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Impact of process parameters (temperature & pH) and salts on kinetics and rheological properties of *Hibiscus sabdariffa* L. leave puree

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Thermal kinetics of *Hibiscus sabdariffa* L. leaves puree (HSLP) were measured to determine the energy of kinetic degradation of chlorophyll a (Chl a) & chlorophyll b (Chl b) of puree at pH (1.4 to 5.8) and temperature (343 K to 363 K). Degradation curves were fitted using first-order reaction kinetic models & the activation energy (*Ea*) for Chl a & Chl b was found to be 44.623±0.18, 39.158±0.25 & 35.775±0.11 kJ mol^{-1} and 6.086±0.14, 5.174±0.24 & 4.328±0.20 kJ mol^{-1} at pH 1.43, 3.8 & 5.8, respectively. Every single rheological estimation was done at different shear rates, temperatures, pH, & in the presence of various salts for the product development, quality control, and engineering applications. Rheological data fitted with the Herschel-Buckley model revealed that puree exhibit non-Newtonian, shear-thinning behavior at all tested concentrations and temperatures. The viscosity reached a maximum value at pH 5.8 and it decreased at lower pH values. Salts caused a reduction in viscosity. Comparatively, NaCl had a more pronounced effect than $CaCl_2$ at a similar concentration.

Keywords: Kinetics, Color, Viscosity; HSLP, Salt

Introduction

Hibiscus sabdariffa L. leaves puree (HSLP) is most commonly used by people as curries, chutneys, pickles, protherbs, salad and is having a good source of vitamins, minerals, sugar, tannins, protein, gums, and different cancer prevention agents[1]. Processing and preservation are important for enhancing the shelf life of these green leafy vegetable. Thermal treatment is a vital and most broadly utilized preservation techniques; however, it severely affects the color, texture, and taste of the food[2,3]. Color (because of chlorophyll) loss can be controlled by pH change, HTST processing or by a combination of both which becomes the variables for understanding the kinetics of color degradation. Rheology (viscosity) is an important property, which is dependent on temperature, pH, salt and constitutional properties of food[4]. There are very few research works on rheology aspects as a function of pH, salt, and temperature. The objective of the study was to investigate a) degradation kinetics of color of HSLP under the influence of temperature (343–363 K) and pH (1.4–5.8); b) effect of pH, temperature & additive (salts) on the rheology of puree.

Material and methods

Preparation of blanched puree

Red stemmed Hibiscus leaves were procured from the market of Raipur, Chhattisgarh (India). The leaves were washed with normal tap water for 2–3 times to remove dust. The blanching was performed for 3 min in hot water (363 K). The purpose of thermal treatment is to inactivate the enzymes responsible for the degradation of pigments and bad smell. Thermal treatment was performed in an open compartment. It is expected that no notable change in moisture content and draining of pigments occur while blanching[5]. Puree (pH=1.4 & TSS=4.3 ^{0}Brix) was obtained and to it, various concentration of NaCl (0.5–1.85 g) and $CaCl_2$ (0.5–1.85 g) were added with constant stirring as per preparatory sensory test permitting adequate time for equilibration**.** Different levels of pH (1.4, 3.8, and 5.8) were separately prepared for a puree of a fixed TSS (4.3 ^{0}Brix) and adjustment was done using a buffer solution.

Method for thermal treatment of HSLP

Thermal degradation (TD) kinetics of HSLP was studied using mild thermal treatment at a temperature of 343 K, 353 K, and 363 K for a residence time of 0, 10, 20, 30 and 60 min using a water bath. Approximately 3 g puree sample were transferred to 5ml brown glass vials & enclosed with a plastic cap to

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keep away from evaporation of thermally sensitive compounds. They were then immersed in a water bath (REMI, India) & preheated to the chosen temperature[5]. For each temperature, 15 vials (five vials for each pH) were arbitrarily taken at desired intervals. Time taken by the sample to reach the desired temperature was considered when the centerline of the vial reaches the exact temperature of the water bath. The sample was withdrawn from the vial after defined times & the reaction was quenched with chilled water.

