INFLUENCE OF LEAF AGE ON GEL RECOVERY AND HEATING ON QUALITYAND SHELF LIFE OF ALOE.
(*Aloe barbadensis* Miller)

BY

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B.Sc. (Horticulture)

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CERTIFICATE

Ms. B. AMARESWARI has satisfactorily prosecuted the course of research and that the thesis entitled "**INFLUENCE OF LEAF AGE ON GEL RECOVERY AND HEATING ON QUALITY AND SHELF LIFE OF ALOE (*Aloe barbadensis* Miller)**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by her for a degree of any university.

Date:
Place: Hyderabad

(Dr. M. PADMA)
Major Advisor

CERTIFICATE

This is to certify that the thesis entitled "**INFLUENCE OF LEAF AGE ON GEL RECOVERY AND HEATING ON QUALITY AND SHELF LIFE OF ALOE(*Aloe barbadensis* Miller)"** submitted in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE IN HORTICULTURE** of the **Andhra Pradesh Horticultural University, Venkataramannagudem**, is a record of the bonafide research work carried out by **Ms. B. AMARESWARI** under our guidance and supervision. The subject of the thesis has been approved by the Students Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

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DECLARATION

I, **B. AMARESWARI** hereby declare that the thesis entitled **"INFLUENCE OF LEAF AGE ON GEL RECOVERY AND HEATING ON QUALITY AND SHELF LIFE IN DIFFERENT ACCESSIONS OF ALOE (*Aloe barbadensis* Mille.)"** submitted to the Andhra Pradesh Horticultural University for the degree of **MASTER OF SCIENCE IN HORTICULTURE** is the result of original research work done by me. I also declare that any material contained in the thesis has not been published earlier in any manner.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	per cent
C	:	Centigrade
CD	:	Critical difference
CFU	:	Colony forming units
CRD	:	Completely Randomised Design
et al.	:	and others
Fig.	:	Figure
g	:	grams
i.e.	:	that is
cm	:	centimeter
mg	:	milli gram
Kg	:	Kilo gram
ml	:	milli litres
MT	:	Million tonnes
N	:	normal
nm	:	nanometer
NS	:	Non-significant
^{o}B	:	Degree brix
^{o}C	:	Degree Celsius
pp	:	page number
ppm	:	parts per million
RH	:	Relative humidity
SEm	:	Standard Error Mean
TSS	:	Total soluble solids
viz.,	:	namely
CMC	:	Carboxy methyl cellulose
AVLP	:	*Aloe vera* leaf powder
EDTA	:	Ethylene diamine tetra acetic acid
mM	:	milli Mohl
TCA	:	Trichloro acetic acid
TBA	:	Thiobarbituric acid
rpm	:	revolutions per minute
NaOH	:	Sodium hydroxide
Hrs	:	hours
TMC	:	Total mould count
TBC	:	Total bacterial count
CRD	:	Completely randomized block design
TBARS	:	Thiobarbituric acid reactive substances

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Date :
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ABSTRACT

Aloe is a succulent, sessile, perennial herb. It is as old as human civilization. It was introduced for ornamental and medicinal purposes. Aloe is widely recognized for containing a number of unique organic phytochemicals in its leaves that favour human health. In the most recent years, many studies have been conducted to evaluate its role to control or cure many human diseases.

An experiment was conducted to study the "Influence of leaf age on gel recovery and heating on quality, shelf life of Aloe gel". The treatments consisted of three accessions of Aloe *viz.,* yellow flowering accession-1, yellow flowering accession-2 and orange flowering accession-3 and three leaf maturity ages *i.e.,* 10 months, 12 months and 14 months. The treatments are replicated thrice in Completely Randomized Design with factorial concept.

In all the three accessions of Aloe, 14 months leaf age has performed better regarding all the physical and physico-chemical parameters like leaf size (553.45 cm^2), leaf weight (480.22 g), gel weight (321.56 g), pH (4.79), TSS (0.78°brix), acidity (0.16%), reducing sugars (0.050%), total sugars (1.84%), antioxidants (64.08% inhibition of peroxidation) and moisture content (90.23%) of gel. The best leaf age in the first experiment *viz.,* 14 months leaf age was selected for conducting the second experiment in all the three accessions. The gel obtained from the 14 months aged leaves of three accessions was subjected to heating at three temperatures *i.e.,* 50°C, 75°C and 100°C. The treatment samples were analysed at every 10 days during storage for the study of quality parameters, storage stability and microbial count in the gel.

During storage, increase in pH, reducing sugars and total sugars was observed in all the treatments while the acidity, TSS, non-reducing sugars, moisture and antioxidants were decreased at all storage intervals up to 30th day of storage.

Yellow flowering accession-1 heated at 75°C has recorded better results in pH (4.67), acidity (0.23%), TSS (1.30°brix) and antioxidant activity while the highest reducing sugars (1.853%), total sugars (2.55%) and moisture content (91.07%) was recorded by yellow flowering accession-1 heated at 50°C followed by the same accession heated at 75°C.

Increase in microbial growth was observed with the storage period in all three accessions at all the temperatures. But, comparatively less microbial growth was observed when the Aloe gel was heated at 75°C. The Aloe gel was stored up to 30 days when heated at 75°C with less microbial count while they could be stored up to 20 days at both 50°C and 100°C temperatures in three accessions with less microbial count.

The overall results indicated that yellow flowering accession-1 with 14 months leaf age heated at 75°C has showed good quality and storage stability of Aloe gel followed by yellow flowering accession-2 and orange flowering accession-3.

INTRODUCTION

CHAPTER I
INTRODUCTION

The herb Aloe is as old as human civilization. It belongs to the family "Liliaceae". The genus is found in Tropical and Southern Africa, Malagasey and Arabia. It was introduced into other parts of the world for ornamental purposes (Reynolds, 1985). Aloe was known to Indians for its medicinal value since time immemorial in the name of Ghrit kumaree or Kanyasara. It has documented evidence of being medicinally used in Vedas, Ayurvedic classics like Charaka Samhita, Susruta Samhita etc. as a laxative, liver tonic, wound healing agent and an adaptogen (Annonymous,1990).

The word 'Aloe' is derived from the Arabic word "Alloeh", means bitter and shiny substance. There are more than 400 identified species of aloe plants, of which a few have medicinal or economic value (Kawai *et al*., 1993). Several species of the genus aloe have been in use under the common name of aloe viz., *Aloe vera, Aloe barbadensis, Aloe ferox, Aloe chinensis, Aloe indica, Aloe peyrii* etc. Among these, *Aloe vera* Linn Syn. *Aloe barbadensis* Miller is accepted unanimously as the correct botanical source of aloe. In most of the references, *Aloe barbadensis* Miller is regarded as the correct name; but according to the WHO monograph (1999) *Aloe vera* (L.) Burm.f. is accepted as the legitimate name for this species. The Aloe is known as "Mussambar" in Indian market (Saroj *et al*., 2004).

Aloe species are perennial succulent plants characterized by lance shaped leaves with jagged edges and sharp points. It is commonly propagated through root suckers and grown in marginal soils (Singh *et al*., 1995).

Aloe is mainly cultivated for its thick fleshy leaves from which the yellow resinous latex or yellow sap or anthraquinones (the bitter yellow liquid between the leaf rind and gel) exudes and can be used as a laxative or purgative. The plant contains about 96 % of water and the remaining is a mixture of several chemical compounds.

The inner most part of the leaf is a clean, soft, moist and slippery tissue where water is held in the form of a viscous mucilage called gel (Newton, 2004). The gel is the rich source of polysaccharides, antioxidants, enzymes, minerals and vitamins (Chauhan *et al.,* 2007).

For centuries, this plant has been used for its medicinal and therapeutic properties. It has a history of use in folk medicine for treating skin and other disorders. In the food industry, it is being used as an ingredient for functional foods, mainly in the development of health drinks and beverages like tea, etc. Other applications of Aloe include healing of wounds and burns, minimizing frost bite damage, protection of skin damage from x-rays, lung cancer, intestinal problems, reducing blood sugar in diabetes etc.

Aloe gel is used as a potential source to develop a wide variety of functional food products and is an ingredient in other food products, health drinks, beverages, cosmetic and toilet industry. It is used in the food products like refreshing juice, ready-to–serve drinks, health drinks, sport drinks, diet drinks, soft drinks, laxative drinks, sherbets etc. The fleshy portion can also be converted into candies, squash,

jam, bar, munch etc. Additionally, it can be incorporated into dairy products eg., Yoghurt, curd, lassi, ice creams etc as a dairy alternatives. The gel can be dried using suitable drying techniques and the dried powder can be used in the development of various products. It is also used in pharmaceutical industry for preparation of ointments, gels, production of tablets and capsules (Hamman, 2008).

Today, the Aloe industry is flourishing and the gel is being used in many products, such as fresh gel juice and other formulations for health, medical and cosmetic purposes. But, the expanding Aloe industry urgently needs a way to regulate itself by developing test procedures and a reliable database, so that a product claims to be tested, verified and certified. These certification procedures reduce fraudulent claims, and also build consumer confidence in aloe products.

Leaf exudates of Aloe contain an array of chemical compounds. It contains aloins, a small amount of volatile oil, resin, gum, emodin, anthraquinone derivatives, chrysophanic acid and traces of coumarins. The active principle of Aloe is a mixture of glycosides called ‘aloin’ and a complex carbohydrate called ‘Acemannan’. The chief constituent of aloin is ‘barbaloin’ which is a glycoside of free anthraquinone compound called Aloe emodin. The purgative principle of Aloe has long been recognized as an anthracnose glycoside (Fair Bairn, 1949) and shown to be C-glycoside of Aloe-emodin anthrone (Birch and Donavan, 1955). Other major components of drug Aloe are 2,5-methyl chromone – C- glucosides, aloesin or aloe resin B; Aloe resin A and the aloesin aglycone, aloesone (Hordsworth, 1972).

The mucilage portion of the leaves contain glucose, galactose, mannose, galacturonic acid and protein with 17 amino acids. Aloe gel or Aloe pulp, a

commercially important constituent of mucilage is a polysaccharide with equal amount of uranic acid.

In Ayurveda, it is described as Bhedinee (purgative), Netrya (beneficial to eyes), Vrishya (promotes virility) Pheeharogaghana (in splenomegaly), Gratighna (cures glandular enlargement), Shwaassaghna (in anti-spasmodic, anti – dysphonic) (Satyavati *et al*., 1976). Besides its use in medicine and cosmetics, the extract of Aloe is used in the preparation of balms, skin creams, body lotions, bath soaps, talc and soft drinks.

The gel or pulp of fresh leaf is a remedy for intestinal worms in children and anti-dysentric, regular use of pulp is useful in prevention of abdominal tumours, dropsy, piles, sciatica, to cure kidney ailments, to enhance sexual excitement, treatment of tuberculosis, cancer and to cure initial stages of AIDS etc (Satyavati *et al*., 1976).

Aloe is a wonderful detoxifier. Its anti-cancer value has been demonstrated in various studies. It is also an immunomodulator which means, it will balance the immune system.

Aloe gel is highly susceptible to oxidation and when exposed to air, the gel rapidly oxidizes, decomposes and looses much of its biological activities (Coats, 1979). The leaves are to be harvested at the right age and cut exactly at right place on the plant to ensure the best gel (Chauhan *et al*., 2007). Wei *et al*. (2004) prepared a health beverage from fresh Aloe leaves. It is known that as the age increase, the size of the leaf increase and also gel content in the leaf. The composition of the gel also may change with increase of leaf age. Processing conditions like temperature, pH,

sucrose, vitamin C and citric acid, on the stability of colour and gelatinoids in Aloe juice showed that stability was negatively affected by increasing sucrose and citric acid concentrations while vitamin C and sodium chloride at low concentrations improved the stability (Jun *et al*., 2002). Heating of gel is an effective method of pasteurization and add better flavour (He *et al*., 2005). Gel heating may change the composition which also has effect on storage. Aloe gel can be stored for more number of days (up to 30 days) at 5°C without any deterioration in quality (Hemalatha *et al*., 2008).

Keeping in view the composition, importance and usage of Aloe gel, it is suggested to standardize the method in the first instance for production of gel to avoid the degradation of contents (Turner *et al.,* 2004). Hence, the present investigation "Influence of leaf age on gel recovery and heating on quality, shelf life of Aloe (*Aloe barbadensis* Miller)" is carried out with the following objectives:

1. **To quantify the gel recovery in different accessions of Aloe as influenced by the leaf age.**
2. **To study the quality components of gel in different accessions of Aloe as influenced by the leaf age.**
3. **To study the effect of heating on gel quality in different accessions of Aloe.**
4. **To study the effect of heating on storage life in respect of microbial content in different accessions of Aloe gel.**

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Aloe vera has gained popularity over the last several decades for its medicinal and nutritional properties. After a thorough reviewing of literature, it was observed that very limited attempts have been made for the use of heat treatments in aloe gel or pulp of the leaf particularly about storage life and microbial affects. Available literature in this area of research on Aloe is very limited. Hence, the main focus of this chapter is to review the literature pertaining to the influence of heating and age of the leaf on the quality components, storage life and microbial count of Aloe gel under the following heads.

2.1 Composition of Aloe leaves

2.2 Composition of Aloe gel

2.3 Age of the Aloe plant

2.4 Extraction of Aloe gel

2.5 Heating of Aloe gel

2.6 Physico-chemical parameters of Aloe gel

2.7 Storage of Aloe gel

2.1 Composition of Aloe leaves:

The leaf pulp was analyzed separately from the rind (Rowe and Park, 1941). The pulp was found to contain 98.5% of water and its alcohol insoluble portion was mucilage with a content of uranic acid, fructose and hydrolysable sugars. Enzymes such as an oxidase, a catalase and amylase were reported to be present, but tannins, pectins and vitamin A and D were absent. Similarly, Roboz and Smith (1948)

examined the chemical components of the gel scrapped from the leaves after the exudates had been drained off. The crude gel had an ash content of 12.9% removed by dialysis. White coloured water soluble mucilage was purified which on hydrolysis was found to contain equal amounts of glucose and mannose as the main constituents, with a small amount of uranic acid (23.7%). They also reported that some aloin was present in the crude gel, despite draining off the exudates from the leaves.

The compound was known as barbaloin and finally characterized as 10β-D-glucopyranosyl -1, 8 dihydroxy-3 hydroxy methyl-9 (10H)-anthracenone (Hay and Haynes, 1956).

