reactor and for the first time encapsulated by spray drying, using maltodextrin as coating agent at different concentrations. In addition, effects of inlet temperature (130 °C and 160 °C) and feed flow (5 mL/min and 10 mL/min) were studied. Physicochemical, antioxidant properties and stability of microparticles were evaluated. High inlet temperature implied lower moisture and bulk density without affecting antioxidant properties. Increasing maltodextrin concentration caused lower bulk density and higher microparticles sizes, while higher feed flow lead to increased moisture content. Spray drying at inlet temperature of 130 °C, maltodextrin concentration of 100 g/L and feed flow of 10 mL/min, resulted in high microencapsulation yield (94%) and encapsulation efficiency (76%) with high polyphenols content (39.5 mg_{CAE}/g_{DP}) and antiradical power (33.8 $mmol_{DPPH^{\cdot}}/L_{extract}$). HPLC and thermogravimetric analysis revealed the thermal protection effect of maltodextrin for phenolic compounds. Microcapsules were stable at 5 °C in dark condition for 70 days, and only 21% were degraded increasing storage temperature up to 25 °C. UV light exposure resulted in a 66% loss in polyphenols after 48 hours of exposure.

Keywords: polyphenols; microparticles stability; antiradical power; hydration properties; stability test.

1. Introduction

Olive pomace (OP), as the main by-product of the olive oil production, is currently treated as an industrial waste or used as combustible material, nonetheless this organic matrix can have potential applications in compost production, heavy-metal absorption or biofuels feedstock (Che, Sarantopoulos, Tsoutsos, & Gekas, 2012; Haddadin, Haddadin, Arabiyat, & Hattar, 2009; Mavros, Xekoukoulotakis, Mantzavinos, & Diamadopoulos, 2008; Michailides, Christou, Akratos, Tekerlekopoulou, & Vayenas, 2011; Miranda et al., 2012; Pagnanelli, Mainelli, Veglio, & Toro, 2003; Tekin & Dalgic, 2000). Moreover, OP is an interesting waste containing polyphenols (Aliakbarian, Casazza, & Perego, 2011; Aliakbarian, Palmieri, Casazza, Palombo, & Perego, 2012; Cioffi et al., 2010), which are bioactive molecules with promising properties (Casazza, Aliakbarian, De Faveri, Fiori, & Perego, 2012; Suarez, Romero, Ramo, Macia, & Motilva, 2009; Tuck & Hayball, 2002), protecting living systems from severe diseases such as cardiovascular dysfunctions (Aliakbarian et al., 2012; Palmieri et al., 2012). Owing to these properties, polyphenols have been considered a promising source of antioxidants, which can be used in pharmaceutical, cosmetic and food industries. Recovering these compounds from agri-food residues has been recognized as a big scientific effort in the past decade (An, Wilhelm, & Searcy, 2011; Champagne, 2007; Makris, Boskou, & Andrikopoulos, 2007). Despite having these benefits, polyphenols show low water solubility and low stability to environmental conditions (exposure to light, oxygen, temperature and enzymatic activities). For these reasons, microencapsulation systems were studied in order to preserve the biochemical functionalities of the components (de Vos, Faas, Spasojevic, & Sikkema, 2010; Fang & Bhandari, 2012).

Spray drying, a common industrial encapsulation technique, has been used in food industry for the preparation of food additives and flavors (Desai & Park, 2005), and consists in the atomization of a liquid product in a hot gas current, generating a powdered product (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Due to the decrease of the water activity, spray drying ensures microbiological stability, avoids degradation processes, reduces storage and transport costs and

enhances instantaneous solubility of the final product (Gharsallaoui et al., 2007). Several studies were performed using spray drying as microencapsulation technology for antioxidant extracts from different plant sources (Fang & Bhandari, 2012; Fazaeli, Emam-Djomeh, Ashtari, & Omid, 2012; Kha, Nguyen, & Roach, 2010; Robert et al., 2010). However, to the best of our knowledge, microencapsulation of polyphenols extracted from OP using spray drying has not been performed yet. The aim of this study was to assess the efficiency of spray drying to microencapsulate phenolic compounds from OP. The extract, characterized in our previous studies, (Aliakbarian et al., 2012; Palmieri et al., 2012), was obtained through high pressure-high temperature extraction (HPTE), which has been recognized as an efficient extraction technology to recover phenolic compounds from different matrices (Aliakbarian et al., 2011, Ben Hamissa et al., 2012; Casazza et al., 2010). Based on its low viscosity at high solid contents, good solubility and notable heat protection capacity (Gharsallaoui et al., 2007; Kha et al., 2010; Robert et al., 2010), maltodextrin has been selected as coating agent. Effects of inlet temperature, feed flow and maltodextrin concentration were investigated, and the physico-chemical properties and encapsulation efficiencies of the powders were evaluated.

2. Materials and methods

2.1 Chemicals

Methanol, acetic acid, ethanol, *n*-hexane, acetonitrile, maltodextrin, Folin–Ciocalteau and 2,2-diphenyl-1-picrylhydrazyl (DPPH˙) reagents and polyphenols standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Standard solutions were prepared with methanol and stored in dark bottles at -20 °C.

2.2 Polyphenols extraction

OP of Taggiasca cultivar was supplied by an Italian olive oil production plant (Raineri S.p.a., Chiusanico, Italy) and stored at -20 °C prior to use.

Before extraction, OP was pre-treated as described by Aliakbarian et al. (2011). The extraction was performed in a high pressure-high temperature agitated reactor (model 4560, PARR Instrument Company, Moline, USA) using ethanol/water 50:50 as solvent, with other parameters set on the best conditions studied by Aliakbarian et al. (2011). Extracts were centrifuged at 6000xg for 10 min (ALC PK131, Alberta, Canada), filtered (0.22 μm) and stored at 4 °C prior to experiments.

2.3 Encapsulation of phenolic extract

Spray drying of OP extract (OPE) was performed using maltodextrins (MD) with 16.5-19.5 dextrose equivalents as coating agent. Fifty milliliters of OPE was mixed with MD until complete homogenization, and the solutions were fed to a Lab Plant SD-04 spray dryer (Huddersfield, UK) with air flow of 30 m^3/h and atomization pressure set at the minimum of the instrument. Spray dryer was run with water for 10 min before and after each experiment. In this work two inlet temperatures (IT) (130 °C and 160 °C, named IT130 and IT160, respectively), two MD concentrations (100 g/L and 500 g/L and named MD10 and MD50, respectively) and two feed flows (5 mL/min and 10 mL/min) were studied. For each sample, the outlet temperature (OT) was registered.

