Clarification of passion fruit juice with chitosan: Effects of coagulation process variables and comparison with centrifugation and enzymatic treatments

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**A B S T R A C T**

Clarification is an important step in the fruit juice processing industry. In this study, chitosan from shrimp shells is proposed as an alternative aid for passion fruit juice clarification being a natural and environmentally friendly adsorbent. Experiments were carried out in Jar tests varying chitosan concentration, pH, and slow velocity speed and time. The obtained results were evaluated in terms of turbidity, color, total soluble solids (TSSs), and viscosity reductions. The best condition found in these tests for chitosan treatment was compared with centrifugation and enzymatic treatments. Two different rotation speeds (4000 and 12,000rpm) were applied for the centrifugation process. Enzymatic treatment was carried out with 1 mL−1 of Pectinex 3XL (Novo Nordisk, Switzerland) for 90 min, at 50 °C. The enzymatic treatment was reliable only for viscosity reduction, while the chitosan treatment after a mild centrifugation showed the best result for passion fruit clarification.

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1. Introduction

Yellow passion fruit juice is marketed worldwide, especially because of its pleasant unique aroma and flavor, making it an attractive ingredient for many formulated beverages and food products.

Clarification is an important step in the processing of fruit juice mainly in order to remove pectin and other carbohydrates which are present in the juice. Generally, clarifying procedures can be achieved by centrifugation, enzymatic treatment or applying clarifying agents such as gelatin bentonite, silica sol, and polyvinyl pyrrolidone [1]. However, these processes can be labor-intensive, time-consuming and discontinuously operated. Besides, the use of additives (fining agents and filter aids) may leave a slight after taste in the juice [2].

Chitosan (deacetylated chitin), being polycationic in nature, nontoxic and biodegradable, has been found to be an effective coagulating agent in aiding the separation of suspended particles from beverages. Several works have reported the successful application of chitosan as a clarifying aid for apple [1,3,4], grape [1], lemon [1], orange [1], and bayberry [5] juices, besides wine [6], and green tea [7]. Chitosan has also been evaluated as a coating material of porous membranes in order to enhance the permeate flux in water treatment processes [8] and as a component for increasing storage life of fresh-cut fruits [9]. Liu et al. [10] successfully applied chitosan to remove arsenic from Laminaria japonica Aresch juice. Moreover, recently studies have been carried out to modify chitosan structure in order to improve its flocculation/coagulation properties [11,12].

In order to better understand the coagulation process, some studies have been carried out to analyze the interaction between chitosan and proteins and carbohydrates. Recently, Boeris et al. [13] showed that there is an electrostatic interaction between the protein pepsin and the chitosan depending on pH values. Marudova et al. [14] studied the interaction between chitosan and pectin, reporting that the action of chitosan as an effective crosslinker occurs at pH 5.6, being the gel behavior dependent on the degree of esterification of the pectin. Hiorth et al. [15] also evaluated the physical features and the temperature dependence of the macro-molecular complexes formed in aqueous mixtures of pectin and chitosan, looking for pharmaceutical formulations.

Although chitosan applications have been evaluated for some purposes in the food industry, at the best of our knowledge, there are no studies reported in the open literature about clarification of tropical fruit juices, such as passion fruit, using chitosan. In this way, the study of chitosan as a clarifying agent for passion fruit juice clarification provides new opportunities to enhance this technique in the juice processing industry.

The scope of the present work is to evaluate the application of chitosan as a clarifying aid to passion fruit juice processing. Moreover, centrifugation and enzymatic treatments were also
applied in the passion fruit juice processing in order to analyze its turbidity, color, total soluble solids, and viscosity reductions.

2. Materials and methods

2.1. Chitosan solution preparation

Chitosan from shrimp shells was purchased by Sigma–Aldrich (Iceland). A 0.01 kg L\(^{-1}\) solution was prepared by hydrolyzing shrimp shell chitosan with a 5% solution of acetic acid (Dinamica, Brazil).

2.2. Preparation of passion fruit juice

The passion fruit pulp was purchased from a local pulp industry (Minas Gerais – Brazil). The pulp was stored at \(-16^\circ\)C and it was defrosted to room temperature before use. The passion fruit juice was used in this work without any dilution.

Juice samples were previously centrifuged at 4000 rpm for 5 min. Preliminary tests showed that this mild centrifugation is necessary to promote chitosan coagulation/floculation. The characteristics of the used juices (raw and centrifuged ones) are presented in Table 1. These data are presented as average of three replications.

