Application of Non Thermal Clarification in Fruit Juice processing - A Review

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Abstract

Juice and juice products represent a very important segment of the total processed fruit industry. Juice clarification is very important process of the juice production industry as it enhances the acceptability of the product. In clarification process semistable emulsion of colloidal plant carbohydrates that support the insoluble cloud material of a freshly pressed juice is broken such that the viscosity is dropped and the opacity of the cloudy juice is changed to an open splotchy look. For clear juices, complete depectinization by addition of enzymes, fine filtration, or high speed centrifugation will be required to achieve visual clarity. Now a days a number of methods are used for clarification of juice i.e. enzymatic clarification, ultrafiltration, centrifugation, earth filtration and cross flow membrane filtration. Enzymatic treatment for juice extraction is most commonly used now a days. Enzymes are an integral component of modern fruit juice manufacturing and are highly suitable for optimizing processes. Their main purposes are: increase extraction of juice from raw material, increase processing efficiency (pressing, solid settling or removal), and generate a final product that is clear and visually attractive. Nonenzymatic clarification involves breaking the emulsion by other means, the most common of which is heat. Other techniques include addition of gelatin, casein, and tannic acid–protein combinations. For juice clarification, ultrafiltration and microfiltration are now commonly used, representing membranes with pore sizes from 10,000 MWCO to 0.6 µm. Membrane filtration processes include reverse osmosis, nanofiltration, ultrafiltration, and microfiltration. Advantages of membrane filtration over traditional clarification methods include reduced processing time, increased juice yield, elimination of filter aid and filter presses, better product quality, and reduced enzyme usage. We can conclude from the technical literature that use of the enzymes i.e. Cellulases, pectinases, combination of these enzymes and some non enzyme process can give better quality in terms of clarity of the fruit juice.

Keywords: Enzymatic clarification, juice extraction, pectinase, clarity, membrane, ultrafiltration

Fruits and vegetables are important sources of essential dietary nutrients such as vitamins, minerals and fiber. Since the moisture content of fresh fruits and vegetables is more than 80%, they are classified as highly perishable commodities. The world fruit production is about 609,213,509 metric ton in 2010 (FAO STAT, 2010-11). In India, out of the total production of fruits and vegetables, nearly 76 percent is consumed in fresh form, while wastage and losses account for 20-22 percent. Only 4 percent of fruit production are being processed (Indian Horticulture Database, 2013).The production of fruit and vegetable juices is important both from the human health and commercial standpoints. The availability of nutritious components from fruits and vegetables to a wide range of consumers is thus facilitated throughout the year by the marketing of their juices. The production of fruit and vegetable juices requires methods for extraction, clarification and stabilization (Bhat, 2000).Clarification is a process by which the semistable emulsion of colloidal plant carbohydrates thatsupport the insoluble cloud material of a freshly pressed juice is "broken" such that the viscosityis dropped and the opacity of the cloudy juice is changed to an open splotchy look. This can beaccomplished in one of two general ways: enzymatically and non-enzymatically (Kilara and Van Buren, 1989).

Nonenzymatic clarification involves breaking the emulsion by other means, the most common of which is heat. Other techniques include addition of gelatin, casein, and tannic acid–protein combinations (Kilara and Van Buren, 1989). Additionally, the uses of honey and combined honey-pectinase treatments have been found to be effective clarification agents. It is believed that the proteinaceous component of honey is responsible for a synergistic effect when honey and pectinase are used in combination (McLellan *et al*., 1985).

Fruit contains pectin and other polysaccharides so it may lead to fouling during filtration through membrane. Enzymatic treatment leads to degradation of pectin. Enzymatically clarified juice resulted in viscosity reduction and cluster formation, which facilitates separation through centrifugation or filtration. As a result, the juice presents higher clarity, as well as more concentrated flavor and colour (Abdullah *et al*., 2007).