Preliminary studies performed in our laboratory (data not shown) indicated that an IT lower than 130 °C causes the formation of a wet film on the walls of the chamber, while an IT higher than 160 °C leads to an excessive polyphenols degradation (da Costa, Barbosa, do Nascimento, & Macedo, 2002). MD concentrations and feed flow ranges were selected based on literature (Fang & Bhandari, 2012; Fazaeli et al., 2012; Kha et al., 2010; Robert et al., 2010). Powders were stored at 4 °C in closed dark vessels before analysis.

2.4 Powder characterization

2.4.1 Moisture content, bulk density and microstructure of particles

Microparticles were dried at 105 °C until a constant weight, and moisture content was calculated based on the loss in weight between before and after drying.

Bulk density (g/mL) was determined as described by Fazaeli et al. (2012) using 1 g of powders. The ratio of mass of microparticles and volume occupied determines the bulk density value.

Particles microstructure were evaluated with scanning electron microscopy (SEM 515, Philips, Netherlands): small amounts of powders were coated with a 30 nm-thick gold layer and observed in secondary electrons (5.0 kV) at 500x magnification. Diameters of particles were measured on SEM images using Sigma Scan Pro 5 software (Systat Software Inc., USA) and were divided in 11 different size ranges. For each range, the percentage of distribution was calculated considering the number of particles with diameter in that range compared to the total number of particles.

2.4.2 Water solubility index, water absorption index and swelling capacity

Water solubility index (WSI) and water absorption index (WAI) were determined according to Ahmed, Akter, Lee, and Eun (2010), by dissolving 1 g of product in 12 mL of water. WSI and WAI were calculated according to equations 1 and 2, respectively.

$$WSI = DW_{sup}/DW_{part} \times 100 \quad (1)$$

$$WAI = PW/DW_{part} \quad (2)$$

where DW_{sup} is the dry weight of the supernatant, DW_{part} is the initial weight of microparticles (dry basis) and PW is the weight of pellet after centrifugation.

The swelling capacity (SC) was calculated according to Lai and Cheng (2004) using equation 3:

$$SC = DW_{sup}/\left[DW_{part} \times (100 - WSI)\right] \quad (3)$$

2.4.3 Thermogravimetric analysis

Thermogravimetric (TG) analysis were carried-out using a TG-DSC Netzsch Gerätebau STA 409 (Germany), equipped with a Netztch410 furnace temperature controller. About 20 mg of microparticles were placed in an alumina crucible and introduced inside the furnace. The test consists

160 in two sections: a dynamic segment, from room temperature to 750 °C at a nominal rate of 10 °C/min
161 in a nitrogen flux of 40 mL/min, and in an isotherm section at 750 °C for 1 h, in an equivalent air
162 flow, in order to measure the weight loss due to the combustion of the sample and the amount of the
163 residual ashes.
164
165 *2.5 Analytical methods*
166 Polyphenols were extracted from microparticles according to Robert et al. (2010) and filtered (0.22
167 μm) prior to analysis.
168 Total polyphenols (TP) content was evaluated using the Folin–Ciocalteu assay, as described by
169 Aliakbarian et al. (2011), using a UV–Vis spectrophotometer (Perkin Elmer, Wellesley, USA) at a
170 wavelength of 725 nm. TP was expressed as milligrams of caffeic acid equivalents (CAE) per gram
171 of dried powder (DP) (mg_{CAE}/g_{DP}).
172 The antiradical power (ARP) of samples were evaluated with the radical scavenging $DPPH^{\cdot}$ method,
173 modified by Casazza et al. (2012). TP and ARP of OPE were measured using the same methods.
174 OPE and extracts from microparticles were also characterized using HPLC-DAD (Hewlett Packard,
175 1100 Series, Palo Alto, CA, USA), equipped with a C18 reverse-phase column (Vydac 201TP54,
176 Hesperia, USA) as described by Aliakbarian et al. (2011). The concentration of each phenolic
177 compound was calculated based on its standard solutions and the results were expressed as percentage
178 ratio between single polyphenol contents and the total amount of identified polyphenols.
179
180 *2.6 Microencapsulation yield and microencapsulation efficiency*
181 The microencapsulation yield was calculated as the percentage of TP in the dried powder compared
182 to the TP of OPE. This parameter is useful to evaluate the degradation of phenolic compounds during
183 spray drying.
184 Surface polyphenols percentages (SP) and microencapsulation efficiencies (ME) of microparticles
185 were calculated according to Robert et al. (2010), using equations 4 and 5, respectively:

$$SP = sp/TP \times 100 \quad (4)$$

$$ME = 100 - SP \quad (5)$$

2.7 Stability tests

Microparticles with highest microencapsulation yield and ME were stored at three different temperatures (5 °C, 25 °C and 45 °C) and at three different light conditions (dark, sunlight and artificial light, ensured by a 62 Watt lamp at 2.1 klux) at room temperature for 70 days. Light intensity was measured using a Lutron LX-107 light meter (Lutron LDV S.r.l, Milan, Italy). Samples were also exposed to UV radiation (280-315 nm), generated from a 20 W UV-B lamp (TL 12, Philips, Holland) at room temperature. During storage tests, microparticles were kept in sealed containers under dry conditions to avoid the effects of humidity on the stability of polyphenols. TP and ARP of samples were analyzed at different storage times.

2.8 Statistical analysis

All the experiments were carried out in triplicate (n =3), except the tests on the bulk density, that were repeated five times. ANOVA and Tukey's post hoc test ($p < 0.05$) were used ("Statistica 10.0", StatSoft, Tulsa, USA).

3. Results and discussion

3.1 OP extract

The OPE obtained by HPTE was characterized in terms of TP and ARP. The phenolic content of OPE was equal to 57.7±0.5 mg_{CAE}/g_{DM}, higher than the one obtained by Aliakbarian et al. (2011) using the same raw material and methanol as solvent (45.2 mg_{CAE}/g_{DM}).

ARP of the extract was equal to 26.9±2.5 $mmol_{DPPH^{\cdot}}/L_{extract}$, lower than the methanolic extract (40.1±1.5 $mmol_{DPPH^{\cdot}}/L_{extract}$) obtained by Aliakbarian et al. (2011). This can be explained considering that different solvents lead to different phenolic profiles: for example, HPTE with methanol shows

212 the presence of hydroxytyrosol and oleuropein, polyphenols with strong antioxidant properties, which
213 were not detected using ethanol/water 50:50 as solvent.
214
215 *3.2 Powder characterization*
216 *3.2.1 Moisture content, bulk density and microstructure of microparticles*
217 The effect of IT, MD concentration and feed flow rate on OT of the process, moisture content and
218 bulk density of microparticles are reported in **Table 1.**
219 **Table 1**
220 All the OTs were comprised between 75±1 °C and 102±1 °C. The highest OT was obtained for the
221 sample in which the driving forces of evaporation were greater (IT160 and lower feed flow), while
222 the lowest OT was obtained in the sample at IT130 and higher feed flow.
223 For all the samples, except for the ones processed at IT130 and MD50, the increase in feed flow
224 results in a significant decrease of OT ($p < 0.05$). This can be explained considering that a higher
225 amount of injected liquid implies a higher heat transfer from hot air to droplets, resulting in a lower
226 OT (Tan, Ibrahim, Kamil, & Taip, 2011).
227 The moisture content of the products decreased at IT160 ($p < 0.05$). This behavior is similar to that
228 found by Fazaeli et al. (2012) in the spray drying of black mulberry juice and by Kha et al. (2010) in
229 the spray drying of Gac fruit extract (with a decrease in the moisture content from 5.3% to 3.9% by
230 increasing IT from 120 to 200 °C), confirming that higher ITs enhance the heat transfer between air
231 and droplets promoting moisture evaporation.
232 At IT160, no significant differences ($p < 0.05$) were observed for samples obtained at different MD
233 concentrations and feed flows. At IT130 and MD10, the moisture content increased changing the feed
234 flow from 5 to 10 mL/min, confirming that increasing solution injection causes a less efficient
235 removal of the solvent, and consequently lower OTs and higher moisture contents.

