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EXTRACTION AND MICROENCAPSULATION OF POLYPHENOLS FROM GRAPE POMACE

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The present research projects aim to optimization extraction and microencapsulation of polyphenols from Indian grape pomace. The effect of increasing concentration of ethanol and methanol (0, 25, 50, 75 and 100%) and contact time (0, 1, 2, 3, 4, and 5 hours) at 25 °C temperature on the extraction of Total Polyphenols (TP) from Thompson Seedless Grape Pomace (TSGP) was examined. The highest phenolic content of 21.60 mg GAE/g was obtained using 75% methanol solutions. The extracts were encapsulated by two types of wall material, which are maltodextrin and gum arabic Core: coating material ratios (1:1 and 1:2), five different Maltodextrin (MD): Gum Arabic (GA) ratios (10:0, 8:2, 6:4, 4:6 and 2:8), and four different inlet temperatures (120, 140, 160, 180 °C) were investigated. Total phenolic contents were evaluated; the most efficient microcapsules were obtained with 8:2 ratio of MD:GA at 140 °C inlet temperature.When maltodextrin was used and the core: coating material ratio was 1:1, total polyphenols was between 9.4-17.4 mg GAE/g and it was calculated as 6.6-11.2 mg GAE/g polyphenols for the ratio of 1:2.

Keywords: Grape pomace, Polyphenols, Solvent extraction, Microencapsulation

INTRODUCTION

Grape is one of the major crops grown in India. The major producer in the country is used as a table purpose and remaining convert in Raisin, Wine and Juice. Grape pomace and other grape processing remains, when released in the environment, can lead to serious pollution, alternating from surface and ground water contamination to foul aromas. Polyphenols are phytochemicals by nature and are present in highest amounts in grapes (Imlak *et al*., 2017).

The polyphenol composition of each part of the grape pomace varies depending on the varieties of grapes and is influenced by the growing location, climate, maturity and the time of fermentation (Fuleki *et al.*, 1997; and Kennedy *et al*., 2000).

'Thompson Seedless Grape' is by a long shot the most planted assortment in India. It is also the most adaptable of grape varieties. While the biggest extent of its land is given to raisin creation (around 70%), a large extent is utilized for fresh table grapes (around 14.5%), squashing for wine, grape juice focus, and refining items (around 14%), and canning (around 1.5%).

Phenolic are the auxiliary metabolites of plants. Artificially, phenolics can be characterized as substances having an aromatic ring bearing at least one hydroxyl group, including their useful byproduct (Yang *et al*., 2009).

The aim of this work was to examine the efficiency of two extraction solvents (ethanol and methanol) and time influence on extraction of total polyphenols from Thompson Seedless Grape Pomace (TSGP) and microencapsulation of total polyphenols.

EXPERIMENTAL DESIGN

Sample Preparation

Solid wastes, i.e., Pomace (skins and stem) was obtained

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from the pressing of Thompsons Seedless Grapes (TSG). They were dried at 40 ºC in a conditioning chamber during 12 hours. The dried pomace was taken for grading. The grinding was carried out with the help of laboratory grinder. Then it was taken for practical analysis and it was performed with the help of sieve shaker. The final size obtained was 70 mm. The powder was then subjected to chemical analysis (Libran *et al.*, 2010).

Extraction Design

In order to fix the extraction time for later experiments, extraction kinetics were performed in duplicate, with the different combination of process conditions having an alcohol percentage (0%, 25%, 50%, 75%, and 100%) of ethanol and methanol each. Extraction kinetics will be monitored by measuring the Total Polyphenols (TP) in the extract at different processing times, i.e., at 0 hr., 1 hr., 2 hr., 3 hr., 4 hr., and 5 hr. After the optimum extraction time and yield of polyphenol was determined. Extractions were performed in duplicate, at the same process conditions commented before. Process yield and extract composition was determined by analyzing the concentration in the extract of total polyphenols.

