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Optimisation of the clarification of kiwifruit juice with tannic acid-modified chitosan

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Abstract: Chitosan (CS) is an effective clarifying agent for fruit juice. However, its low antioxidant ability is a limitation in preserving the antioxidant capacity of the juice during clarification. In this work, an antioxidant CS derivative, tannic acid-modified CS (TA-CS), was used as a clarifying agent to optimise the clarification of kiwifruit juice. By using response surface methodology and the transmittance of the juice as a response, the optimal clarification conditions were obtained as follows: TA-CS concentration of 600 μg mL^{-1}, juice pH of 3.5, and heating temperature of 70 °C. Under the optimal conditions, TA-CS showed an excellent clarification effect on kiwifruit juice, which was confirmed by the high transmittance of 99.3%. Meanwhile, the retention rate of vitamin C in the juice reached 97.0% for the TA-CS treatment, being significantly higher than that for the CS treatment. Our results suggest that TA-CS may be a promising clarifying agent for the production of fruit juice.

Keywords: clarifying agent; response surface methodology; physicochemical analysis; interaction effect; transmittance

Fruit juice is a popular drink in the world due to its beneficial effects on human health (Lettera et al. 2016). Since raw fruit juice has many suspension components, a clarification step is indispensable in juice production (Ullah et al. 2019). Chitosan (CS), a cationic natural polysaccharide, is considered an effective clarifying agent in the production of clear fruit juice (Chatterjee et al. 2004). Moreover, CS has other favourable properties such as antimicrobial, antitumor, and immune-enhancing activity. Thus, CS has been extensively applied in the fields of food and beverages (Khan et al. 2019). Nevertheless, CS has drawbacks of low antioxidant capacity and water-insolubility, which are limitations for the practical applications of CS (Hu and Luo 2016). Fortunately, by conjugating with polyphenols, the antioxidant activity and water-solubility of CS can be improved dramatically. For example, by conjugating with tannic acid (TA), the resulting TA-modified CS (TA-CS) exhibits good water-solubility and possesses stronger antioxidant capacity than CS (Jing et al. 2019). Moreover, CS chains, the backbone of TA-CS, have active and functional amino groups, the protonation of which at acid media makes TA-CS a positively charged molecule. Thus, TA-CS may interact with the charged suspension components in fruit juice and make the juice clear.

Kiwifruit is recognised as the king of fruit due to its high content of vitamin C and strong antioxidant activity (Cassano et al. 2006). Thus, it has received great attention in the market. Compared with whole fruit, the juice retains most of the nutrients of the fruit and is easy to consume. Thus, kiwifruit juice has attracted more and more attention. As a crucial step in kiwifruit juice production, the clarification has been investigated by several methods, such as the membrane process (Cassano et al. 2006). However, to our knowledge, the clarification of kiwifruit juice by TA-CS is not noted in the literature.

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The clarification of fruit juice by a clarifying agent is usually affected by several factors, including clarifying agent concentration, juice pH value, and heating temperature. In food processes, response surface methodology (RSM) is frequently used to study the relationship of responses and variables and to obtain optimal process conditions (Tokatlı and Demirdöven 2017). Therefore, the objective of this paper was to study the clarification of kiwifruit juice by TA-CS and optimise the variables by RSM. In addition, several main compositions of kiwifruit juice were evaluated after clarification.

MATERIAL AND METHODS

Material. Kiwifruit (*Actinidia chinensis* Planch.) was purchased from a local supermarket (Tianjin, China). CS (molecular weight: 54 kDa, deacetylation degree: 90%) was supplied by Aoxing Biotechnology Company, China. Other chemicals were of analytical grade and provided by Jinhai Huaxing Chemicals, China.

