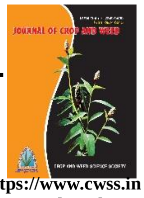
ISSN-O : 2349 9400; P : 0974 6315
Journal of Crop and Weed, 19(3):09-13 (2023)



https://www.cwss.in
www.cropandweed.com

Review Article

Exploring diverse processing techniques for debittering of citrus juice: A mini review

[1,2]D. ADMANE, [2]M. GURJAR, [2]S. MENDKE, [2]V. BANSODE, [2]D. GHOSH AND *[2]S. S. ROY

[1]DSM-Fermenich, Bengaluru, Karnataka, India
[2]ICAR-Central Citrus Research Institute, Nagpur, Maharashtra, India

Received: 17.08.2023; Revised: 05.10.2023; Accepted: 08.12.2023

DOI: https://doi.org/10.22271/09746315.2023.v19.i3.1734

ABSTRACT

Citrus juice bitterness is the main problem in processing industries and is due to two main components limonin and naringin. The bitterness of citrus juice affects its quality, decreases shelf life and lowers consumer acceptance. Many scientists and researchers are attempting to extend the shelf life of citrus processed products by reducing the amount of bitterness-causing compounds. The present review paper discusses the debittering techniques such as lye treatment, by addition of β-cyclodextrin, blanching and enzymatic methods.

Keywords: Citrus fruits, debittering, increased shelf life and processing technologies

Citrus fruit is one of the major horticultural crops traded globally. Citrus fruits can be consumed directly as table fruit, as fresh juice or in the form of different value added products. The genus citrus belongs to the family *Rutaceae* and encompasses various species such as mandarin, grapefruit, orange, pomelo, galgal, lemon, lime, etc. Citrus fruits are well known for its pleasant taste, flavor and aroma. Despite of possessing numerous nutritional properties, industries are facing many challenges in the acceptance of citrus juices for commercialization. Citrus juice bitterness is one of the major problems faced in citrus processing industries all over the world since ancient times. The processing of citrus juice cause "bitterness" and "delayed bitterness" due to the presence of chemical metabolites such as naringin, tangeretin, limonin, nomilin, neohespiridin and quercetin. While naringin is primarily responsible for bitterness; limonin causes delayed bitterness. Hence, de-bittering techniques are required in order for the citrus sector to florish in the market (Purewal and Sandhu, 2021).

Bitterness in juice affects its quality, decreases the value of citrus fruit based products and is unacceptable by the consumers. The concentration of limonin and naringin; primarily responsible for citrus juice bitterness vary in concentration with respect to the fruit parts, type and is affected by growing conditions, maturity and cultivators (Kore and Chakraborty, 2015). In order to improve consumer acceptance, numerous studies have been conducted to develop scientific methods for fruit juice debittering. There are physical, chemical and biological methods available to reduce bitterness; artificial sweeteners are also used to improve the taste.

The basic mechanisms for reduction in bitter compounds are (Kore and Chakraborty, 2015)

i. Removing the bitter compound
ii. Is to remove the physical barrier (pith)
iii. Use of bitter compounds scavengers, such as salts, sugar and florisil
iv. Reduction of bitter compounds using enzymes such as naringinase and alpha-L-rhamnosidase
v. Genetic engineering technique

BITTERNESS CAUSING COMPOUNDS

Citrus fruits contain many phytonutrients having different activities. The predominant phytonutrients are flavonoids, limonoids, ascorbic acid, antioxidants, glucosionates, isothiocynates, etc.

**Email: subhrasaikat@gmail.com*

How to cite: Admane, D., Gurjar, M., Mendke, S., Bansode, V., Ghosh, D. and Roy, S.S. 2023. Exploring diverse processing techniques for debittering of citrus juice: A mini review. *J. Crop and Weed*, 19(3): 09-13.

