INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 3, p. 94-100, 2015

RESEARCH PAPER OPEN ACCESS

Optimization of extraction the red cabbage extract with ultrasound technology, assisted by response surface method

Reihaneh Ahmadzadeh Ghavidel[1*], Zahra Sheikholeslami[2], Saeed Ahmadi[3]

[1]*Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran*

[2]*Technical and Engineering Research Section, Agricultural and Natural Resources Research Center of Khorasan Razavi, Iran*

[3]*Young Researchers and Elite Club, Quchan Branch, Islamic Azad University, Quchan, Iran*

Key words: Red Cabbage, Sonication, Extraction, Anthocyanin, Antioxidants.

http://dx.doi.org/10.12692/ijb/6.3.94-100 Article published on February 07, 2015

Abstract

The issue of free radicals and their effects on biological systems is one of the important issues raised in medical and food industry. Antioxidants are in biological systems to protect against free radicals. In recent years the use of synthetic antioxidants, like other chemical additives restricted due to their probability toxicity and Carcinogenesis. Today, most of the research conducted in these field. Improvements the use of new antioxidant and safely extraction from plant, animal and microbial. In this study, the optimization of the extraction process of the red cabbage extract by ultrasound technology and Evaluation of antioxidant properties was performed with different methods. The process time and temperature were examined in three levels (10, 25, 40 minutes), (40, 55, 70 °C) and the ultrasonic power was 35 kHz. This plan in 2-factor and three-levels which includes 13 tests was performed by the method of response surface. The purposes of this research was to determine the maximum power, free radical scavenging of DPPH, extracted anthocyanin, total phenols, total renewal of the power extraction efficiency. Results of Statistical analyzes to determine the optimal mode showed that, the optimal conditions for red cabbage extract, is 60.94°C for 40 minutes. Amount of anthocyanin concentrate, the rate of extraction efficiency, iron regenerative power, the amount of total phenolic compounds and also the amount of EC50 has inverse relationship with Inhibit power of DPPH free radical in the optimal point Was reported respectively, 9.468158, 8.310664, 421.1768, 4684.69, 5.756068.

* **Corresponding Author:** Reihaneh Ahmadzadeh Ghavidel ✉ reahmadzadeh@yahoo.com

Introduction

Cabbage (*Brassica oleracea* L. var. capitata) is one of the most important vegetables grown worldwide. It belongs to the family Cruciferae, which includes broccoli, and kale. The different cultivated types of cabbage show great variation in respect of size, shape and color of leaves as well as the texture of the head (Rokayya S . *et al.,* 2013). Phenolic compounds with ascorbic acid are major antioxidants of *Brassicaceae* vegetables, while lipid-soluble antioxidants are responsible for only 20% of the total antiradical capacity. Among the analyzed *Brassica* species, Brussels sprouts, broccoli and red cabbage are considered as the vegetables with the most efficient antiradical system (Leja M. *et al.,* 2010). Antioxidant compounds have a very important role in health the antioxidant is a compound that can counteract and mitigate the negative impact of oxidants in the body.

Various scientific evidence indicates that antioxidant compounds reduce risk of chronic disease such as cancer and coronary heart disease. The main character of the antioxidant compound is its ability to capture free radicals. Natural antioxidant compounds found in plants such as vitamin C, vitamin E, carotenoids, phenolic acids, polyphenols and flavonoids known to potentially reduce the risk of degenerative diseases. (Sugiastuti S *et al.,* 2011). During the last decades application of ultrasound to extraction has found increasing attention. Ultrasound was used in extraction of plant materials thanks to enhancedment. (Nguyen T.P., Le V.V.M., 2010). Response surface methodology (RSM) has been applied in the industrial processes for optimization of response of interest. Ultrasound assisted extraction is an inexpensive, simple and efficient alternative to conventional extraction techniques. (Ghafoor K., Choi Y.H., 2009). Many different techniques have been employed for the extraction of antioxidants from plants. The conventional extraction methods, like reflux extraction and maceration extraction have many drawbacks, including the need for long extraction times and the need for relatively large quantities of solvent. In addition, most active ingredients of plants are found in their cells, making it difficult for mechanical crushers to break cells for extraction, and chemical crushing methods may damage the active molecules and inactivate the extract. Compared with conventional and other modern extraction techniques, ultrasonic assisted extraction (UAE) is proposed as an alternative procedure for sample pretreatment and as a greener methodology that allows for a high reproducibility in shorter time, simplified manipulation, significant reduction in organic solvent consumption and temperature, and lower energy input (Lai J. *et al.,* 2013).

