European Journal of Nutrition & Food Safety

12(10): 125-133, 2020; Article no.EJNFS.62204
ISSN: 2347-5641

Enzyme-Assisted Extraction of Anthocyanin from Kokum (*Garcinia indica Choisy*) Rinds

R. C. Ranveer[1*], A. S. Nanadane[2], P. M. Ganorkar[2] and A. K. Sahoo[3]

[1]PG Institute of Post Harvest Management (DBSKKV, Dapoli), Killa – Roha, Raigad District, Maharashtra State-402116, India.
[2]Department of Food Processing Technology, A. D. Patel Institute of Technology, Anand – 388120, India.
[3]Department of Food Technology, Shivaji University, Kolhapur, Maharashtra– 416004, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJNFS/2020/v12i1030309
Editor(s):
(1) Dr. Rasha Mousa Ahmed Mousa, University of Jeddah, Saudi Arabia.
Reviewers:
(1) Tofan Lavinia, Technical University Gheorghe Asachi Iasi, Romania.
(2) Coulibaly Wahauwouele Hermann, University Nangui Abrogoua, Côte d'Ivoire.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62204

Original Research Article

Received 03 September 2020
Accepted 07 November 2020
Published 28 November 2020

ABSTRACT

Background: The kokum rind consists of high amount of anthocyanin. The traditional methods are not capable to extract anthocyanin completely.
Aims: The present study was focused on the optimization of enzyme pretreatment for extraction of anthocyanin from Kokum rind.
Methodology: A central composite design was used to optimize the enzyme-assisted extraction of anthocyanin from the Kokum rinds. Kokum rinds was pretreated with food-grade pectinase and cellulase enzymes separately and then subjected to acidified ethanol extraction. The factors investigated included enzyme concentration (0 – 2%), Incubation time (1-5 h) and pretreatment temperature (15–55ºC).
Results: Overall, 107.17- to 63.71 - fold increase in anthocyanin recovery when treated with pectinase and cellulase, respectively was observed compared to the untreated rinds. From a response surface analysis of the data, a second-degree polynomial equation was developed which provided the following optimal extraction conditions i.e. enzyme concentration 0.5%, Incubation time 2.0h and temperature 35ºC for pectinase pretreatment. Whereas, in case of cellulase the

**Corresponding author: Email: rahul.ranveer@gmail.com;*

optimum conditions was enzyme concentration 1.42%, Incubation time 4.0h and temperature 32ºC. The obtained results strongly support the idea of using cell-wall degrading enzymes as an effective means for recovering anthocyanin from Kokum rinds.

Keywords: Kokum; anthocyanin; pectinase; cellulase; RSM.

1. INTRODUCTION

The Kokum fruit is dark red in colour and consist of mainly three components i.e. Pulp/Juice, rinds/peel and seeds. The fruits are commercially processed for Kokum butter, syrups, squash, Agal (Kokum Juice) and amsul (Kokum rinds) and wastage is generated during processing of these products. The anthocyanins consist of varied groups of intensely colored pigments. These imparts orange, red, purple, and blue colors of many fruits, vegetables, flowers, leaves, roots and other plant organs [1-4]. The fresh fruit kokum contain 2.4 g/100 g anthocyanins [5].

Anthocyanin has been reported for the treatment of dysentery, tumors, heart complaints, stomach acidity, and liver disorders [6-7]. Eye-catching shade and solubility in water allows anthocyanin incorporating in aqueous food systems as well as possible health benefits, which considered anthocyanin as potential replacement for synthetic colour [6-10]. The red color of kokum is ascribed to the presence of anthocyanins such as cyanidin-3-glucoside and cyanidin-3-sambubioside [5,11]. The anthocyanin has high potential as a natural food colorant due to its possible usage in the production of confectionery, jellies, jams, health beverages and squash-like products, red wine, and desserts [6-7].

