***Review Article***

# Enhancing Clarity and Quality: The Role of Clarifying Agents in Horticulture Foods and Formulations

Comment [U1]: Font 18


## Abstract

Comment [U2]: capital

This abstract explores the multifaceted role of clarifying agents in the realm of horticultural foods and their vital functions within formulations. Clarifying agents serve as essential tools in the food industry, aiding in the removal of impurities, cloudiness, and undesirable particles from fruit juices, wines, and various horticultural products. These agents are instrumental in enhancing the visual appeal and quality of these products, improving their marketability. The uses of clarifying agents extend beyond aesthetics; they play a crucial role in stabilizing formulations, preventing sedimentation, and preserving the natural flavors and nutritional value of horticultural foods. Additionally, clarifying agents contribute to product consistency and shelf-life extension, ensuring a consistent and appealing product for consumers. This abstract delves into the diverse types of clarifying agents employed in horticulture and their specific functions within various formulations. It highlights their significance in maintaining product integrity and meeting consumer preferences for clear, visually appealing, and high-quality horticultural foods.


Comment [U3]: the abstract does not comply with the guidelines, 10 font


***Keywords:*** Clarifying agents, Nutritional quality, Food industry, Enzymes and Shelf life


Comment [U4]: italic, 10 font

## Introduction

Comment [U5]: 11 font, capital
1.It is necessary to classify the clarification material, so that the discussion is clear
2.the analysis gap has not yet emerged
3.paragraph 10 font

Clarifying agents are substances that are commonly introduced during or near the end of the brewing process for beverages such as wine, beer, and nonalcoholic juices. These agents serve the purpose of removing organic compounds to enhance clarity, adjust flavor, or influence aroma. The process of clarifying fruit juices, such as apple and grape juice, involves the removal of suspended particles to improve clarity and ensure colloidal stability. To achieve this, an initial enzymatic treatment involving pectinase is sometimes employed for the clarification of fruit juices (Ramezani et al., 2020). This enzymatic treatment helps break down pectin, a key step in the process. The compounds that are typically removed during this clarification process may include sulfides, proteins, polyphenols, benzenoids, or copper ions. Unless these removed compounds create a stable sediment in the final container, the spent fining agents are usually discarded along with the captured target compounds. Various substances can be utilized as fining agents, with some traditional options still in use today, such as egg whites, blood, milk, isinglass, and Irish moss, which are favored by certain producers.However, more contemporary substances have gained wider acceptance and usage in recent years. These modern options encompass a wide range of materials, including bentonite, gelatin, casein, carrageenan, alginate, diatomaceous earth, pectinase, pectolyase, PVPP, kieselsol (colloidal silica), copper sulfate, dried albumen (egg whites), hydrated yeast, and enzyme preparations designed to degrade pectin, which may also contain other enzymes like diastase, among others. For the clarification of fruit juices, gelatin, albumen, methylcellulose, and proteolytic enzyme preparations such as papain and bromelin are highly recommended, particularly for chill-proofing beer. Gelatin treatment, for example, has proven effective in addressing haze formation observed in clarified apple juice during storage. Bentonite is commonly employed in the clarification process to extract proteins from juices, whereas kieselsol is used to eliminate any surplus gelatin that has been added to the juice to target the removal of polyphenols with a molecular weight exceeding 500 Dalton (Dereli et al., 2023). In the fruit juice industry, clarification plays a pivotal role in enhancing the quality of the final product. This critical step aims to eliminate turbidity, undesirable odors, unappealing colors, unwanted flavors, and any gas-related issues from the fruit juice. The clarification process is typically accomplished through various methods, including microfiltration, enzymatic treatment, or the utilization of clarifying agents such as activated carbon, gelatin, bentonite, silica gel, and casein (Venkatachalapathy et al., 2020).

Clarification involves transforming the semi-stable emulsion of colloidal plant carbohydrates that supports the insoluble cloud material present in freshly pressed juice. This transformation results in a reduction in viscosity and a shift in the cloudy juice's opacity to a more open and splotchy appearance. Ultimately, this process leads to a clearer and more visually appealing fruit juice product. Enzymes play a pivotal role in the transformation of colloids, leading to the production of crystal-clear juices that possess the appearance, stability, mouthfeel, taste, and texture characteristics preferred by consumers. Several key enzymes are employed for clarification purposes, including pectinase, amylase, cellulase, hemicellulase, xylanase, glucanase, glucosidase, amyloglucosidase, arabanase, and others. These enzymes collectively contribute to the clarification of fruit juices, and this can be achieved through either enzymatic or non-enzymatic methods (Kilara and Van Buren, 1989). For the removal of phenolic compounds in juices, both protein-based clarifying agents like gelatin, casein, and albumin, as well as carbohydrate-based clarifying agents such as chitosan and xanthan gum, can be employed. Gelatin is particularly effective at eliminating high molecular weight phenolics due to its positive charges, especially at the acidic pH levels commonly found in juices, including RGJ (pH 3.3–3.9). Similarly, chitosan is adept at removing phenolics like tannins, cinnamic acids, and their derivatives, as it carries strong positive charges under acidic juice pH conditions. It's worth noting that different clarification agents can have varying effects on individual phenolic compounds, although they tend to be effective at removing catechin and epicatechin. For instance, casein and albumin are effective in removing catechin and epicatechin, while gelatin efficiently eliminates catechin(Dıblan and Ozkhan, 2021). This diversity of clarifying agents and enzymes provides the juice industry with a range of tools to tailor the clarification process to specific juice characteristics and consumer preferences.