2.3. Clarification of fruit juice with chitosan

The coagulag/floculation process of passion fruit juice with chitosan was carried out in a Jar test apparatus. 200 mL samples of passion fruit juice were put in glass beakers for chitosan addition. A rapid agitation was fixed at 120 rpm during 3 min. Slow mixing jar text parameters were varied according to values presented in Table 2. Jar test parameters (agitation time and speed) were chosen based on a similar work previously published in the scientific literature [12,16]. After jar test experiments, juice samples were kept for sedimentation during 2 h. Preliminary tests showed that this sedimentation time was enough to that the equilibrium was reached. Chatterjee et al. [1] observed that the color of passion fruit juice did not change any more after 90 min of contact with a chitosan solution.

Jar test runs were carried out to evaluate the influence of different variables in the coagulation/floculation process of passion fruit juice with chitosan. The variables chitosan concentration, pH, slow agitation speed, and slow agitation time were analyzed in a 2\(^{4}\) experimental design. The variables and their levels are presented in Table 2.

2.4. Physical chemical analysis

The juice was analyzed for pH, turbidity, viscosity, color, and total soluble solids (TSS). The pH was measured using a Gehaka PC 2000 pH meter (Brazil) standardized to pH 4 and 7. Turbidity was measured with a Nova Organica HD 114 turbidimeter (Brazil). Viscosities were measured using a Brookfield LVDV-III digital rheometer (USA) at 25°C. Color was measured as absorbance at 540 nm in a Shimadzu UV mini 1240 spectrometer (Japan), as suggested by Rai and De [19]. Total soluble solids were measured with a Hanna Instruments HI 96801 refractometer (USA), expressed as Brix.

2.5. Comparison of pretreatments

A study was made in this work to compare the chitosan treatment with other conventional pretreatments applied for juice clarifications. A combination of centrifugation, enzymatic and chitosan treatments were evaluated for passion fruit juice clarification, as shown in Fig. 1.

Centrifugation processes for passion fruit juice were carried out at 4000 and 12,000 rpm for 5 min (T1 and T2). These rotations were chosen in order to verify the obtained juice after mild (4000 rpm) and intense (12,000 rpm) centrifugations. Chatterjee et al. [1] proposed the centrifugation of fruit juices at 8000 rpm for 3 min before clarification with chitosan. Vaillant et al. [20] proposed a mild centrifugation of passion fruit juice at approximately 4000 rpm during 2 min before the microfiltration process.

The enzymatic treatment was carried out with 1 mL L\(^{-1}\) of Pectinex 3X L (Novo Nordisk, Switzerland) for 90 min, at 50°C, as suggested by Vaillant et al. [20] and Domingues et al. [21]. In a previous study, Domingues et al. [21] compared the reduction of viscosity of passion fruit juice using different available commercial enzymes with pectinlytic, cellulase, and amylase activities, concluding that the best viscosity reduction was achieved in the condition mention above. Enzymatic treatments were done after centrifugation at 4000 and 12,000 rpm (T3 and T4).

The chitosan treatment will correspond to the best configuration for pH, chitosan concentration, slow agitation speed, and slow agitation time found in the previous analysis. This treatment was done after both centrifugation and enzymatic pretreatments (T5, T6, T7, and T8).

The obtained responses regarding to turbidity, color, total soluble solids, and viscosity for each proposed treatment were analyzed by ANOVA in a significance level of 95% (p < 0.05).

3. Results and discussion

Jar test runs with chitosan showed a typical behavior of coagulation/floculation processes: flocks were formed as the stirring occurred, and then after the agitation has stopped, the flocks decanted, dragging the suspended particles from the surface to the bottom of the beakers.

Table 3 presents the experimental design and the obtained results after chitosan treatment.
Table 3
Effect of pH, chitosan concentration, and slow agitation time and speed on physical chemical parameters of passion fruit juice according to a 2^4 experimental design.

