Effect of Conventional and Ohmic Pasteurization on Some Bioactive Components of *Aloe vera* Gel Juice

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ABSTRACT: *In this research the effect of conventional and ohmic pasteurization and storage time at different temperatures on some bioactive components of Aloe vera gel juice was investigated. Aloe vera gel juice was pasteurized conventionally and ohmically at 90 ºC for 1 min. The effect of pasteurization on vitamin C, total phenolic content and juice color was evaluated. The samples pasteurized conventionally, stored up to 30 days at 4 and 25 ºC. The effect of storage time on the stated components and also on glucomannan of the juice was evaluated. The results showed that pasteurization reduced vitamin C content and decreasing of vitamin C during ohmic heating was more than conventional heating. Total phenolic content increased during ohmic pasteurization more significant than conventional pasteurization. Browning index of samples after pasteurization increased but there were not any differences between browning index of samples pasteurized conventionally and ohmically. During storage at 4 and 25ºC, total phenolic content remained stable, but vitamin C contents reduced from 84.47 to 54.96 at 4ºC and 46.82 at 25ºC mg vitamin C/100 g d.m and glucomannan contents reduced from 2.11 to 1.77 at 4ºC and 1.71 g/L at 25ºC.. Browning index increased significantly at both storage temperatures, which was more intensive at 25 than 4 ºC.*

KEY WORDS: *Aloe barbadensis miller; Conventional and ohmic pasteurization; Shelf life; Functional properties*.

INTRODUCTION

Aloe barbadensis Miller, also known as *Aloe vera* (L.) Burm. f., or simply, is the most widely used and commercially available Aloe because of its nutritional and therapeutic properties [1] and has often been referred to as the miracle plant, healing plant, wand of heaven and plant of life [2]. It belongs to the Liliaceal [2] or Aloe family [3] and originated from warm and dry climates of Africa, and then transplanted to the Far East and Western hemispheres [4].

The *Aloe vera* leaf has two major liquids including Aloe latex produced by the bundle sheath cells of skin containing mainly anthraquinones, which are used as cathartics, and parenchyma cells containing mucilaginous gel [5]. This clear inner gel has over 75 bioactive components especially phenol compounds, vitamins, bioactive polysaccharide [1-2] Most researchers claim that the observed effects may be due to the synergistic actions of the 75 known ingredients [6]. *Aloe vera* gel

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has been utilized as a resource of functional foods, especially in healthy drinks and beverages, a medicine and also in the cosmetic and toiletry industries [7].

Thermal treatments are the most used methods to extend the shelf-life of liquid foods by the inactivation of microorganisms and enzymes. However, heat causes irreversible losses of nutritional compounds, undesirable changes in physicochemical properties, and alteration of their antioxidant properties [8].

Emerging technologies have been investigated to replace or complement conventional alternatives employed in processing of foods. Such technologies include ohmic heating, High Hydrostatic Pressure (HHP), pulsed electric fields, micro-wave heating, gamma irradiation and ultrasound. The main goal of these modern technologies is to ensure product microbial safety while preserving sensory and nutritional characteristics [6].

Ohmic heating (also referred to as Joule heating) is defined as a process which alternating electric current is passed through food with the primary purpose of heating it. Most foods contain ionic compositions such as salts and acids, therefore, electric current can be made to pass through food and generate heat inside it volumetrically. 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 [9]. One application of ohmic heating in the food production industry is inactivation of microorganisms (pasteurization and sterilization) [10-11]. Thereore, 0hmic heating is an alternative fast heating method for food processing [12].

The aim of this study was to investigate the effect of conventional and ohmic pasteurization and also storage conditions on some bioactive components of *Aloe vera* gel juice.

EXPERIMENTAL SECTION

Materials

All reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany) and all measurements were done in triplicate.

Sample preparation

Leaves of *Aloe vera* (*Aloe barbadensis* Miller) were provided by experimental farm of Tarbiat Modares University, Tehran, Iran. The homogenous leaves were selected according to size, ripeness, color, and freshness. Aloe latex (the yellow-colored liquid) was extracted by cutting the base of the leaves and allowing them to drain for 1 hour. The epidermis was separated; pulp cut into small pieces and blended in a mixer. The blended sample was filtered through a 4-fold cloth to remove every fiber and obtain a clear gel. pH of gel was adjusted to 3.5 with citric acid and kept under cool conditions in a refrigerator (4ºC) (for a short time) until thermal processing. After pasteurization, the best method was selected and the bioactive components were measured during storage at 4 and 25ºC.

