Research Article

Evaluation the Effect of Enzymatic Process on the Edible *Aloe vera* Gel Viscosity Using Commercial Cellulase

Ali Izadi[1], Hamid Rashedi[1*], Reza Ghafarzadegan[2], Reza Hajiaghaee[2]

Abstract

Aloe vera is a plant of the Liliaceae family with a brilliant history in disease treatment. In addition to the pharmaceutical, cosmetic, and health care industries, this plant is used in food industry due to having various nutrients such as amino acids and various vitamins or anti-bacterial compounds. The purpose of this study was to investigate the effects of variables such as temperature, time, and cellulase concentration to reduce the gel viscosity of edible *Aloe vera*. Lowering the gel viscosity increases the gel condensation rate and spray dryer efficiency. So, In this study, the effects of different variables, including temperature, enzymatic process time and cellulase concentration was investigated on *Aloe vera* gel viscosity using a commercial cellulase enzyme. According to the results, temperature, process time and enzyme concentration are the affecting parameters on the gel viscosity. By increasing the temperature, without incorporating the enzyme, a sharp decrease in viscosity was observed so that in 55°C the viscosity decreased to 9.2 cP. By addition of constant amount of enzyme, the gel viscosity reached to 5.1 cP after 34 minutes, with 9.5 g E/100 g SG enzyme concentration, viscosity reached to the minimum value 4.2 cP. The results were statistically significant at the 5% probability level and indicate that the model was significant. Finally, the use of enzymatic processing for the production of *Aloe vera* concentrate or powder is appropriate because by reducing the gel viscosity, gel condensation rate will increase and it's drying is economically efficient.

Keywords: *Aloe Vera*, Cellulase, Enzymatic Process, Viscosity, Temperature

1. School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
2. Department of MedicinalPlants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran

* **Corresponding Author**
Hamid Rashedi
School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
E-mail: hrashedi@ut.ac.ir

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Introduction

Aloe vera is a plant with high therapeutic properties of which thousands of useful compounds are derived. Among different species of Aloe in the world, *Aloe barbadensis* has considerable properties. The gel obtained from this plant has a high viscosity, which its level depends on environmental conditions and the age of plant and typically it is varied from 31 to 52cp. The high gel viscosity causes problems in related industries. About 99% of gel of this plant has composed of water and the remaining 1% contains compounds such as polysaccharides, anthraquinones, chromones, vitamins, amino acids and proteins [1-4]. *Aloe vera* gel is fresh and colorless and has placed in the internal part of the plant while the latex is a yellow liquid and is placed in the vessel elements adjacent to shell of the plant. The latex is laxative, due to the presence hydroxyl anthracene compounds like anthraquinones [5]. The *Aloe vera* gel is highly viscose and this causes many problems in manipulating the gel. The use of enzyme processing is a suitable way for the reduction of gel viscosity.

Cellulase enzyme which is produced from various sources is a suitable enzyme and reduces the viscosity of the gel [6-7]. *Aloe vera* gel contains various polysaccharides such as Mannan, Galactan, Glucomannan, pectin compounds, and Glucuronic acid which all have their own therapeutic properties [8-14]. Among the polysaccharides present in *Aloe vera* gel, Acemannan is the most studied and analyzed polysaccharide due to its therapeutic properties. This polysaccharide is a long chain of acetyl mannose [15-19]. *Aloe vera* gel is commercially presented as a powder. The gel of this plant is used externally, for example, wound healing, burn shooting, etc., and dermal diseases and also applied for internal use such as treatment of coughing, constipation, peptic ulcer, diabetes, headache and immunodeficiency [20-21]. The use of cellulase enzyme has been studied for the breakage of polysaccharides and Aloin deletion process in gel at fixed temperature. Due to performance of enzyme processing, large polysaccharides in *Aloe vera* gel are broken into smaller molecules and as a result, molecular weight of the polysaccharide macromolecules will be reduced to below 80000 Daltons. The gel is condensed and then dried with different methods such as spray or freeze drying and then milling operations are performed for obtaining dry powder [22].