The enzymes like cellulase and pectinase are commercially in food processing for clarification of juices, to improve juice yield and extraction of bioactive components. Many researchers are reported used of enzymes for the extraction of pigments from plant sources such as precarthamine from safflower florets [12]; carotenoid from marigold flowers [13]; Anthocyanin from red grapes [14]; lycopene from tomato [15,16]; anthocyanin from black current [17]; anthocyanin from mulberry [18], Bioactive components from sweet cherry [19].

The present investigation aims to optimize enzyme added extraction of anthocyanin from Kokum rinds using response surface methodology, hence improving its yield.

2. MATERIALS AND METHODS

2.1 Chemicals and Enzymes

Acetone and Hydrochloric acid were purchased from Local supplier. Cellulase and pectinase were procured from Danisco India Pvt. Ltd., Mumbai (India). The cellulase was declared with 1470 U/mL activity at 55ºC optimum temperature and 4.0 – 4.5 pH, whereas pectinase declared with 160 U/mL activity at 50ºC optimum temperature and 4.0 – 5.0 pH.

2.2 Kokum Rinds

The Kokum rinds was procured from Ajramar Food Pvt. Ltd., Sagmeshwar, Ratnagiri district (MS), India. The seeds were separated; the foreign matter was removed packed in polypropylene bags and stored at 15±5°C until up to farther used.

2.3 Enzyme-Assisted Extraction of Anthocyanin

The Kokum rinds were pretreated with enzyme i.e. cellulase and pectinase for anthocyanin extraction. The Kokum rinds (3 g) were added 10 mL of cellulase and pectinase enzyme solution, prepared by dissolving the commercial enzyme in citrate buffer of pH 4.5 and 5.0, respectively [20]. The reaction mixture was continuously stirred and after completion of Incubation time, the enzymes were deactivated by heating at 80ºC for 5 min. afterwards, subjected to anthocyanin extraction by acidified ethanol.

2.4 Anthocyanin Assay

The total anthocyanin was determined by the procedure suggested by Abdel-Aal and Hucl [21], A macerated Kokum rind sample (3 g) was weighed in a 50-mL centrifuge tube, and 24 mL of acidified ethanol (ethanol and HCl 1.0*N*, 85:15, v/v) was added. The solution was mixed and adjusted to pH 1 with 4*N* HCl. The resulting solution was shaken for 15 min, readjusted to pH 1 if necessary, and the solution was shaken for

an additional 15 min. The tube was centrifuged at 7500 RPM for 15 min, and the supernatant was poured into a 50-mL volumetric flask and made up to volume with acidified ethanol. Absorbance was measured at 535 nm against a reagent blank. Cyanidin 3-glucoside (Sigma-Aldrich, India) was used as a standard pigment. Total anthocyanin content per sample (mg/kg) was calculated as cyanidin 3-glucoside:

$$C\ (mg/kg) = \frac{\mathrm{A} \times \mathrm{Volume\ made} \times \mathrm{MW} \times 10^6}{e \times 1000 \times wt\ of\ sample}$$

Where,

A is absorbance reading, e is molar absorptivity (cyanidin 3-glucoside 25,965 $cm^{-1}\ M^{-1}$) [21], MW is molecular weight of cyanidin 3-glucoside = 449.

2.5 Experimental Design and Statistical Analysis

A central composite design (CCRD) [22] was used to study the effect of three independent variables i.e. enzyme concentration (X1), Incubation time (X2) and pretreatment temperature (X3) on experimental response of anthocyanins yield. The statistical software package Design –Expert 8.0. The coded and actual values of independent variables are as mentioned in Table 1.

Overall, the experimental design consisted of total of 20 runs (8+6+6). The layout of experimental design of pectinase and cellulase pre treatment experiment obtained through the software and the experimental values of response are given in Tables 2 and 3, respectively.