**Methods of clarification of juices**

**Straining or screening:** It is an essential step in the clarification of fruit juices. Unclarified fruit juices often contain various suspended particles, including broken fruit tissue, seeds, skin fragments, pectic substances, and proteins in colloidal suspension. These particles can negatively impact the quality of the juice. In small-scale industries and manual processes, straining or screening is typically carried out using muslin cloth or stainless-steel mesh sieves. The purpose of this step is to remove coarse particles from the juice. Muslin cloth or fine stainless-steel mesh sieves are used to physically filter out these undesired components, leaving behind a smoother and clearer juice.

**Comment [U6]:** 1.materials and methods are not clear, it seems mixed with the results of the discussion
2. Materials and methods must comply with the guidelines
3. paragraph 10 font
4.separated by results and discussion

This manual method is effective in preliminary clarification and helps improve the overall quality and appearance of the fruit juice.

**Finishing:** It is a crucial step in the processing of citrus juices, particularly for separating cloudy but otherwise clean juice from pulp, rag, and seeds. This step ensures that the final juice product is clear and pulp-free. In the finishing process, a machine called a finisher is used. The finisher works by employing a rotating auger inside a cylindrical screen. The primary function of the finisher is to separate the pulpy matter from the juice. The juice passes through the screen while the pulp, rag, and seeds are retained and separated. The size of the holes in the screen can vary and is typically in the range of approximately 0.020 to 0.030 inches in diameter. The choice of hole size depends on the condition and softness of the fruit being processed. The effectiveness of the finishing process is typically assessed by measuring the pulp content in the orange fruit juice. A lower pulp content indicates a more successful finishing process, resulting in a clearer and more desirable citrus juice product.

**Decantation:** It is indeed one of the simplest methods of clarification used in various liquid processing industries, including fruit juice production. It involves allowing a mixture of liquid and solids to stand undisturbed so that the solid particles settle at the bottom, and then the clear liquid is carefully poured or siphoned off from the top. In the context of fruit juice production, decantation can be used to separate the juice from any solid particles or sediment that may be present. To facilitate this process, it's often beneficial to keep the juice at a low temperature for an extended period. Lower temperatures can promote the settling of solids and enhance the clarification process. Decantation is a relatively gentle method of clarification and is suitable for juices with relatively low levels of suspended solids. It's particularly useful when a more natural and less processed juice is desired, as it avoids the use of chemicals or mechanical processes that may alter the juice's characteristics. However, for juices with higher levels of suspended particles, other more efficient clarification methods, such as filtration or enzymatic treatment, may be necessary to achieve the desired clarity.

**Centrifugation:** It stands as a highly efficient method within the fruit juice industry for achieving juice clarification by effectively separating solid particles. The process hinges on the principles of centrifugal force, which leverages the varying densities of the components present in the juice. When juice, along with its suspended solids, enters a specialized centrifuge equipped with a basket or disc configuration, it undergoes rapid spinning. This high-speed rotation generates a centrifugal force that pushes heavier particles, such as solids and larger substances, towards the centrifuge's walls, while concurrently directing the clarified juice towards the center. This centrifugal action results in the efficient separation of the clarified juice from undesirable solids and particles. This technique has proven to be exceptionally effective, as demonstrated by its ability to reduce immediate turbidity in freshly pressed juice by an impressive 95% when subjected to centrifugation at 10,000g for 15 minutes. Moreover, this process has the added advantage of minimizing haze development in the juice during cold storage, all while having a minimal impact on the juice's overall composition and quality, including its total phenol levels (Landbo et al., 2006).