Among these, flavonoids (naringin, tangeretin, nobiletin, nomilin, quercetin) and limonoids (limonoid aglycones) are the main compounds causing bitterness of citrus juice (Malta *et al*., 2013; Arruda *et al*., 2017; Neri-Numa *et al*., 2018; Arruda *et al*., 2018; Pereira *et al*., 2020). Naringin is an important disaccharide derivative comprising of rhamnose and glucose residue attached to its aglycone position called naringenin at the 7th position (Chen *et al*., 2010). Puri *et al*. (1996) analysed the naringin content in different parts of grapefruit *viz*., albedo, flavedo, pith, seeds and juice. The results reported that flavedo consist of 270-431 mg100^{-1}g, albedo have 130-1559 mg100^{-1}g, pith have 1328-1760 mg100^{-1}g, whereas seeds and juice contains 29-267 mg100^{-1}g and 30-70 mg100^{-1}g, respectively. Citrus fruits have been found to contain over 30 different limonoids which are oxygenated metabolites; a product of limonoid aglycones. Limonoids have two groups, first is aglycone and second is glucosides. Limonin, nomilinic acid, nomilic, limonin glucoside are some limonoids present in citrus fruits (*Rutaceae* family). One significant limonoid found in citrus fruits is limonin (Yang *et al*., 2020; Yaqoob *et al*., 2020). The limonin content quantified in different citrus species i.e. lemon, grapefruit, tangerine and orange was found to be 12 mgL^{-1}, 11.4 mgL^{-1}, 34 mgL^{-1}and 9.7 mgL^{-1}, respectively (Drewnowski and Gomez-Carneros, 2000).

The major factor for consumer acceptance of various citrus based products is its bitterness. Naringin and limonin are the main flavonoids responsible for bitterness in citrus fruits. Naringin was first abundantly found in grapefruit in immature fruits. Naringenin is the aglycone form of naringin. Naringinase is an enzyme complex consisting of β-D-glucosidase and α-L-rhamnosidase which catalyzes the hydrolysis of naringin; the main component of bitterness to rhamnose and prunin. Further, pruning is hydrolyzed to naringenin (tasteless) and glucose. Thus, juice processing industries after enzymatic action can process the juice into stable processed products with acceptable organoleptic quality (Igbonekwu, 2018). Puri et al. (1996) reported the action of α-L-Rhamnosidase and β-D-glucosidase on the conversion of bitter compound naringin to bitterless compounds naringenin as shown in fig 1.