Physico-chemical & kinetic parameter determination

Chlorophyll (a & b) content, total soluble solids (TSS (^{0}Brix)), pH, rate constants (k), half-life value ($t_{1/2}$) & activation energy (Ea) were determined by the standard procedures[5]. The acidic pH (1.3–5.3) of purees were maintained for physicochemical & rheological analysis by the buffer solution.

Rheological measurement

All the rheological test were conducted as per desired experimental temperature (278, 303 & 318 K) by using a modular compact Rheometer MCR 102(Anton Paar, GmbH, Germany) & was mounted with a four-bladed vane geometry (ST22-4V-40). The experiment was performed within shear rate range of 1–100 s^{-1} as per standard procedure[4]. The rheological properties as a function of pH & salt were determined just at a specified shear rate, 100 s^{-1} and temperature=303K.

Rheological models

The rheological estimations of HSLP were measured by fitting shear rate against shear stress value to the Power law & Herschel Buckley (HB) model by using Equation (1& 2):

Ostwald-de-Waelle (Power Law) $\tau = K\,(\Upsilon)^n$ … (1)

Herschel-Buckley $\tau^{0.5} = \tau_0 + K\,(\Upsilon)^n$ … (2)

Where, τ, Υ, K, n, τ_0 is Shear Stress (Pa), shear rate (s^{-1}), consistency index (Pa.s), flow behavior index (dimensionless), yield stress (Pa), respectively. All the statistical analysis was performed as per standard procedure followed by our previous work on curry leaves puree[5].

Results and Discussion

Kinetic studies on HSLP

It was observed that the TD of chlorophyll (a & b) noticeably followed the first-order reaction kinetic model (Table 1). The kinetic rate constant (k) is an indicator that enables the estimation of the TD of chlorophyll. The lesser the k value, the better the chlorophyll stability. The k values of chlorophyll (a & b) significantly increased ($p < 0.05$) with increase in temperature. pH has a more pronounced effect in lowering TD rate at a fixed temperature. The reaction rate constant of Chl a degradation decreased from 0.011 to 0.004 min^{-1} as pH increased from 1.4 to 5.8 at 343K. Similar trends were observed among the rates of chlorophyll (a & b) degradation at the other treatment temperatures (Table 1). Comparative patterns were seen among the rates of chlorophyll b degradation at different treatment temperatures and pH conditions[6]. The highest k value was observed at all pH value (at 363K) ($p < 0.05$) for Chl a. It was found that Chl a degraded faster than Chl b at each pH and temperature ranges applied. Chl a degraded approximately 2.5 times faster than Chl b, regardless of pH. In addition, high pH causes lesser color degradation. Half-life values ($t_{1/2}$) increased, as pH was raised from 1.4 to 5.8 (Table 1). Higher $t_{1/2}$ confirmed that both chlorophylls (a & b) had the lower degradation rates at the natural pH (1.4). The increase in the stability of chlorophyll pigment with a decline in the $t_{1/2}$ was also less significant ($p < 0.05$). The greatest $t_{1/2}$ value of the

Table 1 — Kinetic parameters for chlorophyll (a & b) degradation at different pH values in HSLP at

	pH	Chlorophyll a*				Chlorophyll b*			
Temperature		k±s.d	E_a±s.d	$t_{1/2}$±s.d.	R^2	k±s.d.	E_a±s.d	$t_{1/2}$±s.d.	R^2
343	1.4	0.010^b±0.04	44.623 ±0.18	60.802±0.01	0.98	0.024^a±0.05	6.086 ±0.14	27.837^c±0.08	0.95
353		0.010^a±0.01		39.160±0.02		0.026^b±0.02		25.863^a±0.09	
363		0.027^c±0.03		25.672±0.01		0.028^b±0.02		24.755^c±0.06	
343	3.8	0.008^a±0.02	39.158 ±0.25	82.517±0.02	0.96	0.018^c±0.00	5.174 ±0.24	38.295^b±0.05	0.89
353		0.012^c±0.01		55.898±0.01		0.019^c±0.08		36.101^a±0.04	
363		0.017^b±0.04		38.723±0.02		0.02^a±0.02		34.657^c±0.04	
343	5.8	0.004^a±0.02	35.775 ±0.11	119.58±0.01	0.94	0.013^b±0.02	4.328 ±0.20	50.228^b±0.03	0.96
353		0.005^b±0.03		154.02±0.08		0.014^a±0.02		47.475^a±0.06	
363		0.009^c±0.03		77.016±0.05		0.015^b±0.01		46.209^c±0.02	