Table 1. The coded and actual values of independent variables for enzymatic extraction of anthocyanins

Independent Variables	Code	Level in code form				
		-2	-1	0	1	2
Enzyme concentration (%)	X1	0	0.5	1	1.5	2
Incubation time (h)	X2	1	2	3	4	5
Pretreatment temperature (°C)	X3	15	25	35	45	55

Table 2. The layout of experimental design in terms of coded values for pectinase

Std. Order*	Run Order**	X1	X2	X3	Yield (%)#
1	16	-1	-1	-1	1.4524
2	11	1	-1	-1	1.5874
3	19	-1	1	-1	1.5223
4	3	1	1	-1	2.0325
5	9	-1	-1	1	1.4652
6	7	1	-1	1	1.5912
7	6	-1	1	1	1.5347
8	5	1	1	1	2.0354
9	18	-2	0	0	1.1807
10	8	2	0	0	1.7348
11	17	0	-2	0	1.5046
12	13	0	2	0	2.0142
13	15	0	0	-2	1.6522
14	12	0	0	2	1.6701
15	14	0	0	0	1.5784
16	20	0	0	0	1.5874
17	2	0	0	0	1.6012
18	1	0	0	0	1.5489
19	10	0	0	0	1.5645
20	4	0	0	0	1.5987

** The formal order of runs, ** Randomized order in which experiments are conducted; # Percentage yield of extracted Anthocyanins*

Table 3. The layout of experimental design in terms of coded values for cellulase

Std. Order*	Run Order**	X1	X2	X3	Yield (%)#
1	20	-1	-1	-1	1.1336
2	11	1	-1	-1	1.2851
3	14	-1	1	-1	1.2954
4	18	1	1	-1	1.5429
5	6	-1	-1	1	1.0188
6	12	1	-1	1	1.3115
7	1	-1	1	1	1.2653
8	17	1	1	1	1.3867
9	13	-2	0	0	0.9931
10	9	2	0	0	1.4955
11	4	0	-2	0	1.2114
12	16	0	2	0	1.5351
13	2	0	0	-2	0.9878
14	5	0	0	2	0.9687
15	19	0	0	0	1.6014
16	15	0	0	0	1.5724
17	7	0	0	0	1.5124
18	3	0	0	0	1.5262
19	10	0	0	0	1.5561
20	8	0	0	0	1.5756

** The formal order of runs, ** Randomized order in which experiments are conducted; # Percentage yield of extracted Anthocyanins*

3. RESULTS AND DISCUSSION

3.1 Effect of Pectinase and Cellulase Pretreatment on Extraction of Anthocyanins

The Kokum processing rinds was pretreated with pectinase and cellulase and its effect on the extraction yield of the anthocyanin was monitored and obtained results are presented in Tables 2 and 3, respectively. The untreated Kokum processing rinds samples showed 0.989 g/100 g yield of anthocyanin. Anthocyanin content of different varieties in the range of 1.90 to 2.35 g/100 g anthocyanin content [23]. The rind contains seeds and along with other foreign materials, which may be responsible for lower anthocyanin content. The anthocyanin yield was increased by 107.17- and 63.71- fold, when treated with pectinase and cellulase, respectively. The increase in enzyme concentration increases the yield of anthocyanins in both enzyme i.e. cellulase and pectinase treatments. The higher recovery of anthocyanins was obtained at 0.5% and 1.4% concentration of pectinase and cellulase enzymes, respectively. Low concentration of enzyme is able to open mainly the cells of the pulp, still attached to the peel, keeping most of the peel intact. Which would showed that the lower anthocyanins concentration of the collected chromoplasts.