As shown in **Table 1**, an increase in IT and MD concentration is associated to a decrease in the bulk density of the powders (e.g. for samples spray dried at MD50 and feed flow 5 mL/min, the bulk density passed from 0.13 g/mL to 0.04 g/mL increasing IT).

These parameters also influence the microstructure of the particles (**Fig. 1**). In particular, IT160 causes a higher degradation of the spherical structure of the particles (**Fig. 1a-c** and **Fig. 1b-d**), which leads to a higher porosity in the product, and consequently to a lower bulk density of the microparticles.

In all the samples, aggregation of the microparticles has been noted, similarly to Fazaeli et al. (2012) in the spray drying of black mulberry juice, in which this phenomenon is related to low glass transition temperatures of the coating agents, which can be easily overcame by OT.

Fig.1

Fig. 2 shows the percentage of particles with a diameter comprised in 11 different size ranges.

Fig.2

An increase in MD concentration leads to an increasing size. This is particularly evident for the samples spray dried at IT130 and feed flow at 10 mL/min: at MD10 (**Fig. 2a**), 99% of the microcapsules show a diameter in the range 0-20 μm, while at MD50 (**Fig. 2b**), 53% of the diameters are in the range 20-50 μm, 19% are in the range 50-100 μm, and only 29% are in the range 0-20 μm. This difference is evident also comparing SEM images of the two samples (**Fig. 1a-b**).

The operative conditions that generate a smaller microparticles size were MD10 and feed flow 10 mL/min, with 99% and 97% of the particles in the range 0-20 μm for IT130 and IT160, respectively. For all the samples, average diameters are smaller than 100 μm, with a high percentage under 50 μm. These results are similar to those obtained by Ersus and Yurdagel (2007) operating at IT between 160 and 200 °C, feed flow rate at 5 mL/min and MD concentration of 6% solid content, but higher than those obtained by Fazaeli et al. (2012), operating at IT between 110 and 150 °C, feed flow rate at 150 mL/h and carrier agent concentrations between 1.5-16% (w/w).

3.2.2 WSI, WAI and swelling capacity

Rehydration properties of microparticles were evaluated in terms of WSI, WAI and SC. As can be seen in **Table 2**, for all the samples WSI is comprised in the range 79-87%.

Table 2

These values are similar to those calculated by Abadio, Domingues, Borges, and Oliveira (2004) for the spray drying of pineapple juice with MD (82%), but higher than those obtained by Kha et al. (2010) (37-38%) and Ahmed et al. (2010) (42-57%) for the encapsulation of Gac fruit extract and purple sweet potato, respectively. These differences can be attributed mainly to a higher content of liposoluble substances in the raw material, which leads to high levels of insoluble components in the extract (Kha et al., 2010) and consequently to a lower WSI.

At IT130, WSI seems to be not affected by MD concentration and feed flow ($p < 0.05$), similarly to Kha et al. (2010), while Ahmed et al. (2010) stated that a lower WSI was related to a lower MD concentration. Passing from IT130 to IT160, for the samples at MD10 a significant decrease in WSI was noted (from 84±1.7% to 79±2.2% and from 85±2.2% to 80±1.6%), while for the samples at MD50 differences were not statistically significant ($p < 0.05$).

WAI were comprised between 0.28 and 0.58 g/g_{DP}, considerably lower than the results obtained by Ahmed et al. (2010), which reported WAI comprised in the range 0.86-1.20 g/g_{DP}. The variation in WAI can be related to different degrees of engagement of hydroxyl groups to form hydrogen and covalent bonds between starch chains (Ahmed et al., 2010). Moreover, an increase in WAI has always been associated with the loss of crystalline structure (Ahmed et al., 2010; Gunaratne & Hoover, 2002). Increasing IT from IT130 to IT160, WAI significantly increased, suggesting a formation of mainly amorphous MD structures during drying phase.

This behavior is also confirmed by the high SCs that characterize the powder products, considering that the presence of a large number of crystallites, which increase granular stability, can be related to low SC (Ahmed et al., 2010; Gunaratne & Hoover, 2002). For OPE microparticles, SC are comprised between 2.10±0.14 and 3.12±0.14 g/g_{DP}, similar to those obtained by Ahmed et al. (2010), comprised

between 1.92 g/g_{DP} and 2.56 g/g_{DP}, and none of the parameters tested seems to have a significant influence on SC. Only the sample spray dried at IT160, MD50 and feed flow 5 mL/min resulted statistically different from other microparticles.

3.2.3 Thermogravimetric analysis

All the samples shown a weight loss between room temperature and 150 °C, due to powder moisture (**Fig. 3a-h**). As reported in literature (Bilici, Doğan, Yıldırım, & Kaya, 2013; Dilek, Doğan, Bilici, & Kaya, 2011), a more evident change in the TG curve begins at 185-220 °C until 500-550 °C, leading to a global weight loss of 71-78%. Only samples spray dried at MD10 and feed flow of 5 mL/min, for both IT tested, show a minor value, equal to 62 and 67% respectively. Residual ashes after the combustion at 750 °C are included between 3 and 11%.

Fig.3

More interesting results were obtained by the analysis of differential thermogravimetric (DTG) curves. In the temperature range of 200-300 °C, all the microparticles show a double peak. A first endothermic reaction, shown in the differential thermal analysis curves (DTA), starts at 195 °C for MD10 samples (**Fig. 3a-b**) and at 215 °C for MD50 samples (**Fig. 3c-d**). This difference is reasonable assignable at the presence of MD, and it proves their thermal protection on the polyphenols. This assumption is confirmed by the thermal analysis of polyphenol without MD, where already at IT160 is evident a weight loss due to decomposition (**Fig. 3j)**. At a temperature of about 250 °C, a second reaction starts, with a maximum rate at 280-290 °C, depending on the MD amount. This second reaction is predominant when higher is the MD concentration as well as when higher is the feed flow, and it can be ascribed at the MD decomposition (**Fig. 3i**).