Extraction Procedure

The sample/solvent ratio was 1:25 (g/ml). Extraction was carried out under agitation on an orbital shaker at a speed of 150 rpm. All experiments were carried out at room temperature (25±2 ºC). Extraction time was determined and fixed after performing previous extraction kinetics. After treatments the extracts were separated from the residual solids and stored at -20 ºC overnight until further use (Libran *et al.*, 2013).

Determination of Total Polyphenols (TP)

The total phenolic content was determined by using the Folin-Ciocalteuassay (Singleton *et al.*, 1965). An aliquot (1 ml) of extracts or standard solution of Gallic acid was added to 25 ml of volumetric flask, containing 9 ml of distilled water. Reagent blank using distilled water was prepared. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes 10 ml of 7% Na_2CO_3 solution was added to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined with a UV-Visible spectrophotometer. Total phenolic content was expressed as mg Gallic Acid Equivalents (GAE).

Effect of Extraction Procedures and Different Parameters

Solvents Extraction

Three distinct solvents were utilized to decide the most appropriate one for the extraction recuperation of polyphenols. The solvents utilized as a part of this analysis were: refined water, methanol and ethanol, these three later solvents were tried as a blender with water at various dilutions of various alcoholic dilutions (0, 25%, 50%, 75%, and 100%) and that at 25°±2 ºC. The best extraction solvent was chosen by the estimation of TP, mg GAE/100 g) (Spigno *et al.*, 2007; and Karacabey *et al.*, 2008).

Extraction Time

Samples were removed utilizing the best dissolvable sort and the best dissolvable dilution concentration, as decided in the initial step, for 0, 1, 2, 3,4, and 5 hours by fixing the extraction temperature steady at 25±2 ºC. The best extraction time was chosen by the best estimation of TP (mg GAE/100 g).

Microencapsulation of Polyphenols

Sample preparation for coating materials, i.e., Maltodextrin and Gum Arabic were dispersed individually in water till attaining 9.0% solid content under magnetic agitation. To prepare coating material solutions, Maltodextrin and Gum Arabic were mixed together at certain ratios (E1, E2, E3, E4 and E5, i.e., 10:0, 8:2; 6:4, 4:6 and 2:8 v/v). The prepared coating material solutions were then combined with phenolic extract (core), which was concentrated up to 9.0% solid content, at certain core: coating ratios (1:1 v/v). They were stirred with laboratory homogenizer at 7000 rpm for 30 min. (Tolun *et al.*, 2016; and Boonchu *et al.*, 2013).

Spray Drying

The microencapsulation was carried by spray drying method suggested with slight modification. In brief the above prepared emulsions were spray – dried on spray drier (LU-222, Labultima, Mumbai). The drying chamber of 150 cm height and 80 cm diameter with two cyclone separator, hot air blower and a exhaust blower. The mixture of core and wall materials was fed at the speed of 2 ml/min into the drying chamber, entry air temperatures of (120, 140, 160, 180 °C), respectively, air pressure of 2 kgf/cm^2 from the blower in parallel flow whereas microcapsules after spray drying were collected in the cyclone. During drying processes, the temperature of the feed mixture was 25 °C (Shu *et al.*, 2006).

Statistical Analysis

Data are presented as mean±SE (Standard error). Coefficient of correlation was calculated for intragroup variations. Significance of inter-group differences was determined by analysis of variance (ANOVA). A p-value of $p < 0.05$ was considered statistically significant (Steel *et al.*, 1981). Response surface methodology was applied to optimize the yield of phenolic compound bioactive compounds using stat graphics software version 8.0.

RESULTS AND DISCUSSION

Chemical analysis of TSG varieties were carried out with average observations, i.e., Moisture content 82%, crude protein 0.60% fat %, total soluble solids and total sugarwere determined in fresh TSG berries respectively 18.6% and 16.20%. Total Polyphenols present in TSG was 172.16 mg of GAE 100 g^{-1}. Freshly harvested Thompson seedless grapes (Vitisvinifera) were used because as per previsesreferences shows that after harvest start the decrease of phenolic compound[1]. Polyphenol composition varies with grape berries skin, cultivar, species, environmental condition and post harvest management skills (Yang *et al.*, 2009; and AOAC, 2006).

