Optimization of extraction and microencapsulation of bioactive compounds from red grape (*Vitis vinifera* L.) pomace

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Abstract Grape (*Vitis vinifera* L.) pomace constitutes a promising source of phenolic compounds, gallic acid, flavan-3-ols, flavonoids, stilbene and anthocyanins that are beneficial for human health. The objectives of this study were to optimize the extraction and microencapsulation of red grape pomace. Central composite designs with two factors were conducted for optimization using response surface methodology. The temperature (45–85 °C), and the time (2–8 h) were designed for the extraction. The results indicated that the extraction temperature and time introduced the increasing the extraction yield, total phenolic content, anthocyanin and resveratrol, but the long extraction time reduced the tannin content. The results showed that the optimize condition was the extraction at 80±1 °C for 2 h 53 min. This provided the highest content of polyphenolic compounds. The next experiment was microencapsulation of the extract which studied the amounts of maltodextrin (7–28 % w/v) and carboxymethyl-cellulose (CMC) (0–1.4 % w/v). The results showed that the optimized microencapsulation used 10.21 % w/v maltodextrin and 0.21 % w/v CMC to maximize all polyphenolic compounds, and also to minimize bitterness and astringency. This study illustrated that the optimal conditions for extraction and microencapsulation of the red grape extract have a high potential to produce functional ingredients.

Keywords *Vitis vinifera* L · Grape pomace · Extraction · Microencapsulation · Optimization

Introduction

The grape is one of the world's major fruit crops, which can grow in nearly every climate. In 2007, production was about 60 million tons, about 80 % of which was used in wine-making (Ruberto et al. 2008; Maier et al. 2008). The wine-making process results in 20 % grape by-products such as seeds and pomace. Grape pomace can be used as fertilizer, animal feed, or fuel. Red grape (*Vitis vinifera* L.) pomace contains a large amount of polyphenolic compounds. The major polyphenolic compounds in red grapes are phenolic acid, gallic acid, flavan-3-ols, flavonoids, stilbene and anthocyanins (González-Paramás et al. 2004), which have been reported to inhibit human low-density lipoprotein (LDL) oxidation, and to act as anti-inflammatory agents and free-radical scavengers (Shahidi and Naczk 2004). These polyphenolic compounds can be recovered by extraction. The grape extract can be used as food additives, natural food colorants, or health supplements (Liang et al. 2008). Solid–liquid extraction is a separation process involving the transfer of solutes from a solid phase to a liquid phase. The selection of a solvent is based on several factors, such as its physicochemical properties, cost and toxicity. Some solvents, such as ethanol, water and their mixtures, are designated as “generally recognized as safe” (GRAS) and are the preferred solvent systems currently used for natural products (Meireles 2009). The most efficient solvent for the extraction of phenolic compounds from grape pomace is 50 % ethanol. However, these methods focused on giving short extraction times and high temperature with rise to possible degradation. The extraction conditions were chosen upon literature data and previous extraction experiences of the research group. (Spigno et al. 2007; Vatai et al. 2009;

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Table 1 Experimental design: 2^2 central composite designs with two center points, of extraction and microencapsulation

Treatments	Code	Extraction		Microencapsulation	
		Temperature (°C); X_1	Time; X_2	Maltodextrin (%w/v), X_1	CMC (%w/v), X_2
1	(1)	50.86	2 h 53 min	10.0	0.2
2	a	79.14	2 h 53 min	25.0	0.2
3	b	50.86	7 h 7 min	10.0	1.2
4	ab	79.14	7 h 7 min	25.0	1.2
5	−αa	45.00	5 h	7.0	0.7
6	+αa	85.00	5 h	28.0	0.7
7	−αb	65.00	2 h	17.5	0.0
8	+αb	65.00	8 h	17.5	1.4
9	Cp1	65.00	5 h	17.5	0.7
10	Cp2	65.00	5 h	17.5	0.7

Vongchitpinyo et al. 2009). As a result, the extraction temperatures and times should be suitably adjusted for the optimum condition of the most active compounds.