During the early 1930s, when fruit industries began to produce juice, the yields were low, and many difficulties were encountered in filtering the juice to an acceptable clarity (Uhlig, 1998). Subsequently, research on industrially suitable pectinases, cellulases and hemicellulases from food-grade micro-organisms (*Aspergillus niger* and *Trichoderma* sp.), together with increased knowledge on fruit components, helped to overcome these difficulties (Grassin and Fauquembergue, 1996a).Enzymatic treatment for juice extraction and clarification is most commonly used now a days. Enzymatic hydrolysis of the cell walls increases the extraction yield, reducing sugars, soluble dry matter content and galacturonic acid content and titrable acidity of the products (Joshi *et al.,* 1991). The resultant pulp has a lower viscosity and the quantity of waste pomace is reduced (Dorreich, 1996). Enzymatic degradation of the biomaterial depends upon the type of enzyme, incubation time, incubation temperature, enzyme concentration, agitation, pH and use of different enzyme combinations (Baumann, 1981).

Currently, pectinases, cellulases and hemicellulases collectively called macerating enzymes are used for improvement in pressing, extraction and clarification of fruit and vegetable juices (Galante *et al.*, 1998b). In addition, α-amylase and amyloglucosidase, active at acidic pH, were used to process starch containing fruits, especially apples harvested during the early stages in order to prevent haze formation (Grassin and Fauquembergue, 1996a; Uhlig, 1998).

Enzymatic clarification of juices

Fruit juices are naturally cloudy, yet in different degrees, especially due to presence of polysaccharides (pectin, cellulose, hemicelluloses, lignin and starch), proteins, tannins and metals (Vaillant *et al*., 2001). As the juice clear appearance is a determinant factor for consumers, the fruit juice industry has been investing in methods that optimize this feature (Tribess and Tadini, 2006). The high concentration of pectin leads to colloid formation, which constitutes one of the main problems during the processing of clear fruit juices. However, although the suspended pulp particles can be removed through filtration, the presence of pectin may make this method difficult (Sulaiman *et al*., 1998). The depectinisation of fruit juices through the use of pectinases has been presented as an efficient alternative to reduce turbidity, in many studies (Kashyap *et al*., 2001; Landbo *et al.,* 2007). Pectinases degrade pectin hence resulting in viscosity reduction and cluster formation, which facilitates separation through centrifugation or filtration. As a result, the juice presents higher clarity, as well as more concentrated flavour and colour (Abdullah *et al*., 2007; Kaur *et al*., 2004). Pectinase enzymes used in grape juice macerate increased the juice clarity and filterability by 100% according to Brown and Ough (1981). For clarified fruit juices, a juice that has an unstable cloud or whose turbidity is considered ‘‘muddy’’ is unacceptable to be marketed as clear juices (Floribeth *et al*., 1981).

Enzymatic treatment leads to increase the clarity of juice. Juice clarity can be determined in terms of absorbance and transmittance at 660 nm using UV visible spectrophotometer.Increase in enzymatic concentration increase the rate of clarification by exposing part of the positively charged protein beneath thus reducing electrostatic repulsion between cloud particles which caused these particles to aggregate into larger particles and eventually settled out (Sin *et al.,* 2006). Clarity showed the lowest absorbance values at highest enzyme concentration, where lower absorbance indicates a clearer juice is being produced. It was also observed that the absorbance values decreased with increasing incubation time at fixed temperature. In general, the time required to obtain a clear juice is inversely proportional to the concentration of enzyme used at constant temperature (Kilara, 1982). At the lowest level of temperature, the clarity of banana juice was found to increase rapidly at the beginning but with a slower rate towards the end, with an increase in enzyme concentration. The temperature increases the rate of enzymatic reactions, hence the rate of clarification, as long as the temperature is below denaturation temperature for the enzyme. A similar behaviour for the clarity was observed for the changes in incubation time in case of banana (Lee *et al.,* 2006). The clarity of centrifuged litchi juice increased with an increase in enzyme concentration.