Ladbo and Meyers [24] was tested five different processing enzymes those had pectinase, macerase, or protease activity, with different degrees of extraction recovery from black currant press cake residue. Enzyme added extractions resulted more polyphenolics, but were not observed to increase anthocyanins when judge against a control (no addition of enzyme). Particle size has shown to affect the quantity of phytochemicals extractable from pomace obtained from black currant, as the particle size reduced the amount extracted was increased. In addition, fine grinding was more influential than the use of commercial enzymes or heating in extraction of phytochemicals from blueberry juice–processing waste [25]. Cellulase and pectinase in phenolic extraction of grape pomace and reported that pectinase had optimistic effects; however cellulase had a negative effect in the extraction of polyphenolics [26].

The fruits peel consist of cellulose, hemicelluloses and pectin, which can be breakdown with the enzymes those have cellulolytic, hemicellulolytic and pectinolytic activities [27]. The yield of anthocyanin may be enhance when the cell-wall polysaccharides breakdown with the enzyme action. Plant cell-wall consist of cellulose, linear polymer of 1, 4 – linked glucose and hemicelluloses [28]. Bundles of cellulose molecules are aggregated together in the form of microfibrils composed of highly

ordered crystalline domains and extended amorphous regions.

3.2 Model Fitting

The central composite design was used to analyze the effect of process variables on anthocyanin extraction yields. The experimental results presented in Tables 2 and 3 were correlated by the following full 2nd-order polynomial equation:

$$y = a_0 + \sum_{i=1}^{3} a_i x_i + \sum_{i=1}^{3} a_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=1}^{3} a_{ii} x_i x_j \text{ ---- (1)}$$

Where, y is the Anthocyanin extraction yield, a0 is the intercept, ai ,aii and aij are the linear, pure quadratic and interaction regression coefficients, respectively, and xi are the coded independent variables.

With the help of stepwise regression method, with independent variables entered and eliminated at a confidence level of 90%, the following regression equation was established.

$$y = a_0 + a_1 x_1 + a_2 x_2 + a_{12} x_{12} + a_{11} x_{11}^2 + a_{22} x_{22}^2 \text{ --- (2)}$$

The estimates of the regression coefficients for pectinase and cellulase pretreatments obtained by the least squares method together with their standard errors, t statistics and corresponding p-values. Eq. 2 was found to be adequate to fit all experimental data with a coefficient of determination (R2) of 0.96 and 0.97, respectively. Furthermore, In case of pectinase enzyme pretreatment, the mean sum of squares for pure error (MSPE) was (0.0004 with 5 degrees of freedom) was compared to the mean sum of squares for lack of fit (MSLoF = 0.002 with 9 degrees of freedom) of the above polynomial, their ratio (MSLoF /MSPE = 5.0) (Table 5a) was less than the percentage point of the F distribution (5.04) at the 95% confidence level. Whereas, in case of cellulase enzyme pretreatment, the mean sum of squares for pure error (MSPE = 0.0011 with 5 degrees of freedom) was compared to the mean sum of squares for lack of fit (MSLoF = 0.0021 with 8 degrees of freedom) of the above polynomial, their ratio (MSLoF /MSPE = 1.90) (Table 5b) was approx. equal to the percentage point of the F distribution (1.9) at the 95% confidence level. Thus indicating that lack of fit was not significant and that the simplified polynomial was a reasonable approximation to the mean structure in the region of experimentation. Therefore, the overall error variance of this composite design was estimated by combining the mean sum of squares for lack of fit and pure error as equal to 0.0024 with 14 degrees of freedom and 0.0032 with 13 degree of freedom for pectinase and cellulase pretreatments, respectively. Lastly, by referring to the last column in Table 4, it was possible to assess the significance of the individual regression coefficients as, that the linear coefficients a_0, a_1, a_2 and the quadratic coefficient a_{11} and a_{22} were significant at the 99% confidence level.