**Enzymes:** In the realm of commercial-scale juice production, the enzymatic approach to juice clarification has garnered broad acceptance. This method involves the application of key enzymes such as cellulases, pectinases, hemicellulases, laccases, and amylases in large-scale juice clarification processes. In commercial operations, it's common to use a combination of pectic enzymes in conjunction with laccase or cellulase to achieve effective juice clarification (Narnoliya et al., 2020). In freshly extracted fruit juice, colloidal suspensions are formed due to the presence of plant carbohydrates like pectin, starch, and proteins. To enhance juice recovery and attain clarification, pectinase enzymes are frequently employed. These enzymes function by breaking down pectin into a soluble form, releasing suspended particles that subsequently settle down, resulting in clear juice. Additionally, proteolytic enzymes and starch-liquefying enzymes, such as amylases, are deployed to eliminate proteins and starch from fruit juices. It's worth noting that Pectinol is particularly effective in acidic juices. The duration required for fruit juice clarification varies with temperature. Generally, fruit juices can be clarified in approximately 1-2 hours at temperatures ranging from 40-50°C, but this duration extends to 20 hours at 20°C. The effectiveness of clarification and the reduction of haze can vary when treated separately with five different acidic proteases. However, it has been consistently observed that one of these protease preparations, Enzeco, which is derived from *Aspergillusniger*, tends to yield the most favorable results (Landbo et al., 2006).

**Physical fining:** These agents, such as kaolin, diatomaceous earth, Spanish clay, and bentonite (also known as China clay), are known for their mechanical actions and are commonly referred to as filter aids. In the juice processing industry, these substances play a crucial role in achieving clarity and removing unwanted particles. Typically, a small percentage of these earth-based fining agents, usually ranging from 0.5 to 1 percent, is mixed with the fruit juice. The mixture is then passed through a filter press. This process effectively separates solid particles from the juice, contributing to its clarity. Another advanced technique known as ultrafiltration is also employed in juice processing. Ultrafiltration operates based on molecular weight, enabling the separation of particles with differing molecular sizes. One notable advantage of ultrafiltration is its ability to retain a higher proportion of the nutrients present in the juice, as compared to some other clarification methods. However, it's important to note that before ultrafiltration, it is often necessary to enzymatically degrade pectin. This enzymatic treatment helps reduce the viscosity of the juice, making it more amenable to the ultrafiltration process and ensuring a satisfactory result in terms of juice clarity and quality (Gokmen et al., 2001).

**Chemical finings:** Gelatin and casein are both commonly used in the clarification of fruit juices, and they serve a dual role in this process. First, they work to neutralize the electrically charged particles present in the juice. Second, they form insoluble precipitates when they interact with various constituents of the juice. Specifically, gelatin binds with tannins, while casein interacts with the acidic components of the juice. However, it's important to note that an excessive amount of gelatin can lead to cloudiness in the juice. To achieve effective clarification, the gelatin solution is mixed with the juice and allowed to stand for a period of 18 to 24 hours. During this time, the precipitated matter clots together and settles at the bottom. Subsequently, the clarified juice is separated and extracted from the settled particles. In the process of juice clarification, albumin, which is derived from egg whites, can also serve as an effective clarifying agent. Another method for removing proteins from juice or wine involves the introduction of proteolytic enzymes or the use of bentonite. Among these methods, bentonite is widely recognized as the most commonly used clarification aid for protein removal. Bentonite plays a critical role in preventing haze formation, especially during the storage of grape juice or wine (Dıblan and Ozkhan, 2021).

**Mechanism**

Clarifying agents operate based on the fundamental principle that all particles responsible for cloudiness or haze in wine or beer carry an electrical charge. Gelatin, for instance, possesses a positive charge, allowing it to attract negatively charged materials present in the beverage. When gelatin binds to these negatively charged materials, the combined weight of the particles increases, leading to their settling or precipitation. In practice, it's often necessary to add fining agents with different charges sequentially to the wine to effectively remove materials of various charges present in the liquid. Particle agglomeration, a key aspect of the clarification process, involves the formation of groupings in a liquid suspension. This mechanism results in the functional destabilization of colloidal systems. In this process, dispersed particles within the liquid phase naturally come together and form irregular clusters known as flocs or agglomerates. This phenomenon is commonly referred to as coagulation or flocculation. As a result of these interactions, the suspension becomes less stable and allows for easier removal of unwanted particles. To initiate particle aggregation, salts or other chemicals known as coagulants or flocculants are introduced into the suspension. These substances play a crucial role in facilitating the clumping together of particles, aiding in the clarification process (Erkan-Koç et al., 2015).

