Tannins in Fruit Juices and their Removal

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ABSTRACT

Tannins are groups of phenolic compounds that cause astringency and turbidity in fruit juices. Many factors had an influence on the concentration of tannins in fruit juices, including cultivar, parts and maturity stages, as well as pH and viscosity of juices. Many methods were investigated for tannins removal from fruit juices. Physical methods included heat treatment and membrane filtration. Chemical methods were based on clarifying agents included polysaccharides (starch and chitosan), proteins (gelatin and casein) and synthetic polymers (polyvinyl polypyrolidone). The enzymatic method was based on the application of enzyme tannase to hydrolyze tannins into soluble compounds. Tannins removal efficiency of each method depended on fruit juice, type and parameters of the method. Downstream process should be considered especially for application of clarifying agents.

Keywords: Fruit juice, Tannin, Gelatin, Polysaccharides, Tannase

INTRODUCTION

Tannins are phenolic compounds with molecular weight from 500 to more than 3,000 Da. They are plant secondary metabolites which have a role in plant defense mechanism against microbial infection, insect and animal herbivores. Tannins are classified into 2 groups: hydrolysable and condensed tannins. The latter is the major tannins in the plant and are flavonoid polymers. Tannins are commonly found in most plant tissues, including leaf, bud, seed, root, stem, bark and fruit (Barbehenn and Constabel, 2011; Hassanpour et al., 2011). Many fruit juices such as apple, grape and berry juices are all high in tannins. (Smeriglio et al., 2017). The high concentration of tannins affected organoleptic properties of the juices, including astringency, brown color and turbidity. Therefore, the removal of tannins from fruit juice is required for consumer acceptance and storage stability. This article review current knowledge on the removal methods including chemical, physical and enzymatic techniques for reduction of tannins in the fruit juices.

TANNINS IN FRUIT JUICE

Tannins can be found in many fruit juices, e.g. apple, banana, guava, cashew apple, guava, grape, persimmon, pomegranate, myrobalan and sugarcane juices (Table 1). Pomegranate and cashew apple juices were widely studied because the presence of tannins obviously resulted in astringent mouthfeel. The reported values for a single fruit juice may vary from one study to another study due to many factors, including fruit cultivar, parts of the fruit used in extraction, maturity stage, processing conditions, a presence of other taste affecting compounds in the juice, as well as types and assays for analysis of tannins.

Table 1. Tannins concentration in fruit juices.

Fruit juice	Tannin type	Concentration (mg/L)	References
Apple	Total tannins	238	Youn et al. (2004)
	Total tannins	275	Araya-Farias et al. (2008)
Banana	Total tannins	838-979	Kyamuhangire et al. (2006)
Cachew apple (*Anacadium occidentale* L.)	Condensed tannins	1,950	Dedehou et al. (2015)
	Total tannins	2,900-3,800	Soro (2012
	Total tannins	49-148	Dedehou et al. (2015)
	Total tannins	840-3,040	Dedehou et al. (2016)
	Total tannins	28	Emelike and Ebere
	Total tannins	19	2016
Guava (*Psidium guajava* L.)	Condensed tannins	1,960	Brasil et al. (1995)
Grape (*Vitis vinifera* L.)	Total tannins	5,190	Lima et al. (2014)
Jamun (*Syzygium cumini* L.)	Total tannins	12	Jana et al. (2015)

Table 1. Cont.

Fruit juice	Tannin type	Concentration (mg/L)	References
Persimmon *(Diospyros kaki* Thunb.*)*	Total tannins	10,390-11,040	Taira et al. (1997)
Pomegranate *(Punica granatum L.)*			
From fresh aril	Total tannins	150-320	Mousavinejad et al. (2009)
From fresh aril	Punicalagin	68	Gil et al. (2000)
From whole fruit		1,500-1,900	
From fresh aril	Hydrolyzable tannin	539	
From whole fruit		417	
From whole fruit	Hydrolyzable tannin	3,041	Erkan-Koç et al. (2015)
From fresh aril	Protein precipitable tannins	4,000	Rout and Banerjee (2006)
From fresh aril	Punicalin	703	Valero et al. (2014)
	Punicalagin	555	
	Ellagic acid	322	
Myrobalan *(Phyllanthus emblica)*	Total tannins	1,125	Srivastava and Kar (2010)
Sugarcane *(Saccharum officinarum)*	Total tannins	250-3,200	Qudsieh et al. (2002)

Effect of cultivars

Fruit juices obtained from different cultivars may contain a varied amount of tannins. A comparison between pomegranate juice of 8 Iranian cultivars reported that the juice from Sweet Alak cultivar had the highest concentration of total tannins (32 mg/100 g juice), while those from other cultivars had tannins between 15 and 22 mg/100 g juice) (Mousavinejad et al., 2009). A similar result was also found in other literature (Orak et al., 2012).