The aim of this study was to optimization of the extraction process of the red cabbage extract by ultrasound technology and Evaluation of antioxidant properties was performed with different methods. The process time and temperature were examined in three levels (10, 25, 40 minutes), (40, 55, 70 °C) and the ultrasonic power was 35 kHz.

Materials and methods

Material

Early Preparation

Red cabbage from *oleracea Brassica* species in May 2013 was puchased from Sabzevar farms. Fresh red cabbage was divided into four parts. Each part located in four layers of plastic cover and frozen at -18 °C. All experiments were performed by using chemical compound from Merck (Germany).

Ultrasound-assisted extraction

Samples were removed from the freezer. The product Surface was removed about 1cm and discarded, then the sample was cut as slice. Samples with ethanol - hydrochloric acid (15:85) solvent were mixed together in the ratio of 1 to 4., and inside ultrasonic device (BANDELIN SONOREX digitec Model 510 H DT Germany) at different temperatures (40, 55 and 70 °C) and time (10, 25 and 40 min) were placed to a constant intensity ultrasound (the device power was KHZ 35). Then extracted by using Whatman filter paper No. 5 (Slow) and a vacuum pump was isolated from plant material. Afrter that, in order to remove

the solvent, the extracts were placed in rotary machine (Model Laborota 4002/4003 control company Haydvlf, Germany) for distillation operations at vacuum. The temperature in this stage was 40-45 ° C to minimize the damage of antioxidant compounds and phenolic compounds. Finally the extract transferred to a glass plate on a water bath at a temperature of 45-50 ° C. The remaining solvent was removed and the extracts were dried until they was not the fluid. Then the plate door closed and covered with aluminum foil to prevent light and placed in 4 layer plastic cover in the freezer at -18 °C and stored until tests time.

Measuring the intensity of anthocyanin with differential pH method

In 1968 Fuleki and Fransis were used differences in wavelength absorption method at two different pH that seems to be good method for anthocyanin measurement. This method based on the difference absorption between the dominant anthocyanin wavelength and 700 nm wavelength in pH=1 (potassium chloride) and pH = 4/5 (sodium acetate). The dominant anthocyanins in red cabbage was cyanidin-3.5-glycoside 520 nm landa maximum.

$\Delta a = [(A_{520} - A_{700})_{pH=1}] - [(A_{520} - A_{700})_{pH=4/5}]$

$C = \Delta a \times M \times D / \varepsilon \times L$

C= the intensity of anthocyanin
M= Molecular mass of the dominant anthocyanin
D= Dilute Factor
L= Cell Length
Ɛ= Molar absorption

Measurement of phenolic compounds amount

5 mg of extract were dissolved in methanol and 2.5 ml Folin's reagent was added to them (Fulin/distilled water=1/10). After mixing the mixture was placed in stasis situation until the reaction be done. Then 5 ml of sodium carbonate 7.5% was added to aqueous phase and after a minute was completed to a volume of 50 mL with distilled water. Samples were kept overnight (24 hours) and then the absorption wavelength of 765 nm was read and the amount of phenolic compounds based on milligrams per kilogram of sample was calculated according to the following formula (Fig. 1)

$P = Y / W \times 1000$

P= the amount of phenolic compounds of sample mg/ml
W= Sample weight

X= sample absorbance –control absorbance
Y= 1/0776 x2 + 0/2644 x + 0/0099

X= sample absorbance –control absorbance
Y= 1/0776 x2 + 0/2644 x + 0/0099

Measurement of iron renewal power

The FRAP solution was prepared as follows:
Acetate buffer, TPTZ reagent solution and 20 mmolar iron chloride III (6H2O) with ratio of 1:1:10 were mix together and kept in a dark place; this solution should be prepared fresh.

