Microencapsulation of polyphenolic compounds recovered from red wine lees: Process optimization and nutraceutical study

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Abstract:	Bioactive polyphenolic compounds, directly recovered by nanofiltration of Cabernet sauvignon wine lees were encapsulated with maltodextrin to obtain a spray dried micro powder with enhanced nutritional value. The spray drying process was optimized by a quadratic factorial design, settling the optimal process parameters using a commercial grape–extract phenols dissolved in water and considering maltodextrin (carrier) concentration and the inlet temperature of the spray drier as the independent variables of the experiment. The microcapsules were characterized according to the drying yield and moisture, total bioactive compounds, surface bioactive compounds, microencapsulation efficiency, polyphenols recovery, and antioxidant activity. The stability of bioactive microcapsules obtained under optimal conditions was investigated using the stress– heat test (isothermal conditions 50±1°C for 22 days) and obtaining the degradation rate constants of total bioactive compounds and antioxidant activity. Further evidence about stability of the spray dried product raised by measuring the water activity of micro powders obtained under optimized processing conditions. Lastly, an in–vitro simulated digestion was performed under physiological conditions to investigate the bioaccessibility of microencapsulated polyphenols. Results indicate that the filtration can represent a valuable one–step low– cost green technology for separation of bioactive compounds from wine lees to exploit cheap source for functional food formulations.
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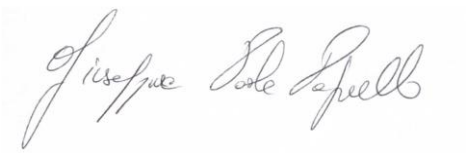
Dear Editor,

On behalf of all co-authors I submit to your attention the original manuscript titled "**Microencapsulation of polyphenolic compounds recovered from red wine lees: process optimization and nutraceutical study"** by **Ricci, A., Arboleda Mejia, J.A, Versari, A, Chiarello E., Bordoni, A and Parpinello, G.P.** This study investigates the possibility of obtaining microencapsulated with high bioaccessibility value from nanofiltrates of red wine lees, through the application of the spry-drying technique. We believe the study provides new and helpful knowledge for research and industry, as it demonstrates that bioactive compounds from enological wastes have potential health benefits and are valuable to be exploited for pharmaceutical, cosmetic and food applications.

We think this original research falls well within the scope of **Food and Bioproducts Processing** Journal.

Best regards,



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Highlights

1) Nanofiltration coupled to spry-drying allowed to exploit enological biproducts
2) Central Composite Design allowed to determine the best process conditions for microencapsulation
3) Microencapsulates were characterized by high nutraceutical values
4) Enological byproducts can be valorized by separation of bioactive compounds with high bioacessibility.

Microencapsulation of polyphenolic compounds recovered from red wine lees: process optimization and nutraceutical study

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Abstract Bioactive polyphenolic compounds, directly recovered by nanofiltration of Cabernet sauvignon wine lees were encapsulated with maltodextrin to obtain a spray dried micro powder with enhanced nutritional value. The spray drying process was optimized by a quadratic factorial design, settling the optimal process parameters using a commercial grape–extract phenols dissolved in water and considering maltodextrin (carrier) concentration and the inlet temperature of the spray drier as the independent variables of the experiment. The microcapsules were characterized according to the drying yield and moisture, total bioactive compounds, surface bioactive compounds, microencapsulation efficiency, polyphenols recovery, and antioxidant activity. The stability of bioactive microcapsules obtained under optimal conditions was investigated using the stress–heat test (isothermal conditions 50±1°C for 22 days) and obtaining the degradation rate constants of total bioactive compounds and antioxidant activity. Further evidence about stability of the spray dried product raised by measuring the water activity of micro powders obtained under optimized processing conditions. Lastly, an *in–vitro* simulated digestion was performed under physiological conditions to investigate the bioaccessibility of microencapsulated polyphenols. Results indicate that the filtration can represent a valuable one–step low–cost green technology for separation of bioactive compounds from wine lees to exploit cheap source for functional food formulations.

Keywords: Wine lees; Antioxidants; Polyphenolic compounds; Functional food; Bioaccessibility; *in vitro* digestion.

1. Introduction

Phenolic compounds are largely exploited in the nutraceutical industry due to their antioxidant activity; several studies evidenced that an increased intake of these compounds from the diet contributes to the reduction of oxidative damages and chronic diseases (Stanner et al., 2004; Goodman et al., 2011; Cianciosi et al. 2020; Chen et al., 2020). The winery by–products are primary sources of polyphenolic compounds, for this reason

there is a consolidated benefit in their functional exploitation, both establishing virtuous production chains and minimizing the impact of several tons industry wastes generated every year by the winemaking industry (Teixeira et al., 2014; Kalli et al., 2018).

Alongside the primary sources of polyphenols, i.e., grape pomaces including skin, seeds, and occasionally stems/ vine leaves, some interest in exploiting the wine lees (also known as 'dregs') has been recently observed (Giacobbo et al., 2019; De Iseppi et al., 2020). According to the EEC Regulation 337/79, lees are defined as the residue accumulated at the bottom of wine tanks following fermentation or other technological treatments (centrifugation, filtration) and during wine storage; they are mainly composed by cell walls matters from yeast autolysis, organic acids, and phenolic compounds. Focused studies on the selective recovery of polyphenols from the dregs have been published (Wu et al., 2009; Kopsahelis et al., 2018;); nevertheless, simple separative filtration of the liquid fraction retained by lees is a viable alternative for low–impact polyphenolic recovery (Giacobbo et al., 2017; Meija et al., 2019).

Despite a large part of the research involves the characterization of the liquid extract obtained from the natural sources, dried extracts are recognized for their advantage in terms of higher stability of active substances over time and lower storage costs (Barbosa et al., 2015; Moayyedi et al., 2018).

Spray drying is probably the drying technique most widely used in the food industry, due to highly available technology, versatility, and reduced operational costs (Piñón–Balderrama et al., 2020). Spray drying allow to process liquid polyphenolic sources to obtain microencapsulated powders with high polyphenolic content; in this context, the process parameters and the additive used as the inert carrier are key factors affecting both the spray drying yield and the physical–chemical properties of the micropowder (Moreno et al., 2016; Aliakbarian et al., 2018; González et al., 2019).

Maltodextrin (MD) is a widespread biopolymer used as the carrier for polyphenolic encapsulation, due to several advantages including low viscosity at high solid contents, good solubility and notable heat protection capacity, long–term resistance, and pleasant flavor (Gharsallaoui et al., 2007, Tuyen et al., 2010, Robert et al., 2010). The low moisture content of MD–based encapsulates ensures the stability of microcapsules over time if adequate storage conditions are applied, mainly a suitable water concentration gradient between the product and air interface (Tonon et al., 2009). The usage of emulsifier is not envisaged to achieve optimal spray drying of polyphenolic compounds, due to their relative hydrophilicity; nevertheless, it has been demonstrated that the combined used of maltodextrin, either alone or in combination with alternative biopolymers (i.e., gum Arabic, soy/whey proteins) might improve the encapsulation especially for hydrophobic polyphenols like flavan–3–ols and tannins (Busch et al., 2017; Tan et al. 2015; Tolun et al. 2016).

The present study combines spray drying multifactorial optimization design with a standardized grape extract and maltodextrin, followed by processing of lees filtrates and characterization of relevant physical–chemical properties of micro powders.

Bioaccessibility, i.e., the release and solubility of bioactive compounds during gastrointestinal digestion for further uptake, is a considerable factor for bioavailability, which is a main determinant for biofunctional properties and possible beneficial effects of polyphenols (Parada and Aguilera, 2007). Since incorporation

within micro–/nanoparticle delivery systems can modify bioaccessibility (Ozkan et al., 2020), microcapsules underwent *in vitro* digestion, and the bioaccessibility, and antioxidant capacity were evaluated.

2. Materials and methods

2.1. Materials and chemicals

Liquid wine lees obtained from Cabernet sauvignon vinification were kindly provided by Cantina di Terre Naldi (Faenza, Emilia–Romagna, Italy) and stored at -20°C until usage.

The extract used to settle the experimental spry-drying design was a commercial grape tannin from Laffort (Bordeaux, France); it was supplied as a liophilized powder and directly dissolved in Milli–Q water (Millipore Corp., Bedford, MA, USA) at the 1 g/L concentration. Maltodextrin Dridex 13–17 (MD 13–17 DE, Merk Group, Darmstadt, Germany) was used as the natural carrier for microencapsulation of bioactive polyphenols. The gallic acid and (+)–catechin standards, anhydrous sodium carbonate and the Folin–Ciocalteu's reagent used for polyphenols quantitation were purchased from Sigma–Aldrich (St. Louis, USA). All chemicals and enzymes used for in vitro digestion were from Sigma–Aldrich (St. Louis, USA).

