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Optimization of extraction and microencapsulation of polyphenols from pomace of Indian grapes

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Abstract : The objective of this study is to improve extraction parameters of polyphenols from pomace of one Indian table grape cultivar. i.e. Tas-A-Ganesh Grape(TAGG).The impact of various solvents i.e.ethanol, (0, 25, 50, 75 and 100%) and methanol (0, 25, 50, 75 and 100%), with extraction time (0, 1, 2, 3, 4 and 5 hr.) were tried. It was found that the dissolvable nature and time significantly affect add up to polyphenols (TP) recuperated from the grape pomace. The best extraction conditions were as per the investigation methanol 75% at room temperature during 4 hr. furthermore, six back to back extractions with content of TP extracted of 22.62 mg GAE/100 g of Tas-A-Ganesh Grape Pomace (TAGGP). This shows the rate of polyphenols removed from grapes pomace relies on upon the 75% of Ethanol solvent. This study on the optimization of the extraction parameters of polyphenols from grape pomace is exceptionally unique on scale of India.

A lab scale spray-dryer was opted to engender microcapsules of polyphenols utilizing different dextrose equipollents of Maltodextrin and Gum Arabic as a coating material. Core: coating material ratios (1:1), five different Maltodextrin (MD): Gum Arabic (GA) ratios (10:0, 8:2, 6:4, 6:4 and 10:0), and four different inlet temperatures (120, 140,160, 180^{0}C) were investigated. Total phenolic contents were evaluated; the most efficient microcapsules were obtained with an 8:2 ratio of MD: GA at 140^{0}C inlet temperature.

Key words : Tas-A-Ganesh Grapes, Pomacs, Polyphenols, Extraction, Microencapsulation.

Introduction:

Grapes (*Vitis vinifera*) are an important fruit crop in India. Grapes are the third most widely cultivated fruit after citrus and banana**.** Grape pomace (GP) is a by-product of wine/Juice industry.GP consists mainly of peels (skins), seeds and stems and accounts for about 20–25% of the weight of the grape crushed for wine or juice production. The waste is facing disposal problem at one end and to follow the stipulated parameters laid by the Central and State Pollution Control Board, India. The agro-industrial residues of grape are commonly solid by-products such as stalks, pomace and the liquid filtrate. Depending on the situations of the grapes when they are picked, the remains may speak from 15 to 30% of the total volume of grapes.[1,2]

Winemaking produces an assortment of buildups comprising of seeds, skins, and stems that cause natural and temperate issues, which could be limited by the abuse and valorization of those items. Grape pomace is perceived as an imperative wellspring of phenolic mixes (flavonoids and non-flavonoids). The fundamental subclasses of phenolic compound in white grape pomace are flavanols, flavonols and phenolic acids.[3]

These residues are composed of water, proteins, lipids, carbohydrates, vitamins, minerals, and compounds with important biological properties such as fiber, vitamin C and phenolic compounds depending on the type of waste, the cultivar and climatic and cultivation conditions.[4]

The objective of this work was to study the solid-liquid extraction of polyphenols from grape wastes (pulp, skins and stem) of the Indian cultivars and examine microencapsulation of these phenolic compounds.

Experimental Design

Sample preparation

Solid wastes i. e. Pomace (skins and stem) was obtained from the pressing of Tas-A-Ganesh (TAG) grapes. They were dried at 50 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.[5]

Extraction design:

In order to fix the extraction time for later experiments, extraction kinetics was performed in duplicate, with the different combination of process conditions having 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 process times i.e. at 0 hr., 1hr., 2hr., 3hr., 4hr., and 5hr. After the optimum extraction time and yield of polyphenol was determined. Extractions were performing 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 extraction of liquid was prepared by adding to the ethanol/water mixture the necessary amounts of HCl or KOH. 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±2ºC). Extraction time was determined and fixed after performing previous extraction kinetics. After treatments the extracts were separated from the residual solids and stored at -20ºC overnight until further use.[5]

Determination of total polyphenols (TP)

The total phenolic content was determined by using the Folin-Ciocalteu assay[6]. 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 an 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 blend with water at various dilutions of various alcoholic dilutions (0, 25%, 50%, 75%, and 100%) and that at 25°±2ºC. The best extraction solvent was chosen by the estimation of TP, mg GAE/100 g).[7,8]

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±2ºC. 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 (10:0, 8:2; 6:4, 4:6, 2:8 and 0:10v/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 (1:1v/v). They were stirred with laboratory homogenizer at 7000 rpm for 30 min.[9-10]

Spray drying

The microencapsulation was carried by spray drying method suggested[11]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, entrance air temperatures of (120, 140, 160, 180^{0}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^{0}C.

