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Phenolic Compounds and Antioxidant Properties of Grape Pomaces Fermented by *Aspergillus oryzae*

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***Abstract* – A central composite design (CCD) was used to evaluate the effect of temperature, time, solvent concentration and whey protein powder on the phenolic compounds content, flavonoids content, FRAP and DPPH radical scavenging activity of grape pomace fermented by *A. oryzae* . The phenolic compounds content ranged from 18.04-90.23mg/g, the flavonoids content from10.5-71.81mg/g, FRAP from 85-203µmol/g and DPPH from 18.19-87.9%. Results of the CCD showed that temperature had a positive effect on phenolic compounds and flavonoids content and a negative effect on DPPH and FRAP. Time and solvent concentration had a positive effect on all of the responses. Whey protein powder had a negative effect on all of the responses.**

***Keywords* – Grape Pomace, Total Phenolic, Antioxidant Activity, Aspergillus Oryzae.**

I. INTRODUCTION

The use of synthetic antioxidant in food such as butylated hydroxyanisol, butylated hydroxytoluene, and tertiary butylhydroquinone, is discouraged because of their toxicity and carcinogenicity (1).

Some of the natural antioxidant compounds are as effective as synthetic antioxidants in different models(2).grapes contain high amount of phenolic compounds such as gallic acid, catechin , resveratrol, and a wide variety of procyanidins.

A wide range of biological activities of these phenolic compounds has recently been reported: inhibition oxidation of human low – density lipoproteins (3), antioxidant, radioprotective (4), anti – inflammatory (5) antihyperglycemic effect (6), and therapy of cancer (7).

Each year the processing of grapes for juice leaves thousands tons of press residue berry skins and seeds are where most phenolic compounds accumulate. For this reason, grape residue has become popular in recent years as a nutritional supplement.

In some cases the antioxidant effect could be significantly enhanced through fermentationusing aspergill. A significant high concentration of phenolics was obtained after fermentation when compared to unfermented soybeans.(8) limited information is available regarding the extraction of antioxidant compounds in fermented fruits.

The aim of this study was to determine phenolic and flavonoid content in grape pomace fermented by aspergillus oryzae and their potential antioxidant activity.

II. MATERIALS AND METHODS

Preparation and extraction of samples fruit samples of Vitis vinifera cv.rashe was supplied by the Agricultural and Natural Research center of west Azarbaijan province, Iran.

A. oryzae was originally obtained from the Persian Type Culture Collectio. A distilled water suspension of the fungi spores was kept at -30 ˚C until used. The volume of inoculum was 1.5ml with a cell concentration of $1.2*10^8$ cells/ml.

Whey protein powder (10-50gr) were added to grape pomace (150gr) then they were both mixed with the spore suspension and manually shaken for 5 min to homogenise the inoculum. The inoculated grape pomace incubated at 30 ˚C for 5 days.

Detailed extraction conditions of temperature, time and concentration of ethanol are shown in Table 1.extraction was carried out in an ultrasonic bath(Ultra cleaning 1400, 40hz, 80W, Unique, Ind. e Com. Ltd., Brazil). samples (10g) were placed into erlenmeyer flasks (125ml) with 100ml of extraction solvent. ethanol, a safe solvent for the extraction of bioactivies in grape (9) was used .after extraction , samples were cooled with tap water and filtered under vacuum through whatman No.1 paper.subsequently the samples were placed in a 100ml volumetric flask, which was then filled to the mark, and used in antioxidant determination tests.

Total phenolic compounds content

The total phenolic compounds content of each extract was determined spectrophotometrically (Hewlett-Packard 8452 A Spectrophotometer) according to the Folin – Ciocalteau method (10). Absorbance was read at 765 nm and results were expressed in dry weight (dw) of pomace, as mg/g gallic acid equivalent (GAE).

Total flavonoids content

The total flavonoid content was determined using the colourimetric method described previously by Dewanto et al(11). The results were calculated and expressed as micrograms of rutin equivalents (mg RAE/g DM) using the calibration curve of rutin.

DPPH radical scavenging activity

Total antioxidant capacity (free radical scavenging activity) was measured from the bleaching of the purple colored methanol solution of free stable radical (diphenyl picrylhydrazyl [$DPPH^{\bullet}$]) inhibition, following the method of Tepe et al.(12). $DPPH^{\bullet}$ radical is a stable radical with a maximum absorbtion at 517 nm that can readily undergo reduction by an antioxidant. The percentage inhibition of

free radical DPPH•(I%) was calculated in the followin way:

$I\% = (A_{blank} - A_{sample} / A_{blank}) *100$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound.