The large amounts of phenolic compounds in red grape pomace (RGP) extract are mainly responsible for its astringency and bitterness; hence taste-masking is necessary (Kosaraju et al. 2009). The spray-drying method of encapsulation was chosen, with maltodextrin and carboxymethylcellulose (CMC)

used as wall material. They were selected because maltodextrin and CMC are commonly used in the food industry, and because they demonstrate an acceptable level of solubility in water. Astringency was reduced (and viscosity increased) by CMC, so CMC was determined to be the best astringency masker (Gouin 2004; Lesschaeve and Noble 2005; Troszynska et al. 2010). The use of a blend of maltodextrin and CMC could lead to the development of new products with interesting performance characteristics. Since both wall materials determine key product parameters, they should be studied for predictive modeling and optimization.

The aim of this research was to optimize the solid–liquid extraction process and microencapsulation in order to maximize the phenolic compounds obtained from RGP. This can produce high value-added products for future application in functional foods or nutraceuticals.

Material and methods

Material and reagents

Red grape (*Vitis vinifera* L. cv. Black queen) pomace was obtained from Siam Winery Co. Ltd., Samut Sakhon province, Thailand, in April 2009. It consisted of skin, seeds and a small amount of stems. Pomace samples were sealed in polyethylene bags and stored at -20°C until used.

Fig. 1 Chromatogram of red grape pomace extract analyzed by HPLC at 280 nm (a), 320 nm (b) where are 1=catechin, 2=epicatechin and 3=resveratrol

Table 2 Total phenolic content (TPC), tannin content (TN), total anthocyanin content (TA), yield of extraction, catechin, epicatechin and resveratrol extracted from red grape pomace and from microencapsulation of red grape pomace extract