2.2. Proximate composition and antioxidant activity of grape extract and wine lees filtrates

The grape extract (1 g/L in 12% hydroalcoholic solution) used to optimize the spray drying process was analysed in terms of total bioactive compounds (Singleton & Rossi, 1965; concentration expressed as mg GAE/100 mg dw), iron–reactive polyphenols and proanthocyanidins (Harbertson et al., 2002; concentration expressed as mg CE/100 mg dw).

Polysaccharides in the grape extract were measured according to the colorimetric method after Segarra et al. (1995).

The filtrates of Cabernet sauvignon wine lees (first racking) used for microencapsulation, were obtained using a laboratory–scale filtration device previously described (Arboleda et al., 2020). Four distinctive samples including bioactive compounds were obtained from the filtration process: i) retentate (hereafter referred to as RET); ii) permeate from thin–film composite nanofiltration commercial membrane NF90 (hereafter referred to as PERM1) supplied by Dupont Filmtec (USA); iii) permeate from a cellulose acetate laboratory–made membranes coded as CA400–22 (hereafter referred to as PERM2); iv) permeate from a cellulose acetate laboratory–made membranes coded as CA316–70 (hereafter referred to as PERM3). The laboratory–made membranes were prepared according to the phase inversion method reported by Kunst and Sourirajan (1974). Retentate and filtrates were analyzed in terms of total polyphenols content by means of the Folin–Ciocalteu's colorimetric assay (Singleton & Rossi, 1965), with results expressed as mg gallic acid equivalent/L (mg GAE/L) of the liquid lees fed to the filtration system. The total polyphenols content of retentate and filtrates was as follows: RET: 587±22 mg GAE/L; PERM1 82±4 mg GAE/L; PERM2: 102±8 mg GAE/L; PERM3: 71±1 mg GAE/L.

All samples contained approximate 12% ethanol (v/v), which was trapped into an appropriate condenser during the drying process (see paragraph 2.3).

The antioxidant activity (AA) of grape extract and wine lees filtrates were determined by the 2,2 –azino–bis(3–ethylbenzothiazoline–6–sulfonic acid) (ABTS•+) – based colorimetric assay (Re et al., 1999); results were expressed as (%) scavenging activity.

2.3. Spray drying process

2.3.1. Optimization of the spray drying conditions

A Mini Spray Dryer B–191 (Büchi Laboratoriums–Technik, Flawil, Switzerland) equipped with a 0.7 mm nozzle was employed for obtaining maltodextrin (MD) microencapsulation of the grape extract–derived polyphenols. The following operational parameters were kept constant throughout the experiments: aspiration rate 100 %, compressed air flow 800 NI/h, pressure 50 mbar, percentage of the peristaltic pump 20%. Outlet temperatures in the experimental design were 79, 100 and 124 °C, corresponding to inlet temperatures of 110, 135 and 160 °C, respectively.

A Central Composite Design (CCD) including two replications in the central point was used to optimize the spray drying conditions (Design Expert 11.0v, USA). The experimental design included 10 experiments and their replications, with overall 20 determinations (**Table 1**). Variable factors were: inlet temperature of the spray drier (Ti, variability range 110–160 °C) and MD concentration levels (MD, variability range: 5–15 g/100 mL). MD was added directly in the grape tannin solution at the different concentration levels provided by the experimental design (5%, 10%, 15%, respectively). A large excess of natural carrier was applied for ensuring high microencapsulation efficiency; the carrier/extract ratios varied between 5:0.1, 10:0.1 and 15:0.1 (i.e., 5 g of carrier per 0.1 g of grape extract, thereof containing 67.7% total polyphenols).

The equipment was carefully washed with water between different spray drying processes. All spray–dried powders were collected, weighed, sealed in plastic vials and immediately used for the analytical determinations.

Observations	Maltodextrin (g/100 mL)	Temperature (°C)
1	15	135
2	10	135
3	10	135
4	15	110
5	10	110
6	15	160
7	10	160
8	5	135
9	5	110
10	5	160

Table 1. The central composite design (CCD) experiment used to optimize the spray drying process parameters.

Optimal conditions from DoE were applied for MD microencapsulation of the grape extract and of the wine lees filtrates. The presence of ethanol in filtered lees was managed by using a Mini spray drier Büchi B290 equipped with Inert loop B295 (condenser) and a 0.7 mm nozzle (Büchi Laboratoriums–Technik, Flawil, Switzerland) under the following operational parameters: 6 mL/min feed rate (20%); 100% aspiration; 600 NL/h dry nitrogen flow to provide enhanced protection against undesirable oxidations (residual oxygen levels during the experiments: 0.35±0.10 %); outlet temperatures ranging 63–70°C.

2.3.2. Drying yield (DY)

Spray drying yield was evaluated according to Fazaeli et al. (2012) and expressed as the percentual ratio between the total mass of product recovered by the mass of extract fed to the system (dry basis).

2.4. Physical–chemical characterization of the spray–dried powders

2.4.1. Moisture content (Mo)

The moisture content was determined using the official AOAC method (AOAC, 1990), with minor modifications. Duplicate samples of microencapsulate powder (1 g each) were weighed and then dried in a vacuum oven at 50 °C. The monitoring of weight loss was repeated on the daily basis and the moisture content (Mo) was expressed as the percentage of weight reduction at the time when constant weight was obtained (*1*), according to Mohammed et al. (2017):

Moisture (%) = ((W1-W2)/W1) x 100 (*1*)

With:

W1 = weight of the sample before oven–dried (g)

W2 = weight of the sample after oven–dried (g)

2.4.2. Total bioactive compounds (TBC)

The analysis of total bioactive compounds (TBC) of microencapsulates was performed according to Robert et al. (2010) with minor modifications: briefly, the microcapsules were destructed by adding 25 mg of the dried powder in 1 mL of methanol: acetic acid: water solution (50:8:42 v/v/v). Microcapsules were dissolved by vortex (1 min) followed by ultrasonication (20 min); the procedure was repeated twice. Samples were then centrifuged at 14500 rpm for 5 minutes then the supernatant was collected and filtered using a 0.22 µm cellulose acetate syringe filter.

The TBC value was determined through the Folin–Ciocalteau method according to Singleton and Rossi (1965) and results were expressed as mg GAE/100 mg microencapsulated powder.

2.4.3. Surface bioactive compounds (SBC) and microencapsulation efficiency (ME)

The analysis of bioactive compounds adsorbed to the microcapsules surface (SBC) was performed according to Robert et al. (2010) with minor modifications: briefly, 25 mg of microcapsules were added to 1 mL of a mixture of ethanol and methanol (1:1 v/v); samples were vortexed for 1 min then filtered using a 0.22 µm cellulose acetate syringe filter. The SBC value was determined through the Folin–Ciocalteau method (Singleton & Rossi, 1965) and results were expressed as mg GAE/100 mg microencapsulated powder.
The following equations (*2, 3*) were applied to obtain SBC and ME percentages (Robert et al., 2010):

SBC (%) = (SBC/TBC) x 100 *(2)*

ME (%) = 100 – SBC (%) *(3)*

2.4.4. Polyphenol's retention (PR)

Polyphenol's retention (PR) after spray drying was calculated according to the method described by Fang and Bhandari (2011), based on dry matter measurements (*4*):

TR(%) = (TBC(%) in spray dried powder (mg/100 mg)/ TBC(%) in feed solution (mg/100 mg)) x 100 *(4)*

2.4.5. Antioxidant activity (AA)

The antioxidant activity of the dried powder was evaluated by means of the ABTS•+ colorimetric assay (Re et al., 1999) following preliminary treatment of the microcapsules described in the Section 2.4.2. Results were expressed as percentage scavenging activity by using the following equation (*5*):

$$AA\ (\%) = ((Abs\text{–}734nm_{\ reagent\ blank} - Abs\text{–}734nm_{\ sample})/\ Abs\text{–}734nm_{\ reagent\ blank)} \times 100 \quad (5)$$

2.4.6. Water activity (Aw)

The water activity (A_w) of microcapsules obtained following the spray drying from cabernet sauvignon wine lees was measured in water activity meter AquaLab 3T (Pullman, Washington) at 25 °C. Samples (1–2 g of each) were analyzed in triplicate and A_w were reported as mean values.

