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# Effect of Encapsulating Agent on Physical-Chemical Characteristics of Olive Pomace Polyphenols-Rich Extracts

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During the last years, olive industry wastes are considered as a potential source of extracts with antioxidant properties rich in polyphenols. The recovery of these added-value compounds can be interesting both for their beneficial properties and from an environmental point of view and several studies have been performed for the optimization and characterization of the phenolic compounds present in olive pomace liquid extracts. However, dried forms of natural extracts are associated with several advantages over liquid forms, such as limited storage costs, higher concentration and stability of target compounds.
Spray drying is one of the most common techniques for the transformation of a liquid extract, or food formulation, into dried powder, due to low operative costs respect to other methods, and high flexibility, related to the large number of operative parameters that can be optimized, such as coating agent type and concentration, inlet temperature, feed flow and aspiration rate. In this study, an ethanolic extract rich in phenolic compounds from olive pomace (Taggiasca cultivar) was obtained by high pressure and temperature extraction and spray dried with different ratios of gum arabic and maltodextrin as coating agents (0:100, 20:80, 40:60, 60:40, 80:20 and 100:0 % w/w). The total amount of coating agent was maintained constant and equal to 10 % w/v (100 g/L). Inlet temperature, feed flow and aspiration rate were 160 °C, 5 mL/min and 30 $m^3$/h, respectively. For all products, moisture content, water solubility index, total polyphenol content, antiradical power and microencapsulation yield were determined. The results of this study show that the coating agent and process conditions led to the production of microencapsulated powders with improved water dissolution rate and a minimal loss in phenolics during the drying phase. The obtained microparticles, due to their high content in bioactive compounds and ease of handling, can have potential industrial applications as functional components for foods or nutraceuticals purposes.

## 1. Introduction

Olive pomace, as the main by-product of the olive oil industry, is currently burnt to obtain energy or treated as an industrial waste, despite to its potential applications as heavy-metal absorption (Pagnanelli et al., 2003) or biofuel-production feedstock (Che et al., 2012; Miranda et al. 2012). This biomass was also considered as one of the most interesting agro-food waste containing bioactive compounds such as polyphenols (Aliakbarian et al., 2011). Polyphenols are secondary metabolites present in plants, which show a wide spectrum of biological activities such as antioxidant, anti-inflammatory, antibacterial and antiviral properties (Paini et al., 2015). These properties led to a potential use of polyphenols as therapeutic agents against diseases like cancer, diabetes and cardiovascular disorders (Aliakbarian et al., 2012), acting against reactive oxygen species (Georgetti et al., 2008) and preventing cellular oxidative stress and the relative damages (Moure et al., 2001). For all these beneficial effects on human health, the recovery of these high added-value compounds from agri-food residues has been recognized as a big scientific effort (An et al., 2011). However, several limitations have been associated with the use of these compounds in traditional formulations, due to their low bioavailability, low stability in environmental conditions, low water solubility and rapid catabolism and excretion (Serrano-Cruz et al., 2013). To overcome these drawbacks, several microencapsulation techniques have been developed in order to preserve the biochemical functionalities of polyphenols (Fang and Bhandari, 2012).

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Among them, spray drying is one of the most common, due to its ability to decrease the water activity, ensure microbiological stability, avoid degradation processes, reduce storage and transport costs and enhance the instantaneous solubility of the final product (Gharsallaoui et al., 2007).
Different coating agents have been proposed for the encapsulation of bioactive compounds via spray drying (Gharsallaoui et al., 2007), and among them maltodextrin (a polysaccharide derived from the hydrolysis of starch) and gum arabic (a polymer consisting of D-glucuronic acid, L-rhamnose, D-galactose and L-arabinose, with approximately 2 % protein, according to Dickinson (2003)) are interesting for potential application in food and nutraceutical industry.
The aim of this work was to evaluate the effects of different combinations of maltodextrin and gum arabic on the physico-chemical properties of spray dried polyphenols from olive pomace, obtained by a high pressure and temperature extraction. In particular, microparticles were characterized in terms of moisture content, solubility properties, polyphenols content and antiradical power. Moreover, the microencapsulation yield of each sample was calculated, in order to evaluate the potential degradation of phenolic compounds due to the operative parameters of the drying phase.