Treatment ¹	Code	TPC ²	TN ³	TA ⁴	Catechin ⁵	Epicatechin ⁵	Resveratrol ⁵	Yield of extract (%) ⁶
Red grape pomace extract								
1	(1)	88.4±1.91 ^e	7.7±0.04 ^d	1.3±0.08 ^{a,b}	10.3±0.57 ^d	1.6±0.22 ^d	0.0069±0.0011 ^{a,b}	22.0±1.15 ^{d,e}
2	a	104.7±2.79 ^b	8.6±0.10 ^c	1.3±0.05 ^{b,c,d}	12.9±2.05 ^b	2.1±0.37 ^{b,c}	0.0059±0.0005 ^{a,b,c}	24.1±0.43 ^b
3	b	87.9±4.38 ^c	7.3±0.44 ^c	1.3±0.06 ^{a,b}	10.2±0.81 ^d	1.6±0.17 ^d	0.0074±0.0014 ^a	21.1±0.10 ^{c,f}
4	ab	106.8±7.10 ^b	9.2±0.13 ^b	1.1±0.05 ^c	16.1±1.24 ^a	2.5±0.23 ^a	0.0033±0.0003 ^d	23.7±0.24 ^b
5	-αa	86.3±3.23 ^c	7.1±0.30 ^c	1.3±0.05 ^{a,b,c}	10.5±0.56 ^{c,d}	1.8±0.11 ^d	0.0071±0.0015 ^{a,b}	20.1±0.05 ^g
6	+αa	117.8±5.77 ^a	9.7±0.41 ^a	1.2±0.01 ^{c,d}	12.2±0.87 ^{b,c}	1.9±0.17 ^{b,c,d}	0.0020±0.0001 ^d	27.6±0.59 ^a
7	-αb	94.8±1.63 ^d	7.7±0.03 ^d	1.4±0.08 ^a	9.9±0.58 ^d	1.7±0.09 ^d	0.0066±0.0008 ^{a,b}	20.9±0.14 ^f
8	+αb	103.4±4.17 ^b	8.6±0.18 ^c	1.2±0.01 ^d	13.5±0.92 ^b	2.3±0.14 ^{a,b}	0.0054±0.0006 ^{b,c}	22.8±0.20 ^c
9	Cp1	98.4±3.61 ^{c,d}	7.7±0.33 ^d	1.2±0.04 ^d	9.5±0.45 ^d	1.6±0.10 ^d	0.0050±0.0007 ^c	21.7±0.24 ^{d,e,f}
10	Cp2	102.0±2.16 ^{b,c}	8.8±0.24 ^c	1.3±0.03 ^a	10.9±0.81 ^{c,d}	1.9±0.12 ^{c,d}	0.0060±0.0007 ^{a,b,c}	22.2±0.45 ^{c,d}
Microencapsulation of red grape pomace extract								
1	(1)	27.8±1.97 ^b	16.6±0.04 ^a	0.2±0.02 ^c	2.0±0.77 ^a	0.3±0.13 ^a	0.00093±0.00013 ^a	
2	a	14.5±1.64 ^c	9.5±0.04 ^g	0.2±0.01 ^f	0.6±0.12 ^d	0.1±0.02 ^c	0.00064±0.00002 ^{b,c}	
3	b	22.6±3.53 ^c	12.0±0.04 ^d	0.3±0.02 ^c	0.9±0.46 ^{c,d}	0.2±0.11 ^{b,c}	0.00042±0.00003 ^e	
4	ab	13.5±2.13 ^c	8.9±0.05 ^h	0.1±0.02 ^g	0.6±0.14 ^d	0.1±0.04 ^c	0.00066±0.00009 ^b	
5	-αa	41.6±4.39 ^a	13.4±0.11 ^b	0.3±0.01 ^b	1.4±0.27 ^b	0.3±0.11 ^{a,b}	0.00096±0.00007 ^a	
6	+αa	13.2±0.33 ^e	7.2±0.05 ⁱ	0.1±0.01 ^g	0.6±0.13 ^d	0.1±0.02 ^c	0.00054±0.00005 ^{c,d}	
7	-αb	23.0±2.89 ^c	9.7±0.02 ^f	0.8±0.01 ^a	1.2±0.37 ^{b,c}	0.2±0.07 ^{a,b}	0.00042±0.00001 ^e	
8	+αb	20.4±2.74 ^{c,d}	6.4±0.03 ^j	0.3±0.02 ^b	0.6±0.14 ^d	0.1±0.02 ^c	0.00048±0.00004 ^{d,e}	
9	Cp1	18.3±0.98 ^d	11.7±0.04 ^e	0.2±0.01 ^e	0.6±0.15 ^d	0.1±0.02 ^c	0.00051±0.00001 ^{d,e}	
10	Cp2	17.9±1.00 ^d	13.0±0.04 ^c	0.2±0.01 ^{e,f}	0.6±0.07 ^d	0.1±0.03 ^c	0.00045±0.00005 ^{d,e}	

Values are means±SD ($n=3$). Data were analyzed by ANOVA ($P<0.05$). Within each column different letters indicate statistically different values according to post-hoc comparison (Duncan's multiple range test) at $P<0.05$

¹ treatments are in Table 1

² total phenolic content, mg GAE/g dry sample

³ tannin content, mg tannic acid/g dry sample

⁴ total anthocyanins mg/g dry sample

⁵ mg/g dry sample

⁶ yield of extraction

The chemicals used in this research were food-grade ethanol (95 %) from the Liquor Distillery Organization Excise Department (Chachoengsao, Thailand), Folin-Ciocalteu reagent (Merck, Darmstadt, Germany), maltodextrin DE 10–13 (CP Kelco, Skensved, Denmark), alum (McCormick, Hunt Valley, MD), carboxymethylcellulose (Daicel Chemical Industries, Osaka, Japan) and caffeine (Sigma-Aldrich, St. Louis, MO).