*Data with different superscript letters (a, b and c) in the same column were significantly different (ANOVA, $p < 0.05$)

Table 2 — Effect of different levels of temperatures & pH on HB & Power law model fitted to the HSLP

	Heschel-Buckley						Power law		
	Temperature effect (At pH 1.43)			pH effect (At 303 K)					
Parameters	278 K	303 K	318 K	1.43	3.80	5.80	278 K	303 K	318 K
σ_0 (Pa)	100.400	80.490	72.41	80.084	85.599	87.457	-	-	-
n	0.157	0.355	0.523	0.353	0.361	0.361	0.131	0.150	0.159
K(Pa.s^n)	16.040	14.254	11.561	16.226	14.782	14.782	99.045	80.862	73.704
η_{100}(Pas)	3.21	1.65	0.75	1.650	2.617	3.405	3.080	1.630	0.410
R^2	0.990	0.970	0.980	0.965	0.985	0.955	0.970	0.980	0.960
RMSE	2.541	3.145	1.051	2.111	1.854	1.594	3.360	3.394	4.125

chlorophyll was observed in Chl a (154.02 min), followed by Chl b (50.228 min). This observation showed that Chl a was more stable than the Chl b for the HSLP. *Ea* was calculated at different pH values, the slopes of linear regression lines resulted in *Ea* of 44.623±0.18, 39.158±0.25 & 35.775±0.11 kJ mol^{-1} for Chl a at pH 1.4, 3.8 &5.8, respectively. While the Chl a degradation was easily affected by changes in temperatures at pH 1.4, as compared to 5.8. The higher *Ea* at lower pH (1.4) signifies greater the degradation of Chl a. *Ea* for Chl b at pH 1.4, 3.8 & 5.8 was found to be 6.086±0.14, 5.174 ±0.24 and 4.328±0.20 kCal mol^{-1}, respectively. The *Ea* for degradation of Chl b was lower than that for Chl a. *Ea* exhibited almost the same trend of temperature dependence for both chlorophylls (a & b). It was found that susceptibility of Chl a was more than Chl b during TD in acidic conditions (Table 1). So the puree at pH 5.8 shows the higher value of *Ea* for Chl a than Chl b during the degradation process. Therefore, it is recommended that buffer solution having the pH (5.8) seems to preserve more Chl a than Chl b[5].

Rheological characteristics

The steady state flow curves of HSLP (TSS=4.3 ^{0}Brix) at the shear rate range of 1–100 s^{-1} with varying temperature (278–318 K) are shown in Figure 1. As expected, the HSLP showed a shear-thinning behavior (n < 1) with a representative yield stress (Table 2). All the mModels (Eqs. 1 & 2) fit satisfactorily at all temperature ranges examined & the R^2 value was found to be greater than 0.97 but while considering the parameter (yield stress), HB model was more appropriate to describe the rheological flow behavior of the puree.

