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OPTIMIZATION OF PROTEIN EXTRACTION AND SOLUBILIZATION, FOR MATURE GRAPES

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ABSTRACT

Protein extraction from grape berries has been challenging, but when it is in mature state, it can have the sugar concentrations as high as 26%. Grape skins and seeds contain large amounts of polyphenols, which can also interfere with efficient protein extraction. It is difficult to extract proteins from cluster stems because, they are highly lignified. In reference article, the Sarry protocol is used to obtain the maximum yield. In this work, the response surface methodology is used with two factors: pH and temperature (independent variable), to attain the recovery (dependent variable) yield % of proteins.

KEYWORDS: Sugar Concentrations, Cluster Stems & PH and Temperature

INTRODUCTION

Performing an optimal protein extraction from grape berry clusters is complex. It is difficult to extract proteins from cluster stems because, they are highly lignified. In addition, the concentration of soluble proteins can increase up to five times during berry maturation, yet remains low compared toot her components. Approximately71% of world grape production issued for wine, 27%asfreshfruit, and 2%as dried fruit. A portion of grape production goes to producing grape juice to be reconstituted for fruits canned "with no added sugar" and 100% natural". Grapes are a non climacteric type of fruit, generally occurring in clusters. Grapes is a type of fruit that grow in clusters of 15 to 300, and can be crimson, black, dark blue, yellow, green, orange, and pink."White" grapes are actually green in color, and are evolutionarily derived from the purple grape.

The volume of the berry increases so much that the concentration of soluble proteins becomes highly diluted and making it difficult to fully recover the proteins prior to 2-DE. The high concentration of sugars at maturity also impairs protein extraction from berry clusters. Polysaccharides co-precipitate with soluble proteins, hence interfering with protein purification. A wide variety of tissues compose berry clusters, and protein content varies from one tissue to another. Stem and seeds, with low water content, probably have a higher protein concentration per fresh weight than the flesh. Consequently, finding a unique protein extraction protocol suitable for all tissues composing the grape cluster is challenging.

LITERATURE REVIEW

Grapes can be eaten fresh as table grapes or they can be used for making wine, jam, juice, jelly, grape seed extract, raisins, vinegar, and grapes seed oil. Grapes are a non-climacteric type of fruit, generally occurring in clusters.

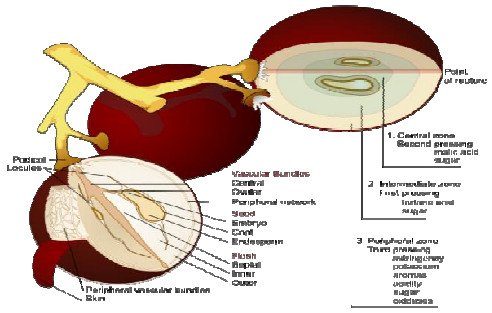


Figure 1

Anthocyanins and other phenolics: Anthocyanins tend to be the main polyphenolics in purple grapes, where asflavan-3-ols (i.e. catechins) are the abundant phenolic in white varieties. Total phenolic content, a laboratory index of antioxidant strength, is higher in purple varieties due, almost entirely to anthocyanin density in purple grape skin, compared to absence of anthocyanins in white grape skin. It is these anthocyanins, that are attracting the efforts of scientists, to define their properties for human health. Phenolic content of grape skin varies with cultivar, soil composition, climate, geographic origin, and cultivation practices or exposure to diseases, such as fungal infections. Red wine may offer health benefits more so than white because potentially beneficial compounds are present in grape skin, and only wine is fermented with skins. The amount of fermentation time a wine spends in with grape skins is an important determinant of its resveratrol content. Ordinary non-muscadinered wine contains between 0.2 and 5.8 mg/L, depending on the grape variety, because it is fermented with the skins, allowing the wine to absorb the resveratrol. By contrast, a white wine contains lower phenolics contents because, it is fermented after removal of skins. Wines produced from muscadine grapes may be more than 40 mg/L, an exceptional phenoliccontent.

Seed Constituents

Biochemical and preliminary clinical studies have demonstrated potential biological properties of grape seed oligomericprocyanidins. For example, laboratory tests indicated a potential anticancer effect from grape seed extract. According to the American Cancer Society, "there is very little reliable scientific evidence available at this time that drinking red wine, eating grapes, or following the grape diet can prevent or treat cancer in people". Grapes seed oil from crushed seeds is used in cosmeceuticals and skin care products for perceived health benefits.

MATERIALS AND METHODOLOGY

Protein Extraction

Frozen whole berry clusters were pulverized with dry ice using a stainless steel blender (Hamilton Beach, Model990). Dry ice was used to pre-chill the blender and to prevent melting of the frozen tissue during pulverization. Following pulverization, the mixture of pulverized grape clusters and dry ice was stored overnight at 2 C to allow for dry ice sublimation, leaving only frozen pulverized plant material. This material was then further ground in liquid nitrogen with mortar and pestle. This finely ground powder was used for all protein extraction protocols (approximately 4 g *per* tube). All extraction steps were performed in 15 ml Falcon tubes until the last step. When not otherwise specified, each centrifugation step with the 15 ml Falcon tubes was performed using the following parameters: 32106*g* at 2107C for 30 min. Just prior to drying, the samples were transferred into 2 ml Eppendorf tubes, to allow for easier re-suspension of the dried pellets. The Eppendorf tubes were centrifuged at14 0006*g*, 07C for 10 min. Eppendorf tubes were weighed first