Statistical analysis

The results obtained were subjected to statistical analysis of variance (ANOVA) using complete randomized design. The critical difference at P<0.05was estimated for significant difference.[12]

Result and Discussion

The chemical composition of fresh Tas-A-Ganesh Grapes (TAGG)clusterwas analyses the results are shown in Table 1.

Table.1 Chemical composition of Tas-A-Ganesh Grape (TAGG)

Parameters	**Values**
Moisture (%)	85±0.90
Acidity (%)	0.62±0.08
TSS (^{o}Brix)	20.00
Crude Protein (%)	0.68 ±0.18
Brix-acid ratio	35.71±0.26
Total sugars (%)	19.42±0.40
Reducing sugars (%)	18.02±0.42
Non reducing sugars (%)	1.40±0.08
Ascorbic acid (mg 100 g-1)	2.52±0.10
Results are mean ± SD of 3 determinations	

The moisture 85%, Total soluble solid 20.00 ^{o}Brix, crude protein 0.68 %, total sugar was 19.91% (Reducing sugars 18.02%, Non reducing sugars 1.40%), ascorbic acid was2.52 mg/ gm.

Table 2. Physicochemical composition of TAGGP

Parameters	Results
Moisture (%)	4.20±0.40
Ash (%)	4.60±0.60
Fat (%)	6.14±0.08
Protein (%)	9.10±0.20
Carbohydrate (%)	30.96±0.32
Glucose (%)	7.95±0.18
Fructose (%)	8.90±0.10
Total Fiber (%)	44.20±0.80
Total Phenolic compound (mg GAE/100 g)	24.10

Pomace yield depend on pressing grape method, bunch type, harvesting parameter and types of grape. On dry matter (pomace powder) basis the physicochemical composition of TAGGPwas showed in Table no. 2moisture content (4.20%),Ash (4.60%) Fat (6.14%) Protein (9.10, Carbohydrate (30.96) and Totalfiber was(44.20%), Note that phenolic compounds in GP are influenced by many factors, including grape variety, growth climate and location, harvest time, as well as processing and storage conditions.[13-14]

Table.3 Total Polyphenols in Tas-A-Ganesh Grape Pomace using different extracting solvents.

Solvent Concentration	Extraction Time (hrs.)	Total Polyphenols(mg of Gallic acid equivalents /g)	
		Ethanol Solvent	Methanol Solvent
0%	0	10.20±0.09d	10.20±0.09d*
	1	10.90±0.10d	10.90±0.10d*
	2	12.33±0.15c	12.33±0.15c*
	3	14.13±0.23b	14.13±0.23b*
	4	16.16±0.35a	16.16±0.35a*
	5	15.43±0.40a	15.43±0.40a*
25%	0	11.06±0.41c	11.43±0.25e
	1	12.76±0.15bc	13.27±0.17d
	2	14.63±.0.20b	15.56±0.33c
	3	17.30±1.41a	17.43±0.40b
	4	18.40±0.20a	18.96±0.32a
	5	17.93±0.30a	17.96±0.05ab
50%	0	12.96±0.32c	14.50±0.36c
	1	13.78±0.29c	16.45±0.43b
	2	15.63±0.35b	17.94±0.62b
	3	16.58±0.33b	20.27±0.25a
	4	19.21±0.22a	20.49±0.61a
	5	18.44±0.03a	20.43±0.49a
75%	0	14.48±0.36d	14.94±0.04e
	1	16.36±0.17c	17.30±0.32d
	2	18.26±0.05b	19.36±0.18c
	3	20.16±0.02a	20.06±0.50bc
	4	20.90±0.13a	22.62±0.15a
	5	20.40±0.29a	21.14±0.22ab
100%	0	14.05±40c	14.12±0.02d
	1	17.07±0.0b6	17.20±0.02c
	2	18.46±0.46b	18.95±0.12b
	3	19.80±0.35a	19.60±0.42ab
	4	19.72±0.43a	20.41±0.42a
	5	19.10±0.26a	19.69±0.42ab