Several free anthraquinones are reported in roots and leaves of Aloe species (Fairbairn and Simic, 1963).

According to Tyler *et al.* (1976), the term '*Aloe*' is derived from the Arabic word '*alloeh*' which means a shining bitter substance with reference to the exudates.

Aloe-emodin is a typical leaf constituent and is a wide spread anthraquinone in this genus. The anthraquinones in leaves may be present as O-glycosides like aloe emodin-11-O-rhamnoside and Nataloe-emodin-2-O-glucoside. Chrysophenol was another anthraquinone which is a common constituent of both roots and leaves (Yagi *et al.*,1977).

Aloe plicatalis (L.) Miller. was found to contain a single type of unbranched acetylated glucomannan (Paulsen *et al.,* 1978).

Later the work in Aloe involved in the separation of the gel carbohydrate polymers into their polysaccharide components. Gowda *et al.* (1979) separated the gel polysaccharides from the *Aloe vera* plant into four partially acetylated glucomannans,

the whole leaf having an average glucose and mannose ratio of 1:6, although the individual ratios varied from 1.5:1 to 1:19. The molecules were linear with β-(1,4)-linkages between the sugar units. They also found traces of galacturonic acid, galactose, xylose and arabinose.

A study by Mandal and Das (1980) on Aloe showed quite a different constitution. The principal component of the *Aloe vera* fillet was a pectic substance containing mainly galacturonic acid and was accompanied by lesser amounts of a galactan and non-acetylated glucomannan. They suggested that the apparent chemical differences were due to the existence of different plant types within the species and seasonal variations. Plants harvested in April were found to contain 85% galacturonic acid, while in October of the same year contained only 70% of galacturonic acid. Mandal *et al.* (1983), hydrolyzed purified pectic acid from *Aloe barbadensis* plants and obtained an acidic oligosaccharide with a 1:5 galacturonic acid and acidigalactose ratio. The main chain consisted of β-(1, 4)-linked galacturonic acid residues.

The pyridine alkaloid, γ-coniceine was reported to occur in six species of aloe whereas coniine was found in *Aloe viguieri* (Dring *et al.*, 1984).

According to Yagi *et al.* (1984), *Aloe saponaria* gel had two polysaccharides. One is an acetylated linear mannan and the other being acetylated branched glucomannan with a glucose and mannose ratio 1:19.

Groom and Reynolds (1987) examined 68 aloe species for barbaloin levels in their exudates (latex). They found that most of the exudates contained barbaloin content between 10-20%. *Aloe ferox*, the species from which drug aloes are generally

obtained, recorded a relatively low barbaloin content of 9.7%. Among the 68 species compared, *Aloe perryi* Baker recorded the highest barbaloin content of 23.5%.

Anthrones are by far the most important of all the classes of compounds present in Aloe. The most outstanding members of this class are aloin A and B which are collectively known as Barbaloin. They were first isolated from *Aloe vera* (formerly Barbados aloe or *Aloe barbadensis*) (Manitto *et al.,* 1990).

A prominent feature of *Aloe vera* fillet is its high water content, ranging from 98.5 to 99.5% of fresh matter. More than 60% of the solid being made of polysaccharides (Mc Annalley, 1990).

Screening of aloe for alkaloids showed that it is positive (21%) for presence of alkaloids viz., N-methyl tyramine and O, N-dimethyl tyramine (Nash *et al.,* 1992).

According to Yaron (1993), the polysaccharides constituted 0.2 to 0.3% of the fresh gel and 0.8 to 1.2% of the dry weight.

According to Davis *et al*. (1994) Aloe is a rich source of polysaccharides of which acemannan and mannose-6-phosphate were the major constituents.

Aloin A and B (Barbaloin) is two diastereomeric C-glucosides that differ in the configuration at C-10 of the aloe emodin anthrone moiety (Zonta *et al.,* 1995).

The term Aloe refers to the dried latex extracted from the leaves of several species of Liliaceae plants like *Aloe ferox*, *Aloe arborescence*, *Aloe perryi* etc, (Zonta *et al*.,1995).

The contribution of aloin, aloe resin A and aloesin in total was 70 to 97% to the total dry weight of leaf exudates with a geographical variation in the aloin content ranging from 9.5 to 31.2% (Van Wyk *et al.,* 1995). A difference in aloin concentration

between leaves within the same plant has also been reported. The highest concentration was found in leaves just below the apex of the plant i.e., younger leaves and lowest in the basal leaves (Okamura *et al.*, 1996).

The yellow exudate from the inner epidermal cell layers was known for its purgative activity due to presence of many phenolic compounds (Okamura *et al.*, 1996).

The concentration of aloin and other closely related anthrone C-glycosides have also been shown to be highest in the top third of a leaf and lowest at its base in *Aloe mutabilis* and *Aloe hereroensis* (Chauser Vlfson and Gutterman, 1997).

The bulk of the gel is mucilage of a polysaccharide nature with smaller amounts of various other compounds. A variety of other organic and inorganic substances were reported to occur in aloe, particularly in the gel of *Aloe vera*. The inorganic minerals in aloe gel were found to be the ions of Ca, Mg, Zn, Fe, Cu, etc. In addition, four saccharides viz., galactose, glucose, mannose and xylose along with 20 amino acids, vitamins viz., B_1, B_2, B_6, B_{12} and C, and enzymes such as amylase, lipase and folic acid were also found (Davis, 1997).

Joshi (1998) studied the chemical constituents and biological activity of *Aloe barbadensis*. He revealed that the mucilagenous jelly isolated from fresh leaves of *Aloe barbadensis* is reported to be a mixture of polysaccharides containing mainly a gluco galactomannan with linear monosaccharide units linked by 1,4 bonds. The polysaccharides constitutes 0.2-0.3% of fresh gel (0.8-1.2% of the dry matter content) posses a wide range of pharmacological activities.

Acemannan is commercially known as Carrysin™, which is a linear polysaccharide composed of β-(1, 4)-linked mannosyl residues, with C_2 or C_3 acelated and some side chains of galactose attached to C_6 (Femenia *et al.,* 1999).

A complete chemical characterization of *Aloe vera* plant (*Aloe barbadensis* Miller) was studied by Feminia *et al.* (1999). According to them, the composition of the main type of the polysaccharides present in the aloe alcohol insoluble residues was mannose and cellulosic glucose (95%). The study also showed that the amount of soluble sugars differed depending on the plant portion of aloe. The skin contained 11.22% of soluble sugars, whereas the fillet and gel contained 16.48% and 27.81% soluble sugars respectively.

The review on the chemistry of aloe species indicated that nearly 137 chemical structures of various compounds were identified in aloe exudates (Dagne *et al.,* 2000). Further, these compounds were grouped into alkaloids, anthraquinones, anthrones, benzene, naphthalene and furan derivatives, chromones, coumarins, pyrans and pyrones, flavonoids, sterols and some miscellaneous compounds.

Further, it was reported that the two main types of anthraquinones present in the roots of Aloe are 1,8-dihyroxy anthraquinone and 1-hydroxy-8-methyl anthraquinone (Dagne *et al.,* 2000).

It is believed that the biological activity of aloe comes from a synergistic action of all these compounds rather than one single ‘magic bullet’. If a single compound is sought to account for the efficacy of *Aloe vera* gel, certainly the polysaccharide-Acemannan is the one that is currently a focus of great deal of attention (Dagne *et al.,* 2000).

These anthrone compounds are believed to be mainly responsible for the bitter and purgative properties of the well known commercial aloe drug (Choi and Chung, 2003) which is principally made up of the leaf exudates of *Aloe ferox* and *Aloe vera.* According to Choi and Chung (2003), the saccharides present in *Aloe vera* are cellulose, glucose, mannose, aldopentose, acetylated mannan (Acemannan), glucomannan, acetylated glucomannan, galacto-galacturan, gluco galactomannan and galacto gluco arabinomannan.

Similarly, Ni *et al.* (2004) isolated and characterized the structural components of *Aloe vera* leaf pulp. They subjected the liquid gel to alcohol precipitation and stated that polysaccharides are alcohol insoluble and their percentage was found to be 7.5% of dry pulp.

Jiang Mei Han, (2004) studied the main chemical components of *Aloe vera* var. *chinensis* leaves and gel, and processing technology for aloe beverages. Higher contents of water and crude polysaccharides, and lower contents of total sugar, reducing sugar, vitamin C, protein, crude fat, and aloin were observed in aloe gel when compared with whole leaves. Optimal processing conditions for aloe beverages included the use of heating- crushing method for extraction of aloe juice, 8% aloe juice, 0.08% compound stabilizer, 6% sucrose, 0.18% citric acid, 0.05% agar and 0.15% CMC (carboxymethylcellulose) – Na.

However, Chow *et al.* (2005) isolated the polysaccharides by alcohol precipitation of *Aloe vera* mucilaginous gel and reported the ratio of mannose : glucose : galactose : galactose-A : fructose : arabinose : xylose as that of 120:9:6:3:2;2:1 with traces of rhamnose and glucose-A.

Chauhan *et al.* (2007) studied the therapeutic and food application of *Aloe vera* and reported that Aloe industry is flourishing and the gel is being used in many products, such as fresh gel, juice and other formulations for health, medicinal and cosmetic purpose.

Growing concern with environmental factors in human health over the last few years has aroused renewed interest in the trace elements. Rajendran *et al.* (2007) studied and analyzed trace elements in Aloe gel and revealed the biological importance. It was found that Aloe gel is rich source of trace elements like Mg, Mn, Ca, Cd, Zn, Fe, Al, Na, K and all these trace elements present in *Aloe vera* have a significant role in anti-diabetic activity.

Hamman (2008) reported that many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides contained in the gel of the leaves. These biological activities include promotion of wound healing, antifungal activity, hypoglycemic or anti diabetic effects, anti - inflammatory, anticancer, immunomodulatory gastro protective properties, enhancement of the intestinal absorption and bioavailability of co-administered compounds as well as enhancement of skin permeation. In addition, important pharmaceutical application such as the use of *Aloe vera* gel powder as an excipient in sustained release of pharmaceutical dosage forms.

Ramchandra and Srinivasa Rao (2008) reported that appropriate processing techniques should be employed during processing in order to extend the use of *Aloe vera* gel. Unfortunately, because of improper processing procedures, many of these so called Aloe products contain, very little or virtually no active ingredients namely

mucopolysaccharides . There is known wide spectrum of biological activities possessed by the leaves of the *Aloe vera* and it has become imperative because of the wide spread use, the leaf should be processed with the aim of retaining essential bio active compounds.

Smita and Pratima (2007) standardized the process of making *Aloe vera* leaf powder (AVLP) and studied its nutritional and physico-chemical characteristics. It was reported that recovery rate of AVLP was 3.2%. AVLP contained 18.5 % crude fiber, 4.8% crude protein, 2.2% crude fat, 14.0% total ash, 48.0% carbohydrate and 23 K cal energy value. It contained substantial amounts of iron (64.8 mg/ 100g) ascorbic acid (27.0mg/100g) and β-carotene (335.8mg/100g).

2.2 Composition of Aloe gel

The purgative principle in aloe gel was recognized as an anthracene glucoside (Fairbairn, 1955) and showed to be a C-glycoside of aloe emodin anthrone (Birch and Donavan, 1955).

Polysaccharides from the gel of other aloe species have been found to contain similar components. Thus, polysaccharides containing galacturonic acid (Ovodova *et al.*, 1975) or mannose (Yagi *et al.*, 1977) have been described in the gel from *Aloe arborescence*.

Aloe gel products appeared to contain anthraquinone and related compounds viz., Barbaloin and aloe emodin in sufficient quantities to act as false substrate inhibitors blocking prostonoid synthesis, since they have a similar chemical structure of prostaglandin substrates (Heggers and Robson, 1983). During commercial extraction of *Aloe vera* gel, it is virtually impossible to prevent contamination by the

leaf exudates as the leaves are cut. Additionally in intact leaves, anthraquinones and their derivatives may diffuse into the gel from the bundle sheath cells.

Chandegara and Varshney (2005) reported that Aloe gel contains 99.8 per cent water or moisture content, 0.2 per cent fibre content, pH of 6.4, total sugar content (1.91 %), reducing sugar (0.026 %) and TSS content (0.93° brix).

2.3 Age of the Aloe plant

Hu Yun Xu Juan Hu QiuHui (2003) reported that three-year-old *Aloe vera* contained significantly higher levels of polysaccharides and flavonoids than two- and four- year-old Aloe, and no significant differences in flavonoid levels were found between three - and four –year - old Aloe. All the aloe extracts showed significant antioxidant activity. The antioxidant activity of Aloe vera extracts and reference compounds followed the order.

2.4 Extraction of Aloe gel

Chandegara and Varshney (2005) reported that the extraction of gel from *Aloe vera* leaves by centrifugation should be carried out at 5°C temperature, 10,000 rpm speed with 30 minutes duration of centrifuge, without addition of acetone to pulp so as to obtain higher gel recovery (50.17%) and good quality of gel.

Aloe gel extractor was developed at Department of Agricultural Process Engineering, Dr. PDKV, Akola, Maharastra, India. It consisted of shaft cum roller, bearing and housing frame and discharge chute and a transmission unit. The cleaned leaf was fed between the roller and rotation of the handle causes the gradual expression of the gel. Extraction capacity of the machine was found to be 3.923 kg / h of leaves giving 91.20% extractability and recovery of gel was 52%. Extracted gel

was preserved by three different methods *viz,* freezing, chemical addition and dehydration.

Roy *et al.* (2007) found that the gel yield is 0.399kg gel/kg of raw leaves. Moisture and ash content of gel was found to be 97.86% and around 15% respectively. During extraction, a little variation of P^H with temperature was observed.

2.5 Heating of Aloe gel

Use of tannic acid and methanol for producing an off white powdered compound of aloe gel at a heating temperature of 30^0 C for two hours has been suggested (Shafi *et al*., 2000).

High Temperature Short Time (HTST) treatment involves heating to $75\text{-}80^0$ C for less than 3 minutes. It is an effective method of pasteurization to prevent deactivation of the gel with minimal denaturing. An advantage of HTST method is that the gel can be processed quickly. High temperature treatments of longer duration, over a number of hours are more likely to change the chemical nature of the gel (Ashleye, 1983). Very recently high temperature short time treatment at 85^0C- 95^0C for 1-2 minutes using vitamin C and citric acid to ensure effective pasteurization and better flavour was also suggested (He *et al*., 2005).

