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# Encapsulated Proanthocyanidins as Novel Ingredients

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Proanthocyanidins (OPC) are polyphenolic compounds, derivatives of flavan-3-ol flavonoids. They are abundant in grape seeds and skins, and contribute to most of the polyphenols in red wine. Proanthocyanidins from grape seed have been reported to show various health as well as technological properties.
Aim of the study was to investigate the coating efficiency of maltodextrin (MD) in different molecular ratios with arabic gum (AG) on encapsulation of phenolic compounds extracted from grape pomace.
The present study was planned to examine the contributions of MD, AG and OPC to the structural architecture of encapsulated OPC by means of scanning electron microscopy (SEM), their encapsulation efficiency and their functionality (antioxidant activity and bioavailability) by spectrophotometric assays and mass spectrometry analysis (MALDI-TOF-MS). The effect of encapsulated OPC on in vitro polyphenol digestibility was also evaluated according to the Infogest protocol.
Encapsulated products were obtained by a mild ultrasonication method in controlled conditions based on the phenomenon of acoustic cavitation, and then freeze-dried. The content of coating material had significant ($p>0.05$) impact on particle morphology of spray-dried suspensions. SEM analysis of samples of AG/MD and AG/MD/OPC were similar and exhibited cracks and sharp edges, but samples with OPC showed a more enclosed structure. Total and Surface phenolic content of microcapsules showed the best encapsulation efficiency for samples with 4% of AG and 12% of MD. MALDI-TOF-MS characterization of encapsulated samples showed integrity of OPC components in the microcapsules with no changes with respect to original OPC. The in vitro digestion experiments showed also that composition and functionality of encapsulated OPC were better preserved along gastrointestinal digestion process.
In conclusion, OPC microcapsules could be utilized both as nutraceuticals and additives in various food applications.

## 1. Introduction

Proanthocyanidins (OPC) are polyphenolic compounds, derivatives of flavan-3-ol flavonoids. They are mainly composed of dimers, oligomers and polymers of catechin, epicatechin and their gallic acid esters (Spranger et al., 2008). Their degree of polymerization is generally distributed between 2 to 15. They are abundant and widely present in the natural plants such as grape and cocoa, particularly in skin and seed of these plants (Georgiev et al., 2014; Esatbeyoglu et al., 2015). Probably, the majority of the proanthocyanidin products found in the market are originated from grape seed (Zhang et al., 2017). OPC from grape seed have been reported to play a positive role in human health, particularly radical scavenger ability, anti-cancer activity, cardiovascular protection.
Microencapsulation is a common technique used to provide a physical barrier between the active ingredient and the other components of the product. In the case of foods, the most common procedure for microencapsulation is spray drying (Gharsallaoui et al., 2007). It consists of “packing” an active ingredient such as flavors, lipids, and carotenoids within a wall material, promoting the conversion emulsions into powders (Desai and Park, 2005; Tonon et al., 2011). Spray drying involves the atomization of emulsions into a drying medium with high temperature, resulting in a very fast water evaporation, which results in a quick crust formation and in a quasi-instantaneous entrapment of the core material (Gharsallaoui et al., 2007).

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Aim of the study was to investigate the coating efficiency of maltodextrin (MD) in different molecular ratios with arabic gum (AG) on encapsulation of phenolic compounds extracted from grape pomace: OPC.
The present study was planned to examine the contributions of MD, AG and OPC to the structural architecture of encapsulated OPC by means of scanning electron microscopy (SEM), their encapsulation efficiency and their functionality (antioxidant activity and bioavailability) by spectrophotometric assays and mass spectrometry analysis (MALDI-TOF-MS).
The effect of encapsulated OPC on in vitro polyphenol digestibility was also evaluated according to the Infogest protocol (Minekus et al., 2014; Romano et al., 2015).

## 2. Materials and Methods

### 2.1 Materials

Maltodextrin (MD, DE 4.0-7.0) and arabic gum (AG), both from Sigma (Italy),were used to prepare the coating materials. Grape proanthocyanidins (OPC) were supplied by INC (The Netherlands). All other reagents, MALDI matrices and solvents were of high purity grade.