**Enzymatic clarification of juices**

Fruit juices naturally possess varying degrees of cloudiness, primarily attributed to the presence of specific components, including polysaccharides like pectin, cellulose, hemicelluloses, lignin, and starch, along with proteins, tannins, and metals, as noted by Vaillant et al. (2001). Recognizing that the visual clarity of juice is a critical factor for consumers, the fruit juice industry has dedicated efforts to enhance this aspect, as emphasized by Tribess and Tadini (2006). One of the primary challenges encountered in achieving clear fruit juices is the elevated concentration of pectin, which gives rise to the formation of colloidal particles. This presents a significant obstacle, especially when attempting to remove suspended pulp particles through filtration, as elucidated by Sulaiman et al. (1998). To effectively address this issue, the utilization of pectinases has emerged as an efficient alternative for serpentinizing fruit juices and reducing turbidity, as substantiated by various studies, including those conducted by Kashyap et al. (2001) and Landbo et al. (2007). Pectinases play a crucial role in breaking down pectin, resulting in a reduction in viscosity and the formation of clusters in fruit juice. This transformation facilitates the separation process through methods like centrifugation or filtration. Consequently, the juice achieves higher clarity and exhibits a more concentrated flavor and color profile (Abdullah et al., 2007; Kaur et al., 2004).In the context of grape juice maceration, the use of pectinase enzymes was found to enhance juice clarity and filterability by a significant 100%, as reported by Brown and Ough (1981) In the production of clarified fruit juices, any juice displaying unstable cloudiness or being perceived as 'muddy' in terms of turbidity is considered unsuitable for marketing as clear juices, as highlighted in the findings of Floribeth et al. (1981). Enzymatic treatment emerges as a pivotal factor contributing to the enhancement of juice clarity.

The degree of clarity can be quantified through the measurement of parameters such as absorbance and transmittance at 660 nm, utilizing a UV-visible spectrophotometer. Higher concentrations of enzymatic agents expedite the clarification process by exposing a portion of the positively charged protein underneath, subsequently reducing the electrostatic repulsion between cloud particles. This results in improved juice clarity. This leads to the aggregation of these particles into larger formations, which eventually settle out of the juice (Sin et al., 2006). At lower temperatures, it was observed that the clarity of banana juice improved significantly, particularly at the start of the process. However, as the concentration of enzymes increased, the rate of improvement in clarity became slower, especially as the process neared its completion.

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

| S.N. | Fruit | Incubation time | Incubation temperature | Enzyme concentration | Clarity | References |
|---|---|---|---|---|---|---|
| 1 | Lichi (*Litchi chinensis* L) | 120 | 40 | 500ppm | 80% T | Vijayanand et al. (2010) |
| 2 | Mosambi (*Citrus sinensis* (L.) | 99.27 | 41.89 | 0.0004 w/v% | 83.97 % T | Rai et al.(2003) |
| 3 | White Grape (*Vitis vinifera*) | 30 | 27-30 | 0.048% | 0.031 Abs | Sreenath and Santhanam, (1992) |
| 4 | Banana (*Musa sapientum* ) | 80 | 43.2 | 0.084% | 0.009 Abs | Lee et al.(2006) |
| 5 | Sapodilla (*Achras sapota*) | 120 | 40 | 0.1% | 0.023 Abs | Sin et al.(2006) |
| 6 | Carambola (*Averrhoa carambola*) | 20 | 30 | 0.10% | 0.019 Abs | Abdullah et al.(2007) |

Incubation time in minutes, Incubation temperature in ℃, Enzyme concentrations in a w/v%: Weight per volume, ppm: parts per million%: Percentage on pulp basis, Clarity in Abs: Absorbance, T: Transmittance.

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

**Comment [U7]:** not according to the guideline/template

**Comment [U8]:** writing table titles according to the template/guideline

| Fruit | Enzymes | Incubation time | Incubation temperature | Enzyme concentration | Turbidity | References |
|---|---|---|---|---|---|---|
| Plum | PME and PG | 120 | 50 | 0.05/kg(2:1) | 590 NTU | Mieszczakowska-Frac (2012) |
| Carambola (*Carambola Averrhoa* L) | Pectinase | 20 | 30 | 0.10% | 20.30 NTU | Abdullah et al., (2007) |
| Sapodilla (*Achras Sapota*) | Pectinase | 120 | 40 | 0.1% | 16.44 NTU | Sin et al.(2006) |
| Banana (*Musa sapientum* cv Berangan) | Pectinase | 80 | 43.2 | 0.084% | 3.62 NTU | Lee et al. (2006) |
| Elderberry (*Sambucus nigra* L) | Pectinase | 50 | 60 | 0.34mg/100g | 154 FNU | Landbo et al. (2007) |
| Date (*Phoenix dectylifera* L.) DegletNour | Pectinases and cellulases | 120 | 50 | 50 U pectinase/ 5U cellulase | 186.45 NTU | Abbes et al. (2011) |

PME:Pectin Methyl Esterase ; PG: Polygalacturonase, Incubation time in minutes, Incubation temperature in ℃, d Enzyme concentrations in% : Percentage on pulp basis, mg/100g: milligram per 100 gram of fruit/pulp. Turbidity in FNU: FormazinNephelometric Units NTU: Nephelometric Turbidity Units