Reducing Power – FRAP method

As described by Benzie and Strain (13), the FRAP method is based on the direct measurement of antioxidant (reducing) ability through the reduction of the complex Fe^{3+}/ tripyridyltriazine (TPTZ) to Fe^{2+} at acid PH (3.6. Absorbance was read at 620nm and the reducing power was expressed (on a dw basis) in µMol/g of TEAC.

Experimental design and statistical analysis

Experimental design was performed using central composite design (CCD). Temperature (55-67 ˚C), processing time (24-32minutes), solvent concentration (37-49%) and whey protein powder content (10-50 gr) were the factors investigated. The experimental responses were analyzed using the "Regressionential 2.210" to determine the effect of the independent variables and the interaction among them on the following responses: total phenolic compounds content, total flavonoids content, DPPH, FRAP. Following quadratic polynomial was fitted to experimental data applying regression analysis methods.

$Y=\beta_0 + \Sigma\beta_i x_i + \Sigma\beta_{ii} x_i^2 + \Sigma\Sigma\beta_{ij} x_i x_j$

Where y= predicted response, β_0 = aconstant, β_i= linear coefficient, β_{ii}=quadratic coefficient and β_{ij} = intraction coefficient.

Regressionential 2.210 was used to statistical analysis of data and creation of plots.

III. Results and Discussion

Among fruits, grapes are a major source of phenolic compounds, specifically flavonoids. phenolic compounds are the most active natural antioxidant in plants (14). They are known to act as antioxidant not only because of their ability to donate hydrogen or electrons but also because they are stable radical intermediates(15).

Free radicals possess an unpaired electron, which makes them highly reactive. Antioxidants neutralize free radicals by donating a hydrogen atom to them.

New environmentally friendly methods have been developed for phenolic compounds extraction. These techniques promote a decrease in solvent consumption with an increase in the extraction ratio (16&17).among these techniques is ultrasound-assisted extraction, which is a simple , efficient and inexpensive alternative(16-20).the greater efficiency achieved with ultrasound- assisted extraction is due to the acoustic cavitations effects produced in the solvent when the ultrasonic wave passes through it. This effect permits better penetration of the solvent into the sample, increasing the release of the analyzed compound from the matrix to the solvent (18). In addition, since ultrasound is a nonthermal process, thermal decomposition of heat sensitive compounds is avoided (21). Ultrasound extraction of phenolic compounds and antioxidants from citrus (21), grape seeds (22), and pomegranate seed (23) were studied by various researchers.

The result summarized in Table 1 show the enfluence of temperature, time,whey protein powder content and solvent concentration on the contents of phenolic compounds, falavonoids, DPPH radical scavenging activity and FRAP, using a central composite design. The effect of each factor and their intraction were obtained with confidence interval of 95% (Table 2).

Table 1: Experimental design of five level, four variable central composite design and total phenols, flavonoids, DPPH and FRAP amounts of ultrasonic assisted grape pomaces fermented by *A.oryzae*

Run	wp	c.s	te	ti	TPmg/g	flavonoids	DPPH%	FRAPµmol/g
1	30	43	61	28	62.21	53.4	53.12	158
2	30	37	61	28	18.04	10.5	18.19	85
3	30	43	61	28	61.18	52.12	54	164
4	20	40	58	30	25.47	19.87	38	139
5	30	43	61	28	60.04	50.89	50.5	157
6	40	40	58	30	24.76	18.7	36.5	134
7	30	43	55	28	20.1	18.04	50.19	155
8	40	40	64	26	38.5	30.85	24.12	95
9	40	46	64	26	76.9	64.8	60.33	172
10	40	46	58	30	30.95	24.8	81.51	203
11	40	46	58	26	28.28	21.29	78.09	198
12	40	40	64	30	39.28	30.9	26.1	99
13	20	46	64	26	80.95	68.83	65.14	175
14	30	49	61	28	70.95	57.53	87.51	197
15	30	43	61	32	65.23	55.58	60.93	165
16	40	40	58	26	22.14	15.19	34.06	124
17	30	43	61	28	55.91	46.49	53.5	155
18	30	43	61	28	58.17	50.1	49	166

