



## Efficacy of Chitosan as Clarifying Agent to Physico-chemical and Organoleptic Attributes of Pineapple Juice during Clarification

Nguyen Phuoc Minh

Faculty of Natural Sciences, Thu Dau Mot University, Binh Duong Province, Vietnam.

### Abstract

Turbidity is a major obstacle in preservation of fruit juice and concentrate. This phenomenon is also not acceptable in handling and distribution of commercial beverage. Fruit juice is a complex of soluble and insoluble multi components in suspension. Clarification is a crucial step to remove pectin, carbohydrate, metal ion as well as solid particles existing in fruit juice to achieve a clear and stable status. As a natural abundant polymer, chitosan has been proven as an alternative approach for juice clarification with diversified manipulations in appropriate cost. Our study focused on the feasibility of chitosan as clarifying agent to physico-chemical and organoleptic properties of pineapple juice during clarification. Different concentrations of chitosan (0.05, 0.075, 0.1, 0.125, 0.15%) and agitation speeds (40, 60, 80, 100, 120 rpm) have been investigated fruit juice to evaluate turbidity (NTU), viscosity (cP), total soluble solid (°Brix), total phenolic (mg GAE/g), flavonoid (mg GE/g) and sensory score after clarification. Our results revealed that 0.125% chitosan; agitation speed 80 rpm were adequate and efficient for juice clarification. As being nontoxic and biodegradable, chitosan could be effectively utilized as an alternative agent for refining of fruit juice.

**Keywords:** Pineapple juice, Clarification, Chitosan, Turbidity, Viscosity, Total soluble solid, Total phenolic, Flavonoid, Sensory score.

### Introduction

Chitosan is the N-deacetylated derivative of chitin; a naturally abundant mucopolysaccharide extracted from crustaceans, insects. Chitosan is a highly insoluble material resembling cellulose in its solubility and low chemical reactivity. Chitosan has been successfully utilized as edible coating to maintain quality of different fruits and vegetables during storage. It will be more useful in combination with essential oils to control pathogen accumulation and proliferation on the food surface.

Non-thermal treatments could be provide a better preservation of the phytochemical property and flavor of the juice. Pineapple is a wonderful tropical fruit having specific juiciness, distinct tropical flavor and diversified health benefits. Pineapple fruit exhibits high moisture, high sugars, soluble solid content ascorbic acid and low crude fibre. The ripen pineapple fruit is consumed fresh and juice as source of essential minerals, vitamins, antioxidants with different therapeutic properties [1, 6].

Pineapple contains a proteolytic enzyme bromelain. Various forms of jam, jelly, pickles are produced from pineapple [7]. Pineapple juice is normally clarified by microfiltration [7, 10], ultra filtration [11], centrifugation [12], osmotic evaporation [13], enzyme [14], clarifying agents like gelatin, bentonite, silica sol, and polyvinyl pyrrolidone [15]. However, there was not any literature mentioned to the clarification of pineapple juice by chitosan.

Chitosan has been proven to be effective as clarifying aid for apple [15, 17], grape [15], lemon [15], orange [15], and bayberry [18] juices, wine [19], and green tea [20]. Therefore, objective of our study focused on the feasibility of chitosan as clarifying agent to physico-chemical and organoleptic properties of pineapple juice during clarification.

### Material and Method

#### Material

Pineapple fruit were collected from Kien Giang province, Vietnam.

After collecting, they must be quickly conveyed to laboratory for experiments. They were washed in clean water having 20 ppm peracetic acid for sanitation. Juice was extracted by squeezing from fresh pineapple fruits, filtered by a sieve of 200 mesh, kept at 4°C in sealed container before clarification by chitosan solution. Chitosan was supplied from **Sigma-Aldrich**.