Table 4. Estimation of regression coefficients of Eq. 4 together with their corresponding standard error (s), t-value and p-value

Coefficient	Pectinase				Cellulase			
	Value	S	t-value	p-value	Value	S	t-value	p-value
a_0	1.614	0.014	33.124	0.000	1.560	0.017	31.48	0.0000
a_1	0.149	0.010	3.766	0.001	0.110	0.011	3.26	0.0046
a_2	0.128	0.010	4.877	0.000	0.087	0.011	4.63	0.0003
a_{11}	0.094	0.015	3.100	0.007	-0.079	0.008	-3.13	0.0064
a_{22}	-0.032	0.008	4.338	0.000	-0.047	0.008	-5.14	0.0001
a_{33}	0.043	0.008	-3.935	0.002	-0.150	0.008	-3.73	0.0015

Table 5a. Analysis of Variance for regression equation of pectinase pretreatment

Source	DF	SS	MS	F	*p*-value
Regression	5	0.800	0.1600	107.10	0.000
Residual Error	14	0.021	0.0014		
Lack-of-fit	9	0.019	0.0020	5.04	0.0588
Pure error	5	0.005	0.0011		
Total	19	0.820			

Table 5b. Analysis of Variance for regression equation cellulase pretreatment

Source	DF	SS	MS	F	*p*-value
Regression	6	0.930	0.1500	87.3	0.000
Residual Error	13	0.023	0.0017		
Lack-of-fit	8	0.018	0.0021	1.9	0.232
Pure error	5	0.005	0.0011		
Total	19	0.95			

3.3 Analysis of Response Surface

Response surface plot for the yield of anthocyanin by enzymatic (Pectinase and cellulase) extraction as a function of two independent variables while the other independent variable is set to their zero level is reported in Fig. 1 (a and b), respectively. Surface plot as a function of enzyme concentration (A) and Incubation time (B) show a plane-like dependence of anthicyanin yield on these variables. In fact, from Eq. 2 it can be seen that the effect of these variables on the response is described by linear terms. Consequently, whatever the values of the levels of the remaining variables, an increase of enzyme concentration or incubation time will result in an increased anthocyanin yield, the regression coefficient estimates of a1 and a2 being positive.

3.4 Model Validation

Several experiments were carried out, as per the optimal solutions to validate the model. The experiments were performed as per formulations given in Table 6. By using Eq. (2) together with the parameter estimates listed in Table 4, it was possible to calculate the predicted anthocyanin yield under the experimental conditions adopted for the validation experiments as well as its 95%-prediction interval (Tables 2 and 3). Furthermore, all the predicted responses were included into the 95%-prediction intervals.

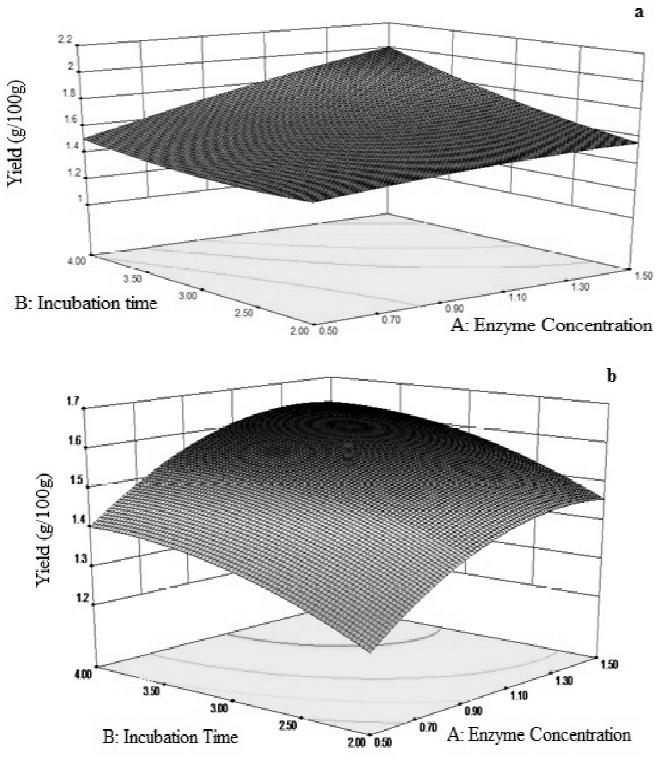

Fig. 1. Response surface plot showing effect of (a) pectinase and (b) cellulase concentration and pretreatment time on extraction yield of anthocyanin when pretreatment time is held at it's central value