Preparation of TA-CS. TA-CS was prepared as follows. In brief, 2 g of CS were fully dissolved in 200 mL of acetic acid solution (0.1 mol L^{-1}), followed by adding 4 mL of hydrogen peroxide solution (1.0 mol L^{-1}) containing 0.216 g of ascorbic acid. Then, TA was added in the CS solution (the molar ratio of TA to CS repeating unit was 0.08 : 1), followed by full blending and subsequent maintenance at room temperature for 24 h. Afterwards, the reaction mixture was transferred into a dialysis tubing (MWCO 8 000–10 000 Da; Spectrumlabs, USA) and dialysed against distilled water at room temperature for 48 h (with eight changes of water to remove the unreacted TA and other chemicals). Finally, the reaction mixture was centrifuged at 8 000 × g for 10 min (CF16RX; Hitachi, Japan) and then freeze-dried to obtain TA-CS (UNICRYO MC 4L; Uniequip, Germany). The TA equivalent content in TA-CS was 267.1 ± 5.4 mg g^{-1}, and the water-solubility of TA-CS was 15.0 ± 0.3 mg mL^{-1}.

Preparation and clarification of kiwifruit juice. Kiwifruit was sanitised with NaOCl solution (1.3 mmol L^{-1}) for 5 min and then washed three times with sterile water [which was prepared by autoclaving distilled water (YXQ-LS-18SI; Boxun, China)]. Then, the kiwifruit was peeled manually with a sterile knife and squeezed with a manual juicer (KOK-D569; KOK, China), followed by filtrating with cheesecloth (grade 90, 44 × 36 weaves; Zhuojie, China) to obtain the raw juice. The raw juice was then centrifuged at 4 000 × g for 5 min (CF16RX; Hitachi, Japan) to promote the flocculation process. Afterwards, the juice was adjusted to a set pH with citric acid, followed by heating for 20 min (501A; Sujin, China). The clarifying agent (TA-CS or CS) was therewith added in the juice. The mixture was stirred at 45 rpm for 8 min with a magnetic stirrer (Model 81-2; Shanghai Sile, China) and then maintained at room temperature for 90 min. Finally, the mixture was centrifuged at 4 000 × g for 20 min, and the supernatant, i.e. the clarified juice, was collected for analysis.

Experimental design. The optimisation of the clarification process was carried out by the one-variable-at-a-time (OVAT) method and RSM. According to the previous work (Rungsardthong et al. 2006), three variables (the clarifying agent concentration, the pH value of juice, and the heating temperature) were selected for the optimisation process. In the OVAT experiments, the clarifying agent concentration was set from 200 μg mL^{-1} to 1 000 μg mL^{-1}; the pH value of the juice was adjusted in a range of 2.5–5; the heating temperature was between 30 °C and 70 °C. The transmittance (%) of the juice was measured at 660 nm using a spectrophotometer (UV-1100; Mapada, China).

To obtain a better clarification effect, the three variables, clarifying agent concentration (600, 800, and 1 000 μg mL^{-1}), juice pH (2.5, 3.0, and 3.5), and heating temperature (50 °C, 60 °C, and 70 °C), were further optimised by a Box-Behnken design (BBD) of RSM using the transmittance of the juice as a response. For each variable, the three tested values at low, basal, and high levels were also coded as −1, 0, and +1 in BBD, respectively.

Physicochemical analysis of juice. The physicochemical analyses of juice were performed by determining the content of several juice compositions, which include protein, total phenols, titratable acid, vitamin C, reducing sugar, and total soluble solids. The protein content of the juice was estimated by the Bradford method (Bradford 1976). The total phenolic content was determined by the Folin-Ciocalteu method (Yeoh and Ali 2017; Abedelmaksoud et al. 2019a). The titratable acid content was assessed with 0.1 mol L^{-1} NaOH solution using phenolphthalein as an indicator (Abdelmalek et al. 2017). The vitamin C and the reducing sugar were measured by using the 2,6-dichloroindophenol titration (Abedelmaksoud et al. 2019b) and 3,5-dinitrosalicylic acid reagent (Saqib and Whitney 2011), respectively. The total soluble solid content was measured by a portable refractometer (WZB20; Jingke, China). The retention rates (%) of the compositions were calculated after clarification and reported.

Statistical analysis. Each experiment was repeated three times; the data were expressed as means ± stan-

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dard deviations (SD). Significant differences ($P < 0.05$) were determined by one-way analysis of variance (ANOVA) in SPSS version 19.0 (SPSS Inc., USA).