Conventional and ohmic heating

Conventional pasteurization

Aloe vera gel juice (about 20 mL) was transferred into Pyrex tubes and thermally treated (pasteurized) in a water bath at 90 ºC for 1 min to ensure the inactivation of spoilage microorganisms [13].

Ohmic pasteurization

The present experiment was performed in a batch ohmic heater, shown in Fig. 1 which was similar to ohmic heater built by *Darvishi et al* [12]. Electrodes were inserted from the two openings of the Poly Tetra Fluro Ethylene (PTFE) cylindrical sample chamber, parallel to each others, the electrode gap (distance between the electrodes in the system) was 4.8 cm, and the diameter of the electrode was 5 cm. *Aloe vera* gel juice samples were poured into the ohmic heater through the thermocouple port. The sample was heated up to 90 °C using alternating current (AC) and stayed at this temperature for 1 min. Temperature at the geometric center of the sample was continuously measured with a K-Type and Teflon coated thermocouple to prevent interference from the electrical field.

Microbiological analysis

After pasteurization, samples were analyzed for numbers of mesophilic aerobic microorganisms, acid resistant bacteria, lactic acid bacteria, moulds and yeasts. Therefore 1 mL of each sample was pour-plated in Plate Count Agar to enumerate mesophilic aerobic microorganisms after incubation at 30 °C for 72 h. Also, 1 mL of each sample was pour-plated in Orange Serum

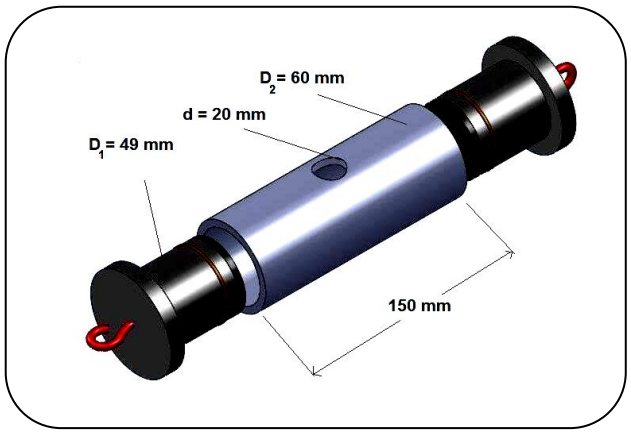

Fig. 1: batch ohmic heater.

Agar to enumerate acid resistant bacteria after incubation at 30 °C for 3-5 days and 0.1 mL of each sample was spread plated on plates to enumerate moulds and yeasts after incubation at 25 °C for 3-5 days [14].

Quality parameters

Vitamin C (L-ascorbic acid)

L-ascorbic acid was determined by the 2, 6 dichlorophenol-indophenol titration method according to AOAC (2000) [15] method no. 967.21. A total of 10 $\pm$ 0.1 g of *Aloe vera* gel was weighed and diluted to the volume of 50 mL. Vitamin C content is expressed as mg vitamin C/100 g dry matter (d.m.)

Determination of TPC

Total Phenolic Content (TPC) was determined colorimetrically using Folin–Ciocalteau reagent (FC) according to *Tezkan et al.* (2009) [16] and *Vega-Galvez et al.* (2011) [6] with some modifications. 300 µL aliquot of the Aloe extract solution was transferred to a glass tube; 1.5 mL of 10-fold-diluted Folin–Ciocalteu reagent and 1.2 mL of 7.5% of sodium carbonate (Na_2CO_3) solution were added. The sample was mixed for 30 s and the reaction proceeded for 90 min at room temperature. The absorbance was measured using the spectrophotometer at 725 nm and compared to the Galic Acid (GA) calibration curve. Results were expressed as mg GA/100 g d.m.

