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Review

Debittering of citrus juice by different processing methods: A novel approach for food industry and agro-industrial sector

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ABSTRACT

Bitterness in citrus fruit juices is the major problem faced by food processing industries. Because bitterness reduce the shelf life of juice and consumer acceptability. The major components responsible for the bitterness in citrus fruit juices are limonin and naringin. Several researchers/scientists are working in the direction to remove bitterness from citrus fruit juices so that shelf life may be enhanced. The major scientific methods used for the reduction of bitterness in the citrus fruit juices are lye treatment, addition of sugars, β-cyclodextrin, hot water treatment, cellulose acetate layers, enzymatic methods using microbial consortia. Capability of microbial consortia for the production of debittering enzymes has been explored and discussed in a systematic manner. The present review paper has its focus on major problems faced during processing of fruit juices, debittering methods, challenging tasks and future prospects.

1. Introduction

Citrus fruits are one of the famous world fruit crops which are rich in specific vitamins, minerals and bioactive compounds. Major citrus fruit grown by Indian farmers are mosambi, kinnow, orange, bitter orange, lemon, lime, galgal, tangerine and grapefruit and they belongs to family *Rutaceae* and Plantae kingdom. Throughout the world they are being consumed either directly or in the form of fresh juice/processed fruit products. Fruits are well known for their specific taste, aroma and oils (Zou et al., 2016; Cai et al., 2004; Ke et al., 2015). Despite seasonal availability, packing of fruit juices and products could be an alternate option to make them available throughout the year. Packing of food materials facilitates the easiest way to transport fruits based products to distant places (Purewal and Sandhu, 2020; Matche, 2018; Ramos et al., 2015). However, the bitterness of citrus juice may create problem during their long term storage. Chemical composition indicates the presence of various metabolites in the fruits which are chemically bitter (Naringin, tangeretin, nobiletin, sinensetin, quercetin, limonin, nomilin and neohesperidin) however only few selected metabolites (limonin and naringin) play an important role in causing bitterness (Singh et al., 2003). During processing of citrus fruits, the major problem is bitter taste (Ley, 2008; Drewnoswki, 2001). Bitterness may results in deterioration of quality, reduced consumer acceptability and economic value of the fruit based products (Kore and Chakraborty, 2015; Mongkolkul et al., 2006). The concentration of the bitterness causing components in citrus fruits may vary with the fruit type, fruit parts, cultivars and conditions under which they are growing. In fruits, a non bitter compound limonoate A-ring lactone is formed which is converted to limonin (bitter compound) in acidic conditions. Scientific studies reported that under low pH conditions the conversion of limonoate-A ring lactone to limonin occurs at faster rate (Hasegawa et al., 1991). Majority of consumer's rejects fruit products having bitter taste. Bitter taste in fruit juices/-products is not desirable so there is a need to eliminate bitterness from citrus juice.

Researchers are focusing on the scientific methods which could be utilized for the debittering of fruit juices to enhance the shelf life of fruits juices as well as to increase the consumer acceptability. Worldwide physical, chemical and microorganisms based biological methods are being screened for the efficacy towards bitterness reduction in citrus fruits. Artificial sweeteners, resins and enzymes are also being used to reduce the bitterness and improve the taste. The basic mechanism behind reduction of bitterness includes i) removal of bitter compounds ii) removal of physical barriers such as pith iii) flavor enhancers and use of bitter compounds scavengers (salt, sugar, florisil) iv) enzymatic (naringinase and α-L-rhamnosidase) reduction of bitter components v) use of genetic engineering techniques for modulating the synthetic pathways of bitter compounds. The present review paper has its focus on major problems in processing of citrus fruit juices, debittering methods,

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challenging tasks, future prospects and the methods using which shelf life of fruit juices may be enhanced.

2. Bitterness causing compounds

Different types of phytonutrients are present in citrus fruits with specific functionality and usefulness (Malta et al., 2013; Arruda et al., 2017; Neri-Numa et al., 2018; Arruda et al., 2018; Pereira et al., 2020). Out of these compounds some are very bitter whereas others are not, depending upon the type of the glycoside chain. Phytonutrients are classified as flavanones, flavones, flavonols, flavans, isoflavones, triterpenes, limonoid aglycones, glucosinolates (organosulphur compounds) and isothiocyanates etc. Naringin, tangeretin, nobiletin, sinensetin, quercetin, limonin and nomilin are bitter compounds present in citrus fruits. Major bitterness causing compounds in kinnow are naringin (Flavanones) and limonin (Limonoid aglycones). The type and concentration of bitterness causing compounds may vary with the specific part, maturity stage, growing conditions and type of fruit.

3. Naringin

Naringin ($C_{27}H_{32}O_{14}$ M.W.: 580.5 g mol^{-1}) is an important disaccharide derivative. It is (*S*)-naringenin substituted by 2-O-(alpha-L-rhamnopyranosyl)-beta-D-glucopyranosyl moiety at 7th position via glycosidic linkage (Alam et al., 2014; Chen et al., 2016). The IUPAC name of naringin is (2S)-5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-3,

Table 1
Bitter compounds present in fruits.