RESULTS AND DISCUSSION

Effects of variables on the clarification. The effect of clarifying agent concentration on the clarification of kiwifruit juice is shown in Figure 1A. When the clarifying agent concentration increased from 200 μg mL^{-1} to 800 μg mL^{-1}, a significant increase ($P < 0.05$) in the transmittance of the juice was observed. However, the further increase in the clarifying agent concentration did not lead to a progressive increase in the juice transmittance. Adversely, a steep descent occurred in the juice transmittance when the clarifying agent concentration rose from 800 μg mL^{-1} to 1 000 μg mL^{-1}. TA-CS and CS are biopolymers. When TA-CS or CS is added to the kiwifruit juice, it can interact with the particulates in the juice. Thus, particle-polymer-particle bridges are formed, resulting in the sedimentation of the particles and subsequent clarification of the juice. Within a critical range, increasing the polymer concentration can form more bridges among particles and wrap the floc, leading to an increase in the clarification effect of the juice. However, when the polymer concentration increases over the critical range, the excessive polymer molecules are attached around the flocculent to form a protective layer, preventing the formation of bridges and thus leading to a reduction in the transmittance (Marudova et al. 2004).

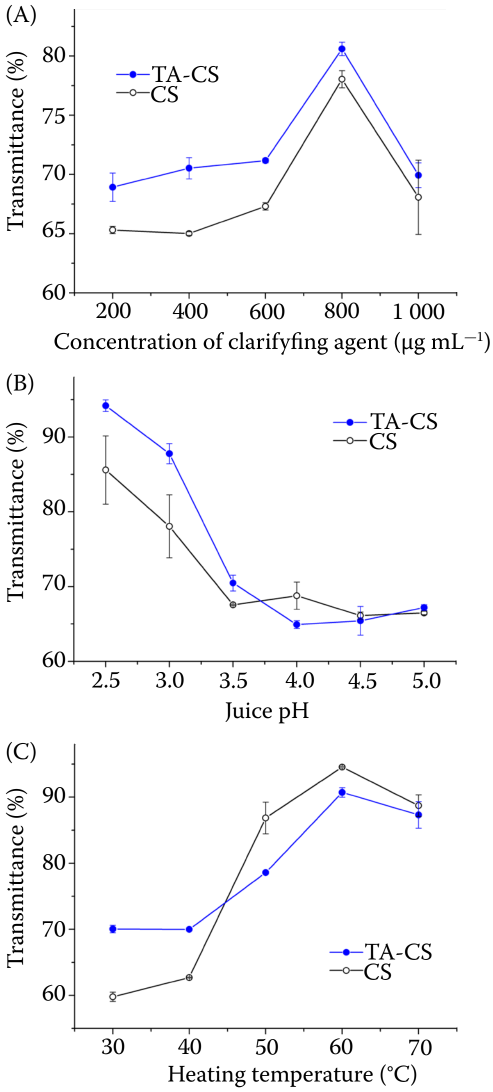

Figure 1. Effects of (A) clarifying agent concentration, (B) juice pH, and (C) heating temperature on the clarification of kiwifruit juice

The effect of pH value on the clarification of kiwifruit juice is shown in Figure 1B. TA-CS and CS showed a similar pattern in the transmittance, i.e. the transmittance of the juice increased with decreasing the pH value. Moreover, the transmittance of TA-CS-treated juice was higher than that of the CS-treated sample when the pH value was < 3.5, which agrees with a previous observation (Jing et al. 2011). At lower pH values, TA-CS and CS have positive charges due to protonation; thus, the positive molecules interact with the negative constituents of the juice, such as pectin, resulting in the sedimentation of particles and subsequent clarification of the juice (Tastan and Baysal 2015). Moreover, TA-CS had a better clarifying effect than CS at lower pH values, which may be a result of more active sites of TA-CS relative to CS. In addition, the natural pH value of kiwifruit juice is in the range of 2.8–3.5. Thus, pH 3.0 was selected for the next experiments, although pH 2.5 showed a better effect than pH 3.0.

The effect of heating temperature on the clarification of kiwifruit juice was also studied in this work. In a temperature range of 30–60 °C, the transmittance of juice increased with temperature (Figure 1C), which is probably because higher temperatures inhibit the enzymatic browning of the juice (Toribio and Lozano 1986). However, when the temperature was beyond 60 °C, the transmittance was adversely declined, which may be a result of the partial denaturation and flocculation of proteins at a high temperature.