### 2.2 Encapsulation procedure

Encapsulation method was based on the phenomenon of acoustic cavitation. Emulsions were prepared from solutions of AG (4%) and MD (10, 12, 16%) in water, which were mixed in the ratio 8: 2 (V:V). 1 g OPC powder was then added into 20 g of coating material (MD:GA 8:2). Further mixing was carried out with high speed homogenizer and then ultrasonication applied for 20min at 160 W power and 20 KHz frequency. During the sonication, the samples were placed in a bath of water at 4 °C to prevent the overheating of emulsion. Finally the emulsions were freeze-dried for 48 h to obtain the final coated material. Diameter of droplets were smaller than 50 μm. The dried content was ground into a fine powder. Each experiment was performed in triplicate.

### 2.3 Microstructural analysis (SEM)

Samples were dried at the critical point and coated with gold particles in an automated critical point drier (model SCD 050, Leica Vienna). The microstructure of the samples was observed and photographed in a LEO EVO 40 scanning electron microscope (SEM Zeiss, Germany) at magnifications of x 500 with accelerating voltage of 20 kV.

### 2.4 Microcapsules characterization

#### 2.4.1 Total and surface content of polyphenols and encapsulation efficiency

The total phenolic content (TPC) was measured by the Folin–Ciocalteau method (Singleton and Rossi, 1965), with the following modifications: 3,5 mg phenolic powder or encapsulated phenolic powder was dispersed in 10 mL ethanol: acetic acid: water (50:8:42 v/v) solution. The mixture was stirred and filtered, then 1ml ample was added with 5 mL 0.2 N Folin-Ciocalteau reagent. After 5 min, 4 mL of 75 g/L sodium carbonate solution was added. Samples were kept in the dark at room temperature (25°C) for 1 hour and then absorption at 760 nm was measured by using UV/VIS spectrometer T70.
Surface phenolic content (SPC) was determined with the same method, but dissolving the encapsulated sample (3,5 mg of microcapsules) in 10 mL ethanol: methanol (50:50 v/v) mixture to preserve capsule integrity All the analyses were carried out in triplicate.
The encapsulation efficiency (EE) in the encapsulated product is defined by Eq. (1):

$$EE\ (\%) = \frac{EPC}{TPC} = \frac{(TPC - SPC)}{TPC} \quad (1)$$

where EPC and TPC are the amount of the encapsulated and the total polyphenols content in the encapsulated product, respectively.

#### 2.4.2 Antioxidant Power

The ABTS free radical-scavenging activity was assayed according to the method described by Miller et al. (1993) with the following modifications. A mixture (5:1, v/v) of ABTS (7.0 mM) and potassium persulfate (140 mM) was allowed to react overnight at room temperature in the dark to form radical cation ABTS+. The mixture was then diluted (1:80) with 95% ethanol, to reach absorbance values between 0,700 and 0,720 at 734 nm. 1g sample was then dissolved in 10ml 95% ethanol (v/v), stirred for 1 h in the dark, then centrifuged at 3000 rpm for 30 min to remove insoluble material. At this stage, a 30μl sample volume was then added to 1ml ABTS+ solution, and the absorbance decrease was measured at 734 nm after 6 min of incubation at 30

°C. The antioxidant capacity was expressed as mM Trolox equivalent antioxidant capacity (TEAC) / g dry weight.

**2.4.3 Bioavailability and mass spectrometry analysis**

The bioavailability were determined by spectrophotometric assays and mass spectrometry analysis (MALDI-TOF-MS). For MALDI TOF-MS analysis, OPC extracted from microcapsules in dilute HCl were desalted on SPE C18 EC cartridges (Isolute from Step-Bio, Bologna, Italy), rinsed with 5 mL methanol and 5 mL Milli-Q water; 2 mL of sample were loaded by gravity, the cartridge was washed with 5 mL of Milli-Q water, dried under vacuum for 30 min. The SPE column was eluted with 5 mL trifluoroacetic acid (TFA) (1% in methanol v/v); the sample was dried under a nitrogen stream at 40°C, then redissolved in 1 mL methanol and analysed by MALDI-TOF-MS. and LC/ESI-Q-TOF-MS/MS.