All the DTA curves show only an endothermic broad peak connected with the melting point of the microparticles (around 230 °C), while to the weight loss do not correspond any significant change, probably due to the presence of exothermic phenomena that offset the endothermic pyrolysis reaction.

No significant differences in the thermal behavior between the samples dried at IT130 and IT160 were evident.

3.3 TP, ARP, microencapsulation yield and efficiency

Table 3 shows TP content, ARP, microencapsulation yield and efficiency of the microparticles.

Table 3

TP content is maximum (39.5±4.9 mg_{CAE}/g_{DP}) at IT130, MD10 and feed flow of 10 mL/min. Except for this case, TP in the products is not influenced by IT, emphasizing the thermo-protective effect of MD (Munin & Edwards-Lévy, 2011). This effect is also evident considering ARP values of microparticles: passing from IT130 to IT160, for the samples spray dried with MD10 a significant decrease ($p < 0.05$) of ARP was noted, while in samples at MD50 this phenomenon is not present. The highest ARPs were found in microparticles with MD10 and feed flow equal to 10 mL/min, for both the IT tested (33.8±4.3 and 19.1±2.1 $mmol_{DPPH\cdot}/L_{extract}$ for IT130 and IT160, respectively). Microencapsulation yields are higher than 50% for all samples, and the maximum yield was obtained at IT130, MD10 and feed flow 10 mL/min. At these conditions, 94±0.4% of the polyphenols of OPE is still present in powders, suggesting that polyphenols were not affected by high inlet temperatures used in the drying process.

This was confirmed by HPLC-DAD, in which only small differences could be noted in the phenolic profiles of microparticles (**Table 4)**. Indeed, an increase in IT leads to a significant difference in the phenolic profiles only between powders obtained at MD10 and feed flow 5 mL/min (from 27.9±1.1 to 21.1±1.2% and from 12.1±1.0 to 18.5±1.8% for tyrosol and apigenin 7-glucoside, respectively) and between samples obtained at MD10 and feed flow of 10 mL/min for caffeic acid (from 9.6±1.0 to 4.4±0.8%).

Table 4

Compared to OPE, before encapsulation microparticles show a different profile of polyphenols. This is evident for tyrosol, luteolin 7-glucoside and luteolin, in which their percentages, compared to the

339 total identified polyphenols, are higher in OPE (equal to 35±1.2, 27±0.5 and 8±0.4%, respectively)
340 than in microparticles, indicating that these compounds are more sensitive to spray drying than other
341 polyphenols.
342 ME resulted higher than 65% for all samples, and at IT160 MEs were similar or lower to the ones
343 obtained at IT130: this can be explained considering that the evaporation rate is higher when IT
344 increases, generating a lower bulk density, as shown in **Table 1**, and consequently a higher
345 microparticles surface exposed to the environment. ME seems to be not influenced by feed flow. The
346 higher ME was obtained at IT130, MD10 and feed flow at 5 mL/min, equal to 77±3.5%.
347
348 *3.4 Stability tests*
349 The best sample in terms of microencapsulation yield and ME was selected for stability tests at three
350 different temperatures and three different light conditions for 70 days (**Fig. 4a-b**). Storage under UV
351 light conditions was also studied for a period of 48 h (**Fig. 4c**). TP and ARP were measured.
352 **Fig.4**
353 The role of storage temperature in the degradation of encapsulated polyphenols at 5, 25 and 45 °C is
354 shown in **Fig. 4a**. At 25 °C, microparticles were stable until 28 days of storage ($p < 0.05$), while at
355 45 °C a statistically significant decrease was found already after 14 days of storage. During the
356 storage, microparticles stored at 5 °C had shown no significant differences compared to the initial
357 sample, suggesting that this condition can be ideal for a long storage period of the powders. After 70
358 days, a reduction of 21% and 34% of the initial TP was noted for the samples stored at 25 °C and 45
359 °C, respectively.
360 **Fig. 4b** shows the effect of different light conditions on TP of the microparticles, at room temperature.
361 Powders stored in dark conditions show a limited degradation during storage, probably due to the
362 temperature, as described above. Samples exposed to sunlight had shown a statistically significant
363 difference after 14 days, decreasing from 40.0±4.0 mg_{CAE}/g_{DP} to 33.1±0.8 mg_{CAE}/g_{DP}. After 70 days,
364 TP of the microparticles was decreased up to 27.7±0.7 mg_{CAE}/g_{DP}, indicated that 31% of the initial

polyphenols have been oxidized. At every time considered, TP contents were similar to those obtained in dark conditions ($p < 0.05$), suggesting that the effect of temperature on the stability of the product is more evident than that of sunlight. Under strong light illuminance (2.1 klux), the degradation of polyphenols is accelerated: after 7 days of storage a reduction in TP content was detected ($p < 0.05$), and after 70 days 44% of the initial polyphenols were degraded. Considering these results, a temperature equal to 5 °C in dark conditions seem to be the best choice for a long-period storage of the spray dried OP polyphenols.

The effect of UV light on microparticles was evaluated during 48 h storage under UV lamp (**Fig. 4c**). After 1 h, 33% of the initial polyphenols were degraded, probably due to oxidation of surface polyphenols, which for that sample are equal to 9.4±0.4 mg_{CAE}/g_{DP}. In the storage period from 1 h to 8 h, microparticles had not shown TP differences ($p < 0.05$), while after 8 h TP decreased up to 13.7±0.8 mg_{CAE}/g_{DP} after 48 h of exposure, with the loss of 66% of initial TP content.

For all the storage conditions tested, ARP values follow the same behaviors of TP values.

4. Conclusions

Results of this study confirmed the efficiency of spray drying to microencapsulate OP polyphenols. The effect of IT, MD concentration and feed flow were studied and microparticles were characterized. An increase from IT130 to IT160 leads to lower moisture content and bulk density, but has not statistically significant ($p < 0.05$) effects on microencapsulation yield and ME. Increasing MD concentration causes lower bulk density and higher size of the powders. WAI and SC values suggests that by increasing IT, the structure of the MD matrix tends to be in an amorphous state, which enhance the final solubility of the samples. The highest microencapsulation yield was obtained at IT130, MD10 and feed flow of 10 mL/min. At these conditions notable TP content (39.5±4.9 mg_{CAE}/g_{DP}) and ARP (33.8±4.3 $mmol_{DPPH^{\cdot}}/L_{extract}$) were obtained, along with a high ME (76±3.3%), and small particle size.

The product obtained shows good stability at storage conditions and remarkable antioxidant properties, and can be considered a potential source for integration in novel food or pharmaceutical products.

