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Clarification of apple juice using activated sepiolite as a new fining clay

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ABSTRACT: The function of acid-activated sepiolite clay was evaluated for use in the clarification of apple juice. Optimizing the clarification process was done using response surface methodology (RSM) considering juice turbidity as a response. The efficiency of sepiolite in terms of clarification was compared to that of bentonite and a combination of these fining agents with gelatin and silica gel at optimal conditions. The conditions of 0.05% clay concentration at a temperature of 50°C for 7 h were deemed optimal in terms of juice clarification. The present results showed that treatment of sepiolite-gelatin-silica gel was the most active fining agent with 99.7% reduction in apple-juice turbidity. The rate of turbidity, the viscosity, the total phenolic contents and the colour changes were fitted to first-, zero-, first- and zero-order kinetic models, respectively.

KEYWORDS: activated clays, bentonite, gelatin, kinetic study, optimization, silica gel, total phenolic content.

Fresh apple juice consists of protein, phenolic compounds, pectic substances and starch and is unstable from a taste, colour and odour point of view (Gökmen & Çetinkaya, 2007; De Souza Bezerra *et al.*, 2015). Phenolic and pectic substances may influence physico-chemical changes in apple juice during concentration and storage. To improve the appearance and marketability of apple juice, it is necessary to remove these compounds during juice production (Koyuncu *et al.*, 2007; Talasila *et al.*, 2012).

In the fruit-juice industry, clarification is one of the most important steps in terms of removal of unfavourable components such as pectin and polyphenols and in the prevention of haze formation during storage (Chatterjee *et al.*, 2004; Tastan & Baysal, 2015). Pectic substances can be removed from apple juice by enzyme clarification (depectinization) using pectinase which can hydrolyse pectin and produce pectin-protein complexes which are settled easily. The adsorption of phenolic and pectic substances on clay minerals has been studied previously (Lynch *et al.*, 1957; Ahmaruzzaman, 2008; Djebbar *et al.*, 2012; Park *et al.*, 2013; Bertolino *et al.*, 2017). El-Hamidi & Zaher (2016) used four different commercial clays namely Fulmont, Tonsil optimum N, Tonsil ACC and factory-grade bentonite as adsorbents of carotenoids, chlorophyll and phenolic compounds from vegetable oils. Furthermore, gelatin, bentonite, activated carbon, silicasol or a combination of these compounds can be used as fining agents in conventional clarification to increase the process yield (Chatterjee *et al.*, 2004; Tastan & Baysal, 2015). Talasila *et al.* (2012) reported that the application of gelatin increased the clarity of apple juice and Tastan & Baysal (2015) reduced the turbidity of pomegranate juice using bentonite and gelatin as fining agents.

Sepiolite, a natural fibrous phyllosilicate clay mineral with the formula $Mg_8Si_{12}O_{30}(OH)_4.nH_2O$ ($n = 10–12$), has been reported to have significant porosity and large specific surface area and adsorption capacity (Sabah *et al.*, 2002; Miura *et al.*, 2012; Suárez & García-Romero, 2012; Alan & Işçi, 2014; Tuler

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et al., 2016). Up to now, sepiolite has been applied in the clarification of olive mill, in the removal of phosphorus from vegetable oil, in the decolouration of crude palm oil and in the decolourization of sugar juice (Ünal & Erdoğan 1998; Sabah & Majdan, 2009; Rytwo *et al.*, 2013; Tian *et al.*, 2014).

In the present study, sepiolite was used in the clarification of apple juice. Response Surface Methodology (RSM) was used to optimize the conditions of juice clarification., Sepiolite and other commercial fining agents were compared with respect to clarification efficiency. The changes in turbidity, colour, viscosity and total phenolic content (TPC) of apple juice with time were fitted to three types of zero-, first- and second-order kinetic models.

MATERIALS AND METHODS

Materials

The pasteurized and unclarified apple juice, Gelatin (Bloom = 80–100), and the Amylase (Alphamyl MG) and Pectinase (Pectofruit XL) enzymes were obtained from Behnoosh Iran Co. (Shahrekord, Iran). Sepiolite (with specific surface area = 105 m^2/g) was purchased from Farapooyan Isatis Yazd Co. (Yazd, Iran). The bentonite used was of Na-Ca type (NaCalit ®PORE-TEC Premium Na-Ca-Bentonite ERBSLÖH, Geisenheim, Germany). The silica gel and chemicals of analytical grade were purchased from Merck Co. (Germany).

Acid activation of sepiolite

Sepiolite was activated with hydrochloric acid according to the method of Mirzaaghaei *et al.* (2016).