Materano *et al*. (2005) reported that leaf extract samples were stored at 4^0C, 6^0C,10^0C and 27^0C for 24 days, the pH of the leaf extract showed significant differences at the temperatures of 10^0C and 27^0C, while the samples at 4^0 and 6^0C maintained their values near 5.2. There was no difference for degrees Brix and titrable acidity. The Luminosity was conserved in the sample stored at 4^0C at an average of 62.3.The treatments at 6^0C, 10^0C and 27^0C showed significant differences. The values

for Hue (H Degrees) did not show changes due to different treatments with an average of 60.37. The Chroma of the samples at 4^0C differs from the rest of the treatments reaching 2.17. This investigation confirms that aloe stored at 4^0 and 6^0C maintains its parameters of quality during 24 days of storage.

Chang XiuLian, (2006) reported that the polysaccharide from *Aloe vera* exhibited a maximal stability at 70^0C decreasing either at higher or lower temperatures. Heating promoted a remarkable decrease in barbaloin content depending on temperature and time, more affected than polysaccharide of the gel juice from *Aloe vera*. Barbaloin is unstable when dessolved in methanol resulting in the transformation in to a series of unidentified compounds, in addition to aloe emodin with the period of storage at 4^0C in refrigerator.

Miranda *et al*. (2009) reported that a drying temperature of 80^0 and 90^0C resulted in significant variation in and/ or loss of the physico- chemical and nutritional properties of the gel; in addition, the antioxidant capacity of the gel was decreased at these temperatures. These effects were also observed as a result of a lengthy drying period (i.e., 810 min at 50^0C). However, minor alterations in the physico- chemical and nutritional properties of Aloe gel were produced at drying temperatures of 60-70^0C resulting in the production of a high quality gel.

2.6 Physico-chemical parameters of Aloe gel

Jiang Mei Han (2004) reported that higher contents of water and crude polysaccharides, and lower contents of total sugar, reducing sugar, vitamin C, protein, crude fat, and aloin were observed in aloe gel compared with whole leaves. Optimal processing conditions for aloe beverages include: the use of heating- crushing method

for extraction of aloe juice, 8% aloe juice, 0.08% compound stabilizer, 6% sucrose, 0.18% citric acid, 0.05% agar and 0.15% CMC (carboxymethylcellulose)-Na.

Roy *et al*. (2007) reported that the gel yield is 0.399 kg gel/ kg of raw leaves. Moisture and ash content of gel was found to be 97.86% and around 15% respectively. During extraction a little variation of pH with temperature was observed. However, there was a change of pH(4.07 to 3.68) after filtration of gel. Chemical characterization of Aloe gel was carried out for assaying various nutrients present in the gel. The skin and fillet fraction contained approximately 90-96% of water. Proteins, aloin, fibres, chlorophyll and soluble sugar in the *Aloe vera* lyophilized portion were characterized. On dry basis, proteins were a minor fraction around 8.92%. The amount of total sugar detected was 29.92% in the gel, being one of the most important components. Glucose amounted for over 95% of the soluble sugar in aloe fraction analyzed.

Smita and Pratima (2007) reported that the recovery rate of *Aloe vera* leaf powder (AVLP) was 3.2%. AVLP contained 18.5% crude fibre, 4.8% crude protein, 2.2% crude fat, 14.0% total ash, 48.0% carbohydrate and 231 k.cal of energy. It contained substantial amounts of iron (64.8 mg/ 100g), ascorbic acid (27.0 mg/ 100g) and β-carotene (335.8mg/100g). Dietary fibre was 21.3% while reducing and non-reducing sugars each were 76mg/10g. The pH of the powder was 4.8, color of the powder was olive green, maximum water retention of the powder was obtained on 40 mesh sieve size.

Waszkiewicz-Robak *et al*.(2007) reported that most of the polyphenol compounds were found in the skin of Aloe (390.8mg/100g), which showed the highest

antioxidant activity (6.6 micro mol of Trolox in 1.0 g of skin). The next position belonged to whole leaves (213.2 mg of GAE in 100g of preparation or 4.2 micro mol of Trolox in 1.0 g of leaves) followed by Aloe gel obtained in the laboratory (94.9 mg of GAE in 100g of gel or 0.9 micro mol of Trolox in 1.0 g of gel). The least polyphenol compounds (34.6 mg of GAE in 100g of preparation) and the lowest antioxidant activity (0.13 micro mol of Trolox in 1.0 g of preparation) were present in the manufactured Aloe gel. After calculating these values in dry mass,a similar antioxidant activity of whole aloe leaf and preparations obtained experimentally (gel and skin) was determined. It was almost 4 times lower in the manufactured gel.

2.7 Storage of Aloe gel

Qian He *et al.* (2005) developed a quality and safety management system for the food industry to ensure the biological integrity, the organoleptic stability and quality of the final product. This study reveals that the control points for safety were addition of vitamin C and citric acid and pasteurization. The control points for quality were reception of raw materials, filleting operation, grinding or homogenization, pectolytic enzyme addition, filtration, addition of vitamin C and citric acid, deaeration, pasteurization, flash cooling and storage.

Maria Serrano *et al.* (2006) revealed the usage of *Aloe vera* gel as the coating material. Table grapes were coated with *Aloe vera* gel and stored for 35 days at 10°C. Uncoated clusters showed a rapid loss of functional compounds such as total phenolics, ascorbic acid, total antioxidant activity and increase in total anthocyanins, sharing an accelerated ripening process.

Frozen mass of *Aloe vera* can be preserved longer when stored at temperature 5-8°C. Propyle paraben methyl paraben, citric acid mixed each concentration with 0.25,0.5, 1.0 and 2.0% can preserve the gel up to 6,3 and 4 days respectively at room temperature. Whereas, storage period at low temperature (5 to 10 °C) increased up to 22, 6 and 30 days respectively. *Aloe vera* gel was dehydrated under drying parameter of 3.8 °C and 19% relative humidity with loading density 0.8kg/m^{-2} took 96 min for drying. Citric acid at 0.5% having storage panel up to 4 days is more suitable as less number of colonies were found in microbial counting (Khambalkar *et al*., 2007).

Shilpa *et al.* (2007) reported that squash of 45^0 Brix and 50^0 Brix was prepared with 25, 30 and 40% pulp. Physico - chemical and sensory characteristics were studied up to 90 days of storage period. It was found that squash of 50 degrees Brix with 25 percent pulp of aloe showed highest taste, aroma, flavour and acceptability score during storage period.

Hemalatha *et al*. (2008) reported that all the parameters, namely gel weight reduction (0.030 and 0.100 %), gel pH (3.380 and 4.195), TSS (0.688 and 0.572 %), titrable acidity (3.968 and 3.771%) and fibre content (0.090 and 0.075%) were best with the treatment composed of sodium benzoate (1000 ppm) and citric acid (1%) up to the 20^{th} day after storage (DAS) at room temperature and 35^{th} day after storage (DAS) at 5^0C, respectively. However the TSS of the gel (0.6 to 0.7%) was maintained only up to the 30^{th} day after storage (DAS) (0.622%) at 5^0C. Hence, aloe gel can be stored for more number of days (up to 30 days) at 5^0C without any deterioration in quality.

MATERIAL AND METHODS

CHAPTER III

MATERIAL AND METHODS

The present study entitled “Influence of leaf age on gel recovery and heating on quality, shelf life of Aloe *(Aloe barbadensis* Miller)” was conducted in Laboratory located at Herbal garden, College of Horticulture, Rajendranagar, Hyderabad during the year 2010. The details of the materials used and the methods adopted during the course of present investigation are elucidated in this chapter under the following heads.

3.1 Collection of Aloe leaves

3.2 Procurement of chemicals

3.3 Extraction of gel

3.4 Experimental details

3.5 Physico chemical analysis of Aloe gel

3.6 Microbiological examination of the Aloe gel

3.7 Statistical analysis

3.1 COLLECTION OF ALOE LEAVES

Aloe leaves used in this experiment were obtained from Herbal garden, Andhra Pradesh Horticultural University, Rajendranagar, Hyderabad. Healthy and matured leaves of different accessions with different ages as per the treatments were harvested manually during January, 2010. Leaves with injuries, spots and damages were discarded.

3.1.1 Characteristics of accessions:

Accession-1: It is yellow flowering variety. Leaves are dark green in color with purple tinge. On an average leaf size (530 cm^2), weight (506 g) and thickness are more than the accession-2 and accession-3. Spines on leaf margin are set closer when compared to accession-2 and accession-3. Gel color is yellow.

Accession-2: It is also yellow flowering variety. Leaves are green in color. On an average leaf size (460 cm^2), weight (413 g) and thickness are less than accession-1. Distance between the spines on leaf margin is medium when compared to accession-1 and accession-3. Gel color is yellow.

Accession-3: It is orange flowering variety. Leaves are green in color as accession-2. On an average leaf size (315 cm^2), weight (315 g) and thickness are less than accession-1 and accession-2. Small white spots appear on lower surface of the leaf. Spines on leaf margin are set widely when compared to accession-1 and accession-2. Gel color is pale yellow and very sticky when compared to yellow flowering accessions.

3.1.2 Age of the leaves:

Ten months matured leaf: Leaf is small in length and diameter than 12 months and 14 months matured leaf. Leaf is pale green in color. In case of orange flowering accession-3, small white spots appear on lower surface of the leaf. Gel content is less.

Twelve months matured leaf: Leaf size and weight are more than 10 months matured leaf. Leaf color turns to light green. Gel weight and quality are also more than 10 months matured leaf.

Fourteen months matured leaf: Leaf size and weight are more than 12 months matured leaf. Leaf color turns to dark green. Small white spots are confined to basal portion of lower surface of leaf in case of orange flowering accession-3. Gel weight and quality are good when compared to 12 months matured leaf.

3.2 PROCUREMENT OF CHEMICALS

All the chemicals used in the present study for analysis were of analytical grade collected from Standard Indian Chemical Companies.

3.3 EXTRACTION OF GEL

Aloe leaves of different accessions with different ages have been collected as per the requirement of the treatments. After harvesting, Aloe leaves are washed thoroughly two times with clean water to remove all the dust and soil micro organisms. The aloetic juices is separated from the leaves by cutting them transversely at the base and keep the cut portion touching the ground and allows the leaf to stand in slanting position for half an hour. Thus, it helps for removal of yellow latex. The leaves are again washed thoroughly and then cut into pieces by stainless steel knife under hygienic conditions. Then the outer peels were separated. Thus, the extracted gel was thoroughly homogenized in a mixer. Then the juice is analyzed for physico-chemical parameters and the observations were recorded.

3.4 EXPERIMENTAL DETAILS

In the present investigation, two experiments were carried out and the experimental details are furnished here under.

3.4.1 Experiment I

"Studies on gel recovery and quality components in different accessions of Aloe leaf at different ages".

Number of accessions : 3, Yellow flowering accession -1 (A_1)

Yellow flowering accession -2 (A_2)

Orange flowering accession -3 (A_3)

Number of maturity ages : 3, 10Months (M_1)

12 Months (M_2)

14 Months (M_3)

Number of treatments : 9

Number of replications : 3

Design : CRD with factorial concept.

Interval of analysis : One.

Treatment details:

Parameters studied: Leaf size, Leaf weight, Gel weight, Peel weight T_1 – M_1A_1 (Aloe leaf of 10 months age + Yellow flowering accession-1)

T_2 – M_1A_2 (Aloe leaf of 10 months age + Yellow flowering accession-2)

T_3 – M_1A_3 (Aloe leaf of 10 months age + Orange flowering accession-3)

T_4 – M_2A_1 (Aloe leaf of 12 months age + Yellow flowering accession-1)

T_5 – M_2A_2 (Aloe leaf of 12 months age + Yellow flowering accession-2)

T_6 – M_2A_3 (Aloe leaf of 12 months age + Orange flowering accession-3)

T_7 – M_3A_1 (Aloe leaf of 14 months age + Yellow flowering accession-1)

T_8 – M_3A_2 (Aloe leaf of 14 months age + Yellow flowering accession-2)

T_9 – M_3A_3 (Aloe leaf of 14 months age + Orange flowering accession-3)

, TSS, pH, Acidity, Reducing sugars, Total sugars, Moisture and Antioxidants.

3.4.2 **Experiment II**

"Effect of heating at different temperatures on gel quality and microbial content in different accessions of Aloe".

Number of accessions : 3, Yellow flowering accession -1 (A_1)

Yellow flowering accession -2 (A_2)

Orange flowering accession -3 (A_3)

Number of Temperatures : 3, 50^0C (t_1)

75^0C (t_2)

100^0C (t_3)

Number of treatments : 9

Number of replications : 3

Design : CRD with factorial concept.

Interval of analysis : 10 days intervals.

Treatment details:

T_1 - A_1 t_1 (Yellow flowering accession-1 + 50^0C temperature)

T_2 - A_1 t_2 (Yellow flowering accession-1 + 75^0C temperature)

T_3 - A_1 t_3 (Yellow flowering accession-1 + 100^0C temperature)

T_4 - A_2 t_1 (Yellow flowering accession-2 + 50^0C temperature)

T_5 - A_2 t_2 (Yellow flowering accession-2 + 75^0C temperature)

T_6 - A_2 t_3 (Yellow flowering accession-2 + 100^0C temperature)

T_7 - A_3 t_1 (Orange flowering accession-3 + 50^0C temperature)

T_8 - A_3 t_2 (Orange flowering accession-3 + 75^0C temperature)

T_9 - A_3 t_3 (Orange flowering accession-3+ 100^0C temperature)

Parameters studied: TSS, pH, Acidity, Reducing sugars, Total sugars, Moisture, Antioxidants, Total Bacterial Count (TBC) and Total Mould Count (TMC)

3.5 OBSERVATIONS RECORDED:

3.5.1 Physical parameters

Leaf size: Five leaves are selected randomly and the length and width of these leaves were measured by using the measuring scale and recorded in centimeters.

Leaf weight: Average weights of the five leaves are recorded in grams.

Gel weight: After peeling of the leaves, the gel and peel were separated. Gel weight is recorded in grams.

Peel weight: Average peel weight recorded in grams.