Among the different concentrations used for the optimization of pectinase, the litchi pulp added with 500 ppm of pectinase resulted in maximum transmittance of 80% at 660 nm. The clarity of mosambi juice decreases with time up to 90 min and increases thereafter. Similarly at constant time and temperature, the clarity decreases with enzyme concentration and remains constant and increases thereafter. From both the observations, it is evident that there exists an optimum enzyme concentration and time for the juice clarity (Rai *et al.,* 2003).Table 1 represents the Optimized conditions for clarification of various fruit juices using Pectinase.

Table 1: Optimized conditions for clarification of various fruit juices using Pectinase

Fruit/ Vegetable	Incubation time[a]	Incubation temperature[b]	Enzyme concentration[c]	Clarity[d]	Referance
Banana (*Musa sapientum* cv Berangan)	80	43.2	0.084%	0.009 Abs	Lee *et al.,*(2006)
Carambola (*Carambola Averrhoa* L.)	20	30	0.10%	0.019 Abs	Abdullah *et al.,(*2007)
White Grape *(Vitis vinifera)*	30	27-30	0.048%	0.031 Abs	Sreenath and Santhanam, (1992)
Sapodilla (*Achras sapota*)	120	40	0.1%	0.023 Abs	Sin *et al.,*(2006)
Mosambi (*Citrus sinensis* (L.) Osbeck)	99.27	41.89	0.0004 w/v%	83.97% T	Rai *et al.,*(2003)
Lichi (*Litchi chinensis* L)	120	40	500ppm	80% T	Vijayanand *et al., (*2010)

[a] Incubation time in minutes, [b] Incubation temperature in ^{0}C, [c] Enzyme concentrations in [a] w/v% : Weight per volume, ppm: parts per million,% : Percentage on pulp basis, [d]Clarity in Abs: Absorbance, T: Transmittance.

Table 2: Effect of Incubation time, Temperature and Enzymatic concentration on Turbidity at optimized condition using enzymatic treatments

Fruit/ Vegetable	Enzymes[a]	Incubation time[b]	Incubation Temperature[c]	Enzyme Concentration[d]	Turbidity[e]	Referances
Elderberry *(Sambucus nigra L)*	Pectinase	50	60	0.34 mg/100gm	154 FNU	Landbo *et al., (*2007)
Banana (*Musa sapientum* cv Berangan)	Pectinase	80	43.2	0.084%	3.62 NTU	Lee *et al.,*(2006)
Sapodilla (*Achras sapota*)	Pectinase	120	40	0.1%	16.44 NTU	Sin *et al.,*(2006)
Carambola (*Carambola Averrhoa* L.)	Pectinase	20	30	0.10%	20.30 NTU	Abdullah *et al., (*2007)
Date (*Phoenix dactylifera* L.) Variety *Deglet Nour*	Pectinase and cellulase	120	50	50U pectinase / 5U cellulase	186.45 NTU	Abbes *et al., (*2011)
Plum	PME and PG	120	50	0.05g/kg (2:1)	590 NTU	Mieszczakowska-Frac (2012)

[a] PME:Pectin Methyl Esterase ; PG: Polygalacturonase, [b] Incubation time in minutes, [c] Incubation temperature in ^{0}C, [d] Enzyme concentrations in% : Percentage on pulp basis, mg/100g: milligram per 100 gram of fruit/pulp. [e] Turbidity in FNU: Formazin Nephelometric Units NTU: Nephelometric Turbidity Units.

In case of elderberry it was observed very clearly from the turbidity data that ranged from 120–161 FNU with enzyme addition, and thus on average turbidity was 30% lower than those of samples produced without enzyme addition that had turbidity levels ranging between 191–212 FNU (Landbo *et al.,* 2007). Since the turbidity in the juices may be due to pectin and other plant cell wall substances released during the enzymatic prepress maceration, it seems logic that elevated turbidities may transiently result during enzyme catalyzed cell wall degradation, which can partly explain the positive effect coefficient of the enzyme dosage on the turbidity. Turbidity in fruit juices can be a positive or a negative attribute depending on the expectation of the consumers (Hutchings, 1999). In the case of orange and tomato juices, the juices are usually cloudy and have colloidal suspensions. However, this cloud is desirable and acceptable by the consumers. Turbidity of juice at optimized condition for enzymatic treatment of various fruits and vegetable shown in Table 2. Increase in enzyme concentration and incubation time might decrease turbidity. Pectin was the main cause of turbidity (Grassin and Fauquembergue, 1996a). As the clarification process took place, the amount of pectin in the juices decreased, therefore reducing the turbidity of the juices (Alvarez *et al*., 1998).