MALDI-TOF MS experiments were carried out on a PerSeptiveBioSystems (Framingham, MA, USA) Voyager DE-Pro instrument, equipped with an N2 laser (337 nm, 3 ns pulse width, 20 Hz repetition rate). Mass spectra acquisition was performed in both positive linear and reflectron mode. The instrument operated with an accelerating voltage of 20 kV. Even if several matrices, 2,5-dihydroxybenzoic acid (DHB) provided clearly superior performances. The matrix solution was prepared by dissolving 10 mg of crystalline DHB (Sigma, St. Luis, MO, USA) in 1 mL methanol containing 0.1% trifluoroacetic acid (TFA, Sigma, Milan, Italy). Typically, 250 laser pulses or more were acquired for each mass spectrum.

**2.5 In vitro digestibility**

The sample for which the best encapsulation efficiency (EE) was observed was submitted to simulated in vitro digestion using the Infogest protocol (Minekus et al., 2014; Romano et al., 2015) with some modifications. Briefly, 1 mg of sample was dissolved in 1mL 5% (v/v) formic acid and incubated (37°C) with pepsin (1:100, enzyme/substrate w/w ratio) for 1h. The pH was then increased to 7.0 with phosphate buffer and incubated with a mixture of pancreatin (1:500, 40 μl), bile salts (1mg/ml, 160μl), for 1h at 37°C. The sample were then stored at –80°C for further analysis.

## 3. Results and Discussion

**3.1 Microstructural analysis**

In order to study the contributions of MD, AG and OPC to the microstructural architecture of encapsulated OPC, the characterization of the microstructures by means of SEM was performed. The microscopic observations of initial microstructures of coating materials, maltodextrin (MD) and arabic gum (AG), and OPC particles are shown in Figure 1.

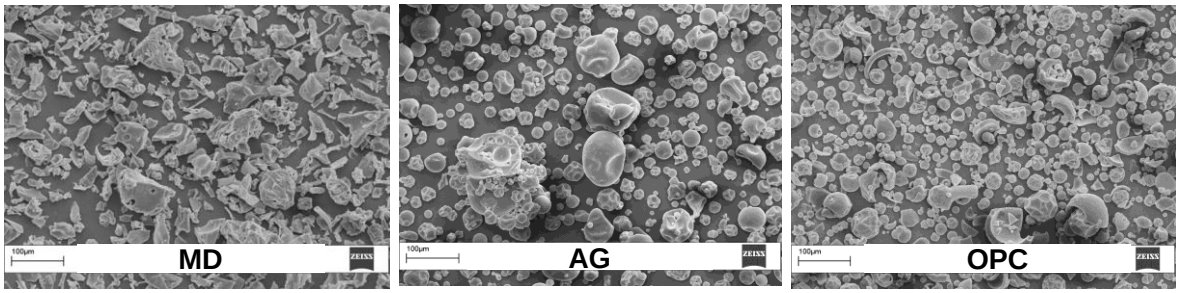


*Figure 1: Scanning electron micrographs of samples of: Maltodextrin (MD); Arabic Gum (AG) and Proanthocyanidins (OPC).*

SEM analysis (Fig. 1) showed that MD produces broken and incomplete particles, whereas AG was a fine powder, consisting of spherical particles with many typically dents on the surface. OPC particles appeared as small deformed and dented spheres with smooth and shrivelled surfaces, being these typical morphologies of polymeric spray-dried powders (Tonon et al., 2011).

The microscopic observations of emulsions of coating materials, maltodextrin (MD) and arabic gum (AG), and with OPC particles are shown in Figure 2. Emulsions were prepared from solutions with different MD concentration(10, 12, 16%) and the same concentration of AG (4%). Gum arabic is in fact one of the wall materials most widely used in the microencapsulation (Fang et al., 2005; Krishnan et al., 2005; Madene et al., 2006).