Pretreatment of fining agents

A pretreatment was conducted to improve the performance of sepiolite, bentonite, gelatin and silica gel according to the method of Mirzaaghaei *et al.* (2016).

Optimization of clarification conditions

Clarification conditions using activated sepiolite were optimized using RSM. The independent factors at three levels were: concentration of activated sepiolite (0.05–0.1% w/v); temperature of clarification (50–60°C); and process time (2–10 h). Juice turbidity was regarded as a response. A second-order polynomial model according to the following equation was used to show the relationship between the response function (y) and coded variables (x_i, where $i = 1$, 2 and 3):

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \tag{1}$$

Where b_0 (constant term), b_1, b_2 and b_3 (linear effects), b_{11}, b_{22} and b_{33} (quadratic effects), and b_{12}, b_{13} and b_{23} (interaction effects) show the coefficients of the polynomial model. The significant terms in the polynomial model were found by using the analysis of variance (ANOVA) at the 95% significance level.

Clarification of apple juice

The samples were clarified using amylase and pectinase enzymes and then fining agents based on the procedure explained by Mirzaaghaei *et al.* (2016) were applied for each run of RSM (Table 1). For kinetic studies, the fining agents were mixed with the juice at optimal conditions determined by the RSM.

Chemical and physical analyses of apple juice

The turbidity of the juice was measured using a portable turbidometer (Martini, Mi 415, Romania) at 20°C and expressed as NTU (nephelometeric turbidity units) (Cerreti *et al.*, 2016). A capillary viscometer (Ubbelohde-Viscometer, Fisher, USA) was used to measure the viscosity of the juice at 20°C (Bermejo-Prada *et al.*, 2015). A Denshoku ZE 6000 colour meter (Japan) was used to measure juice colour at 20°C. The parameters used to characterize colour were L* and a* based on the CIE Lab system (Mirzaaghaei *et al.*, 2016). The TPC (mg of gallic acid equivalent per 100 mL of juice) was measured using Folin-Ciocalteu reagent (Pinelo *et al.*, 2010).

Kinetic modelling of turbidity, viscosity, TPC and colour

To estimate the efficiency of sepiolite in juice clarification, the following fining agents were applied at optimal conditions: sepiolite (S), commercial bentonite (B), and a combination of these agents with gelatin (G) and silica gel (SG) (S + G, B + G, S + G + SG, B + G + SG). The concentrations of bentonite or sepiolite, gelatin and silica gel used were 0.05%, 0.015% and 0.04% (w/v), respectively (Türkyilmaz *et al.*, 2012). Sampling was conducted on an hourly

TABLE 1. Treatments based on D-optimal design; actual and predicted values for turbidity.

Treatments (Run)	Clay concentration (%)	Temperature (°C)	Time (h)	Turbidity (NTU) Actual	Turbidity (NTU) Predicted
1	0.05	53.59	2.00	32.5	33.43
2	0.05	50.00	10.00	14.04	14.86
3	0.05	50.00	10.00	15.24	14.86
4	0.05	60.00	7.16	15.02	14.44
5	0.05	60.00	2.00	25.10	24.71
6	0.06	50.00	2.00	25.75	25.70
7	0.07	50.00	5.22	13.02	11.58
8	0.07	55.97	10.00	27.64	26.93
9	0.07	56.25	5.07	20.28	20.33
10	0.08	53.75	2.00	33.69	33.79
11	0.08	60.00	2.00	21.95	21.56
12	0.08	60.00	2.00	21.66	21.56
13	0.08	60.00	7.42	8.40	10.90
14	0.10	50.00	2.00	29.96	30.28
15	0.10	50.00	2.00	30.28	30.28
16	0.10	50.00	10.00	17.12	17.56
17	0.10	50.00	10.00	17.53	17.56
18	0.10	55.95	5.25	21.06	20.74
19	0.10	60.00	10.00	19.53	18.02
20	0.10	60.00	10.00	17.34	18.02

basis to monitor the changes in juice turbidity, viscosity, colour and TPC during the clarification process. The zero-, first- and second-order kinetic models with the following equations were applied to evaluate the rates of changes:

$$\text{Zero-order model}: A = A_0 \pm \mathrm{k}t$$

$$\text{First-order model}: A = A_0 \times \exp(\pm \mathrm{k}t)$$

$$\text{Second-order model}: \frac{1}{A} = \frac{1}{A_0} \pm \mathrm{k}t$$

where A_0 = initial value of the parameter, A = the value of parameter at a certain time, k = reaction-rate constant, t = time. The model with the greatest correlation coefficient and the smallest value for the residual sum of squares (SS_r) was used to interpret the rate of change during clarification.