2.4.7. Storage stability evaluation

Microcapsules obtained under spray drying optimal conditions were assessed for their stability under accelerated aging. In detail, 1 gram of each of the three replicates was placed in a plastic vessel and stored at 50 ± 1 °C for 22 days. Aliquots (25 mg each) of the microencapsulates were collected with a two–days frequency and measured according to TBC (mg/100 mg) and AA (%) values. The kinetic rates of TBC and AA decrease over time can be written as (*6*):

$$- d[A] / dt = k [A] \quad (6)$$

Rearrangement yields the following (7):

$$d[A] / [A] = - k\, dt \quad (7)$$

To obtain a linear equation we integrate the Eq. (*7*) to obtain (*8*):

$$\ln [A] = - kt + C \quad (8)$$

Considering the general equation y = mx + b, we consider the y–value is ln [A], m equals negative k, the x–value is t, and the y–intercept is ln $[A]_o$. A plot of ln [A] versus t is a line with slope corresponding to negative k (first–order kinetic rate).

2.5. *In vitro* digestion

The spray dried powders obtained by processing wine lees permeates and retentate with maltodextrin under optimal spray drying conditions (PERM1, PERM2, PERM3, RET, see section 3.4) underwent *in vitro* oral–gastric–intestinal digestion according to the INFOGEST standardized protocol (Minekus et al., 2014). Briefly, to simulate the oral phase 1.8 g of each sample were mixed with 1.44 mL of simulated salivary fluid (SSF) containing 15.1 mM KCl, 3.7 mM KH_2PO_4, 13.6 mM $NaHCO_3$, 0.15 mM $MgCl_2(H_2O)_6$, 0.06 mM $(NH_4)_2CO_3$, pH 7. Since the investigated product was starch–free, alpha amylase was not added to SSF (Minekus et al., 2014). Nine µL of 0.3 M $CaCl_2$ and 0.351 mL of water were immediately added and mixed thoroughly. After 2 min of oral digestion, to simulate the gastric phase 2.88 mL of simulated gastric fluid (SGF) (6.9 mM KCl, 0.9 mM KH_2PO_4, 25 mM $NaHCO_3$, 47.2 mM NaCl, 0.12 mM $MgCl_2(H_2O)_6$, 0.5 mM $(NH_4)_2CO_3$, pH 3) and 1.8 µL of 0.3 M $CaCl_2$ were added, and the pH was adjusted to 3 with 0.1 mL of 37% HCl. Then, 4.5 mg of porcine gastric mucosa pepsin (2000 U/mL) dissolved in 0.62 mL of water were added and mixed. After further 120 min, 5.76 mL of simulated intestinal fluid (SIF) (6.8 mM KCl, 0.8 mM KH_2PO_4, 85 mM $NaHCO_3$, 38.4 mM NaCl, 0.33 mM $MgCl_2(H_2O)_6$, pH 7), 220 mg of porcine pancreatin (100 U/mL), 14,4 µL of 0.3 M $CaCl_2$ and 72 mg of 10 mM bile dissolved in 1.43 mL of water were added and mixed. After additional 120 min, the final volume (14.4 mL) of each digested sample was collected in conical tubes and frozen at -20°C until use.

2.6. Statistical analysis

All experiments were conducted in duplicate. Results from the CCD Experiment, including the analysis of significant effects at $p < 0.05$ and the surface response methodology were performed using the Design Expert software (Stat–ease, Minneapolis, USA). The Microsoft Excel program (Microsoft Corporation, Washington, USA) was used to process results from the analytical determinations (expressed as mean values ± SD) as well as for the kinetic studies from the stability test.

3. Results and discussion

3.1. Grape extract characterization and bioactive content

Table 2 reports the proximate compositional information of the grape extract used to develop the CDD experiment. Polyphenols (TPC) constitute approx. 68% of the dry weight of the extract, mostly characterized as iron–reactive polyphenols (98.5% of TPC) with enhanced reactivity against metal catalysts and free radical species. Tannins (proanthocyanidins) constitute the 38.3% of total polyphenols in the extract. The residual dry weight fraction is dominated by polysaccharides, possibly derived from the structural tisses of grapes, representing approx. 25.4% of the dry weight of the extract.

TPC (mg GAE/100 mg dw)	67.7±3.4
Iron–reactive polyphenols (mg CE/100 mg dw)	66.7±1.5
Tannins (mg CE/100 mg dw)	25.9±0.6
Polyphenolic substances/dry weight (%)	67.7
Tannins/TPC (%)	38.3
Polysaccharides (mg Glu/100 mg dw)	25.4±3.7
Radical scavenging (% ABTS•+ scavenging)	57.3±5.6

Legend: TPC: Total phenolic compounds; GAE: Gallic acid equivalents; CE: (+)–Catechin equivalents; Glu: Glucose equivalents.

Table 2. Composition of the liophilized grape pomace extract used in the CCD experiment.

According to previous literature, a relevant content of complex sugars in the extract is considered a potentially advantageous condition for the spray drying experiment. In fact, polysaccharides from grapes (primary pectins) are likely to interact with hydrophobic compounds (i.e. tannins) resulting in colloidal systems with high retention of polyphenols (Carvalhoet al., 2006); moreover, maltodextrins/pectin mixtures improve organoleptic properties and stability of spray–dried powders (Sansone et al., 2011).

3.2. Microencapsulation of grape extract–polyphenols

Table 3 summarizes results from the experimental design, including relevant parameters accounting for the effectiveness of the spray–drying process.

The moisture content (Mo) was considered apart from the experimental design, and it was not included in the model. Mo is a key parameter accounting for the shelf life of powders affecting the drying efficiency, powder flowability, stickiness, and storage stability (Mahdavi et al. 2016). Mo in the microencapsulates obtained from DoE, ranged 0.85 to 2.80% with average 1.40±0.57% value; these values were generally lower than those reported from the literature, even from experiments performed under dehumidified air conditions (Mohammed

et al. 2017; Mahdavi et al. 2016; Goula and Adamopoulos, 2005), falling in typical values reported by Tan et al. (2015) which used a combination of maltodextrin and gum Arabic as encapsulating agent.

The low moisture content positively affects the physico–chemical properties of the microencapsulates limiting the ability of water to act as a plasticizer and to reduce the glass transition temperature (Tg); this is important (1) to ensure reduced surface stickiness of droplets of sugar–enriched formulations, thus enhancing the spray–drying process yield and improving processability, handling properties and stability of powders (Adhikari et al., 2005; Roos, 2002); (2) during storage of the microencapsulates, preventing typical physico–chemical modifications (collapse, caking, agglomeration, browning and oxidation) which may be accelerated when the storage temperature falls above the Tg value of the powder and the amorphous phase of the powder become predominant (Bhandari & Howes, 1999).

DoE was applied to settle optimal conditions for the microencapsulation of polyphenols; linear, quadratic and cross–product forms were considered for the "encapsulating agent" and "inlet temperature" independent variables at $P \leq 0.05$ levels. **Figure 1a–e** show response surfaces for the CCD experimental design.

The drying yield (DY, **Figure 1a**) of the process was not significantly affected ($p > 0.1$) by the factors selected in this DoE for both linear and quadratic terms of the model. The yield of the spray drying process (DY) ranged 44.6–63.4 %, with average 56.6±6.2 % value (**Table 3**); absolute values of powders obtained after spray drying followed an obvious trend related to the initial carrier concentration levels (maltodextrin: 5–10–15 g/100 mL) used in the experiments. In general terms, the loss rates with respect to the initial solid matter fed to the spray drier ranged 32.2–46.5 %, with average 36.9±6.7% decrease.

The micro–powders obtained were partly retained into the spray drying chamber and to the cyclone walls, in an extent which is dependent on the water retention during the drying process and final moisture content of the powder (Roos, 2002; Tolun et al., 2016); in this experiment the retention effects were neglected, considering the spray dried material which was allowed to reach the collector cabin.