## 2. Materials and methods

### 2.1 Chemicals

Ethanol, methanol, acetic acid, n-hexane, maltodextrin, gum arabic, Folin–Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) reagents and caffeic acid standard were purchased from Sigma-Aldrich (St. Louis, MO, USA). Caffeic acid solutions were prepared with methanol and stored in dark bottles at -20 °C.

### 2.2 Extraction of polyphenols from olive pomace

Olive pomace (Taggiasca cultivar) from a three-phase oil extraction decanter was supplied by a local olive oil production plant (Raineri S.p.a., Chiusanico, Italy) and stored at -20 °C prior to use.
A part of samples was utilised for determination of the oil percentage (7.1% ± 0.1) which was carried out by using a Soxhlet apparatus and petroleum ether (b.p. 40-60 °C) as the solvent. Before the extraction, olive pomace was washed with n-hexane to remove the residual oil, oven dried at 60 °C until constant weight, milled and stored at room temperature in dark conditions before use (Aliakbarian e al., 2011).
Aliquots from the above homogenized lot were subjected to quantitative acid hydrolysis (method TAPPI T13m) with 72% sulfuric acid. The solid residue after hydrolysis was expressed as Klason lignin (49.9% ± 3.4). Monosaccharides from hydrolyzates (glucose 13.1% ± 1.2; xylose 15.3% ± 0.9 and arabinose 1.1% ± 0.1) and acetic acid (2.4% ± 0.1) were analyzed by high performance liquid chromatography (HPLC) as reported by Aliakbarian et al. (2011). The results allowed the determination of the sample contents of cellulose and starch (based on the glucose present in liquors), hemicellulosic polysaccharide constituents (based on the xylose and arabinose present in liquors), and acetyl groups (based on the acetic acid present in liquors).
Polyphenols were extracted from the matrix using a high pressure-high temperature agitated reactor (model 4560, PARR Instrument Company, Moline, USA), with ethanol:water 50:50 (v/v) as solvent. All the other parameters are equal to the best conditions studied by Aliakbarian et al. (2011). After this phase, extract was centrifuged at 6000 xg for 10 min (ALC PK131, Alberta, Canada), filtered through a 0.22 µm syringe filter (Sartorius Stedim Biotech GmbH, Göttingen, Germany) and stored at 4 °C before spray drying.

### 2.3 Spray drying of olive pomace extract

Spray drying of olive pomace extract was performed using a Büchi Mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Flawil, Switzerland), with fixed values of inlet temperature, feed flow, aspiration rate and amount of coating agent, equal to 160 °C, 5 mL/min, 30 $m^3$/h and 10 % w/v (100 g/L), respectively. In this work, different coating agents mixtures were tested, varying the ratio between maltodextrin (MD, with 16.5-19.5 dextrose equivalents) and gum arabic (GA). In particular, the ratios 0:100, 20:80, 40:60, 60:40, 80:20 and 100:0 % (w/w) were studied. The spray dryer was run with deionized water for 10 min before and after each experiment, and for each sample the outlet temperature (OT) was registered. Microparticles were stored at 4 °C in closed dark vessels before analysis.

### 2.4 Moisture content and water solubility index

Powder samples were oven-dried at 105 °C until a constant weight. The moisture content of microparticles was calculated based on the loss in weight between powders before and after drying.
In order to evaluate the solubility properties of the microparticles, the water solubility index (WSI) was determined according to Anderson (1982), with some modifications. Briefly, 1 g of product was added to 12 mL of water, mixed and incubated in a water bath at 30 °C for 30 min. After incubation, samples were centrifuged at 3000 xg for 15 min, and the supernatants were collected and evaporated at 105 °C overnight. WSI was calculated according to Eq(1).

