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Rheological behaviour of enzyme clarified Indian gooseberry juice

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A b s t r a c t. The rheological behaviour of enzyme clarified depectinated Indian gooseberry juice and its concentrate was studied as a function of total soluble solids concentration in the range of 8.2 to 35.9°Bx and temperatures from 20 to 80°C using coaxial controlled stress rheometer up to a shear rate of 600 s^{-1}. The results indicated that the enzyme clarified gooseberry juice behaves like a Newtonian fluid with a viscosity ranging from 3.92 to 7.94 mPa s. The effect of temperature on viscosity of different concentration of juice was described by an Arrhenius type relationship (R >0.99). The activation energy for viscous flow was found to be in the range 4.34 to 5.37 KJ mol^{-1} depending upon the concentration of the juice. The activation energy of viscous flow on concentration was modeled by exponential equation (R>0.99). The effect of concentration on viscosity followed an exponential type relationship (R>0.98) at the temperature used.

K e y w o r d s: gooseberry juice, rheology, viscosity, activation energy

INTRODUCTION

Knowledge of rheological properties of agricultural products undergoing industrial transformation is of great importance in the design of industrial equipments in which these changes take place during processing. The rheological properties of fruits and vegetable juice concentrates are of great importance when heat and momentum transfer take place during industrial unit operations such as chilling, evaporation, mixing, concentration, pumping, agitation *etc.* where such products are processed (Rao, 2007). The rheological behaviour of fruits and vegetable pulps/juices and their products are largely influenced by their quantitative and qualitative composition and therefore it depends on the type of fruit and on the treatment to which it is subjected during the manufacturing process (Blahovec, 2007; Burubai *et al*., 2007). Texture of juice also called mouth fell as defined as experience deriving from the sensation of the skin of the mouth after consumption of beverage and it depends on flow properties such as viscosity, specific gravity, surface tension and other related physical properties of the material. The physical properties of fruit juice have gained an important as rheological properties of juices have been developed and quantified (Ingate and Christensen, 2007).

The enzyme clarification is one of the most important techniques to increase the qualitative and quantitative physicochemical characteristics of the juice. If the clarified juice is also depectinated, its behaviour would be Newtonian in nature i.e. the relationship between shear rate and shear stress is linear. Several investigators used Newtonian model to describe the rheological behaviour of clarified juices, liquorice extract and pekmez (concentrated grape juice) (Kaya and Belibagli, 2002; Maskan, 1999).

Indian gooseberry (*Phyllanthus emblica* L.) is one of the most often used herbs and is widely available in most of the tropical and subtropical countries like China, India, Indonesia, and Malaysia. Indian gooseberry is known to have higher content of vitamin C and phytochemicals which lead to higher extent of antioxidant potential. The vitamin C content is highest as compared with any other naturally occurring substance in nature. It is a water soluble antioxidant vitamin which is important in forming collagen, a protein that gives structure to bones, cartilage, muscles and blood vessels and is very sensitive to heat; it will be destroyed during heat treatment. The active extracts of fruits posses several pharmacological properties like antioxidative, chemoprotective, analgesic, and antiinflammatory properties (Khan *et al*., 2002). It has abundant quantity of ascorbic acid,

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phenolics, flavonoids and super oxide dismutase activity (Verma and Gupta, 2004). It is used in many traditional medicinal systems such as Chine, Tibetan and Ayurvedic medicines. Indian gooseberry is reported to have hypolipodemic, hypoglycemic and antimicrobial activities. It is observed that Indian gooseberry fruit extract are being marketed by herbal/medicinal plant retailers for the treatment of various conditions or ailments because of its medicinal significance (Liu *et al*., 2008).

The juice extraction from Indian gooseberry is important in order to prepare several novel beverage products like juice beverage, fermented beverage, squash, cordial, sherbet or syrup and concentrate *etc*. The enzyme clarification is one of most important techniques to increase the qualitative and quantitative quality characteristics of juice for preparation of novel products from Indian gooseberry. Rheological information is necessary to design the processing equipment and process control of several unit operations.

The present investigation was carried out to study the effect of total soluble solid concentration and temperature on the rheological behaviour of Indian gooseberry juice.