The moisture, ash, protein, total sugar, carbohydrate, total phenolic compound and dietary fiber contents of Pomace powder belonging to Thompson Seedless Grapes are given in Table 1. The chemical composition of the TSGP is given in Table 2. Moisture content was (4.5%), Ash (3.9%)

Table 1: Chemical Composition of Thompson Seedless Grapes (TSG)

Parameters	Values
Moisture (%)	82±0.90
Acidity (%)	0.62±0.03
TSS (^{o}Brix)	18.6±0.9
Brix-acid ratio	30
Crude protein (%)	0.60±0.18
Crude fat (%)	0.16±0.08
Crude fiber (%)	1.6±0.10
Total sugars (%)	17.20±0.4
Polyphenols (mg of GAE 100 g^{-1})	164.26±6.0

Note: Results are mean±SD of 3 determinations.

Table 2: Chemical Composition of Thompson Seedless Grapes Pomace (TSGP)

Parameters (g/100 g)	Results
Moisture (%)	4.5±0.22
Ash (%)	3.9±0.18
Fat (%)	3.6±0.46
Protein (%)	8.4±0.08
Carbohydrate (%)	37.48±0.80
Glucose (%)	5.70±0.14
Fructose (%)	6.10±0.36
Crude fiber (%)	42.12±0.10
Total phenolic compound (mg GAE/100 g)	23.80±0.26

Fat (3.6%) Protein (8.4%), Carbohydrate (37.48) and crudefiber (42.12%). This result not perfect but slightly similar (Altan *et al.*, 2009).

Table 1 shows that the different types of solvent has a significant effect ($p<0.05$) on total polyphenols content and they were able to extract phenolic compounds, but methanol 75% was the bestactive solvent as ethanol at the similar concentration.Methanol 75% allows extracting the maximum amount of total polyphenols which was 21.60±0.20, followed by 75% ethanol 20.42±0.36 mg GAE/100 g. The effects of extraction time on the polyphenolic contents of extract are showed in Table 3, the highest polyphenolic contents was obtained in four hours at temperature 25±2 ºC. The same solvent was used by several authors for the extraction of phenolic compounds from grapes Combinations of solvents such as methanol; ethanol and acetone with water improve the extraction of phenolic compounds (Bucic-Kojic *et al.*, 2006 and 2009; and Benmeziane *et al.*, 2014).

Response Surface Plot

The yield of the encapsulated powders in different process circumstances were showedin Figures 1 and 2. Encapsulated Powders yield indicated an increase with the increasing temperature in the event of different coating material ratios (10:0; 8:2; 6:4; 4:6; and 2:8) and the core: coating material ratios (1:1 and 1:2).

When only maltodextrin coating material powders yield increased,the core: coating ratio was increased from 1:1 to

Table 3: Total Polyphenols in Thompson Seedless Grapes Pomace (TSGP) Using Different Extracting Solvents