Fruits	Botanical name	Fruit part	Bitter compound	Amount	References
Grapefruit	*Citrus × paradisi*	Flavedo	Naringin	270.1–431.9 mg 100 g^{-1}	Ortuño et al. (1995); Puri et al. (1996); McIntosh and Mansell (1997); Hsu et al. (1998); Del-Rio et al. (1998)
		Albedo		130.1–1559.2 mg 100 g^{-1}	
		Pith		1328.5–1760.3 mg 100 g^{-1}	
		Seeds		29.5–267.7 mg 100 g^{-1}	
		Juice		30.0–75.0 mg 100 ml^{-1}	
		Whole fruit	Tangeretin	0–3 mg 100 g^{-1}	Del-Rio et al. (1998)
		Juice		0.06 mg 100 ml^{-1}	
Orange	*Citrus × sinensis*	Whole fruit	Nobiletin	1.4–11.2 mg 100 g^{-1}	Veldhuis et al. (1970); Sendra et al. (1988); Pupin et al. (1998)
		Juice		0.27–0.29 mg 100 ml^{-1}	
		Whole fruit	Sinensetin	1.4–4.6 mg 100 g^{-1}	
		Juice		0.1 mg L^{-1}	
Grapefruit	*Citrus × paradisi*	Juice	Quercetin	4.9 mg L^{-1}	Trock et al. (1990)
Lemon	*Citrus limon*	Juice		7.4 mg L^{-1}	
		Juice		12.2 mg L^{-1}	
Orange	*Citrus × sinensis*	Juice		9.7 mg L^{-1}	
Grapefruit	*Citrus × paradisi*	Juice		11.4 mg L^{-1}	
Tangerine	*Citrus tangerina*	Juice		34.7 mg L^{-1}	
Grapefruit	*Citrus × paradisi*	Flabedo	Limonin	0.61−4.2 mg 100 g^{-1}	Puri et al. (1996)
		Albedo		1.16−6.5 mg 100 g^{-1}	
		Pith		10.3−52.5 mg 100 g^{-1}	
		Seeds		118.8−188.5 mg 100 g^{-1}	
Sweet orange	*Citrus × sinensis*		Naringin	0.00−1.73 mg 100 g^{-1}	Peterson et al. (2006); Fisher (1978); Drawert et al. (1980); Galensa and Herrmann (1980); Rouseff et al. (1987); Rouseff (1988); Gamache et al. (1993); Mouly et al. (1993); Ooghe et al. (1994); Fuchs (1994); Bronner and Beecher (1995); Wallrauch (1995); Marini and Balestrieri (1995); Mouly et al. (1997); Justesen et al. (1997), 1998; Pupin et al. (1998a); Berhow et al. (1998); Careri et al. (2000)
			Narirutin	0.00−6.87 mg 100 g^{-1}	
Tangerine	*Citrus tangerina*		Narirutin	7.70 mg 100 g^{-1}	Nogata et al. (1994); Berhow et al. (1998)
Tangor	*C. reticulata × C. sinensis*		Narirutin	3.15−11.17 mg 100 g^{-1}	Rouseff et al. (1987); Berhow et al. (1998)
Tangelo	*Citrus reticulata × Citrus paradisi*		Naringin	0.00−33.73 mg 100 g^{-1}	Rouseff et al. (1987); Berhow et al. (1998)
			Narirutin	0.45−5.82 mg 100 g^{-1}	
			Neohesperidin	65.07 mg 100 g^{-1}	
Sour orange	*Citrus × aurantium*		Naringin	6.10−34.13 mg 100 g^{-1}	Rouseff et al. (1987); Mouly et al. (1993); Berhow et al. (1998)
			Narirutin	0.44 mg 100 g^{-1}	
			Neohesperidin	1.50−21.25 mg 100 g^{-1}	
Kinnow	*Citrus nobilis × Citrus deliciosa*	Pulp residue	Naringin	0.4−7.8 mg g^{-1}	Singla et al. (2019)
			Limonin	3−6 mg g^{-1}	
		Peel	Limonin	8 mg 100 g^{-1}	Mahajan et al. (2018)
		Seeds	Limonin	0.25 g 100 g^{-1}	
		Juice	Limonin	1.5 mg 100 ml^{-1}	

4-dihydro-2H-chromen-7-yl 2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside. Naringin possess 8 hydrogen bond donors, 14 hydrogen bond acceptor, 6 rotatable bonds with topological polar surface area 225 $Å^2$. Naringin is a flavanone-7-O-glycoside which is present in citrus fruits and the major cause of juice bitterness. Naringin is involved in inhibition mechanism of certain drugs dealing with enzymes (drug metabolizing cytochrome P450, CYP1A2 and CYP3A4). Inhalation/ingestion of naringin powder affects absorption of drugs and their circulation. Bitter compounds present in citrus fruits are presented in the form of Table 1. Diversity in amount of bitter compounds may be observed in the different parts of single fruit (Drewnowski and Gomez-Carneros, 2000; Soares et al., 2013; Li et al., 2019). Different parts of grapefruit (Albedo, flavedo, pith, seeds and juice) were analyzed to check the presence of naringin by different workers. Naringin present in different parts of grapefruit was observed as flavedo (270−431 mg 100 g^{-1}); albedo (130−1559 mg 100 g^{-1}); pith (1328−1760 mg 100 g^{-1}); seeds (29−267 mg 100 g^{-1}) and juice (30−75 mg 100 g^{-1}) (Ortuño et al., 1995; Puri et al., 1996; McIntosh and Mansell, 1997; Hsu et al., 1998; Del-Rio et al., 1998). The amount of naringin in sweet orange was 1.73 mg 100 g^{-1}; sour orange (6−34 mg 100 g^{-1}) and Tangelo (33.73 mg 100 g^{-1}) (Gamache et al., 1993; Mouly et al., 1993; Ooghe et al., 1994; Wallrauch, 1995; Marini and Balestrieri, 1995; Mouly et al., 1997; Berhow et al., 1998; Careri et al., 2000).

4. Limonin

Limonin is a derivative of limonoid aglycones which is highly oxygenated metabolites related to triterpene derivatives. They are present in fruits related to *Rutaceae* and *Meliaceae* families (Yang et al., 2020; Yaqoob et al., 2020; Roy and Saraf, 2006). There are two different groups of limonoids: first group includes the aglycones whereas second group includes their corresponding glucosides. Limonoids include limonin, nomilin, limonin glucoside, ichangin, and nomilinic acid etc. (Yang et al., 2019; Montoya et al., 2019; Minamisawa et al., 2017; Russo et al., 2016). Limonin ($C_{26}H_{30}O_8$ M.W. 470.52 g mol-1) is a white colored compound present in citrus fruits. The compound is well known as limonoic acid di-delta-lactone and limonoate D-ring-lactone. Chemically limonin belongs to furanolactones. The IUPAC name of limonin is 7,16-Dioxo-7,16-dideoxylimondiol. Limonin possess 8 hydrogen bond acceptors, 1 rotatable bond with topological polar surface area 105 $Å^2$ and 1 covalently bonded unit. Limonin is slightly soluble in water however soluble in absolute ethanol and glacial acetic acid. Limonin is an important type of limonoids that is present in fruits belonging to *Rutaceae* family. More than 30 different limonoids has been identified from citrus fruits and their hybrid cultivars (Sato, 2013; H. Li et al., 2016; N. Li et al., 2016; Izawa et al., 2010). Hasegawa and Miyake (1996) demonstrated that limonoids are synthesized in citrus fruits via terpenoid biosynthetic pathway which initialize with the squalene cyclization through CAM (cytoplasmatic acetate mevalonate) pathway. They are highly oxygenated compounds which possess furan ring attached with D ring. Further limonoids are classified on the basis of their skeletal arrangement and oxidative reactions (Endo et al., 2002). Limonin concentration in juice extracted from different citrus fruits such as: lemon (12 mg L^{-1}); grapefruit (11.4 mg L^{-1}); orange (9.7 mg L^{-1}) and tangerine (34 mg L^{-1}) (Drewnowski and Gomez-Carneros, 2000). Mahajan et al. (2018) studied kinnow peel, seeds and juice to check the presence of limonin and they found limonin 8 mg $100g^{-1}$ in peel; 0.25 mg $100g^{-1}$ in seeds and 1.5 mg $100g^{-1}$ in juice. Concentration of limonin in different parts of grapefruit was reported as: flavedo (6−42 mg/Kg); albedo (11−65 mg Kg^{-1}); pith (10.3−52.5 mg $100g^{-1}$) and seeds (118.8−188.5 mg $100g^{-1}$) respectively. Nomilin concentration in juice extracted from grapefruit, oroblanco and melogold was reported as 0.1−0.6 mg L^{-1}; 0.4−0.8 mg L^{-1} and 0.9-1.8 mg L^{-1} respectively.