Table 6. Observed (y) and predicted (y) Anthocyanin extraction yields under different experimental conditions together with their 95%-prediction intervals (PI) by the response surface model

Sr. No.	X1	X2	X3	Y(%)	Y_{pred} (%)	PI(%)
For Pectinase						
1	1.50	4.0	35	2.05	2.00	1.9 - 2.1
2	1.50	3.9	35	2.02	1.97	1.8 - 2.0
3	1.35	4.0	35	1.97	1.95	1.8 – 2.0
For Cellulase						
4	1.40	4.0	32	1.60	1.62	1.5 – 1.7
5	1.10	4.0	36	1.62	1.61	1.5 – 1.7
6	1.50	3.5	30	1.62	1.60	1.5 – 1.7

4. CONCLUSION

The results of this study indicate that the recovery of anthocyanin from the Kokum rinds can be greatly enhanced by the use of pectinase and cellulase enzymes. In particular, pretreatment of Kokum rinds by pectinase and cellulase resulted in 107.17- to 63.71 - fold increase in extraction yields. This fact, the comparatively low cost of commercial food-grade enzyme preparations lends strong support to the possible implementation of the process on an industrial scale. We have also shown that the response surface methodology is an appropriate method for determining the optimum values of the factors studied. At present, considerable amounts of Kokum processing rinds is produced as a solid waste. As a result, the exploitation of this material as a source of natural anthocyanin could not only provide economic benefits but also contribute to environmental protection.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Science and Engineering Research Board (SERB) and Ministry of Food Processing Industry, Government of India, New Delhi for financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sharma R, Kaur D, Oberoi DPS, Sogi DS. Thermal degradation kinetics of pigments and visual color in watermelon juice. International Journal of Food Properties. 2008;11:439–449.
2. Righetto AM, Netto FM. Effect of encapsulating materials on water sorption, glass transition and stability of juice from immature Acerola. International Journal of Food Properties. 2005;8:337–346.
3. Jyothi AN, Moorthy SN, Eswariamma CS. Anthocyanins in sweet potato leaves-varietal screening, growth phase studies and stability in a model phases in a model system. International Journal of Food Properties. 2005;8:221–232.
4. Rababah TM, Khalil IE, Majdi AA, Khalid I, Hidar A, Yang W. Total phenolics, antioxidant activities and anthocyanins of different grape seed cultivars grown in Jordan. International Journal of Food Properties. 2008;11:472–479.
5. Nayak CA, Srinivas P, Rastogi NK. Characterization of anthocyanin from *Garcinia indica Choisy*. Food Chemistry. 2010;118:719–724.
6. Bhat JD, Kamat N, Shirodkar A. Compendium and proceedings of the 2nd national seminar on kokum

(*Garcinia indica Choisy*), Goa University; 2005.