Temperature dependence on steady flow properties (Viscosity, K & n) of puree

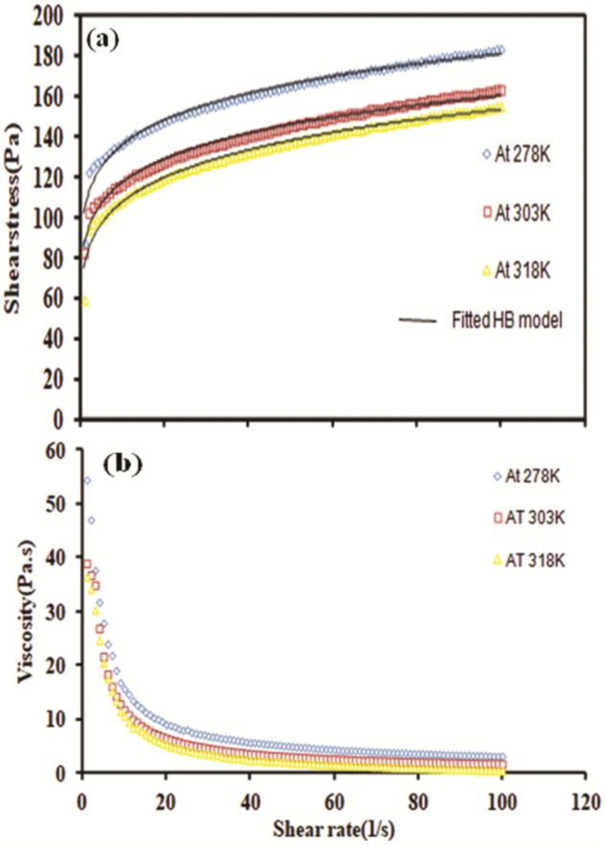

Fig. 1 — Effect of temperatures (278–318K) & shear rates (1–100 s^{-1}) on a) shear stress, b) viscosity of HSLP

Figure 1 shows the viscosity dependence of the HSLP on shear rates & temperatures. For all samples, with increasing shear rate, the apparent viscosity of the HSLP decreased at a specified temperature. The viscosity of the fluid is markedly affected by temperature, the concentration of solute, its molecular weight, pressure & suspended matter[7]. Additionally, the viscosity was affected by changing temperature & shear rate simultaneously & sharply decreased at low temperatures used (278 K & 288 K), & the lower shear rates of 10 s^{-1} (Figure 1)._With increasing temperature, the energy required for molecular mobility of the puree is provided. Therefore, the resistance of a fluid to flow is decreased, leading to

Table 3 — Effect of different concentrations of salts on HB model parameters at shear rate (1–100 s^{-1}) & 303K

Parameters	Control	NaCl (g)			$CaCl_2$(g)		
	0	0.5	1	1.85	0.5	1	1.85
σ_0 (Pa)	80.409	83.499	84.599	89.099	76.599	79.499	81.499
n	0.3755	0.3735	0.372	0.358	0.362	0.357	0.328
k ($Pa.s^n$)	18.992	19.092	24.000	25.061	20.445	26.310	29.578
Viscosity (Pa.s) at 100 s^{-1}	1.630	3.120	2.160	1.895	2.575	2.120	1.875
RMSE	1.124	2.054	1.141	3.054	1.845	0.894	1.098

decrease in viscosity during heating[8]. This behavior may be due to TD of high molecular weight particle of purees into purees having smaller molecular weights during heating. The K value was also affected by the temperature at a constant brix, so that increasing temperature led to decrease of K value. The K reflects the values of the viscosity, so the main reasons for K value variations are the same as those mentioned above for viscosity variation. Generally, the n and K values are changed conversely. As presented in Table 2, a shear thinning behavior (n less than 1) is observed for HSLP at different temperatures. Shear thinning behavior is mainly due to the breakdown of structural units of the puree and rearrangement of its chains in direction of hydrodynamic forces generated during shear[9]. n for all puree solutions was in the range of 0.157–0.523 and tended to be close to Newtonian behavior, as temperature increased[10]. The impact of factors was discovered significant ($P < 0.05$) for all temperature range (Table 2). The temperature had a remarkable inverse effect on n value. With increasing temperature, the average kinetic energy per molecule increases, which can lead to increase in molecular mobility and flow behavior index[9].