5. Mechanism

Although the fresh juice extracted from the fruits don't elicit bitterness in taste, however, after a particular span of time the juice become bitter/not acceptable by consumers. The mechanism behind the appearance of bitter taste in juice is the conversion of limonin glucoside (non-bitter component) in to bitter compound (limonin) by deglycosylation and cyclization (Hasegawa, 2000; Mongkolkul et al., 2006). Premi et al. (1995) reported that seeds of citrus fruits possess maximal amount of limonin followed by peel and juice. McIntosh et al. (1982) reported that bitterness in juice extracted from fruits (bitter and non-bitter) occurs in two different ways: 1) bitterness due to the presence on flavonoids and their derivatives in fruits (pummelo; bitter orange and grapefruit) 2) bitterness due to conversion of tasteless form of compounds in to bitter one. Limonin derived bitterness in juice extracted from citrus fruits generally develops as a result of physical and freezing damage. The reaction starts with the mechanical disruption of juice containing sacs which results in transformation of non-bitter LARL (limonate A-ring monolactone) in to bitter form (limonin). Fong et al. (1992) reported that transformation of tasteless non-bitter form in to bitter form is an enzyme (limonin D-ring lactone hydrolase) catalyzed reaction which occurs in acidic condition and rate of reaction depends on the availability of LARL. With the maturation stage the concentration of limonin glucoside and expression of CitLGT starts increasing while a decrease in concentration of LARL was observed (Endo et al., 2002; Kita et al., 2000; Moriguchi et al., 2003).

6. Debittering methods

So many efforts have been made by researchers/food scientists to reduce the accumulation of bitter compounds during the development and maturing of citrus fruits using chemical sprays, agronomic practices and post-harvest treatment of fruit. Many debittering technologies have been developed based on physical, chemical and biological processes.

7. Physical methods

7.1. Resins

Resins are mixture of heterogeneous fatty acids, waxes, resenes and resin acid ($C_{20}H_{30}O_2$). Commercially resins are extracted from trees which mainly belong to family *Pinaceae* and *Dipterocarpaceae*. Resins are synthesized in specific surface glands/internal ducts of both non-woody and woody plants. They are metabolic byproducts of plants which can be extracted by infection/incision (Dilworth et al., 2017). Chemically resins are water insoluble and organic solvent soluble metabolic byproducts with inert nature. The efficacy of various resins is presented in Table 2. Mishra and Kar (2003) reported the effect of amberlite IR 400 and IR 120 on reduction of bitterness in grapefruit juice. Significant difference was observed in debittering potential of IR 400 and IR 120 as IR 400 resulted in removal of naringin by 69.23 % whereas IR 120 decreases the naringin content by 9%. Kola et al. (2010) use Dowex Optipore L285 and Amberlite XAD-16HP for the removal of limonin based bitterness from orange juice. They observed that application of Dowex Optipore L285 results in reduction of titrable acidity in orange while Amberlite XAD-16HP application does not pose any risk to change in nutritional quality. Nas and Karatas (2017) use Amberlite XAD-7HP for the reduction of bitterness in orange juice. Their results showed that Amberlite XAD-7HP application was successful as the applied method results in reduction of 90–96 % limonin from orange juice.

7.2. Fruit juice extraction methods

Juice extraction method play an important role while determining the bitterness of juice extracted from fruits (Lotha et al., 1994). Premi et al. (1994) reported that a gentle pressing of fruits results in lowest

Table 2
Detailed descriptions of physical and chemical treatments being used for debittering of juice extracted from different natural resources.

Substrate	Chemical used	Conc. Used	Temp.	pH	Exposure duration	Major findings	References
Bitter gourd	β-cyclodextrin	0.25–2%	24 ± 2 °C	3.5	1h	Use of β-cyclodextrin in concentration 1.5 % proved to be useful for debittering of juice extracted from bitter gourd. As compared to untreated counterparts the amount of bioactive compounds in β-cyclodextrin treated juice was observed in higher concentration.	Deshaware et al., 2018
Bitter gourd	Sodium chloride	3.5 %	25 °C	–	1h	Amount of total soluble solids, catechin and chlorogenic acid was significantly higher in sodium chloride treated bitter gourd as compared to untreated bitter gourd.	
Bitter gourd	Carboxymethylcellulose	0.5 %	–	–	–	Authors observed the increment in viscosity and total soluble solids in carboxymethylcellulose and gum Arabic treated bitter gourd. Antioxidant potential in carboxymethylcellulose and gum treated bitter gourd was found significantly higher during ABTS assay.	Rashima et al., 2017
Bitter gourd	Gum arabic	15 %	–	–	–		
Pummelo juice	Sodium hydroxide	1.25–1.75%	82–83 °C	4.05–4.10	40s	Treatment with sodium hydroxide and sodium bicarbonate results in improvement in pH which contributes in decreasing the bitterness of juice. However, the amount of ascorbic acid in juice extracted from treated samples was significantly lower as compared to untreated counterparts.	Kore and Chakraborty, 2015
	Citric acid	1%	Room temp.		–		
Pummelo juice	Sodium bicarbonate	–	Room temp.	4.25–4.75	–		
Tangerine Citrus Reticulata Blanco Juice	β-cyclodextrin	5%	30 °C	–	60 min	Use of β-cyclodextrin in concentration 5% at 30 °C for a period of 60 min. results in reduction of limonin by 80.71 %. The efficiency of β-cyclodextrin retained even at temperature 6 °C with limonin reduction by 80.96 %.	Mongkolkul et al., 2006
Lime Juice	β-cyclodextrin	2%	Ambient temp.	–	10 min	Treatment of juice with β-cyclodextrin in concentration 2% at ambient temp. for a period of 10 min. results in reduction of limonin content from 23.24–19.93 μg ml^{-1}.	Bala et al., 2017
Orange Juice	Amberlite XAD-16HP and Dowex Optipore L285	–	20 °C 35 °C 50 °C	–	–	Orange juice was treated with Amberlite XAD-16HP at temperature range 20–50 °C. Significant reduction in limonin content (ppm) was observed at temp. 20 °C which results in reduction of limonin content (ppm) from 10.67–0.06. Orange juice was treated with Dowex Optipore L285 at temperature range 20–50 °C. Significant reduction in limonin content (ppm) was observed at temp. 20 °C which results in reduction of limonin content (ppm) from 10.67–0.126.	Kola et al., 2010
Orange juice	Amberlite XAD-7HP	–	30 °C 40 °C 50 °C 60 °C	–	–	Treatment of orange juice with Amberlite XAD-7HP results in reduction of limonin content (ppm) from 11.4–5.4 ppm at temperature 40 °C.	Nas and Karatas, 2017