Preparation of reagents TPTZ: 23.4 mg of TPTZ reagent with 7.5 ml of 40 mmolar hydrochloric acid were mixed.

Preparation of acetate buffer solution: to prepare acetate buffer solution (0.3 Molar, pH =3/6), 3.1 g 3H2O sodium acetate were mixed with about 16 ml of acetic acid and was reached with 1 liter of distilled water to ensure was measured its pH.

Depending on the inhibitory power of the sample solution containing 100 mg of sample was prepared in 10 mL of methanol and 30 microliter with 90 ml distilled water and 900 microliters of FRAP reagent were mixed in a test tube, the test tube was vortexed bath after the temperature reached 37 ° C the absorption at 595 nm was read. Fe II was obtained from the following formula.

Y= 1782 x – 9/211
X= absorbance of sample - Absorbance of control
Y=Micromoles Fe II per liter
X= read absorbance at 595 nm

Measurement of free radical inhibitory power

0/006 percent free radical DPPH solution was prepared in methanol then into the test tubes (methanol carrier) and the sample with different concentrations (depending on the inhibitory power of free radicals), 1ml of the above solution was added. The test tubes after becoming vortexes for 15 minutes, its absorbance was read at 512nm wavelength against the control sample, the percentage of inhibited free radical was calculated by the following formula.

$\% A = A_C - A_S / A_C \times 100$

A = Inhibitory percentage of DPPH free radical

A_C= control sample absorbance

A_S= Sample absorbance

After diagram drawing of inhibitory percentage of free radicals against concentration of antioxidant compounds, suitable curve was achieved on this data and then the concentration at which of antioxidant compounds able to inhibiting 50% of free radicals were calculated with EC50 name.

Calculating the extraction efficiency

The solvent available in the extract that obtained by ultrasonic extraction method finally evaporated. By Calculating the initial weight and final weight of plate, extracted total dry material was calculated as a percent (mg/g dry sample).

Statistical analysis

RSM is a collection of statistical techniques that are used in the optimization process of the desired response is influenced by a number of variables. Will be reduced with this statistical design of experiments. In this study, response surface designs two variables for investigating the relationship between answers and the treatments and optimization the combination of the treatments was used. Numeric amounts of independent variables include: time and temperature in three levels.

Result and discussion

RSM Modeling

The optimum extraction conditions were determined by using response surface method and design expert 7.0.0 software.

Selection of the appropriate model

In order to assess the validity of the processed models, Lack of Fit efficient, R2, (adj) R2, were determined. The most important part in statistical analysis table in part of the variance analysis is Lack of Fit and statistically the suitable model is the model no significant lack of Fit. This parameter indicate the appropriate or inappropriate model. If it was less than 0/05, it is unusable. A model with good Lack of Adj R-Squared and R-Squared amounts should be close to 1 more and more (Table 1).

Table 1. Amount of the response for each test at different temperature and time.