Color

The sample color was measured before pasteurization and during storage by Hunter- Lab Color Flex, A60-1005-654 45/0 model colorimeter (HunterLab, Reston, VA). Color value was expressed as L^* (whiteness or brightness/darkness), a^* (redness/greenness) and b^* (yellowness/blueness). Also, browning index (BI) (Eqs (1 a,b)) was calculated from the Hunter L^*, a^*, b^* values and used to describe the color changes during storage [17].

$$BI = [100 (X - 0.31)] / 0.17 \quad (1a)$$

$$X = (a^* + 1.75 L^*) / (5.645 L^* + a^* - 3.012 b^*) \quad (1b)$$

Aloe vera polysaccharide measurement

Preparation of Aloe vera Polysaccharide Standard

The *Aloe vera* polysaccharide standard was provided according to *Eberendu et al* (2005) [18] with some modifications. The mucilaginous parenchyma tissue of *Aloe vera* leaf was filleted, homogenized in a blender and centrifuged at 14000 rpm for 20 min to remove every fiber. The solution was further clarified by filtering through a Whatman 0.45 µm syringe filter to remove every insoluble particle. Clarified gel was transferred into dialysis tubing of 12000 Da molecular weight cut-off. After 48 h of continuous dialysis and several changes of water, the retained polysaccharide was freeze dried and stored in an airtight plastic container at room temperature. This process removed cell walls that contain insoluble fibers including pectin, inorganic salts, and low molecular weight compounds of <8000 Da, such as proteins which make up <2.5% (w/w) of Aloe gel powder. The resulting standard was calculated 100% water-soluble glucomannan.

Preparation of Aloe vera Polysaccharide Calibration Standard

The freeze-dried Aloe polysaccharide standard (15 mg) was weighed on an analytical balance and dissolved in 25 mL De Ionized (DI) water to the concentration of 0.6 mg/mL as stock solution. Standard solutions of 25 to 600 mg/L were prepared by diluting appropriate aliquots of the stock solution with DI water. Calibration standards were plotted according to *Eberendu and McAnalley* (1996) [19], aliquot samples (4 mL) of each concentration were transferred into test tubes. 5 mL of 0.2 M NaOH was added to each tube and slightly vortexed. Then one mL of 2×10^{-4} M aqueous Congo Red reagent solution was added and slightly mixed. The mixture was left at room temperature for 20 min before measuring the absorbance

at 540 nm using spectrophotometer (Spectronic_ 20 GenesysTM131, Illinois, USA). Calibration curve was plotted to give regression equation (Eq.(2)):

$$Y = a X + b$$

Where *Y*, *a*, and *b* are absorbance, slope and intercept, respectively.

Preparation of Sample

First, *Aloe vera* gel samples were filtered through a Whatman 0.45 µm syringe filter. One milliliter of every sample was diluted 4 times and transferred into test tubes. Next, 5 mL of 0.2 M NaOH and then 1 mL of 2×10-4 M aqueous Congo Red solution (sodium 4,4_-diphenyl-2,2_diazo-bis-1-naphtlamino-4-sulfonate) were added and slightly mixed. Finally, the absorbance of samples was measured according to the procedure previously described for the standard. The amount of bioactive polysaccharide of *Aloe vera* gel juice samples was determined using regression equation (2).

Statistical analysis

Results were analyzed by analysis of variance (ANOVA) using SAS 9.1 Statistical Software and the LSD test with 95% confidence interval was used to compare the means of the tests.

RESULTS AND DISCUSSION

Chemical and microbiological properties

The initial moisture content of *Aloe vera* gel juice was about 99.1%. Since the moisture content of this product is very high, so other components are in very low concentrations.

Microbiological assays

Microbiological assays showed that all pasteurized samples during storage at two temperatures exhibited appropriate levels of microbiological safety which should be lower than 1×10^2 CFU/g for both mesophilic aerobic microorganisms and moulds and yeasts. This value is considered as the acceptable limit for *Aloe vera* gel according to the World Health Organization (WHO) [20-21].

Quality assessment after pasteurization

Vitamin C

Fig. 2 shows the effect of thermal treatment on vitamin C content. Initial vitamin C content of *Aloe vera* gel (raw sample) was 81.42 mg /100 g d.m. Results indicated that there is a significant difference ($p < 0.05$) in vitamin C content between thermally treated and untreated (raw) gel juice samples but vitamin C retention after conventional pasteurization was igher thanh ohmic. pasteurization Ascorbic acid is a thermo labile nutrient. It is known that ascorbic acid degrades, following two consecutive or parallel pathways, aerobically and Anaerobically [6]. *Louarme et al* (2012) [22] reported that decreasing of ascorbic acid with aerobic mechanism occurred in presence of oxygen, metal ions, enzymes and sugars and with anaerobic mechanism occurred due to thermal heating. Decreasing of vitamin C content during pasteurization could be due to the high temperatures applied during pasteurization [23].