bitterness in juice as compared to untreated counterparts. Modern juice extractor although results in faster extraction of juice from fruits however during the extraction process crushing of seeds along with juicy sac may contribute in providing bitterness to juice. Manual removal of seeds from fruits before juice extraction results in decreased bitterness in juice. Sandhu and Singh (2001) observed that use of screw type juice extractor was effective to decrease the bitterness in juice. Thakur and Lal Kaushal (2000) demonstrated that the amount of bitterness causing components (naringin and limonin) was minimum in juice extracted from seedless fruits.

7.3. Hot water treatment and filtration

Hot water treatment (HWT) and filtration is the important physical methods which are being applied on variety of fruits to reduce the bitterness problem. During the HWT, fruits are kept in hot water (50 °C) for a specific period (20–30 min) followed by manual peeling and juice extraction. Kore and Chakraborty (2015) reported that HWT of pummelo juice results in decrease in TSS (11.10–9.40°B); acidity (1.32–0.94%) and ascorbic acid (73.9–32.5 mg 100 ml^{-1}). Specific equipments (filter press) and membranes (HFM; hollow fiber membranes)/ultra-filtrations (UF) are in use to avoid the entry of bitterness causing components to extracted juice. Wethern (1991) reported the use of UF for the clarification of juice extracted from grapefruit. The mechanism behind the filtration is: UF membranes have the capability to retain large sized molecules whereas smaller one could pass through the membrane thus resulting in the specific permeability (Cassano and Basile, 2013). HFM and UF helps in clarification of juice without allowing the entry of suspended particles and pulp. Clarified juice will be further processed using chemical as well as other physical methods. Ilame and Singh (2018) demonstrated that UF membrane modules (polysulfone based membrane; 30 kDa) have potential to increase the shelf life of kinnow for a period of 60 days without the requirement of additives.

8. Chemical method

8.1. Lye treatment

Debittering of fruit juices using lye treatment includes the treatment of fruits with sodium hydroxide at temperature 82–83 °C for 40–60 s followed by rinsing in citric acid of known concentration and washing under tap water to remove excess sodium hydroxide (Kore and Chakraborty, 2015). Debittering using lye treatment could be applied on variety of fruits with different ages. During the lye treatment outer creamy-white part of peeled fruit reacts with hydroxyl and carboxylic

group and results in removal of hydrophilic derivative during washing with water. Sogi and Singh (2001) observed that during the lye treatment concentration of sodium hydroxide used acts a determinant factor for debittering. As sodium hydroxide up to a specific concentration results in debittering however beyond specific limits it results in negative effects. Anand et al. (2012) studied the effect of various techniques for the purpose to debitter kinnow juice. Among different methods (lye treatment, florisil and their combinations) they found that lye treatment as best method for debittering of juice. Scientific reports on juice debittering suggest that removal of white papery segment from kinnow fruits during lye treatment results in maximal debittering of juice (Sandhu et al., 1990; Sandhu and Singh, 2001).

8.2. Florisil

Florisil is white colored, odorless compound which is chemically known as activated magnesium silicates. Florisil is used as an important debittering agent to improve the shelf life of fruit juices. Barmore et al. (1986) reported the use of florisil for the purpose to debitter the grapefruit juice. In their study, different concentration of florisil (5–20 %) was used. Increasing concentration of florisil significantly affects the amount of bitterness causing compounds in grapefruit juice. Use of florisil in concentration (20 %) results in reduction of limonin content from 8.8 to 1.7 ppm and naringin from 326ppm-159 ppm. Percentage of total acid in grapefruit juice was reduced from 0.81−0.33%. Kashyap and Anand (2017) reported the effect of florisil on ascorbic acid and reducing sugar of kinnow juice. The observed that florisil use results in decrease in ascorbic acid content from 18−14 mg $100g^{-1}$ and reducing sugar (3.53−2.90%).

8.3. β-cyclodextrin

Scientific studies reported the use of β-cyclodextrin for the removal of bitterness from fruit juices. Deshaware et al. (2018) reported the use of β-cyclodextrin for debittering of bitter gourd juice. They use β-cyclodextrin in concentration 0.25–2% out of which 1.5 % proved to be fruitful for removing the bitterness from bitter gourd (Table 2). Mongkolkul et al. (2006) demonstrated that β-cyclodextrin in concentration 5% at 30 °C for a period of 60 min. results in reduction of limonin content from tangerine Juice by 80.71 %. Bala et al. (2017) reported that treatment of lime juice with β-cyclodextrin (2%, 10 min) decrease the amount of limonin from 23−20 μg ml^{-1}.

9. Biological methods

9.1. Microbial consortia for enzyme production

Microbial strains are continuously being used for the production of specific enzymes (α-L-rhamnosidase and naringinase) for debittering purposes. The strains specifically used by researchers for the production of naringinase are *A. oryzae* (Chen et al., 2010); *A. foetidus* (Mendoza-Cal et al., 2010); *A. niger* (Luo et al., 2019; Igbonekwu et al., 2018; Awad et al., 2016; Shanmugaprakash et al., 2011; Machado et al., 2010); *A. flavus* (Srikantha et al., 2016); *Bacillus* sp. (Patil et al., 2019); *Pseudomonas* sp. (Patil et al., 2019); *Streptomyces* sp.(Patil et al., 2019); *Fusarium solani* (Patil et al., 2019); *Escherichia coli* (Patil et al., 2019); *Aspergillus brasiliensis* (Patil et al., 2019); *Rhizophus Stolonifer* (Karuppaija et al., 2017); *Bacillus cereus* (Pegu et al., 2019). Fungal and bacterial strains commonly utilized for the production of α-L-rhamnosidase are *Clavispora lusitaniae* (Singh et al., 2018); *A. niger* (Petri et al., 2014); *Aspergillus ochraceous* (Yadav et al., 2018); *A. wentii* (Yadav et al., 2018); *A. sydowii* (Yadav et al., 2018) and *A. foetidus* (Yadav et al., 2018).