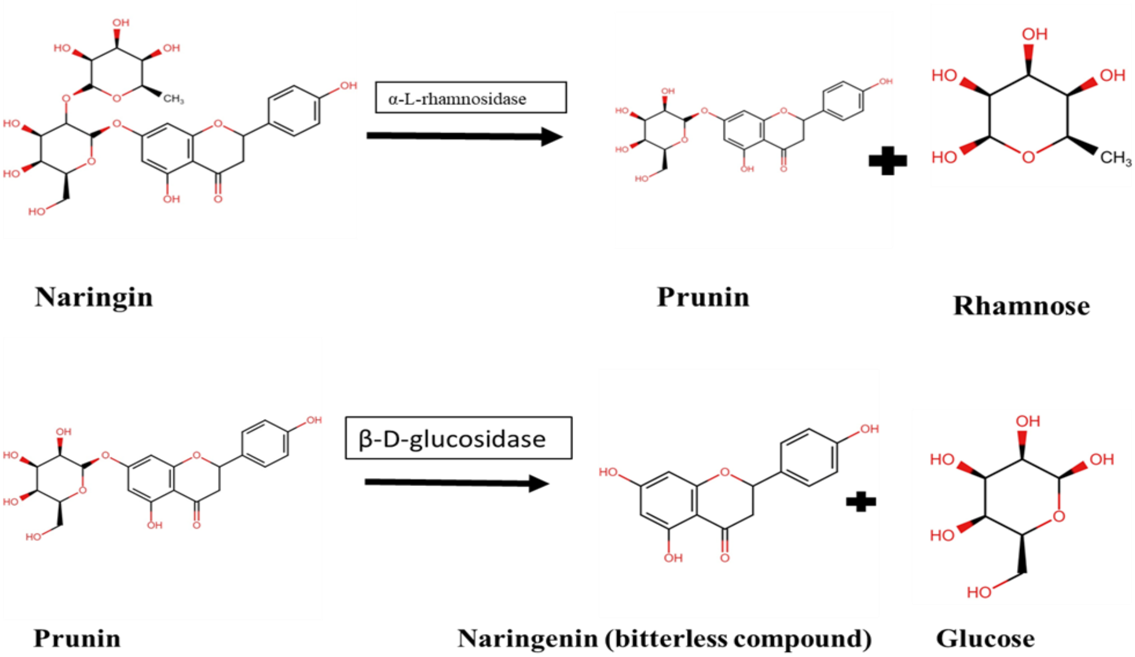


Fig. 1: Enzymatic action on naringin

DEBITTERING METHODS

Physical methods

1. *Resins*

These are byproducts of plants mixture extracted from trees belonging to Pinaceau and Dipterocarpaceae family (Dilworth *et al*., 2017). Resins are composed of mixture of fatty acids, resin acid, waxes and the resenes that are generally soluble in water and organic solvent. The reduction in grapefruit juice was performed by using resin of amberlite IR 400 and IR 120 and resulted that debittering potential of IR 400 removes naringin by 69.23% and 9% reduction of naringin by IR 120 Mishra and Kar (2003). Researchers have employed a variety of resins, including IR 40, IR 120, IR 400, Dowex Optipore L285 and Amberlite XAD-16HP and Amberlite XAD-7HP, to effectively debitter citrus juices by eliminating the content of naringin and limonin.

2. *Extraction of juice*

Extraction of juice plays an important role to debitter the juice. During juice extraction, seeds get crushed liberating bitter compounds (Premi *et al*., 1994). Screw type juice extractor is useful to extract juice with low limonin and narinign content (Sandhu and Singh, 2001; Bala *et al*., 2017).

3. *Blanching and filtration*

One physical method to lessen bitterness in fruits is hot water treatment, which involves immersing the fruits in 50°C hot water for 20 to 30 minutes. To prevent the entry of bitter compounds into the juice that has been extracted, filter presses and membranes like hollow fiber membranes (HFM) and ultrafiltration are also utilized.

Chemical methods

1. *Lye treatment*

In lye treatment, fruits are treated with sodium hydroxide (Lye) at temperature of 80–85°C for 40–60 seconds, after which they are rinsed in citric acid and then washed under water to remove any remaining NaOH. The hydroxyl and carboxylic groups reacts with the outside of peeled fruits during treatment, causing the hydrophilic compounds to be washed away (Kore and Chakraborty, 2015). Debittering of kinnow juice was performed by lye treatment with water (Anand *et al*., 2012; Sandhu *et al*., 1990).

2. *Florisil*

Florisil is activated magnesium silicates (a white coloured and odourless compound). It is used as a debittering agent for juices. Barmore *et al*. (1986) carried out a study to determine the best concentration of florisil (5–20%) to use for debittering of grapefruit juice. The greatest percentage of florisil (20%) causes the limonin content to decrease from 8.8 ppm to 1.7 ppm while the naringin content decreased from 326 ppm to 159 ppm, respectively.

3. *β-cyclodextrin*

Several studies have reported the use of β-cyclodextrin in citrus juice debittering. A range of β-cyclodextrin concentrations have been tested; of which 5% concentration for 30 minutes have been found to reduce the amount of limonin in tangerine juice by 80% (Mongkolkul *et al*., 2006). Additionally, β-cyclodextrin at 2% concentration for 10 min decreased the amount of limonin from 23–20 ug ml^{-1} when added to lime juice.

Biological methods

Enzymatic process have been gaining interest nowadays for debittering of fruit juices by converting of bitterness causing compounds into non bitter metabolites to enhance the shelf life of juices. Their actions on compounds causing bitterness are much higher than other techniques. Microorganisms are used for the production of juice debittering enzymes. These enzymes are produce depending on specific substrate, conditions of incubations and microbial strains (Purewal and Sandhu, 2021). Orange juice was debittered using enzyme limonite dehydrogenase, which decreased the amount of limonin from 21- 3 ppm (Brewster *et al*., 1976).

Debittering enzymes like α-L-rhamnosidase and naringinase are produced by a variety of microbial strains. *A. oryzae*, *A. foetidus*, *A. niger*, *A. flavus*, *Bacillus* sp., *Pseudomonas* sp., *Streptomyces* sp., *Fusarium solani*, *Escherichia coli*, *Aspergillus brasiliensis*, *Rhizophus stolonifer*, and *Bacillus cereus* are the strains used to produce naringinase. A variety of bacterial and fungal strains, including *Aspergillus ochraceous*, *Clavispora lusitaniae*, *A. wentii*, *A. sydowii*, and *A. foetidus*, are utilized in the production of α-L-rhamnosidase.