Time	Temperatur	Anthocyanin	yeild	Frap	Fulin	EC50
40	40	6.6716	7.53	703.59	2821.692	7.0306
40	70	11.4314	8.91	256.31	6889.73	8.5313
25	55	7.1841	7.03	584.19	2821.692	5.4624
25	70	9.7461	7.37	395.51	3959.7413	7.4905
25	55	7.7455	6.73	452.95	2587.7579	4.7565
10	70	8.1328	6.41	648.87	3629.6077	4.2116
10	55	6.5245	5.87	405.99	2416.833	3.5547
25	40	6.8238	6.67	666.17	2533.77	7.2595
10	40	6.1652	5.74	248.35	2360.72	3.4976
25	55	7.0726	6.83	409.77	3062.522	6.3493
25	55	6.6644	6.81	438.07	2762.56	6.1683
25	55	6.8772	6.51	423.81	3310.2501	6.2889
40	55	8.8122	8.06	632.31	3247.671	3.8086

The amount of extracted anthocyanin

The quadratic model for the extraction of anthocyanins was statistically significant (p>0/01), but the weakness of fit test was not significant (p>0/05) that indicating the model is appropriate. Significant phrases in the model were time (A, p>0/01) and temperature (B, p>0/01). The results Obtained from table 2 (R-Squared=0.9578 and Adj R-Squared=0.9277) indicates good matching Computational Model with tested Points and model accuracy (Table 2).

Table 2. Results of statistical analysis of the quadratic model fitted on anthocyanin of red cabbage.

R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision	Std. Dev.	Mean	C.V.	PRESS
0.9578	0.9277	0.8277	18.975	0.41	7.68	5.30	4.75

The amount of extraction Efficiency

The quadratic model for the efficiency of extraction was statistically significant (p>0/01) and lack of fit test was not significant (p>0/05) that indicate the model is appropriate. Significant phrases in the model were time (p>0/01, A) and temperature (P>0/01, B). Results in Table 3 (R-Squared=0.9798 and Adj R-Squared=0.9653) indicates good matching Computational Model with tested Points and model accuracy (Table 3).

Table 3. Results of statistical analysis of the the quadratic model fitted on the extraction efficiency red cabbage.

R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision	Std. Dev.	Mean	C.V.	PRESS
0.9798	0.9653	0.9339	28.298	0.16	6.96	2.30	0.59

Power of iron renewal (FRAP test)

The quadratic model for power of iron renewal was statistically significant (p<0/05) and lack of fit test was not significant (p>0/05), that indicate the model is appropriate. Significant phrases in the model were time (p<0/05,A) and temperature (p<0/05,B). Results in Table 4(0/7686= R-Squared and 0.6033 = Adj R-Squared) indicates good matching Computational Model with tested Points and model accuracy (Table 4).

Table 4. Results of statistical analysis of the quadratic model fitted power of iron renewal on the red cabbage.

R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision	Std. Dev.	Mean	C.V.	PRESS
0.7686	0.6033	-0.5968	8.191	95.19	481.99	19.75	4.376E+005

The amount of total phenolic compound

The quadratic model for the amount of total phenolic compound was statistically significant (p<0/01) and lack of fit test was not significant (p>0/05) that indicate the model is appropriate. Significant phrases in the model were time (p<0/05,A) and temperature (p<0/05,B). Results in Table 5(0/9070= R-Squared and 0.8406 = Adj R-Squared) indicates good matching Computational Model with tested Points and model accuracy (Table 5).

Table 5. Results of statistical analysis of the the quadratic model fitted power of iron renewal on the red cabbage.

R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision	Std. Dev.	Mean	C.V.	PRESS
0.9070	0.8406	0.2940	12.544	474.85	3261.89	14.56	1.198E+007

Inhibitory power of free radical DPPH (EC_{50})

The quadratic model for Inhibitory power of *DPPH* free radical was statistically significant (p<0/05) and lack of fit test was not significant (p>0/05) that indicate the model is appropriate. Significant phrases in the model were time (p<0/05,A) and temperature

(p<0/05,B). Results in Table 6(0/7898= R-Squared and 0.6397 = Adj R-Squared) indicates good matching Computational Model with tested Points and model accuracy (Table 6).

Table 6. Results of statistical analysis of the quadratic model fitted on Inhibitory power of free radical DPPH of the red cabbage.