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19	20	40	64	26	35.92	28.91	30.18	114
20	20	46	58	26	29	22	83.8	202
21	30	43	61	28	59.18	47.6	48	165
22	40	46	64	30	86.42	71.28	65.95	178
23	20	40	58	26	24.69	16.62	35.41	131
24	20	46	58	30	33.8	26.88	87.9	203
25	20	46	64	30	90.23	71.81	70.03	185
26	30	43	67	28	72.61	60.77	28.5	107
27	20	40	64	30	41.42	32.59	32.19	120
28	50	43	61	28	47.14	38.7	44.98	144
29	30	43	61	24	43.8	35.19	50.21	154
30	10	43	61	28	50.23	41.29	53.18	157

Table 2: Regression coefficient and analysis of the model

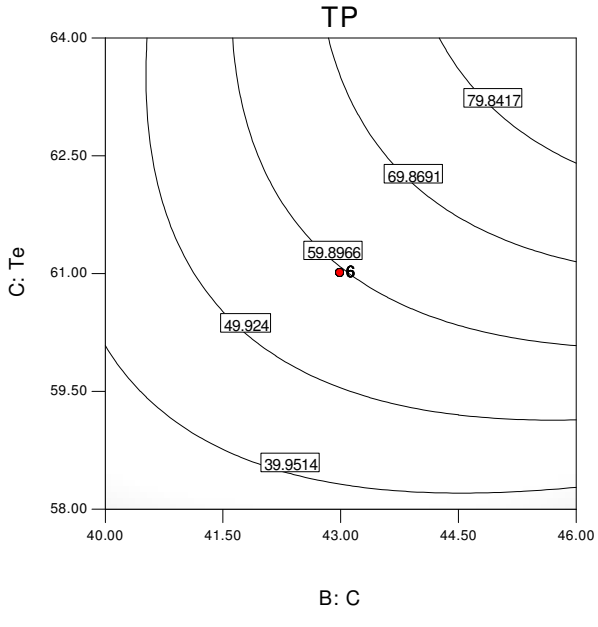
Response	R^2	R^2-adjusted	R^2-pridicted	Regression equation
Total Phenol	0.995	0.993	0.989	TP = 59.44833-0.85125*A+14.74306*B+16.99396*C +2.161042*D+9.650625*B*C+1.036875*B*D+0.888125* C*D-2.77667*A^2-3.82417*B^2-5.37823*C^2-3.00323*D^2
Flavonoids	0.994	0.991	0.988	flavonoid = 50.1-0.62*A+11.33833*B+16.99396*C +1.691944*D+8.055*B*C-2.60556*A^2-4.10056*B^2- 4.86264*C^2-3.04014*D^2
DPPH	0.978	0.971	0.948	DPPH=52.44938-2.18292*A+19.78458*B-6.02542*C +2.020417*D-2.40438*B*C-2.49398*C^2+1.562266*D^2
FRAP	0.989	0.985	0.975	FRAP = 161.5278-3.83333*A+35.65278*B-12.1667*C +3*D+2.375*A*B-2.125*A*C-1.91667*A^2-6.79167*C^2

Effect of independent variables on phenolic compounds content

The assay with the highest phenolic compounds content was number 25(Table1) with 90.23 mg GAE /g where the variable temperature, time,whey protein powder content and solvent concentration were adjusted at 64 ˚C, 30min, 20 gr and 46% respectively. The lowest phenolic copmpound extraction was observed in assay number 2 with 18.04 mg GAE /g where the variable temperature, time, whey protein powder content and solvent concentration were adjusted at 61 ˚C, 28min, 30 gr and 37% respectively.

Sanchez, et al evaluated the total polyphenol content extracted from grape pomace of the Airen variety and obtained a value of 78.5mg/g (23). There are significant diffrences between the total content of phenolic compounds in the extract of different varieties, because the phenolic concentration in grapes is dependent on genetic, environmental and cultural characteristics (24).

The results reported in Table 2 show that the model for phenolic compound content has a R-square of 0.99. The R-square value is an indicator of how well the model fits the data , i.e.. an R-square close to 1.0 indicates that almost all of the variability was accounted with the variables specified in the model. All variables had significant liner and quadratic effects ($p<0.05$). In addition, interaction between the variables had a significant effect on phenolic compounds content (fig1).