Effect of fruit parts

Different parts of the same fruit contain a varied concentration of tannins. Sugarcane juice obtained from a top portion of the stem had higher concentration of tannins compared to those obtained from middle and bottom parts (Qudsieh et al., 2002). Pomegranate juice obtained from fresh aril had 68 mg/L of punicalagin while commercial pomegranate juice from whole fruit contained 1,562 mg/L of the tannins. This was due to a different distribution of punicalagin in aril and rind of the fruit. In commercial practice, the rind was not separated before squeezing and water-soluble tannins were therefore diffused into the juice (Gil et al., 2000). Moreover, it was also reported that peel and seed extracts of pomegranate had

16.6- and 4.5-folds higher tannins content than the juice extract (Orak et al., 2012).

Effect of plant maturity

Immature sugarcane had higher tannin concentration than a mature counterpart. During the growth of sugarcane, tannins concentration increased at the early stage of maturity up to 5 months (around 0.4%). Afterward, tannins rapidly decreased to less than 0.1% within 8 months and slowly decreased to 0.025% at the end of maturity stage at 10 months (Qudsieh et al., 2002).

Effect of juice compositions and viscosity

Perception of astringency can be affected by compositions of the juice. In a model wine solution, panelist could perceive lower astringency at higher pH or higher ethanol concentration (Fontoin et al., 2008). Similarly, cranberry juice with lower pH had higher astringent perception. Adjusting pH of cranberry juice from 2.65 to 3.00 significantly decreased astringent perception without affecting tannins (Peleg and Noble, 1999).

Viscosity of the juice also affected astringency perception. Cranberry juice stored at 5°C provided lower astringency compared to 25°C. The lower temperature increased viscosity of the juice and decreased reaction rate between tannins and salivary protein. However, higher viscosity due to the presence of polysaccharide, e.g. carboxymethylcellulose (CMC) could further decrease astringent perception. This was caused by an additional mechanism that the hydrocolloid formed a complex with tannins which reduce interaction between tannins and salivary protein (Peleg and Noble, 1999). Complexation between polysaccharide and tannins was also observed in intact fruit. During postharvest softening of persimmon, pectin formed a complex with soluble tannins and mitigated astringent perception of the fruit (Taira et al., 1997).

Effect of analytical methods

Total tannins analyzed by Folin-Denis method might differ from those analyzed by protein precipitation method. Phenol reagent in Folin-Denis method reacted with both free tannins and tannins that form complex with protein or polysaccharide. However, only free tannins can form complex with albumin in protein precipitation method. The latter was more correlated with astringency score of the juice (Taira et al., 1997).

IMPACT OF TANNINS IN FRUIT JUICE

The presence of tannins affected organoleptic properties of fruit juice in both appearance (turbidity and color) and flavor (astringency) (Qudsieh et al., 2002).

For fresh juice, tannins and other phenolic compounds could be substrates for polyphenol oxidase (PPO) in catalyzing enzymatic browning reaction. Moreover, tannins could also form complex with anthocyanins to produce brown color in pomegranate juice (Turfan et al., 2011).

Turbidity of fruit juice can occur in 2 stages, namely, after pressing and during storage. Turbidity of fresh fruit juice after pressing is due to suspended pectin particle from plant cell wall (Pinelo et al., 2010). Turbidity during storage or haze is caused by interaction between pectin, polyphenols (include tannins), protein, starch, cellulose or hemicellulose (Echavarria et al., 2011). Cloudy appearance may be a consumer expectation for some juices, e.g. orange juice, while for the others, e.g. pomegranate juice, the clear appearance is required.