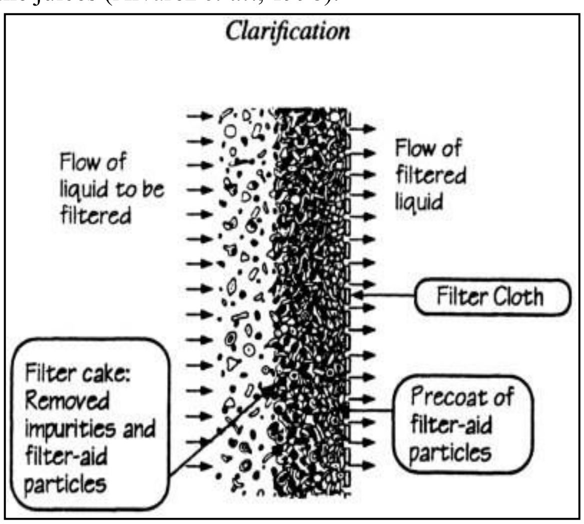

Fig.1: Diatomaceous earth (DE) filtration

The use of filter aids in filtration operations is one of the most traditional techniques to achieve a clarified juice. It is not as popular as in the past due to safety restrictions in handling the material and cost of waste disposal. It involves a three-step operation in element (paper, cloth, or screen), and then filtration is conducted using the continuous addition of filter aid to the juice.

Non-enzymatic clarification of juices

Nonenzymatic clarification involves breaking the emulsion by other means, the most common of which is heat (Smock and Neubert, 1950). Other techniques include addition of gelatin, casein, and tannic acid–protein combinations (Kilara and Van Buren, 1989). Additionally, the use of honey and combined honey–pectinase treatments have been found to be effective clarification agents (Kime, 1982). It is believed that the proteinaceous component of honey is responsible for a synergistic effect when honey and pectinase are used in combination (McLellan *et al.,* 1985).A high-solids stream can be partially clarified using decanters and finishers. Both pieces of equipment operate on the same principle with a spinning central cone, drum, and set of paddles pushing the juice through a screen of some type. The unit is typically mounted horizontally, and throughput is relatively high.

A very common unit used for removal of juice-insoluble solids is the centrifuge. A centrifuge places the juice under high gravimetric force induced by centrifugal action. This is effective in producing a juice that is opaque but free of visible solids. Modern centrifuges are highly automated and run continuously with timed solids ejection. Centrifuges with a high force of gravity are capable of producing clear juice under optimized conditions. Operation of the centrifuge must be done in a way that minimizes the introduction of excessive oxygen in the product.

which a precoat of filter aid is built up on a filtration

Membrane filtration in the fruit juice industry has grown from a novel approach into a reliable and economically attractive standard unit operation. Membrane processing has been used for concentrating, clarifying/fractionating and/or purifying fruit juices and for enhancing process efficiency and profitability. This technique has provided an excellent alternative for manufacturing high quality fruit juices under more hygienic conditions. Microfiltration (MF) and ultrafiltration (UF) are cross flow filtration processes, which retain particles in a wide range from 10,000 Da to 500,000 Da. MF and UF have been widely used for clarification of fruit juices such as pear and apple. However, studies suggest that UF juices are more susceptible to post bottling haze (PBH) than traditionally clarified juices (Nagel and Schobinger, 1985). Membrane filtration processes include reverse osmosis, nano filtration, ultra filtration, and microfiltration. Advantages of membrane filtration over traditional clarification methods include reduced processing time, increased juice yield, elimination of filter aid and filter presses, better product quality, and reduced enzyme usage (Cheryan, 1998).