9.2. Substrate used in enzymes production

The potential of microbial strains for the production of α-L-rhamnosidase and naringinase are being screened throughout the world using different substrates. Substrates chosen for microbial fermentation should be easily available at low cost and may be season independent so that enzymatic productions may continue throughout the year. Specific substrates are being utilized as solid platform during fermentation process as they could serve as best carbon and energy source. For the production of α-L-rhamnosidase commonly used substrates are orange peel, rice bran, wheat bran, corn cob (Yadav et al., 2018); sugarcane bagasse (Yadav et al., 2018; Petri et al., 2014); soybean hull (Petri et al., 2014); and rice straw (Petri et al., 2014). For the production of naringinase substrate that were used during fermentation process are pomelo pericarp powder (Chen et al., 2010); grapefruit rind (Mendoza-Cal et al., 2010); rice bran, wheat bran, sugarcane bagasse, citrus peel, press mud (Shanmugaprakash et al., 2011); orange rind (Shehata and Abd-El-Aty, 2014; Awad et al., 2016); Citrus fruit and peel (Luo et al., 2019; Patil et al., 2019; Srikantha et al., 2016; Machado et al., 2010) and lemon peel (Igbonekwu et al., 2018).

Although various substrates could be used for enzymatic productions however, the capability of microbial strains that can grow on specific substrates may vary depending on the nutritional profile of substrate; water retention potential; surface area of fermentation chamber/flask and incubation conditions. Substrate being fermented should be capable enough to imbibe moisture for sustaining microbial growth and metabolic reactions during the fermentation period (Purewal et al., 2019; Salar et al., 2017; Postemsky et al., 2017; Sandhu et al., 2016). Food grade GRAS (generally recognized as safe) cultures is one of important choice for the enzymatic production as they are safe. As compared to bacterial strains fungal strains are preferably used because of their capability to grow under minimal presence of water. For the better results during fermentation process it is necessary to maintain the softness of substrates as they ease the penetration of fungal hyphae in them and helps in boosting mycelial growth (Purewal et al., 2020; Acosta-Estrada et al., 2019; Mansor et al., 2019; Aita et al., 2019; Salar and Purewal, 2016; Salar et al., 2012). Physiology of starter culture (microbial strains) and incubation conditions (moisture content; temp.; pH etc.); aeration, particle size of substrate and porosity are the factors which ultimately determine the amount of enzymes produced during fermentation process. Ncube et al. (2012) reported that sometimes during the fermentation process if substrate doesn't fulfill the necessity of starter culture, addition of medium supplements (external supportive sources) could helps to achieve desirable changes. List of starter cultures along with substrates used are reported in Table 2.

9.3. Debittering enzymes and their production under different conditions

Researchers are working throughout the world to debitter the juice extracted from citrus family fruits. The keen focus of their research is to convert the bitterness causing components in to non-bitter metabolites so that the shelf life of juice may be extended. Enzymatic methods for debittering fruit juices are gaining interest from researchers/scientists/industries as their action on bitterness causing components are much higher as compared to chemical reagents. Further the enzymatic methods are cheaper than chemical treatments as enzymes can be immobilized on suitable surface to ensure their long term repetitive uses. For this purpose screening of microorganisms are being carried out for their potential to produce specific enzymes so that they can be used at industrial level for debittering the juice. Depending on the specific substrate, microbial strains and incubation conditions the type and amount of enzymes produced may vary accordingly. Production of debittering enzymes under different set of conditions using microbial consortia is represented in the form of Table 3. Brewster et al. (1976) reported that use of enzyme limonite dehydrogenase on orange juice results in reduction of limonin content of juice from 21−3 ppm. Johnson

Table 3
Production of debittering enzymes under different set of conditions using microbial consortia.