Astringency is a dry sensation in the mouth which occurs during drinking beverages, such as tea and wine. General explanation of this phenomenon is that tannins bind with salivary protein which results in loss of lubrication in the mouth. However, a recent study suggested that astringency was also caused by interaction of tannins with oral tissue, membrane-bound protein, epithelial cells and receptors in the tongue as well as oral tissue (Rossetti et al., 2009). Astringency was also common sensation from drinking raw pomegranate or cashew apple juice.

REDUCTION OF TANNINS IN FRUIT JUICE

General method for processing cashew apple juice includes fruit selection, washing, cutting, juice extraction (e.g. pressing), addition of clarifying agent (to remove tannins and clarify the juice), filtration and heat treatment (Dedehou et al., 2015). Many methods were investigated for mitigation of tannins in fruit juices, including chemical, physical and enzymatic methods.

Chemical clarifying agents

Clarifying agents used in the mitigation of tannins from fruit juice are proteins (gelatin and casein), polysaccharides (starch and chitosan) or synthetic polymer (polyvinyl polypyrolidone) (Dedehou et al., 2015). Processing conditions and efficiency for each method in the decrease of tannins is shown in Table 2.

Table 2. Conditions and tannins mitigation efficiency for each clarifying agent in selected fruit juice.

Juice	Tannin mitigation method	Tannin mitigation efficiency	References
Apple juice	Bentonite (0.5%)	23.11% tannins	Youn et al. (2004)
	Pectinase (0.03%) + amylase (0.003%)	28.99% tannins	
	PVPP (0.2%)	30.67% tannins	
	Activated carbon (1%)	26.89% tannins	
	Microfiltration	40.34% tannins	
	Ultrafiltration	47.90% tannins	
	Bentonite (0.5%) + microfiltration	45.38% tannins	
	Pectinase (0.03%) + amylase (0.003%) + microfiltration	35.71% tannins	
	PVPP (0.2%) + microfiltration	59.24% tannins	
	Activated carbon (1%) + microfiltration	43.70% tannins	
	Gelatin (200 mg/L) with electroflotation	26-47% tannins	Araya-Farias et al. (2008)
Pomegranate juice	PVPP, 2 g/L, 16 h, 5°C	18.09% total phenolics	Vardin and Fenercioğlu (2003)
	Gelatin, 2 g/L, 16 h, 5°C	26.60% total phenolics	
	Gelatin, 0.375 g/L, 16 h, 4°C	69.35% hydrolysable tannins	Erkan-Koç et al. (2015)
	Chitosan, 0.5 g/L, 16 h, 20°C	60.54% hydrolysable tannins	
	Casein, 0.375 g/L, 16 h, 4°C	70.24% hydrolysable tannins	
	Albumin, 0.125 g/L, 16 h, 4°C	58.99% hydrolysable tannins	
	Xanthan gum, 0.375 g/L, 16 h, 2°C	57.38% hydrolysable tannins	
	Ultrafiltration (Polyethersulfone 0.1 μm)	43.10% punicalagins; 94.10% ellagic acid	Valero et al. (2014)
	Microfiltration (Polysulfone 0.2 μm)	34.60% punicalagins; 65.40% ellagic acid	
Guava juice	Gelatin + silica sol	73.70%	Brasil et al. (1995)
Cherry juice	Gelatin 0.063 g/L + silica sol 0.625 g/L	84.30% turbidity	Meyer et al. (2001)
Cashew apple juice	Cassava starch (21% amylose) at 6.2 mL of 5% starch solution/ 1 L of juice for 300 min	65.80% tannins	Dedehou et al.(2015)
	Rice starch (24% amylose) at 10 mL of 5% starch solution/ 1 L of juice for 193 min	57.86% tannins	Dedehou et al. (2015)

Table 2. Cont.

Juice	Tannin mitigation method	Tannin mitigation efficiency	References
	Starch		Talasila et al. (2012)
	2 g/L	15.38% tannins	
	4 g/L	13.18% tannins	
	Sago		
	2 g/L	42.85% tannins	
	4 g/L	24.17% tannins	
	Gelatin 2.7-3.0 g/L	24.00% tannins	Lam Quoc et al. (1998)
	Gelatin		Talasila et al. (2012)
	2 g/L	26.37% tannins	
	4 g/L	36.26% tannins	
	PVP		Talasila et al. (2012)
	2 g/L	25.27% tannins	
	4 g/L	31.86% tannins	
	Celite bed filtration (particle size 20-45 μm) 40% w/v	10.98% tannins	Talasila et al. (2012)
	Hot water on cashew apple, 20 min	96.20% tannins	Emelike and Ebere (2016)
	Gelatin 50 g/kg cashew apple, 2 h	30.43% tannins	

Note: PVPP – Polyvinyly polypyrrolidone.