<table>
<thead>
<tr>
<th>Run</th>
<th>pH</th>
<th>Chitosan concentration (ppm)</th>
<th>Slow agitation speed (rpm)</th>
<th>Slow agitation time (min)</th>
<th>Turbidity (NTU)</th>
<th>TSS (°Brix)</th>
<th>Color (540 nm)</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>300</td>
<td>20</td>
<td>3</td>
<td>1051</td>
<td>10.50</td>
<td>2.25</td>
<td>3.23</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>300</td>
<td>20</td>
<td>3</td>
<td>23</td>
<td>11.20</td>
<td>0.24</td>
<td>2.57</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1000</td>
<td>20</td>
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<td>0.14</td>
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<td>50</td>
<td>3</td>
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<td>0.11</td>
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<td>7</td>
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<td>6.90</td>
<td>3.05</td>
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<td>6</td>
<td>1000</td>
<td>50</td>
<td>3</td>
<td>25</td>
<td>11.10</td>
<td>0.17</td>
<td>2.46</td>
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<td>1093</td>
<td>10.10</td>
<td>2.30</td>
<td>4.64</td>
</tr>
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<td>300</td>
<td>20</td>
<td>10</td>
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<td>0.25</td>
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<td>0.76</td>
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<td>10</td>
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<td>0.17</td>
<td>2.56</td>
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<td>50</td>
<td>10</td>
<td>1088</td>
<td>9.90</td>
<td>2.40</td>
<td>4.58</td>
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<td>50</td>
<td>10</td>
<td>36</td>
<td>11.60</td>
<td>0.19</td>
<td>2.52</td>
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<td>1000</td>
<td>50</td>
<td>10</td>
<td>372</td>
<td>9.60</td>
<td>0.89</td>
<td>4.56</td>
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<td>1000</td>
<td>50</td>
<td>10</td>
<td>42</td>
<td>10.80</td>
<td>0.16</td>
<td>2.44</td>
</tr>
</tbody>
</table>

Eqs. (1)–(4) show the obtained first-degree polynomial models for turbidity ($Y_1$), total soluble solids ($Y_2$), color ($Y_3$), and viscosity ($Y_4$) responses, respectively, in relation to the significant variables and to the interaction between them at $p < 0.05$. Values of $R^2$ represent the fit between the experimental data and the proposed model.

$$Y_1 = 376.10 - 327.14X_1 - 177.45X_2 + 16.19X_3 + 180.79X_2X_3 \quad (R^2 = 0.9978) \quad (1)$$

Numerical coefficients in Eq. (1) shows that the increase in pH values ($X_1$) and in chitosan concentration ($X_2$) decreases the juice turbidity ($Y_1$), while the slow agitation time ($X_4$) has a slight positive effect on the turbidity response. Results presented in Table 3 show that all samples at pH 6 presented higher turbidity removal after the treatment with chitosan. Renault et al. [17] stated that chitosan acts as coagulant and/or flocculant at neutral pH condition. According to Rao et al. [7] chitosan can coagulate the anionic components such as pectin and protein and then chitosan can separate the suspended particles from beverage decreasing its turbidity. This behavior is associated with the physico-chemical properties of chitosan related to the presence of amine functions [14]. Rao et al. [7] observed that the optimal pH for green tea clarification with chitosan was at pH 5.5. Similar results were also observed by Marudova et al. [14] who reported that chitosan has an effective effect on pectin networks at pH 5.6.

Regarding to chitosan concentration, an increase in chitosan concentration from 300 to 1000 ppm also leads to a decrease in the juice turbidity. Similarly, Runsgardthong et al. [4] observed a gradual decrease of turbidity when apple juice was treated with chitosan from 100 to 700 ppm. However, Runsgardthong et al. [4] observed that the addition of chitosan at 1000 ppm increased the turbidity of apple juice. This behavior was not observed in this work within the analyzed concentration range. The saturation point probably depends on the fluid to be treated, besides the other operational variables (pH, temperature, agitation).

$$Y_2 = 10.63 + 0.63X_1 - 0.27X_2 + 0.23X_1X_2 \quad (R^2 = 0.9387) \quad (2)$$

Eq. (2) shows that the decrease in pH ($X_1$) and the increase in chitosan concentration ($X_2$) decrease the total soluble solids response ($Y_2$), while slow agitation speed ($X_3$) and time ($X_4$) do not influence this response at $p < 0.05$. Chatterjee et al. [1] also observed a reduction in soluble solid values of fruit juices treated with chitosan. Fang et al. [5] justify this occurrence since some soluble components could have been flocculated and then removed by the filtering operation.

$$Y_3 = 0.86 - 0.67X_1 - 0.41X_2 + 0.03X_4 + 0.39X_1X_2 - 0.04X_1X_3 \quad (R^2 = 0.9974) \quad (3)$$