Optimisation of clarification by RSM. RSM is a useful approach widely used in process optimisation in the fields of food and beverages (Yolmeh and Jafari 2017). Based on the OVAT experiments, the levels of three variables were further optimised

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by a BBD of RSM. The clarifying agent concentration was set from 600 μg mL^{-1} to 1 000 μg mL^{-1}; the juice pH was in a range of 2.5–3.5; the heating temperature was at 50–70 °C. The BBD matrix and the results are presented in Table 1. Both TA-CS and CS exhibited excellent clarifying effects on kiwifruit juice, with a maximum transmittance of 96.0% for TA-CS and 98.5% for CS. The correlations of the transmittance of the juice with three variables (in terms of coded values) were expressed as Equations 1 and 2 for TA-CS and CS treatments, respectively.

$$y_{\text{TA-CS}} = 93.04 + 1.46x_1 - 0.19x_2 + 3.10x_3 - 0.60x_1x_2 - 1.43x_1x_3 + 2.83x_2x_3 - 0.37x_1^2 - 0.37x_2^2 - 2.39x_3^2 \quad (1)$$

$$y_{\text{CS}} = 94.00 - 0.11x_1 - 6.58x_2 + 7.82x_3 + 7.93x_2x_3 - 4.65x_3^2 + 9.93x_1^2x_2 - 9.35x_1^2x_3 \quad (2)$$

The ANOVA results of these two models are shown in Table 2. The *P*-values less than 0.05 indicate the model term is significant (Zheng et al. 2014). Herein, the *P*-values of models 1 and 2 were 0.0088 and 0.0002, respectively, suggesting that both the models were significant for the clarification of kiwifruit juice. Furthermore, the lack of fit is more than 0.05 for both models, indicating that the regression equations could predicate the experimental data well. In addition, the determination coefficient $R^2 > 0.75$ generally means the acceptance of a model, and $R^2 > 0.80$ implies a good fit of the model (Kontogiannopoulos et al. 2016). In this work, the R^2 values for models 1 and 2 were 0.900 and 0.926, respectively, confirming the good fitness of the models with the experimental data again. Therefore, these results suggested that models 1 and 2 could be applied to predict the transmittance of the juice after treatment with TA-CS and CS, respectively. As shown in Table 1, the predicted values of transmittance obtained from the models were in good agreement with the experimental data.

In addition, three variables exhibited different effects on the clarification of the juice with TA-CS, and CS. For TA-CS treatment, the TA-CS concentration (x_1) and heating temperature (x_3) were significant as their *P*-values were < 0.05, while the juice pH value (x_2) was not significant. From the *P*-values, the order in which the variables affected the TA-CS clarification was the heating temperature (x_3) > TA-CS concentration (x_1) > juice pH (x_2). For CS treatment, however, different patterns were observed. The significant vari-

Table 1. BBD matrix and results of RSM for the clarification of kiwifruit juice

No.	Variables			Transmittance (%)			
	x_1: TA-CS or CS concentration (μg mL^{-1})	x_2: juice pH	x_3: heating temperature (°C)	$y_{\text{TA-CS, exp}}$	$y_{\text{TA-CS, pred}}$	$y_{\text{CS, exp}}$	$y_{\text{CS, pred}}$
1	600 (–1)	2.5 (–1)	60 (0)	89.7	90.4	91.6	90.8
2	1 000 (+1)	2.5 (–1)	60 (0)	93.4	94.6	91.8	90.6
3	600 (–1)	3.5 (+1)	60 (0)	92.4	91.3	98.5	97.5
4	1 000 (+1)	3.5 (+1)	60 (0)	93.7	93.0	98.3	97.2
5	600 (–1)	3.0 (0)	50 (–1)	85.0	84.3	92.1	91.0
6	1 000 (+1)	3.0 (0)	50 (–1)	91.2	90.1	91.7	90.8
7	600 (–1)	3.0 (0)	70 (+1)	92.2	93.3	89.1	87.9
8	1 000 (+1)	3.0 (0)	70 (+1)	92.7	93.4	88.6	87.7
9	800 (0)	2.5 (–1)	50 (–1)	90.2	90.2	95.0	96.0
10	800 (0)	3.5 (+1)	50 (–1)	82.3	84.2	66.0	67.0
11	800 (0)	2.5 (–1)	70 (+1)	92.6	90.7	94.8	95.8
12	800 (0)	3.5 (+1)	70 (+1)	96.0	96.0	97.5	98.5
13	800 (0)	3.0 (0)	60 (0)	93.5	93.0	87.1	94.0
14	800 (0)	3.0 (0)	60 (0)	93.1	93.0	94.6	94.0
15	800 (0)	3.0 (0)	60 (0)	94.2	93.0	94.0	94.0
16	800 (0)	3.0 (0)	60 (0)	92.8	93.0	94.1	94.0
17	800 (0)	3.0 (0)	60 (0)	91.6	93.0	96.0	94.0