**Researching Method**

Chitosan powder (0.05, 0.075, 0.1, 0.125, 0.15 g) was dissolved in 100 mL of acetic acid 1.0% to get different concentration 0.05, 0.075, 0.1, 0.125, 0.15%. 100 mL of pineapple juice were put in glass beakers for chitosan addition. Various agitation speeds (40, 60, 80, 100, 120 rpm) were also examined to evaluate turbidity (NTU), viscosity (cP), total soluble solid (°Brix), total phenolic (mg GAE/g), flavonoid (mg GE/g) and sensory score of juice after flocculation.

**Physico-chemical, Sensory and Statistical Analysis**

Turbidity (NTU) was measured by turbidimeter. Viscosity (cP) was evaluated by digital rheometer. Total soluble solid (°Brix) was measured by refractometer. Total phenolic content (mg GAE/g) was evaluated using Folin-Ciocalteu assay [21]. Total flavonoid content (mg GE/g) was a valuated by the aluminum calorimetric method [22]. Sensory score was estimated by a group of panelists using 9-point Hedonic scale. The experiments were run in triplicate with three different lots of samples. Statistical analysis was performed by the Stat graphics Centurion XVI.

**Result & Discussion**

**Effect of Chitosan Concentration in Clarification to Physico-chemical and organoleptic Attributes of Pineapple Juice**

The phenolics were popularly existed in the cloudy juice, especially gallic acid, chlorogenic acid and caffeic acid [23]. In our research, different concentrations (0.05,

0.075, 0.1, 0.125, 0.15%) of chitosan were examined (see Table 1). It’s obviously to see that 0.125% chitosan was appropriate for coagulation of pineapple juice. Our findings were similar to other literatures. Total phenolic was 2.8 times higher in the cloudy than in the clarified apple juice. Meanwhile, the cloudy apple juice possessed significantly more 2.5 times of antioxidant activity compared to the clarified sample [23].

Chitosan can flock the anionic constituents like pectin and protein by dragging the suspended particles in juice to decrease its turbidity and viscosity [20, 24]. The increase in chitosan concentration decreased the passion juice turbidity [12].A gradual decrease of turbidity when apple juice was treated with chitosan from 100 to 700 ppm. Rungsardthong et al [17]. Suggested the implementation of chitosan concentrations from 100 to 1000 ppm for apple juice clarification.

A reduction in soluble solid values was observed on fruit juices treated with chitosan. The fungal chitosan at 0.7 g/l was highly effective in reducing the apple juice turbidity [25].Chatterjee et al [15].Proved that an increase in chitosan concentration in more than 2000 ppm did not enhance the clarification efficiency. Soluble constituents could be coagulated then separated by fining [18]. Zero turbidity for apple juice could be achieved by addition of 0.08% chitosan [26]. Total soluble solids were not significantly changed during clarification [12]. Clarification of acaí pulp by pectinase and chitosan resulted in a 50 % loss of total anthocyanin and 29 % reduction in antioxidant capacity [27].

Loss of phenolic compounds during clarification was the result of oxidation of phenolic and clarifying agents based on polysaccharides such as chitosan lowering effect on the amount of phenolic as precipitator [28, 29].

**Table 1: Effect of chitosan concentration (%) in clarification to physico-chemical and organoleptic attributes of pineapple juice**