3.5.2 Physico- chemical analysis of Aloe juice

In first experiment, the fresh juice was analyzed for its physico- chemical parameters. The total soluble solids, pH, Acidity, Reducing sugars, Non- reducing sugars, Total sugars, Moisture, Antioxidants were recorded. In second experiment, in all three accessions only one maturity leaf age was taken, which has performed best in first experiment. The fresh gel samples were heated at different temperatures as per the treatments for 15 minutes and stored at room temperature. Standard preservative (Sodium benzoate 1000ppm + Citric acid 1%) was added to Aloe gel and all physico-chemical parameters which are mentioned above are recorded at 10 days intervals. Microbial count was also recorded during the storage at 10 days intervals.

3.5.2.1 pH

The pH of the aloe gel was estimated in each treatment with the help of digital pH meter.

3.5.2.2 Acidity

Homogenized sample size of 10 ml was taken and made up to 100 ml volume in a volumetric flask. The contents were filtered with Whatman No 1 filter paper. An aliquot of 10 ml was taken for titration against 0.1 N NaOH using phenolphthalein as an indicator. The turn of aliquot to light pink colour which persists for 15 seconds was considered as an end point and the titrable acidity was estimated in terms of per cent citric acid (Ranganna, 1986).

$$\text{Acidity} = \frac{\text{Titre value} \times \text{Normality of NaOH} \times 0.064 \times 100}{\text{Volume of aliquot taken}}$$

3.5.2.3 Total Soluble Solids

The per cent of TSS was determined by using hand refractometer and the values were corrected at 20ºC with the help of temperature correction table (Mazumdar and Majumder, 2003). The Total soluble solid content in each treatment was determined by using hand refractometer by placing a drop of the filtered juice on the prism of the refractometer and observing the coincidence of the sample with the reading on the scale and expressed as degree brix (^{0}B)

3.5.2.4 Reducing sugars

Reducing sugars were determined by following the method of Lane and Eyon method (Ranganna 1986). Sample of 10 ml was transferred to a 250 ml volumetric flask using a little amount of distilled water. Lead acetate was added to the flask for precipitation of colloidal matter and kept aside for 10 minutes. Then potassium oxalate was added to this solution to remove excess lead and volume was made upto 250 ml with distilled water. The contents were then filtered through Whatman No. 1

filter paper. Reducing sugars in the lead free solution were estimated by taking this solution into a burette and titrating against 10 ml of standard Fehling solution mixture A and B (1:1) using methylene blue as an indicator and formation of brick red color precipitate was noted as end point. The titration was carried out by keeping the Fehling solution boiling on the heating mantle.

$$\text{Reducing sugars} = \frac{\text{Invert sugar (mg)} \times \text{Dilution} \times 100}{\text{Titre} \times \text{wt (or) volume of sample} \times 100}$$

3.5.2.5 Total sugars

Total sugars were determined in the sample following the method of Lane and Eyon method (Ranganna, 1986). For estimation of total sugars, 50 ml of filtered sample was taken from the above sample in 250 ml conical flask to which 50 ml water and 5g citric acid was added. The sample was boiled gently for 10 minutes to complete the inversion of sucrose. Then transferred to 250 ml volumetric flask and neutralized with 1N NaOH. The volume was made upto the mark and determined the total sugars as invert sugars.

Sucrose (%) = % total invert sugars - % reducing sugars × 0.95

Total sugars = % reducing sugars + % sucrose.

3.5.2.6 Non- Reducing Sugars

The non-reducing sugars present in the samples were determined mathematically by deducting the values of reducing sugars from total sugars using following formula.

Non- reducing sugars (%) = Total sugars (%) - Reducing sugars (%)

3.5.2.7 Moisture

Moisture content was estimated by using moisture meter and expressed as moisture per cent. Sample size of 1ml is taken into the moisture free methanol solution and titrated with Karl Fischer's reagent. The consumed Karl Fischer's reagent is counted for the estimation of per cent of moisture in aloe using the following formula.

Percent (%) of moisture = KF factor × Consumed Karl Fischer's reagent.

3.5.2.7 Total antioxidants

Total antioxidant activity was determined by following the TBARS method (Nickos *et al.,* 1994). Sample size of 1ml was homogenized with 10 ml of 0.1 M phosphate buffer (pH 7.8) one per cent of EDTA 0.05 M and centrifuged at 4000 rpm for 15 minutes at 5^0C. The clear supernatant extract was used for analysis.

The reaction mixture contained 2-3 ml of aliquot of sample, coconut oil (0.24 ml) in phosphate buffer (0.26ml, 0.1 M, pH 7.8), ferrous sulphate (0.05 mM), ascorbic acid (0.4 mM), potassium hydrogen phosphate (100 mM, pH 6.0), BHT (25 mM in 5ml hexane) in a final volume of 2.4 ml. Contents of the tube were incubated for 30min at 37^0C. TCA (0.75 ml, 20%) was added and centrifuged at 10,000 rpm for 30 minutes at 4^0C, followed by addition of TBA (0.5% in 0.1 M NaOH). Distilled water was added to equalize the final volume to 3.24 ml. This was heated at 95^0C in water bath for 30 minutes followed by immediate cooling in ice pack for 5 minutes. Finally, the reaction mixture was submitted to read the absorbance at 532 nm against TBA.

3.6 MICROBIOLOGICAL EXAMINATION OF THE ALOE GEL

Total Bacterial Count (TBC)

For estimating the microbial population in different sample products, dilution plate method was followed (Cruik Shank *et al*., 1975). One ml of sample was thoroughly mixed in 9 ml of sterile saline water; 1ml of sample was transferred through a sterile pipette to a screw cap tube containing 9ml sterile saline water. This gave dilution of 10^{-2}. Similarly, serial dilutions were made. 1ml of the serially diluted sample was placed in the sterile petridish to which cooled plate count agar medium was added and mixed thoroughly with the suspension and then allowed to set and then incubated at 28 ± 2^0 C for 48 hrs. Individual colonies were counted and multiplied with the dilution factor to get the microbial population. The counts are reported as Colony Forming Units (cfu's) per ml of the sample.

Total Mould Count (TMC)

Dilution plate method was followed for estimation of yeast /mould population (Cruik Shank *et al*., 1975), potato dextrose agar medium was used for estimating the fungal population. One ml of sample was thoroughly mixed in 9 ml of sterile saline water. One ml of sample was transferred through a sterile pipette to a screw cap tube containing 9ml sterile saline water. This gave dilution of 10^{-2}. Serial dilutions were made similarly. One ml of diluted sample was placed in the sterile petridish to which cooled potato dextrose agar medium was added and mixed thoroughly with the suspension and then allowed to set and then incubated. Individual colonies were counted and multiplied with the dilution factor to get the microbial population. The counts are reported as Colony Forming Units (cfu's) per ml of the sample.

3.7 STATISTICAL ANALYSIS

Completely randomized design with Factorial concept (Factorial CRD) was adopted for the experiment. Observations on various parameters were recorded with three replications and the data were analyzed with 5% level of significance. Critical differences were worked out for different parameters and the effects which were significant were presented (Sundararaj *et al.*, 1972).

RESULTS

CHAPTER IV

RESULTS

The data obtained during the present investigation on the **"Influence of leaf age on gel recovery and heating on quality and shelf life of Aloe (*Aloe barbadensis* Miller)"** was statistically analyzed and the results obtained are presented in this chapter.

The results on physico-chemical parameters, storage life and microbial count of Aloe gel as influenced by the leaf age and heating at different temperatures in different accessions are presented here under.

4.1. Experiment I: Studies on gel recovery and quality components at different ages in different accessions of Aloe.

4.1.1 Physical parameters:

4.1.1.1 Leaf size (cm^2)

The data on the leaf size as influenced by leaf age of different Aloe accessions are presented in table 1and figure 1. There were significant differences in leaf size due to different leaf ages in various Aloe accessions studied.

Among the three ages of the leaf, highest leaf size (553.45 cm^2) was recorded with 14 months aged leaves which was significantly superior to 12 months aged leaves (445.67 cm^2) while the lowest leaf size (305.56 cm^2) was recorded with 10 months aged leaves. With regard to accessions, highest leaf size (530.11cm^2) was recorded with yellow flowering accession-1 which was significantly superior to yellow flowering accession-2 (460.22 cm^2) while the lowest leaf size (314.33 cm^2) was recorded with orange flowering accession-3.

The interaction between leaf ages and Aloe accessions was significant with respect to leaf size. Maximum leaf size (686.67 cm^2) was recorded in yellow flowering accession-1 at 14 months leaf age which was significantly superior to yellow flowering accession-2 at 14 months leaf age (569.0 cm^2) and the minimum leaf size (234.0 cm^2) was recorded with orange flowering accession-3 at 10 months leaf age.

The yellow flowering accession-1 was found to be superior at all the leaf ages compared to the other two accessions, followed by yellow flowering accession-2 while the orange flowering accession-3 has recorded the lowest leaf size at all the stages.

4.1.1.2 Leaf weight (g)

The data on the leaf weight as influenced by leaf age of different Aloe accessions are presented in table 2 and figure 2. There were significant differences in leaf weight due to different leaf ages in various Aloe accessions.

Among the three ages of the leaf studied, 14 months aged leaf recorded the highest leaf weight (480.22 g) which was significantly superior to 12 months aged leaf (422.78 g) while the 10 months aged leaf recorded the lowest leaf weight (331.33 g). Among the accessions, leaf weight differed significantly with different accessions. Yellow flowering accession-1 recorded the highest leaf weight (506.67 g) which was significantly superior to yellow flowering accession-2 (413.78 g) while the lowest leaf weight (313.89 g) was recorded with orange flowering accession-3.

The interaction between leaf ages and Aloe accessions showed significant differences between the treatments with respect to leaf weight. Yellow flowering

accession-1 at 14 months leaf age recorded the highest leaf weight (559.0 g) which was significantly superior to yellow flowering accession-1 at 12 months leaf age (514.67 g) and the lowest leaf weight (216.67 g) was recorded with orange flowering accession-3 at 10 months leaf age.

Significantly superior leaf weight was recorded by yellow flowering accession-1 irrespective of leaf age compared to other accessions while the orange flowering accession-3 has recorded the lowest leaf weight at all three stages.

4.1.1.3 Gel weight (g)

The data recorded on the gel weight as influenced by leaf age of different Aloe accessions are presented in table 3 and figure 3. There were significant differences in gel weight due to different leaf ages in various Aloe accessions.

The results indicated that among the three ages of the leaf studied, 14 months aged leaf recorded highest gel weight (321.56 g) which was significantly superior to 12 months aged leaf (276.0 g) while the 10 months aged leaf showed lowest gel weight (207.33 g). In respect of different accessions, maximum gel weight (350.78 g) was recorded with yellow flowering accession-1 which was significantly superior to yellow flowering accession-2 (256.56 g) and the lowest gel weight (197.56 g) was recorded with orange flowering accession-3.

The interaction between leaf ages and Aloe accessions was found to be significant with respect to gel weight. Maximum gel weight (402.67g) was recorded in yellow flowering accession-1 at 14 months leaf age which was significantly superior to yellow flowering accession-1 at 12 months leaf age (354.0 g) and the minimum gel

weight (132.33 g) was recorded with orange flowering accession-3 at 10 months leaf age.

Yellow flowering accession-1 at 14 months and 12 months leaf age was found to be superior followed by yellow flowering accession-2 at 14 months leaf age.

4.1.1.4 Peel weight (g)

The data recorded on the leaf peel weight presented in table 4 and figure 4 indicated that there were significant differences among the different leaf ages in various Aloe accessions.

Among the three ages of the leaf studied, maximum peel weight (158.22 g) was recorded with 14 months aged leaf which was significantly superior to 12 months aged leaf (142.33g) and the 10 months aged leaf recorded the lowest peel weight (112.56 g). With regard to accessions, yellow flowering accession-2 showed maximum peel weight (161.89g) which was significantly superior to yellow flowering accession-1 (141.78 g) while the orange flowering accession-3 recorded lowest peel weight (109.67 g).

The interaction between leaf ages and Aloe accessions was significantly different between the treatments with respect to peel weight. Maximum peel weight (175.67 g) was recorded in yellow flowering accession-2 at 14 months leaf age followed by the same accession at 12 months leaf age (171.33 g) and the lowest peel weight (76.0 g) was recorded with orange flowering accession-3 at 10 months leaf age.

Yellow flowering accession-2 was found to be superior at all leaf ages compared to the other two accessions, followed by yellow flowering accession-1

while the orange flowering accession-3 has recorded the lowest peel weight at all the stages.

4.1.2 Physico-chemical parameters:

4.1.2.1 Gel pH

The data on the gel pH as influenced by leaf age of different Aloe accessions are presented in table 5 and figure 5. There were significant differences in gel pH due to different leaf ages in various Aloe accessions studied.

Among the three ages of the leaf, 14 months aged leaf recorded the maximum gel pH (4.79) which was significantly superior to 12 months aged leaf (4.39) while the lowest gel pH (3.60) was recorded with 10 months aged leaves. With regard to accessions, maximum gel pH (4.52) was recorded with orange flowering accession-3 which was significantly superior to yellow flowering accession-2 (4.22) while the lowest gel pH (4.03) was recorded with yellow flowering accession-1.

The interaction between leaf ages and Aloe accessions was significantly different between the treatments with respect to gel pH. Maximum gel pH (5.17) was recorded in orange flowering accession-3 at 14 months leaf age which was significantly superior to yellow flowering accession-2 at 14 months leaf age (4.70) and the lowest gel pH (3.47) was recorded with yellow flowering accession-1 at 10 months leaf age.

Significantly superior pH was recorded by orange flowering accession-3 at all leaf ages, followed by yellow flowering accession-2 while the lowest pH was recorded with yellow flowering accession-1 at all stages.

4.1.2.2 Acidity (%)

The data on the gel acidity are presented in table 6 and figure 6. Gel acidity has significantly varied among the different leaf ages and various Aloe accessions studied.

Among the three ages of the leaf, the maximum gel acidity (0.22%) was recorded with 10 months aged leaves which was significantly superior to 12 months aged leaf (0.19) while the lowest gel acidity (0.16%) was recorded with 14 months aged leaves. With regard to accessions, maximum gel acidity (0.28%) was recorded with yellow flowering accession-1 which was significantly superior to yellow flowering accession-2 (0.19%) while the lowest gel acidity (0.09%) was recorded with orange flowering accession-3.

The interaction between leaf ages and Aloe accessions showed that there were significant differences between the treatments with respect to gel acidity. Maximum gel acidity (0.32%) was recorded by the combination of yellow flowering accession-1 with 10 months leaf age which was significantly superior to yellow flowering accession-1 with 12 months leaf age (0.28%) and the lowest gel acidity (0.06%) was recorded with orange flowering accession-3 at 14 months leaf age.