BBD – Box-Behnken design; RSM – response surface methodology; CS – chitosan; TA-CS – tannic acid-modified CS; exp – experimental data; pred – predicted values

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ables were juice pH (x_2) and heating temperature (x_3), whereas the CS concentration (x_1) was not significant. The order for three variables was the heating temperature (x_3) > juice pH (x_2) > CS concentration (x_1). Among three variables, the heating temperature was the most important factor for both TA-CS and CS treatments, which is mainly due to its great influence on the enzymatic browning of juice.

In process optimisation, the interaction effects of variables are also critical for responses. Herein, the interaction effect of juice pH and heating temperature (x_2x_3) was highly significant for both TA-CS and CS treatments as their *P*-values were < 0.01. Figure 2A and 2B present the interaction effects of juice pH and heating temperature on the clarification of kiwifruit juice with TA-CS, and CS. With increasing the juice pH value, the optimal temperature for the clarification of the juice increased. Thus, the optimal juice pH value and the heating temperature were 3.5 and 70 °C, respectively. The results were different from the OVAT data, indicating that the interaction of juice pH value and the heating temperature had a great influence on the clarification of kiwifruit juice. However, the interactions of clarifying agent concentration with the other two variables (x_1x_2 and x_1x_3) were not significant. Thus, the concentration of 600 μg mL^{-1} was selected as the optimal concentration. In addition, the perturbation plots for TA-CS and CS treatments, presented in Figure 2C and 2D, respectively, reveal the influence sequence of three variables in agreement with the ANOVA results.

Based on the RSM results, the optimal conditions for the clarification of kiwifruit juice were as follows: clarifying agent concentration of 600 μg mL^{-1}, juice pH of 3.5, and heating temperature of 70 °C. The transmittance of kiwifruit juice was measured under the optimal

Table 2. ANOVA for the clarification of kiwifruit juice

Clarifying agent	Source of variation	Sum of square	Degree of freedom	Mean of square	*F*-value	*P*-value
TA-CS	model	162.11	9	18.01	7.03	0.0088
	x_1	17.11	1	17.11	6.68	0.0362
	x_2	0.28	1	0.28	0.11	0.7501
	x_3	76.88	1	76.88	30.02	0.0009
	x_1x_2	1.44	1	1.44	0.56	0.4778
	x_1x_3	8.12	1	8.12	3.17	0.1182
	x_2x_3	31.92	1	31.92	12.46	0.0096
	x_1^2	0.58	1	0.58	0.23	0.6497
	x_2^2	0.58	1	0.58	0.23	0.6497
	x_3^2	24.15	1	24.15	9.43	0.0180
	residual	17.93	7	2.56	–	–
	lack of fit	14.24	3	4.75	5.14	0.0738
	pure error	3.69	4	0.92	–	–
	cor total	180.04	16	–	–	–
CS	model	814.94	7	116.42	16.13	0.0002
	x_1	0.10	1	0.10	0.014	0.9083
	x_2	172.92	1	172.92	23.97	0.0009
	x_3	244.92	1	244.92	33.94	0.0003
	x_2x_3	251.22	1	251.22	34.82	0.0002
	x_3^2	91.58	1	91.58	12.69	0.0061
	$x_1^2x_2$	197.01	1	197.01	27.30	0.0005
	$x_1^2x_3$	174.84	1	174.84	24.23	0.0008
	residual	64.94	9	7.22	–	–
	lack of fit	16.49	5	3.30	0.27	0.9069
	pure error	48.45	4	12.11	–	–
	cor total	879.88	16	–	–	–