**Effect of enzymatic treatment on juice yield and juice clarification**



Kashyap et al. (2001) emphasized that the production of specific juices requires enzymatic depectinization due to their elevated pectin content. This high pectin content tends to persist as dissolved colloidal residues, resulting in a dense and challenging-to-clarify juice. Enzymes added during the maceration step prove to be invaluable in simplifying the subsequent clarification, filtration, and concentration processes. In a study conducted by Granada et al. (2001), pectinolytic enzymes were employed during the cold pressing extraction of clarified blackberry juices (Rubus spp. L.) from three different cultivars: Guarani, Tupi, and Brazos. The primary objective of this approach was to enhance juice yield, and the study's findings were compared with those of juices produced without the addition of enzymes.Furthermore, Rai and De (2009) noted that the ultrafiltration clarification process proves highly effective for addressing pectin-rich juices. This method offers a valuable alternative to conventional heat treatment or the addition of preservatives to achieve an adequate shelf life through cold sterilization. In a study conducted by Bump (1989), comprehensive research was undertaken to evaluate the extraction yield and various physical, chemical, and organoleptic characteristics of clarified apple juice. The primary objective was to enhance juice extraction using three distinct treatments: the use of pectinolytic enzymes alone, the introduction of rice husk as fining agents during pressing, and a combination of both treatments. The consistent findings from the study indicated that the treatment involving pectinolytic enzymes was more effective compared to the treatment with rice husk alone.

Yamaski et al. (1964) demonstrated that the clarification of apple juice could be successfully achieved using a combination of polygalacturonase and polymethylesterase enzymes, obviating the need for additional apple contaminant enzymes. Enzymes are also pivotal in the production of both white and red grape juice, where they play a crucial role in depectinization, increasing yield, and ensuring clarification, as highlighted by Oliveira et al. (2006). In the case of pomegranate juice, achieving optimal clarification was largely contingent on the concentration of complex enzymes. Cerreti et al. (2017) graphically determined the optimum operational conditions, which were found to be a temperature range of 25-30°C for 100-110 minutes, employing an enzyme amount of 0.22-0.25 grams of protease-pectinase complex per 100 grams of juice, with a protease-to-pectinase ratio of 1:2.

**Non-enzymatic clarification of juices**

Nonenzymatic methods for juice clarification involve disrupting emulsions through various techniques, with heat being the most commonly used approach, as noted by Smock and Neubert (1950). Alternative methods for juice clarification involve the introduction of substances such as gelatin, casein, and combinations of tannic acid and proteins, as emphasized by Kilara and Van Buren (1989). Furthermore, the utilization of both honey and combined honey-pectinase treatments has exhibited effectiveness as clarification agents, as discussed by Kime (1982). It is posited that the proteinaceous component of honey contributes to a synergistic effect when honey and pectinase are employed in tandem, as suggested by McLellan et al. (1985). In the context of partial clarification for high-solids streams, the industry commonly employs decanters and finishers. These pieces of equipment operate on a shared principle, featuring a central spinning cone, drum, and a set of paddles that facilitate the passage of juice through a screen. Typically installed in a horizontal configuration, these units offer a relatively high throughput capacity. Membrane filtration has evolved from a novel concept into a dependable and cost-effective standard operation in the fruit juice industry. It serves various crucial purposes, including concentration, clarification/fractionation, and purification of fruit juices. As a result, it contributes to enhanced process efficiency and profitability. Membrane filtration presents a compelling alternative for the production of high-quality fruit juices under more hygienic conditions. Within the realm of membrane filtration, both microfiltration (MF) and ultrafiltration (UF) are cross-flow filtration methods capable of retaining particles within a wide range, encompassing sizes from 10,000 Da to 500,000 Da. These methods, MF and UF, have garnered widespread acceptance and are commonly employed in the clarification of fruit juices. Among these techniques, ultrafiltration stands out as a widely adopted method for juice clarification, offering numerous advantages such as increased juice yield, cost savings, and the production of high-quality products, as noted by Gokmen et al. (2001). The optimal conditions for juice clarification have been determined to involve a 0.05% (w/v) clay concentration at a temperature of 50°C for 2 hours, as highlighted by Mirzaaghaei et al. (2016). Regarding clarification agents, there are positively charged substances like gelatin, egg albumen, isinglas, and Sparkalloid, as well as negatively charged ones, including bentonite and Kieselsol. These agents play a crucial role in the clarification process.