MATERIALS AND METHODS

Indian gooseberries were purchased from the local market in Mysore, India. The fruits were washed thoroughly with water, the seed removed using sharp stainless steel knife and cut into 6-8 pieces. The fruit pieces were steam blanched at 103.4 KPa pressure for 2 min in a pressure controlled autoclave. The blanched material was pulverised into paste by 5% distilled water using commercial blender (Waring Lab., Torrington, CT). The clarification of gooseberry paste was done by treating with enzyme pectinex ultra SPL (Novozyme, Denmark). The concentration of enzyme is 0.2% and incubated for 4 h at 45°C in a constant temperature water bath. The enzyme was heat inactivated by placing in a water bath maintained at 95°C for 2 minutes and cooled immediately in ice cold water. The paste was filtered through 4 fold muslin cloth and pressed in tincture press (Hafio, Germany). Then the juice was centrifuged at relative centrifugal force 6285.75 × g using continuous centrifuge (CEPA, Lahr/Baden, Germany). The clarified gooseberry juice was subjected to concentration by reverse osmosis.

The enzyme clarified depectinated gooseberry juice was concentrated by reverse osmosis using DDS lab model-20, 0.72 m^2 plate and frame UF-RO system (De Danske Sukker, Nakaaskov, Denmark). The gooseberry juice was concentrated at 4.5 MPa pressure using thin film composite polypropylene HR98PP membranes and the average water permeate flux was 15.3 l m^{-2} h^{-1}. During the concentration process gooseberry juice was collected at different concentrations for rheological measurements.

The total soluble solids was determined by refractometry using digital refractometer (Atago Co., Ltd., Tokyo, Japan) and the total soluble solid content was expressed as ° Brix. A digital pH-meter (Cyber scan, India) was used to measure the acidity of gooseberry juice at 25°C. The acidity was determined by titration method with standard 0.01N NaOH solution using phenolophthalein as indicator and expressed as % citric and ascorbic acids content of the juice was determined by titration method using 2,6-dichlorophenolindophenol dye as indicator and expressed as mg/100 ml of juice (Ranganna, 1986).

The colour parameters of clarified gooseberry juice was measured using colourmeter (Mini scan XE plus, model 45/0-S Hunter Lab. Inc., Baton). Measurement was carried out at 10^0 observations, D65 illuminant source and expressed as L*, a* and b* values in Hunter scale. L* refers to lightness, +a* refers to redness and +b* refers to yellowness.

Total phenolics content was determined by spectrophotometrically using Folin-Ciocalteu method (Singleton *et al*., 1999). The total phenolics content was expressed as mg equivalent of gallic acid/100 ml and total flavonoids content was determined spectrophotometrically (Zhishen *et al*., 1999) and expressed as mg equivalent of catachin/100 ml using UV-visible spectrophotometer (Model, UV-1601, Simadzu, Japan).

The rheological measurements were carried out using MCR 100 controlled stress rheometer (Paar Physica, A. Paar Gmbh, Germany) equipped with coaxial cylinders (CC 27) and the radii ratio of coaxial cylinders was 1.08477. The rheometer is equipped with an electric temperature controlled Peltier system (TEZ-15P-C) to control the experimental temperature and to maintaining constant temperature, a circulating water bath was used (Viscotherm VT-2, Paar Physica, A. Paar GmBh, Germany). The rheological parameter shear stress (Pa) was measured linearly up to shear rate of 600 s^{-1} with 10 min duration of time. Thirty data points collected and analyzed using universal software US200 (Paar Physica, A. Paar Gmbh, Germany).The rheological measurements were carried out at 20, 35, 50, 65 and 80°C temperatures. All the measurements were carried out in triplicate and fresh sample was used in each measurement. The viscosity values were obtained by experimental shear rate and shear stress data fitting with Newton equation:

$$\sigma = \eta\gamma , \quad (1)$$

where: σ is shear stress (Pa), η is viscosity (Pa s), and γ is the shear rate (s^{-1}).

The experimental results, data analysis and different mathematical models were fitted using statistical software (Statistica 7.0, Stat Soft Tulsa, USA). The fitting and estimates were calculated at 95% significance level.

RESULTS AND DISCUSSIONS

Semiconcentrates of juices need to have desired flow properties and the physicochemical constituents play an important role in rendering requisite rheological behaviour.