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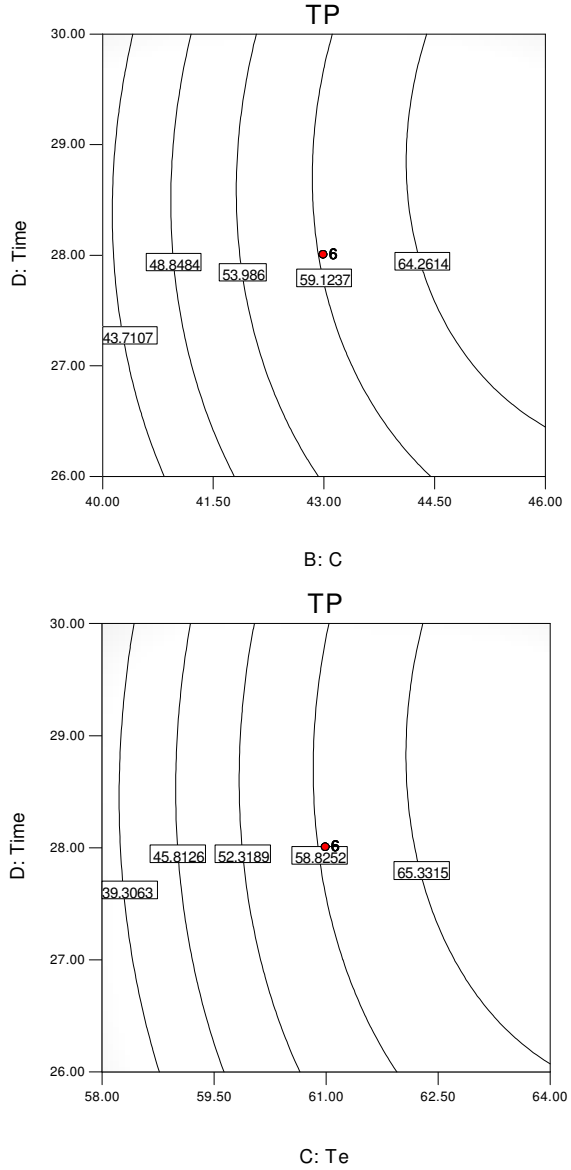

Fig.1: Phenolic compound content as affected by ethanol concentration, time and temperature

Effect of independent variables on flavonoids content

The assay with the highest flavonoids content was number 25(Table1) with 71.81 mg quercitin /g where the variable temperature, time,whey protein powder content and solvent concentration were adjusted at 64 ˚C, 30min, 20 gr and 46% respectively. The lowest flavonoids extraction was observed in assay number 2 with 10.5 mg /g where the variable temperature, time,whey protein powder content and solvent concentration were adjusted at 61 ˚C, 28min, 30 gr and 37% respectively.

The results reported in Table 2 show that the model for flavonoids content has a R-square of 0.99. The R-square value is an indicator of how well the model fits the data , i.e.. an R-square close to 1.0 indicates that almost all of the variability was accounted with the variables specified in the model. All variables had significant liner and quadratic effects ($p<0.05$). In addition, interaction between the temperature and solvent extraction had a significant effect on flavonoids content (fig2).

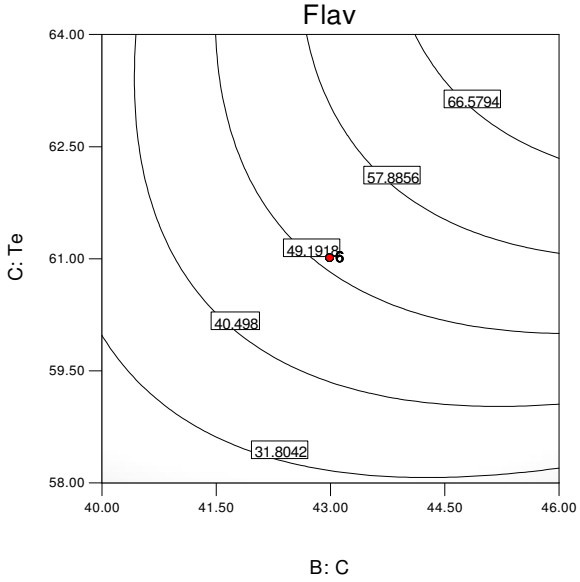

Fig.2. Flavonoids content as affected by ethanol concentration and temperature

Effect of independent variables on DPPH radical scavenging activity

The highest amount of DPPH% (87.9%) was observed in assay number 24, where where the variable temperature, time,whey protein powder content and solvent concentration were adjusted at 58 ˚C, 30min, 20 gr and 46% respectively. On the other hand, the lowest percent of DPPH (18.19%) was observed in assay number 2 where the solvent concentration were set at the lowest level. The model adjusted for DPPH had a R-square 0.98, and all of the variable had a significant linear effect ($p<0.05$), but only temperature and time had a significant quadratic effect. Whey protein powder content and temperature had a negative effect but solvent concentration and time had a positive effect). In addition, interaction between the temperature and solvent extraction had a significant effect on DPPH radical scavenging activity (fig3).