Statistical analysis

To optimize the conditions of the three independent factors affecting the clarification process, a D-optimal design was deployed in RSM using *Design Expert* (version 7.0) software (State-Ease Inc., Minneapolis, USA). The non-linear regression procedure of the IBM *SPSS Statistics* (version 20) software was used to estimate the parameters of the kinetic models. Three replicates were done for each analysis and the Duncan method was applied to compare the means at the 95% significance level.

RESULTS AND DISCUSSION

Model fitting

The second-order polynomial (quadratic) model was suggested as best describing the optimum conditions of clarification with insignificant lack of fit and high R^2 (0.9845). Table 2 shows the ANOVA data for the selected model. A minimum R^2 value of 0.8 is necessary for a good fit of a model (Jahed *et al.*, 2014). The signal to noise ratio, measured by adequate precision (a parameter defined by the *Design Expert* software (v. 7.0)), was 27.039 for this model.

Influence of process variables on turbidity

The RSM was used to estimate the influence of independent variables: clay concentration (A),

TABLE 2. ANOVA (analysis of variance) data for the selected second-order polynomial model.

Sources	Sum of squares	Degrees of freedom	Mean square	Coefficient	F value	p value
Model	910.62	9	101.18		70.61	<0.0001 significant
Residual	14.33	10	1.43			
Lack of fit	11.03	5	2.21		3.35	0.1054 not significant
Pure error	3.30	5	0.66			
Corrected total R^2	924.95	19		0.9845		
Adjusted R^2				0.9706		
Predicted R^2				0.9392		
CV				5.61		
PRESS				56.23		
Standard deviation				1.20		
Adequate precision				27.039		
A				–0.16	0.17	0.6852
B				–0.019	2.904E−003	0.9581
C				–3.44	85.05	<0.0001
AB				–2.09	22.95	0.0007
AC				–0. 58	2.08	0.1801
BC				2.34	37.94	0.0001
AA				1.08	2.36	0.1553
BB				–9.11	169.83	<0.0001
CC				10.50	189.44	<0.0001

A: clay concentration (%); B: temperature (°C); C: time (h).

temperature (B) and time (C) of the clarification process on the dependent variable (turbidity, y). The p values ($p < 0.05$) (Table 2) were considered to determine the significant terms in the equation. Equation 5 shows how each parameter affects turbidity.

$$\text{Turbidity(NTU)} = +19.50 - 3.44\text{C} - 2.09\text{AB} + 2.34\text{BC} - 9.11\text{B}^2 + 10.50\text{C}^2 \quad (5)$$

Based equation 5, the effect of process time on turbidity was significant ($p < 0.05$) in linear (C) and quadratic (C^2) terms. The quadratic term of temperature (B^2) had a negative effect on turbidity. The effect of interaction between temperature and clay concentration (AB) and between temperature and time (BC) was significant (Fig. 1).

When the temperature increased to 55°C, the turbidity of the juice increased with the quadratic curve, decreasing thereafter up to 60°C (Fig. 1a). Therefore, the temperature at either end of the temperature range was better and 50°C was chosen by the software as the optimum temperature. This result is attributed to the fact that, with increasing temperature, the kinetic energy of particles would increase, and the turbidity agents could not be adsorbed on the clay surface. Moreover, the adsorbed molecules may be released from the surface of sepiolite into the juice at high temperature (Gökmen & Serpen, 2002). Also, some turbidity agents may be produced by non-enzymatic reactions at elevated temperature (Farmani *et al.*, 2006). High temperature may also decrease the turbidity due to the greater solubility or breaking down of components, such as proteins, pectin and starch, however, and could also have a role in the reduction of juice viscosity (Qiu *et al.*, 2007). Mirzaaghaei *et al.* (2016) reported similar results for the effects of temperature on the clarification of pomegranate juice.

Minimum turbidity was observed at clay concentrations of <0.09% and at temperatures of 50–51°C or at clay concentrations >0.08% and temperatures of ~60° C (Fig. 1b). The effect of clay concentration was dependent on the temperature while the clay concentration at a temperature of 55°C did not affect turbidity. The smallest turbidity values were observed at the highest and lowest temperatures. By increasing the clay concentration, the specific surface area increased (Qiu