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Figure Captions

Fig.1 SEM images of the microcapsules obtained at different inlet temperature (IT), maltodextrins (MD) concentrations and feed flows: a) IT 130°C, MD 100 g/L, feed flow 10 mL/min; b) IT 130°C, MD 500 g/L, feed flow 10 mL/min; c) IT 160°C, MD 100 g/L, feed flow 10 mL/min; d) IT 160°C, MD 500 g/L, feed flow 5 mL/min

Fig.2 Percentage of microparticles with diameter comprised in different size ranges, calculated as the ratio between the number of particles in the range and the number of total measured particles. a)

Microparticles obtained at IT130, MD10, feed flow 5 mL/min (■) and IT130, MD10, feed flow 10 mL/min (□). b) Microparticles obtained at IT130, MD50, feed flow 5 mL/min (■) and IT130, MD50, feed flow 10 mL/min (□). c) Microparticles obtained at IT160, MD10, feed flow 5 mL/min (■) and IT160, MD10, feed flow 10 mL/min (□). d) Microparticles obtained at IT160, MD50, feed flow 5 mL/min (■) and IT160, MD50, feed flow 10 mL/min (□).

Fig.3 Thermogravimetric analysis (▬ TG), differential thermogravimetric analysis (⋯ DTG) and differential thermal analysis (▬ DTA) of the microparticles obtained at different conditions: a) IT130, MD10, feed flow 5 mL/min; b) IT130, MD10, feed flow 10 mL/min; c) IT130, MD50, feed flow 5 mL/min; d) IT130, MD50, feed flow 10 mL/min, e) IT160, MD10, feed flow 5 mL/min; f) IT160, MD10, feed flow 10 mL/min; g) IT160, MD50, feed flow 5 mL/min; h) IT160, MD50, feed flow 10 mL/min; i) MD without extract at IT130, MD10, feed flow 10 mL/min; j) Extract without MD.

Fig.4 Effect of different storage conditions (temperature, light, UV exposure) on microparticles. a) TP of stored microparticles at 5 °C (▬), 25 °C (▬ ▬) and 45 °C (⋯) during a 10 weeks storage. b) TP of stored microparticles in dark conditions (▬), sunlight (▬ ▬) and lamp light (⋯) for a 10 weeks storage. c) TP of stored microparticles under UV exposure during a 48 h storage. Different letters indicate significant difference between data at $p < 0.05$.