Various substrates that are inexpensive are utilized to produce the enzymes α-L-rhamnosidase and naringinase during microbial fermentation. Certain substrates are also used in the fermentation process because of their carbon and energy source. Grapefruit rind, pomelo peel powder, orange peel, citrus peel, orange rind, citrus fruit peel and lemon peel was used as a substrate for the production of α-L-rhamnosidase and naringinase (Igbonekwu *et al*., 2018; Patil *et al*., 2019; Srikantha *et al*., 2016).

The surface area of the fermentation tank, the incubation conditions, the water retention capacity, and the nutritional profile of the substrate are some of the factors that affects the ability of microbial strains to grow on particular substrates. The substrates used for microbial production of enzyme should also be able to absorb moisture in order to support metabolic processes and microbial growth (Purewal *et al*., 2019; Salar *et al*., 2017). Physiology of microbial strains, aeration, the particle size of substrate and porosity and incubation conditions such as moisture content, temperature, pH are the factors used which are responsible for the amount of enzymes production during fermentation. Table 1 reports the starter culture with the substrate used in the production of debittering enzymes. Orange juice treated with limonase dehydrogenase has lower limonin content (Brewster *et al*., 1976). According to Soares and Hotchkiss (1988), grapefruit's naringin content was reduced by 23% through the use of naringinase enzymes. Chen *et al*. (2010) has reported the using of starter culture *Aspergillus oryzae* for the production of naringinase (408.28 IU m).

Table 1: Production of debittering enzyme under different conditions

Source	Microorganism used	Temp (°C)	pH	Enzyme	Amount	Reference
Orange peel	*A. wentii* *A. sydowii* *A. foetidus*	30	4.5	α-L-rhamnosidase	39 U ml^{-1} 105 U ml^{-1} 92 U ml^{-1}	Yadav *et al*., 2018
Pomelo peel powder	*A. oryzae*	28	6	Naringinase	408.25 IU ml^{-1}	Chen *et al*., 2010
Grapefruit rind	*A. foetidus*	35	5.4	Naringinase	2.58 U	Mendoza-Cal *et al*., 2010
Grapefruit rind	*A. niger*	35	5.4	Naringinase	2.06 U	Mendoza-Cal *et al*., 2010
Orange rind	*A. niger*	28	7.5	Naringinase	4.42 U ml^{-1}	Shehata and Abd-El-Aty, 2014
Orange rind and grapefruit powder	*A. niger*	28	7.5	Naringinase	32-899 U ml^{-1}	Awad *et al*., 2016
Citrus fruit and peel	*A. flavus*	Room temp	4.5	Naringinase	449.58 U ml^{-1}	Srikantha *et al*., 2016
	A. niger	28			178.6 U ml^{-1}	Machado *et al*., 2010
	Bacillus sp.	28			197.3 U	Patil *et al*., 2019
	Pseudomonas sp.	28			186.8 U	Patil *et al*., 2019
Lemon peel	*A. niger*	50	3.5	Naringinase	157.70 U	Igbonekwu *et al*., 2018

CONCLUSION

To summarize, the Indian citrus sector, especially the small and medium scale foodpreneurs, faces significant challenges in commercializing citrus juices due to bitterness induced by chemicals like naringin and limonin. The harsh flavor reduces the quality and value of citrus-based products, resulting in consumer displeasure. To solve this issue, different debittering approaches have been investigated, including physical methods such as resin extraction, juice extraction, blanching, and filtration. Chemical treatments, including lye treatment, florisil, and β-cyclodextrin, have been used to lessen bitterness. Microbial enzymes such as α-L-rhamnosidase and naringinase have the potential to convert bitter substances into non-bitter metabolites. Microbial fermentation using low-cost substrates also improves the efficiency of debittering processes. These multiple approaches provide a holistic plan for the citrus sector to increase the acceptance of citrus-based products, resulting in improved organoleptic quality and longer shelf life. As technology advances, the development and implementation of these debittering processes will be critical to citrus's continued success in the worldwide market.

ACKNOWLEDGEMENT

Authors gratefully acknowledge the guidance and necessary facilities provided by the Director, ICAR-Central Citrus Research Institute, Nagpur during the course of investigation.

REFERENCES

Anand, S., Dhaliwal, Y.S. and Badyal, J. 2012. Effect of debittering techniques on the chemical characteristics of stored kinnow juice. Innovation in food science and technology to fuel the growth of the Indian food industry (abstracts). *Proceedings of the XXI Indian Convention of Food Scientists and Technologists*, Pune, Maharashtra, pp. 146.