R-Squared	Adj R-Squared	Pred R-Squared	Adeq Precision	Std. Dev.	Mean	C.V.	PRESS
0.7898	0.6397	-0.6145	8.112	0.99	5.72	17.28	52.63

Optimal condition

The optimum operating conditions for the extraction of red cabbage was performed by ultrasound technology by using Numerical optimization technique. This technique was applied on models obtained for anthocyanin concentration, extraction efficiency, the amount of total phenolic compounds, DPPH free radical, scavenging power and power of iron renewal. In this study, the purposes of the optimization was to maximize the concentration of anthocyanin extract, extraction efficiency, total phenolic compounds content, free radical scavenging power, DPPH (or set minimums EC50) and power Iron renewal (Table 7, 8) and Fig 1.

Table 7. The amounts using for optimization of red cabbage extract.

Goal	Upper Limit	Lower Limit	Variables and Answers
is in range	40	10	Time
is in range	70	40	Temperature
Is target = 11.4314	11.4314	6.1652	Anthocyanin
Is target = 8.91	8.91	5.74	Yeild
Is target = 703.59	703.59	248.35	Frap
Is target = 6889.73	6889.73	2360.72	Folin
Is target = 3.4976	8.5313	3.4976	EC_{50}

Table 8. Results obtained from the optimization process red cabbage.

Four optimal Points	Time	Temperature	Anthocyanin	yield	Frap	Folin	EC_{50}
1	40	60.94	9.468158	8.310664	421.1768	4684.69	5.756068
2	40	60.74	9.429838	8.30024	424.5321	4653.773	5.729512
3	40	61.42	9.563872	8.336681	412.9127	4761.975	5.824267
4	40	60.4	9.364099	8.282353	430.3536	4600.77	5.684961

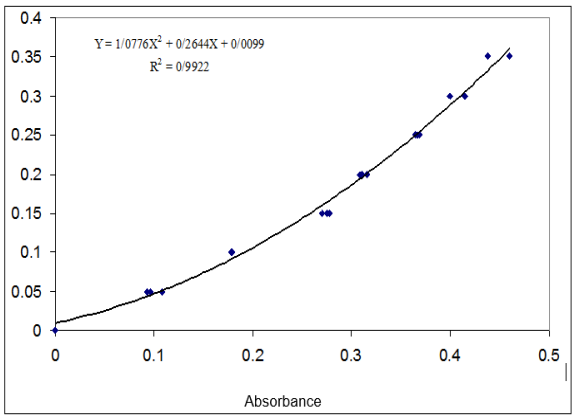

Fig. 1. The calibration curve of concentration of poly phenolic compounds against, absorbance. Read in 765 nm wavelength.

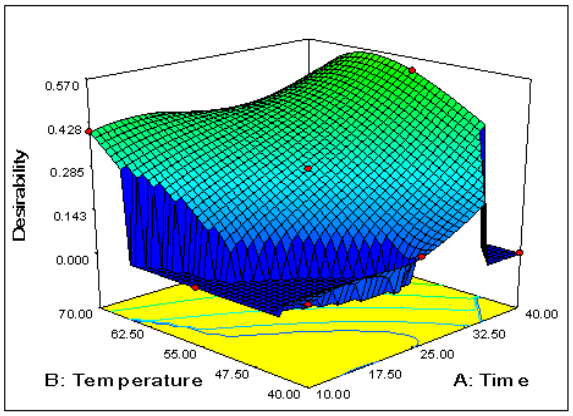

Fig. 2. Flow Diagram response surface and optimal red cabbage extract as a function of temperature and extraction time.