**Chitosan**

The utilization of chitosan for pomegranate juice clarification has proven to be a practical and efficient method with versatility for various applications. Through the conducted analysis, it became evident that the quality of pomegranate juice is notably influenced by storage time and temperature (Li et al., 2014). Specifically, samples stored at 4°C exhibited superior preservation of quality characteristics when compared to those stored at 20°C, as elucidated by the findings of Tastan and Baysal (2015). In the context of apple juice clarification, optimal conditions were identified, encompassing a chitosan concentration of 191.6 mg/100 ml of juice, a process temperature of 20°C, and a processing time of 30 min.In the case of palmyra palm sap, the utilization of chitosan resulted in the clearest juice. To further enhance clarity and improve attributes such as color, flavor, and physical stability during storage, it is recommended to explore combinations of different types of clarifying agents, as suggested by Naknean et al. (2014).

**Casein**

Casein, whether sodium or potassium caseinate, constitutes the principal protein in milk and is derived from milk through a precipitation process involving acid. In wine clarification, caseinates play a crucial role by binding to haze particles and diminishing undesirable polyphenolic substances like tannins. When the wine's acidity interacts with alkali casein, it causes the casein to lose its counterion, reducing its solubility. This phenomenon leads to turbidity and facilitates the removal of haze particles, contributing to the overall clarification process.

**Gelatin**

These clarifying agents are derived from animal collagen and positively charged proteins. However, they tend to cause food discoloration, which is why tannins are often used in conjunction with them. For example, this combination is used in the clarification of juices like apple and cashew apple. Tropical fruit juices subjected to treatment with two different clarifying agents, namely hide buffalo gelatin (HBG) and commercial bovine gelatin (CBG), were subjected to analysis, focusing on turbidity, pH, and total solid properties. The results revealed no significant differences in water-holding capacity (WHC) and oil-holding capacity (OHC) properties between these two clarifying agents, as reported by Mulyani et al. (2021). In another study, the optimal conditions for the removal of pyrethroid residues from apple juice were determined. The most effective treatment involved the use of gelatin at a dosage of 500 mg/L, as outlined by Wongmaneepratip et al. (2023).

**Colloidal Silica and Chitosan**

This combination of colloidal silica and chitosan is a viable replacement for isinglass in white wines and gelatin in red wines. Colloidal silica effectively binds with smaller proteins, causing them to aggregate. Chitosan, sourced from shellfish and crustacean shells, acts as a comprehensive flocculating agent, efficiently removing all solids, including the larger proteins that are brought together by colloidal silica. For the clarification of red grape juice, it is recommended to use a combination of bentonite with casein and then bentonite with albumin, rather than relying solely on casein or albumin alone, as advised by (Dıblan and Ozkhan, 2021). The chitosan clarification method is well-regarded for its compatibility, complete degradation, production of low-toxicity degradation metabolites, and favorable biological properties. It finds extensive application in the clarification of fruit and vegetable juices during production. In the processing of blackberry juice, carboxymethyl chitosan has been proven effective in reducing the content of proteins and polyphenols in the juice compared to chitosan treatment alone. Additionally, it helps prevent turbidity during storage or any subsequent precipitation, as demonstrated by Li et al. (2014).

**Table 3 Non-enzymatic clarifying agents effects and uses**

| **S.N.** | **Clarifying agent** | **Nature** | **Mechanism** | **Optimum Temperature** | **Effect** | **Uses** |
|---|---|---|---|---|---|---|
| 1 | Bentonite | Protein ( montmorillonite clay) | Negative chage attract with positive charge | 10-25℃ | Loss of color red wine Increased alkalinity | Protein removal Clarification of juice |
| 2 | Gelatin | Protein & higher polyphenol | Hydrolysis of fiberous insoluble protein | 10-25℃ | Lower tannin Reduce astringency Remove colour | Positively charged fining agent |
| 3 | Enolophin 700 | Protein nature | Protein reactant Increase floc size rapid sedimentation | 10-25℃ | | Fining agent for beer and wine |
| 4 | Egg White | Higher polyphenol | Albumin protein positive charge on surface | 10-25℃ | Not suitable for white wine | Separate egg white from egg yolk |
| 5 | Isinglass | Polyphenol | Positively charge on | | Suitable for white | Fining agent beer |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | tanin | | wine. Brilliant color | and wine |
| 6 | Sparkolloid | Protein/ metal ion | Attract negatively charged particle | 10-25℃ | Broad spectrum haze removal | Beer ,wine and juices |
| 7 | Activated carbon | Polyphenol | Adsorbent of benzoic compound | 10-25℃ | Decolorized and deodorized juices | Juices ,wine |

Source: (Damasceno et al., 2008)