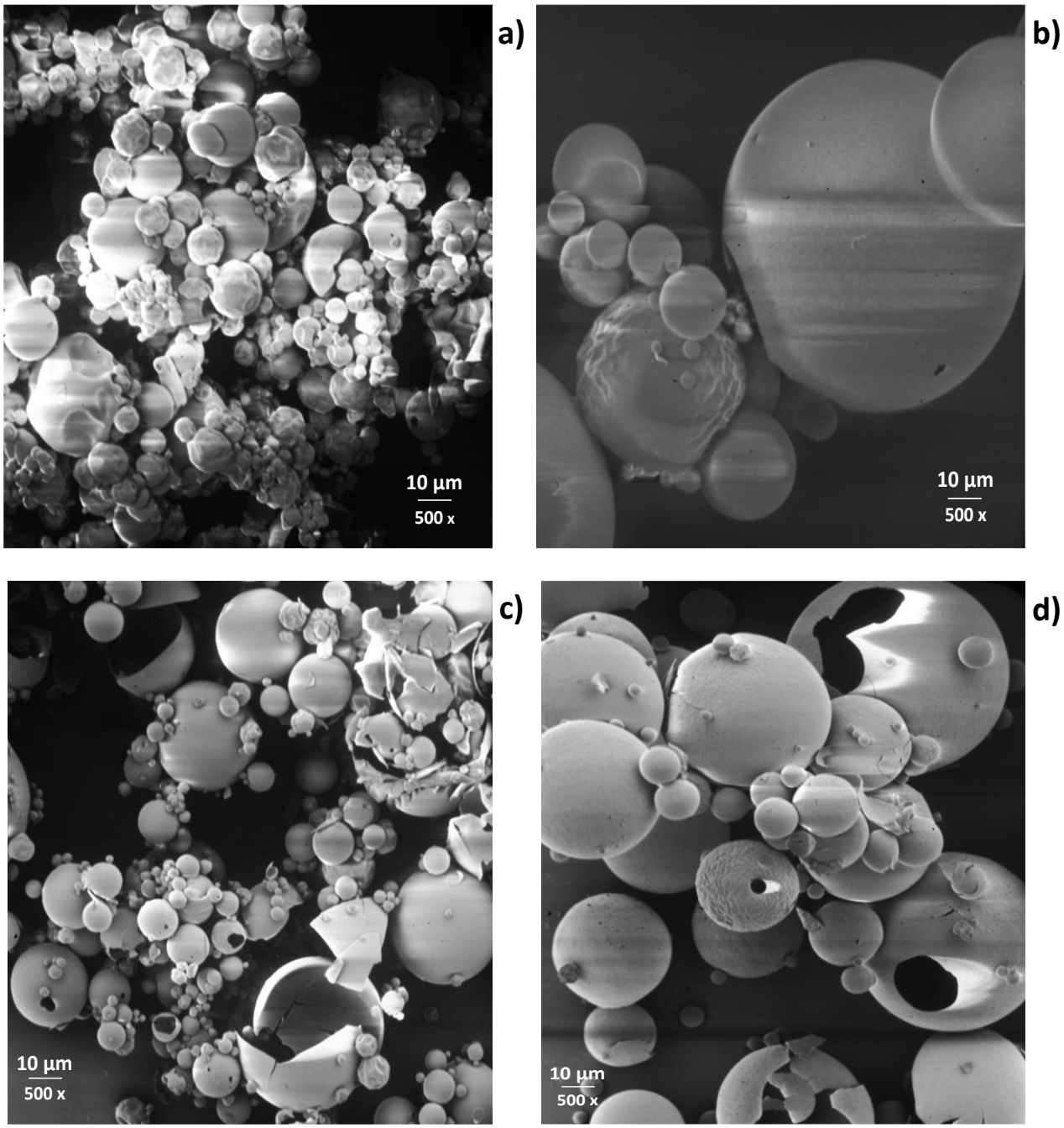

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Fig.1

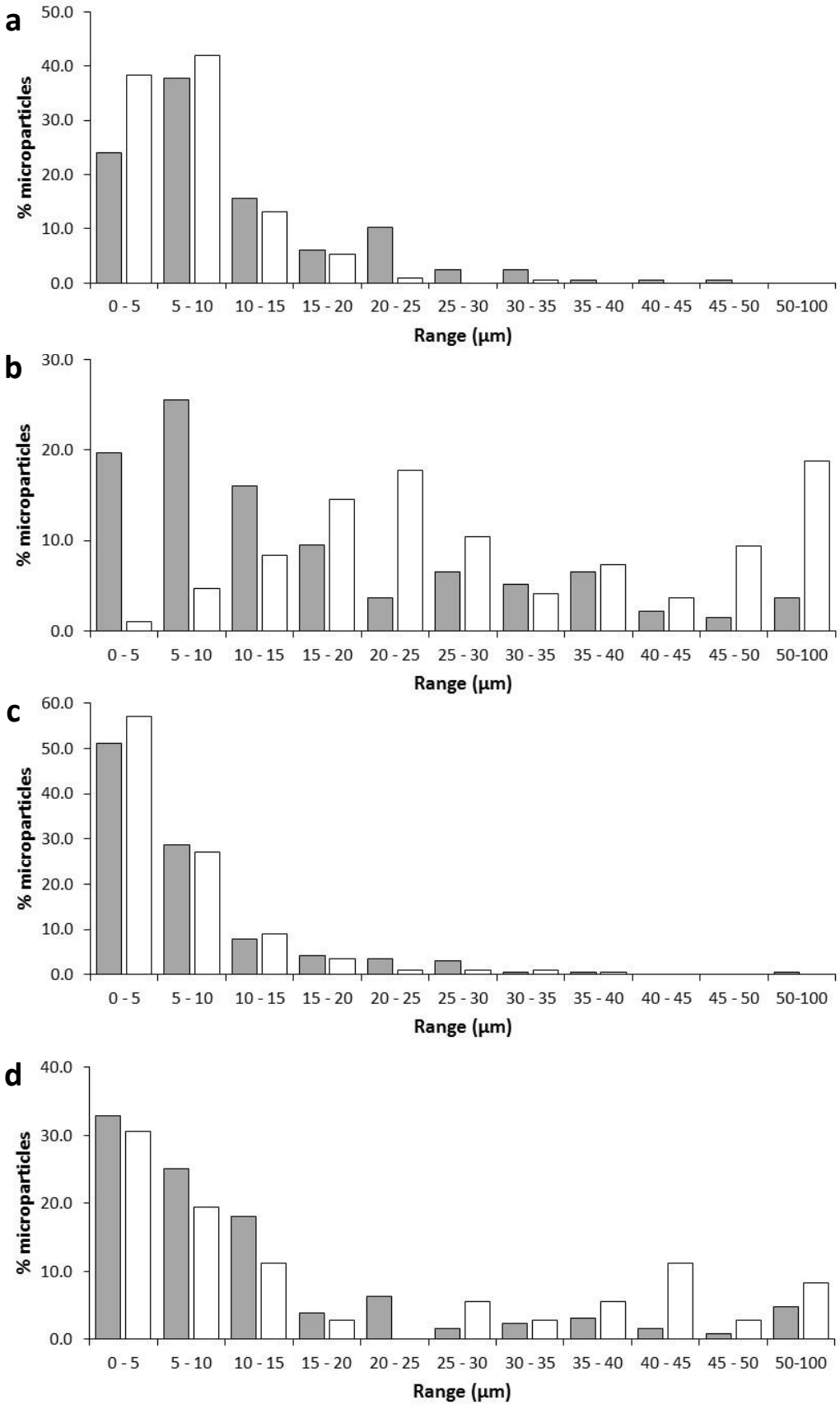

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Fig. 2

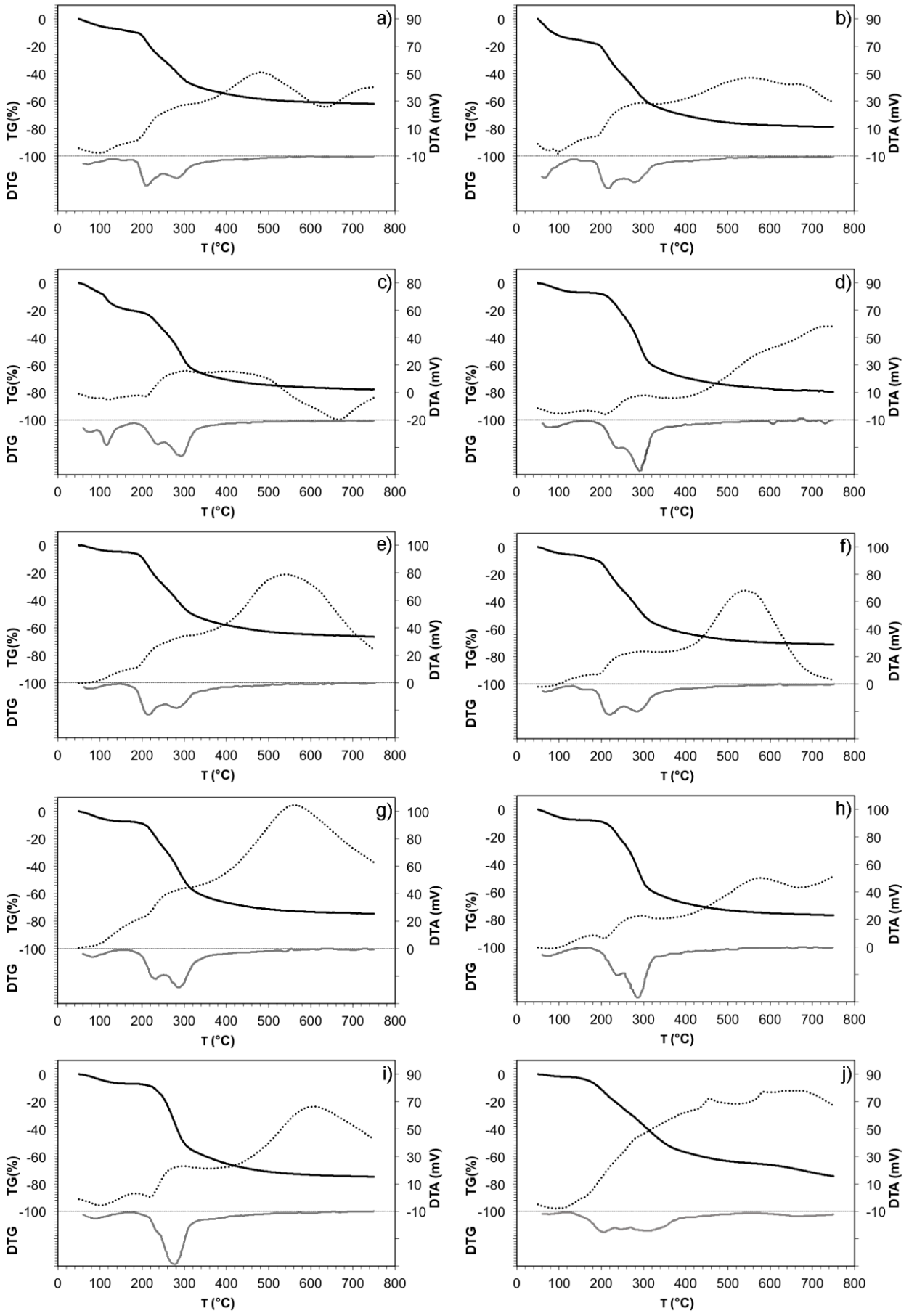
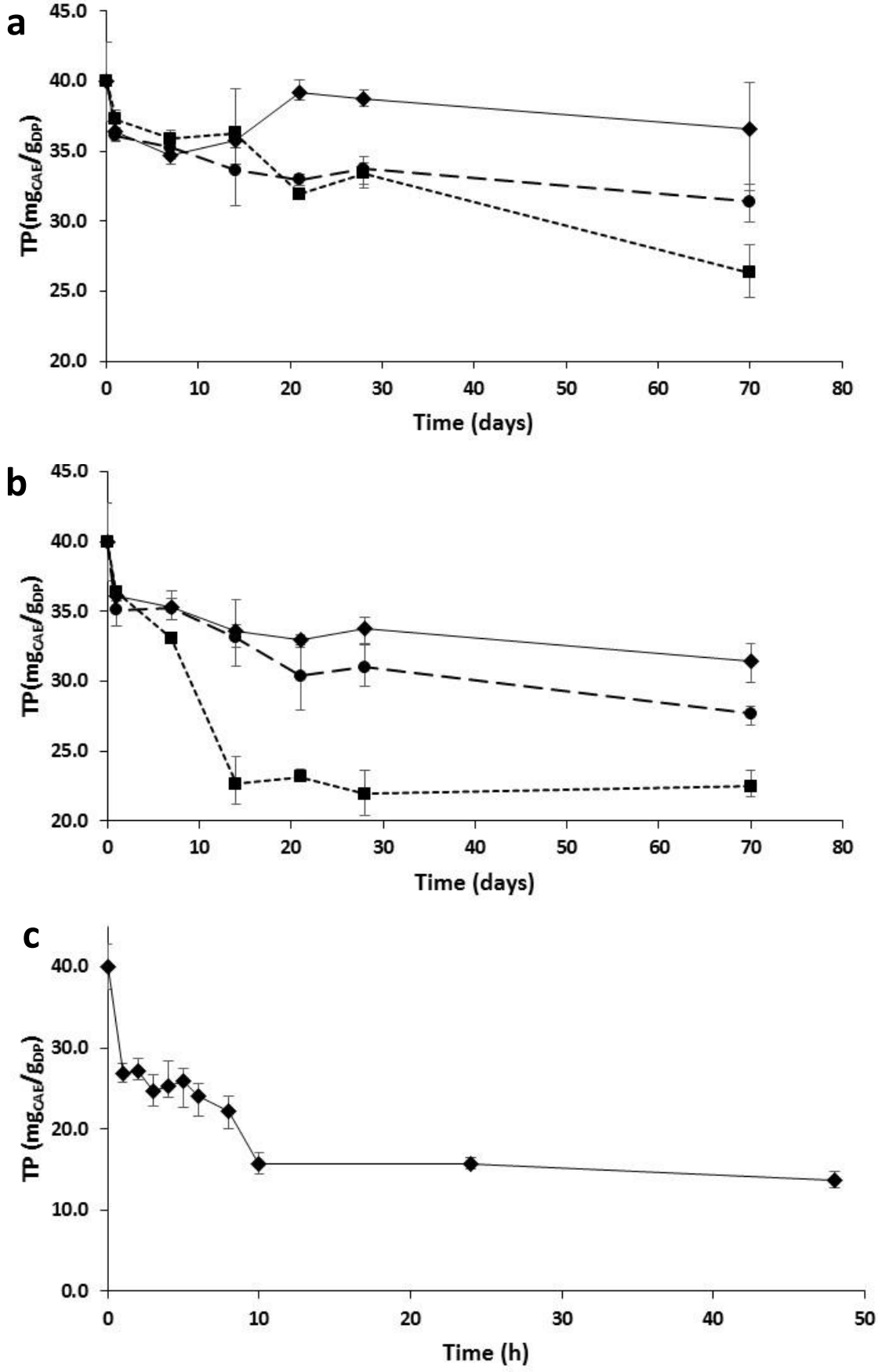


Fig.3



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528 **Fig.4**
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Table 1

Physical characterization of the microcapsules obtained by SD of OP extract using MD as coating agent.

IT (°C)	MD (g/L)	Feed flow (mL/min)	OT (°C)	Moisture content (%)	Bulk density (g/mL)
130	100	5	86 ± 1 [a,b]	6.2 ± 0.2 [b]	0.19 ± 0.01 [b]
		10	75 ± 1 [c]	7.0 ± 0.4 [c]	0.19 ± 0.02 [b]
	500	5	87 ± 1 [a,b]	5.9 ± 0.4 [b]	0.13 ± 0.01 [e]
		10	83 ± 1 [a]	6.1 ± 0.2 [b, c]	0.16 ± 0.01 [f]
160	100	5	95 ± 1 [d]	2.3 ± 0.3 [a]	0.07 ± 0.01 [c]
		10	84 ± 1 [a]	2.1 ± 0.5 [a]	0.10 ± 0.01 [d]
	500	5	102 ± 1 [e]	2.4 ± 0.3 [a]	0.04 ± 0.01 [a]
		10	89 ± 1 [b]	2.4 ± 0.4 [a]	0.05 ± 0.01 [a]