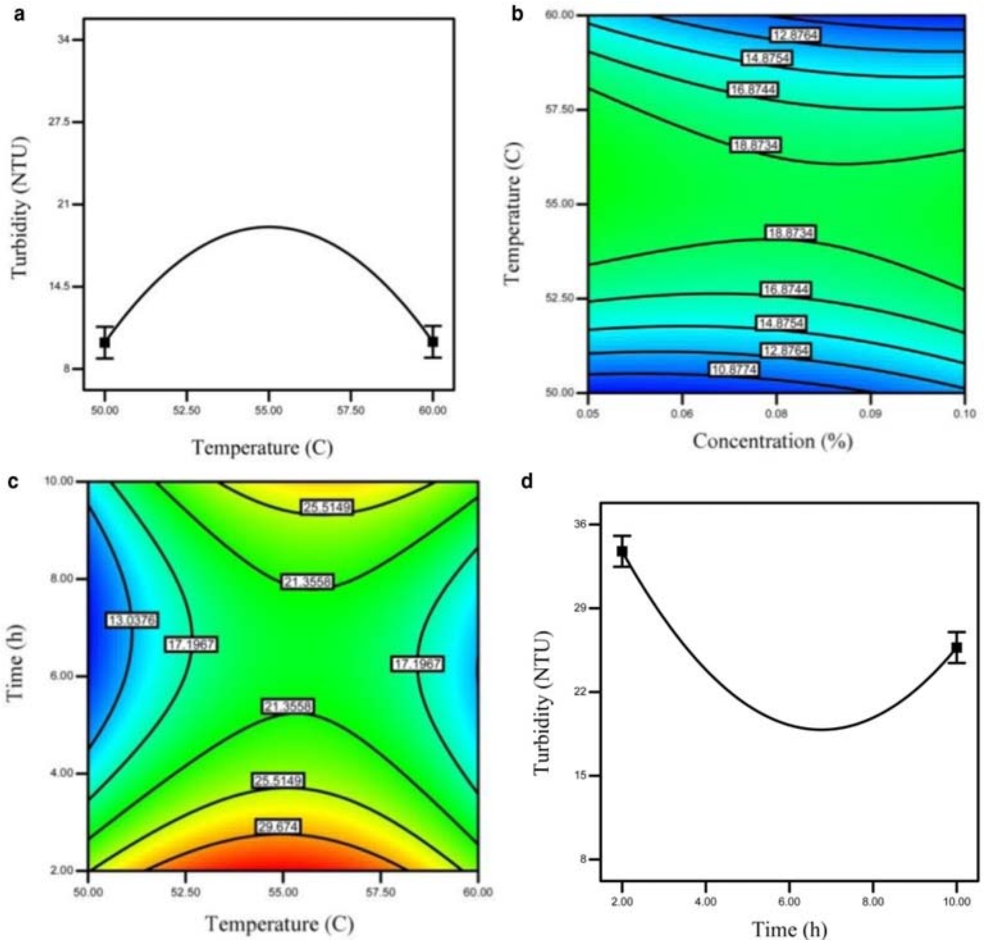

FIG. 1. The effects of: (a) temperature, (b) interaction between temperature and clay concentration, (c) interaction between temperature and time, and (d) time, on the turbidity of apple juice.

et al., 2007), but excess clay might act as a turbidity agent itself (Jahed *et al.*, 2014) due to the presence of fine particles which were not removed by centrifugation. Increasing the amount of bentonite and sepiolite may increase the turbidity of sugar beet and pomegranate juice, respectively (Jahed *et al.*, 2014; Mirzaaghaei *et al.*, 2016).

The smallest turbidity value was obtained for a time of 5–9 h and temperatures of 50 or 60°C (Fig. 1c). The single effect of time on turbidity is presented in Fig. 1d. By increasing the reaction time to 7 h, the turbidity of the juice decreased in accordance with the quadratic curve, but after that, when the time increased to 10 h, the turbidity of the juice increased again. Farmani *et al.* (2006) reported that in the refining of sugarcane juice with bentonite, using a refining time of 0–60 min enhanced turbidity reduction. After that, reduction of the turbidity is reversed until 150 min.

Optimization of juice clarification

The optimum points for apple-juice clarification were obtained at 0.05% clay concentration and temperature of 50°C for 7 h. The actual and predicted values of turbidity at optimum conditions were 10.44 and 8.88 NTU, respectively.

Clarification capacity of various fining agents

Turbidity. Changes in turbidity during clarification of apple juice using various fining agents are shown in Fig. 2a. In enzyme clarification, the turbidity decreased

TABLE 3. Changes in quality parameters of apple juice using various fining agents.