Significantly superior gel acidity was recorded with yellow flowering accession-1 at all stages followed by yellow flowering accession-2 at all leaf ages.

4.1.2.3 Total Soluble Solids (^{0} Brix)

The data recorded on the gel TSS as influenced by leaf ages of different Aloe accessions are presented in table 7 and figure 7. There were significant differences in gel TSS due to different leaf ages in various Aloe accessions studied.

Among the three ages of the leaf studied, maximum gel TSS (0.78) was recorded with 14 months aged leaf which was on par with 12 months aged leaf (0.63) while the lowest gel TSS (0.54) was recorded with 10 months aged leaves. With regard to accessions, maximum gel TSS (0.76) was recorded with yellow flowering accession-1 and was on par with yellow flowering accession-2 (0.63). The lowest gel TSS (0.57) was recorded with orange flowering accession-3.

The interaction between leaf ages and Aloe accessions recorded significant differences between the treatments with respect to gel TSS. Maximum gel TSS (0.90) was recorded in yellow flowering accession-1 at 14 months leaf age which was on par with yellow flowering accession-2 at 14 months leaf age (0.77), yellow flowering accession-1 at 12 months leaf age (0.73), orange flowering accession-3 at 14 months leaf age (0.67), yellow flowering accession-1 at 10 months leaf age (0.63). The lowest TSS (0.47) was recorded with orange flowering accession-3 at 10 months leaf age.

Highest gel TSS was recorded with yellow flowering accession-1 at all leaf ages, followed by yellow flowering accession-2 while the lowest gel TSS was recorded with orange flowering accession-3 at all stages.

4.1.2.4 Reducing Sugars (%)

The data recorded in table 8 and figure 8 indicated that there were significant differences in reducing sugars of gel due to different leaf ages in various Aloe accessions.

Out of the three ages of the leaf studied, 14 months aged leaf recorded the maximum percentage of reducing sugars (0.050%) which was significantly superior to 12 months aged leaf (0.028%) while the 10 months aged leaf recorded the lowest

reducing sugars (0.017%). Among the accessions, maximum reducing sugars (0.035%) was recorded with yellow flowering accession-1 which was significantly superior to yellow flowering accession-2 (0.031%) while the minimum reducing sugars (0.028%) was recorded with orange flowering accession-3.

The interaction between leaf ages and Aloe accessions indicated that there was significant difference between the treatments with respect to reducing sugars. Maximum reducing sugars (0.054%) was recorded in yellow flowering accession-1 at 14 months leaf age followed by yellow flowering accession-2 at the same leaf age (0.050%) and the minimum amount of reducing sugars (0.014%) was recorded with orange flowering accession-3 at 10 months leaf age.

Significantly superior reducing sugars was recorded with all the three accessions at 14 months leaf age while the lowest reducing sugars observed at 10 months leaf age with all the three accessions.

4.1.2.5 Non-Reducing Sugars (%):

The data recorded in table 9 indicated that there were significant differences in non reducing sugars of gel due to different leaf ages in various Aloe accessions.

The maximum percentage of non reducing sugars (1.79%) was recorded with 14 months aged leaf but on par with 12 months aged leaf (1.63%) while the minimum percentage of non reducing sugars (1.45%) was recorded with 10 months aged leaf. In respect of different accessions, maximum percentage of non reducing sugars (1.68%) was recorded with yellow flowering accession-1 which was on par with yellow flowering accession-2 (1.61%) while the minimum non reducing sugars (1.58%) was recorded with orange flowering accession-3.

The interaction effect showed that there was a significant difference between the treatments with respect to the percentage of non reducing sugars. Yellow flowering accession-1 at 14 months leaf age has recorded the maximum percentage of non reducing sugars (1.87%) which was significantly superior to the rest of the treatments but on par with yellow flowering accession-2 at 14 months leaf age (1.76), orange flowering accession-3 at 14 months leaf age (1.75), yellow flowering accession-1 at 12 months leaf age (1.70). The minimum percentage of non reducing sugars (1.43%) was recorded with orange flowering accession-3 at 10 months leaf age.

Highest non-reducing sugars were recorded at 14 months leaf age with all the three accessions while the lowest non-reducing sugars were recorded with all the three accessions at 10 months leaf age.

4.1.2.6 Total Sugars (%)

The data recorded in table 10 and figure 10 indicated that there was a significant difference in total sugars of gel due to leaf ages in various Aloe accessions.

Among the different ages of the leaf studied, 14 months aged leaf recorded maximum percentage of total sugars (1.84%) which was significantly superior to the rest of the treatments followed by 12 months aged leaf (1.66%) while the 10 months aged leaf recorded the lowest percentage of total sugars (1.47%). With respect to different accessions, maximum percentage of total sugars (1.72%) recorded with yellow flowering accession-1 followed by yellow flowering accession-2 (1.64%) while the lowest percentage of total sugars (1.61%) was recorded with orange flowering accession-3.

The interaction effect showed that there was a significant difference between the treatments with respect to total sugars (%). Maximum percentage of total sugars (1.93%) was recorded in yellow flowering accession-1 at 14 months leaf age followed by yellow flowering accession-2 at 14 months leaf age (1.81%) and the lowest percentage of total sugars (1.45%) was recorded with orange flowering accession-3 at 10 months leaf age.

All the three accessions at 14 months leaf age have recorded significantly superior total sugars followed by the 12 months leaf age while the lowest total sugars were recorded by the all the three accessions at 10 months leaf age.

4.1.2.7 Percentage of moisture in gel (%)

The data on moisture percentage of gel are presented in table 11 and figure 11. There were significant differences in gel moisture due to leaf ages in various accessions.

With regard to different ages of the leaf studied, 14 months aged leaf recorded the maximum moisture content (90.23%) which was significantly superior to other ages followed by 12 months aged leaf (87.0%) while the 10 months aged leaf recorded the lowest moisture content (84.91%). In respect of accessions, yellow flowering accession-1 showed maximum moisture content (95.67%) followed by yellow flowering accession-2 (88.73%) while the orange flowering accession-3 recorded the lowest moisture content (77.74%).

The interaction between leaf ages and Aloe accessions showed that there were significant differences between the treatments with respect to gel moisture content (%). Maximum gel moisture content (98.23%) was recorded in yellow flowering

accession-1 at 14 months leaf age which was significantly superior to other combinations followed by yellow flowering accession-1 at 12 months leaf age (95.30%) while the lowest gel moisture content (75.07%) was recorded with orange flowering accession-3 at 10 months leaf age.

Significantly superior gel moisture was recorded by yellow flowering accession-1 at all leaf ages, followed by yellow flowering accession-2 while the lowest gel moisture was recorded by orange flowering accession-3 at all stages.

4.1.2.8 Antioxidants:

There is an inverse relationship between %TBARS and % inhibition of peroxidation. If the %TBARS is low, the % inhibition of peroxidation is high and also the antioxidant activity is high and vice-versa.

4.1.2.8 (a) Thiobarbituric acid reactive substances (%TBARS)

The data recorded on the per cent TBARS as influenced by leaf age of different Aloe accessions are presented in table 12 and figure 12. The per cent TBARS have differed significantly due to different leaf ages in various Aloe accessions.

The highest per cent TBARS (482.78%) was recorded with 10 months aged leaf which was significantly superior to 12 months aged leaf (445.33%) and 14 months aged leaf (423.67%). In respect of accessions, maximum per cent TBARS (483.33%) was recorded in orange flowering accession-3 which was significantly superior to yellow flowering accession-2 (439.56%) while the lowest per cent TBARS (428.90%) was recorded in yellow flowering accession-1.

The interaction effect revealed that there were significant differences between the treatments with respect to per cent TBARS. Orange flowering accession-3 at 10

months leaf age recorded maximum per cent TBARS (517.33%) which was significantly superior to same accession at 12 months leaf age (475.0%) while the yellow flowering accession-1 at 14 months leaf age recorded the lowest per cent TBARS (401.67%).

Orange flowering accession-3 was found to be superior at all leaf ages compared to the other two accessions, followed by yellow flowering accession-2, while the yellow flowering accession-1 has recorded the lowest per cent TBARS at all stages.

4.1.2.8 (b) Per cent inhibition of peroxidation (%)

The data recorded on the per cent inhibition of peroxidation are presented in table 13. There were significant differences in per cent inhibition of peroxidation due to different leaf ages in various Aloe accessions.

Out of three ages of the leaf studied, maximum per cent inhibition of peroxidation was recorded with 14 months aged leaf (64. 08%) followed by 12 months aged leaf (47.0%) and minimum was recorded with 10 months aged leaf (42.10%). With regard to accessions, yellow flowering accession-1 recorded maximum per cent inhibition of peroxidation (53.16%) followed by yellow flowering accession-2 (51.30%) while the orange flowering accession-3 recorded minimum per cent inhibition of peroxidation (48.71%).

The interaction effect showed that there was a significant difference between the treatments with respect to per cent inhibition of peroxidation of gel. The highest per cent inhibition of peroxidation (66.47%) was recorded in yellow flowering accession-1 at 14 months leaf age but was on par with yellow flowering accession-2 at

14 months leaf age. The lowest per cent inhibition of peroxidation (40. 43%) was noticed with orange flowering accession-3 at 10 months leaf age.

The highest per cent inhibition of peroxidation was recorded with all the three accessions at 14 months leaf age while the lowest was recorded by all the three accessions at 10 months leaf age.

4.2 Experiment II: Effect of heating at different temperatures on gel quality and microbial Content in different accessions of Aloe.

4.2.1 Gel pH

The data on the gel pH as influenced by heating of the gel at different temperatures in different Aloe accessions are presented in table 14 and figure 14. There were significant differences in gel pH during the storage due to heating at different temperatures in various Aloe accessions.

The results indicated that the gel pH has increased gradually with increase in storage period from day 1 to 30^{th} day in all the treatments. Out of the three accessions of Aloe, the highest pH (5.17) on day 1 was recorded with orange flowering accession-3 and has increased to maximum (5.30, 5.63, 5.89 at 10^{th}, 20^{th}, 30^{th} day of storage respectively) which was significantly superior to yellow flowering accession-2 (4.76, 4.83, 5.11, 5.41 at all storage intervals). But the lowest gel pH (4.41) on day1 was recorded with yellow flowering accession-1 and increased to 4.48, 4.64, and 4.77 at 10^{th}, 20^{th} and 30^{th} day respectively.

Regarding temperatures, the highest gel pH on day 1 (4.79) was recorded in case of both the temperatures 50°C and 100°C which has increased to maximum due to heating (5.46) at 30^{th} day of storage but was on par with lowest gel pH (4.76) at

75°C temperature which has increased to 4.82, 5.0, 5.16 at 10th, 20th, 30th day of storage respectively.

Among the interactions, there were significant differences between the treatments with respect to gel pH. Orange flowering accession-3 which recorded the highest gel pH (5.20) on day 1 has increased to maximum (5.30,5.80, 6.07 at 10th, 20th, 30th day of storage respectively) due to heating at 100°C followed by the same accession at 50°C (5.17, 5.33, 5.67,6.0 at 1st, 10th, 20th and 30th day of storage respectively). These treatments were on par at 1st and 10th day of storage but there was a significant difference between the treatments at 20th and 30th day of storage. Lowest gel pH on day 1 (4.40) was recorded by yellow flowering accession-1 and has increased to 4.43, 4.53, 4.67 at 10th, 20th, 30th day of storage on heating at 75°C.

Significantly superior gel pH was recorded by orange flowering accession-3 at all temperatures at all storage intervals followed by yellow flowering accession-2 while the lowest gel pH was recorded with yellow flowering accession-1 at all stages during the storage.

4.2.2 Acidity (%)

The data recorded on the gel acidity are reported in table 15. There were significant differences in gel acidity due to heating at different temperatures in various Aloe accessions during the different days of storage.

The results revealed that there was a decreasing trend in gel acidity during the storage period from day 1 to day 30th. Among the different accessions of Aloe, yellow flowering accession-1 has recorded the highest gel acidity initially on day 1 (0.24%) but has decreased (0.24, 0.22, 0.22% at 10th, 20th and 30th days respectively) during

storage and was significantly superior to yellow flowering accession-2 (0.16, 0.16, 0.15, 0.14% at 1st,10th, 20th and 30th day of storage respectively) while the lowest gel acidity (0.07%) on day 1 was recorded with orange flowering accession-3 and has decreased to 0.07, 0.07,0.06% at 10th, 20th and 30th day of storage respectively.

In respect of different temperatures, 75^{0}C temperature which has recorded the highest gel acidity (0.16%) initially has decreased to 0.16,0.15, 0.15% at 10th, 20th and 30th day of storage respectively and was on par with heating at 50°C (0.15, 0.15, 0.14, 0.14%) and 100^{0}C (0.15, 0.15, 0.14, 0.14% at day 1, 10th, 20th and 30th days respectively).

With regard to interactions, there were significant differences between the treatments with respect to gel acidity during the storage period. Yellow flowering accession-1 which has recorded the maximum acidity (0.24%) on day 1, has decreased to 0.24, 0.23,0.23% at 10th, 20th and 30th day of storage respectively and was on par with the same accession heated at 50°C and 100°C (0.24, 0.23, 0.22, 0.22% at 1st, 10th, 20th and 30th days of storage respectively). But the lowest gel acidity (0.07%) on day 1 was recorded with orange flowering accession-3 upon heating at 50°C and 100^{0}C has decreased to 0.07, 0.06, and 0.06% at 10th, 20th and 30th day of storage respectively.

Highest gel acidity was recorded by yellow flowering accession-1 at all the three heating temperatures in storage followed by yellow flowering accession-2 while the lowest gel acidity was recorded with orange flowering accession-3 at all stages.

4.2.3 Total Soluble Solids (^{0} Brix) The data on the gel TSS are presented in table 16. There were significant differences in gel TSS due to different temperatures in various Aloe accessions studied during the storage.

The TSS of the gel has gradually increased with increase in storage period up to 30th day in all the treatments. In respect of different accessions of Aloe studied, the highest gel TSS on day 1 (0.90) was recorded in yellow flowering accession-1 which has increased to 0.97, 1.04 and 1.20 at 10th, 20th and 30th day of storage respectively but was on par with yellow flowering accession-2 (0.77, 0.81, 0.89, and 1.07 at day 1, 10th, 20th and 30th day of storage respectively). But the lowest gel TSS on day 1 (0.62) was recorded with orange flowering accession-3 which has increased to 0.67, 0.74, and 0.97 at 10th, 20th and 30th day of storage respectively.