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

where $DW_{sup}$ is the dry weight of the supernatant and $DW_{part}$ is the initial weight of microparticles (dry basis).

**2.5 Total polyphenols content, microencapsulation yield and antiradical power**

Microparticles obtained with different MD:GA ratios were also analysed for evaluate the content of phenolic compounds effectively encapsulated. As described by Robert et al. (2010), 0.2 g of microparticles were dissolved in 2 mL of methanol:acetic acid:water (50:8:42 v/v/v), mixed for 1 min and sonicated twice for 20 min (FALC UTA 90 ultrasonic bath, Treviglio, Italy). Samples were then centrifuged at 6000 *x*g for 15 min and filtered through a 0.22 µm syringe filter.

Total polyphenols content (TP) was evaluated using the Folin-Ciocalteu assay (Gutfinger, 1981): 0.2 mL of sample and 0.5 mL of Folin-Ciocalteu reagent were added to 4.8 mL of deionized water, then the contents were mixed and 1 mL of a saturated $Na_2CO_3$ solution was added. Deionized water was added in order to reach a final volume of 10 mL. Solutions were mixed and left at room temperature in dark conditions for 60 min. Aliquots of samples were then used for the determination of total phenols concentration using a UV-Vis spectrophotometer (Perkin Elmer, Wellesley, USA) at a wavelength of 725 nm. TP was expressed as milligrams of caffeic acid equivalents (CAE) per gram of dried powder (DP) ($mg_{CAE}/g_{DP}$). The same assay was used for the calculation of TP of the olive pomace extract before spray drying. The method response was described with a linear equation Eq(2), using standard methanolic solutions of caffeic acid (10 – 1000 µg/mL), with a $R^2$ of 0.996.

$$ABS_{725} = 0.002 \times TP - 0.004 \quad (2)$$

In order to evaluate the potential degradation of polyphenols due to the spray drying operative conditions, microencapsulation yield was calculated as the percentage of total amount of polyphenols in the dried powder and the total amount of polyphenols in the initial extract.

The antiradical power (ARP) of microparticles and coating agents without extract was evaluated with the radical scavenging $DPPH^{\cdot}$ method, described by Brand-Williams et al. (1995). For each extract, 0.1 mL of nine different dilutions in methanol (in the range 1:2-1:128) were prepared and mixed with 3.9 mL of $DPPH^{\cdot}$ methanolic solution (9.15 x 10-5 mol/L). The reaction mixtures were shaken and incubated for 1 h in the dark at room temperature, and afterwards the absorbance was read at 515 nm. The $DPPH^{\cdot}$ concentration in the reaction medium ($C_{DPPH^{\cdot}}$) was calculated from a calibration curve (Eq(3)) using standard solutions of $DPPH^{\cdot}$ in the range 3 – 44 $\mu g_{DPPH^{\cdot}}$/mL. The ratio of $mL_{extr}/mg_{DPPH}$ was plotted against $\%DPPH_{rem}$, calculated as the ratio of final (after 1 h) to initial concentrations of $DPPH^{\cdot}$. The amount of extract necessary to decrease the initial $DPPH^{\cdot}$ concentration by 50% (EC50 in $mL_{extr}/mg_{DPPH}$) was determined from the resulting exponential equation. Finally, antiradical power (ARP) was defined as $1/EC_{50}$.

$$ABS_{515} = 0.023 \times C_{DPPH^{\cdot}} - 0.008 \quad (3)$$

**2.6 Statistical analysis**

All the experiments were carried out in triplicate. Influences of the various parameters were assessed by analysis of variance (ANOVA) and Tukey's post hoc test ($p < 0.05$), using ''Statistica'' software version 11.0 (StatSoft, Tulsa, USA). The statistically significant differences were illustrated in tables and figures by different letters.