Treatments	Turbidity (NTU)	Viscosity (cp)	TPC (mg of gallic acid/100 mL of juice)	Colour	
				L* parameter	a* parameter
S	10.44 ± 0.00^{a}	1.023 ± 0.003^{c}	22.02 ± 0.49^{a}	90.48 ± 0.00^{d}	0.19 ± 0.01^{a}
S + G	6.9 ± 0.01^{c}	1.014 ± 0.004^{d}	21.29 ± 0.24^{b}	90.61 ± 0.05^{c}	0.14 ± 0.02^{a}
S + G + SG	4.87 ± 0.02^{f}	1.000 ± 0.006^{e}	20.58 ± 0.00^{cd}	91.07 ± 0.03^{a}	-0.16 ± 0.02^{c}
B	9.15 ± 0.01^{b}	1.043 ± 0.003^{a}	21 ± 0.14^{bc}	90.22 ± 0.1^{e}	-0.05 ± 0.01^{b}
B + G	5.93 ± 0.02^{d}	1.033 ± 0.002^{b}	20.32 ± 0.24^{d}	90.53 ± 0.01^{cd}	-0.13 ± 0.09^{c}
B + G + SG	4.93 ± 0.00^{e}	1.017 ± 0.003^{d}	19.84 ± 0.25^{e}	90.73 ± 0.01^{b}	-0.26 ± 0.02^{d}

S: sepiolite; G: gelatin; SG: silica gel; B: bentonite. The data are expressed as mean ± SD of triplicate measurements; the superscripted letters indicate significant difference at the 5% level.

from 1646 NTU (in unclarified juice) to 284.58 NTU. Pectinase and amylase enzymes hydrolyse pectin and starch, respectively, and produce pectin–protein, protein-carbohydrate or other complexes which may be settled easily. Hence, enzyme-clarified juice has smaller amounts of pectin and starch leading to lower viscosity and turbidity (Lee *et al.*, 2006). The S + G + SG was statistically the best treatment and reduced the turbidity from 284.58 NTU to 4.87 NTU (Table 3), although B + G + SG also decreased turbidity to 4.93 NTU (for commercial apple juice, a turbidity value of <5 NTU is acceptable). The greatest turbidity value was related to sepiolite treatment (10.44 NTU). Gelatin may react with negatively charged phenolics such as tannins or other polyphenol compounds and remove high molecular-weight polyphenols because of its positive charge in the low-pH range of fruit juice (Onsekizoglu Bagci, 2014). The silica gel may remove the excess gelatin because it is negatively charged and because of its ability to react with some positively charged particles (Türkyılmaz *et al.*, 2012). Hence, maximum reduction in turbidity may be achieved when all fining agents were used simultaneously. In a similar study, Lee *et al.* (2007) reported a reduction in turbidity of banana juice using two treatments with bentonite and a combination of bentonite and gelatin.

Viscosity. Some compounds including pectin, starch and other soluble solids may influence the juice viscosity (Costa *et al.*, 2015; Sassi *et al.*, 2016). The enzyme-clarified juices have less viscosity due to the pectin and starch hydrolysed using pectinase and amylase enzymes, respectively. Maktouf *et al.* (2014) reported that using fungal pectinolytic enzymes decreased the viscosity of lemon juice. In the present study, the viscosity of apple juice decreased during the clarification process (Fig. 2b). The application of enzymes reduced juice viscosity from 3.1 cp to 1.631 cp. The best treatment which may cause maximum reduction in viscosity was S + G + SG with a final viscosity of 1 cp (Table 3). The best reduction in viscosity was obtained using bentonite (1.043 cp). Sepiolite and bentonite mainly removed proteins and some of the phenolics that formed complexes with proteins (Lee *et al.*, 2007).

TPC. Phenolic materials are the main cause of turbidity in apple juice and may produce haze and undesirable colour and taste through the formation of polymeric complexes or condensation during concentration and storage of juice (Alper *et al.*, 2011). In the present study, the TPC decreased during clarification (Fig. 3). As mentioned previously, enzyme clarification may form pectin–protein and protein-carbohydrate complexes, and some phenolics may be bonded to these proteins so that the enzyme clarification may eliminate these compounds. During this step, therefore, TPC was reduced from 44.38 to 40 mg of gallic acid per 100 mL of juice. The B + G + SG treatment was the best treatment for reducing TPC to 19.84 (mg/100 mL), while sepiolite treatment yielded juice with maximum TPC (22.02 mg/100 mL) (Table 3). The efficiency of the combined treatment produced better results compared to others because positively charged gelatin can remove negatively charged phenolics and clay can alone remove some phenolic compounds which form complexes with proteins. Erkan-Koç *et al.* (2015) reported that gelatin was one of the most efficient agents for reduction of TPC in pomegranate juice.