*indicate that the same value i. e. 0% solvent means 100% refined water

Statistical analysis indicated that both solvent concentration and time highly effect on extraction of polyphenol compound from TAGGP. It depends on the extraction conditions 0%, 25%, 50%, 75% and 100% of Ethanol and Methanol concentration. It was observed an increase the total polyphenols extraction with increase solvent concentration but 100% solvent concentration used for extraction that time decrease polyphenols as compared with 75% solvent concentration. Table 3shows that the different types of solvent has a significant effect ($p < 0.05$) on TP content and they were able to extract polyphenols, but methanol 75% was the most effective solvent as ethanol at the same concentration. Methanol 75% allows extracting the highest quantity TP which was22.62±0.15 mg GAE/100gfollowed by ethanol (75%) 20.90 ± 0.13mg GAE/100 g.as shown in the Table no.3, the highest TP content is obtained after an extraction time of 4 hours. In the literature, a few solvents are utilized for extraction of polyphenols and regularly blended with water at various amount. [15,16,17]

Hence, excessive extraction time was no longer useful to extract more phenolic compounds from TAGGP. From an economic point of view and also taking into account the yield of phenolic compounds from the TAGGP extract, 4 hours can be considered as optimal extraction time at room temperature i. e. 25°±2ºC

Microencapsulation of Total Polyphenols

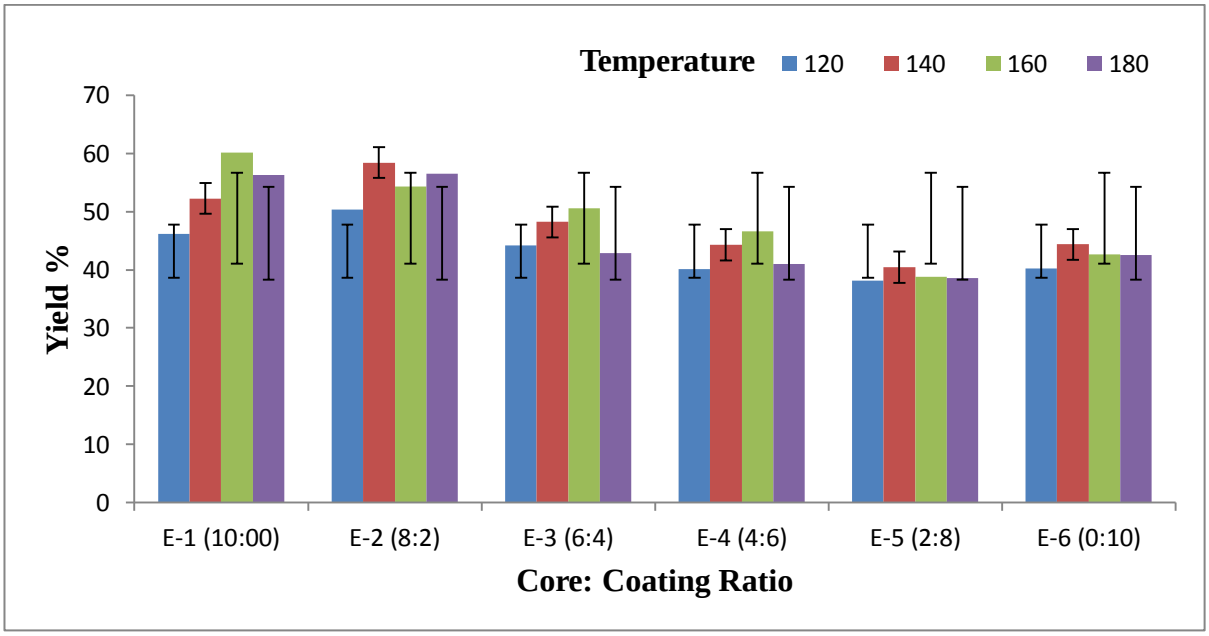

Figure1. Yield of microencapsulated powders with Maltodextrin and Gum Arabic ratio at different inlet temperatures.