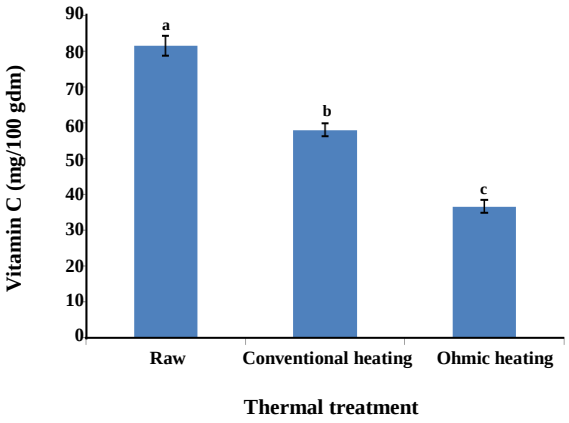

Fig. 2: The effect of thermal treatment on vitamin C content.

Asirry et al (2003) [24] reported that degradation of ascorbic acid under ohmic heating conditions may be broken down into the following phenomena:

(i) Chemical oxidation (either catalysed or uncatalysed) in the manner typical of most heating reactions.

(ii) Chemical degradation via the anaerobic pathway

(iii) Electrochemical degradation by reactions at the electrode.

They explained that in the presence of oxygen, oxidative degradation is the dominant mechanism exceeding the anaerobic degradation rate. The presence of metal ions catalyses the above reaction; in particular, ions such as Fe^{3+} and Cu^{2+} which may accelerate the reaction by several orders of magnitude. The third mechanism, electrochemical degradation is very important

during ohmic heating conditions and any differences in reaction rate between conventional and ohmic heating are likely due to this pathway.

They discussed some of the important reactions that may influence the degradation of ascorbic acid such as electrolysis of water, yielding molecular oxygen which can accelerate oxidation of ascorbic acid and electrode corrosion, either by direct metal oxidation (as shown below) or by electrochemical generation of corroding chemicals. In particular, for stain less steel electrodes, the following reactions could occur [24]:

$$2H_2O(l)\,(\text{Catode reaction})\,2H^+(aq)+2e^-\rightarrow H_2(g)$$

$$(\text{Anode reactions})\rightarrow O_2(g)+4H^+(aq)+4e^-$$

$$(\text{Overall reaction})\,2H_2(g)+O_2(g)\,2H_2O\rightarrow$$

$$(\text{Catode reaction})\;2H^+(aq)+2e\rightarrow H_2(g)$$

$$(\text{Anode reactions})\,Fe(s)\rightarrow Fe^{2+}(aq)+2e^-$$

$$Cr(s)\rightarrow Cr^+(aq)+3e^-$$

$$Ni(s)\rightarrow Ni^{2+}(aq)+2^{e-}$$

$$Mo(s)\rightarrow Mo^{3+}(aq)+3e^-$$

Therefore, it seems that the presence of three factors including high temperature, oxygen and metal ions all with together had made synergistic effect on degradation of Vitamin C of samples which were pasteurized ohmically.

TPC and color

Antioxidant capacity of fruit juices is related to the composition and concentration of bioactive compounds such as vitamins, phenols, carotenoids or flavonoids [25]. Initial TPC of *Aloe vera* gel juice (raw sample) was 418 mg GA/100 dm which is higher than the amount (96.81 mg GA/100 dm) reported by *Vega-Galvez et al.* (2011) [6]. The main reasons for differences in TPC among different fruit juices are the ripening degree and the environmental growing conditions of the fruits used for the formulation, as well as the storage time [26-27]. Absence of any fiber in the gel juice samples in comparison with their gel samples, which contained a lot of fibers, is another reason of this difference. After thermal treatment, two methods of pasteurization increased TPC but TPC of samples pasteurized ohmically was significantly higher than conventional heated samples (Fig. 3). The increase of TPC during heating

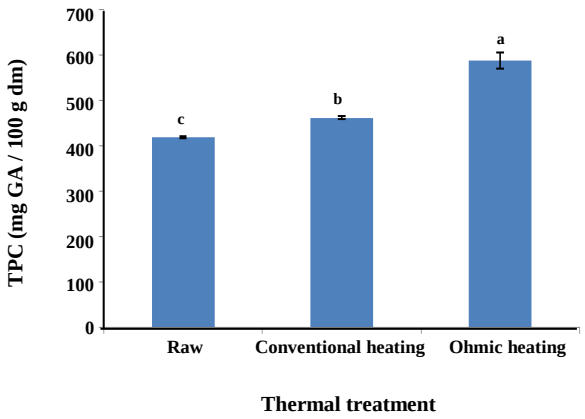

Fig. 3: The effect of thermal treatment on TPC.