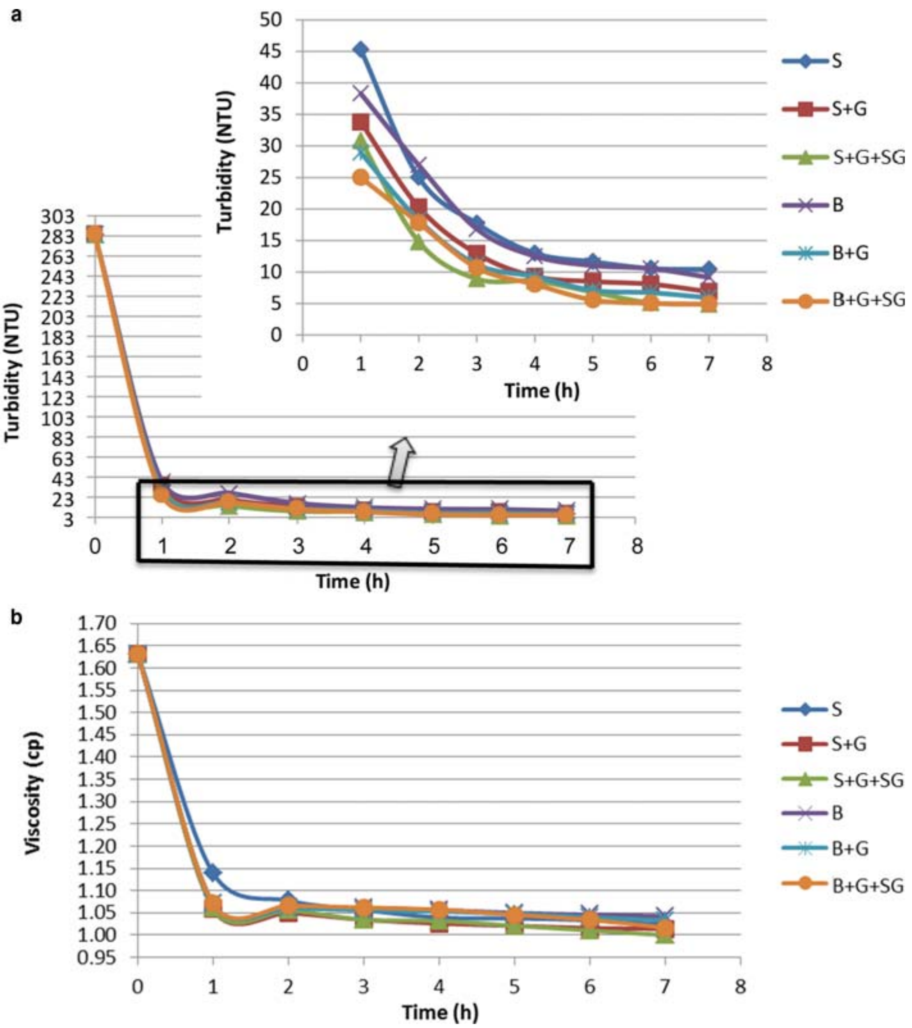

FIG. 2. Changes in (a) turbidity and (b) viscosity during clarification of apple juice.

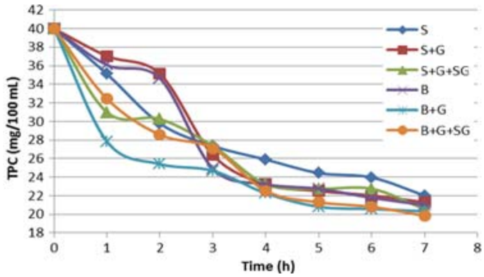

FIG. 3. Changes in TPC (total phenolic content) during clarification of apple juice.

Colour. Clarification increased the L* parameter of the apple juice (Fig. 4a). Enzyme clarification increased the L* parameter from 2.98 to 63.27. Hydrolysing the pectin and starch in this step produced clarified juice with greater lightness. The maximum increase in lightness was obtained using S + G + SG (91.07), whereas the minimum lightness was observed when using bentonite only (90.22).