Table 1 shows the physicochemical characteristics of enzyme clarified depectinated Indian gooseberry juice. It is found that gooseberry juice has significant amount of ascorbic acid content to an extent and is also rich in phenolics and flavonoids compounds which contribute to high antioxidant activity (Poltanova *et al*., 2009). The Hunter colour values L*, a* and b* are very low indicate that the juice was clear after enzyme clarification. The total soluble solids (TSS) of the juice were 8.2°Bx which were low compared to that of native fruit largely due to the dilution phenomenon during juice extraction and also because of loss of solids during the blanching and filtration processes (Gopalan *et al*., 2000; Goyal *et al*., 2008).

Figure 1 shows the shear stress/ viscosity *vs* shear rate of enzyme clarified depectinated Indian gooseberry juice of concentration of 16.4°Bx at temperature 65°C. There was a linear increase in shear stress with respect to increase in shear rate and the curve passes through the origin, which indicates that the flow is Newtonian in behaviour. The viscosity *vs* shear rate curve shows that flow curve is almost parallel to x-axis which again indicates that the fluid is Newtonian. The viscosity of juice was evaluated using Newtonian model (Eq. (1)) by fitting the experimental shear stress-shear rate and the correlation coefficient of Newtonian model is greater than 0.97. The viscosity of enzyme clarified depectinated Indian gooseberry juice at different soluble solids and temperatures is shown in Table 2 and the viscosity was in range of 3.92 to 7.94 mPa s. The results indicate that viscosity of enzyme clarified juice decreases significantly ($p \leq 0.05$) with increase in temperature. The viscosity of water solutions strongly depend on the water-solutes interaction and the intermolecular forces, which results from the intermolecular spacing and the strength of hydrogen bonds. Both are strongly affected by temperature and concentration. The viscosity of juice increases significantly ($p \leq 0.05$) with increase in soluble solids concentration and it was due to the increase in hydrated molecules and hydrogen bonding with the hydroxyl groups of solutes. The sugar content plays an important role in the magnitude of the viscosity of juices (Rao *et al*., 1984). Several authors have reported similar type of results for cherry, pomegranate, pineapple and orange juices (Ibarz *et al*., 2009; Juszczak and Fortuna, 2004; Kaya and Sozer, 2005; Shamsudin *et al*., 2007).

The temperature has a major effect on the Newtonian viscosity similar to the effect on the consistency coefficient for non-Newtonian fluids. The effect of temperature on the viscosity of enzyme clarified depectinated Indian gooseberry juice with different soluble solid contents was describe using the Arrhenius equation:

$$\eta = \eta_{\infty} \exp\left(E_a / RT\right), \qquad (2)$$

where: η – viscosity (Pa s), η_{∞} – material constant/pre-exponential coefficient/frequency factor (Pa s), E_a – flow activation energy (J mol^{-1}), R – gas constant (J mol^{-1} K^{-1}), and T – temperature (K).

Table 1. Physicochemical characteristics of enzyme clarified depectinated Indian gooseberry juice

Parameter	Quantity
Total soluble solids (° Bx)	8.20 ± 0.00
pH	2.90 ± 0.004
Acidity (% citric acid)	1.91 ± 0.016
Ascorbic acid (mg 100 ml^{-1})	233.8 ± 2.1
Colour values	
L*	13.65 ± 0.21
a*	0.60 ± 0.01
b*	1.54 ± 0.09
Total phenolics (mg 100 ml^{-1} as gallic acid)	253.4 ± 2.1
Total flavonoids (mg 100 ml^{-1} as catachin)	165.5 ± 1.5

Mean ± SD (n = 3).

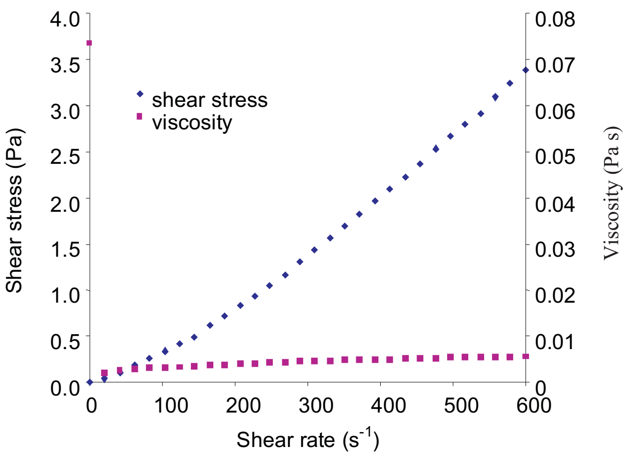

Fig. 1. Typical flow curve of enzyme clarified depectinated Indian gooseberry juice at soluble solid content 16.4°Bx and temperature 35°C.