pH dependency

The K and n values were significantly affected by changing pH value. Generally, these were changed inversely. As evident in Table 2, the maximum K was obtained at pH of 5.8, whereas the n value tends to be the lowest at this pH values. The K value significantly changed with varying pH values, while for the n value, no significant change was seen in acidic condition. As the initial puree prepared from Hibiscus leaves is having pH of 1.43, so the puree is an anionic polysaccharide containing a great number of negative charge-bearing groups such as carboxyl groups, which may undergo different degrees of ionization with changing pH value, leading to changes in viscosity and rheological properties[11]. In addition, the pH-dependence of viscosity may be due to change in puree confirmation. At low pH, polysaccharide chains tend to appear in coil state with acid groups in free acid form. With increasing pH, acid groups of coils are gradually ionized and the coils are expanded due to increase in electrostatic repulsion between functional groups, leading to more intermolecular interactions among the coils and consequent higher viscosity of the puree[12,13]. The maximum viscosity (303 K & 100 s^{-1}) was obtained at around the pH 5.8 and it decreased at lower pH 1.4 for HSLP (Table 2). At pH 5.8, the shape of chains is close to rod conformational state[13]. This condition usually appears at where acid groups are ionized and electrostatic repulsion reaches a maximum and consequently, tends to keep the molecules in an extended form, leading to a high viscous solution and higher K values. The decrease of viscosity from pH 3.8 to 5.8 may be explained by the neutralization effect of added alkali on the negative charges of the puree, which reduces the hydrodynamic volume of the puree and consequent viscosity.

Salts dependency

The yield stress of the HSLP was in the range of 76.599 to 89.099 kJ mol^{-1} depending upon the concentration and types of salt added to the puree (Table 3 & Figure 2). The lower concentration of salt in the puree samples showed lower values of stress than the concentrated form. The values of yield stress and K were higher in concentrated samples. The effects of salts on the apparent viscosity and HB parameters of puree at a specific shear rate (the 100s^{-1}) and 303 K are summarized in Table 3. The apparent viscosity clearly decreased from 3.120 to 1.895 Pa.s and 2.575 to 1.875 for NaCl and $CaCl_2$, respectively with increasing each of the salts concentration[14]. Comparatively, NaCl had a more pronounced effect than that observed for $CaCl_2$ at a similar concentration of the salt used. For example, the inclusion of 0.5 g NaCl to 4.3 ^{0}Brix HSLP increased apparent viscosity value up to about 47%, while it dropped to about 37% in the presence of 0.5 g $CaCl_2$. Rheological properties

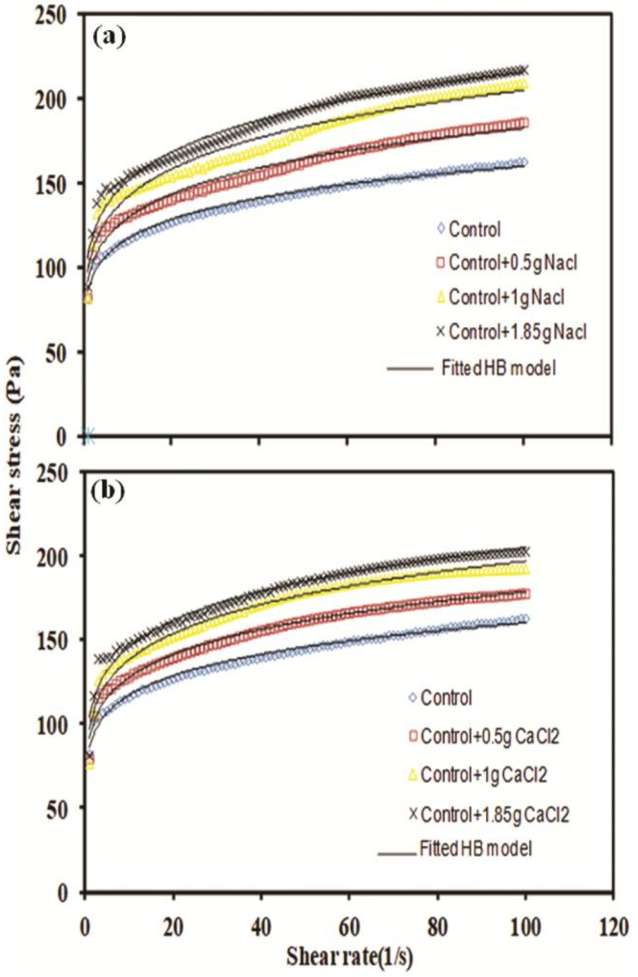

Fig. 2 — Effect of shear rates & salt concentrations on the shear stress of a) NaCl, b) $CaCl_2$ of HSLP at 303 K