Gelatin at the concentration levels of 2.7 to 3.0 g/L provided 94% mitigation of tannins in cashew apple juice which were higher than those obtained by PVPP (24%) and XAD-16 (4.3%). Application of these treatments also reduced nutrients, e.g. ascorbic acid, especially if multiple treatments were used in combination (Lam Quoc et al., 1998). In another study, optimal condition for precipitation of tannins could be accomplished by mixing of gelatin at 0.67 g/100 mL cashew apple juice for 15 min, which removed 79% of tannins from the juice (Prommajak et al., 2018).

Casein at 0.375 g/L could remove 70.24% of hydrolysable tannins in pomegranate juice which was not significantly different from that obtained by gelatin at the same concentration (69.35% removal). However, albumin at 0.125 g/L provided a lower efficacy in tannins mitigation (58.99%), which was similar to clarification by 0.5 g/L chitosan (60.54% mitigation) and 0.375 g/L xanthan gum (57.38% mitigation) (Erkan-Koç et al., 2015)

Gelatin in combination with silica sol was also used for clarification and mitigation of tannins in fruit juices, e.g. guava and cherry juices. In industrial practice, clarification of fruit juice with gelatin-silica sol might take up to 6-18 h until sedimentation of colloidal particles was completely formed (Meyer et al., 2001). Moreover, downstream processing, especially filtration, was also required to obtain clarified juice (Pinelo et al., 2010).

Tannin removal by protein-based clarifying agents also depends on pH level of fruit juice. Complexation was due to positive charge of protein and negative charge of phenolic compounds. Generally, proteins had a positive charge at pH level lower than their isoelectric points (pI). Gelatin, albumin, and casein had pI of 7.0, 4.7 and 4.7, while pomegranate juice had pH level of 3.15. Therefore, gelatin was more effective than the other proteins in mitigation of tannins due to its higher difference between pI of protein and pH level of the juice (Erkan-Koç et al., 2015).

Tannin removal can be accomplished by addition of polysaccharides, e.g. starch, agar and chitosan. Starch was usually gelatinized or dissolved in lukewarm water before adding to the juice. Optimal condition for cassava starch (21% amylose) was by adding 6.2 mL of 5% w/v starch solution in 1 L of juice for 300 min. This could mitigate tannins content by 34.2%. For rice starch (24% amylose), an optimal condition of 10 mL 5% w/v starch solution in 1 L of juice for 193 min could decrease tannins content to 42.14%. Efficacy of starch depended on amylose and amylopectin ratio. Starch with more amylose content could strongly interact with proanthocyanidins compared to starch with more amylopectin. Amylose provided more hydrophobic core for monomeric phenols while amylopectin trapped polymeric tannins without chemical interaction (Dedehou et al., 2015).

Sago, a commercial starch preparation of cassava (*Manihot esculenta*), at 2 g/kg juice was more effective in mitigation of tannins in cashew apple juice than PVP at 1.4 g/kg and gelatin at 4 g/kg juice. Sago also provided high visual clarity compared to other compounds (Jayalekshmy and John, 2004).

In addition to the previously mentioned clarifying agents, chitosan was also proved for clarifying passion fruit and apple juices. However, the effects on tannin content in these studies were not investigated (Domingues et al., 2012; Taştan and Baysal, 2017).

Protein and polysaccharide formed complex with tannins by hydrogen bonding between phenolic hydroxyl group of tannins and carbonyl group of the polymer. The complex bound into floc and created the heavier complex that sank to the bottom of the container. During floc formation, pectin and other cell wall components might also be trapped within the floc (Pinelo et al., 2010). In case of starch, amylose could also form an inclusion complex with condensed tannins. If raw starch was used, condensed tannins were also adsorbed in the surface pores of starch granules (Dedehou et al., 2015).