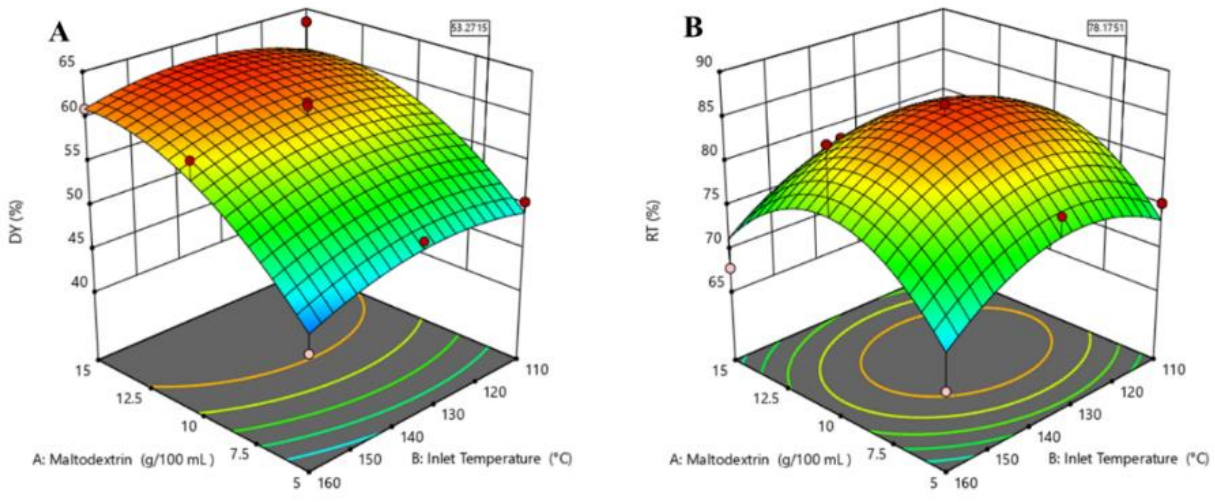
The polyphenolic compounds retained after the spray drying process (PR, **Figure 1b**) were not significantly affected by the carrier concentration, the inlet temperature, and their combined effects in this case study. On the opposite, the total bioactive content (TBC, **Figure 1c**) of the dried powders ranged 0.30–1.04 % of the dry weight of the microencapsulated powders (**Table 3**) and was significantly affected by the quadratic term of the content of wall material ($p = 0.041$); the other linear and quadratic terms failed to display a significant influence on the total bioactive content ($p > 0.05$). Therefore, the encapsulating agent was the most significant variable for maximizing the total polyphenols content of the powders under the experimental conditions, and it was connected to a dilution effect observed when the maltodextrin concentration was raised. Same result was obtained by Robert et al. (2010) in the microencapsulation of pomegranate juice with maltodextrin as the carrier agent, and by Mishra et al. (2014) in the encapsulation of *Emblica officinalis* extracts.

The surface bioactive compounds (SB) parameter accounts for bioactive compounds which were not effectively encapsulated; in this DoE they constitute a minor fraction of the total bioactive content, laying in the range 0–9.22% of TBC, resulting in high microencapsulation efficiencies (ME> 90%) regardless the carrier concentration and the inlet temperature values; as a confirmation, none of the factors and level combinations

settled by the experiment showed significant effect on SBC and ME ($p > 0.10$). The ME (**Figure 1d**) guarantees the stability and controlled release of the bioactive compounds (Ozkan et al., 2019) and performances were very high in this DoE compared to previous literature: typical ME values ranged 65–77% (Paini et al., 2015), 53.5–71.0% (Robert et al., 2010), 69.6–73.4% (McNamee et al., 2001), 69–75% (Pasrija et al., 2015) and 18.4–45.0% (Sun–Waterhouse et al., 2013). In the present study high ME yield may be discussed in view of the large excess of maltodextrin involved, also resulting in a limited polyphenolic content per unit weight (up to 1.04 % of the dry weight of the powder).

The antioxidant activity (AA, **Figure 1e**) displayed by microencapsulates (range 27.6–93.1 %) was significantly affected by the experimental factors selected in this DoE: main contributions raised from the quadratic term of the carrier (maltodextrin) concentration in the feed solution (p= 0.003), followed by a significant contribution of the maltodextrin – inlet temperature binary combination (p= 0.0156) and of both linear (p= 0.025) and quadratic (p= 0.014) terms of the inlet air temperature. Targeted analyses on aqueous solutions containing maltodextrin at the different concentration levels (i.e., 5–15 g/100 mL) proved that the carrier did not display any ability to neutralize the ABTS•+ radical as such (radical scavenging activity < 2% in all cases), confirming previous results from the literature (Sahin–Nadeem et al., 2013; Mishra et al., 2014). Peak values were reached when the higher active compound/carrier ratio occurs (i.e., maltodextrin level 5 g/100 mL), confirming the trend highlighted in previous studies (Tuyen et al., 2010; De Souza et al., 2014; Silva et al., 2013).

The inertness of maltodextrin with respect to reducing and redox mechanisms is possibly the basis for the enhanced antiradical and storage stability of the maltodextrin microencapsulated; higher retention of their antioxidant properties during storage was observed compared to performances of different carrier agents, i.e., HP–β–cyclodextrin and Arabic gum (Wilkowska et al., 2016). All these stated, we concluded that on the one hand microcapsules have a protective action towards the inner polyphenolic compounds, and on the other they do not trigger side–reactions with them preserving their original bioactivity in time.



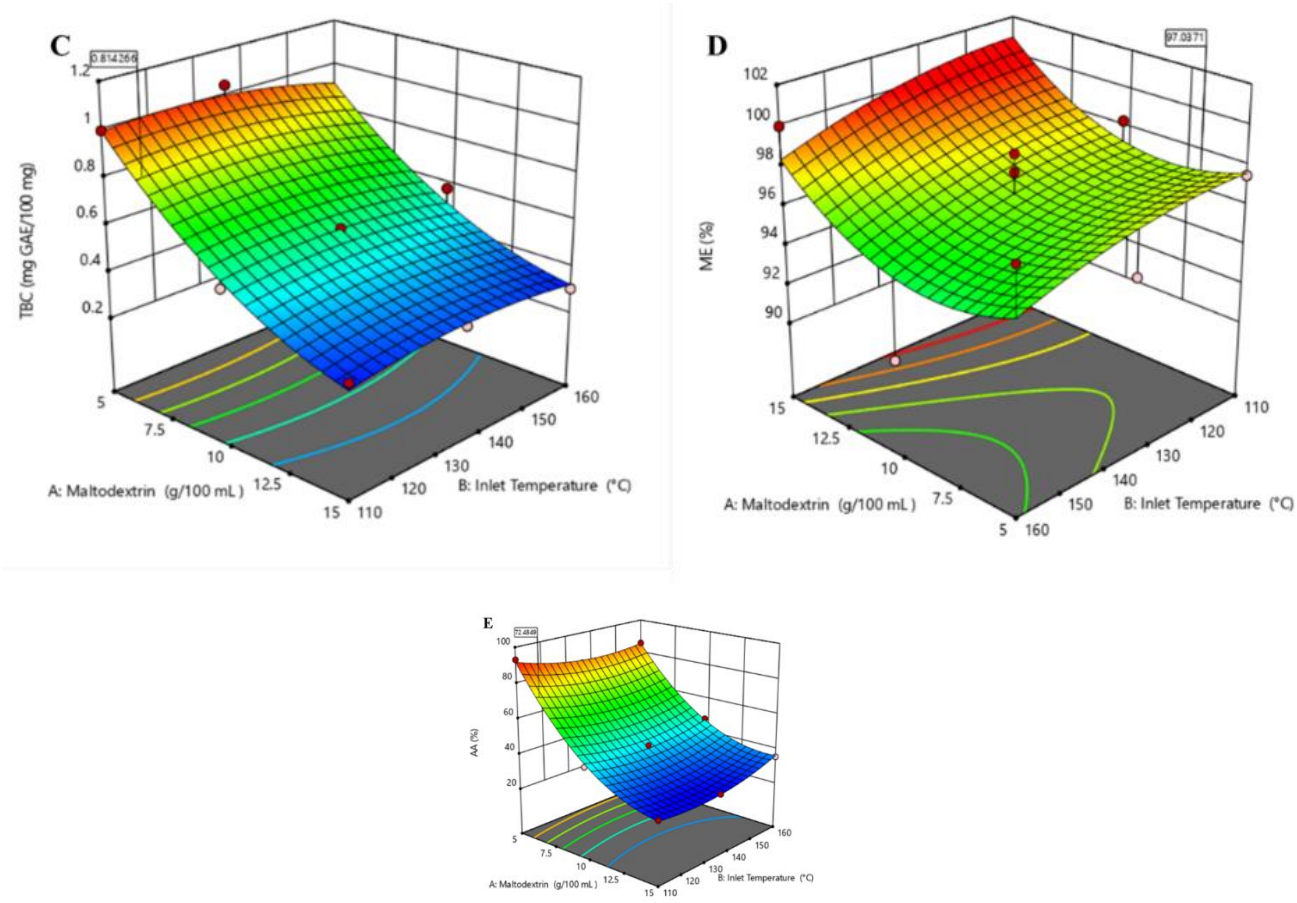


Figure 1. Response surfaces obtained for the CCD experimental design showing the effect of the maltodextrin concentration and inlet temperature on selected properties of microencapsulated. **A.** Drying yield (DY, %); **B.** Polyphenols retention (PR, %); **C.** Total bioactive compounds (TBC, mg GAE/100 mg dw); **D.** Microencapsulation efficiency (ME, %); **E.** Antioxidant activity (AA, %).

The following optimal conditions were settled by DoE to optimize performances of the spray drying process: (a) Maltodextrin concentration: 7 g/100 mL; (b) Inlet temperature: 110°C. Such conditions were applied to the same grape extract and to Cabernet sauvignon wine lees filtrates.