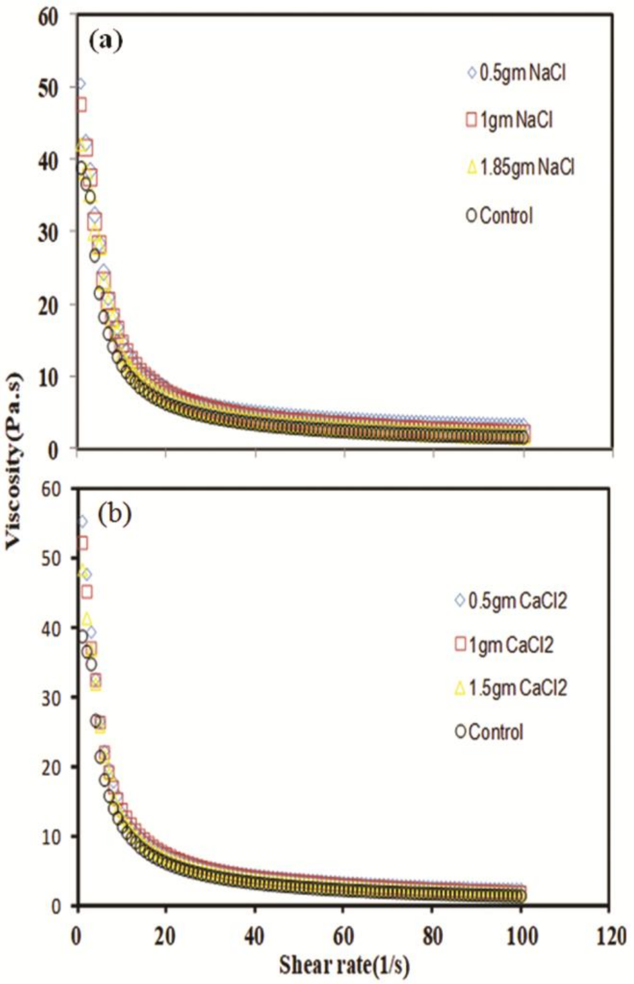

Fig. 3 — Effect of shear rates & salt concentrations on the viscosity of a) NaCl, b) $CaCl_2$ of HSLP at 303 K

of *E. contortisilliquum* gum in the presence of Na^+, Ca^{2+}, and Al^{3+} at a same ionic strength (I=1) was studied in literature and it is concluded that lower value of charge to ionic radius ratio induces lower chain contraction of molecules in the sample[15,16]. Hence, puree containing Na^+ produce the highest, while Ca^{2+} exerts lower chain contraction and viscosity. Variations in apparent viscosity and K values were significantly increased in all concentration of $CaCl_2$ and NaCl, while the n value significantly decreased (Figure 3 and Table 3).

Conclusion

At higher pH and lower temperatures, it was observed that HSLP followed first-order kinetics. Degradation of Chl a was higher than Chl b. The rate constant increased with temperature and the dependence could be described using the Arrhenius equation. The *Ea* was found to be higher at low pH for both chlorophyll molecules and signified greater heat sensitiveness of total color during thermal processing of puree. The flow behavior of HSLP exhibited shear-thinning behavior (pseudoplastic) with the n (0.157–0.523) and the HB model adequately described. The temperature, pH variation and adding salts significantly affected rheological properties of the puree. The maximum viscosity (3.405 Pa.s) found to be at pH 5.8, whereas it was the least under acidic conditions (pH 1.4). Comparatively, NaCl had a more pronounced effect than $CaCl_2$ at a fixed TSS on its rheological parameters. The flow behavior is results obtained are potentially useful for the optimizing the thermal processing and its flow behavior of the vegetable puree.

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