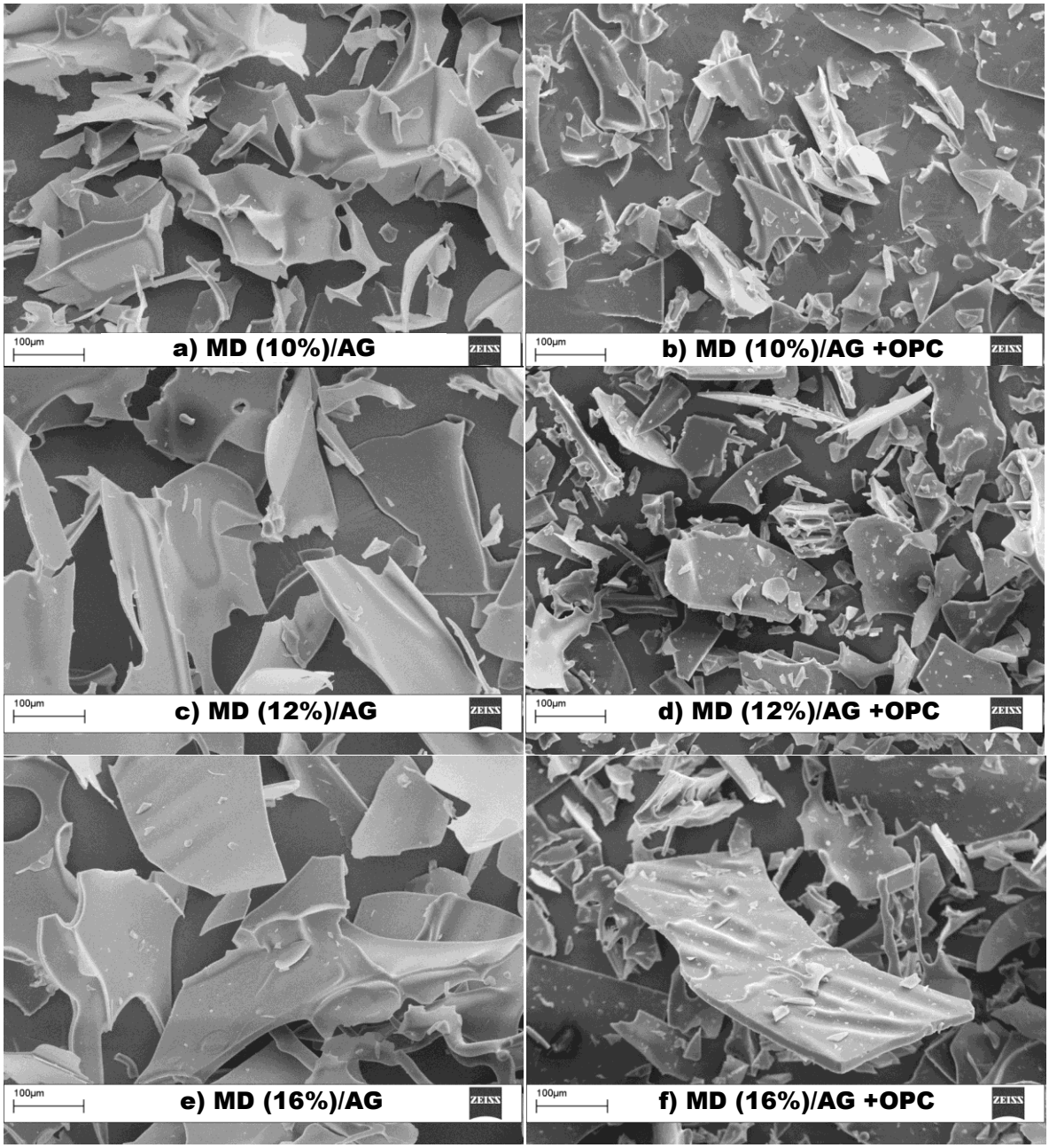


*Figure 2: Scanning electron micrographs of samples of: a) MD/AG (10 / 4%); b) MD/ AG (10/ 4%) with OPC; c) MD/AG (12 / 4%); d) MD/ AG (12 / 4%) with OPC; e) MD/AG (16 / 4%); f) MD/AG (16 /4%) with OPC.*

SEM micrographs of samples of AG / MD and AG / MD + OPC (Fig. 2a - f) were similar and exhibited cracks and sharp edges, but samples with OPC showed a more enclosed structure (Fig. 2b, d, f).

### 3.2 Microcapsules characterization

Table 1 shows the total (EPC) and Surface (SPC) phenolic content and encapsulation efficiency (EE) of microcapsules as a function of the MD concentration.