Observation	**MDX** *(g/100 mL)*	**T** *(°C)*	**SDP** *(g (fw))*	**Mo** *(%)*	**DY** *(%)*	**TBC** *(mg GAE/100 mg dw)*	**SBC** *(mg GAE/100 mg dw)*	**SBC** *(%)*	**ME** **(***%)*	**PR** *(%)*	**AA** *(%)*
1	15	135	9.50±0.13	1.27±0.09	59.4±0.8	0.35±0.01	0.003±0	0.86±0.08	99.5±0.6	78.4±2.3	27.6±3.1
2	10	135	6.73±0.32	0.99±0.01	61.2±2.9	0.54±0.02	0.007±0.002	1.37±0.32	98.6±0.3	80.8±3.0	41.3±2.02
3	10	135	6.78±0.05	0.89±0.06	61.6±0.4	0.58±0.01	0.013±0.002	2.28±0.38	97.7±0.4	86.4±1.2	43.6±1.2
4	15	110	10.15±0.06	1.13±0.06	63.4±0.4	0.34±0.01	0.000	0.0	100.0±0.0	76.8±2.7	28.7±2.9
5	10	110	5.91±0.43	2.41±0.55	53.7±3.9	0.52±0.01	0.009±0	1.72±0.02	98.3±0.0	77.2±0.9	44.3±2.3
6	15	160	9.75±0.88	0.89±0.05	60.9±5.5	0.30±0.00	0.000	0.0	100±0.0	67.8±0.0	35.3±2.4
7	10	160	6.57±1.76	1.36±0.45	59.7±16.0	0.58±0.02	0.053±0.006	9.22±0.62	90.8±0.6	86.3±3.7	47.6±3.4
8	5	135	3.06±0.13	1.48±0.30	51.0±2.2	1.04±0.08	0.053±0.020	5.15±2.62	94.8±2.6	78.7±6.3	82.5±1.1
9	5	110	3.03±0.12	2.34±0.26	50.4±2.0	1.00±0.02	0.025±0.002	2.47±0.16	97.5±0.2	75.2±1.4	93.1±1.2
10	5	160	2.68±0.49	1.24±0.33	44.6±8.1	0.87±0.01	0.017±0	2.01±0.01	98.0±0.0	65.5±0.5	85.8±2.4

Legend: MDX: Maltodextrin; T: Temperature; SDP: Spray dried powder; Mo: Moisture content; DY: Drying yield; TBC: Total bioactive compounds; SBC: Surface bioactive compounds; ME: Microencapsulation efficiency; PR: Polyphenol's retention; AA: Antioxidant activity; GAE: Gallic acid equivalents.

Table 3. Experimental outcomes from the CCD experiment (mean ±SD).

3.3. Application of optimal spray drying parameters: characterization of the microencapsulates and stability test

Table 4 summarizes the experimental results for microencapsulates obtained under optimized spray drying conditions; the commercial grape extract was dissolved in the same concentration of the experimental design (1 g/L) to verify performances of the CCD outcomes.

Mo *(%)*	**DY** *(%)*	**TBC** *(mg GAE/ 100 mg*	**SBC** *(mg GAE/ 100 mg dw)*	**SBC** *(%)*	**ME** *(%)*	**PR** *(%)*	**AA** *(%)*
1.40±0.24	70.0±2.7	0.73±0.06	0.056±0.038	7.6±5.3	92.4±5.3	87.4±7.7	61.8±1.7

Legend: Mo: Moisture content; DY: Drying yield; TBC: Total bioactive compounds; SBC: Surface bioactive compounds; ME: Microencapsulation efficiency; PR: Polyphenol's retention; AA: Antioxidant activity; GAE: Gallic acid equivalents.

Table 4. Characterization of the spray dried powder (mean ±SD) obtained with optimal process parameters from DoE (maltodextrin: 7 g /100 mL; inlet temperature: 110°C).

The microencapsulates showed an average 29.1±2.7% reduction of solid matter with respect to the solid content of the feed solution (approx. 7 g considering the maltodextrin content), and, contextually, a high yield of the drying process (DY 70.0±2.7 %, **Table 4**). At a glance, this result is contradictory compared to the experiment 4 of the CCD, where the higher maltodextrin level, 15 g/100 mL of the feed solution provided best performances in terms of drying yield (63.4±0.4 %, **Table 3**); nevertheless, solid matter recovery in the CCD experiments showed a stochastic trend connected to the amount of solid matter which was allowed to reach the collector cabin (spray drier performances), more than to the experimental conditions settled.

Moisture content (Mo) of the microencapsulates (1.40±0.24%, **Table 4**) was aligned with average values observed along the CCD experiment (1.40±0.57%) with potentially positive impact in the storage stability.

The TBC value felt within the limits settled by the experimental design (0.30–1.04 %), with compromise results between ensuring high microencapsulation efficiency (ME 92.4±5.3%, **Table 4**) and maximizing the content of bioactive compounds (TBC 0.73±0.06 mg GAE/100 mg dw, **Table 4**). The antioxidant activity displayed by microcapsules showed satisfactory results (61.8±1.7%, **Table 4**), aligned with the availability of polyphenols (TBC value) in the powders.

The retention of polyphenols (RP) during the spray drying process was high in this experiment (87.4±7.7%, **Table 4**); nevertheless, the experiments failed to minimize the surface bioactive compounds (SBC 7.6%), meaning that a not negligible fraction of polyphenols is not embedded as core compounds but simply adsorbed to the surface of maltodextrin capsules. High standard deviation (± 5.3%) informed about the limited control over the encapsulated polyphenols under these experimental conditions. The SB compounds do not advantage

of the protective effect of the carrier material; for this reason, a detailed study on the stability of microcapsules was settled to predict storage stability and retention of bioactive properties of the powders obtained (heat–stress test).

Figure 2 shows the natural log the total bioactive compounds measured in the microencapsulates under accelerated aging conditions; the degradation kinetic, resulting in a decrease of TBC content over time, fits a first order kinetic equation. The kinetic rate values (K_{obs} = 1.05 x 10^{-2}±0.10 x 10^{-2} $days^{-1}$) resembles previous findings from the literature (Robert et al., 2010; Tolun et al., 2016), with storage temperature of 60°C.

Despite the good linearity of the graph (R^2 = 0.949, **Figure 2(a)**), it was observed that a second–order polynomial curve provided an enhanced fitting of experimental data (R^2 = 0.987, **Figure 2(b)**). This is consistent with previous observations (Tolun et al., 2016), reporting that the pseudo–first order degradation graph of polyphenolic compounds showed two distinctive steps, including a second slope with a slower rate. Authors referred this peculiar trend as the consequence of degradative effects involving the surface bioactive compounds (SBC in this study), corresponding to a faster rate, followed by a slowing down of the curve in correspondence of degradation of the polyphenolic compounds (Tolun et al., 2016); this is a further confirmation of the protective effect displayed by the carrier material on the encapsulated bioactive compounds.

The limited SBC content observed in this study did not allow us to appreciate significant differences in the SBC degradation over time in the accelerated aging test (*data not shown*), for this reason we assumed that the first–order linear equation could provide satisfactory kinetic description to account for the shelf life of microcapsules.

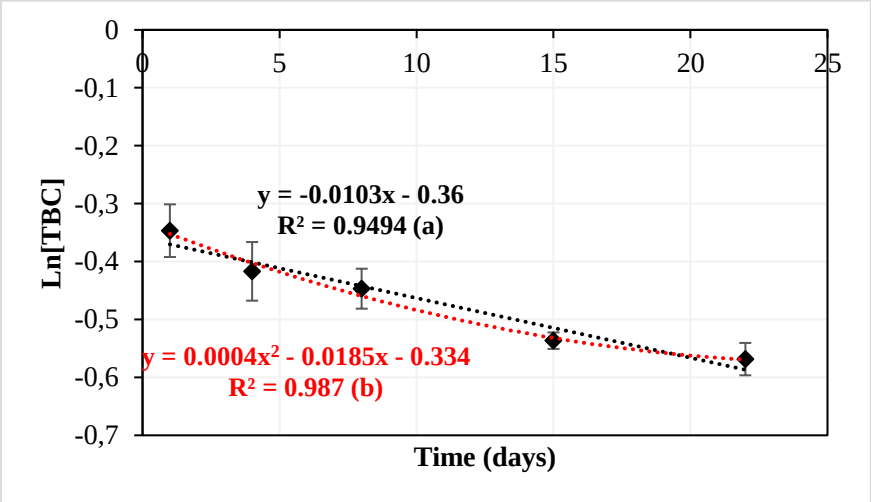


Figure 2. TBC degradation of microcapsules obtained under optimal conditions and stored at 50 ± 1°C. (a) pseudo–first order kinetic graph; (b) second–order polynomial curve.