Solvent Concentration	Extraction Time (hrs.)	Total Polyphenols (mg of Gallic Acid Equivalents/g)	
		Ethanol Solvent	Methanol Solvent
0%	0	9.06±0.11^{d}	9.06±0.11^{d}
	1	10.20±0.20^{c}	10.20±0.20^{c}
	2	10.66±0.11^{c}	10.66±0.11^{c}
	3	11.73±0.30^{b}	11.73±0.30^{b}
	4	13.93±0.11^{a}	13.93±0.11^{a}
	5	13.760.23^{a}	13.760.23^{a}
25%	0	9.73±0.74^{d}	10.28±0.09^{c}
	1	10.76±0.63^{cd}	11.54±0.11^{d}
	2	12.53±0.65^{c}	12.80±0.35^{b}
	3	13.60±0.66^{b}	14.56±0.30^{b}
	4	15.16±1.35^{ab}	17.00±0.17^{a}
	5	14.86±1.03^{a}	16.80±0.17^{a}
50%	0	11.20±0.20^{e}	11.47±0.38^{e}
	1	12.80±0.20^{d}	13.26±0.30^{d}
	2	14.33±0.41^{c}	15.13±0.41^{e}
	3	16.40±0.34^{b}	17.13±0.41^{b}
	4	19.13±0.11^{a}	19.15±0.50^{a}
	5	18.53±0.11^{a}	19.40±0.12^{a}
75%	0	12.60±0.20^{d}	12.20±0.52^{d}
	1	16.13±0.30^{d}	15.86±0.11^{c}
	2	18.20±0.40^{c}	16.66±0.61^{b}
	3	19.36±0.15^{c}	19.73±0.46^{c}
	4	20.42±0.36^{b}	21.60±0.20^{b}
	5	20.30±0.41^{ab}	20.93±0.11^{a}
100%	0	11.80±0.20^{e}	13.93±0.11^{d}
	1	14.26±0.43^{d}	14.13±0.11^{d}
	2	16.40±0.30^{c}	16.80±0.40^{c}
	3	19.20±0.13^{b}	18.03±0.68^{b}
	4	20.10±0.54^{a}	20.80±0.41^{a}
	5	20.12±0.12^{a}	20.46±0.40^{a}

1:2. The combination of maltodextrin and gum arabic as a coating material to reduction of powders yield if the core: coating ratio was changed from 1:1 to 1:2. The use of only maltodextrin resulted in varying yield between 34.02-50.4%

Figure 1: Response Surface Plot for the Effect of Temperature and MD:GA Ratio on Yield of Encapsulated Powder at 1:1 Core Coating Ratio

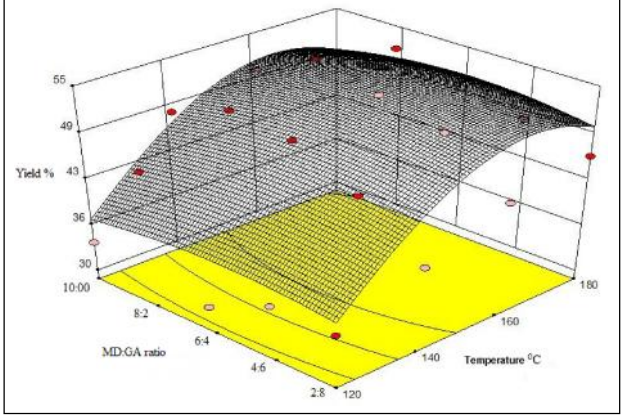
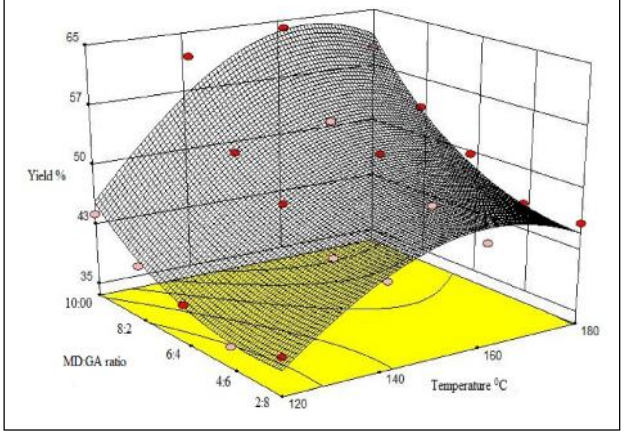


Figure 2: Response Surface Plot for the Effect of Temperature and MD:GA Ratio on Yield of Encapsulated Powder at 1:2 Core Coating Ratio



for 1:1 core: coating ratio and 44.2-64.4% for 1:2 core: coating ratio.Whereas the range was observed between 46.6-52.4% in the case of utilizing MD:GA ratio of 8:2 for 1:1 core:coating ratio and 41.2-52.3% for 1:1 core: coating ratio. Moreover, it was found that increasing the gum arabic amount in the coating material (6:4) has decreased this value. This result was similar to that of Tolun *et al.* (2016) who has encapsulated grape polyphenols.