With regard to different temperatures, the highest gel TSS on day 1 (0.81) was recorded at 75^{0}C which has increased to 0.89, 0.97 and 1.16 at 10th, 20th and 30th day of storage respectively and was on par with 50°C temperature (0.76, 0.80, 0.88 and 1.08 at different storage intervals) while the lowest gel TSS on day 1 (0.72) was recorded at 100^{0}C which has increased to 1.0 at 30th day of storage.

The interaction between the Aloe accessions and different temperatures showed that there was significant difference between the treatments with respect to gel TSS during the storage period. Highest initial gel TSS on day 1 (0.93) was recorded by yellow flowering accession-1 which on heating at 75^{0}C has increased to 1.03, 1.10 and 1.30 at 10th, 20th and 30th day of storage respectively and was on par with the same accession heated at 50°C (0.90, 0.97, 1.03 and 1.17 at all storage intervals) while the lowest initial gel TSS (0.57) was recorded with orange flowering accession-3 which on heating at 100^{0}C has increased to 0.60, 0.67 and 0.87 at 10th, 20th and 30th day of storage respectively.

Significantly superior gel TSS was recorded by yellow flowering accession-1 at all heating temperatures during the storage period followed by yellow flowering accession-2 while the lowest gel TSS was recorded with yellow flowering accession-3 at all temperatures.

4.2.4 Reducing Sugars (%)

The data on the gel reducing sugars as influenced by heating of the gel at different temperatures in different Aloe accessions are presented in table 17. There were significant differences in gel reducing sugars due to different temperatures in various Aloe accessions studied during the storage.

The data revealed that there was a considerable increase in reducing sugars during the storage period. Out of the three accessions of Aloe, maximum reducing sugars on day 1 (0.050%) was recorded by yellow flowering accession-1 which has increased to maximum (1.838%) on 30th day of storage but was on par with yellow flowering accession-2 (0.048 on day 1 and 1.788% on 30th day). The lowest initial reducing sugars (0.044%) were recorded with orange flowering accession-3 which has increased to 1.784% on 30th day of storage.

In respect of different temperatures, the highest reducing sugars on day 1 (0.049%) has increased to maximum (1.827%) at 30th day of storage due to heating at 50°C which was on par with 75°C (0.047 to 1.799 on day 1 to 30th day) during storage while the lowest initial reducing sugars (0.046%) has increased to 1.784% due to heating at 100°C on 30th day of storage.

With regard to interactions, there were significant differences between the treatments with respect to reducing sugars during the storage period. Yellow flowering

accession-1 which recorded the maximum reducing sugars on day 1 (0.053%) on heating at 50°C has resulted in (0.687, 1.310 and 1.853% at 10^{th}, 20^{th} and 30^{th} day of storage respectively) followed by the same accession at 75°C (0.05, 0.647, 1.287 and 1.833% at day 1, 10^{th}, 20^{th} and 30^{th} day respectively).

However, the orange flowering accession-3 which recorded the lowest initial reducing sugars (0.043%) has also recorded the lowest reducing sugar per cent at all intervals on heating at 100°C (0.570, 1.197 and 1.760 at 10^{th}, 20^{th} and 30^{th} day of storage respectively).

Yellow flowering accession-1 at all temperatures has recorded the highest reducing sugars followed by yellow flowering accession-2 while the orange flowering accession-3 has recorded the lowest reducing sugars compared to the other two accessions irrespective of heating temperature and storage intervals.

4.2.5 Non-reducing Sugars (%)

The data recorded on the non-reducing sugars are presented in table 18 and figure 18 indicated that there were significant differences among the treatments due to heating temperature and accessions.

The data indicated that there was a decreasing trend in non-reducing sugars during the storage period. Among the different accessions of Aloe studied, yellow flowering accession-1 has recorded the maximum non-reducing sugars (1.86%) on day1 which has significantly decreased (1.44, 0.98 and 0.69% at 10^{th}, 20^{th} and 30^{th} day of storage respectively) compared to yellow flowering accession-2 (1.76, 1.29, 0.88 and 0.61% at day 1, 10^{th}, 20^{th} and 30^{th} day during storage while the lowest initial non-

reducing sugars (1.73%) was recorded by orange flowering accession-3 and has decreased to 0.60% at 30th day of storage.

With regard to different heating temperatures, the highest non-reducing sugars on day 1 i.e., 1.79% has decreased to 0.64% at 30th day of storage due to heating at 50°C which was on par with 75°C (1.78% decreased to 0.64% on day 1 to 30th day respectively) and 100^{0}C (1.78 and 0.63% at 1st and 30th day of storage respectively.

Regarding interactions, there were significant differences between the treatments with respect to non-reducing sugars. Yellow flowering accession-1 which recorded the highest non-reducing sugars on day1 (1.87%) has decreased to 1.44, 1.0 and 0.70% at 10th, 20th and 30th day of storage due to heating at 50°C but was on par with the same accession at 75°C (1.86, 1.45, 0.97 and 0.69%) and 100^{0}C (1.86, 1.44, 0.97 and 0.67% at 1st, 10th, 20th and 30th day during storage respectively.

Significantly superior non-reducing sugars were recorded by yellow flowering accession-1 at all temperatures compared to yellow flowering accession-2 while the lowest non-reducing sugars were recorded with orange flowering accession-3 irrespective of heating temperatures.

4.2.6 Total Sugars (%)

The data recorded on the total sugars are reported in table 19 and figure 19. The total sugars have differed significantly due to different temperatures in various Aloe accessions.

The result indicated that the total sugars have increased gradually with increase in storage period up to 30th day in all the treatments. Out of three different accessions of Aloe, the highest total sugars (1.91%) on day 1 were recorded in yellow flowering

accession-1 which has increased to maximum (2.09, 2.27 and 2.53% at 10^{th}, 20^{th} and 30^{th} day of storage respectively) which was significantly superior to yellow flowering accession-2 (1.81, 1.92, 2.12 and 2.41% at all storage intervals) while the lowest total sugars on day 1 (1.78%) was recorded with orange flowering accession-3 and increased to maximum 1.89, 2.09 and 2.39% at 10^{th}, 20^{th} and 30^{th} day respectively.

Regarding temperatures, the highest total sugars content on day 1 (1.84%) was recorded at 50^0C which has increased to maximum due to heating (2.46%) at 30^{th} day of storage and was on par with 75°C (1.83, 1.97, 2.16 and 2.44% at day 1, 10^{th}, 20^{th} and 30^{th} day of storage respectively). The lowest initial total sugars content (1.82%) was recorded at 100^0C which has increased to maximum (2.42%) at 30^{th} day of storage.

The interaction effect showed that there was a significant difference between the treatments with respect to total sugars of gel during the storage period. Highest initial total sugars content (1.92%) was recorded in yellow flowering accession-1 on heating at 50^0C which has increased to maximum (2.55%) at 30^{th} day of storage period which was on par with 75°C (1.91 to 2.53%) while the lowest initial total sugars (1.76%) was recorded with orange flowering accession-3 on heating at 100^0C which has increased (2.36%) during the storage period.

Highest total sugars were recorded by yellow flowering accession-1 at all the temperatures followed by yellow flowering accession-2 while the lowest total sugars were recorded with orange flowering accession-3 at all the temperatures.

4.2.7 Moisture (%)

The data on the gel moisture content as influenced by heating of the gel at different temperatures in different Aloe accessions are presented in table 20 and figure 20. There were significant differences in gel moisture content due to different temperatures in various Aloe accessions during the storage.

The results revealed that there was a decreasing trend in gel moisture during the storage period from day 1 to day 30th. Among the different accessions of Aloe studied, the highest moisture content on day 1 (95.78%) was recorded by yellow flowering accession-1 which was significantly superior and was decreased (94.73, 92.80 and 90.63% at 10^{th}, 20^{th} and 30^{th} day of storage respectively) during storage followed by yellow flowering accession-2 (88.97, 87.42, 85.81 and 83.07% at 1^{st}, 10^{th}, 20^{th} and 30^{th} day of storage respectively) while the lowest moisture content on day1 (78.57%) was recorded with orange flowering accession-3 and has decreased to 77.19, 74.74 and 73.02% at 10^{th}, 20^{th} and 30^{th} day of storage respectively.

Among different temperatures, the highest moisture content on day1 (88.07%) was recorded on heating at 50^0C which has decreased to 86.82, 84.78 and 82.68% at 10^{th}, 20^{th} and 30^{th} day of storage respectively and was on par with 75°C temperature (87.77, 86.46, 84.47 and 82.27% at different storage intervals) while the lowest moisture content on day 1 (87.48%) was recorded at 100^0C which has decreased to 86.07, 84.11 and 81.78% at 10^{th}, 20^{th} and 30^{th} day of storage respectively.

The interaction between the Aloe accessions and different temperatures showed that there was a significant difference between the treatments with respect to gel moisture during the storage period. Highest gel moisture on day 1 (96.07%) was

recorded by yellow flowering accession-1 which on heating at 50^0C on day1 has decreased to 95.10, 93.13 and 91.07% at 10^{th}, 20^{th} and 30^{th} day of storage respectively and was on par with the same accession heated at 75°C (95.83, 94.70, 92.80 and 90.67% at all storage intervals). While the lowest moisture content (78.30%) was recorded with orange flowering accession-3 which on heating at 100^0C has decreased to 76.80, 74.30 and 72.53% at 10^{th}, 20^{th} and 30^{th} day of storage respectively.

Yellow flowering accession-1 at all heating temperatures recorded the highest moisture content followed by yellow flowering accession-2. But the lowest moisture was recorded by orange flowering accession-3 at all temperatures.

4.2.8 Antioxidants:

4.2.8 (a) Thiobarbituric acid reactive substances (% TBARS)

The data recorded on the per cent TBARS is presented in table 21. The %TBARS has differed significantly due to different temperatures in various Aloe accessions studied.

The results indicated that there was a significant increase in %TBARS during the storage period. Among the different accessions of Aloe, the maximum % TBARS on day 1 (453.22) was recorded in orange flowering accession-3 which has increased (460.11, 467.56 and 475.44 at 10^{th}, 20^{th} and 30^{th} day of storage respectively) and was on par with yellow flowering accession-2 (427.56, 433.78, 443.44 and 447.22% at day 1, 10^{th}, 20^{th} and 30^{th} day of storage respectively). Minimum %TBARS on day 1 (404.11%) was recorded with yellow flowering accession-1 which has increased to 404.11, 412.33, 422.89 and 435.89 at 10^{th}, 20^{th} and 30^{th} day of storage respectively.

With regard to different temperatures, the maximum %TBARS on day1 (430.44) was recorded on heating at 100^0C which has increased to 438.44, 447.22 and 455.67 at 10^{th}, 20^{th} and 30^{th} day of storage respectively and was on par with 50°C temperature (427.89, 435.22, 44.78 and 452.89% at 1^{st}, 10^{th}, 20^{th} and 30^{th} day of storage). Minimum %TBARS on day 1 (426.56%) was recorded at 75^0C and increased to maximum of 450.00% at 30^{th} day of storage.

The interaction effect revealed that there was a significant difference between the treatments with respect to %TBARS during the storage period. Maximum %TBARS on day1 (455.33%) was recorded in orange flowering accession-3 on heating at 100^0C has increased to 462.67, 469.00 and 478.33 at 10^{th}, 20^{th} and 30^{th} day of storage respectively and was on par with orange flowering accession-3 at 50°C (452.33, 460.0, 467.67 and 475.67% at all storage intervals). Minimum %TBARS on day 1 (401.67%) was recorded with yellow flowering accession-1 on heating at 75^0C has increased to 409.33, 419.0 and 433.33% at 10^{th}, 20^{th} and 30^{th} day of storage respectively.

Maximum %TBARS was recorded by orange flowering accession-3 at all heating temperatures during all storage intervals followed by yellow flowering accession-2 while the minimum %TBARS was recorded with yellow flowering accession-1.

4.2.8 (b) Percent inhibition of peroxidation (%)

The data on the per cent inhibition of peroxidation as influenced by heating of the gel at different temperatures in different Aloe accessions are presented in table 22

and figure 22. There were significant differences in per cent inhibition due to different temperatures in various Aloe accessions during the storage.

The results indicated that the per cent inhibition of peroxidation was significantly decreased during the storage period up to 30th day in all the treatments. Out of the different accessions of Aloe studied, the highest per cent inhibition of peroxidation on day 1 (66.33) was recorded in yellow flowering accession-1 which has decreased (64.32, 62.76 and 61.72) at 10th, 20th and 30thday of storage respectively) and was significantly superior to yellow flowering accession-2 (64.26, 63.16, 61.49 and 60.43 at 1st, 10th, 20th and 30th day of storage respectively). The lowest per cent inhibition of peroxidation on day 1 (60.98) was recorded with orange flowering accession-3 and has decreased to 60.11, 58.80 and 57.21 at 10th, 20th and 30th day of storage respectively.

In respect of different temperatures, the highest per cent inhibition of peroxidation (64.30) was recorded on heating at 75^{0}C which has decreased (62.80, 61.26 and 60.08 at all storage intervals respectively). The was on par with 50°C temperature (63.78, 62.47, 60.99 and 59.77% at day 1, 10th, 20th and 30th day of storage respectively) and the lowest per cent inhibition of peroxidation (63.49) was recorded on heating at 100^{0}C which has decreased to 62.32, 60.80 and 59.52 at 10th, 20th and 30th day of storage respectively.

With regard to interactions, the Aloe accessions and different temperatures showed that there was a significant difference between the treatments with respect to per cent inhibition of peroxidation during the storage period. Highest per cent inhibition on day 1 (66.90) was recorded by yellow flowering accession-1 on heating

at 75^0C which has decreased (64.60, 62.97 and 61.90 at 10^{th}, 20^{th} and 30^{th} day of storage respectively) and was on par with yellow flowering accession-1 on heating at 50°C (66.17, 64.27, 62.70 and 61.70% at all storage intervals). The lowest per cent inhibition on day 1 (60.43) was recorded with orange flowering accession-3 on heating at 100^0C which has decreased to 59.87, 58.60 and 56.93 at 10^{th}, 20^{th} and 30^{th} day of storage.

Highest antioxidant activity was recorded by yellow flowering accession-1 at all heating temperatures during the storage followed by yellow flowering accession-2 and orange flowering accession-3.