Extraction experiment

Grape pomace was ground, and then subjected to extraction in a water bath shaker (Memmert, Hamburg, Germany) at a constant shaking rate of 90 strokes/min. Ten g samples were placed in 250 mL flasks containing 30 mL solvent consisting of 50 % ethanol and distilled water (solvent-to-solid ratio

Table 3 The predicted models of extraction parameters

Extraction parameters	Model	P-value	Adj. R ²
Total phenolic content	$49.14+0.70X_1+0.82X_2$	0.00001	0.90
Tannin content	$6.87+0.01X_1-0.45X_2+8.50\times10^{-3}X_1X_2$	0.0046	0.81
Total anthocyanin content	$1.10+4.43\times10^{-3}X_1+0.08X_2-1.58\times10^{-3}X_1X_2$	0.0260	0.65
Resveratrol	$1.89\times10^{-4}+2.65\times10^{-4}X_1+4.48\times10^{-4}X_2-1.87\times10^{-6}X_1^2+8.33\times10^{-5}X_2^2-2.50\times10^{-5}X_1X_2$	0.00121	0.88
Yield of extraction	$13.79+0.14X_1$	0.0021	0.68

X_1 =Temperature °C

X_2 =Time (h)

[L/S]=3:1). Two factors were studied: extraction temperature (45–85 °C) and time (2–8 h) using 2^2 central composite designs with two center points (Table 1). The extracts were filtered and kept at -20 °C until analyzed.

Microencapsulation experiment

Encapsulation with both maltodextrin and carboxymethyl-cellulose (CMC) were prepared as follows: the 1 % freeze-

Fig. 2 Contour plot and response surface curve of regression models of extraction: TPC (a), TN (b), TA (c), resveratrol (d) and yield of extraction (e)

dried extract was mixed with maltodextrin and CMC with constant stirring; then the mixture was swollen for 12 h. The treatment combination was designed by varying maltodextrin (7–28 % w/v) and CMC (0–1.4 % w/v) using 2^2 central composite designs with two center points (Table 1). Each treatment preparation was homogenized using a Silverson L4R homogenizer (Silverson Machines, Chesham, England) at 5,000 rpm for 5 min. The solutions were fed into a spray dryer (JCM Best Technology, Nonthaburi, Thailand) operated at 140 ± 2 °C inlet temperature and 80 ± 2 °C outlet temperature. The powder was kept at -20 °C until analyzed.

Determination of physical properties

Yield of extraction

The yield of evaporated dried extracts (on a dry weight basis) was calculated from Eq. (1) shown below, where W_1 is the weight of extract after evaporation of ethanol and W_2 is the dry weight of the fresh plant sample (Maisuthisakul et al. 2007):

$$\text{Yield}(\%) = (W_1 \times 100) / W_2 \quad (1)$$

Solubility

Solubility was determined using a modified method based on the Thai Industrial Standards Institute (1987). Briefly, 2 g powder samples were slowly mixed with 20 mL distilled water, then filtered and washed with 200 mL hot water. The solubility of powder (dry weight basis) was calculated from Eq. (2) shown below, where W is the weight of powder, W_1 is the weight of the filter paper (no. 41) and the residue of the powder sample, and W_2 is the dry weight of the filter paper.

$$\text{Solubility}(\%) = 100 - \{[(W_1 - W_2) \times 100] / W\} \quad (2)$$

Determination of chemical properties

Moisture content

Partially air-dried samples were kept in a hot-air oven at 105 °C until they attained a constant weight, calculating moisture content in terms of weight loss (AOAC 2000).

Total phenolic content (TPC)

The total phenolic contents of grape pomace were determined using a modified Folin-Ciocalteu colorimetric method (Singleton and Rossi 1965). One hundred μ L of the appropriate diluted extract was mixed with 1 mL phenol reagent, 1 mL 10 % sodium bicarbonate and 4 mL distilled water. The mixture was allowed to stand for 2 h in the dark. The total

phenolic concentration was calculated from a calibration curve by plotting known solutions of gallic acid against absorbance at 760 nm. Results were reported as mg of gallic acid equivalents.