The increasing ratios of MD and GA have different effect on the yield. The highest yield (64.9%) between all coating material ratios was achieved when the MD:GA ratio was 10:0 and the temperature was 160^{0}Cin the conditions of 1:2 core: coating material ratio (Benmeziane *et al.*, 2014; Mishra *et al.*, 2014; and Tolun *et al.*, 2016).

Figure 3: Total Polyphenols of Microencapsulated Powders at 1:1 Core:Coating Ratio

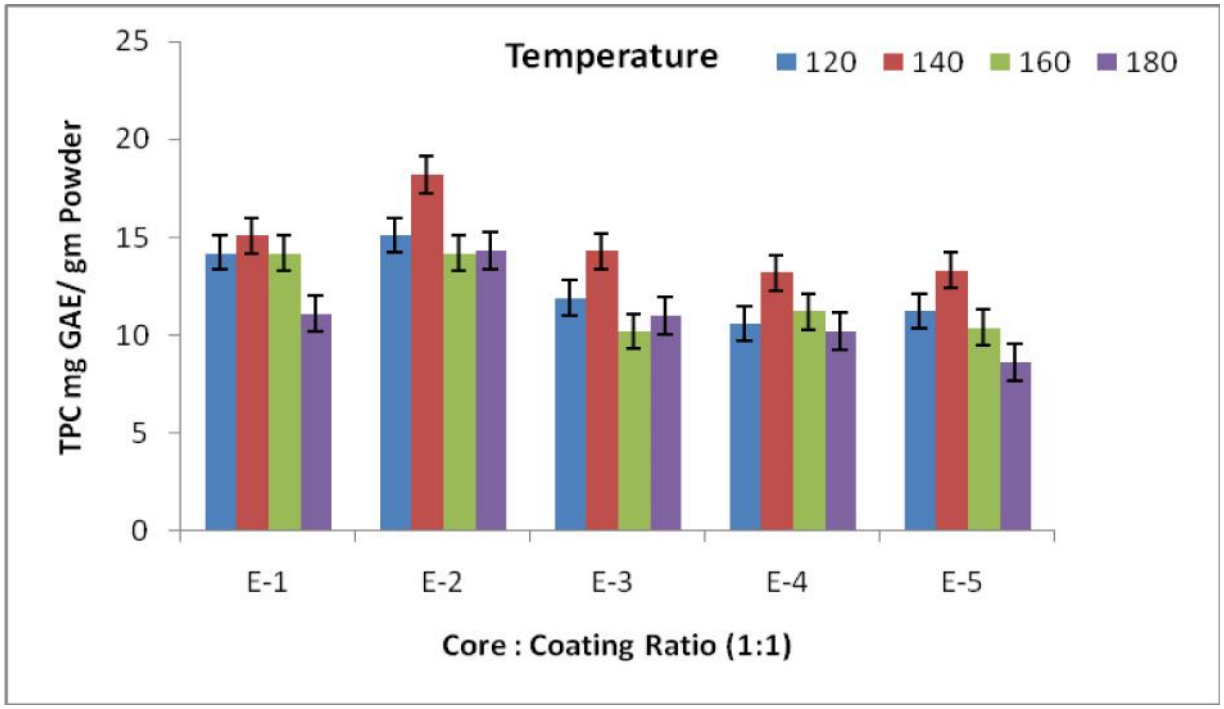
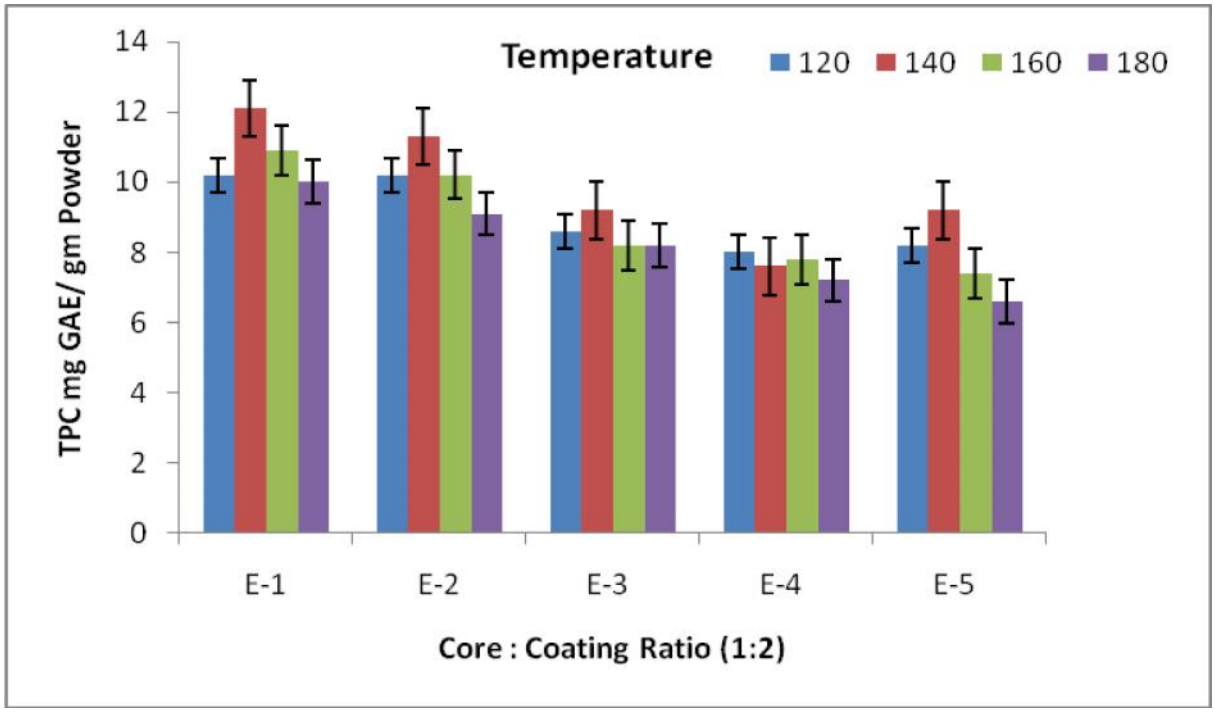


Figure 4: Total Polyphenols of Microencapsulated Powders at 1:2 Core:Coating Ratio



As shown in Figures 3 and 4 Maximum total polyphenols content 18.2 mg GAE/g encapsulated powder was found in 1:1 core:coating material ratio and 12.3 mg GAE/g for 1:2 core:coating material ratio. The TPC concentration demonstrated a significant decrease when the coating material ratio was increased from 1:1 to 1:2. Core:coating material ratio 1:1, MD:GA ratio 10:0 was highest phenolic content, but in 1:2 core: coating material ratio, 8:2 MD:GA ratio was highest phenolic content. This result was similar to that of Tolun *et al.* (2016) who has encapsulated grape polyphenols (Mote *et al.*, 2017).

CONCLUSION

In this study,the waste obtained from TSG pomace was the best source of total fiber, Carbohydrate and Total polyphones. The yield of extracting polyphenols was affected by the concentration of ethanol and methanol solvent and extraction time.Increasing the extraction time, the total polyphenols yields increased, but more than four hours extraction time, total polyphenols yields decrease or constant. The polyphenols content decreased as a result of the increasing concentration of coating material in the core: coating mixture.

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