In addition, in recent years there has been an increasing demand for natural, free-additive products, motivating the juice processing industry to develop and employ free-additive clarification techniques. Ultra filtration is the mostly widespread free additive clarification method due to many advantages, including higher juice yield, cost reduction and high quality products (Gokmen *et al.,* 2001). Ultra filtration (UF) is a membrane filtration process that separates particles based on molecular weight (Milnes, 1984; Cheryan, 1986). The process uses a cross-flow method of operation, as opposed to depth filtration used in DE filtration. It can be utilized to clarify apple juice, as well as other fruit juices (Heatherbell *et al.,* 1977). It has been used commercially for this purpose in several plants in Europe, the U.S., and South Africa (Cheryan, 1998; O'Sullivan *et al.,*(1988); Möslang, 1984). Baumann *et al.,* (1986) reported that apple juice ultra filtered with membranes of pore sizes between 10 kDa and 0.22 mm presented large variations only in color. Veleirinho *et al.,* (2008) reported that apple juice clarification was achieved by adding 0.5 g/L of gelatin and 2.5g/L of bentonite to the enzymatically treated juice, at 50°C for 2 h. The precipitate was removed by centrifugation (4000 rpm, 10 min) and the clear juice was stored at 4°C. In order to produce the clear juice, 150mL of enzymatically treated juice were ultrafiltered through a regenerated cellulose membrane (Millipore, cut off 100 kDa), under N_2 pressure. The clarified juice was stored until analysis at 4°C.

In a study of clarification process of pectin-containing juice using ultrafiltration it has been observe that a typical pectin rich juice, clarified by ultrafiltration (cold sterilization) can have adequate shelf life without any heat treatment or addition of preservatives (Rai and De, 2009). In Lemon juice clarification process by ultrafiltration the optimum treatment conditions were: enzyme concentration 600 U/L, time 45 min and temperature 30°C. Their application led to a 77% and 47% reduction of viscosity and turbidity, respectively. The enzymatic treatment was followed by ultrafiltration (cutoff value = 15 kDa). Analysis of the clarified juice indicated that enzyme depectinization permitted a higher permeate flux and a higher juice quality. The lemon juice obtained was clear, stable and characterized by viscosity = 0.7 m Pa s, turbidity = 0.17 NTU, clarity (*A*650nm) = 0.063 and color (*A*420nm) = 0.232. Microbiological study showed that lemon juice was free from aerobes, molds, enterobacteriaceae and coliforms and was microbiologically stable during 3 months storage (Maktouf *et al.,* 2014). Cassano *et al.,* (2007) observed that, In a membrane-based process for the clarification of the cactus pear juice, the rejection of the UF membrane towards betaxanthins was lower than the rejection measured for betacyanins. Only a 4% loss in the total antioxidant activity was found in the UF permeate with respect to the fresh juice. In osmotic distillation process the clarified juice with a TSS content of about 11°Brix was concentrated up to 61 °Brix. An initial evaporation flux of 1.16 kg/m^2 h was obtained using a calcium chloride dehydrate solution at 60 w/w% as stripper.

Conclusion

Now a days a number of methods are used for clarification of juice i.e. enzymatic clarification, ultrafiltration, centrifugation, earth filtration and cross flow membrane filtration. Enzymatic treatment for juice extraction is most commonly used now a days. Enzymes are an integral component of modern fruit juice manufacturing and are highly suitable for optimizing processes. Advantages of membrane filtration over traditional clarification methods include reduced processing time, increased juice yield, elimination of filter aid and filter presses, better product quality, and reduced enzyme usage. We can conclude from the technical literature that use of the enzymes i.e. Cellulases, pectinases, combination of these enzymes and some non enzyme process can give better quality in terms of clarity of the fruit juice.

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