The best EE (79.3%) was observed for microcapsules prepared with 12% of MD and 4% AG. This high EE is significant in protecting OPC from degradation events such as oxidative polymerization, as confirmed also by the measurement of antioxidant power of encapsulated samples (see Table 2). These data also highlight the protective role of microcapsules obtained using GA as a coating material compared to those consisting of MD alone, as also suggested by previous studies (Dattanand et al., 2007).

*Table 1: EPC,SPC and EE of microcapsules*

| MD (%) | Ratio coating material (AG/MD, w/w) | EPC (mg GAE/g dry weight) | SPC (mg GAE/g dry weight) | EE*(%) |
|---|---|---|---|---|
| 10 | 1.60 | 343.3±0.1 | 314.8±0.3 | 51.97±0.03 |
| 12 | 1.33 | 523.6 ±0.1 | 177.9 ±0.4 | 79.26±0.02 |
| 16 | 1.00 | 302.5 ±0.1 | 199.48 ±0.3 | 45.79±0.03 |

Data are expressed as mean ± standard deviation (n=3)
*OPC standard : 660,63±1,06 mg GAE/g

The antioxidant power of microcapsules determined by Trolox equivalent antioxidant capacity (TEAC) method is shown in Table 2.

*Table 2: Antioxidant power of microcapsules with OPC by TEAC method*

| Sample | INIBITION (%) | µmoliTrolox/L solution | µmoli/g dry weight |
|---|---|---|---|
| control | 85.88 ±0.5 | 1251.9 ±7.4 | 18592.07 ±4.17 |
| AG /MD (4/ 10%) + OPC | 61.79 ±1.2 | 900.71 ±17.7 | 891.79 ±2.39 |
| AG /MD (4/ 12%) + OPC | 77.04 ±2.9 | 1121.8 ±41.9 | 1097.50 ±3.22 |
| AG /MD (4/ 16%) + OPC | 47.14 ±0.5 | 687.1 ±7.6 | 680.29 ±1.42 |

Data are expressed as mean ± standard deviation (n=3)

The antioxidant activity of OPC was well preserved in the process of coating. Antioxidant assay were also repeated 60 days after preparation of microcapsules, without significant changes of the measured values, showing that encapsulation was effecting in preserving OPC activity.
Again, the sample with 12% MD showed the best antioxidant efficiency, comparable to the value of the not encapsulated OPC (control). Thus, it can be assumed that these type of microcapsule are stable against OPC oxidative degradation. This effect is likely to be attributed to the protective function which the combination of gum arabic and maltodextrins develop as a result of encapsulation. Besides, these data confirmed the results of encapsulation efficiency.
MALDI-TOF-MS characterization of encapsulated samples showed integrity of OPC components in the microcapsules with no changes with respect to original OPC. Prior to analysis, a SPE purification step was necessary to remove salts and other contaminants from coating materials. In the pure OPC sample, an oligomeric series of catechin / epicatechin units and their gallic acid ester derivatives (sodium adductions, MNa+), up to the decamer was detected. Additionally, masses corresponding to a series of polygalloylpolyflavans were also detected in the samples extracted from microcapsules.

### 3.3 In vitro digestibility

The effect of simulated gastrointestinal digestion on the polyphenol availability of sample for which either the best EE or antioxidant activity were observed (12% MD/ 4% AG) was evaluated according to the Infogest protocol (Minekus et al., 2014; Romano et al., 2015) with some modifications.
The polyphenol content of the encapsulated sample after the gastric and intestinal digestion was 97.3 % and 75.5 %, respectively as percent of the initial values. It can be observed that while the level of non-encapsulated OPC, taken as control, was nearly maintained after the gastric phase (90.0 %), it was significantly decreased (26.5 %, about -74%) after the intestinal phase. This is expected considering the harsh conditions of digestion. On the contrary, in the encapsulated samples, the OPC levels after both phases of digestion were much higher. This demonstrates the protection provided by the coating matrix to the polyphenol core against oxidative and other chemical reactions and by the temperature and enzyme actions during the whole process of gastrointestinal digestion.

## 4. Conclusions

Antioxidant capacity resulted well preserved in the encapsulated OPC along time, showing high potential to protect the nutritional value of foods. In vitro digestion experiments showed that composition and functionality of encapsulated OPC were better preserved along gastrointestinal digestion process.
These results underline the effectiveness of the use of OPC microcapsules either as nutraceuticals or as additives in various food applications.

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