Polyvinyl polypyrolidone (PVPP) is a cross-linked polyvinylpyrolidone (PVP) which has a relatively high absorption capacity toward tannins and low-molecular-weight phenolic compounds. PVPP is insoluble in water and can be used in beverage industry (Bagci, 2014). PVP removed tannins by chelation mechanism. General concentration for PVPP was 2 to 4 g/L which provided up to 31.86% mitigation of tannins in cashew apple juice (Talasila et al., 2012).

For most clarifying agent, e.g. gelatin and PVP, tannin mitigation was dose-dependent. Addition of more agents could decrease more tannins proportionately. However, starch had an optimal dose, in which adding more starch beyond certain concentration level would provide lower efficacy in tannin mitigation. Tannins in cashew apple juice could be further removed by addition of sago or starch at 2 g/L more than addition of the same compound at 4 g/L. This was due to the greater soluble property of starch which might form soluble complex with tannins instead of settling down at a higher concentration (Talasila et al., 2012).

Celite (filter aid) and activated charcoal (adsorbent) were also tested for tannin removal from cashew apple juice. However, tannin mitigation was not greater than 11% for individual or combined treatments (Talasila et al., 2012).

Application of polysaccharides also provided an advantage over gelatin as it was plant origin with less difficulty in product labeling. If gelatin was used, the product cannot be claimed as for vegetarians. Moreover, some animal sources of gelatin may not be suitable for some group of consumer (Jayalekshmy and John, 2004). Many clarifying agents, e.g. starch and gelatin were usually prepared in form of solution while PVP was a water-soluble compound and can be directly added into the juice. Dilution effect should be noticed on tannin removal by addition of compounds dissolved in water. For example, addition of starch or gelatin solution increased the total volume of the fruit juice and therefore dilute natural strength of the juice (Dedehou et al., 2015). Labeling of juice product should be in accordance with the legislation of each country.

Negative effect of some clarifying agents could be noticed as some compounds did not only decrease tannins but also mitigate other beneficial compounds in the juice. For example, gelatin solution at 0.5% could remove 4-7% of total anthocyanins in pomegranate juice obtained from juice sac while gelatin solution at 1% removed 19-22% of total anthocyanins in the juice obtained from whole pomegranate fruit (Turfan et al., 2011). Similar effect was also observed in juice clarification with other protein-based agents, e.g. casein and albumin. Addition of gelatin and casein resulted in higher mitigation of hydrolysable tannins in pomegranate juice compared to polysaccharide-based agent. They also caused a higher decrease in antioxidant activity and a slight decrease in titratable acidity as well as total soluble solids. In contrast, polysaccharide-based clarifying agent, e.g. chitosan and xanthan gum, could decrease tannins without significant reduction on antioxidant activity (Erkan-Koç et al., 2015).

Downstream process after flocculation should also be considered. Membrane filtration is a conventional method for separation of floc and clarified juice. However, disadvantage of this method is membrane fouling which depreciates permeate flux over time. Clarification method based on flotation has gained attention in beverage industry in recent years. Gas bubbles, e.g. nitrogen, hydrogen or oxygen, are passed into the juice and made floc or suspended particle float to the surface. Electroflotation (EF) is a novel flotation method based on application of electrodes to produce homogeneous bubbles. EF treatment reduced 26-47% of tannins in apple juice. If the juice was flocculated with gelatin and the floc was then separated with EF, tannins content could be further reduced (Araya-Farias et al., 2008).

Physical methods

Physical methods, namely microfiltration (MF) and ultrafiltration (UF), could also reduce tannins in fruit juice to some extent. MF and UF are membrane technologies used for clarification of fruit juice and other beverages. The difference between these methods was pore size of the membrane, 0.01-0.1 μm for UF and 0.1-10 μm for MF. For the removal of tannins in fruit juice, molecular weight cut-off (MWCO) of the membrane should not more than 25,000 Da (Echavarria et al., 2011). Application of MF and UF reduced 40.34% and 47.90% tannins from the apple juice, respectively. Further reduction of tannins could be obtained by using clarifying agents, followed by membrane filtration. Apple juice pretreated with 0.2% PVPP and filtered by MF and UF had 58-59% reduction of tannins, which was more effective than using bentonite, activated carbon, pectinase and amylase in combination with membrane filtration (Youn et al., 2004).