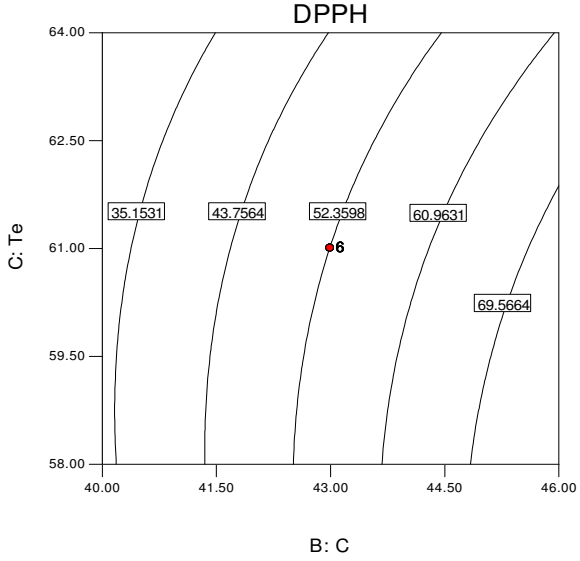

Fig.3. DPPH as affected by ethanol concentration and temperature

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Effect of independent variables on FRAP

The highest amount of FRAP (203µmol/g) was observed in assay number 24, where the variable temperature, time,whey protein powder content and solvent concentration were adjusted at 58 ˚C, 30min, 20 gr and 46% respectively. On the other hand, the lowest FRAP (85µmol/g) was observed in assay number 2 where the solvent concentration were set at the lowest level. The model adjusted for FRAP had a R-square 0.98, and all of the variable had a significant linear effect ($p<0.05$), but only temperature and whey protein powder had a significant quadratic effect. Interaction between whey protein powder and solvent concentration had a positive effect and Interaction between whey protein powder and temperature had negative effect on FRAP.

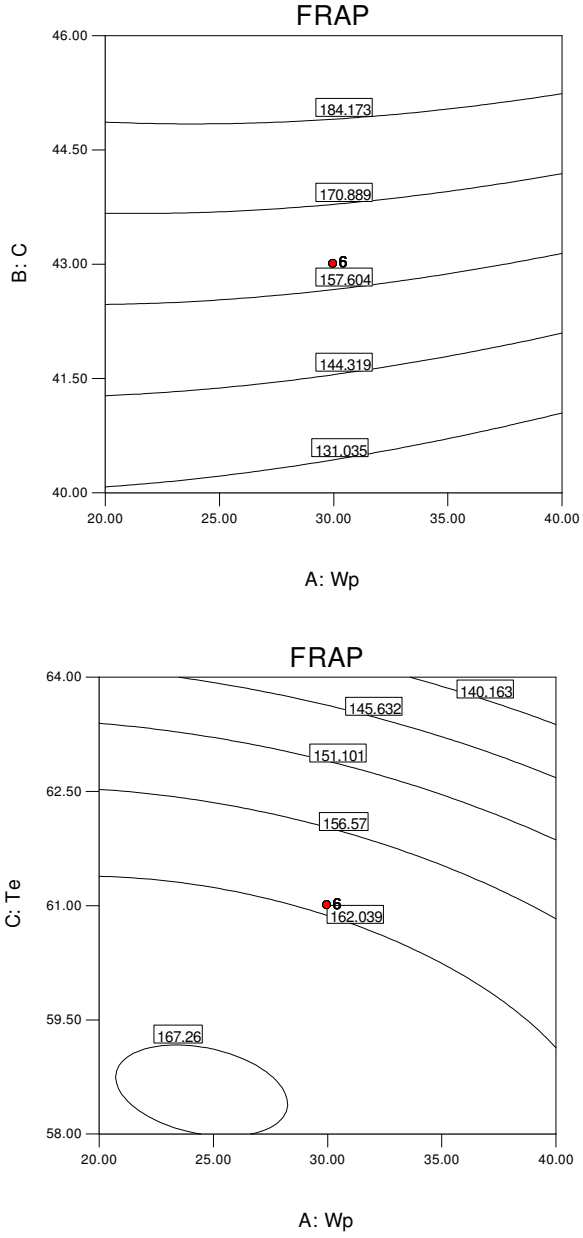

Fig.4. FRAP as affected by ethanol concentration, whey protein powder and temperature

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