Determination of total anthocyanin content (TA)

The total anthocyanin content of grape pomace was determined using a modified method of Fuleki and Francis (1968). Briefly, 1 mL extract was diluted with 50 mL ethanol and 1.5 N hydrochloric acid (85:15), and the absorbance measured at 535 nm after 2 h. Total anthocyanin content was calculated with the aid of extinction coefficients. Results were reported as mg/g.

Determination of tannin content (TN)

The tannin content of grape pomace was determined using the Folin-Denis colorimetric method (AOAC 2000). The absorbance was measured at 760 nm. Results were reported as mg of tannin acid.

HPLC analysis

HPLC analysis was modified from Kammerer et al. (2004), using an Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA) apparatus with ChemStation software, a model G1322A degasser, G1311A binary gradient pump, G1329A thermo-autosampler, G1316A column oven, and a G1315A diode array detector. A Spherisorb (Waters Corp., Milford, MA) C18 ODS column (250 $\times$ 4.6 mm i.d.; 5 μ m particle size) was operated at 25 °C.

Fig. 3 Optimization of extraction of red grape pomace

Table 4 Verification of optimized model of extraction

Extraction parameters	Predicted value	Experimental value ^a	Error ^b (%)
Total phenolic content (mg GAE/g dry sample)	106.96	101.79±1.33	5.07
Tannin content (mg/g dry sample)	8.58	9.14±0.53	6.08
Total anthocyanin content (mg/g dry sample)	1.31	1.39±0.17	5.69
Resveratrol (mg/g dry sample)	0.0054	0.0049±0.0003	9.63
Catechin (mg/g dry sample)	11.92	12.13±1.53	1.92
Yield of extraction (%)	24.56	24.04±3.19	2.17

^a Mean±SD with 3 replications^b Error (%) = experimental value – predicted value × 100/experimental value

The gradient was composed of two solvents: (A) 2 % v/v acetic acid in water, and (B) 0.5 % acetic acid in water and acetonitrile (50:50, v/v). The gradient program was as follows: from 10–24 % B (20 min), from 24–30 % B (20 min), from 30–55 % B (20 min), from 55–100 % B (15 min), 100 B isocratic (8 min), and from 100–10 % B (2 min). Total run time was 90 min. The injection volume for all samples was 10 µL. Flow rate was set at 1 mL/min, and detection was carried out using 280 nm (flavonols) and 320 nm (stilbenes). Standards used for identification and quantification, respectively, were as follows: gallic acid (Fluka, Buchs, Switzerland); (+)-catechin, (–)-epicatechin (Sigma, St. Louis, MO); and resveratrol (*Calbiochem*, La Jolla, CA). Quantification was accomplished using external calibration with pure standards. The calibration

curves (seven data points) were linear, with $R^2=0.994$, 0.981 and 0.994 for catechin, epicatechin and resveratrol, respectively.

Sensory descriptive analysis

Ten trained panelists (8 female and 2 male) were selected from graduate students in the Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University. Sensory descriptive analysis was used for this study. Panelists were trained to evaluate bitterness and astringency using a 150 mm unstructured line scale with anchors at 12.5 and 137.5 mm for an intensity scale. The references for bitter tastes were 0.05 % caffeine (bitterness 2), 0.08 % caffeine (bitterness 5) and 0.15 % caffeine (bitterness 10); the references for astringency were 0.02 % alum (astringency 2) and 0.10 % alum (astringency 10) (Meilgaard et al. 2007; Beecher et al. 2008). Individual testing booths were equipped with a computerized program SUSense (Silpakorn University, Nakhon Pathom, Thailand) for experimental setting and data collection. Formal evaluations of all tastes took place in booths, with samples at room temperature. Panelists were provided with unsalted crackers and deionized water to cleanse the palate between samples.