**Limitations of clarifying agents**: The application of clarifying agents in fruit juice processing, while beneficial in many aspects, does come with certain limitations. One significant limitation is that the choice of clarifying agent and its effectiveness can vary depending on the type of fruit juice being processed. Some fruits may contain specific compounds or particles that are more challenging to clarify, making it necessary to select the appropriate clarifying agent carefully. Additionally, the process of clarifying fruit juices can sometimes lead to the removal of certain desirable compounds, such as flavor compounds and antioxidants, which may impact the overall taste and nutritional value of the juice. Furthermore, the cost associated with clarifying agents and the need for specialized equipment can be a limiting factor for smaller-scale fruit juice producers. Balancing the benefits of improved clarity and stability with the potential drawbacks in terms of flavor and cost is a crucial consideration in fruit juice production. To address the challenges of high production costs, operational instability, separation difficulties, and limited reusability, enzyme immobilization onto solid support has been employed as an alternative solution. (Akbarbaglu*et al*., 2019).

## Conclusion

**Comment [U9]:** must answer the goal, while the goal is not clear in the text

In contemporary juice production, various techniques are utilized to clarify juice, encompassing enzymatic clarification, ultrafiltration, centrifugation, earth filtration, and cross-flow membrane filtration. Among these, enzymatic treatment has risen to prominence in modern fruit juice manufacturing, assuming a central role in process optimization. Membrane filtration, on the other hand, presents a range of clear advantages over conventional clarification methods. These benefits encompass shortened processing durations, heightened juice yields, elimination of the necessity for filter aids and filter presses, enhanced product quality, and reduced enzyme consumption. A comprehensive review of technical literature suggests that the combination of enzymes like cellulases and pectinases, alongside non-enzyme processes, can significantly enhance both the clarity and quality of fruit juices. This approach represents a valuable innovation in the industry.

## Future Scope

The future scope of enhancing clarity and quality in horticulture foods and formulations is a promising frontier, with clarifying agents poised to play a pivotal role. These agents, whether derived from natural sources or advanced technological processes, hold the key to transforming the horticulture industry. By harnessing cutting-edge filtration techniques and innovative additives, horticultural products can attain unprecedented levels of purity and transparency. This evolution is not only beneficial for consumers who seek healthier and more trustworthy food options but also aligns with sustainability goals. Natural clarifying agents can reduce the reliance on synthetic chemicals, promoting eco-friendly practices. Moreover, by enhancing clarity and removing impurities, these agents extend the shelf life of products, reducing food waste. As the horticulture industry continues to prioritize quality, transparency, and sustainability, the role of clarifying agents is set to expand. They will be instrumental in shaping a future where horticultural foods and formulations are not only more nutritious and visually appealing but also contribute to a more environmentally responsible and resource-efficient food system.

**Comment [U10]:** not in the guideline/template

## References

**Comment [U11]:** have not followed the guideline/template


Abbes, F., Bouaziz, M., Blecker, C., Masmoudi, M., Attia, H., and Besbes, S. (2011). Date syrup: Effect of hydrolytic enzymes (pectinase/cellulase) on physicochemical characteristics, sensory and functional properties. *Food Sci. Technol*. 44: 1827-1834.

Akbarbaglu, Z., Jafari, S. M., Sarabandi, K., Mohammadi, M., Heshmati, M. K., & Pezeshki, A. (2019). Influence of spray drying encapsulation on the retention of antioxidant properties and microstructure of flaxseed protein hydrolysates. *Colloids and Surfaces B: Biointerfaces*, *178*, 421-429.

Bump, V.L. Apple pressing and juice. In: Processed Apple Products (Eds). New York, AVI.1989. pp. 53–57

Damasceno, L. F., Fernandes, F. A., Magalhães, M. M., & Brito, E. S. (2008). Non-enzymatic browning in clarified cashew apple juice during thermal treatment: Kinetics and process control. *Food Chemistry*, *106*(1), 172-179.

Dereli, B. O., Türkyılmaz, M., & Özkan, M. (2023). Clarification of pomegranate and strawberry juices: Effects of various clarification agents on turbidity, anthocyanins, colour, phenolics and antioxidant activity. *Food Chemistry*, *413*, 135672.

Dıblan, S., & Özkan, M. (2021). Effects of various clarification treatments on anthocyanins, color, phenolics and antioxidant activity of red grape juice. *Food Chemistry*, *352*, 129321

Erkan-Koç, B., Türkyılmaz, M., Yemiş, O., & Özkan, M. (2015). Effects of various protein-and polysaccharide-based clarification agents on antioxidative compounds and colour of pomegranate juice. *Food chemistry*, *184*, 37-45.