To inactivate the enzyme produced from *Trichoderma* fungus source, temperature of 80°C and time of 10 min are needed. According to previous studies, the activity of enzyme at 50-60°C which reduces from 80% to 10% and increasing the temperature to 80°C inactivates the enzyme completely [23]. The factors that effect on the activity of the enzyme are temperature, enzymatic processing time and concentration of enzyme [24]. In this research, effects of temperature, enzymatic process time and the concentration of cellulase enzyme on viscosity of the *Aloe vera* gel have been studied.

Material and Methods

Preparation of Aloe vera gel

In this research, the fresh and ripen leaves of the *Aloe vera* plant were prepared from *Department of Medicinal Plants Research Center, Institute of Medicinal Plants*. After washing leaves, the fillet was isolated from its shell and yellow latex containing Anthraquinones was washed with distilled water and the fillet wascompletely crushed with an extruder. The resulting gel was filtered for the isolation of coarse pulps, because the presence of these pulps causes error in measurement of viscosity. Since this gel contains many nutrients and is attacked by microorganisms, to prevent the growth of aerobic bacteria and also for avoiding the oxidation and browning the gel, 1% (w/w) of vitamin C was added to the gel.

Enzyme

Commercial cellulase enzyme was prepared from Biolife Company as a bright brown liquid. It was derived from Trichoderma and had the activity of 10-700 units/mg. Cellulase enzyme activity was measured with Bernfeld method and by preparing standard glucose curve and reading absorption of the sample prepared in wavelength of 540 nm [25]. Optimal condition for the function of cellulase enzyme was pH=3.5-6 and the temperature of 50°C. The enzyme was kept at 4°C before use.

Performance of enzymatic processing

To perform tests, 400 g of *Aloe vera* gel was used inside reactor suspended in water bath with stirring by a speed of 100rpm and cellulase enzyme was added to the gel in different concentrations. By considering that 0.5 g of dry solid is obtained from 100 g of gel, the effects of temperature, time of enzymatic processing and the concentration of cellulase enzyme on viscosity of the gel was investigated. Based on various studies and primary tests, these three variables are the most effective variables on enzyme processing. To measure the viscosity of the samples, temperature of the sample increased to 80°C for 10 min to inactivate cellulase enzyme and prevention of enzymatic process. Considering the equality of acidity of enzyme and gel (pH=4.5), this variable remains fixed during the reaction.

This reaction can be divided into two parts: viscosity reduction, due to temperature increase, and enzyme hydrolysis. To study the effect of temperature on viscosity, pure gel was used and changes in gel viscosity were studied. Then, cellulase was used with suitable temperature obtained from the previous step for enzyme hydrolysis to measure the reduction of viscosity due to enzymatic process.

Based on pretests conducted at 55°C, 7-10 gr enzyme/100 gr Solid Gel concentration had the highest effect on gel viscosity and for this reason; so the effect of enzyme process on viscosity of *Aloe vera* gel at 55°C and 7gr enzyme/100 gr Solid Gel concentration was measured. To study effect of concentration of cellulase enzvme, tests were performed at 55°C within 34 min.

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Viscosity

Viscosity of *Aloe vera* gel was measured after thermal processing and enzymatic hydrolysis by Brookfield DV-II+Pro viscometer.

Statistical analysis

All tests were performed in three replications and the statistical analysis was performed with Eviews7 software and linear regression model. Significance of data was studied in probability level of 5%. To draw the related diagrams, Excel software was used. *Aloe vera* gel viscosity changes were fitted in relation to variables of temperature, time and enzyme concentration of with third order linear equation.

(1)

$$vis = a + bx + cx^2 + dx^3$$

Where *vis* is the viscosity, a ،b and c are equation's coefficients and x is the studied variable (temperature, time and enzyme concentration).

Results

Effect of temperature on the viscosity of Aloe vera gel

According to Figure 1, the effect of temperature on gel viscosity were studied and viscosity changes due to increase of temperature were considerable. Findings indicate that viscosity is reduced due to the increase in temperature and when the temperature reaches 55°C, the viscosity is reduced to 9.2 cP and there was no change in viscosity after this temperature. The highest reduction of viscosity is in 35-45°C and above 45°C, the temperature effect was reduced gradually. In Table 1, the statistical analysis performed for studying the effect of temperature on *Aloe vera* gel has been shown and the results indicate the significance of model (like coefficient of determination, R^2=99%).

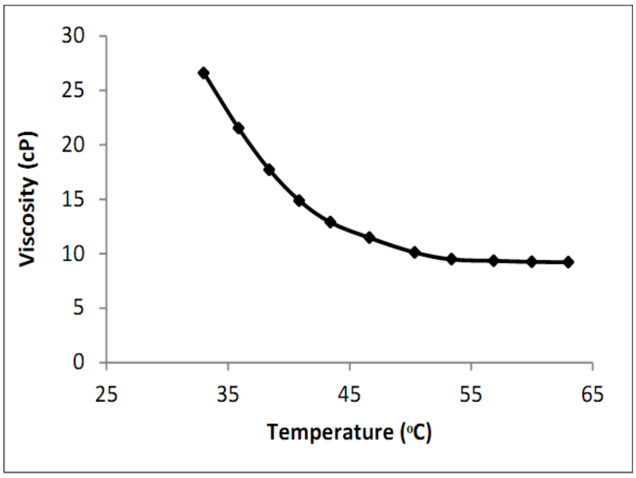

Figure 1. Changes in viscosity of *Aloe vera* gel in relation to temperature.

Table 1. Statistical analysis of temperature effects on *Aloe vera* gel viscosity.

Temperature (°C)	Viscosity (cP)	Parameter	Coefficient	Standard Error	Static-t
33	26.6	c	221.362	8.183	27.051
36	21.5	T	-10.955	0.532	-20.576
38	17.7	T^2	0.1891	0.011	16.719
40	14.8	T^3	-0.001	7.85E-05	-13.884
43	12.8				
46	11.5	**R^2**	0.99	**S.E. of regression**	0.17
50	10.1	**Adj R^2**	0.99	**F-Statistic**	4023.87
53	9.5				
56	9.3				
60	9.2				
63	9.2				

Effect studying of enzymatic process time on viscosity of Aloe vera gel

As shown in Figure 2, the viscosity of the *Aloe vera*gel is reduced with elapse of enzymatic process time by using of 7 gr cellulase enzyme/100 gr Solid Gel and constant temperature of 55°C until viscosity reaches 5.1 cP after 34 min. After this time, the gel viscosity slightly increased to a fixed value. In Table 2, results of the statistical analysis of the effect of time on viscosity have been shown and it is clear that the regression has been acceptable and the performed fitting is suitable.

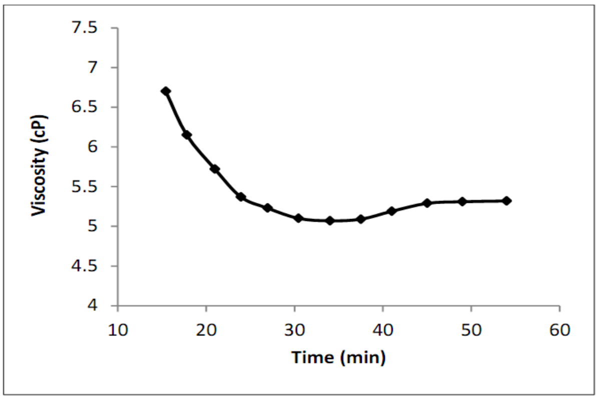

Figure 2. Changes in viscosity of *Aloe vera* gel in relation to time of enzymatic process.

Effect studying of concentration of cellulase enzyme on viscosity of Aloe vera gel

Considering Figure 3, increase in concentration of cellulase reduces the viscosity enzyme due to the hydrolysis of polysaccharides in *Aloe vera* gel and the release of water molecules. When the concentration of cellulase enzyme reaches to 9.5gr enzyme/100 gr Solid Gel, viscosity will reaches to its minimum level, 4.2 cP and after this point, the viscosity of gel will be increased partially. The obtained results can be fitted with the third order linear equation considering Table 3 and considering that coefficient of determination (R^2) and Adjusted coefficient of determination (Adj R^2) is 99%, fitting will be done well and the model is significant.

Table 2. Statistical analysis of reaction time effects on *Aloe vera* gel viscosity.

Time (min)	Viscosity (cP)	Parameter	Coefficient	Standard Error	Static-t
15.5	6.7	c	12.650	0.231	54.668
17.8	6.2	t	-0.586	0.023	-25.509
21.0	5.8	t^2	0.015	0.001	20.797
24.0	5.4	t^3	-0.000	6.77E-06	-17.306
27.0	5.2				
30.0	5.1				
34.0	5.0	**R^2**	0.99	**S.E. of regression**	0.31
37.5	5.0	**Adj R^2**	0.99	**F-Statistic**	897.22
41	5.2				
45	5.3				
49	5.3				
54	5.3				

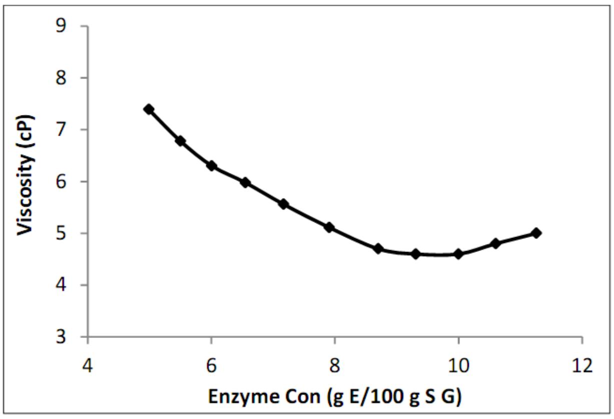

Figure 3. Changes in viscosity of *Aloe vera* gel in relation to concentration of Cellulase enzyme.

Discussion

In this research, the effect of temperature, enzymatic process time, and the concentration of cellulase enzyme on the viscosity of *Aloe vera* gel were studied. Considering that viscosity in liquids results from intermolecular forces and high viscosity indicates high intermolecular interactions. Due to temperature increasment, intermolecular bonds are reduced and macromolecular interaction is weakened and the viscosity of gel is reduced until the viscosity value is minimized at 55°C and after this point, intermolecular forces may not change or some intermolecular bonds are broken and some of them are enhanced and as a result, the viscosity will remain fixed.

At very high temperatures, thermal decomposition of the constituent parts of gel will occur which is not desirable and will have undesirable effect on properties of the gel. Based on the researches, polysaccharides will be less decomposed at temperatures below 80°C and times shorter than 1 hour. Gel decomposition will occur at temperatures above 90°C [26].

Table 3. Statistical analysis of cellulase concentration effects on *Aloe vera* gel viscosity.

Concentration (gr En/100 gr SG)	Viscosity (cP)	Parameter	Coefficient	Standard Error	Static-t
5.0	7.4	C	12.695	1.641	7.74
5.5	6.8	C	-0.971	0.645	-1.51
6	6.3	C^2	-0.058	0.082	-0.71
6.5	6.0	C^3	0.007	0.003	2.22
7.2	5.6				
8.0	5.1				
8.7	4.7	**R^2**	0.99	**S.E. of regression**	0.06
9.3	4.6	**Adj R^2**	0.99	**F-Statistic**	725.02
10.0	4.6				
10.6	4.8				
11.3	5				

By enzymatic processing, glycosidic bonds will be broken and polysaccharides will be decomposed and viscosity will be reduced. Cellulase enzyme completely decomposes the small pulps remaining in the filtered gel and homogenizes the gel. Reduction of viscosity continues until it reaches to the minimum value and then there is a partial increase in viscosity value which can be as a result of increase in intermolecular forces due to the inhibition of the product or substrate and as a result, it causes the viscosity to reach to a fixed value. Increasing the concentration of enzyme, enzymatic hydrolysis is enhanced by cellulase and viscosity of *Aloe vera* gel is reduced until viscosity is minimized in 9.5 gr enzyme/100 gr solid and then increasing of gel viscosity is observed. This increase of viscosity can also be as the result of creation of intermolecular bonds and creation of macromolecules or enzyme inhibition by the product or substrate. The use of enzyme process for improvement of physical properties of *Aloe vera* gel and other plant is a suitable and efficient method [27-28].

Conclusion

Variables of temperature, time of enzymatic process and concentration of cellulase enzyme are the most effective variables on viscosity of *Aloe vera* gel which is reduced due to increase of temperature because of reduction of intermolecular forces until it reaches to a fixed value. With elapse of time and increase of enzyme concentration, viscosity also decreases and there was partial increase in viscosity after reaching to the minimum point due to increase of intermolecular forces. Therefore, the use of enzymatic process is a suitable method for reduction of viscosity of *Aloe vera* gel.

Acknowledgements

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