Statistical analysis

Data were expressed as mean±SD for three replications. Only variables with a confidence level greater than 95 % were considered as significant. Duncan's multiple range test was used for post-hoc comparison, at $P<0.05$. Statistical analysis was performed using SPSS software (SPSS, Chicago, IL), and

Table 5 Yield of production, moisture content, a_w , solubility, and intensity of bitterness and astringency of microencapsulation of red grape pomace extract

Treatment ¹	Code	Yield of production (%)	Moisture content	a_w	Solubility (%)	Intensity ²	
						Bitterness	Astringency
1	(1)	9.2±0.98 ^{a,b}	2.91±0.03 ^a	0.31±0.01 ^a	98.4±0.09 ^a	2.5±0.8 ^a	1.9±0.6 ^{a,b}
2	a	10.1±0.78 ^a	2.58±0.05 ^{b,c}	0.25±0.01 ^b	98.0±0.03 ^d	0.8±0.4 ^e	0.8±0.5 ^e
3	b	6.2±0.88 ^d	2.86±0.10 ^{a,b}	0.24±0.01 ^{b,c}	97.7±0.01 ^f	2.0±0.5 ^{a,b}	1.6±0.7 ^{a,b,c}
4	ab	7.2±0.78 ^d	2.44±0.06 ^{c,d}	0.22±0.00 ^{c,d}	98.2±0.04 ^{b,c}	1.4±0.6 ^{c,d}	1.5±0.6 ^{a,b,c,d}
5	–αα	6.0±0.78 ^d	2.74±0.39 ^{a,b,c}	0.20±0.01 ^{d,e}	98.1±0.08 ^c	2.4±0.7 ^a	1.9±0.7 ^{a,b}
6	+αα	10.6±0.76 ^a	2.07±0.07 ^e	0.18±0.00 ^{f,g}	98.3±0.03 ^{a,b}	1.3±0.5 ^{d,e}	1.1±0.7 ^{b,c,d,e}
7	–αb	9.1±0.84 ^{a,b}	2.12±0.01 ^{d,e}	0.19±0.00 ^{e,f}	98.2±0.01 ^c	1.8±0.5 ^{b,c}	1.4±0.6 ^{c,d,e}
8	+αb	6.4±0.83 ^d	2.19±0.04 ^{d,e}	0.17±0.01 ^{g,h}	97.5±0.03 ^g	2.0±0.4 ^{a,b}	1.0±0.6 ^{d,e}
9	Cp1	8.3±0.12 ^{b,c}	2.12±0.04 ^{d,e}	0.15±0.01 ^h	97.8±0.03 ^{e,f}	2.2±0.3 ^{a,b}	2.1±0.5 ^a
10	Cp2	9.1±0.95 ^{a,b}	1.97±0.00 ^e	0.15±0.00 ^h	97.9±0.03 ^e	2.1±0.6 ^{a,b}	2.0±0.6 ^a

Values are means±SD ($n=3$). Data were analyzed by ANOVA ($P<0.05$). Within each column different letters indicate statistically different values according to post-hoc comparison (Duncan's multiple range test) at $P<0.05$

¹ treatments are in Table 1² values are means±SD ($n=10$) rated on a 15 cm unstructured line scale with anchors at 1.25 and 13.75 cm