Global Health and Education Foundation (2007). "Conventional Coagulation-Flocculation-Sedimentation". *Safe Drinking Water is Essential*. National Academy of Sciences. Retrieved 2007-12-01

Gokmen, V., Artik, N., Acar, J,, Kahraman, N., and Poyrazoglu, E. (2001). Effects of various clarification treatments on patulin, phenolic compound and organic acid compositions of apple juice. Europ.*Food Res.Technol*. 213: 194–199

Granada, G.L., Vendruscolo, J.L. & Treptow, R.O. Revista Brasileira de Agrocieˆncia, 2001, 7, 143–147.

Kashyap, D. R., Vohra, P. K., Chopra, S., and Tewari, R. (2001). Applications of pectinases in the commercial sector: a review. *Bioresource Technol*. 77: 215–227.

Kaur, G., Kumar, S., and Satyanarayana, T. (2004). Production, characterization and application of a thermostablepolygalacturonase of a thermophilicmoulSporotrichumTermophile. *Bioresource Technol*., 94, 239-243.

Landbo, A. K. R., Pinelo, M., Vikbjerg, A. F., Let, M. B., & Meyer, A. S. (2006). Protease-assisted clarification of black currant juice: synergy with other clarifying agents and effects on the phenol content. *Journal of Agricultural and Food Chemistry*, *54*(18), 6554-6563.

Lee, W.C., Yusof, S., Hamid, N.S.A., and Baharin, B.S. (2006). Optimizing conditions for enzymatic clarification of banana juice using response surface methodology (RSM). *J. Food Engg.* 73: 55–63.

Li, Z. X., Zhou, X. Y., Tian, Z. H., Li, H., & Wang, S. S. (2014). Application of modified chitosan in fruit juice clarification. *Applied mechanics and materials*, *651*, 211-214.

McLellan, M.R., Kime, R.W., and Lind, L.R. (1985). Apple juice clarification with the use of honey and pectinase. *J. Food Sci*.50: 206-2

Mieszczakowska-Frac, M., Markowski, J., Zbrzezniak, M., and Plocharski, W. (2012). Impact of enzyme on quality of blackcurrant and plum juices. *Food Sci.Technol*. 49: 251-25.

Mulyani, S., Bintoro, V. P., Legowo, A. M., &Setiani, B. E. (2021, July). Functional properties comparison of hide buffalo gelatin and commercial bovine gelatin as clarifying agent for the tropical fruit juice. In *IOP Conference Series: Earth and Environmental Science* (Vol. 803, No. 1, p. 012038). IOP Publishing.

Naknean, P., Juntorn, N., &Yimyuan, T. (2014). Influence of clarifying agents on the quality of pasteurisedpalmyra palm sap (B orassusflabellifer L inn.). *International Journal of Food Science & Technology*, *49*(4), 1175-1183.

Narnoliya, L. K., Jadaun, J. S., Chownk, M., & Singh, S. P. (2020). Enzymatic systems for the development of juice clarification strategies. In *Biomass, Biofuels, Biochemicals* (pp. 397-412). Elsevier.

Oliveira, M.C.S., Silva, N.C.C., Nogueira, A. & Wosiacki, G. Cieˆncia e Tecnologia de Alimentos, 2006, 26, 906–915.

Rai, P., Majumdar, G.C., DasGupta, S., and De, S. (2003). Optimizing pectinase usage in pretreatment of mosambi juice for clarification response surface methodology.*J. Food Engg*.64: 397–403.

Ramezani, M., Ferrentino, G., Morozova, K., Kamrul, S. H., & Scampicchio, M. (2020). Clarification of apple juices with vegetable proteins monitored by multiple light scattering. *Journal of food science*, *85*(2), 316-323.

Sreenath, H. K. and Santhanam, K. (1992). The use of commercial enzymes in white grape juice clarification. *J. Fermen. Bioengg*. 73(3): 241-24.

Tastan, O., & Baysal, T. (2015). Clarification of pomegranate juice with chitosan: Changes on quality characteristics during storage. *Food chemistry*, *180*, 211-218.

Venkatachalapathy, R., Packirisamy, A. S. B., Ramachandran, A. C. I., Udhyasooriyan, L. P., Peter, M. J., Senthilnathan, K., ... & Muthusamy, S. (2020). Assessing the effect of chitosan on pesticide removal in grape juice during clarification by gas chromatography with tandem mass spectrometry. *Process biochemistry*, *94*, 305-312.

Vijayanand, P., Kulkarni, S. G., and Prathibha G. V. (2010). Effect of pectinase treatment and concentration of litchi juice on quality characteristics of litchi juice. *J. Food Sci.Technol.* 47(2): 235–23.

Wongmaneepratip, W., Tongkhao, K., &Vangnai, K. (2023). Effect of clarifying agent type and dose on the reduction of pyrethroid residues in apple juice. *Food Control*, 109909.

Yamaski, M., Yasui, T. & Arima, K. Agriculture and Chemistry, 1964, 28, 779–787.