Figure 3 highlights a similar trend for the reduction of the microcapsules' AA in time and under heat–stress conditions. The reduction of the ABTS•+ radical scavenging capacity followed a first order kinetic (K_{obs} = 2.37 x 10^{-2}±0.99 x 10^{-2} $days^{-1}$), and same observations as from the TBC degradation can be applied about the potential two–step mechanism involved in the thermal degradation (**Figure 3–(a), (b)**).

In absolute terms, the thermal treatment induced a percentage reduction of the antiradical capacity of 31.4±4.4 % with respect to the values obtained at the time zero, meaning that approx. 70% of the original antiradical capacity is retained under extreme storage conditions.

Results confirmed that an improved protection of the polyphenolic compounds encapsulated is observed when using an excess of carrier material in the spray drying procedure, with retention of their antiradical capacity over time. In particular, the spray dried polyphenols exhibit enhanced stability in comparison to alternative storage method (freeze–drying or simple aqueous extraction) due to the protective effect of the inert carrier (De Souza et al., 2014).

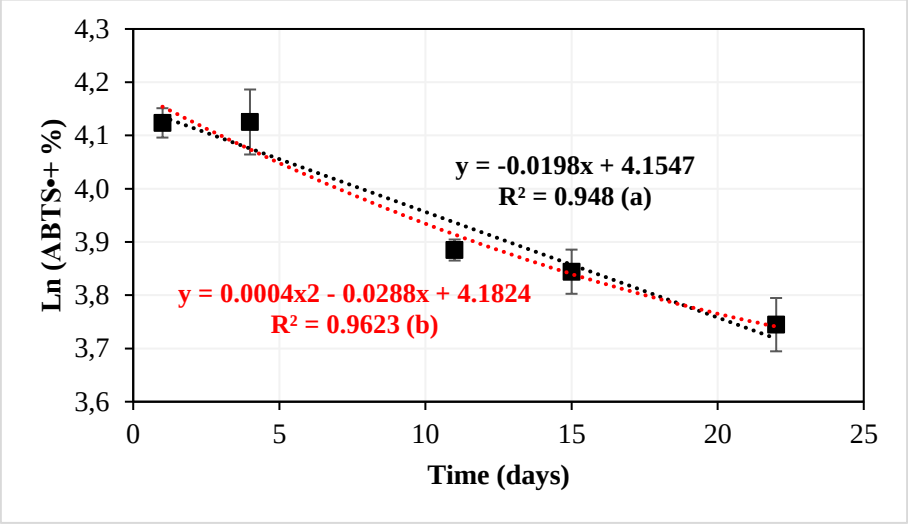


Figure 3. AA decrease of microcapsules obtained under optimal conditions and stored at 50±1°C. (a) pseudo–first order kinetic graph; (b) second–order polynomial curve.

3.4. Microencapsulation of polyphenols from Cabernet sauvignon wine lees filtrates

The optimized spray drying process was applied to the encapsulation of polyphenols obtained by filtering first racking liquid lees obtained from Cabernet sauvignon vinification; this experiment aimed at hypothesizing a virtuous chain for the recovery of a by–product of the wine industry, obtaining a food supplement with high nutraceutical value. **Table 5** summarizes the technological outcomes of microencapsulation.

Sample	Mo *(%)*	DY *(%)*	TBC *(mg GAE/100 mg dw)*	SBC *(%)*	ME *(%)*	PR *(%)*	AA *(%)*	Aw (25 °C)
PERM1	1.52	73.9	0.10±0.01	30.6±10.4	69.4±10.4	82.7±4.9	8.5±2.8	0.31±0.0
PERM2	0.81	69.1	0.09±0.01	34.5±16.9	65.5±16.9	57.4±5.0	7.8±1.1	0.30±0.0
PERM3	1.27	62.9	0.08±0.01	5.8±2.9	94.2±2.9	81.7±7.5	7.2±1.7	0.36±0.02
RET	1.32	82.0	0.57±0.03	8.2±1.3	91.8±1.3	68.1±3.1	30.5±2.7	0.27±0.0

Legend: Mo: Moisture content; DY: Drying yield; TBC: Total bioactive compounds; SBC: Surface bioactive compounds; ME: Microencapsulation efficiency; PR: Polyphenol's retention; AA: Antioxidant activity; GAE: Gallic acid equivalents; A_w: Water activity.

Table 5. Characterization of the spray dried powder obtained by processing wine lees permeates and retentate with maltodextrin under optimal spray drying conditions.

All samples exhibited a low Mo, ranging 0.81–1.52% with an average of 1.23%, aligned with the result obtained by microencapsulation of the commercial grape extract (1.40%, **Table 4**). The drying yield of the wine lees microencapsulated ranged 62.9–82.0% with average 71.9%; in particular, the mean drying yield value resembles the result obtained in the experiment for optimization of operating conditions (**Table 4**).

The TBC values of the microcapsules ranged 0.08–0.57 mg GAE/100 mg dw, with an average value of 0.21±0.24 mg GAE/100 mg dw. The bioactive compounds retained in the surface of microcapsules (SBC) ranged 5.8–34.5% with a mean value of 19.7±14.8%; these results showed higher values if compared to microencapsulation of polyphenols from the grape extract in aqueous solution. The potential impact of ethanol removal during spray drying of lees filtrates in the SBC parameter will be evaluated in future works.

As a consequence of enhanced SBC values, the average microencapsulation efficiency (ME) obtained in the spray dried powder obtained by processing wine lees (80.22±14.8%, **Table 5**) was reduced with respect to the previous experiment, ranging 65.5–94.2 %. The polyphenols retention (PR) is within the range 57.4–82.7 with respect to the solutions fed to the drying system, with a mean value of 72.4±12.0% (**Table 5**).

Among samples, spray drying of PERM1, PERM3 and RET resulted in microcapsules with higher ME and PR; contrariwise, the sample PERM2 presented a low retention of polyphenols (57.4%) resulting in higher surface bioactive compounds level (SBC 34.5%, **Table 5**).

The AA of the microcapsules ranged 7.2–30.5%, with a mean value of 13.5±11.3% (**Table 5**); results are aligned with the total availability of polyphenolic compounds in the micro powders (TBC value).

The water activity (Aw) for the spray dried powder obtained by processing wine lees permeates and retentate ranged 0.272–0.362 with a mean value of 0.3125±0.03. The results obtained in this experiment are within the range of 0.2–0.7 which represents water availability for some biological and chemical reactions such as the Maillard reaction (non–enzymatic browning) and enzymatic activities. However, all values fall below the

maximum acceptable value to prevent the decomposition of food matrices by microorganisms, according to the food stability diagram after Labuza (1980).

These values, together with the reduced moisture content exhibited by micro powders, ensure good stability over time under suitable storage conditions.

3.5. Bioaccessibility

One of the main issues related to the beneficial effects of polyphenols in nutraceutical application is their bioavailability, which depends on bioaccessibility, and the efficiency of the transepithelial passage (Manach et al., 2005). Bioaccessibility is the term used to define the amount of components released from the matrix in the gastrointestinal lumen, which is required for their further intestinal absorption and therefore bioavailability (Saura–Calixto et al., 2007). During digestion, food is exposed to three digestive phases (oral, gastric and intestinal phase); *in vitro* digestion systems are used to mimic the physiological conditions of the upper gastrointestinal tract (Brodkorb et al., 2019), and thus to provide information about the digestibility of controlled release systems and the bioavailability of functional compounds (Alminger et al.,, 2014).

Microcapsules obtained from spray drying of the PERM1, PERM2, PERM3, and RET underwent *in vitro* digestion (see section 2.6), and total polyphenol concentration and AA were determined in each sample before and after digestion (**Table 6).**

	Total polyphenols concentration *(mg GAE/100 mg dw)*		**Polyphenol bioaccessibility** *(%)*	**AA** *(% scavenging)*	
Sample	**Before digestion**	**After digestion**		**Before digestion**	**After digestion**
PERM1	0.10±0.01	0.07±0.0	70	8.5±2.8	18.8±4.0
PERM2	0.09±0.01	0.07±0.02	78	7.8±1.1	18.0±1.1
PERM3	0.08±0.01	0.04±0.0	50	7.2±1.7	17.0±3.2
RET	0.57±0.03	0.36±0.03	63	30.5±2.7	61.3±6.5

Legend: AA*: Antioxidant activity*; *GAE: Gallic acid equivalent*

Table 6. Total polyphenol concentration and bioaccessibility, and AA before and after digestion

Although a reduction of the initial polyphenol content was evidenced after in vitro digestion in the order PERM3 (50%) > RET (36.8%) > PERM1 (30%) > PERM2 (22.5%), RET had the highest concentration of polyphenols even after digestion. *In vitro* bioaccessibility of polyphenols in the different samples was calculated according to the following equation:

$$\% \text{ Bioaccessibility} = (C_{POST}/C_{PRE}) \times 100$$

where C_{POST} and C_{PRE} correspond to the polyphenol concentration after and before the digestion process, respectively. *In Vitro* bioaccessibility of polyphenols exceeded 50% in all samples, and it was ≥ 70% in PERM1 and PERM2.