CS – chitosan; TA-CS – tannic acid-modified CS; ANOVA – analysis of variance

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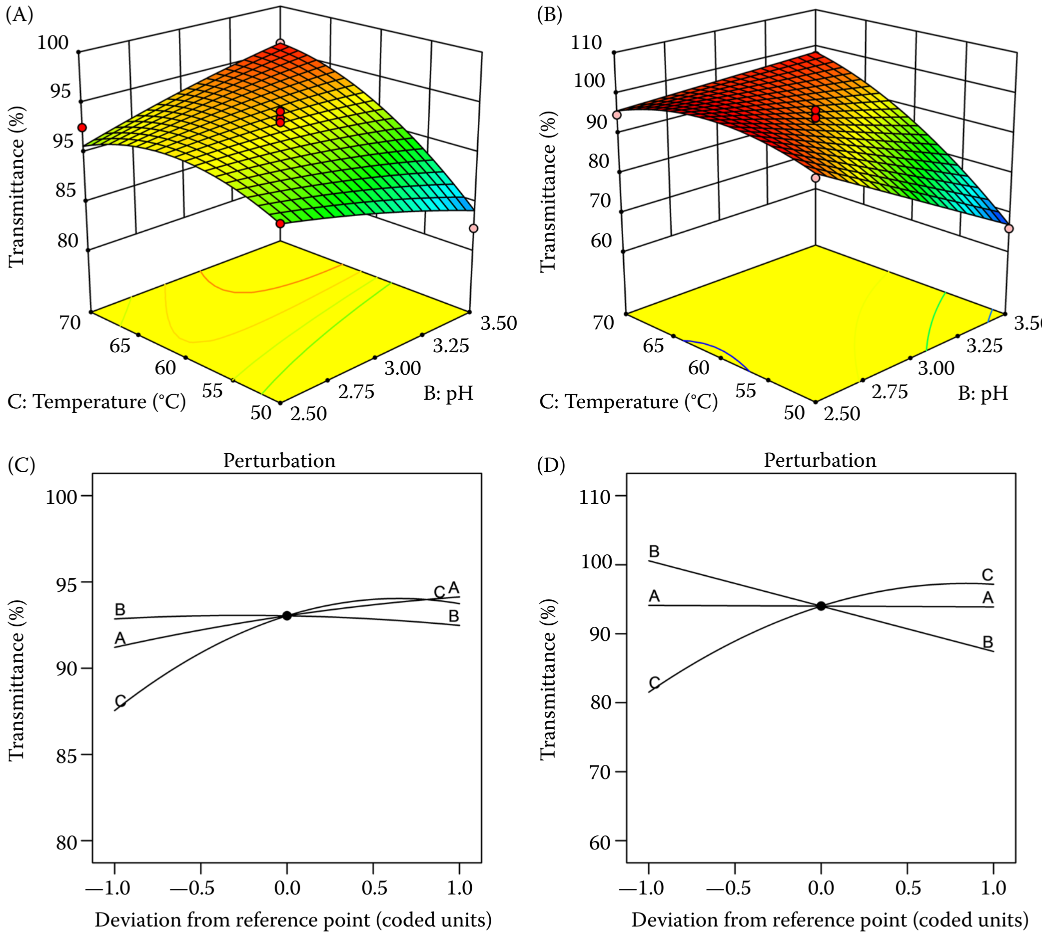

Figure 2. Interaction of juice pH value and heating temperature on the clarification of kiwifruit juice by (A) TA-CS and (B) CS along with the perturbation plots for (C) TA-CS and (D) CS treatments

A – x_1; B – x_2; C – x_3; CS – chitosan; TA-CS – tannic acid-modified CS

conditions obtained by RSM to confirm the effectiveness of the optimisation procedure. In the validation experiments, the transmittance of 99.3 ± 0.3% and 99.1 ± 0.4% was obtained for the juice treated with TA-CS and CS, respectively, which well agreed with their respective predicted values (96.2% for TA-CS treatment and 99.0% for CS treatment). These results indicate that TA-CS, and CS had a good clarifying effect for kiwifruit juice without a significant difference.