Chitosan concentration (%)	0.05	0.075	0.10	0.125	0.15
Turbidity (NTU)	1038.71±0.02 <sup>a</sup>	624.35±0.03 <sup>b</sup>	187.32±0.02 <sup>c</sup>	38.69±0.00 <sup>d</sup>	11.64±0.03 <sup>e</sup>
Viscosity (cP)	4.29±0.00 <sup>a</sup>	4.15±0.01 <sup>ab</sup>	4.04±0.00 <sup>ab</sup>	3.99±0.03 <sup>b</sup>	3.98±0.01 <sup>b</sup>
Total soluble solid (°Brix)	11.35±0.01 <sup>a</sup>	11.31±0.00 <sup>a</sup>	11.29±0.03 <sup>a</sup>	11.28±0.01 <sup>a</sup>	11.27±0.02 <sup>a</sup>
Total phenolic (mg GAE/g)	39.24±0.03 <sup>a</sup>	38.71±0.02 <sup>ab</sup>	38.46±0.01 <sup>b</sup>	38.21±0.03 <sup>bc</sup>	38.09±0.00 <sup>c</sup>
Flavonoid (mg GE/g)	17.58±0.00 <sup>a</sup>	17.29±0.03 <sup>ab</sup>	17.05±0.01 <sup>ab</sup>	16.94±0.00 <sup>b</sup>	16.92±0.00 <sup>b</sup>
Sensory score	5.42±0.02 <sup>c</sup>	7.28±0.01 <sup>b</sup>	7.69±0.00 <sup>ab</sup>	7.95±0.02 <sup>a</sup>	7.97±0.03 <sup>a</sup>

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ( $\alpha = 5\%$ )

### Effect of Agitation Speed in Clarification to Physico-chemical and Organoleptic Attributes of Pineapple Juice

Flocculation happened as the agitation appeared, and then after the agitation has ceased, the coagulation decanted, separating

the suspended elements from the top to the bottom of the beakers. In our research, different agitation speeds (40, 60, 80, 100, 120 rpm) were examined thoroughly. It's clearly noticed that agitation speed 80 rpm was adequate for juice flocculation. In another report, a faster centrifugation at 12,000 rpm didn't reduce viscosity [12].

**Table 2: Effect of agitation speed (rpm) in clarification to physico-chemical and organoleptic attributes of pineapple juice**

Agitation speed (rpm)	40	60	80	100	120
Turbidity (NTU)	11.48±0.00 <sup>a</sup>	7.25±0.02 <sup>b</sup>	3.69±0.01 <sup>c</sup>	3.67±0.02 <sup>c</sup>	3.66±0.01 <sup>c</sup>
Viscosity (cP)	3.95±0.03 <sup>a</sup>	3.81±0.00 <sup>ab</sup>	3.70±0.02 <sup>b</sup>	3.68±0.01 <sup>b</sup>	3.67±0.00 <sup>b</sup>
Total soluble solid (°Brix)	11.24±0.02 <sup>a</sup>	11.22±0.01 <sup>a</sup>	11.21±0.00 <sup>a</sup>	11.20±0.03 <sup>a</sup>	11.20±0.03 <sup>a</sup>
Total phenolic (mg GAE/g)	38.03±0.01 <sup>a</sup>	37.89±0.03 <sup>ab</sup>	37.62±0.02 <sup>b</sup>	37.51±0.00 <sup>bc</sup>	37.39±0.03 <sup>c</sup>
Flavonoid (mg GE/g)	16.80±0.02 <sup>a</sup>	16.63±0.01 <sup>ab</sup>	16.58±0.00 <sup>ab</sup>	16.45±0.02 <sup>b</sup>	16.44±0.01 <sup>b</sup>
Sensory score	8.02±0.03 <sup>b</sup>	8.19±0.02 <sup>ab</sup>	8.45±0.01 <sup>a</sup>	8.46±0.03 <sup>a</sup>	8.47±0.00 <sup>a</sup>

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant ( $\alpha = 5\%$ )

### Conclusion

Chitosan (poly-b (1-4) N-acetyl-glucosamine) has a variety of potential applications as an adhesive, chelating agent for metal ions, and as fruit-juice clarifying aids. Being polycationic in nature, nontoxic and biodegradable; chitosan (deacetylated chitin) has been demonstrated to be an effective coagulating agent in supporting the removal

of suspended particles from beverages. Pineapple fruit is highly perishable and seasonal. It can be consumed as fresh, cooked, juiced, and fermented forms. Clarification is an important step in juice production. In this research, we have demonstrated the effectiveness of chitosan as clarifying agent to physico-chemical and organoleptic properties of pineapple juice during clarification.