Arruda, H.S., Pereira, G.A. and Pastore, G.M. 2017. Optimization of extraction parameters of total phenolics from Annona crassiflora Mart. (Araticum) fruits using response surface methodology. *Food Anal. Methods*, **10:**100–10.

Arruda, H.S., Pereira, G.A. and Pastore, G.M. 2018. Brazilian Cerrado fruit araticum (*Annona crassiflora* Mart.) as a potential source of natural antioxidant compounds. *Int. Food Res. J.*, **25**: 2005-12.

Awad, G.E.A., Abd El. Aty, A.A., Shehata, A.N., Hassan, M.E. and Elnashar, M.M. 2016. Covalent immobilization of microbial naringinase using novel thermally stable polymer for hydrolysis of naringin. *3 Biotech*, **6**: 14.

Barmore, C.R., Fisher, J.F., Fellers, P.J. and Rouseff, R.L. 1986. Reduction of bitterness and tartness in grapefruit juice with florisil. *J. Food Sci.*, **51**: 415-16.

Brewster, L.C., Hasegawa, S. and Maier, V. P. 1976. Bitterness prevention in citrus juices. Comparative activities and stabilities of the limonoate dehydrogenases from *Pseudomonas* and *Arthrobacter*. *J. Agric. Food Chem.*, **24**: 21-24.

Bala, A., Kaushal, B.B.L., Joshi, V. K. and Kaushal, M. 2017. Comparisons of juice extraction methods, determination of bittering principles and standardization of debittering of lime juice. *Indian J. Nat. Prod. Resour.*, **8**: 263–68.

Chen, D., Niu, T. and Cai, H. 2010. Optimizing culture medium for debittering constitutive enzyme naringinase production by Aspergillus oryzae JMU316. *Afr. J. Biotechnol.*, **9**: 4970-78.

Dilworth, L.L., Riley, C.K. and Stennett, D.K. 2017. Plant constituents: carbohydrates, oils, resins, Balsams, and plant hormones. Pharm. *Fund. Appl. Strat.*, 61–80.

Drewnowski, A. and Gomez-Carneros, C. 2000. Bitter taste, phytonutrients, and the consumer: a review. *Am. J. Clin. Nutr.*,**72**: 1424–1435.

Kore, V.K. and Chakraborty, I. 2015. Efficacy of various techniques on biochemical characteristics and bitterness of pummelo juice. *J. Food Sci. Technol.*,**52**: 6073–77.

Igbonekwu, A., Omeje, K.O., Ezugwu, A.L., Soo, E., Njoku, O.U. and Chilaka, F.C. 2018. Characterization of nariginase obtained from *Aspergillus niger* by submerged fermentation using naringin extracted from lemon peels. *Res. Dev. Mater. Sci.*,**4**: 420-24.

Machado, R.A.M., Buzato, J.B., Dias, C.D. and Celligoi, M.A.P.C. 2010. Naringinase production by *Aspergillus niger* in the fermentation of different carbon and nitrogen sources. *Biotechnol.: An Indian J.*, **4**: 201-06.

Mendoza-Cal, A., Cuevas-Glory, L., Lizama-Uc, G. and Ortiz-Varquez, E. 2010. Naringinase production from filamentous fungi using grapefruit rind in solid state fermentation. *African J. Microbiol. Res.*, **4**: 1964-69.

Mongkolkul, P., Rodart, P., Pipatthitikorn, T., Meksut, L. and Sanguandeekul, R. 2006. Debittering of Tangerine (*Citrus reticulata* Blanco.) juice by β-Cyclodextrin polymer. *J. Incl. Phenom. Macrocycl. Chem.*, **56**: 167–70.

Mishra, P. and Kar, R. 2003. Treatment of Grapefruit Juice for bitterness removal by amberlite IR 120 and amberlite IR 400 and alginate entrapped naringinase enzyme. *J. Food Sci.*, **68**: 1229-33.

Malta, L.M., Tessaro, E.P., Eberlin, M., Pastore, G.M. and Liu, R. H. 2013. Assessment of antioxidant and antiproliferative activities and the identification of phenolic compounds of exotic Brazilian fruits. *Food Res. Int.*, **53**: 417–25.