## 3. Results and discussion

**3.1 Spray drying and physical properties of microparticles**

The olive pomace extract was obtained with high pressure and temperature extraction and spray dried using different mixtures of MD and GA as coating agents. After spray drying, microparticles were characterized in terms of moisture content and WSI. As can be seen in Table 1, OTs of the microparticles show no statistically significant differences ($p < 0.05$), indicating that the variation in the coating agent composition does not affect the evaporation of the solvent from the solution.

The moisture content of the powders significantly ($p < 0.05$) increases with the increasing of the GA fraction (MD:GA equal to 20:80 and 0:100, in particular), reaching a value of 29.3 ± 0.1 % when only GA was used as coating agent, while the addition of a small amount of GA (MD:GA 80:20) does not increase significantly the moisture content of the microparticles.

*Table 1: Outlet temperatures (OT), moisture contents and water solubility indexes (WSI) of spray dried polyphenol-rich particles.*

| MD:GA | OT (°C) | Moisture (%) | WSI (%) |
|---|---|---|---|
| 100:0 | 103 ± 1[a] | 11.2 ± 0.3[a] | 80.76 ± 3.26[a] |
| 80:20 | 102 ± 1[a] | 12.2 ± 0.2[a] | 76.14 ± 1.24[a,b] |
| 60:40 | 104 ± 2[a] | 15.7 ± 0.1[b] | 69.35 ± 0.87[b,c] |
| 40:60 | 102 ± 1[a] | 15.6 ± 0.8[b] | 67.65 ± 1.62[b,c] |
| 20:80 | 103 ± 2[a] | 25.3 ± 0.1[c] | 66.18 ± 2.17[c] |
| 0:100 | 105 ± 1[a] | 29.3 ± 0.1[d] | 62.89 ± 2.76[c] |

The addition of GA as coating agent had shown a significant decrease in WSI from 80.76 ± 3.26 % for the sample in absence of GA to 76.14 ± 1.24 % when 20 % of GA was added to the coating agent mixture. The lower WSI was recorded for the microparticles with only GA as coating agent, equal to 62.89 ± 2.76 %.

**3.2 Total polyphenols content, microencapsulation yield and antiradical power**

Total polyphenols in the microparticles were quantified with the Folin-Ciocalteu assay, and expressed as milligrams of caffeic acid equivalents (CAE) per gram of dried powder ($mg_{CAE}/g_{DP}$). For the calculation of the microencapsulation yield, the same assay was used for the evaluation of TP of the olive pomace extract before spray drying. As shown in Table 2, no statistically significant ($p < 0.05$) differences were noted between the TP of microparticles, indicating that the modification of the coating agent composition does not imply a change in the phenolic content of the final product. Microencapsulation yields of the powders show that mixtures of MD and GA are able to better protect polyphenols from oxidation during spray drying. Indeed, if no statistical significant differences were noted between the samples in which only MD or GA were used as coating agent, the use of both the materials as coating agent leads to a slightly (but not statistically significant) increase in the microencapsulation yield. This is particularly evident for the microparticles with MD:GA ratio of 60:40, in which microencapsulation yield is 87.3 ± 1.8 %, significantly different from the samples spray dried with only MD or GA.

*Table 2: Total polyphenols yield (TP) and microencapsulation yield of spray dried polyphenol-rich particles.*

| MD:GA | TP ($mg_{CAE}/g_{DP}$) | Microencapsulation yield (%) |
|---|---|---|
| 100:0 | 29.0 ± 3.5 [a] | 70.5 ± 10.7 [a] |
| 80:20 | 35.1 ± 1.9 [a] | 83.0 ± 4.5 [a,b] |
| 60:40 | 36.9 ± 0.8 [a] | 87.3 ± 1.8 [b] |
| 40:60 | 30.0 ± 4.8 [a] | 77.6 ± 1.9 [a,b] |
| 20:80 | 28.5 ± 4.5 [a] | 72.0 ± 10.5 [a] |
| 0:100 | 29.2 ± 1.2 [a] | 69.2 ± 2.9 [a] |

The antioxidant properties of the microparticles were evaluated in terms of hydrogen-donating or radical-scavenging ability to reduce the radical DPPH. Values are expressed in $mg_{DPPH^{\cdot}}/mL$ of extract from microparticles and are presented in Figure 1.