Contrary to the trend with polyphenolic content, an increase in AA was evidenced in all samples following *in vitro* digestion. These results are in agreement with previous studies carried out in several food matrices, including grapes (Danesi et al., 2020; Tagliazucchi et al., 2010; Vázquez–Sánchez et al., 2018). However, this can be due to the addition of bile during the digestion procedure, since bile pigments are potent peroxyl radical scavengers (Bulmer et al., 2008).

4. Conclusion

A Central Composite Design allowed setting up the best spry–drying condition using a commercial grape extract–polyphenol as a standard. The encapsulating agent was the most significant variable for maximizing the total bioactive content of the dried powders, with a dilution effect observed at higher concentration. Conversely, le microencapsulation efficiency was not affected by the carrier or temperature in the range tested and maltodextrin did not to affect antioxidant activity of the microencapsulated. The optimized operative condition were then applied to encapsulate bioactive compounds in several nanofiltrates of wine lees. The microencapsulates were characterized by high recovery of polyphenols, remarkable antioxidant activity and good stability under storage conditions. *In vitro* digestion evidenced a different bioaccessibility of polyphenols among samples, highlighting their potential to be absorbed in the gastrointestinal tract or have beneficial effects at the intestinal level. Although results are promising, further investigation are needed to assess whether this high bioaccessibility is also found *in vivo.* Based on the presented results, nanofiltrates coupled to spry–drying can be considered a valuable approach for selecting compounds with potential health benefits from enological wastes to be exploited in pharmaceutical, cosmetic and food industries.

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References

Aliakbarian, B., Sampaio, F.C., de Faria, J.T., Pitangui, C.G., Lovaglio, F., Casazza, A.A., Converti, A. and Perego, P. (2018). Optimization of spray drying microencapsulation of olive pomace polyphenols using response surface methodology and artificial neural network. LWT–Food Science and Technology, 93, 220-228.

Adhikari, B., Howes, T., Lecomte, D. and Bhandari, B.R. (2005). A glass transition temperature approach for the prediction of the surface stickiness of a drying droplet during spray drying. Powder Technology, 149, 168-179.

Alminger, M., Aura, A. M., Bohn, T., Dufour, C., El, S.N., Gomes, A., Karakaya, S., Martínez-Cuesta, M.C., Mcdougall, G.J., Requena, T., et al. (2004). In vitro models for studying secondary plant metabolite digestion and bioaccessibility, Comprehensive Reviews in Food Science and Food Safety, 13, 413–436. doi: 10.1111/1541-4337.12081.

AOAC (1990). In K. Helrich (Ed.), Official Methods of Analysis (15th ed.). Arlington, VA, USA: Association of Official Analytical Chemists, Inc.

Arboleda Mejia, J.A., Parpinello, G.P., Versari, A., Conidi, C. and Cassano, A. (2019). Microwave-assisted extraction and membrane-based separation of biophenols from red wine lees. Food and Bioproducts Processing, 117, 74-83.

Arboleda Mejia, J.A., Ricci, A., Figueiredo, A.S., Versari, A., Cassano, A., Parpinello, G.P. and De Pinho, M.N. (2020). Recovery of phenolic compounds from red grape pomace extract through nanofiltration membranes. Foods, 9, 1649.

Barbosa, J., Borges, S., Amorim, M., Pereira, M.J., Oliveira, A., Pintado, M.E. and Teixeira, P. (2015). Comparison of spray drying, freeze-drying and convective hot air drying for the production of a probiotic orange powder. Journal of Functional Foods, 17, 340-351.

Bhandari, B.R. and Howes, T. (1999). Implication of glass transition for the drying and stability of dried foods. Journal of Food Engineering, 40, 71-79.

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Balance, S., Bohn T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., et al. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. Nature Protocols, 14, 991–1014. doi: 10.1038/s41596-018-0119-1.

Bulmer, A.C., Ried, K., Blanchfield, J.T., Wagner, K.H. (2008). The anti-mutagenic properties of bile pigments. Mutation Research, 658, 28–41. doi: 10.1016/j.mrrev.2007.05.001.

Busch, V.M., Pereyra-Gonzalez, A., Šegatin, N., Santagapita, P.R., Ulrih, N.P. and Buera, M.D.P. (2017). Propolis encapsulation by spray drying: Characterization and stability. LWT - Food Science and Technology, 75, 227-235.

Carri, F. (2014). A standardised static in vitro digestion method suitable for food– an international consensus. Food and Function, 5, 1113–1124.

Carvalho, E., Mateus, N., Plet, B., Pianet, I., Dufourc, E. and De Freitas, V. (2006). Influence of wine pectic polysaccharides on the interactions between condensed tannins and salivary proteins. Journal of Agricultural and Food Chemistry, 54, 8936-8944.

Chen, Y., Wen, J., Deng, Z., Pan X., Xie, X. and Peng, C. (2020). Effective utilization of food wastes: Bioactivity of grape seed extraction and its application in food industry. Journal of Functional Foods, 73. 104113.

Cianciosi, D., Forbes-Hernández, T.Y., Ansary, J., Gil, E., Amici, A., Bompadre, S., Simal-Gandara, J., Giampieri, F. and Battino, M. (2020). Phenolic compounds from Mediterranean foods as nutraceutical tools for the prevention of cancer: The effect of honey polyphenols on colorectal cancer stem-like cells from spheroids. Food Chemistry, 325, 126881.

Danesi, F., Calani, L., Valli, V., Bresciani, L., Del Rio, D., Bordoni, A. (2020). (Poly)phenolic content and profile and antioxidant capacity of whole-grain cookies are better estimated by simulated digestion than chemical extraction, Molecules, 25(12):2792. doi: 10.3390/molecules25122792.

De Iseppi, A., Lomolino, G., Marangon, M. and Curioni, A. (2020). Current and future strategies for wine yeast lees valorization. Food Research International, 137,109352.

de Souza, V.B., Fujita, A., Thomazini, M., da Silva, E.R., Lucon Jr, J.F., Genovese, M.I. and Favaro-Trindade, C.S. (2014). Functional properties and stability of spray-dried pigments from Bordo grape (Vitis labrusca) winemaking pomace. Food Chemistry, 164, 380-386.

Fang, Z. and Bhandari, B. (2011). Effect of spray drying and storage on the stability of bayberry polyphenols. Food Chemistry, 129, 1139-1147.

Fazaeli, M., Emam-Djomeh, Z., Ashtari, A.K. and Omid, M. (2012). Effect of spray drying conditions and feed composition on the physical properties of black mulberry juice powder. Food and Bioproducts Processing, 90, 667-675.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A. and Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Research International, 40, 1107-1121.

Giacobbo, A., Bernardes, A.M. and de Pinho, M.N. (2017). Sequential pressure-driven membrane operations to recover and fractionate polyphenols and polysaccharides from second racking wine lees. Separation and Purification Technology, 173, 49-54.

Giacobbo, A., Dias, B.B., Onorevoli, B., Bernardes, A.M., de Pinho, M.N., Caramão, E.B., Rodrigues, E. and Jacques, R.A., 2019. Wine lees from the 1st and 2nd rackings: valuable by-products. Journal of Food Science and Technology, 56, 1559-1566.

González, E., Gómez-Caravaca, A.M., Giménez, B., Cebrián, R., Maqueda, M., Martínez-Férez, A., Segura-Carretero, A. and Robert, P. (2019). Evolution of the phenolic compounds profile of olive leaf extract encapsulated by spray-drying during in vitro gastrointestinal digestion. Food Chemistry, 279, 40-48.

Goodman, M., Bostick, R.M., Kucuk, O. and Jones, D.P. (2011). Clinical trials of antioxidants as cancer prevention agents: past, present, and future. Free Radical Biology and Medicine, 51, 1068-1084.

Goula, A.M. and Adamopoulos, K.G. (2005). Spray drying of tomato pulp in dehumidified air: II. The effect on powder properties. Journal of Food Engineering, 66, 35-42.

Harbertson, J.F., Kennedy, J.A. and Adams, D.O. (2002). Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. American Journal of Enology and Viticulture, 53, 54-59.

Kalli, E., Lappa, I., Bouchagier, P., Tarantilis, P.A. and Skotti, E. (2018). Novel application and industrial exploitation of winery by-products. Bioresources and Bioprocessing, 5, 1-21.