### References

- Ogunmefun TO, Asoso SO, Olatunji BP (2018) Nutritional values, chemical compositions and antimicrobial activities of fruit juice from pineapple (*Ananas comosus* L.) and coconut (*Cocos nucifera* L.) blends. *J. Food Sci. Nutr.*, 1: 40-46.
- Hemalatha R, Anbuselvi S (2013) Physicochemical constituents of pineapple pulp and waste. *J. Chem. Pharm. Res.*, 5: 240-242.
- Yong Ker Loon, Mieke Hemiawati Satari, Warta Dewi (2018) Antibacterial effect of pineapple (*Ananas comosus*) extract towards *Staphylococcus aureus*. *Padjadjaran Journal of Dentistry*, 30: 1-6.
- Dabesor AP, Asowata-Ayodele AM, Umoiette P (2017) Phytochemical compositions and antimicrobial activities of *Ananas comosus* peel (M.) and *Cocos nucifera* kernel (L.) on selected food borne pathogens. *American Journal of Plant Biology*, 2: 73-76.
- Biradar Balasaheb Gunwantrao, Sonawane Kaveri Bhausahab, Barge Sagar Ramrao, Kharade Sachin Subhash (2016) Antimicrobial activity and phytochemical analysis of orange (*Citrus aurantium* L.) and pineapple (*Ananas comosus* (L.) Merr.) Peel extract. *Annals of Phytomedicine*, 5: 156-160.
- Jaanvi Kaushik, Namrata Kundu (2018) Phytochemical screening, anti-oxidant and antimicrobial activity of polyphenolic flavonoids isolated from fruit of *Ananas comosus* in various solvents. *International Journal of Scientific and Research Publications*, 8: 31-55.
- Farid Hossain, Shaheen Akhtar, Mustafa Anwar (2015) Nutritional value and medicinal benefits of pineapple. *International Journal of Nutrition and Food Sciences*, 4: 84-88.
- Carneiro L, Iralla dos Santos Sa, Flávia dos Santos Gomes (2002) Cold sterilization and clarification of pineapple juice by