could be attributed to increased extractability of antioxidant components due to the changes in the tissue matrix induced by high temperatures, resulting in the release of compounds with antioxidant activity from viscous gel to be exposed to Folin–Ciocalteu reagent [28]. *Girgin & El* (2014) [29] reported when cauliflower samples were steamed, phenol content increased by 20.36%, explaining this by enhanced extractability due to disruption of the polyphenol–protein complex. Phenols in vegetables also exist in both free and conjugated forms as they could be in soluble forms as well as in combination with cell wall components. The apparent increase in polyphenols was most likely due to disruption of complexes between polyphenols and proteins which result in better availability of these compounds to extraction from steamed cauliflower as compared with fresh one. Also, *Roy et al.* (2009) [30] stated that steam-processing can release more bound phenolic acids from the breakdown of cellular constituents. Therefore, it seems that during ohmic heating, alternative current has been synergistic effect on releasing TPC which could be exposed to Folin–Ciocalteu reagent.

Color is the first quality factor of a food that a consumer appreciates and has an important influence on its acceptance. Color is also an indicator of changes of foods that occur during storage or processing [31]. As shown in Fig. 4, BI of *Aloe vera* gel juice pasteurized at both methods increased and this increase was similar. In some fruit juice concentrates, the accumulation of brown color during thermal processing is attributed to enzymatic browning, but during storage, it is mainly

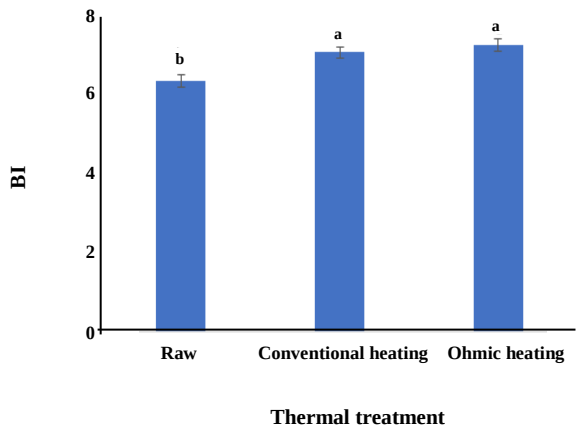

Fig. 4: The effect of pasturization on BI.

due to nonenzymatic browning, which include caramelization, ascorbic acid degradation and Maillard reaction [32]. *Khoshgozaran-Abras et al* (2011) [33] reported that the addition of *Aloe vera* gel into film-forming chitosan solution at higher levels yielded blend films with darker appearance (brown), which can be attributed to the anthraquinones oxidation from the skin, because they are difficult to be completely removed -34] .

Quality assessment during storage

Glucomannan

An assay for qualitative and quantitative determination of glucomannan, a bioactive polysaccharide which is the special polysaccharide of *Aloe vera* gel and Konjac [18] was used. This method uses a complex agent, namely Congo Red to react with β-1, 4 link of glucomannan present in the *Aloe vera* gel and gives a color change. The color change is compared with standard color changes and the difference between color changes is dependent on the amount of glucomannan. *Eberendu and McAnalley* (1996) [19] reported that this method can determine the amount of glucomannan available in the *Aloe vera* gel in the range of up to about 500 parts per million (ppm). In this study, the absorbance of color against the standard solutions of glucomannan (25-600 mg/L) was plotted, which showed a very good linearity (0.98) (Fig. 5a). *Eberendu et al* (2005) [18] used various activators, stabilizers and other ingredients to develop the linearity of the absorbance plot against glucomannan standard concentrations.