Table 3 shows the parameters of Arrhenius equation determined by least square method and the correlation coefficient was greater than 0.9899. The flow activation energy (E_a) is defined as minimum energy required to overcome energy barrier before the elementary flow can occur and the viscous flow occurs as a sequence of events which are shifts of particles in the direction of shear force action from one equilibrium position to another overcoming a potential energy barrier whose height determines the free activation energy of viscous flow. The flow activation energy values increases with increase in soluble solid content of the samples, therefore temperature has a greater effect on the sample with higher soluble solid contents. When the temperature increases, the value of viscosity decreases because thermal energy of the molecules and intermolecular spacing increases (Rao *et al*., 1984).

T a b l e 2. Viscosity values of enzyme clarified depectinated Indian gooseberry juice at different soluble solids and temperature

Newtonian model $\sigma = \eta\gamma$			
Total soluble solids (°Bx)	Temperature (°C)	Viscosity (mPa s)	R
8.2	20	5.30 ± 0.037	0.9914
	35	4.73 ± 0.016	0.9936
	50	4.42 ± 0.015	0.9936
	65	4.11 ± 0.015	0.9949
	80	3.92 ± 0.015	0.9953
16.4	20	5.79 ± 0.017	0.9857
	35	5.14 ± 0.026	0.9917
	50	4.74 ± 0.024	0.9945
	65	4.38 ± 0.009	0.9944
	80	4.18 ± 0.008	0.9952
26.5	20	6.62 ± 0.015	0.9796
	35	5.88 ± 0.011	0.9878
	50	5.33 ± 0.011	0.9920
	65	4.92 ± 0.011	0.9933
	80	4.71 ± 0.008	0.9942
35.9	20	7.94 ± 0.018	0.9882
	35	6.87 ± 0.014	0.9797
	50	6.26 ± 0.013	0.9838
	65	5.76 ± 0.017	0.9883
	80	5.53 ± 0.017	0.9910

Explanations as in Table 1.

T a b l e 3. Parameters of the Arrhenius equation for enzyme clarified concentrated gooseberry juice at different soluble solids content

Soluble solids content (°Bx)	η_∞ (mPa s)	E_a (KJ mol^{-1})	R
8.2	8.81 ± 0.40	4.34 ± 0.122	0.9946
16.4	8.11 ± 0.17	4.76 ± 0.050	0.9947
26.5	8.30 ± 0.11	5.04 ± 0.036	0.9946
35.9	8.62 ± 0.08	5.37 ± 0.024	0.9899

Explanations as in Table 1.

The effect of soluble solids on flow activation energy (E_a) of enzyme clarified Indian gooseberry juice was described by two equations namely, power law and exponential type equations:

$$E_a = \mathrm{a}\,(C)^{\mathrm{b}}\,, \tag{3}$$

$$E_a = \mathrm{a}\exp(\mathrm{b}\,C), \tag{4}$$

where: a and b are constants, E_a is flow activation energy (J mol^{-1}) and C is the soluble solid content (° Brix).

The values of E_a and their corresponding concentration were fitted using Eqs (3) and (4) by the method of least squares to obtain estimations of the parameters of the model as shown in Table 4. It was found that the exponential model was better able to describe the effect of soluble solids on flow activation energy because correlation coefficient for exponential model (R> 0.9954) is high compared to that of power law model (R< 0.9875).

The concentration of the soluble solids and insoluble solids has a strong non-linear effect on the viscosity of the Newtonian fluids (Krokida *et al*., 2001). The viscosity of juice increases with increase in soluble solids concentration and it was due to the increase in hydrated molecules and hydrogen bonding with the hydroxyl groups of solutes. It is observed that, irrespective of temperature an increase in soluble solids content increases the viscosity. It is due to the concentration which increases the aggregation between the particles; this increases interaction between the particles. Two different equations were used to study the variation of the viscosity with soluble solid content at particular temperatures as described by several authors (Altan and Maskan, 2005; Juszczak and Fortuna, 2004; Juszczak *et al*., 2009):

power law:

$$\eta = \mathrm{a}\,(C)^{\mathrm{b}}\,, \tag{5}$$

exponential type:

$$\eta = \mathrm{a}\exp(\mathrm{b}\,C)\,. \tag{6}$$

The values of the parameters of both the equations at different temperatures were estimated using least square approximation method (Table 5). The exponential model gives a good fit compared to that of power law model, since the correlation coefficient values are from 0.9887 to 0.9951 for