Source	Microorganisms used	Process type	Temp.	pH	Static/ Shaking	Enzyme extraction phase	Enzyme	Amount Of enzyme	Material for enzyme immobilization	References
Corn cob	*Aspergillus ochraceous* *A. wentii* *A. sydowii* *A. foetidus*	Solid state fermentation	30 °C	4.5	Static, Shaking	1 ml of sodium acetate / acetic acid buffer solution	α-L-rhamnosidase	69 U ml^{-1} 73 U ml^{-1} 142 U ml^{-1} 39 U ml^{-1}	–	Yadav et al., 2018
Rice bran	*Aspergillus ochraceous* *A. wentii* *A. sydowii* *A. foetidus*	Solid state fermentation	30 °C	4.5	Static, Shaking	1 ml of sodium acetate / acetic acid buffer solution	α-L-rhamnosidase	34 U ml^{-1} 43 U ml^{-1} 73 U ml^{-1} 115.9 U ml^{-1}	–	Yadav et al., 2018
Sugarcane bagasse	*Aspergillus ochraceous* *A. wentii* *A. sydowii* *A. foetidus*	Solid state fermentation	30 °C	4.5	Static, Shaking	1 ml of sodium acetate / acetic acid buffer solution	α-L-rhamnosidase	186 U ml^{-1} 153 U ml^{-1} 198 U ml^{-1} 309 U ml^{-1}	–	Yadav et al., 2018
Wheat bran	*Aspergillus ochraceous* *A. wentii* *A. sydowii* *A. foetidus*	Solid state fermentation	30 °C	4.5	Static, Shaking	1 ml of sodium acetate / acetic acid buffer solution	α-L-rhamnosidase	52 U ml^{-1} 60 U ml^{-1} 39 U ml^{-1} 201 U ml^{-1}	–	Yadav et al., 2018
Orange peel	*Aspergillus ochraceous* *A. wentii* *A. sydowii* *A. foetidus*	Solid state fermentation	30 °C	4.5	Static, Shaking	1 ml of sodium acetate / acetic acid buffer solution	α-L-rhamnosidase	69 U ml^{-1} 39 U ml^{-1} 105 U ml^{-1} 92 U ml^{-1}	–	Yadav et al., 2018
pomelo pericarp powder	*A. oryzae*	Solid state fermentation	28 °C	6.0	Shaking	–	Naringinase	408.28 IU ml^{-1}	–	Chen et al., 2010
Grapefruit rind	*A. foetidus*	Solid state fermentation	35 °C	5.4	Static	sodium acetate buffer 0.1 M	Naringinase	2.58 U	–	Mendoza-Cal et al., 2010
Grapefruit rind	*niger*	Solid state fermentation	35 °C	5.4	Static	sodium acetate buffer 0.1 M	Naringinase	2.06 U	–	Mendoza-Cal et al., 2010
Rice bran	*Aspergillus niger*	Solid state fermentation	27 °C	4.5	Static	acetate buffer 0.1 M	Naringinase	58.1 U g^{-1}	–	Shanmugaprakash et al., 2011
Wheat bran	*Aspergillus niger*	Solid state fermentation	27 °C	4.5	Static	acetate buffer 0.1 M	Naringinase	48.4 U g^{-1}	–	Shanmugaprakash et al., 2011
Sugarcane bagasse	*Aspergillus niger*	Solid state fermentation	27 °C	4.5	Static	acetate buffer 0.1 M	Naringinase	43.2 U g^{-1}	–	Shanmugaprakash et al., 2011
Citrus peel	*Aspergillus niger*	Solid state fermentation	27 °C	4.5	Static	acetate buffer 0.1 M	Naringinase	3.3 U g^{-1}	–	Shanmugaprakash et al., 2011
Press mud	*Aspergillus niger*	Solid state fermentation	27 °C	4.5	Static	acetate buffer 0.1 M	Naringinase	54.76 U g^{-1}	–	Shanmugaprakash et al., 2011
sugar bagasse, soybean hulls and rice straw	*Aspergillus niger*	Solid state fermentation	28 °C	4.5	Static	sodium acetate buffer 50mM	α-L-rhamnosidase	1.92 U ml^{-1}	–	Petri et al., 2014
Orange rind	*Aspergillus niger*	Solid state fermentation	28 °C	7.5	Static	sodium acetate buffer 0.1 M	Naringinase	4.42 U ml^{-1}	–	Shehata and Abd-El-Aty, 2014
Orange rind and grape fruit powder	*niger*	Solid state fermentation	28 °C	7.5	Static	sodium acetate buffer 0.1 M	Naringinase	32–899 U g^{-1}	Alginate beads	Awad et al., 2016
–	*Aspergillus niger*	–	40 °C	3.5	–	–	Naringinase	517.43 U ml^{-1}	Silica material	Luo et al., 2019
Citrus fruit and peel	*Aspergillus flavus*	Liquid state fermentation	Room temp.		Shaking	–	Naringinase	449.58 U g^{-1}	–	Srikantha et al., 2016
–	*Aspergillus niger*	Liquid state fermentation	28 °C	4.5	Shaking	–	Naringinase	178.6mUI/ mL	–	Machado et al., 2010
–	*Bacillus sp.*	Liquid state fermentation	28 °C	–	Shaking	–	Naringinase	197.3 U	–	Patil et al., 2019
–	*Pseudomonas sp.*	Liquid state fermentation	28 °C	–	Shaking	–	Naringinase	186.8 U	–	Patil et al., 2019
–	*Streptomyces sp.*		28 °C	–	Shaking	–	Naringinase	168.4 U	–	Patil et al., 2019

(*continued on next page*)

Table 3 (*continued*)

Source	Microorganisms used	Process type	Temp.	pH	Static/ Shaking	Enzyme extraction phase	Enzyme	Amount Of enzyme	Material for enzyme immobilization	References
		Liquid state fermentation								
–	*Fusarium solani*	Liquid state fermentation	28 °C	–	Shaking	–	Naringinase	178.9 U	–	Patil et al., 2019
–	*Escherichia coli*	Liquid state fermentation	28 °C	–	Shaking	–	Naringinase	89.4 U	–	Patil et al., 2019
–	*Aspergillus brasiliensis*	Liquid state fermentation	28 °C	–	Shaking	–	Naringinase	194.7 U	–	Patil et al., 2019
Palmyrah Fruit Pulp	*Rhizophus Stolonifer*	Liquid state fermentation	Room temp.	4.0	Shaking	–	Naringinase	3.125 μmol ml^{-1}	–	Karuppaija et al., 2017
–	*Bacillus cereus*	Liquid state fermentation	35 °C	4–9	Shaking	–	Naringinase	7.8 U ml-1	–	Pegu et al., 2019
–	*Clavispora lusitaniae*	Liquid state fermentation	35 °C	4.0	Shaking	–	α-L-rhamnosidase	0.106 IU ml-1	–	Singh et al., 2018
Lemon peel	*Aspergillus niger*	Submerged fermentation	50 °C	3.5	–	–	Naringinase	157.70 U	–	Igbonekwu et al., 2018

and Chandler (1982) demonstrated the absorptive potential of cellulose acetate films for limonin. The smoother surface of cellulose acetate films helps in maintaining the activity of enzymes at low temperature comparable to free enzymes. Due to the presence of naringin reduction potential cellulose acetate films are used in packing materials for storing citrus fruit juices. Immobilization of industrially important enzymes are currently in trend as the process results in maintenance of enzymatic activity for longer time and facilitate their repetitive use. Immobilization could be achieved in many ways which includes a) Use of inert material for immobilization of enzymes b) Entrapment of enzymes with in polymerizes gel lattice c) Cross linking of active proteins with multifunctional reagents d) covalent bonding of enzymes on insoluble supporting materials. The success rate of immobilization depends on activity of enzymes under different pH and temperature, presence of specific prosthetic and functional groups; molecular mass and enzymatic purity. Hasegawa et al. (1982) observed that application of immobilized cells could reduce the limonin content of serum up to 70 %. Soares and Hotchkiss (1988) reported that naringinase enzymes immobilized using cellulose acetate film was capable to reduce the level of naringin in grapefruit by 23 %. Puri et al. (1996) reported the action of α-L-Rhamnosidase and β-D-glucosidase on conversion of naringin to bitterless compound naringenin. The mechanisms of action of both enzymes are represented in the form of Fig. 1. Yadav and Yadav (2004) studied 6 MTCC certified fungal strains for their potential to produce α-L-rhamnosidase under pH range 4.0–5.5 and temperature range 53–60 °C. The conditions under which the fungal strains gave their optimal response was as follows; *Aspergillus foetidus* MTCC-508 (K_m value: 0.17 mM; Peak value: 7.74; pH: 4.0 and temp. 56 °C); *Aspergilus terreus* MTCC-3566 (K_m value: 0.13 mM; Peak value: 4.60; pH: 4.0 and temp. 55 °C); *Aspergillus terreus* MTCC-3375 (K_m value: 0.42; Peak value: 6.97; pH: 4.5 and temp. 57 °C); *Aspergillus ochraceus* MTCC-4643 (K_m value: 0.36; Peak value: 4.32; pH: 5.0 and temp. 60 °C); *Aspergillus flavipus* MTCC-4644 (K_m value: 0.48; Peak value: 7.92; pH: 5.5 and temp. 55 °C) and *Aspergillus fumigatus* MTCC-3376 (K_m value: 0.18; Peak value: 3.75; pH: 4.5 and temp. 53 °C).