The a* parameter changes during clarification (Fig. 4b). Redness increased from 1.11 to 11.03 in enzyme-clarified apple juice. The maximum reduction in the a* colour parameter was observed

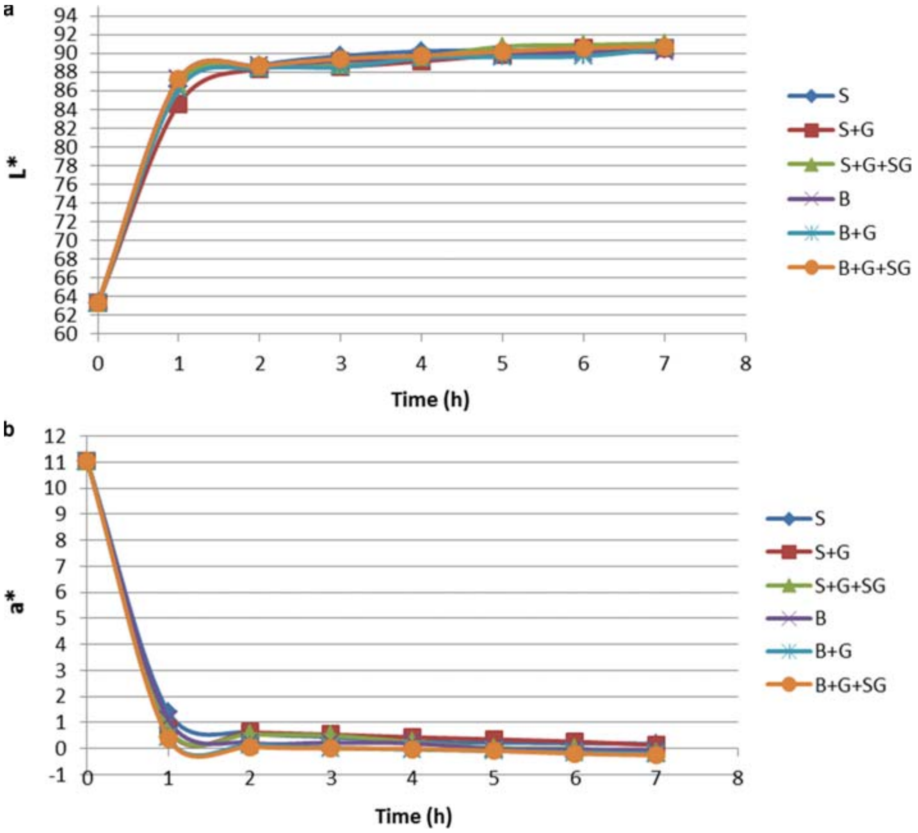

FIG. 4. Changes in the L* (a) and a* (b) parameters during clarification of apple juice.

under the treatment with S + G + SG (compared with other sepiolite treatments) and B + G + SG (among the treatments containing bentonite), which decreased the redness to −0.16 and −0.26, respectively. Some dark and brown-coloured compounds present in apple juice, can be removed by trapping in complexes that are produced during clarification (such as protein-pectin, protein-carbohydrate or gelatin-silica gel) (Türkyılmaz *et al.*, 2012). Hence, the treatments which yielded products with high clarity showed minimum redness compared to other samples. As mentioned previously, the combination of fining agents may produce juice with maximum clarity, so that this treatment led to maximum reduction in redness. Indeed, in a similar study, Oszmiański & Wojdyło (2007) reported that using combination of B + G + SG increased the L* parameter and decreased the a* parameter in clarified apple juice.

Kinetic modeling of turbidity, viscosity, TPC and colour

The modification rate of turbidity, viscosity, TPC and colour was evaluated by application of zero-, first- and second-order kinetic models. The model with the greatest R value and smallest SS_r (residual sum of squares) value was selected to describe the reaction rate. The R and SS_r of the kinetic models for these analyses are listed in Table 4. During clarification, reduction in turbidity followed a first-order kinetic model, in agreement with Mirzaaghaei *et al.* (2016) for modification of turbidity in pomegranate juice during clarification. The data for viscosity changes were in agreement mainly with a zero-order kinetic model. Deshmukh *et al.* (2015) reported that the viscosity changes of enzyme-clarified sapota (*Achras sapota L*) juice could be described by a second-order kinetic model.

Table 4. R (correlation coefficient) and SS_r (residual sum of squares) values of three types of kinetic models for turbidity, viscosity and TPC reduction and changes in colour during clarification.