Different letters within the column indicate significant difference between data at $p < 0.05$. Data are expressed as mean value ± standard deviation, $n = 3$. For bulk density measurements, $n = 5$. (SD: Spray Drying, OP: Olive Pomace, MD: Maltodextrins, IT: Inlet Temperature, OT: Outlet Temperature).

Table 2

WSI, WAI and SC of the microparticles obtained by SD of OP extract using MD as coating agent.

IT (°C)	MD (g/L)	Feed flow (mL/min)	WSI (%)	WAI (g/g_{DP})	SC (g/g_{DP})
130	100	5	84 ± 1.7 [a]	0.34 ± 0.02 [a]	2.13 ± 0.27 [a]
		10	85 ± 2.2 [a]	0.33 ± 0.02 [a,b]	2.31 ± 0.49 [a]
	500	5	85 ± 0.9 [a]	0.38 ± 0.01 [a,c]	2.49 ± 0.18 [a,b]
		10	87 ± 1.6 [a]	0.28 ± 0.02 [b]	2.10 ± 0.14 [a]
160	100	5	79 ± 2.2 [b]	0.49 ± 0.02 [d]	2.43 ± 0.33 [a,b]
		10	80 ± 1.6 [b]	0.58 ± 0.03 [e]	2.85 ± 0.16 [a,b]
	500	5	83 ± 0.5 [a,b]	0.53 ± 0.01 [d]	3.12 ± 0.14 [b]
		10	85 ± 1.2 [a]	0.40 ± 0.02 [c]	2.76 ± 0.31 [a,b]

Different letters within the column indicate significant difference between data at $p < 0.05$. Data are expressed as mean value ± standard deviation, $n = 3$ (WSI: Water Solubility Index, WAI: Water Absorption Index, SC: Swelling Capacity, SD: Spray Drying, OP: Olive Pomace, MD: Maltodextrins, DP: Dried Powder).