Neri-Numa, I.A., Sancho, R.A.S., Pereira, A.P.A. and Pastore, G.M. 2018. Small Brazilian wild fruits: nutrients, bioactive compounds, health-promotion properties and commercial interest. *Food Res. Int.*,**103**: 345–60.

Puri, M., Marwaha, S.S., Kothari, R.M. and Kennedy, J.F. 1996. Biochemical basis of bitterness in citrus fruit juices and biotech approaches for debittering. *Crit. Rev. Biotechnol.*, **16**: 145–55.

Purewal, S.S., Sandhu, K.S., Salar, R.K. and Kaur, P. 2019. Fermented pearl millet: a product with enhanced bioactive compounds and DNA damage protection activity. *J. Food Meas. Charact.*,**13**: 1479–88.

Pereira, G.A., Arruda, H.S., Morais, D.R., Araujo, N.M.P. and Pastore, G.M. 2020. Mutamba (*Guazuma ulmifolia* Lam.) fruit as a novel source of dietary fibre and phenolic compounds. *Food Chem.*, **310**: 125857.

Patil, S.V., Koli, S.H., Mohite, B.V., Patil, R.P., Patil, R.R., Borase, H.P. and Patil, V.S. 2019. A novel screening method for potential naringinase producing microorganisms. *Biotechnol. Appl. Biochem.*, 323–27.

Shehata, A.N. and Abd-El-Aty, A. 2014. Optimization of process parameters by statistical experimental designs for the production ofnaringinase enzyme by marine fungi. *Int. J. Chem. Eng.*, 1–10.

Srikantha, K., Kapilan, R. and Seevaratnam, V. 2016. Characterization of best naringinase producing fungus isolated from the citrus fruits. *Int. J. Biol. Res.*, **4**:83-87.

Salar, R.K., Purewal, S.S. and Sandhu, K.S. 2017. Fermented pearl millet (*Pennisetum glaucum*) with in vitro DNA damage protection activity, bioactive compounds and antioxidant potential. *Food Res. Int.*, **100**: 204–10.

Sandhu, K. S., Bhatia, B. S. and Shukla, F. C. 1990. Effect of lye treatment on the quality of Kinnow mandarin juice. *Indian J. Hortic.*, **47**: 55.

Sandhu, K.S. and Singh, N. 2001. Studies on factors affecting the physicochemical and organoleptic properties of Kinnow juice. *J. Food Sci. Technol.*,**38**; 266–69.

Soares, N.F.F. and Hotchkiss, J.H. 1988. Naringinase immobilization in packaging films for reducing naringin concentration in grapefruit juice. *J. Food Sci.*, **63**: 61–65.

Srikantha, K., Kapilan, R. and Seevaratnam, V. 2016. Characterization of best naringinase producing fungus isolated from the citrus fruits. *Int. J. Biol. Res.*, **4**: 83–87.

Purewal, S. S. and Sandhu, K. S. 2021. Debittering of citrus juice by different processing methods: A novel approach for food industry and agro-industrial sector. *Sci. Hortic.*, **276**:109750.

Premi, B.R., Lal, B.B. and Joshi, V.K. 1994. Distribution pattern of bittering principle in Kinnow fruits. *J. Food Sci. Technol.*,**31**: 140–41.

Puri, M., Marwaha, S.S., Kothari, R.M. and Kennedy, J.F. 1996. Biochemical basis of bitterness in citrus fruit juices and biotech approaches for debittering. *Crit. Rev. Biotechnol.*, **16**: 145–55.

Yadav, S., Kumar, D., Yadav, K.D.S. 2018. α-L-rhamnosidases produced under solid state fermentation by few Aspergillus strains. *Biotechnol. J. Int.*, **21**:1–8.

Yang, R., Song, C., Chen, J., Zhou, L., Jiang, X., Cao, X., Sun, Y. and Zhang, Q. 2020. Limonin ameliorates acetaminophen-induced hepatotoxicity by activating Nrf2 antioxidative pathway and inhibiting NF-κB inflammatory response via upregulating Sirt1. *Phytomedicine*, **69**:153211.

Yaqoob, M., Aggarwal, P., Aslam, R. and Rehal, J. 2020. Extraction of Bioactives from Citrus. *In. Green Sustainable Process for Chemical and Environmental Engineering and Science*, pp. 357-77.