Table 6 Regression model of attributes from microencapsulation

Attributes	Model	P-value	Adj. R ²
Total phenolic content	$39.68 - 1.05X_1$	0.0012	0.72
Catechin	$4.17 - 0.23X_1 - 2.70X_2 + 3.58 \times 10^{-3}X_1^2 + 0.63X_2^2 + 0.08X_1 X_2$	0.0028	0.94
Epicatechin	$0.70 - 0.04X_1 - 0.45X_2 + 7.26 \times 10^{-3}X_1^2 + 0.14X_2^2 + 9.52 \times 10^{-3}X_1 X_2$	0.0003	0.98
Yield of production	$7.45 + 0.15X_1 - 2.65 X_2$	0.0011	0.82
Solubility	$99.86 - 0.17X_1 - 1.40X_2 + 3.75 \times 10^{-3}X_1^2 + 0.02 X_2^2 + 0.06X_1 X_2$	0.84	
Bitterness ^a	$2.98 - 0.06X_1$	0.0018	0.69
Astringency ^a	$1.41 + 0.07X_1 + 1.05X_2 - 4.54 \times 10^{-3}X_1^2 - 1.63X_2^2 + 0.07X_1 X_2$	0.0241	0.83

X_1 = Maltodextrin (%w/v)

X_2 = CMC (%w/v)

^a Sample prepared 5 g/L. Rated on a 15 cm unstructured line scale with anchors at 1.25 and 13.75 cm

optimization technique with Design-Expert 6.0.10 (Stat-Ease, Minneapolis, MN).

Results and discussion

Optimization of extraction of red grape pomace

The red grape pomace was extracted and then analyzed by HPLC. Figure 1 illustrate chromatogram of grape pomace extract at 280 nm for catechin and epicatechin and at 320 nm

for resveratrol. Table 2 presents the significant differences ($P < 0.05$) of total phenolic content (TPC), tannin content (TN), total anthocyanin content (TA), yield of extraction (%), catechin, epicatechin and resveratrol. These results showed the relationship of extraction temperature and time on TPC, TN, TA, resveratrol and yield of extraction with predicted regression models (Table 3). It was in general noticed that by increasing the extraction temperature and time, the amount of total extracted phenols was increased. TPC and TA were higher than in the reports by Vongchitpinoy et al. (2009) and Khanal et al. (2009). The more temperature could

Fig. 4 Contour plot and response surface curve of regression models of encapsulation: TPC (a), catechin (b), epicatechin (c), yield of production (d), solubility (e), bitterness (f) and astringency (g)

promote the phenolic extraction, but not on purity, by increasing both solubility and diffusion coefficient of phenolic compounds in extraction solvent and also able to release both the cell wall phenolics and bounded phenolics by breaking down of cellular constituents (Cacace and Mazza 2003). The present study revealed that the grape (*Vitis vinifera* L. cv. Black queen) pomaces contained higher total phenolics compared to five wild grapes and two hybrids native to Japan (Poudel et al. 2008). TN and TA decreased by increasing the extraction time and temperature, respectively. This is probably because water in the solvents is another source of (hydrolytic) degradation when tannin is extracted within 2–5 h (Cork and Krockenberger 1991). The temperature of extract has a considerable impact on the stability of monomeric anthocyanins (Morais et al. 2002). Based on the results of extraction temperature and time on TPC, TN, TA, resveratrol and yield of extraction of RGP extracts, these parameters were taken into account in the experimental design using a response surface methodology approach (Fig. 2).

The optimal extraction condition, with a maximum for all parameters, was at 80 °C and 2 h 53 min (Fig. 3). Table 4 shows the verification of the optimized model of extraction, with less than 10 % error. The grape extract contained a high content of total phenolic compounds (101.79 ± 1.33 mg GAE/g), total anthocyanin (1.39 ± 0.17 mg/g), resveratrol (0.0049 ± 0.0003 mg/g) and catechin (12.13 ± 1.53 mg/g). According to the results of a study by Rockenbach et al. (2011), red grape extract (Cabernet Sauvignon variety) provided total phenolic compounds of 75.75 mg GAE/g, total anthocyanin (1684.11 mg/100 g), resveratrol (4.02 mg/100 g) and catechin (150.16 mg/100 g). Therefore, this optimized extraction could be used to extract highly active compounds from RGP.