Glucomannan could not be spectrophotometrically measured before thermal processing because after NaOH addition, it precipitated alone or along with other colloids, which reached their isoelectric point and formed the yellow sediment. However, after pasteurization, this phenomenon did not occur because the thermal treatment probably changed the electrostatic charge of colloids, which prevented the sediment formation after NaOH addition. The results showed that the amount of glucomannan after pasteurization (time = 1st day) was 2.11 $\pm$ .035 g/L (288 $\pm$ 4.8 mg glucomannan/g d.m. of fresh gel juice) that is similar to the glucomannan content (268 mg glucomannan/g d.m. of fresh Aloe filets) reported by *Femenia et al* (2003) [35], but higher than the glucomannan content (113-139 mg glucomannan/g d.m of fresh Aloe filets) reported by *Rodrıguez-Gonzalez et al* (2011) [36]. Several factors such as geographic location, annual season, climate or exposure to light and applied irrigation treatment could be key factors to explain such differences [37]. It seems logical that *Aloe vera* gel without fibers has a higher amount of glucomannan than *Aloe vera* fillet because the fillet has a lot of fibers, but in the clear gel fibers were removed. The bioactive polysaccharide of *Aloe vera* gel decreased significantly during storage at both temperatures, which this decline was much higher at 25ºC than 4ºC (Fig. 5b). After 30 days storage at 4 and 25ºC, the glucomannan content decreased by 8.30% and 26% respectively, indicating three times higher losses at 25ºC than at 4ºC. *Chang et al* (2006) [38] reported that enzymes in *Aloe vera* gel juice lead to the degradation of the polysaccharide. After pasteurization of *Aloe vera* gel, its enzymes as well as some bacteria and fungi probably remained and produced enzymes that could hydrolyse glucomannan. Meanwhile, microbes can grow and produce enzymes at 25 ºC better than 4ºC resulting higher losses of the glucomannan.

Vitamin C

Vitamin C retention has been used as an indicator of fruit juice quality as well as a shelf-life marker for chilled fruit juices. When vitamin C retention decreases to 50% of its initial amount, shelf life ends [25]. The analysis of vitamin C content during storage showed that there is a soft deterioration at both temperatures. *Vega-Galvez et al* (2011) [6] reported that vitamin C of *Aloe vera* gel decreased during storage at refrigerator. The retention of vitamin C after 30 days of storage at 4ºC and 25°C were 65% and 55%, respectively indicating that vitamin C

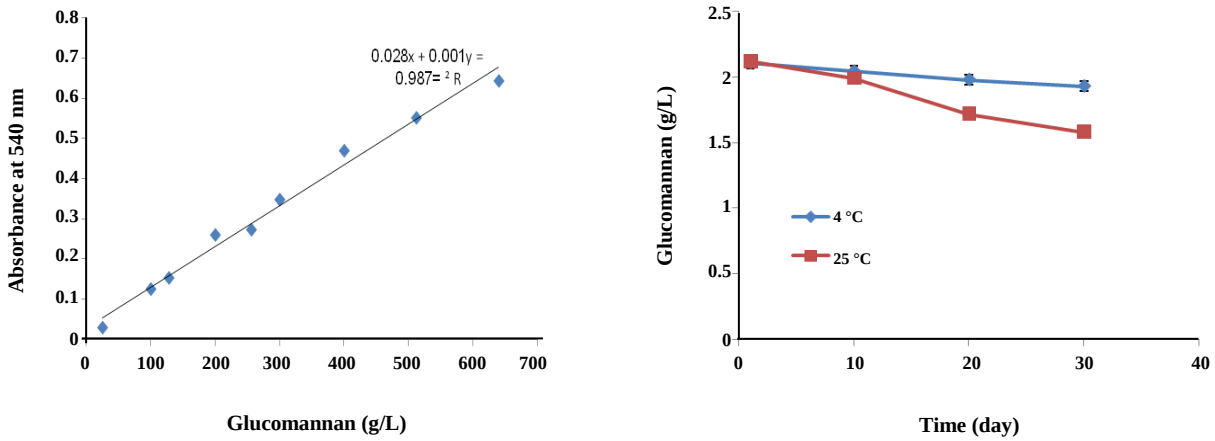

Fig. 5: a) Aloe polysaccharide standard curve, b) Effect of storage time at 4 and 25°C on the glucomannan of conventional pasteurized Aloe vera gel.