4.2.1.8 Microbial count

The data recorded on gel microbial count as influenced by heating of the gel at different temperatures in different Aloe accessions are presented in table 23.

The results indicated that the gel microbial count has increased gradually with increase in storage period up to 30^{th} day in all the treatments. On day 1, no microbial count was observed in all the treatments. On 10^{th} day of storage highest microbial count was observed in orange flowering accession-3 heated at 50°C (bacterial count-40, mould count-30) which was increased to maximum (bacterial count- >100, mould count- >100) at 30^{th} day of storage followed by the same accession heated at 100°C (bacterial count- 35, mould count- 17) on 10^{th} day of storage which was increased to maximum (bacterial count- >100, mould count- >100) at 30^{th} day of storage respectively. The lowest microbial count was recorded with yellow flowering accession-1 heated at 75°C temperature on 10^{th} day of storage (bacterial count-10,

mould count- 5) which was increased to maximum (bacterial count- 87, mould count- 75) at 30^{th} day of storage respectively.

Highest microbial count was recorded by orange flowering accession-3 at all temperatures in storage followed by yellow flowering accession-2 and the lowest microbial count was recorded with yellow flowering accession-1 at all stages.

Table 1: Influence of leaf age on leaf size (cm^2) in different accessions of Aloe.

Treatments	**Accessions**			
	A_1	**A_2**	**A_3**	**Mean**
Maturity ages				
M_1 (10 months)	350.33	332.33	234.00	**305.56**
M_2 (12 months)	553.33	479.33	304.33	**445.67**
M_3 (14 months)	686.67	569.00	404.67	**553.45**
Mean	**530.11**	**460.22**	**314.33**	

	Accessions	**Maturity ages**	**Interaction**
SEm ±	18.0489	18.0489	31.2615
CD at 5%	37.9207	37.9207	65.6805

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 2 : Influence of leaf age on leaf weight (g) in different accessions of Aloe.

Treatments	**Accessions**			
	A_1	**A_2**	**A_3**	**Mean**
Maturity ages				
M_1 (10 months)	446.33	331.00	216.67	**331.33**
M_2 (12 months)	514.67	431.33	322.33	**422.78**
M_3 (14 months)	559.00	479.00	402.67	**480.22**
Mean	**506.67**	**413.78**	**313.89**	

	Accessions	**Maturity ages**	**Interaction**
SEm ±	10.7163	10.7163	18.5612
CD at 5%	22.5150	22.5150	38.9971

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 3 : Influence of leaf age on gel weight (g) in different accessions of Aloe.

Treatments / Maturity ages	Accessions A_1	A_2	A_3	Mean
M_1 (10 months)	295.67	194.00	132.33	**207.33**
M_2 (12 months)	354.00	264.67	209.33	**276.00**
M_3 (14 months)	402.67	311.00	251.00	**321.56**
Mean	**350.78**	**256.56**	**197.56**	

	Accessions	Maturity ages	Interaction
SEm ±	7.8499	7.8499	13.5965
CD at 5%	16.4927	16.4927	28.5662

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 4 : Influence of leaf age on peel weight (g) in different accessions of Aloe.

Treatments / Maturity ages	Accessions A_1	A_2	A_3	Mean
M_1 (10 months)	123.00	138.67	76.00	**112.56**
M_2 (12 months)	139.33	171.33	116.33	**142.33**
M_3 (14 months)	160.00	175.67	136.67	**158.22**
Mean	**141.78**	**161.89**	**109.67**	

	Accessions	Maturity ages	Interaction
SEm ±	3.9969	3.9969	6.9299
CD at 5%	8.3975	8.3975	14.5449

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 5: Influence of leaf age on gel pH in different accessions of Aloe

Treatments	**Accessions**			
	A_1	**A_2**	**A_3**	**Mean**
Maturity ages				
M_1 (10 months)	3.47	3.57	3.77	**3.60**
M_2 (12 months)	4.13	4.40	4.63	**4.39**
M_3 (14 months)	4.50	4.70	5.17	**4.79**
Mean	**4.03**	**4.22**	**4.52**	

	Accessions	**Maturity ages**	**Interaction**
SEm ±	0.1122	0.1122	0.1944
CD at 5%	0.2358	0.2358	0.4084

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 6 : Influence of leaf age on gel acidity (%) in different accessions of Aloe.

Treatments	**Accessions**			
	A_1	**A_2**	**A_3**	**Mean**
Maturity ages				
M_1 (10 months)	0.32	0.21	0.13	**0.22**
M_2 (12 months)	0.28	0.19	0.08	**0.19**
M_3 (14 months)	0.25	0.16	0.06	**0.16**
Mean	**0.28**	**0.19**	**0.09**	

	Accessions	Maturity ages	Interaction
SEm ±	0.0074	0.0074	0.0127
CD at 5%	0.0154	0.0154	0.0267

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 7: Influence of leaf age on gel TSS in different accessions of Aloe.

Treatments	**Accessions**			
	A_1	**A_2**	**A_3**	**Mean**
Maturity ages				
M_1 (10 months)	0.63	0.53	0.47	**0.54**
M_2 (12 months)	0.73	0.60	0.57	**0.63**
M_3 (14 months)	0.90	0.77	0.67	**0.78**
Mean	**0.76**	**0.63**	**0.57**	

	Accessions	Maturity ages	Interaction
SEm ±	0.0880	0.0880	0.1523
CD at 5%	0.1848	0.1848	0.3201

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 8 : Influence of leaf age on reducing sugars (%) of gel in different accessions of Aloe.

Treatments	**Accessions**			
	A_1	**A_2**	**A_3**	**Mean**
Maturity ages				
M_1 (10 months)	0.020	0.017	0.014	**0.017**
M_2 (12 months)	0.032	0.026	0.025	**0.028**

M_3 (14 months)	0.054	0.050	0.045	**0.050**
Mean	**0.035**	**0.031**	**0.028**	

	Accessions	**Maturity ages**	**Interaction**
SEm ±	0.0015	0.0015	0.0026
CD at 5%	0.0032	0.0032	0.0056

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 9 : Influence of leaf age on non- reducing Sugars (%) of gel in different accessions of Aloe.

Treatments	**Accessions**			
	A_1	**A_2**	**A_3**	**Mean**
Maturity ages				
M_1 (10 months)	1.48	1.45	1.43	**1.45**
M_2 (12 months)	1.70	1.62	1.57	**1.63**
M_3 (14 months)	1.87	1.76	1.75	**1.79**
Mean	**1.68**	**1.61**	**1.58**	

	Accessions	**Maturity ages**	**Interaction**
SEm ±	0.0960	0.0960	0.1662
CD at 5%	0.2016	0.2016	0.3492

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 10 : Influence of leaf age on total sugars (%) of gel in different accessions of Aloe.

Treatments	Accessions			
	A_1	A_2	A_3	Mean
Maturity ages				
M_1 (10 months)	1.50	1.47	1.45	**1.47**
M_2 (12 months)	1.73	1.64	1.60	**1.66**
M_3 (14 months)	1.93	1.81	1.80	**1.84**
Mean	**1.72**	**1.64**	**1.61**	

	Accessions	Maturity ages	Interaction
SEm ±	0.0235	0.0235	0.0406
CD at 5%	0.0493	0.0493	0.0854

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 11: Influence of leaf age on gel moisture (%) in different accessions of Aloe.

Treatments	Accessions			
	A_1	A_2	A_3	Mean
Maturity ages				
M_1 (10 months)	93.47	86.20	75.07	**84.91**
M_2 (12 months)	95.30	88.57	77.13	**87.00**
M_3 (14 months)	98.23	91.43	81.03	**90.23**
Mean	**95.67**	**88.73**	**77.74**	

	Accessions	Maturity ages	Interaction
SE (m)±	0.6730	0.6730	1.1656
CD at 5%	1.4139	1.4139	2.4889

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 12 : Influence of leaf age on gel thiobarbituric acid reactive substances (% TBARS) in different accessions of Aloe.

Treatments	Accessions			
	A_1	A_2	A_3	Mean
Maturity ages				
M_1 (10 months)	460.00	471.00	517.33	**482.78**
M_2 (12 months)	425.00	436.00	475.00	**445.33**
M_3 (14 months)	401.67	411.67	457.67	**423.67**
Mean	**428.90**	**439.56**	**483.33**	

	Accessions	Maturity ages	Interaction
SE (m)±	1.7662	1.7662	3.0591
CD at 5%	3.7107	3.7107	6.4271

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 13: Influence of leaf age on % inhibition of peroxidation in different accessions of Aloe.

Treatments	Accessions			
	A_1	A_2	A_3	Mean
Maturity ages				
M_1 (10 months)	43.43	42.43	40.43	**42.10**
M_2 (12 months)	49.60	46.93	44.47	**47.00**
M_3 (14 months)	66.47	64.53	61.23	**64.08**
Mean	**53.17**	**51.30**	**48.71**	

	Accessions	Maturity ages	Interaction
SE (m)±	0.5425	0.5425	0.9397
CD at 5%	1.1398	1.1398	1.9743

A_1 : Yellow flowering accession-1
A_2 : Yellow flowering accession-2
A_3 : Orange flowering accession-3

Table 14: Effect of heating at different temperatures on gel pH in different accessions of Aloe.

Treatments	Days of storage			
	Fresh	10th day	20th day	30th day
Accessions				
A_1 Yellow flowering accession-1	4.41	4.48	4.64	4.77
A_2 Yellow flowering accession-2	4.76	4.83	5.11	5.41
A_3 Orange floweringAccession-3	5.17	5.30	5.63	5.89
SEm ±	**0.0471**	**0.0497**	**0.0537**	**0.0595**
CD at 5%	**0.0991**	**0.1044**	**0.1128**	**0.1250**
Temperatures				
$T_1 – 50^0$ C	4.79	4.90	5.20	5.46
T_2 - 75^0 C	4.76	4.82	5.00	5.16
T_3 - 100^0C	4.79	4.89	5.19	5.46
SEm ±	**0.0471**	**0.0497**	**0.0537**	**0.0595**
CD at 5%	**0.0991**	**0.1044**	**0.1128**	**0.1250**
Accessions×Temperatures				
A_1t_1 (Yellow flowering accession-1+50°C)	4.43	4.50	4.77	4.83
A_1t_2 (Yellow flowering accession-1+75°C)	4.40	4.43	4.53	4.67
A_1t_3 (Yellow flowering accession-1+100°C)	4.40	4.50	4.63	4.80
A_2t_1 (Yellow flowering accession-2 +50°C)	4.77	4.87	5.17	5.53
A_2t_2 (Yellow flowering accession-2+75°C)	4.73	4.77	5.03	5.20
A_2t_3 (Yellow flowering accession-2+100°C)	4.77	4.87	5.13	5.50
A_3t_1 (Orange flowering accession-3+50°C)	5.17	5.33	5.67	6.00
A_3t_2 (Orange flowering accession-3+ 75°C)	5.13	5.27	5.43	5.60
A_3t_3 (Orange flowering accession-3+100°C)	5.20	5.30	5.80	6.07
SEm ±	**0.0817**	**0.0861**	**0.0930**	**0.1030**
CD at 5%	**0.1716**	**0.1808**	**0.1953**	**0.2165**

Table 15: Effect of heating at different temperatures on gel acidity (%) in different accessions of Aloe.

Treatments	Days of storage			
	Fresh	10th day	20th day	30th day
Accessions				
A_1 Yellow flowering accession-1	0.24	0.24	0.22	0.22
A_2 Yellow flowering accession-2	0.16	0.16	0.15	0.14
A_3 Orange flowering accession-3	0.07	0.07	0.07	0.06
SEm ±	**0.0057**	**0.0057**	**0.0057**	**0.0056**
CD at 5%	**0.0120**	**0.0120**	**0.0120**	**0.0117**
Temperatures				
$T_1 – 50^0$ C	0.15	0.15	0.14	0.14
T_2 - 75^0 C	0.16	0.16	0.15	0.15
T_3 - 100^0C	0.15	0.15	0.14	0.14
SEm ±	**0.0057**	**0.0057**	**0.0057**	**0.0056**
CD at 5%	**0.0120**	**0.0120**	**0.0120**	**0.0117**
Accessions×Temperatures				
A_1t_1 (Yellow flowering accession-1+50°C)	0.24	0.23	0.22	0.22
A_1t_2 (Yellow flowering accession-1+75°C)	0.24	0.24	0.23	0.23
A_1t_3 (Yellow flowering accession-1+100°C)	0.24	0.23	0.22	0.22
A_2t_1 (Yellow flowering accession-2+50°C)	0.16	0.16	0.15	0.14
A_2t_2 (Yellow flowering accession-2+75°C)	0.16	0.16	0.15	0.14
A_2t_3 (Yellow flowering accession-2+100°C)	0.15	0.15	0.15	0.14
A_3t_1 (Orange flowering accession-3+50°C)	0.07	0.07	0.06	0.06
A_3t_2 (Orange flowering accession-3+75°C)	0.08	0.08	0.07	0.07
A_3t_3(Orange flowering accession-3+100°C)	0.07	0.07	0.06	0.06
SEm ±	**0.0099**	**0.0099**	**0.0099**	**0.0097**
CD at 5%	**0.0208**	**0.0208**	**0.0208**	**0.0203**

Table 16 : Effect of heating at different temperatures on gel TSS (^{0} Brix) in different accessions of Aloe.