- tangential microfiltration. *Desalination*, 148: 93-98.
9. Lucia Maria Jaeger de Carvalho, Carlos Alberto Bento da Silva (2012) Clarification of pineapple juice by microfiltration. *Ciência e Tecnologia de Alimentos*, 30: 828-832.
  10. Aporn Laorko, Sasitorn Tongchitpakdee, Wirote Youravong (2013) Storage quality of pineapple juice non-thermally pasteurized and clarified by microfiltration. *Journal of Food Engineering*, 116: 554-561.
  11. Lucia Maria Jaeger de Carvalho, Carlos Alberto Bento da Silva, Anna Paola Trindade R Pierucci (1998) Clarification of pineapple juice (*Ananas comosus* L. merryl) by ultra filtration and microfiltration: physicochemical evaluation of clarified juices, soft drink formulation, and sensorial evaluation. *J. Agric. Food Chem.*, 46: 2185-2189.
  12. Rui Carlos Castro Domingues, Sebastião Braz Faria Junior, Rafael Bernardes Silva, Vicelma Luiz Cardoso, Miria Hespagnol Miranda Reis (2012) Clarification of passion fruit juice with chitosan: Effects of coagulation process variables and comparison with centrifugation and enzymatic treatments. *Process Biochemistry*, 47: 467-471.
  13. CA Hongvaleerat, LMC Cabral, M Dornier, M Reynes, S Ningsanond (2005) Concentration of clarified and pulpy pineapple juice by osmotic evaporation. *Information and Technology for Sustainable Fruit and Vegetable Production*, 5: 12-16.
  14. Harsh P Sharma, Hiral Patel, Sugandha Sharma (2014) Enzymatic extraction and clarification of juice from various fruits-A review. *Trends in Post Harvest Technology*, 2: 1-14.
  15. Chatterjee S, Chatterjee S, Chatterjee BP, Guha AK (2004) Clarification of fruit juice with chitosan. *Process Biochem.*, 39: 2229-2232.
  16. Oszmianski J, Wojdylo A (2007) Effects of various clarification treatments on phenolic compounds and color of apple juice. *Eur. Food Res. Technol.*, 224: 755-762.
  17. Rungsardthong V, Wonputtanakul N, Kongpien N, Chotiwaranon P (2006) Application of fungal chitosan for clarification of apple juice. *Process Biochem.*, 41: 589-593.
  18. Fang ZX, Zhang M, Tao GJ, Sun YF, Sun JC (2006) Chemical composition of clarified bayberry (*Myrica rubra* Sieb. et Zucc.) juice sediment. *J. Agric. Food Chem.*, 54: 7710-7716.
  19. Spagna G, Pifferi PG, Rangoni C, Mattivi F, Nicolini G, Palmonari R (1996) The stabilization of white wines by adsorption of phenolic compounds on chitin and chitosan. *Food Res Int.*, 29: 241-248.
  20. Rao L, Hayat K, Lv Y, Karangwa E, Xia SQ, Jia CS (2011) Effect of tiltrafiltration and fining adsorbents on the clarification of green tea. *J. Food Eng.*, 102: 321-326.
  21. Nizar Sirag, Elhadi MM, Algaili M Algaili, Hozeifa Mohamed Hassan and Mohamed Ohaj (2014) Determination of total phenolic content and antioxidant activity of roselle (*Hibiscus sabdariffa* L.) calyx ethanolic extract. *Standard Research Journal of Pharmacy and Pharmacology*, 1: 034-039.
  22. Formagio ASN, Ramos DD, Vieira MC, Ramalho SR, Silva MM, Zárata NAH, Foglio MA, Carvalho JE (2015) Phenolic compounds of *Hibiscus sabdariffa* and influence of organic residues on its antioxidant and antitumoral properties. *Braz. J. Biol.*, 75: 69-76.
  23. Candrawinata VI, Blades BL, Golding JB, Stathopoulos CE, Roach PD (2012) Effect of clarification on the polyphenolic compound content and antioxidant activity of commercial apple juices. *International Food Research Journal* 19: 1055-1061.
  24. Marudova M, MacDougall AJ, Ring SG (2004) Pectin-chitosan interactions and gel formation. *Carbohydr Res.*, 339: 1933-1939.
  25. Vilai Rungsardthong, Nijarin Wongvuttanakul, Nilada Kongpien, Pachara Chotiwaranon (2006) Application of fungal chitosan for clarification of apple juice. *Process Biochemistry*, 41: 589-593.
  26. Sotoperalta NV, Muller H, Knorr D (1989) Effects of chitosan treatments on the clarity and color of apple juice. *J. Food Sci.*, 54: 495-496.
  27. Leiliane Teles Cesar, Marília de Freitas Cabral, Geraldo Arraes Maia, **Raimundo**

Wilane de Figueiredo, Maria Raquel Alcântara de Miranda, Paulo Henrique Machado de Sousa, Isabella Montenegro Brasil, and Carmen Luiza Gomes (2014) Effects of clarification on physicochemical characteristics, antioxidant capacity and quality attributes of açaí (*Euterpe oleracea* Mart.) juice. *J. Food Sci. Technol.* 51: 3293-3300.

28. Erkhan-Koc B, Turkyilmaz M, Yemis O, Ozkan M (2013) Effect of various protein and polysaccharide-based clarification agents on anti-oxidative compounds and color of pomegranate juice. *Food Chemistry*, 184: 37-45.
29. Saba Belgheisi, Reza EsmailZadeh Kenari (2019) Improving the qualitative indicators of apple juice by Chitosan and ultrasound. *Food Sci. Nutr.*, 7: 1214-1221.