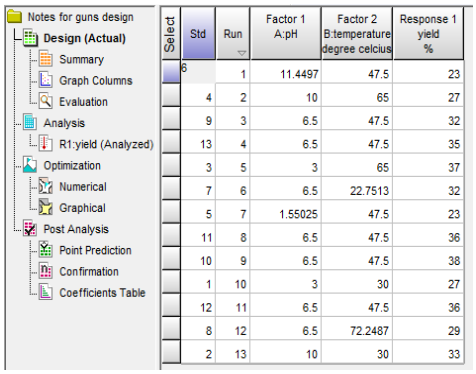
without and then with the pellet. The pellet weight was calculated by subtracting the weight of the empty tube from the weight of the same tube containing the dry pellet. Protein extracts were obtained by adding a defined volume of re-suspension buffer (RB) *per* milligram of pellet and the protein content was evaluated (see Protein assay in Section 2). Extracts were stored at 2807Cuntil further use. Each extraction procedure was replicated three times.

Optimization Method

In this report, Response Surface Methodology of central composite method was used with 13 runs. Here the factors were the pH, temperature and the recovery is yield (%) as shown in the fig 2.

Independent variable: pH, temperature (◦c).

Dependant variable: yield%



Select	Std	Run	Factor 1 A:pH	Factor 2 B:temperature degree celcius	Response 1 yield %
	6	1	11.4497	47.5	23
	4	2	10	65	27
	9	3	6.5	47.5	32
	13	4	6.5	47.5	35
	3	5	3	65	37
	7	6	6.5	22.7513	32
	5	7	1.55025	47.5	23
	11	8	6.5	47.5	36
	10	9	6.5	47.5	38
	1	10	3	30	27
	12	11	6.5	47.5	36
	8	12	6.5	72.2487	29
	2	13	10	30	33

Figure 2

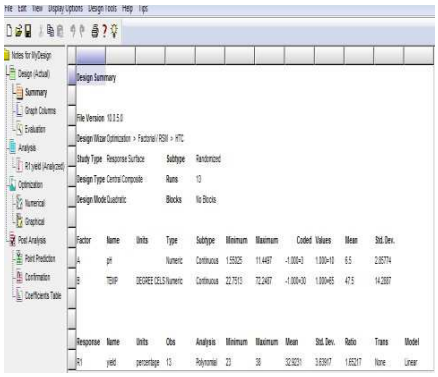


Figure 3

RESULTS

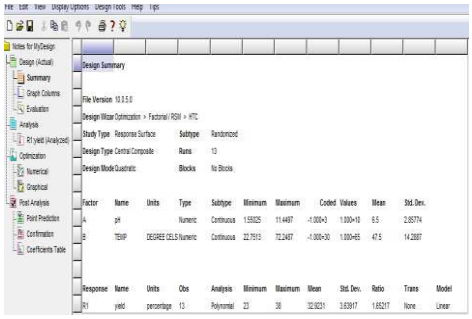


Figure 4

In the fig 3, the two factor values in which the minimum value for pH is 1.55 to the maximum value of 11.44 were used. And for the temperature minimum value is 22.75 and maximum value is 72.24. The linear design model was used in the Response Surface Methodology as shown in the fig4.

ANOVA FOR RESPONSE SURFACE MODEL

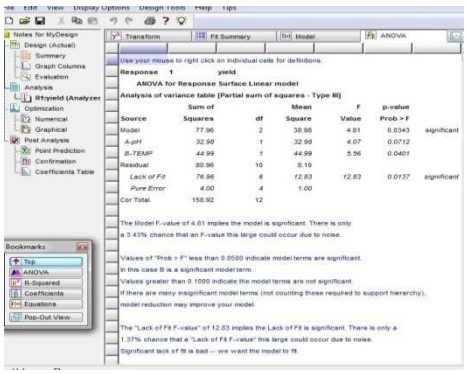


Figure 5

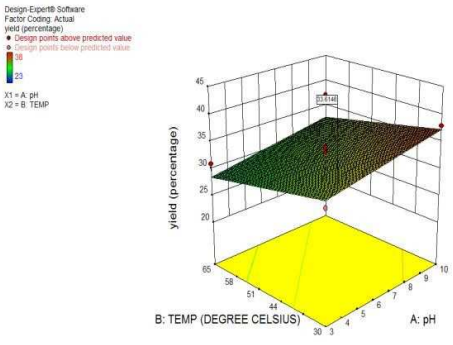


Figure 6

The significant value is carried out for the corrective action. In fig 5, the model F-value of 4.81 implies the model is significant. The lack of fit F value of 12.83 implies the lack of fit is significant. There is only a 1.37% chance that a lack of fit f value this large could occur due to noise.

The equation obtained in coded terms, Yield = 31.38 -0.50 *A -0.030 B

Where, A –pH, B –temperature

DISCUSSIONS

The extraction of protein was increased when the temperature reached up to c and in the pH range greater than 3. The higher levels of protein were extracted at pH 5 to 8 as shown in the fig6.

CONCLUSIONS

The overall optimized values obtained from the Response Surface et odolo (central compo ite) were pH:, temperature: c and t e recover yield (%): 31.4. The reference article used two protocols (Damerval et al. and Sarry et al.) in which, the protein extracted were around 30%. So, the Response Surface Methodology holds good for protein extraction.

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