	Turbidity		Viscosity		TPC		L*		a*	
Treatments	R	SSr	R	SSr	R	SSr	R	SSr	R	SSr
Zero-order										
S	0.849	269.400	0.903	0.002	0.946	12.121	0.875	4.755	0.831	0.353
S + G	0.867	141.093	0.964	0.000	0.908	45.227	0.907	7.232	0.998	0.001
S + G + SG	0.834	153.767	0.901	0.003	0.956	8.768	0.963	1.564	0.986	0.020
B	0.896	138.968	0.982	0.000	0.907	43.641	0.935	1.221	0.812	0.335
B + G	0.886	90.598	0.984	0.000	0.959	4.197	0.931	2.623	0.935	0.031
B + G + SG	0.914	58.163	0.960	0.000	0.958	11.189	0.845	13.092	0.931	0.035
First-order										
S	0.955	85.286	0.907	0.002	0.993	8.672	0.872	4.855	0.97	0.067
S + G	0.968	35.579	0.965	0.000	0.925	31.498	0. 904	7.461	0.928	0.010
S + G + SG	0.957	43.024	0.897	0.003	0.946	7.208	0.962	1.628	0.982	0.097
B	0.975	34.434	0.982	0.000	0.924	31.982	0.934	1.248	0.970	0.058
B + G	0.976	19.908	0.983	0.000	0.968	3.160	0.929	2.686	0.857	0.071
B + G + SG	0.989	7.560	0.958	0.000	0.979	7.054	0.843	13.265	0.764	0.116
Second-order										
S	R < 0.5	2399.355	0.903	0.002	R < 0.5	1690.927	R < 0.5	15309.071	0.831	0.353
S + G	R < 0.5	1392.666	0.964	0.000	R < 0.5	2050.196	R < 0.5	14641.652	0.998	0.001
S + G + SG	R < 0.5	1070.560	0.901	0.003	R < 0.5	1505.674	R < 0.5	15196.135	0.986	0.020
B	R < 0.5	1944.671	0.982	0.000	R < 0.5	1958.391	R < 0.5	15488.008	0.812	0.335
B + G	R < 0.5	1052.611	0.984	0.000	R < 0.5	1124.510	R < 0.5	15112.655	0.935	0.031
B + G + SG	R < 0.5	862.485	0.960	0.000	R < 0.5	1545.535	R < 0.5	15045.496	0.931	0.035

TABLE 5. Values of k and A_0 for different fining agents.

Treatments	Turbidity (First-order)		Viscosity (Zero-order)		TPC (First-order)		L* (Zero-order)		a* (Zero-order)	
	A_0	K	A_0	k	A_0	k	A_0	k	A_0	k
S	58.79	0.347	1.126	0.017	35.79	0.074	86.71	0.659	1.15	0.167
S + G	45.37	0.358	1.062	0.008	40.55	0.110	84.79	0.967	0.83	0.097
S + G + SG	44.46	0.445	1.089	0.017	33.64	0.073	86.40	0.752	0.91	0.157
B	49.90	0.305	1.074	0.005	39.51	0.108	87.23	0.488	0.85	0.151
B + G	38.76	0.347	1.075	0.006	28.86	0.057	86.16	0.667	0.38	0.090
B + G + SG	35.10	0.353	1.085	0.009	34.58	0.089	85.93	0.805	0.35	0.091

The first-order kinetic model expressed better the TPC changes. In a similar study, Wang & Xu (2007) observed that the first-order reaction models described successfully anthocyanin degradation during the storage of blackberry juice. Moreover, the variation in L* and a* parameters were described by a zero-order kinetic model (Table 4), in agreement with the work of Tiwari *et al.* (2008) on orange juice.

Two kinetic models parameters, *i.e.* k and A_0 for each analysis, which were obtained from the best model and experimental data, are shown in Table 5. The k parameter (reaction rate constant) expresses the reaction rate. Among the treatments containing sepiolite, the S and S + G + SG treatments had smallest and largest values of k, respectively. Similarly, among the treatments containing bentonite, the B and B + G + SG treatments had smallest and largest values of k, respectively. It can be concluded, therefore, that treatments with S or B only could reduce the turbidity at a lower rate than the other treatments. In addition, a relationship holds between k for turbidity and lightness of colour implying a direct relationship between turbidity and the lightness of colour of the apple juice (Table 5). The value of k only may describe the rate of reaction but it cannot predict the final performance of the treatment in question. Hence, the value of k in the a* parameter for treatment with B was greater than the B + G + SG treatment, whereas, the performance of B + G + SG treatment for reduction of redness (increasing of greenness) in apple juice was greater than the B treatment.

CONCLUSIONS

The results of the present study showed that increasing the sepiolite content and temperature had a negative effect on the clarification of apple juice, whereas, increasing the processing time to 7 h had a positive effect on turbidity reduction. The optimum clarification conditions for producing apple juice with maximum clarity were obtained at 0.05% clay concentration, temperature of 50°C and reaction time of 7 h. The rate of change in turbidity, viscosity, TPC and colour during the clarification process followed the first-, zero-, first- and zero-order kinetic models, respectively. The use of sepiolite-gelatin-silica gel provided optimum treatment with 99.7% reduction in the turbidity of apple juice. Sepiolite may, therefore, be used successfully instead of commercial bentonite in the fruit-juice industry, although additional studies on its dosage, yield, separation from clarified juice and cost are required.

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