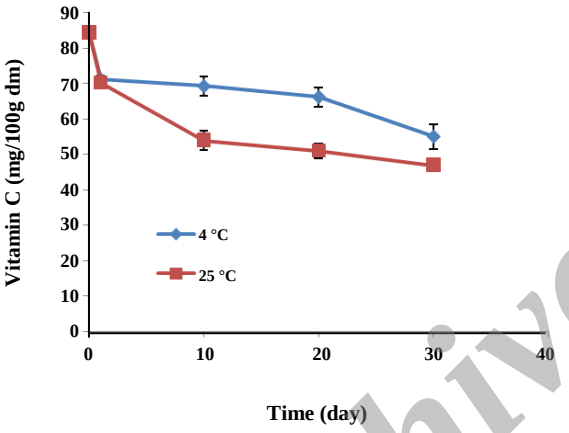

Fig. 6: Effect of storage time at 4 and 25 °C on vitamin C content (after conventional pasteurization).

degradation at room temperature is higher than refrigerated temperature (Fig. 6). Ascorbic acid retention in citrus juices after storage at 28ºC, 37ºC and 45ºC was about 54.5–83.7%, 23.6–27% and 15.1–20.0%, respectively due to the formation of Hydroxyl Methyl Furfural (HMF) which is one of the decomposition compounds of ascorbic acid degradation [23]. *Kabasakalis et al* (2000) [39] reported higher ascorbic acid losses of a commercial orange juice kept outside the refrigerator than in the refrigerator for 10 days. Similar results were reported by *Esteve et al* (2005) [31] that ascorbic acid of a Spanish orange juice varied with storage time more significantly at 10ºC than 4ºC.

TPC and color

During storage at two temperatures, there was not significant difference between samples (data are not showed). *Kevers et al*. (2007) [40] reported that the phenolic compounds of many fruits and vegetables remain stable during storage. It is shown that heat treatments applied to beverages may inactivate enzymes such as polyphone oxidase and as a result, TPC of the beverage remains stable throughout the storage period [25].

As shown in Fig. 7, BI of *Aloe vera* gel juice stored at both temperatures increased significantly ($p < 0.05$), but this increase was considerably higher at 25 than 4ºC. In some fruit juice concentrates, the accumulation of brown color during storage is mainly due to nonenzymatic browning, which include caramelization, ascorbic acid degradation and Maillard reaction [32]. Hydroxy Methyl Furfural (HMF) is one of the decomposition products of ascorbic acid, which are suggested to act as a precursor of brown pigments. *Burdurlu et al* (2006) [23] reported that after an eight-week storage, HMF content of citrus juice concentrates at 28 ºC increased more significantly than 45ºC which had a significant correlation with the ascorbic acid loss. Therefore, it seems that the most important reasons for color changes after heating were more vitamin C degradation, Mailard reaction and anthraquinones oxidation.

CONCLUSSIONS

The results showed that ohmic pasteurization reduced vitamin C content more than conventional heating

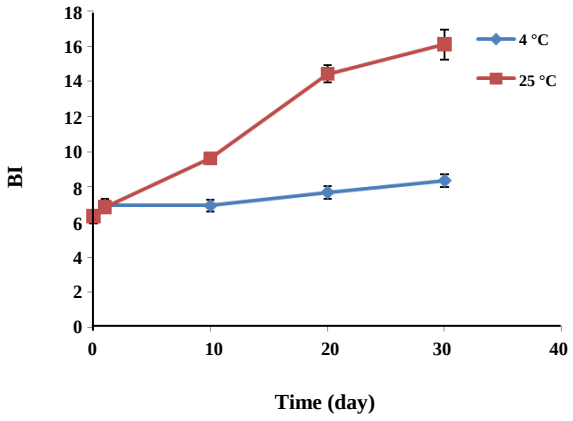

Fig. 7: Effect of storage time at 4 and 25ºC on BI (after conventional pasteurization.

because the presence of three factors including high temperature, oxygen and metal ions all with together had made synergistic effect on degradation of Vitamin C and therefore, ohmic pasteurization has been more destructive effect on vitamin C than conventional pasteurization. During ohmic pasteurization TPC increased more significant than conventional pasteurization. There were not any differences between BI of samples pasteurized conventionally and ohmically. As regard that ascorbic acid is a thermo labile nutrient and is known as quality factor and because its decrease during ohmic pasteurization was higher than conventional pasteurization, the changing of bioactive component of *Aloe vera* gel was studied only after conventional pasteurization. During storage at 4 and 25ºC glucomannan significantly decreased due to the hydrolysis of bioactive polysaccharide by enzymes. Vitamin C content decreased because of ascorbic acid degradation and Mailard reaction. Also BI was increased at both storage temperatures due to vitamin C degradation, Mailard reaction and anthraquinones oxidation. The rate of changes was much more considerable at 25 than 4ºC due to direct influence of higher temperature. Unlike the other components, TPC was stable probably due to polyphenoloxidase inactivation.

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