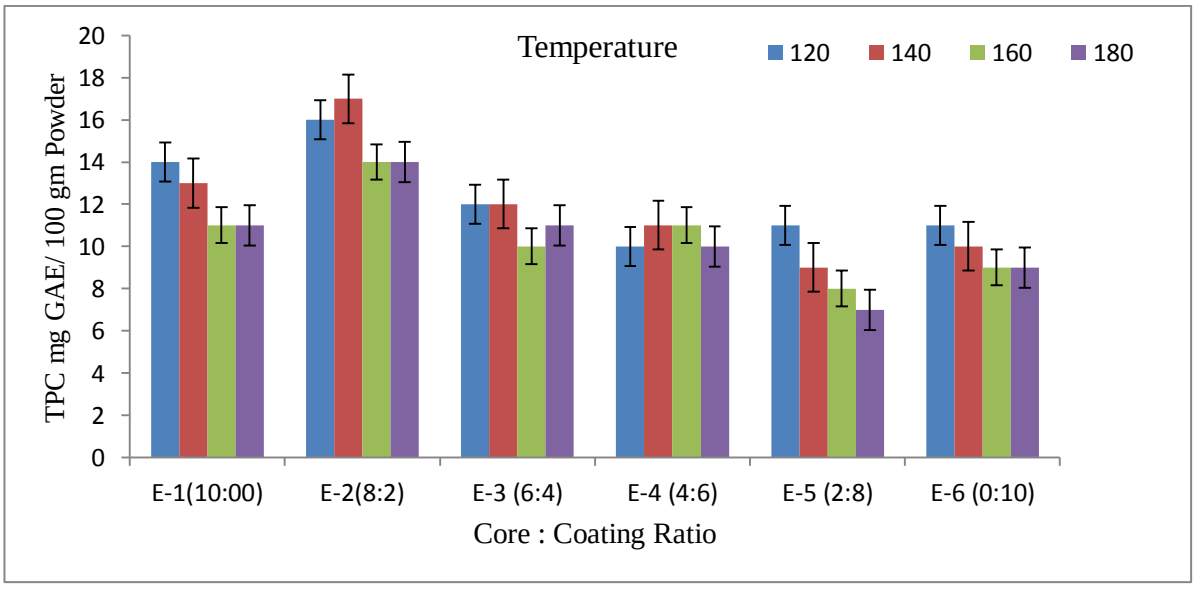

Figure 2.Total polyphenols of microencapsulated powders

Total polyphenol content yield showed an increase with the rising temperature in the event of different coating material ratios (10:0; 8:2; 6:4,4:6, 2:8 and 0:10) and the core: coating material ratios (1:1). The increasing temperatures led to greater process yield, which can be attributed to the greater efficiency of heat and mass transfer processes occur in the case of using higher inlet air temperatures.

The use of only Maltodextrin resulted in variable yield between 46.02–60.12%, whereas the range was observed between 50.3–58.4% in the case of utilizing MD: GA at ratio of 8:2. Moreover, it was found that increasing the gum Arabic amount in the coating material has decreased this value up to 38.12–58.4%. Therefore, it can be concluded that the increasing ratios of MD and GA have contrary influence on the yield. The highest yield (60.12%) among all coating material ratios was achieved when the MD: GA ratio was 10:0 and the temperature was 160^0Cin the conditions of 1:1 core: coating material ratio with maltodextrin.

Applications of different temperatures were shown to have an effect on the amount of polyphenols. The polyphenols an increased when the MD: GA ratio was 8:2 at 140^0C, but their amounts decreased when the temperature was increased above 140^0C. This might be due to polymerization and synthesis of polyphenols after 140^0C. A further increase in temperature caused those phenolic components to degrade. The highest amount of total polyphenols 17 mg GAE/100 gwas obtained with a MD: GA ratio of 8:2 at 140^0C.

The amount of phenolic compound increased in general when temperature was increased from 120^0C to 140^0C, but started to decrease when the temperature continued increasing (140^0C to 180^0C). Comparing all process conditions, the powders obtained with maltodextrin resulted in higher amounts of polyphenols with compare between different ratios MD: GA.[18]

Conclusion

The aim of the current study was to get a higher insight into optimisation of solvent extraction of total polyphones from TAGGP.The waste obtained from TAGGP was good source of total fiber, Carbohydrate and Total polyphones(22.60 mg GAE/100 g), whereas it is fair source of fat. Among the solvent used for the extraction the methanol gives better extractability at 75% concentration with 4 h extraction time. During the encapsulation study of the total phenolic it was observed that, highest retention was observed when MD to GA ratio was 8:2 at 140^0C.