Kopsahelis, N., Dimou, C., Papadaki, A., Xenopoulos, E., Kyraleou, M., Kallithraka, S., Kotseridis, Y., Papanikolaou, S. and Koutinas, A.A. (2018). Refining of wine lees and cheese whey for the production of microbial oil, polyphenol-rich extracts and value-added co-products. Journal of Chemical Technology and Biotechnology, 93, 257-268.

Labuza, T.P. (1980). The effect of water activity on reaction kinetics of food deterioration. Food Technology, 34, 36-41.

Mahdavi, S.A., Jafari, S.M., Assadpoor, E. and Dehnad, D. (2016). Microencapsulation optimization of natural anthocyanins with maltodextrin, gum Arabic and gelatin. International Journal of Biological Macromolecules, 85, 379-385.

Manach, C., Williamson, G., Morand, C., Scalbert, A. and Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. The American Journal of Clinical Nutrition, 81, 230S-242S.

McNamee, B.F., O'Riorda, E.D. and O'Sullivan, M. (2001). Effect of partial replacement of gum Arabic with carbohydrates on its microencapsulation properties. Journal of Agricultural and Food Chemistry, 49, 3385-3388.

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carriere, F., Boutrou, R., Corredig, M., Dupont, D. and Dufour, C. et al. (2014). A standardised static in vitro digestion method suitable for food–an international consensus. Food & Function, 5, 1113-1124.

Mishra, P., Mishra, S. and Mahanta, C.L., 2014. Effect of maltodextrin concentration and inlet temperature during spray drying on physicochemical and antioxidant properties of amla (Emblica officinalis) juice powder. Food and Bioproducts Processing, 9, 252-258.

Moayyedi, M., Eskandari, M.H., Rad, A.H.E., Ziaee, E., Khodaparast, M.H.H. and Golmakani, M.T. (2018). Effect of drying methods (electrospraying, freeze drying and spray drying) on survival and viability of microencapsulated Lactobacillus rhamnosus ATCC 7469. Journal of Functional Foods, 40, 391-399.

Mohammed, N.K., Tan, C.P., Abd Manap, Y., Alhelli, A.M. and Hussin, A.S.M. (2017). Process conditions of spray drying microencapsulation of Nigella sativa oil. Powder Technology, 315, 1-14.

Moreno, T., De Paz, E., Navarro, I., Rodríguez-Rojo, S., Matías, A., Duarte, C., Sanz-Buenhombre, M. and Cocero, M.J. (2016). Spray drying formulation of polyphenols-rich grape marc extract: evaluation of operating conditions and different natural carriers. Food and Bioprocess Technology, 9, 2046-2058.

Ozkan, G., Franco, P., De Marco, I., Xiao, J. and Capanoglu, E. (2019). A review of microencapsulation methods for food antioxidants: Principles, advantages, drawbacks and applications. Food Chemistry, 272, 494-506.

Ozkan, G., Kostka, T., Esatbeyoglu, T., Capanoglu, E. (2020). Effects of Lipid-Based Encapsulation on the Bioaccessibility and Bioavailability of Phenolic Compounds. Molecules, 25(23):5545.

Paini, M., Aliakbarian, B., Casazza, A.A., Lagazzo, A., Botter, R. and Perego, P. (2015). Microencapsulation of phenolic compounds from olive pomace using spray drying: A study of operative parameters. LWT-Food Science and Technology, 62, 177-186.

Pasrija, D., Ezhilarasi, P.N., Indrani, D. and Anandharamakrishnan, C. (2015). Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. LWT-Food Science and Technology, 64, 289-296.

Parada, J. and Aguilera, J.M. (2007). Food microstructure affects the bioavailability of several nutrients. Journal of Food Science, 72, R21-32.

Piñón-Balderrama, C.I., Leyva-Porras, C., Terán-Figueroa, Y., Espinosa-Solís, V., Álvarez-Salas, C. and Saavedra-Leos, M.Z. (2020). Encapsulation of active ingredients in food industry by spray-drying and nano spray-drying technologies. Processes, 8, 889.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26, 1231-1237.

Robert, P., Gorena, T., Romero, N., Sepulveda, E., Chavez, J. and Saenz, C. (2010). Encapsulation of polyphenols and anthocyanins from pomegranate (Punica granatum) by spray drying. International Journal of Food Science & Technology, 45, 1386-1394.

Roos, Y.H., 2002. Importance of glass transition and water activity to spray drying and stability of dairy powders. Le Lait, 82, 475-484.

Şahin-Nadeem, H., Dinçer, C., Torun, M., Topuz, A. and Özdemir, F. (2013). Influence of inlet air temperature and carrier material on the production of instant soluble sage (Salvia fruticosa Miller) by spray drying. LWT-Food Science and Technology, 52, 31-38.

Sansone, F., Mencherini, T., Picerno, P., d'Amore, M., Aquino, R.P. and Lauro, M.R. (2011). Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts. Journal of Food Engineering, 105, 468-476.

Saura-Calixto, F., Serrano, J., Goñi I. (2007). Intake and bioaccessibility of total polyphenols in a whole diet. Food Chemistry, 101, 492–501.

Segarra, I., Lao, C., López-Tamames, E. and De La Torre-Boronat, M.C. (1995). Spectrophotometric methods for the analysis of polysaccharide levels in winemaking products. American Journal of Enology and Viticulture, 46, 564-570.

Silva, P.I., Stringheta, P.C., Teófilo, R.F. and de Oliveira, I.R.N. (2013). Parameter optimization for spray-drying microencapsulation of jaboticaba (Myrciaria jaboticaba) peel extracts using simultaneous analysis of responses. Journal of Food Engineering, 117, 538-544.

Singleton, V.L. and Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144-158.

Stanner, S.A., Hughes, J., Kelly, C.N.M. and Buttriss, J. (2004). A review of the epidemiological evidence for the 'antioxidant hypothesis'. Public Health Nutrition, 7, 407-422.

Sun-Waterhouse, D., Wadhwa, S.S. and Waterhouse, G.I. (2013). Spray-drying microencapsulation of polyphenol bioactives: a comparative study using different natural fibre polymers as encapsulants. Food and Bioprocess Technology, 6, 2376-2388.

Tagliazucchi, D., Verzelloni, E., Bertolini, D. and Conte, A. (2010). In vitro bio-accessibility and antioxidant activity of grape polyphenols. Food Chemistry, 120, 599-606.

Tan, S.P., Tuyen, C.K., Parks, S.E., Stathopoulos, C.E. and Roach, P.D. (2015). Effects of the spray-drying temperatures on the physiochemical properties of an encapsulated bitter melon aqueous extract powder. Powder Technology, 281, 65-75.

Teixeira, A., Baenas, N., Dominguez-Perles, R., Barros, A., Rosa, E., Moreno, D.A. and Garcia-Viguera, C., (2014). Natural bioactive compounds from winery by-products as health promoters: a review. International Journal of Molecular Sciences, 15, 15638-15678.

Tolun, A., Altintas, Z. and Artik, N. (2016). Microencapsulation of grape polyphenols using maltodextrin and gum arabic as two alternative coating materials: Development and characterization. Journal of Biotechnology, 239, 23-33.

Tonon, R.V., Baroni, A.F., Brabet, C., Gibert, O., Pallet, D. and Hubinger, M.D. (2009). Water sorption and glass transition temperature of spray dried açai (Euterpe oleracea Mart.) juice. Journal of Food Engineering, 94, 215-221.

Tuyen, C.K., Nguyen, M.H. and Roach, P.D. (2010). Effects of spray drying conditions on the physicochemical and antioxidant properties of the Gac (Momordica cochinchinensis) fruit aril powder. Journal of Food Engineering, 98, 385-392.

Vázquez-Sánchez, K., Martinez-Saez, N., Rebollo-Hernanz, M., Del Castillo, M.D., Gaytán-Martínez, M., Campos-Vega, R. (2018). In vitro health promoting properties of antioxidant dietary fiber extracted from spent coffee (Coffee arabica L.) grounds. Food Chemistry, 261, 253-259.

Wilkowska, A., Ambroziak, W., Adamiec, J. and Czyżowska, A. (2017). Preservation of antioxidant activity and polyphenols in chokeberry juice and wine with the use of microencapsulation. Journal of Food Processing and Preservation, 41, 1-9.

Wu, J.J., Lin, J.C., Wang, C.H., Jong, T.T., Yang, H.L., Hsu, S.L. and Chieh-ming, J.C. (2009). Extraction of antioxidative compounds from wine lees using supercritical fluids and associated anti-tyrosinase activity. The Journal of Supercritical Fluids, 50, 33-41.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: