Original Research Article

Enzymatic Clarification and Preservation of *Aloe vera* Juice by Ohmic Heating

ABSTRACT

Aims: The aim of this study was to optimize clarification process of the Aloe vera juice followed by its preservation by ohmic heating as no systematic study has been conducted on these aspects.
Study design: The enzymatic clarification method was used for clarification of Aloe vera juice by using the enzyme pectinase. The enzyme concentration, incubation temperature and time were optimized for clarification of juice. The Aloe vera juice was treated at different Time (min) gradients, current, initial temperature and after temperature at particular current gradient and the ohmic heated juice was then stored in sterilized bottles for further analysis.
Place and Duration of Study: Experiments were done in Department of Food Science and Technology, Shivaji University, Kolhapur, M.S. (India) and completed within 12 months.
Methodology: The optimal conditions for the enzymatic treatment of Aloe vera juice were investigated in order to minimize the turbidity of the juice and maximize the TSS of the juice. The clarified Aloe vera juice was then treated with ohmic heating at different time and current combinations and stored for 60 days to study the physic-chemical and microbial parameters of stored juice.
Results: The recommended enzymatic treatment conditions were: enzyme concentration 1% incubation time 6 h and incubation temperature 45^0C and the TSS, acidity and Turbidity under these conditions were 3.5^0Brix, 0.30% and 206.66 NTU respectively. During storage, increased in TSS value from 2.1 to 2.6^0Brix, acidity from 0.21 to 0.33 % were recorded in ohmic treated juice samples. A very high TPC ($102x10^5$ CFU/ml) and yeast and mold count ($68x10^5$ CFU/ml) was recorded in untreated sample at 30 days of storage whereas the juice samples treated with ohmic heating at different time and current gradients were observed to be within the limit of standard requirement of microbial quality even up to 60 days of storage.
Conclusion: Enzymatic treatments can reduce the turbidity in Aloe vera juice. Ohmic treatment at different time and current gradients can preserve the clarified juice with respect to its microbial quality for more than 60 days.

Keywords: *Aloe vera juice, enzymatic clarification, pectinase, ohmic heating*

1. INTRODUCTION

The Aloe plant is considered to be of *Asphodelaceae (Liliaceae)* family, which has numerous different species. Among these species, one variety '*Aloe vera*' has a medical reputation. It has another botanical name '*Barbadensis Miller*' which is used as a synonym. Aloe plant is very much prevalent in hot and dry climates. It is among the oldest known medicinal plants gifted by nature. The aloe plant has long (up to 20 inches long and 5 inches wide), triangular, fleshy mucilaginous leaves that have soft spikes along the edges. The fresh parenchyma gel from the center of the leaf is clear and has numerous medicinal and therapeutic properties[1].

The *Aloe vera* leaf gel contains about 98% water. On dry matter basis aloe gel consists of polysaccharides, sugars, minerals, proteins, lipids and phenolic compounds. The *Aloe vera* gel contains many vitamins including the important antioxidant vitamins A, C and E. Vitamin B1 (thiamine), niacin, Vitamin B2 (riboflavin), choline and folic acid are also present [2]. *Aloe vera* is basically used in various forms such as fresh gel, juice and other formulations for health, medicinal and cosmetic purposes. Chicago-based Mintel's Global New Products Database (GNPD) reports that more than 225 beverages containing *Aloe vera* were launched in various locations around the world in the year 2013[3]. *Aloe vera* drinks are gaining popularity internationally due to their beneficial health effects. Work has been initiated by many food scientists to incorporate *Aloe vera* as an ingredient for supplementation in various products such as tea, sparkling water, flavored water and juice. According to Korean Food and Drug administration (KFDA) functional health foods containing aloe when taken orally support immune function[3].

Aloe vera gel is a highly viscous and it must be properly treated before subsequent stabilization process. *Aloe vera* gel juice due to the presence of pectin substances produces turbidity, precipitation and other phenomena which seriously affect the stability of the product. Enzymatic clarification of *Aloe vera* gel offers the stability to the juice, retains the nutrients and unique flavor.

Conventional food heating methods require heat energy to be generated externally and then transferred to the food product by convection, conduction, or radiation. But that causes degradation of the outer portion. There is therefore considerable need for technologies that perform rapid, uniform heating those results in desired microbial lethality without altering or degrading the overall food quality[4].

Ohmic heating, or Joule heating or resistance heating is a type of electro thermal technique in which the food gets heated up by the passage of electric current. The food is heated due to the generation of heat energy by the passage of electric current. The amount of heat generated depends on the current induced by the voltage gradient and the electrical conductivity. Ohmic heating can be distinguished from other electro thermal techniques due to the presence of electrodes in contact with food, the frequency range and waveform[5]. Ohmic heating provides rapid and uniform heating and a high quality product with minimal changes of structure, nutrition, or organoleptic. Moreover, the use of ohmic heating for food processing is cleaner and more environmentally friendly[6]. One application of ohmic heating in the food production industry is inactivation of microorganisms (pasteurization and sterilization). Therefore, Ohmic heating is an alternative fast heating method for food processing[7].
Several researches have worked on different medicinal properties of *Aloe vera* leaves and other parts of this plant. The aim of this study was to optimize clarification process of the *Aloe vera* juice followed by its preservation by ohmic heating as no systematic study has been conducted on these aspects.

2. MATERIALS AND METHODS

The *Aloe vera* leaves were collected from botanical garden of Department of Botany, Shivaji University, Kolhapur (Maharashtra, India). The enzyme *pectinase,* extracted from malt were procured from Hi- Media Lab, Mumbai. Enzyme activity was 1:2000 I.P. Units. All the chemicals used in this investigation were of analytical grade.

2.1 Physico- chemical Analysis of *Aloe vera* Leaves

Aloe Vera leaves were evaluated for compositional parameters such as moisture, protein, fat, ash and carbohydrates following procedures as described by A.O.A.C. (1990)[8].

2.2 Extraction *Aloe vera* Juice

The leaves were washed under high pressure water. As base and tip will not contribute for juice they were removed with the help of sharp knife and leaves were separated into sections to facilitate pulp removal. The pulp was scooped out by removing rind of the leaves. Pulp of *Aloe vera* is then pressed through sterilized 4 layered muslin cloth and filtered through filter paper. Juice was stored at 4^0C.

2.2.1 Physico- chemical Analysis of Extracted Aloe vera Juice

Extracted *Aloe vera* juice was evaluated for some physico-chemical parameters. Total soluble solids (TSS) in the juice were recorded by using hand refractometer (A32 Erma, Tokyo) and the values were calibrated to 20°C with the help of temperature correction chart[8]. Acidity by titration method using 0.1% NaOH solution and pH of the juice was recorded by using pH meter (model 290A, Orion, Boston, Mass, Italy). Turbidity of the *Aloe vera* juice was estimated with the help of digital turbidity meter (EI model number 331).

2.2.2 Clarification of *Aloe Vera* Juice

The enzymatic clarification method was used for clarification of *Aloe vera* juice by using the enzyme pectinase. The enzyme concentration, incubation temperature and time were optimized for clarification of juice. Parameters such as TSS, acidity and turbidity of the clarified juice were used as a basis for optimization of enzymatic conditions.

2.2.2.1 Optimization of enzyme concentration

Aloe vera juice was treated with enzyme pectinase with varying concentrations viz. 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 % and kept for 4 h of incubation at room temperature. *Aloe vera* juice without any enzymatic treatment was considered as a control. After completion of incubation time, enzyme was inactivated at 60^0C for 4-5 min.

2.2.2.2 Optimization of incubation time

The optimized concentration (i.e. 1.0%) of pectinase was used to study the optimization of incubation time. To study the effect of incubation time, *Aloe vera* juice treated with optimized pectinase concentrations were hold for 2, 4, 6 and 8 hours at room temperature. In each juice sample after completion of particular incubation time, enzyme was inactivated at 60^0C for 4-5 min.

2.2.2.3 Optimization of temperature for clarification of juice

To study the effect of incubation temperature, the juice samples were treated with the optimized concentration (1%) and incubation time (6 h). Optimization of incubation temperatures such as 25℃, 35℃, 45℃ and 55^0C was carried out. After completion of incubation period enzyme was inactivated by above method.

2.3 Preservation by Ohmic Heating

A laboratory scale batch ohmic heating system was used to perform the experiment. The ohmic heating system comprises of ohmic heating cell with stainless steel electrodes, ammeter, voltmeter, coated thermocouple to record the temperature. The ohmic heating chamber, made of polytetrafluroethane, has a capacity of 650 ml. The *Aloe vera* juice was poured between the electrodes in the ohmic heating cell. In order to ensure a uniform temperature profile, the temperature was monitored in different points within the cell; like in the center of the cell and near the electrodes [9].

The Aloe vera juice was treated at different Time (min) gradients of 3 min, 5 min and 10 min, current was 0.5A, 0.25A and 0.15A, initial temperature to 18℃, 16℃ and 16℃, after temperature was 24-25℃, 19-20℃ and 21℃ for 3 min, 5 min and 10 min at particular current gradient as shown in Table 1.

Table 1. Combination of time and current applied for ohmic treatments

Time (min)	Current (A)	Initial Temperature (˚C)	After Temperature (˚C)
3	0.5	18	24-25
5	0.25	16	19-20
10	0.15	16	21

The ohmic heated juice was then stored in sterilized bottles for further analysis. The treated juice was filled in pre-sterilized glass bottle and analyzed for physico-chemical parameters such as TSS, pH and acidity at regular interval of 10 days and for microbial quality such as Total Plate Count, Yeast and Mold count. Therefore 1 ml of each sample was pour-plated in Plate Count Agar to enumerate mesophilic aerobic microorganisms after incubation at 30℃ for 72 h. Also, 1 ml of each sample was pour-plated in Orange Serum Agar to enumerate acid resistant bacteria after incubation at 30 ℃ for 3-5 days and 0.1ml of each sample was spread plated on plates to enumerate mould and yeasts after incubation at 25℃ for 3-5 days [10].

3. RESULTS AND DISCUSSION

3.1 Physico- Chemical Analysis of *Aloe vera* Leaves and Extracted Juice

Proximate composition generally represents the nutritional quality of product. Initially procured *Aloe vera* leaves were evaluated for their proximate composition viz. moisture, protein, fat, ash and carbohydrates and the results obtained were depicted in Table 2. It can be accessed from the Table 2 that *Aloe vera* leaf contain a very high moisture i.e. approximately 96.12% on fresh weight basis. Earlier investigations[11] indicated 90-98% moisture in *Aloe vera* leaf. The other constituent includes 6.4% protein, 2.6 % fat, 17.9% ash and 73.02 % carbohydrates on dry matter basis. These results were partially in accordance with the findings of Femenia *et al.* (1999)[12].

Table 2. Proximate analysis of *Aloe vera* leaves

Constituents	*Aloe vera* leaves*
Moisture (% fresh weight basis)	96.12±0.13

Protein %	6.4± 0.06
Fat %	2.6± 0.09
Ash %	17.9± 0.04
Carbohydrate %	73.02±0.02

**The values mean ±SD of three determinations; except moisture all parameters are determined on dry matter basis*

The *Aloe vera* juice was extracted from the leaves and analyzed for various physico-chemical parameters. TSS, acidity, pH and turbidity of the extracted juice were determined and presented in Table 3. TSS of the *Aloe vera* juice was observed to be 1.6^0Brix, acidity 0.21% and pH 5. *Aloe vera* juice observed to be more turbid with recorded turbidity 561 NTU. It confirms the earlier findings by Kaur *et al.* (2015)[13].

Table 3. Physico-chemical Parameters of *Aloe vera* juice

Parameters	***Aloe vera* juice***
TSS (^{0}Brix)	1.6±0.1
Acidity (%)	0.21±0.1
pH	5±0.2
Turbidity (1000 NTU)	561±12

****** The data are average of three replications*

3.2 Enzymatic Clarification of *Aloe vera* Juice

The enzyme *pectinase* was used for clarification of *Aloe vera* juice. The optimization of pectinase treatment was carried out for enzyme concentration, incubation time and incubation temperature. TSS, acidity, and turbidity of the treated juice were used as a basis for optimization of enzymatic conditions.

3.2.1 Effect of pectinase concentration on clarification of *Aloe vera* juice

The extracted *Aloe vera* juice was treated with different concentrations of pectinase ranging from 0.2 to 1.2% and their effect on various parameters viz. TSS, acidity, turbidity and colour value of clarified juice were studied. It can be accessed from the Table 4 that increased in pectinase concentrations observed to be increase the TSS and acidity of the juice whereas the turbidity was decreased. Significant increase in TSS from 1.6^0Brix to 3.2^0Brix and in acidity from 0.21% to 0.27% occurred when the enzyme concentration increased up to 1% level. Similarly up to this level of 1% enzyme concentration, significant decreased in turbidity was recorded i.e. from 561 NTU to 365 NTU. This indicated that the juice was significantly clarified with increased in *pectinase* concentration up to 1% level. This may be due to the pectinase acts on the pectic substances and breakdown into simple ones, which reduces turbidity and improves TSS[14]. In this enzymatic breakdown unesterified galacturonic acid units are released [15]. This may be the reason why there was increased in acidity of enzyme treated juices than in untreated one.

Similar results were reported in other fruit juices by Kyamuhangire *et al.* (2002)[16] who showed that treatment of banana pulp with enzymes resulted in increased in TSS in the juice. In other fruits like apple, pears, apricots and carrot juice also same trend were reported by Pilink *et al.* (1975) [17] and Mc Lellan *et al.,* (1985)[18].

Table 4. Effect of enzyme concentration on clarification of *Aloe vera* juice

Enzyme concentration (%)	TSS (^{0}Brix)	Acidity (%)	Turbidity at 1000 NTU
0.0	1.6±0.1^a	0.21±0.1^a	561±12^f
0.2	2.0±0.1^b	0.22±0.2^{ab}	510±8^e
0.4	2.0±0.2^b	0.22±0.2^{ab}	490±7^d
0.6	2.5±0.1^c	0.23±0.1^{ab}	470±5^c
0.8	3.0±0.1^d	0.24±0.1^b	440±10^b
1.0	3.2±0.1^d	0.27±0.1^c	365±5^a
1.2	3.3±0.2^d	0.28±0.1^c	360±6^a

In each column, means followed by the same superscript letter are not significantly different ($p \leq 0.05$)

3.2.2 Effect of incubation time on *Aloe vera* juice

Optimized level of enzyme concentration i.e. 1.0 % was selected to study the effect of different incubation time on clarification of juice. From Table 5 it is revealed that a significant increase in TSS and acidity and decreased in turbidity was observed up to the incubation time of 6 h. Further incubation period observed to be slightly affect these parameters. The results are in line with observations of Choudhari and Ananthanarayan (2007)[19] for incubation time in case of tomato tissues.

Table 5. Effect of incubation time on clarification of *Aloe vera* juice

Incubation time (h)	TSS (^{0}Brix)	Acidity (%)	Turbidity at 1000 NTU
2	2.9±0.1^a	0.23±0.02^a	420.66±6^a
4	3.2±0.1^b	0.27±0.0.2^{ab}	366±6.57^b
6	3.4±0.1^{bc}	0.28±0.02^b	287±5.57^c
8	3.5±0.1^c	0.29±0.02^b	279.66±4.51^c

In each column, means followed by the same superscript letter are not significantly different ($p \leq 0.05$)

3.2.3 Effect of incubation temperature on *Aloe vera* juice

After optimizing the process parameters viz. enzyme concentration and incubation time, the effect of incubation temperatures on the clarification of juice were studied.

Aloe vera juice were kept at different incubation temperatures viz. 25℃, 35℃, 45℃ and 55^{0}C by treating with optimized enzyme concentration (1%) and incubation time (6 h). Results depicted in Table 6 shows that increased in incubation temperature (up to 45^{0}C) increased the TSS and acidity of clarified juice but further increased in temperature decreased the TSS and acidity. Turbidity of the juice was observed to be decreased from 338.33 NTU at 25^{0}C to 206.66 NTU at the incubation temperature of 45^{0}C. But further increased in incubation temperature increased the turbidity. This may be due to denaturation of enzymes at high temperature. The temperature increases the rate of enzymatic reactions,

hence the rate of clarification, as long as the higher temperature adversely affects the activity of enzyme[20]. Previous researchers[16,21,22] had reported a similar phenomenon after adding commercial enzyme preparations to various fruit mashes at different temperatures and enzyme dosages.

Table 6. Effect of incubation temperature on *Aloe vera* juice

Incubation temperature (ºC)	TSS (^{0}Brix)	Acidity (%)	Turbidity at 1000 NTU
25	2.6±0.1^a	0.24±0.02^a	338.33±4.72^c
35	3.0±0.2^b	0.28±0.01^b	289.66±5.50^b
45	3.5±0.2^c	0.30±0.01^b	206.66±4.51^a
55	2.4±0.2^a	0.29±0.01^b	449.33±7^d

In each column, means followed by the same superscript letter are not significantly different ($p \leq 0.05$)

3.3 Effect of Ohmic Heating on Physico-chemical Properties of Stored *Aloe vera* Juice

The clarified *Aloe vera* juice was treated with ohmic heating at different time and current gradients and stored for 60 days. The stored juice samples were analyzed at regular interval of 10 days for their physico-chemical parameters. It can be assessed from the Table 7 that in all the ohmic heated samples of different combinations of current and time TSS and acidity of the juice was increased than the juice sample without treatment. Increase in treatment time and decrease in current improve the TSS value from 2.1 to 2.6^{0}Brix, which was higher than the sample without treatment. Similar trend for acidity was observed in all the treated samples. pH values decreased from 5.06 to 3.75, when samples were treated for 3,5,10 min. The probable reason for increase in acidity may be due to conversion of some sugars to acids [23]. The increase in acidity may also be attributed to increase in release of hydrogen ions during storage[24].

During storage it has been observed that the sample without treatment was spoiled and discarded after 30 days storage whereas no spoilage occurred in treated samples. In all the ohmic heated juice samples, the TSS and acidity were increased with increase in the storage period whereas the pH value was decreased with increased in storage period.

Table 7. Changes in physic-chemical properties of ohmic heated juice during storage

Storage Days	Time (min)	Current (A)	TSS (^{0}Brix)	pH	Acidity (%)
0	Control	----	1.60±0.1^a	5.06±0.02^d	0.21±0.01^a
	3	0.5	2.10±0.1^b	4.11±0.05^c	0.21±0.01^a
	5	0.25	2.22±0.1^b	3.81±0.02^b	0.22±0.01^a
	10	0.15	2.61±0.1^c	3.75±0.02^a	0.23±0.01^a
10	Control	----	1.53±0.1^a	4.80±0.01^c	0.22±0.01^a
	3	0.5	2.12±0.1^b	4.10±0.01^b	0.22±0.01^a
	5	0.25	2.20±0.1^b	3.71±0.01^a	0.23±0.01^a
	10	0.15	3.10±0.2^c	3.69±0.01^a	0.23±0.01^a

20	Control	----	1.53±0.1[a]	4.71±0.02[d]	0.23±0.01[a]
	3	0.5	2.23±0.1[b]	4.03±0.03[c]	0.24±0.01[a]
	5	0.25	2.41±0.05[c]	3.57±0.03[b]	0.25±0.01[a]
	10	0.15	3.42±0.03[d]	3.39±0.03[a]	0.25±0.02[a]
30	Control	----	1.41±0.08[a]	4.52±0.03[d]	0.24±0.01[a]
	3	0.5	2.31±0.07[b]	3.91±0.02[c]	0.25±0.01[ab]
	5	0.25	2.34±0.09[b]	3.41±0.02[b]	0.26±0.01[b]
	10	0.15	3.40±0.07[c]	3.23±0.04[a]	0.27±0.01[b]
40	Control	Sample was Discarded			
	3	0.5	2.36±0.03[a]	3.70±0.04[c]	0.32±0.02[a]
	5	0.25	2.72±0.09[b]	3.31±0.03[b]	0.35±0.01[a]
	10	0.15	3.83±0.08[c]	3.13±0.03[a]	0.42±0.03[b]
50	Control	Sample was Discarded			
	3	0.5	2.40±0.09[a]	3.20±0.09[b]	0.35±0.01[a]
	5	0.25	2.91±0.10[b]	3.11±0.04[b]	0.38±0.02[a]
	10	0.15	3.80±0.11[c]	2.91±0.05[a]	0.46±0.02[b]
60	Control	Sample was Discarded			
	3	0.5	2.51±0.06[a]	3.0±0.01[c]	0.40±0.02[a]
	5	0.25	3.03±0.11[b]	2.7±0.01[b]	0.46±0.01[b]
	10	0.15	3.91±0.12[c]	2.4±0.02[a]	0.53±0.03[c]

In each column of particular storage period, means followed by the same superscript letter are not significantly different ($p \leq 0.05$)

3.4 Effect of ohmic heating and storage on microbial quality of juice

The *Aloe vera* juice sample with and without ohmic treatment were stored in sterilized bottles and subjected to microbial analysis at regular interval of 10 days. Results presented in Table 8 indicated that at fresh conditions both the samples did not show any microbial growth. During storage, the sample without any ohmic treatment was observed to spoil at 30 days of storage. Compared to ohmic treated samples, a very high TPC ($102x10^5$ CFU/ml) and yeast and mold count ($68x10^5$ CFU/ml) was recorded in untreated sample at 30 days of storage. Whereas even at 60 days of storage, the samples treated with ohmic heating at different time and current were observed to be within the limit of standard requirement of total colony count ($78x10^5$ to $98x10^5$ CFU/ml) and yeast and mold count ($42x10^5$ to $85x10^5$ CFU/ml). This indicated that ohmic treatment can preserve the juice with respect to its microbial quality for more than 60 days. Similar effects of preservation by ohmic heating

were recorded in guava juice orange juice[25]. The main mechanism of microbial inactivation by ohmic heating is the thermal effect on destruction of membrane structure and enzymes of the microorganisms[26] regardless of the current effect[27]. In addition, most studies suggest that electroporation is the main non-thermal mechanism of cell death during ohmic heating which leads to pore formation in the membrane and changes in cell permeability[28].

Table 8. Effect of ohmic heating and storage on microbial quality of juice

Storage days	Time (min)	Current (A)	Total Plate count (CFU/ml)	Yeast and mold (CFU/ml)
0	Control	----	---	ND	ND
	3	0.5	ND	ND
	5	0.25	ND	ND
	10	0.17	ND	ND
10	Control	----	---	52×10^5	38×10^5
	3	0.5	12×10^5	8×10^5
	5	0.25	$8x\ 10^5$	$6x\ 10^5$
	10	0.17	$7x\ 10^5$	$4x\ 10^5$
20	Control	----	---	68×10^5	53×10^5
	3	0.5	$17x\ 10^5$	$12x\ 10^5$
	5	0.25	$12x\ 10^5$	8×10^5
	10	0.17	$9x\ 10^5$	6×10^5
30	Control	----	----	102×10^5	68×10^5
	3	0.5	$38x\ 10^5$	$27x\ 10^5$
	5	0.25	$25x\ 10^5$	$15x\ 10^5$
	10	0.17	$18x\ 10^5$	$11x\ 10^5$
40	Control	Sample was Discarded		
	3	0.5	$58x\ 10^5$	$51x\ 10^5$
	5	0.25	$34x\ 10^5$	$33x\ 10^5$
	10	0.17	$28x\ 10^5$	$21x\ 10^5$

Control	Sample was Discarded			
50	3	0.5	75x 10^5	65x 10^5
	5	0.25	58x 10^5	45x 10^5
	10	0.17	40x 10^5	32x 10^5
Control	Sample was Discarded			
60	3		98x 10^5	85x 10^5
	5	0.5	87x 10^5	67x 10^5
	10	0.25	78x 10^5	42x 10^5

4. CONCLUSION

Pectic substances contribute to turbidity in *Aloe vera* juice and enzyme pectinase with optimal enzymatic conditions can be used to clarify the *Aloe vera* juice. The present study concluded that total soluble solids, acidity and turbidity of *Aloe vera* juice are the functions of different enzymatic treatment conditions viz. enzyme concentration, incubation time and temperature. The recommended enzymatic clarification conditions for *Aloe vera* juice are 1% enzyme concentration at 45^0C for 6 h to achieve maximum TSS (3.5^0Brix) and acidity (0.3%) and minimum turbidity (206.66 NTU). Ohmic treatment at different time and current gradients can preserve the clarified *Aloe vera* juice with respect to its microbial quality for more than 60 days.

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