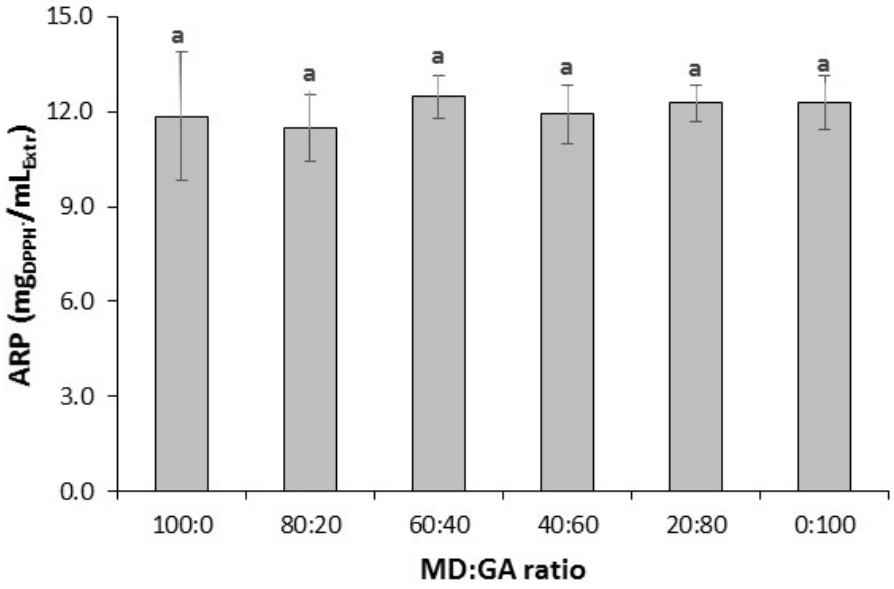


*Figure 1: Antiradical power (ARP) of spray dried microparticles with different MD:GA ratios, expressed as mg$_{DPPH^{\cdot}}$/mL.*

As can be seen from Figure 1, similar to TP values, also ARP shows no statistically significant ($p$ < 0.05) differences between the samples, indicating that the produced powder can have a potential role as ingredient rich in antioxidants in food or nutraceutical formulations. ARP were comprised, for all the samples, between 11.5 ± 1.5 and 12.5 ± 0.9 mg$_{DPPH^{\cdot}}$/mL, while MD and GA without olive pomace extract show negligible values of ARP (data not shown), indicating that the coating agents does not contribute to the final ARP of the powders.

## 4. Conclusions

In this study, microparticles of an olive pomace polyphenol-rich extract were produced by spray drying, varying the ratio of MD and GA as coating agents. The produced powders were subsequently characterized from a physical (moisture content, solubility in water) and a chemical point of view (polyphenols content, antiradical power, microencapsulation yield).

Results shown that an increase of the GA fraction in the coating agent mixture leads to an increasing moisture content in the microparticles, and a decrease in the solubility of the final product (WSI). The lower WSI was found in the sample with MD:GA ratio of 0:100, in which only 63 % of the product is able to solubilize in warm water.

TP and ARP do not show any significant difference between the samples, suggesting that MD and GA, independently from their fractions, are able to efficiently protect phenolic compounds from oxidation during spray drying. The sample with MD:GA 60:40 had shown the highest value of microencapsulation yield, equal to 87.3 ± 1.8 %, significantly higher than those obtained with only MD or GA.

The selected coating agents and process conditions led to stable and handling microencapsulated powder forms of the olive pomace ethanolic extract with improved water dissolution rate. The made up powder can be suitable for successive manufacturing to produce functional components for food or nutraceutical purposes.

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