Optimization of microencapsulation of red grape extract

The results of physiochemical and sensory evaluations are shown in Tables 2 and 5; all parameters were significantly different ($P < 0.05$). This data could generate the predicted regression models of TPC, catechin, epicatechin, yield, solubility, bitterness and astringency of red grape microencapsulation (Table 6). It was obvious that maltodextrin affected total phenolic content (Fig. 4a). As maltodextrin increased, total phenolic content decreased; but CMC had no effect on phenolic content. A higher maltodextrin and solid content contributed a greater effect on the reactions between molecules of anthocyanins lost during the drying process (Nielsen et al. 1993). As shown in Fig. 4b and c, catechin and epicatechin increased with an increase in maltodextrin and CMC. The mixture of maltodextrin and CMC was useful as a drying aid in spray drying. These results were similar to those of Beatus et al. (1985), and Wagner and Warthesen (1995). Figure 4d shows that the yield of the products increased when maltodextrin increased. The addition of maltodextrin increased the

Fig. 5 Optimization of microencapsulation of grape extract

glass transition state temperature, which resulted in a drastic decrease in moisture absorption, but with no adhesion to the dryer's wall (Adhikari et al. 2004; Goula and Adamopoulos 2008). On the other hand, the yield decreased when CMC increased. Also, as shown in Fig. 3e, the solubility decreased when CMC increased, because the low pH of the solution had precipitated CMC (BeMiller and Whistler 1996). Bitterness decreased when the proportion of maltodextrin increased (Fig. 4f). Certainly, the sweetness of maltodextrin decreased

Table 7 Verification of optimized model of microencapsulation of red grape extract

Parameters	Predicted value	Experimental value ^a	Error ^b (%)
Yield (%)	8.49	7.73±0.06	9.84
Solubility (%)	98.37	97.29±0.92	1.11
Total phenolic content (mg GAE/g dry sample)	29.05	31.56±0.40	7.95
Tannin content (mg/g dry sample)	15.39	14.14±0.09	8.84
Total flavonoids (mg Q/g dry sample)	1.23	1.27±0.08	3.15
Catechin (mg/g dry sample)	1.86	1.74±0.47	6.41
Epicatechin (mg/g dry sample)	0.31	0.28±0.09	9.12
Bitterness ^c	2.33	2.27±0.90	2.64
Astringency ^c	1.96	1.91±0.89	2.62

^a Mean±SD with 3 replications

^b Error (%)=experimental value – predicted value×100/experimental value

^c Sample preparation with 5 g of microencapsulated red grape extract per 1 L of distilled water

the perception of bitterness (Keast and Breslin 2002). Astringency decreased with increased amounts of maltodextrin and CMC in the mixture (Fig. 4g). This may be due to the effect of viscosity on astringency, as astringency decreases with a rise in viscosity (Troszynska et al. 2010). As reported by Smith et al. (1996), there is a decrease in the intensity of astringency of grape seed tannin solution when thickened with CMC. This is also the case with cranberry juice and soy milk (Courregelongue et al. 1999; Peleg and Noble 1999).

The optimal encapsulation – with a maximum of TPC, catechin, epicatechin and yield, and a minimum of bitterness and astringency – was with 10.10 % w/v maltodextrin and 0.21 % w/v CMC (Fig. 5). The optimal model was validated with less than 10 % error: 7.95 % TPC, 6.41 % catechin, 9.12 % epicatechin, 9.84 % yield, 1.11 % solubility, 2.64 % bitterness and 2.62 % astringency (Table 7). Therefore, microencapsulation provided the optimal conditions for the extraction of bioactive compounds from RGP.

Conclusions

Temperature and contact time showed critical effects of the yield of extraction, total phenolic content, and total anthocyanin, tannin, catechin and resveratrol. A study of the optimal variables could provide insight on the economic feasibility of the overall spray-drying process. According to these results, maltodextrin and CMC blends appear to compare favorably with other encapsulating agents, and may serve as acceptable and suitable replacements. This is of particular importance, as the mixtures could be an economical alternative to conventional encapsulating agents.

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