Pore size of the membrane is the main parameter determining reduction of tannins from fruit juice. UF was more effective than MF in removal of total punicalagins from pomegranate juice, which provided 43.1% and 34.6% reduction of the compounds, respectively. Ellagic acid was also reduced by UF (94.1%) and MF (65.4%). However, punicalins were not significantly removed by both filtration methods (Valero et al., 2014).

Thermal processing was a commonly used physical method in food preservation (Yousef and Balasubramaniam, 2013). Simple heat treatment could also be used to reduce tannins in fruit juice. Immersion of cashew apple in hot water for 20 min before juice pressing reduced 96.20% of tannins due to degradation of hydrolysable tannins at high temperature. This treatment was more effective in tannins reduction compared to treatment of cashew apple with gelatin, salt and sodium carbonate. However, astringency scores were not significantly different between gelatin-treated and hot water-treated juices (Emelike and Ebere, 2016).

Enzymatic methods

Tannase (tannin acyl hydrolase; EC 3.1.1.20) is an enzyme that hydrolyses ester bond of hydrolysable tannins, e.g. gallotannins and ellagitannins, and releases gallic acid and glucose (Beniwal et al., 2013). Tannase can be produced by fungi in the genus *Aspergillus*, *Penicillium* and *Rhizopus* and the bacterium *Bacillus*. Source of enzyme and its application in fruit juices are shown in Table 3.

Table 3. Source of tannase and its application in the decrease of tannins from fruit juices.

Tannase source	Optimal condition	Tannin mitigation efficiency	References
Aspergillus niger	Immobilized enzyme tannase 36.6 U/mL, pH 5.4, 40°C, 150 rpm,180 min	73.6% tannins in myrobalan juice	Srivastava and Kar (2010)
	Soluble enzyme enzyme 36.6 U/mL, 37°C, 150 rpm, 180 min	45.2% tannins in myrobalan juice	
	2% tannase (2.97 U/mL), 37°C, 60 min	45.2% tannins in guava juice	Sharma et al. (2014)
Aspergillus foetidus and *Rhizopus oryzae*	1 mL tannase (35.6 U/mL) per 10 mL juice, 37°C, 120 min 35.6 U/mL tannase with 0.1% gelatin at 1:1 ratio, 90 min	25% tannins in pomegranate juice 49% tannins in pomegranate juice	Rout and Banerjee (2006)
Penicillium montonanse	2 mL tannase (41.54 U/mL) per 10 mL juice, 37°C, 150 rpm, 120 min	46% tannins in grape juice	Lima et al. (2014)
Bacillus subtilis	134 U tannase/10 mL juice, 40°C, 120 rpm, 120 min	60% tannins in jamun juice, 51% tannins in cashew apple juice	Jana et al. (2015)

Addition of tannase into fruit juice could decrease tannins concentration which in turn reduced the bitterness of the juice. The enzyme could be added directly into the juice or immobilized by different media. Enzyme immobilization in the form of alginate bead could retain 93.6% of the enzyme. Immobilized enzyme had higher efficiency in the decrease of tannins in myrobalan juice (73.6%) compared to soluble enzyme (45.2%). Moreover, immobilized enzyme could retain more than 99% of its activity until the third cycle and more than 84% until the 6^{th} cycle of utilization (Srivastava and Kar, 2010).

A combination of tannase with gelatin addition improved tannin reduction. Utilization of tannase from *A. foetidus* and *Rhizopus oryzae* mitigated tannin content in pomegranate juice at 25% after 120 min while application of tannase with gelatin solution could decrease tannin content at 49% after 90 min (Rout and Banerjee, 2006). Tannase could lower tannin content and removed some haze of the juice. However, high clarity was obtained by further step of ultrafiltration or using clarifying agents (Dedehou et al., 2015).

CONCLUSION

Tannins are found in many fruit juices at varying concentration. Many methods were investigated for tannins removal from fruit juices. Chemical methods were based on clarifying agents included polysaccharides, proteins and synthetic polymers. Downstream processing, especially the separation of floc, should be considered if clarifying agents were used. Physical methods included heat treatment and membrane filtration. Enzymatic method was based on application of tannase to hydrolyse tannins into soluble compounds. Tannins removal efficacy of each method depended on type, parameters and fruit juices.

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