T a b l e 4. Effect of soluble solids on flow activation energy (E_a) of enzyme clarified Indian gooseberry juice

Model	a (J mol^{-1})	b (brix^{-1})	R
$E_a = \mathrm{a}\,(C)^{\mathrm{b}}$	3 243.93 ±149.5	0.1384 ± 0.015	0.9875
$E_a = \mathrm{a}\exp(\mathrm{b}\,C)$	4 164.14 ± 66.9	0.0072 ±0.0006	0.9954

Explanations as in Table 1.

exponential model, where as 0.9377 to 0.9542 for power law model. Exponential model is suitable for describing the effect of soluble solid content on viscosity of enzyme clarified gooseberry juice. Similar results were reported for different juices (Juszczak *et al*., 2009; Kaya and Sozer, 2005). According to Krokida *et al*. (2001) the power law model tends to give good result in puree type liquid foods and the exponential one gives good results in concentrated juices. The parameter b has decreased with increase in temperature up to 65°C. This indicates that viscosity increases more slowly at higher temperatures up to 65°C as compared to that seen at lower temperatures. This may be due to the increase in mobility of the molecules and increase in inter molecular spacing. However between 65 and 80°C the parameter b value increased marginally. This deviation in parameter b may be due to small amount of starch present in clarified gooseberry juice and the increase in b value may be due to the gelatinization of starch which influences the viscosity. Several authors reported that the gelatinization temperature of starch is around 65°C and it was confirmed by means of rheological measurements (Singh and Singh, 2001; Yusup *et al*., 2003).

In order to design and modify processing machinery used for processing of fruit juices and their products, it is very useful to obtain a simple equation describing the combined effect of temperature and concentration on viscosity of Indian gooseberry juice. Figure 2 shows the combined effects of temperature and concentration on viscosity of clarified Indian gooseberry juice. The figure shows that at low temperature the effect of total soluble solid content on viscosity of gooseberry juice is high compared to that at higher temperatures. The following two equations were used to evaluate the viscosity of gooseberry juice for different of concentrations and temperature of the study. Different authors have used the following equations for describing the combined effect of soluble solids content and temperature on viscosity of clarified juices (Altan and Maskan, 2005; Ibarz *et al*., 2009; Kaya and Sozer, 2005):

exponential model:

$$\eta = \text{a} \exp (E_a/RT + \text{b}\, C), \tag{7}$$

power law model:

$$\eta = \text{a}\, (C)^{\text{b}} \exp (E_a/\text{R}T). \tag{8}$$

The values of viscosity shown in Table 2 were fitted to the above two equations by least squares approximation and the estimates of equation parameters are significant ($p \leq 0.05$). The Table 6 shows the model parameter for the two models tested. The correlation coefficient for exponential model (Eq. (7)) is 0.9907 where as power law type model (Eq. (8)) is 0.9641. This indicates that the exponential model is better to describe the combined effects of soluble solid content and temperature on the viscosity of enzyme clarified depectinated Indian gooseberry juice. Several authors have reported exponential model to better fit than that of power law type model for different juices at combination of concentration and temperature (Altan and Maskan, 2005; Ibarz *et al*., 2009; Kaya and Sozer, 2005).

In order to validate the model, the theoretical viscosities were calculated from Eqs (7) and (8) was compared with those obtained by experiment (Table 2), the both viscosities (theoretical and experimental) were compared by a linear equation:

T a b l e 5. Parameters of the power law model relating total soluble solids to viscosity of enzyme clarified Indian gooseberry juice at different temperatures

Temperature (°C)	a (mPa s)	b (Brix^{-1})	R
	Power law model $\eta = \text{a}\,(C)^{\text{b}}$		
20	2.782 ± 0.44	0.2800 ± 0.0051	0.9468
35	2.601 ± 0.27	0.2612 ± 0.0029	0.9542
50	2.550 ± 0.33	0.2387 ± 0.0036	0.9405
65	2.409 ± 0.19	0.2314 ± 0.0022	0.9377
80	2.266 ± 0.16	0.2373 ± 0.0019	0.9379
	Exponential model $\eta = \text{a} \exp (\text{b}\, C)$		
20	4.581 ± 0.25	0.01490 ± 0.0002	0.9918
35	4.143 ± 0.02	0.01389 ± 0.0002	0.9951
50	3.888 ± 0.27	0.01264 ± 0.0003	0.9898
65	3.623 ± 0.15	0.01252 ± 0.0001	0.9887
80	3.444 ± 0.11	0.01282 ± 0.0001	0.9887

Explanations as in Table 1.