As well as for turbidity response, the increase in pH values ($X_1$) and in chitosan concentration ($X_2$) decreases the juice color ($Y_3$), while the slow agitation time ($X_4$) has a slight positive effect on the color response. Jiang et al. [12] observed higher color removal at pH 7 than at pH 4 related to electrostatic interactions. The results obtained in this study (Table 3) showed that the effect of chitosan concentration was more expressive at lower pH values, since in an acidic solution charge neutralization can be achieved just with higher chitosan contents [12].

$$Y_4 = 3.02 - 0.67X_1 - 0.06X_2 + 0.34X_4 - 0.33X_1X_4 \quad (R^2 = 0.9946) \quad (4)$$

According to Eq. (4), pH ($X_1$) and chitosan concentration ($X_2$) are negatively related to viscosity ($Y_4$), while the slow agitation time ($X_4$) has a positive effect on the viscosity response. This response shows that chitosan addition is also able to reduce juice viscosity, besides color and turbidity, especially at pH 6. Viscosity reduction is an important step for further process like membrane filtrations [22]. Marudova et al. [14] confirmed that chitosan interacts with pectin, reducing fluid viscosity.

Generally speaking, the pH ($X_1$) was the most significant variable for all the analyzed responses, as this variable presented higher regression coefficients among the others. The pH is negatively related to turbidity, color, and viscosity. All samples treated at pH 6 presented turbidity and color reductions superior to 90% and viscosity reduction of almost 40%, regardless the chitosan concentration. Within the analyzed range, the chitosan concentration ($X_2$) showed a negative effect on all the analyzed responses. However, the effect of chitosan concentration is more evident at pH 3. According to Rizzo et al. [23] pH and chitosan concentration, as well as the initial turbidity of the solution to be treated, are important variables for coagulation process using chitosan. Slow agitation speed ($X_3$) did not present significant effect over any of the analyzed variables. Slow agitation time ($X_4$) did not present significant effect over color response and presented a positive effect for turbidity, color, and viscosity responses. In general, the obtained results showed that the analyzed Jar test parameters are less important than pH and chitosan concentration for the coagulation process. Sánchez-Martín et al. [16] also observed that rapid
Table 4
Combination results of centrifugation and addition of enzyme and chitosan on physical chemical parameters of passion fruit juice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Enzyme</th>
<th>Chitosan</th>
<th>Centrifugation</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>No</td>
<td>No</td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>No</td>
<td>No</td>
<td>12,000</td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>Yes</td>
<td>No</td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>Yes</td>
<td>No</td>
<td>12,000</td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>Yes</td>
<td>Yes</td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>No</td>
<td>Yes</td>
<td>12,000</td>
<td></td>
</tr>
<tr>
<td>T7</td>
<td>Yes</td>
<td>Yes</td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>T8</td>
<td>Yes</td>
<td>Yes</td>
<td>12,000</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2.** Turbidity reduction after the proposed treatments (in comparison to raw and to 4000 rpm centrifuged juices).

**Fig. 3.** Color reduction after the proposed treatments (in comparison to raw and to 4000 rpm centrifuged juices).

**Fig. 4.** Total soluble solids (TSS) reduction after the proposed treatments (in comparison to raw and to 4000 rpm centrifuged juices).

**Fig. 5.** Viscosity reduction after the proposed treatments (in comparison to raw and to 4000 rpm centrifuged juices).

and slow agitation times in jar test, as well as agitation intensity, are less important than tannin-based coagulant dosage for water treatment.

During the experiments 6 and 14 the formation of bigger flocks was observed, leading to a faster clarification. The total decantation of the suspended particles in these experiments happened in less than 30 min. It was assumed that the combination of low chitosan concentration and high pH induced the best condition for the formation of flocks. Thus, pH 6, 300 for ppm chitosan concentration, 50 rpm for slow agitation speed and 3 min for slow agitation time were used for further chitosan experiments.

3.1. Comparison of pretreatments

Table 4 shows the obtained results (average of three replications) after the pretreatments proposed in Fig. 1.

An analysis of variance (ANOVA) were carried out with these results and p-values presented in Table 5 shows that chitosan treatment is significant for all the evaluated responses, centrifugation is significant for turbidity and color reductions, while the enzymatic treatment is significant only for viscosity, at p < 0.05.