Machado et al. (2010) reported that combination of naringin and molasses results in enhancement of naringinase activity produced by *Aspergillus niger*. The optimum experimental conditions for the production of naringinase was KH_2PO_4 (1 g L^{-1}); KCl (0.5 g L^{-1}); $MgSO_4.7H_2O$ (0.5 g L^{-1}) and $FeCl_3$ (0.1 g L^{-1}); peptone (10 g L^{-1}); naringin (0.5 g L^{-1}) and molasses (3 g L^{-1}). Experimental conditions were temperature (28 °C); pH (4.5); spore count (10^6 spores ml^{-1}) and shaking condition (180 rpm). Under these conditions the amount of naringinase produced by *Aspergillus niger* was 178.6 mUI ml^{-1}. Mendoza-Cal et al. (2010) screened twelve different fungal strains for their potential to produce naringinase enzyme. During their study they used naringin as an inductor and observed the hydrolysis of naringin in range 79–81 %. The optimal conditions for the naringinase producing fungal strains were pH 5.4; temperature 35 °C and 40 °C. *Aspergillus foetidus* was the best fungal strain which produce 2.58 U ml^{-1} of enzyme. Chen et al. (2010) use

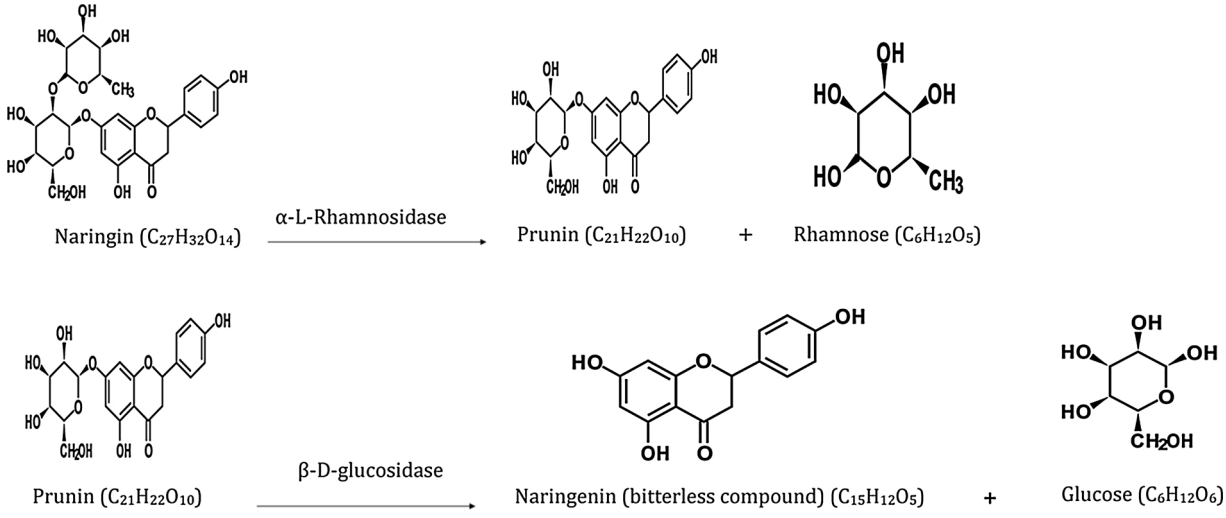

Fig. 1. Enzymatic action on naringin (Puri et al., 1996).

Aspergillus oryzae as starter culture on pomelo pericarp powder for the naringinase production. The optimal conditions they found for the maximum production of naringinase enzyme was: pomelo pericarp powder (15 g); peptone (12 g); NaCl (0.4 g); $MgSO_4.7H_2O$ (2 g); $CaCl_2$ (0.2 g) and K_2HPO_4 (1 g). The amount of naringinase enzyme produced under optimized conditions was 408.28 IU ml^{-1}.

Shanmugaprakash et al. (2011) use *Aspergillus niger* (MTCC-1344) as starter culture on different substrates (sugarcane bagasse; rice bran; citrus peel; press mud; wheat bran and citrus peel) for the production of naringinase enzyme. Amount of naringinase produced by starter culture on different substrates was as follows: rice bran (58 U g^{-1}); wheat bran (48 U g^{-1}); sugarcane bagasse (43 U g^{-1}); press mud (3 U g^{-1}) and citrus peel (54.76 U g^{-1}). Shehata and Abd-El-Aty (2014) optimized the experimental parameters for the production of naringinase from marine fungi. The output of their study indicates *Aspergillus niger* as promising strain for the naringinase production. During optimization strategy TOAD (Taguchi's orthogonal array design) and PBFD (Plackett-Burman factorial design) design was used to verify the significance of experimental parameters. The optimized conditions for the naringinase production from *Aspergillus niger* was orange rind (15 g); $FeSO_4$ (5 mM); $MgSO_4$ (5 mM); $NaNO_3$ (1%); grape fruit (1%); K_2HPO_4 (0.5 %) and pH (7.5). Naringinase activity observed under optimized experimental conditions was 3.14 fold higher as compared to routine cultivation conditions.

Awad et al. (2016) reported the immobilization of naringinase from *Aspergillus niger* on biocatalytically active gel beads. Under optimized conditions the loading capacity improved by 28 folds (32−899 U g^{-1}). Srikantha et al. (2016) studied use of paddy husk on 5 fungal strains for the screening of their potential to produce naringinase enzyme. The fungal strain with maximual naringinase production potential was identified as *Aspergillus flavus*. The enzyme activity observed for *Aspergillus flavus* was 450 U g^{-1}.

Karuppaija et al. (2017) reported naringinase activity in strain *Rhizopus stolonifer* isolated from Palmyrah fruit pulp. They observed that enzyme produced by *Rhizopus stolonifer* remains active with V_{max}: 3.125 μmol ml^{-1} at temperature 60 °C and pH 4.5. Yadav et al. (2018) compare the α-L-rhamnosidase production capability of *A. foetidus;, A. wentii; A. sydowii* and *Aspergillus ochraceous*. They observed maximum enzyme production at 30 °C with substrate:moisture ratio (1:1 w/v). They demonstrated that use of naringin as substrate during the fermentation process results in enhancement of enzyme production. Sucrose proved to be an efficient carbon source which results in α-L-rhamnosidase activity in *A. foetidus* (738 U ml^{-1}), *A. wentii* (397 U ml^{-1}); *A. sydowii* (596 U ml^{-1}) and *Aspergillus ochraceous* (363.6 U ml^{-1}) comparable to other carbon sources (rhamnose, fructose and glucose) which produces lesser enzymes unit.