Reference

1. R.G. Somkuwar, J. Satisha and S.D. Ramteke. Berry weight, quality and cane biochemistry changes in relation to cane thickness of own-rooted and grafted ‘Tas-A-Ganesh’ grape. Journal of. Horticultural. Science., 2013, Vol. 8(1):30-34,
2. Sousa, E., Uchôa-Thomaz, A., Carioca, J., Morais, S., Lima, A., & Martins, C. et al. Chemical composition and bioactive compounds of grape pomace (Vitisvinifera L.), Benitaka variety, grown in the semiarid region of Northeast Brazil. Food Science and Technology (Campinas), 2014, 34(1), 135-142.
3. Jara-Palacios, M. José et al. "Study of Zalema Grape Pomace: Phenolic Composition And Biological Effects In Caenorhabditis Elegans". Journal of Agricultural and Food Chemistry 61.21 (2013): 5114-5121.
4. Sunitha N. Seenappa. Chemical Analyses of Vermicomposted Red Pomace Waste from a Winery. World Journal of Applied Environmental Chemistry. 2012, Volume 1, Issue 1: 13-17
5. Libran, C., Mayor, L, Garcia-Castello, E., Gras, M.L., Arguelles, A., Vidal-Brotons, D. Assessment of process conditions on the extraction of antioxidants from grape marc. International conference on food innovation. 2010, October 25 -29.
6. Singleton, V. L.; Rossi, Jr J.A. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965, 16, 144-158.
7. Spigno, G., Tramelli, L., & De Faveri, D. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering, 81(1), 200-208.

8. Karacabey, E. &Mazza, G. (2008). Optimization of Solid−Liquid Extraction of Resveratrol and Other Phenolic Compounds from Milled Grape Canes (Vitisvinifera). Journal Of Agricultural And Food Chemistry, 56(15), 6318-6325. http://dx.doi.org/10.1021/jf800687b
9. Tolun, A., Altintas, Z., &Artik, N. Microencapsulation of grape polyphenols using maltodextrin and gum arabic as two alternative coating materials: Development and characterization. Journal of Biotechnology, 2016, 239, 23-33.
10. Boonchu, Thapakorn and NiramonUtama-ang. "Optimization Of Extraction And Microencapsulation Of Bioactive Compounds From Red Grape (VitisVinifera L.) Pomace". Journal of Food Science and Technology., 2013, 52.2: 783-792.
11. Shu, B., Wenli, Yu, Yaping, Zhao and Xiaoyong Liu (2006). Study onmicroencapsulation of lycopene by spray-drying. *J. Food Eng.* 76: 664–669.
12. Steel R, Torrie J (1981) Principles and Procedures of Statistics: A Biometrical Approach. 2nd edition. McGraw-Hill Book Co., New York, 633 p.
13. Lafka, T.-I., Sinanoglou, V., &Lazos, E. S. On the extraction and antioxidant activity of phenolic compounds from winery wastes. Food Chemistry., 2007, 104(3), 1206-1214.
14. Thimothe, J., Bonsi, I. A., Padilla-Zakour, O. I., & Koo, H. Chemical characterization of red wine grape (vitisvinifera and vitis interspecific hybrids) and pomace phenolic extracts and their biological activity against streptococcus mutans. Journal of Agricultural and Food 18 Chemistry, 2007, 55(25), 10200-10207
15. Naczk, M. and Shahidi, F. Extraction and analysis of phenolics in food. Journal of Chromatography., 2004, 1054: 95–111.
16. Hismath, I., Wan Aida, M. and Ho, C.W. Optimization of extraction conditions for phenolic compounds from neem, Azadirachtaindica) leaves. International Food Research Journal., 2011, 18(3): 931-939.
17. Benmeziane, F., Djamai, R., Cadot, Y. and Seridi, R. Optimization of extraction parameters of phenolic compounds from Algerian fresh table grapes, (VitisVinifera). International Food Research Journal., 2014, 21(3): 1061-1065.
18. Aizpurua-Olaizola, O., Navarro, P., Vallejo, A., Olivares, M., Etxebarria, N., Usobiaga,A. Microencapsulation and storage stability of polyphenols from Vitisvinifera grape wastes. Food Chem., 2016, 190, 614–621.

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