7. Nayak CA, Rastogi NK, Raghavarao KSMS. Bioactive constituents present in *Garcinia indica Choisy* and its potential food applications. International Journal of Food Properties. 2009;13:441–453.
8. Francis FJ, Markakis PC. Food colorants: Anthocyanins. Critical Reviews in Food Science and Nutrition. 1989;28(4):273–315.
9. Mazza G and Miniati E. Anthocyanins in fruits, vegetables and grains; CRC Press: London; 1993.
10. Nayak CA, Chethana S, Rastogi NK, Raghavarao KSMS. Enhanced mass transfer during solid-liquid extraction of gamma irradiated red beetroot. Radiation Physics and Chemistry. 2006;75: 173–178.
11. Krishnamurthy N, Lewis YS, Ravindranath B. Chemical constituents of kokum fruit rind. Journal of Food Science and Technology. 1982;19:97–100.
12. Saito K. A new enzymatic method for the extraction of precarthamine from dyer's saffron florets. Zeitschrift für Lebensmittel-Untersuchung und Forschung. 1993;197: 34–36.
13. Barzana E, Rubio D, Santamaria RI, Garcia Correa, Garcial F, Ridaura Saaz VE and Lopez MA. Enzyme mediated solvent extraction of carotenoids from marigold flower (*Tagetes erecta* L). Journal of Agriculture Food Chemistry. 2002;50(16): 4491–4496.
14. Nicolini G, Mattivi F. Red grapes vinification with exogenous pectolytic enzymes. Enotechnico. 1997;33(3):65–71.
15. Ranveer RC, Patil SN, Sahoo AK. Effect of different parameters on enzyme-assisted extraction of lycopene from tomato processing waste. Food and Bioproducts Processing. 2013;91(4):370-375.
16. Laudio Lombardelli, Katia Liburdi, Ilaria Benucci, Marco Esti. Tailored and synergistic enzyme-assisted extraction of carotenoid-containing chromoplasts from tomatoes. Food and Bioproducts Processing. 2020;121:43-53.
17. Buchert J, Koponen JM, Suutarinen M, Mustaranta A, Lille M, Torronen R, Poutanen K. Effect of enzyme-aided pressing on anthocyanin yield and profiles in bilberry and blackcurrant juices. Journal of the Science of Food and Agriculture. 2005;85(15):2548–2556.
18. Lixia Zhang, Gongjian Fan, Muhammad Ammar Khan, Zheng Yan, Trust Beta. Ultrasonic-assisted enzymatic extraction and identification of anthocyanin components from mulberry wine residues. Food Chemistry. 2020;323: 126714.
19. Gloria Domínguez-Rodríguez, María Luisa Marina, Merichel Plaza. Enzyme-assisted extraction of bioactive non-extractable polyphenols from sweet cherry *(Prunus avium L.)* pomace. Food Chemistry. 2020; 339:128086.
Available:https://doi.org/10.1016/j.foodchem.2020.128086
20. Ranveer RC, Sahoo AK. Effects of cellulase and pectinase combinations on the recovery of lycopene from tomato processing industry waste. Agro Food Industry Hi- Tech. 2015;26(1):30-33.
21. Abdel-Aal ESM, Hucl P. A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. Cereal Chemistry. 1999;76(3):350–354.
22. Montgomery DC. Design and analysis of experiments. 6th edn. John Wiley and Sons, New York; 2005.
23. Braganza M, Shirodkar A, Jayarama Bhat D, Krishnan S. Resource book on kokum, western ghats kokum foundation, Panaji – Goa. India; 2012.
24. Ladbo AK, Meyer AS. Enzyme – assisted extraction of antioxisantive phenols from black currant juice press residues (Ribes nigrum). Journal of Agriculture Food Chemistry. 2001;49(7):3169-3177.
25. Stanley R, Miller S. Enzymatic processing for the release of phenolic antioxidants from fruit waste. In Biologically-Active Phytochemicals in Food, Edited by Pfannhauser, W., Fenwick, G. R. and Khokhar, S. Published by Royal Society of Chemistry, USA. 2001;552–556.
26. Meyer AS, Jepsen SM, Sorensen NS. Enzymatic release of antioxidants for human-low-density lipoprotein from grape pomace. Journal of Agriculture Food Chemistry. 1998;46:2439–2446.

27. Gross KC. Fractionation and partial characterization of cell walls from normal and non-ripening mutant tomato fruit. Physiologia Plantarum. 1984;62: 25–32.

28. Zuorro A, Lavecchia R. Mild enzymatic method for the extraction of lycopene from tomato paste. Biotechnology and Biotechnological Equipment. 2011;24: 1854–7.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/62204