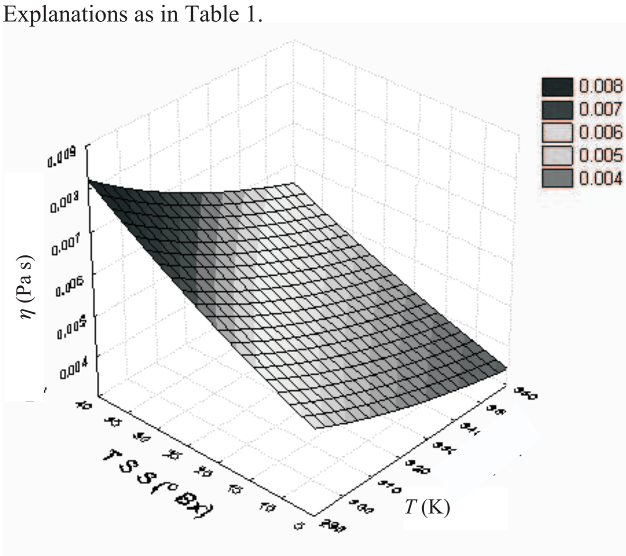

Fig. 2. Surface plot for combined effect of total soluble solid (TSS) content and temperature (T) on viscosity (η) of enzyme clarified depectinated Indian gooseberry juice.

T a b l e 6. Combined effect of temperature and soluble solid concentration on viscosity of enzyme clarified Indian gooseberry juice

Model equation	a	E_a/R	b	R
Exponential $\eta = a \exp(E_a/RT + b\,C)$	0.000604 ± 0.000004	599.33± 1.36	0.01362 ± 0.000076	0.9907
Power law $\eta = a\,(C)^b \exp(E_a/RT)$	0.000386± 0.000003	599.04± 1.27	0.25430 ± 0.001900	0.9641

Explanations as in Table 1.

T a b l e 7. Parameters to represent the goodness of fit for models $\eta_{\exp} = m\eta_{theo} + n$

Model equation	Slope (m)	Intercept (n)	R
Exponential	0.9892	5.99 10^{-5}	0.9908
Power law type	0.9788	9.73 10^{-5}	0.9643

Explanations as in Table 1.

$$\eta_{\exp} = m\eta_{theo} + n\,, \qquad (9)$$

where: $\eta_{\exp}$ – experimentally determined viscosity, η_{theo} – theoretically calculated viscosity. The validity of fit was determined based on slope (m) and intercept (n) of the linear equation, if slope (m) of linear equation tend to be 1 and intercept (n) tends to 0 the model describes as best fit between experimental and theoretical values. Table 7 shows the parameters of linear equation which was determined by linear regression analysis and shows that the slope (m) is 0.9892 and intercept (n) is 5.99 10^{-5} for exponential model where as 0.9788 and 9.73 10^{-5} for power law type model. This indicates that the exponential model (Eq. (8)) better describes the combined effect of soluble solid content and temperature on viscosity of enzyme clarified depectinated Indian gooseberry juice. The final combined equation is:

$$\eta = 6.04\,10^{-4} \exp(599.33/T + 0.01362C)\,. \qquad (10)$$

CONCLUSIONS

1. The enzyme clarified depectinated Indian gooseberry juice behave like Newtonian liquid

2. The magnitude of viscosities of Indian gooseberry juice was in the range from 3.92 to 7.94 mPa s depending upon total soluble solid content and temperature.

3. Effect of temperature on viscosities of Indian gooseberry juice was described by Arrhenius equation having flow activation energy from 4.34 to 5.37 KJ mol^{-1} depending upon the concentration of juice.

4. Effect of soluble solid content on flow activation energy and viscosity of Indian gooseberry juice was described by an exponential type relationship.

5. The combined equation in which dynamic viscosity depends on temperature and concentration is given by:

$$\eta = 6.04\,10^{-4} \exp(599.33/T + 0.01362C)\,.$$

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