Table 3

TP, ARP, encapsulation yield and ME of the microparticles obtained by SD of OP extract using MD as coating agent.

IT (°C)	MD (g/L)	Feed flow (mL/min)	TP (mg_{CAE}/g_{DP})	ARP ($mmol_{DPPH^{\cdot}}/L_{extract}$)	Microencapsulation Yield (%)	ME (%)
130	100	5	27.7 ± 1.7^{b}	20.4 ± 2.7^{c}	$66 \pm 4.1^{a, b}$	77 ± 3.5^{b}
		10	39.5 ± 4.9^{c}	33.8 ± 4.3^{d}	94 ± 0.4^{c}	$76 \pm 3.3^{a, b}$
	500	5	4.5 ± 0.3^{a}	3.8 ± 0.6^{a}	54 ± 4.1^{a}	$72 \pm 1.4^{a, b}$
		10	6.2 ± 0.4^{a}	6.8 ± 1.0^{a}	73 ± 0.6^{b}	$75 \pm 1.0^{a, b}$
160	100	5	26.0 ± 1.2^{b}	14.8 ± 0.7^{b}	$62 \pm 2.9^{a, b}$	65 ± 3.5^{a}
		10	26.0 ± 1.5^{b}	19.1 ± 2.1^{c}	$60 \pm 3.6^{a, b}$	$66 \pm 3.1^{a, b}$
	500	5	4.8 ± 0.5^{a}	5.0 ± 0.4^{a}	$58 \pm 7.1^{a, b}$	$73 \pm 5.2^{a, b}$
		10	4.4 ± 0.3^{a}	3.6 ± 0.3^{a}	51 ± 4.8^{a}	$71 \pm 6.8^{a, b}$

Different letters within the column indicate significant difference between data at $p < 0.05$. Data are expressed as mean value ± standard deviation, $n = 3$ (TP: Total polyphenols, ARP: Antiradical Power, ME: Microencapsulation Efficiency, SD: Spray Drying, OP: Olive Pomace, MD: Maltodextrins, IT: Inlet Temperature).

Table 4

Percentage of each phenolic compound respect to the total amount of identified polyphenols in the olive pomace (OP) extract before spray drying and in microparticles spray dried at different inlet temperatures (IT), maltodextrins concentration (MD) and feed flows.

IT (°C)	MD (g/L)	Feed flow (mL/min)	[1]Tyr	[2]VA	[3]SA	[4]CA	[5]*p*-CA	[6]Lut 7-G	[7]Api 7-G	[8]Lut	[9]Api
130	100	5	27.9 ± 1.1 ^{b}	16.5 ± 1.3 ^{a}	3.6 ± 0.6 ^{a}	6.3 ± 1.0 ^{b}	3.2 ± 0.4 ^{a}	10.0 ± 1.2 ^{a}	12.1 ± 1.0 ^{b}	5.9 ± 0.5 ^{a}	14.6 ± 1.3 ^{c}
		10	19.5 ± 3.9 ^{a}	16.5 ± 0.9 ^{a}	4.7 ± 0.5 ^{a}	9.6 ± 1.0 ^{c}	1.9 ± 0.7 ^{a}	13.6 ± 2.1 ^{a}	14.9 ± 1.8 ^{b,c}	6.0 ± 0.4 ^{a}	13.3 ± 1.0 ^{c}
	500	5	21.9 ± 1.0 ^{a}	14.1 ± 0.5 ^{a}	4.0 ± 0.4 ^{a}	6.0 ± 1.2 ^{b}	3.0 ± 1.0 ^{a}	22.7 ± 1.3 ^{b}	10.7 ± 1.0 ^{b}	6.3 ± 0.4 ^{a}	11.1 ± 0.6 ^{b}
		10	24.6 ± 1.8 ^{a}	18.5 ± 1.0 ^{a}	4.9 ± 0.6 ^{a}	4.9 ± 1.4 ^{b}	3.6 ± 1.0 ^{a}	15.0 ± 1.9 ^{a}	13.7 ± 1.1 ^{b}	4.4 ± 0.8 ^{a}	10.4 ± 0.5 ^{b}
160	100	5	21.1 ± 1.2 ^{a}	14.8 ± 1.8 ^{a}	3.3 ± 1.0 ^{a}	6.8 ± 1.5 ^{b}	3.5 ± 0.3 ^{a}	12.2 ± 1.0 ^{a}	18.5 ± 1.8 ^{c}	5.5 ± 0.4 ^{a}	14.2 ± 1.0 ^{c}
		10	18.4 ± 3.9 ^{a}	18.6 ± 1.4 ^{a}	5.4 ± 1.4 ^{a}	4.4 ± 0.8 ^{b}	1.7 ± 0.9 ^{a}	13.7 ± 0.9 ^{a}	17.3 ± 2.0 ^{c}	6.5 ± 0.6 ^{a}	14.0 ± 0.9 ^{c}
	500	5	23.9 ± 0.8 ^{a}	14.3 ± 1.9 ^{a}	3.8 ± 1.2 ^{a}	4.4 ± 1.0 ^{b}	2.9 ± 0.5 ^{a}	22.2 ± 1.0 ^{b}	12.9 ± 1.5 ^{b}	5.4 ± 0.7 ^{a}	10.2 ± 0.7 ^{b}
		10	25.2 ± 1.0 ^{a}	14.3 ± 1.6 ^{a}	4.1 ± 1.1 ^{a}	4.4 ± 1.1 ^{b}	3.3 ± 0.6 ^{a}	19.4 ± 2.5 ^{a,b}	13.4 ± 0.8 ^{b}	5.7 ± 0.5 ^{a}	10.4 ± 0.4 ^{b}
		OP extract	35.1 ± 1.2 ^{c}	12.9 ± 1.3 ^{a}	2.9 ± 0.8 ^{a}	2.9 ± 0.5 ^{a}	1.1 ± 0.8 ^{a}	26.5 ± 0.5 ^{c}	4.5 ± 0.8 ^{a}	8.1 ± 0.4 ^{b}	5.9 ± 0.6 ^{a}

Different letters within the column indicate significant difference between data at $p < 0.05$. Data are expressed as mean value ± standard deviation, $n = 3$.

[1]Tyr: Tyrosol.
[2]VA: Vanillic acid.
[3]SA: Syringic acid.
[4]CA: Caffeic acid.
[5]*p*-CA: *p*-coumaric acid.
[6]Lut 7-G: Luteolin 7-glucoside.
[7]Api 7-G: Apigenin 7-glucoside.
[8]Lut: Luteolin.
[9]Api: Apigenin.