Changes in juice compositions. In the clarification process, the clarifying agent not only removes the particulates but also interacts with the other compositions in the juice, resulting in a decrease in nutrients. Thus, several main compositions of kiwifruit juice, i.e. total soluble solids, protein, total phenols, titratable acidity, vitamin C, and reducing sugar, were determined after clarification with TA-CS, and CS. The retention rates of these compositions are present in Figure 3. Both TA-CS and CS have charged groups and protein-binding ability; thus, the protein content of the juice reduced dramatically after clarification; specifically, the retention rate of protein was < 50%. The protein content is one of the main reasons for an increase in juice turbidity (Pinelo et al. 2010); the reduction in protein content facilitates the clarification of juice and subsequently improves the juice appearance. Herein, the TA-CS treatment showed a significantly lower retention rate of protein ($P < 0.05$) than the CS treatment, which is favourable for the clarification of kiwifruit juice with TA-CS. Furthermore, more nutrients remaining in the juice after clarification is favourable for juice production. In this case, the TA-CS-treated juice exhibited significantly higher retention rates of vitamin C and reducing sugar than the CS-treated one, suggesting that TA-CS is more suitable for the clarification of kiwifruit juice than CS. Especially, a much higher retention rate of vitamin C of 97.0% was obtained for TA-CS treatment

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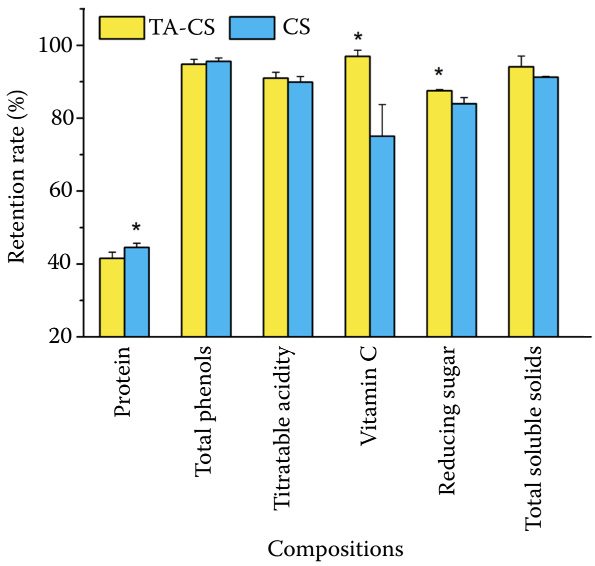

Figure 3. Retention rates of the main compositions of kiwifruit juice after clarification. Clarification conditions: Clarifying agent concentration of 600 μg mL^{-1}, juice pH of 3.5, and heating temperature of 70 °C

*Indicates significant differences ($P < 0.05$); CS – chitosan; TA-CS – tannic acid-modified CS

relative to CS treatment (75.9%), which indicates that TA-CS-treated juice is more favourable to human health. In addition, the total phenol, titratable acidity, and total soluble solid content of the juice treated with TA-CS were similar to those of the sample treated with CS. These results indicate that TA-CS not only has good clarification ability but also retains the nutrients in the kiwifruit juice.

CONCLUSION

Clarification is a major step in the production of fruit juice. The use of a clarifying agent is an effective and easy approach for juice clarification. In this study, TA-CS was used as a clarifying agent for the clarification of kiwifruit juice. By using RSM, the optimal clarification conditions were obtained for TA-CS treatment: a clarifying agent concentration of 600 μg mL^{-1}, juice pH of 3.5, and a heating temperature of 70 °C. Under optimal conditions, the transmittance of the juice reached up to > 99% after clarification with TA-CS. Nevertheless, the retention rates of vitamin C and reducing sugar in the TA-CS-treated juice were significantly higher than those in the CS-treated sample. Our results suggest that TA-CS may be a promising clarifying agent in the production of fruit juice.

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