According to the optimization results the amount of anthocyanin concentration of red cabbage in the optimal point was equal to 9.468158 and the amount of anthocyanin in the control treatment was equal to 3.2014. Red cabbage extraction efficiency at the optimal point was equal to 8.310664 and this amount in the control treatment was equal to 4.81845. Power renewal iron at the optimal point was equal to 421.1768 but this amount in the control treatment was equal to 258.631. The amount of total phenolic compounds at the optimum point was equal to 4684.69 and this amount in the control treatment was equal to 2086.62. The amount of EC50 has negatively correlated with DPPH free radical scavenging ability at optimal point (5.756068) but control treatment was equal to 2.1513, which show lower inhibitory power than control treatment (Fig. 3).

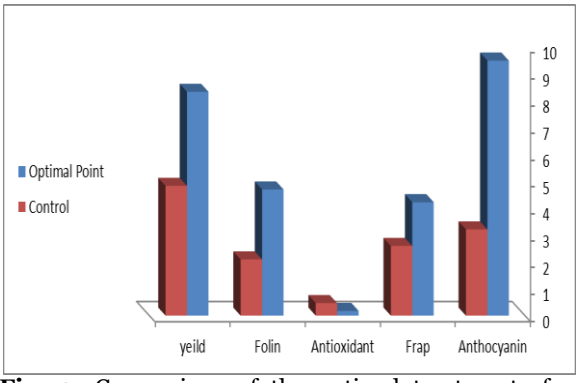

Fig. 3. Comparison of the optimal treatments for each control test (without sonication) to red cabbage plant.

Conclusion

The purpose of this study was scavenging the power of DPPH free radical, anthocyanin extraction, total phenols, and total power of iron renewal and red cabbage plant extraction efficiency in the ethanol - Hydrochloric Acid (15:85) solvent with ultrasound technology. For this aim two factors, time (10, 25, 40 min) and temperature (40, 55, and 70) was used. The results of the Statistical analysis in the anthocyanin extraction test indicated that, concentration of anthocyanin at optimal point and the rate of extraction efficiency was equal to 9.468158, 8.310664 respectively. The amount of iron renewal power and the amount of total phenolic compounds at the optimal point was equal to 421.1768, 4684.69 respectively. The amount EC50 has negatively correlated with DPPH free radical scavenging ability at the optimal point of the cabbage was 5.756068. The results of statistical analysis to determine the optimal mode, considering all 5 tests indicated that the optimal conditions for red cabbage extraction is 40 minutes and 60.94C. Therefore, red cabbage is introduce as an antioxidant source due to the presence of phenolic compounds in this plant.

References

Nguyen TP, Lee VVM. 2012. Application of ultrasound to pineapple mash treatment in juice processing. International Food Research Journal, P. 547-552.

Leja M, Kaminski I, Kolton A. 2010. Phenolic compounds as the major antioxidants in red cabbage, 19-24 P.

Ghafoor K, Hee Choi Y. 2009. Optimization of Ultrasound Assisted Extraction of Phenolic Compoundsand Antioxidants from Grape Peel through Response Surface Methodology 295-300 P. http://dx.doi.org/10.3839/jksabc.2009.052

Sugiastuti S, Farida Y, Putri Puspita Sari D. 2011. Antioxidant Activity of White and Red Cabbage (*Brassica oleracea* L. var capitata L) Using DPPH.

Ahmadi F, Kadivar M, Shahedi M. 2007. Antioxidant activity of Kelussia odoratissima Moza, in model and food systems, Food Chemistry **105,** 57- 64.

Borges GD. 2011. Optimization of the extraction of flavanols and anthocyanins from the fruit pulp of *Euterpe edulis* using the response surface methodology. Food Research Internationa, l **44**(2011) 708 –715. http://dx.doi.org/10.1155/2013/810547

Rokayya S, Chun-Juan Li, Zhao Y, Li Y, Sun CH. 2013. Cabbage (Brassica oleracea L. var. capitata) Phytochemicals with Antioxidant and Anti-inflammatory Potential, Asian Pacific Journal of Cancer Prevention **14,** 2013. http://dx.doi.org/10.7314/APJCP.2013.14.11.6657