The results presented in Table 4 are analyzed in Figs. 2–5 in terms of percentage of reduction for turbidity, color, TSS, and viscosity, respectively, of the passion fruit juice after the proposed treatments in relation to the raw juice and to the juice centrifuged at 4000 rpm.

Fig. 2 shows that the treatments employing chitosan (T5–T8) ensures a turbidity reduction of almost 100% regarding to the raw juice or even to the juice centrifuged at 4000 rpm. Similar behavior is observed regarding to color removal (Fig. 3), suggesting that chitosan coagulation is the main responsible for juice clarification. The action of chitosan on color and turbidity reductions was also reported by several authors. Sotoperalta et al. [24] achieved zero turbidity for apple juice treated with 0.8 kg m⁻³ of chitosan. Chaterjee et al. [11] observed a reduction of 73, 76, 72, and 61% of color using

Table 5
p values for the analyzed pretreatments and responses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Turbidity</th>
<th>Color</th>
<th>TSS</th>
<th>Viscosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme</td>
<td>0.3303</td>
<td>0.7225</td>
<td>0.2637</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Chitosan</td>
<td>&lt;0.0001*</td>
<td>&lt;0.0001*</td>
<td>0.0084⁷</td>
<td>0.0009⁷</td>
</tr>
<tr>
<td>Centrifugation</td>
<td>0.0013⁵</td>
<td>0.0003⁵</td>
<td>0.1094</td>
<td>0.1575⁷</td>
</tr>
</tbody>
</table>

Significant at p < 0.05.
chitosan to clarify apple, grape, lemon and orange juices, respectively. Rungsadthong et al. [4] achieved turbidity values of 2.80 NTU in the clarification of apple juice with chitosan extracted from shrimp cells.

Chitosan addition slightly increased the measured °Brix in 3–5%. A similar behavior was observed by Chatterjee et al. [1]. The other pretreatments did not present a significant change in TSS, as it can be observed in Fig. 4. This result is indicating that nutritional characteristics of the raw juice may be preserved after the proposed treatments, since the total soluble solids are not greatly changed after the proposed processes.

Fig. 5 shows the viscosity reduction after the proposed pretreatments for passion fruit juice. The action of the enzymes on viscosity reduction of juices is widely reported in the scientific literature [22,25,26]. Pectolytic enzymes break down the pectin molecules that facilitate the formation of pectin–protein flakes. As this process takes place, the amount of pectin in the juice decreases, while galacturonic acid monomers and oligomers remain in the juice. During the enzymatic treatment, pectin is degraded, and hence, is lowered its water-holding capacity. The viscosity of the fruit juice is then reduced because of free water released to the system [25]. Jiraratananon and Chanachai [22] observed a viscosity reduction of 18% with enzymatic treatment of passion fruit juice with pectinase.

Fig. 5 shows that the viscosity reductions are higher than 90% comparing to the raw juice after all the proposed process. However, the comparison between the treatments T3 and T4, both employing enzyme, shows that a faster centrifugation at 12,000 rpm do not ensure higher viscosity reductions. Moreover, the addition of chitosan after centrifugation and enzymatic treatments increased the viscosity reduction. Related to the centrifugation process at 4000 rpm, the enzymatic treatment (T3) reduced 54% on viscosity, while the chitosan treatment working alone (T5) reduced 43%. Marudova et al. [14] confirmed that chitosan interacts with pectin.

4. Conclusion

In this study we carried out clarification tests for passion fruit juice using chitosan at different concentration and pH values, as well as varying slow agitation time and speed. The obtained results showed that pH and chitosan concentration are negatively related to turbidity, color, and viscosity. Moreover, the pH has the major effect between the analyzed variables in the clarification of passion fruit with chitosan. Samples treated at pH 6 achieved turbidity and color reductions of almost 100%, regardless the chitosan concentration. Passion fruit pulp was also treated by centrifugation and enzymatic treatments. The obtained results showed that centrifugation is significant for turbidity and color reductions, while the enzymatic treatment is significant only for viscosity reduction. Comparison of different treatments showed that a mild centrifugation at 4000 rpm followed by a coagulation/floculation process with chitosan at 300 ppm and at pH 6 can be a suitable process for clarifying passion fruit juice. In this way, chitosan can be proposed as an alternative aid for passion fruit juice clarification with high performance and low cost, since it is a natural abundant polymer. Chitosan is a natural and abundant polymer, and due to its high performance and low cost, it can be a suitable and a more economic process for passion fruit juice clarification.

Acknowledgments

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