Singh et al. (2018) studied the effect of C-sources (glucose, fructose, lactose, sucrose and rhamnose) and reported the maximum production of rhamnosidase by *Clavispara lusitaniae* (KF633446) under experimental conditions rhamnose (0.6 g 100 ml^{-1}); yeast extract (0.4 g 100 ml^{-1}) and naringin (0.2 g 100 ml^{-1}). Temperature and pH during the experimental work was 35 ± 5 °C and 4 respectively. Luo et al. (2019) use silica material for the purpose of naringinase immobilization. The starter culture used by them was *Aspergillus niger*. Silica material with variable pore size (MCM-41 2 nm; SBA-15 7.7 nm and silica gel 80 nm) was screened for the efficiency to retain naringinase activity. As compared to free enzymes (89 U ml^{-1}); they found SBA-15 as efficient carrier for retaining enzyme activity (467.62 U g^{-1}; 40 °C for 4 h) up to 8 consecutive cycles. Storage period of 30 days results in residual naringinase activity 81 %. Patil et al. (2019) reported the presence of naringinase activity in 6 microbial strains namely: *Bacillus* sp. (197 U); *Pseudomonas* sp. (186 U); *Streptomyces* sp. (168 U); *Fusarium solani* (179 U); *Escherichia coli* (89 U) and *Aspergillus brasiliensis* MTCC-1344 (195 U). The maximum naringinase activity was shown by *Bacillus* sp. (197U).

10. Other debittering method

10.1. Addition of syrup

Bitterness in kinnow juice depends on the sugar-acid ratio in the juice sacs. Environmental conditions especially temperature, light irradiance and agricultural practice significantly affects the sugar content in juice sacs of kinnow fruit. During ripening phase, increase in temperature resulted in decreased acid level and increased sugar content of kinnow fruit (Lado et al., 2016; Benjamin et al., 2013; Marsh et al., 1999). Total soluble solids (TSS) present in kinnow pulp may vary from 9.5–16% (Goldenberg et al., 2014; Shen et al., 2013; Ladaniya, 2011; Xu et al., 2008). Sugar syrups are being used to overcome the bitterness of juice. Bala et al. (2017) reported the reduction in amount of bitterness causing components present in kagzi lime juice. They observed 60.38 % and 39.76 % reduction in limonin (μg ml^{-1}) during treatment T_1 (juice TSS 65°B) and T_2 (juice TSS 45°B). Further, 63.35 % and 49.16 % reduction in naringin content was also observed in both T_1 (juice TSS 65°B) and T_2 (juice TSS 45°B) treatments. Kore and Chakraborty (2015) studied the effect of syrup treatment on debittering of pummelo juice. In their study pummelo juice was mixed with sugar syrup to achieve final TSS value (15°B, 30°B and 45°B). They reported that addition of syrup results in modulation of bio-chemical properties of pummelo juice as indicated by change in acidity from 1.32 to 0.50 %; reducing sugar (4.61–18.47 %) and ascorbic acid (73.97−42.35 mg 100 ml^{-1}). Further, addition of sugar syrup in juice results in improvement of consumer acceptability from 3.33 to 7.88 (Sensory analysis, hedonic scale).

10.2. Challenges

Besides chemical methods which is a costly method for adoption at industrial scale, one of the attracting and challenging approach to combat bitterness in fruit juice is the utilizing the potential of genetic engineering techniques. Transgenic plants could be able to solve the problem of bitterness in juice. Enzyme coding genes whose action result in formation of non bitter intermediate complexes in the juice may be synthesized. Suitable changes in genome at specific locations could arrest the formation of limonin in such fruits where the problems of bitterness in juice mainly due to limonin. Enzymes which could be one of the target for genetic engineering is: 1) Nomilin Deacetylase 2) Limonate dehydrogenase 3) Glucosyltransferase. Insertion of specific enzymes like Nomilin deacetylase in citrus fruit could modulate the pathway of limonin synthesis. In routine way the metabolic pathways results in formation of limonin from nomilin which results in bitterness in juice extracted from citrus fruits. The action of enzyme results in formation of deacetylnomilin from nomilin which is a bitterless compound. Another important enzyme that could be a boon in debittering direction is limonate dehydrogenase which has potential to convert bitter limonin in to bitterless 17-dehydrolimonate A-ring lactone. Glucosyltransferase catalyze the conversion of limonin aglycones to bitterless 17β-D-glucopyranoside derivatives.

10.3. Future prospects and conclusions

Nowadays, cost effective, easy to perform, reliable and user friendly methods are gaining more interest. Pulpy part of citrus and seeds are mainly responsible for the bitterness of juice therefore decreasing their shelf life and consumer acceptance. It is important to develop a juice extraction method or use of specific equipment like screw type juice extractor which allow minimal addition of pulp particles and seeds to improve shelf life with consumer acceptance. The methods which could results in debittering of juice with lesser effects on organoleptic properties of fruits are needs to be developed. Scientific studies on debittering of juice indicate that screw type juice extractor is more efficient to extract juice with less bitter components as compared to other methods. Application of specific growth promoting hormones such as 2-(4-

cholorophenyl thio) tri-ethylamine, ethylene and GA3 could improve the nutritional profile of citrus fruits (Sandhu et al., 2012; Berhow, 2000). The amount of bitterness causing components starts decreasing with the age of fruit and seasonal variation. Seasonal changes in bitterness causing components of citrus fruits should be kept in mind before preparing any food product based on them. Lye treatment, addition of sugar, florisil and naringinase are in use to combat bitterness of juice and improvement of taste. Blending of juice is also useful as it improve the taste and aroma. Enzymes immobilized on suitable material could be preferable as they could be used again and again for debittering purposes. Naringinase has been used mainly for debittering of citrus juices and in biotransformation processes. Enzymes are sufficient enough to reduce the bitterness causing compounds up to a significant level. However with many advantages, enzymatic use also has some limitation. Industries are not adopting the use of enzymes for debittering purpose as the enzymatic use raise the cost of products prepared from debittered juice. Further, enzymes purification/immobilization is a tedious process and inactivation of enzymes/leaching limits the efficacy level half time.

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Declaration of Competing Interest

Authors declare no conflict of interest.

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