Treatments	Days of storage			
	Fresh	10th day	20th day	30th day
Accessions				
A_1 Yellow flowering accession-1	0. 90	0.97	1.04	1.20
A_2 Yellow flowering accession-2	0.77	0.81	0.89	1.07
A_3 Orange flowering accession-3	0.62	0.67	0.74	0.97
SEm ±	**0.0952**	**0.0816**	**0.0806**	**0.0846**
CD at 5%	**0.1999**	**0.1715**	**0.1694**	**0.1778**
Temperatures				
$T_1 – 50^0$ C	0.76	0.80	0.88	1.08
T_2 - 75^0 C	0.81	0.89	0.97	1.16
T_3 - 100^0C	0.72	0.76	0.83	1.0
SEm ±	**0.0952**	**0.0816**	**0.0806**	**0.0846**
CD at 5%	**0.1999**	**0.1715**	**0.1694**	**0.1778**
Accessions×Temperatures				
A_1 t_1 (Yellow flowering accession-1+ 50°C)	0.90	0.97	1.03	1.17
A_1 t_2 (Yellow flowering accession-1+ 75°C)	0.93	1.03	1.10	1.30
A_1 t_3 (Yellow flowering accession-1+ 100°C)	0.87	0.90	1.0	1.13
A_2 t_1 (Yellow flowering accession-2+ 50°C)	0.77	0.80	0.87	1.07
A_2 t_2 (Yellow flowering accession-2+ 75°C)	0.80	0.87	0.97	1.13
A_2 t_3 (Yellow flowering accession-2+ 100°C)	0.73	0.77	0.83	1.0
A_3 t_1 (Orange flowering accession-3+ 50°C)	0.60	0.63	0.73	1.0
A_3 t_2 (Orange flowering accession-3+ 75°C)	0.70	0.77	0.83	1.03
A_3 t_3 (Orange flowering accession-3+ 100°C)	0.57	0.60	0.67	0.87
SEm ±	**0.1648**	**0.1414**	**0.1397**	**0.1466**
CD at 5%	**0.3463**	**0.2971**	**0.2934**	**0.307**

Table 17: Effect of heating at different temperatures on reducing sugars (%) of gel in different accessions of Aloe.

Treatments	Days of storage			
	Fresh	10th day	20th day	30th day
Accessions				
A_1 Yelow flowering accession-1	0.050	0.652	1.288	1.838
A_2 Yellow flowering accession-2	0.048	0.631	1.244	1.788
A_3 Orange flowering accession-3	0.044	0.589	1.209	1.784
SEm ±	**0.0018**	**0.0284**	**0.0245**	**0.0256**
CD at 5%	**0.0037**	**0.0597**	**0.0515**	**0.0539**
Temperatures				
$T_1 – 50^0$ C	0.049	0.647	1.264	1.827
T_2 - 75^0 C	0.047	0.622	1.246	1.799
T_3 - 100^0C	0.046	0.603	1.231	1.784
SEm ±	**0.0018**	**0.0284**	**0.0245**	**0.0256**
CD at 5%	**0.0037**	**0.0597**	**0.0515**	**0.0539**
Accessions×Temperatures				
A_1 t_1 (Yellow flowering accession-1 + 50°C)	0.053	0.687	1.310	1.853
A_1 t_2 (Yellow flowerimg accession-1 + 75°C)	0.050	0.647	1.287	1.833
A_1 t_3 (Yellow flowering accession-1 + 100°C)	0.048	0.623	1.267	1.827
A_2 t_1 (Yellow flowering accession-2 + 50°C)	0.050	0.647	1.260	1.817
A_2 t_2 (Yellow flowering accession-2 + 75°C)	0.048	0.630	1.243	1.780
A_2 t_3 (Yellow flowering accession-2 + 100°C)	0.047	0.617	1.230	1.767
A_3 t_1 (Orange flowering accession-3 + 50°C)	0.046	0.607	1.223	1.810
A_3 t_2 (Orange flowering accession-3 + 75°C)	0.044	0.590	1.207	1.783
A_3 t_3 (Orange flowering accession-3 + 100°C)	0.043	0.570	1.197	1.760
SEm ±	**0.0030**	**0.0492**	**0.0424**	**0.0444**
CD at 5%	**0.0064**	**0.1034**	**0.0891**	**0.0933**

Table 18: Effect of heating at different temperatures on non-reducing sugars (%) of gel in different accessions of Aloe.

Treatments	Days of storage			
	Fresh	10th day	20th day	30th day
Accessions				
A_1 Yellow flowering accession-1	1.86	1.44	0.98	0.69
A_2 Yellow flowering accession-2	1.76	1.29	0.88	0.61
A_3 Orange flowering accession-3	1.73	1.30	0.88	0.60
SEm ±	**0.0188**	**0.0270**	**0.0195**	**0.0391**
CD at 5%	**0.0395**	**0.0568**	**0.0409**	**0.0822**
Temperatures				
$T_1 – 50^0$ C	1.79	1.35	0.93	0.64
T_2 - 75^0 C	1.78	1.35	0.91	0.64
T_3 - 100^0C	1.78	1.34	0.90	0.63
SEm ±	**0.0188**	**0.0270**	**0.0195**	**0.0391**
CD at 5%	**0.0395**	**0.0568**	**0.0409**	**0.0822**
Accessions×Temperatures				
A_1 t_1 (Yellow flowering accession-1 + 50°C)	1.87	1.44	1.00	0.70
A_1 t_2 (Yellow flowering accession-1 + 75°C)	1.86	1.45	0.97	0.69
A_1 t_3 (Yellow flowering accession-1 + 100°C)	1.86	1.44	0.97	0.67
A_2 t_1 (Yellow flowering accession-2 + 50°C)	1.76	1.30	0.89	0.61
A_2 t_2 (Yellow flowering accession-2 + 75°C)	1.76	1.29	0.88	0.62
A_2 t_3 (Yellow flowering accession-2 + 100°C)	1.76	1.29	0.86	0.61
A_3 t_1 (Orange flowering accession-3+ 50°C)	1.75	1.30	0.89	0.60
A_3 t_2 (Orange flowering accession-3+ 75°C)	1.73	1.30	0.88	0.60
A_3 t_3 (Orange flowering accession-3+ 100°C)	1.71	1.30	0.87	0.60
SEm ±	**0.0326**	**0.0468**	**0.0337**	**0.0678**
CD at 5%	**0.0685**	**0.0984**	**0.0709**	**0.1425**

Table 19: Effect of heating at different temperatures on total sugars (%) of gel in different accessions of Aloe.

Treatments	Days of storage			
	Fresh	10th day	20th day	30th day
Accessions				
A_1 Yellow flowering accession-1	1.91	2.09	2.27	2.53
A_2 Yellow flowering accession-2	1.81	1.92	2.12	2.41
A_3 Orange flowering accession-3	1.78	1.89	2.09	2.39
SEm ±	**0.0187**	**0.0223**	**0.0222**	**0.0214**
CD at 5%	**0.0393**	**0.0469**	**0.0467**	**0.0449**
Temperatures				
$T_1 – 50^0$ C	1.84	2.00	2.19	2.46
T_2 - 75^0 C	1.83	1.97	2.16	2.44
T_3 - 100^0C	1.82	1.94	2.13	2.42
SEm ±	**0.0187**	**0.0223**	**0.0222**	**0.0214**
CD at 5%	**0.0393**	**0.0469**	**0.0467**	**0.0449**
Accessions×Temperatures				
A_1 t_1 (Yellow flowering accession-1 + 50°C)	1.92	2.13	2.31	2.55
A_1 t_2 (Yellow flowering accession-1 + 75°C)	1.91	2.10	2.26	2.53
A_1 t_3 (Yellow flowering accession-1 + 100°C)	1.90	2.06	2.24	2.50
A_2 t_1 (Yellow floweing accession-2 + 50°C)	1.81	1.95	2.15	2.43
A_2 t_2 (Yellow flowering accession-2 + 75°C)	1.81	1.92	2.12	2.40
A_2 t_3 (Yellow flowering accession-2 + 100°C)	1.81	1.90	2.09	2.39
A_3 t_1 (Orange flowering accession-3 + 50°C)	1.80	1.91	2.12	2.41
A_3 t_2 (Orange flowering accession-3 + 75°C)	1.77	1.89	2.09	2.38
A_3 t_3 (Orange flowering accession-3 + 100°C)	1.76	1.87	2.06	2.36
SEm ±	**0.0324**	**0.0387**	**0.0385**	**0.0370**
CD at 5%	**0.0681**	**0.0813**	**0.0809**	**0.0778**

Table 20: Effect of heating at different temperatures on moisture (%) in different accessions of Aloe.

Treatments	Days of storage			
	Fresh	10th day	20th day	30th day
Accessions				
A_1 Yellow flowering accession-1	95.78	94.73	92.80	90.63
A_2 Yellow flowering accession-2	88.97	87.42	85.81	83.07
A_3 Orange flowering accession-3	78.57	77.19	74.74	73.02
SEm ±	**0.2254**	**0.3448**	**0.2508**	**0.2867**
CD at 5%	**0.4735**	**0.7244**	**0.5269**	**0.6023**
Temperatures				
$T_1 – 50^0$ C	88.07	86.82	84.78	82.68
T_2 - 75^0 C	87.77	86.46	84.47	82.27
T_3 - 100^0C	87.48	86.07	84.11	81.78
SEm ±	**0.2254**	**0.3448**	**0.2508**	**0.2867**
CD at 5%	**0.4735**	**0.7244**	**0.5269**	**0.6023**
Accessions×Temperatures				
A_1 t_1 (Yellow flowering accession-1 + 50°C)	96.07	95.10	93.13	91.07
A_1 t_2 (Yellow flowering accession-1 + 75°C)	95. 83	94.70	92. 80	90.67
A_1 t_3 (Yellow flowering accession-1+ 100°C)	95. 43	94.40	92. 47	90.17
A_2 t_1 (Yellow flowering accession-2 + 50°C)	89.30	87.83	86.07	83.47
A_2 t_2 (Yellow flowering accession-2 + 75°C)	88.90	87.43	85.80	83.10
A_2 t_3 (Yellow flowering accession-2 + 100°C)	88.70	87.00	85.57	82.63
A_3 t_1 (Orange flowering accession-3 + 50°C)	78.83	77.53	75.13	73.50
A_3 t_2 (Orange flowering accession-3 + 75°C)	78.57	77.23	74.80	73.03
A_3 t_3 (Orange flowering accession-3 + 100°C)	78.30	76.80	74.30	72.53
SEm ±	**0.3904**	**0.5972**	**0.4343**	**0.4965**
CD at 5%	**0.8202**	**1.2548**	**0.9126**	**1.0432**

Table 21: Effect of heating at different temperatures on thiobarbituric acid reactive substances (%TBARS) of gel in different accessions of Aloe.

Treatments	Days of storage			
	Fresh	10th day	20th day	30th day
Accessions				
A_1 Yellow flowering accession-1	404.11	412.33	422.89	435.89
A_2 Yellow flowering accession-2	427.56	433.78	443.44	447.22
A_3 Orange flowering accession-3	453.22	460.11	467.56	475.44
SEm ±	**4.1564**	**3.7963**	**3.6863**	**3.6840**
CD at 5%	**8.7326**	**7.9759**	**7.7448**	**7.7401**
Temperatures				
$T_1 – 50^0$ C	427.89	435.22	444.78	452.89
T_2 - 75^0 C	426.56	432.56	441.89	450.00
T_3 - 100^0C	430.44	438.44	447.22	455.67
SEm ±	**4.1564**	**3.7963**	**3.6863**	**3.6840**
CD at 5%	**8.7326**	**7.9759**	**7.7448**	**7.7401**
Accessions×Temperatures				
A_1 t_1 (Yellow flowering accession-1 + 50°C)	404.67	412.33	423.00	435.67
A_1 t_2 (Yellow flowering accession-1 + 75°C)	401.67	409.33	419.00	433.33
A_1 t_3 (Yellow flowering accession-1 + 100°C)	406.00	415.33	426.67	438.67
A_2 t_1 (Yellow flowering accession-2 + 50°C)	426.67	433.33	443.67	447.33
A_2 t_2 (Yellow flowering accession-2 + 75°C)	426.00	430.67	440.67	444.33
A_2 t_3 (Yellow flowering accession-2 + 100°C)	430.00	437.33	446.00	450.00
A_3 t_1 (Orange flowering accession-3 + 50°C)	452.33	460.00	467.67	475.67
A_3 t_2 (Orange flowering accession-3 + 75°C)	452.00	457.67	466.00	472.33
A_3 t_3 (Orange flowering accession-3 + 100°C)	455.33	462.67	469.00	478.33

SEm ±	**7.1991**	**6.5753**	**6.3848**	**6.3809**
CD at 5%	**15.1253**	**13.8147**	**13.4144**	**13.4063**

Table 22 : Effect of heating at different temperatures on % inhibition in different accessions of Aloe.

Treatments	**Days of storage**			
	Fresh	**10th day**	**20th day**	**30th day**
Accessions				
A_1 Yellow flowering accession-1	66.33	64.32	62.76	61.72
A_2 Yellow flowering accession-2	64.26	63.16	61.49	60.43
A_3 Yellow flowering accession-3	60.98	60.11	58.80	57.21
SEm ±	**0.5393**	**0.4051**	**0.2731**	**0.1776**
CD at 5%	**1.1332**	**0.8511**	**0.5737**	**0.3731**
Temperatures				
$T_1 - 50^0$ C	63.78	62.47	60.99	59.77
T_2 - 75^0 C	64.30	62.80	61.26	60.08
T_3 - 100^0C	63.49	62.32	60.80	59.52
SEm ±	**0.5393**	**0.4051**	**0.2731**	**0.1776**
CD at 5%	**1.1332**	**0.8511**	**0.5737**	**0.3731**
Accessions×Temperatures				
A_1 t_1 (Yellow flowering accession-1 + 50°C)	66.17	64.27	62.70	61.70
A_1 t_2 (Yellow flowering accession-1 + 75°C)	66.90	64.60	62.97	61.90
A_1 t_3 (Yellow flowering accession-1 + 100°C)	65.93	64.10	62.60	61.57
A_2 t_1 (Yellow flowering accession-2 + 50°C)	64.10	63.07	61.47	60.43
A_2 t_2 (Yellow flowering accession-2 + 75°C)	64.57	63.40	61.80	60.80
A_2 t_3 (Yellow flowering accession-2 + 100°C)	64.10	63.00	61.20	60.07
A_3 t_1 (Orange flowering accession-3 + 50°C)	61.07	60.07	58.80	57.17
A_3 t_2 (Orange flowering accession-3 + 75°C)	61.43	60.40	59.00	57.53
A_3 t_3 (Orange flowering accession-3 + 100°C)	60.43	59.87	58.60	56.93
SEm ±	**0.9342**	**0.7016**	**0.4729**	**0.3076**
CD at 5%	**1.9627**	**1.4751**	**0.9936**	**0.6463**

Table 23 : Effect of heating at different temperatures on microbial count in different accessions of Aloe.

Treatments	**Microbial count**							
	1st day		**10th day**		**20th day**		**30th day**	
	B	**Y/M**	**B**	**Y/M**	**B**	**Y/M**	**B**	**Y/M**
Yellow flowering accession-1 + 50°C	-	-	28	15	63	38	>100	>100
Yellow flowering accession-1 + 75°C	-	-	10	5	35	27	87	75
Yellow flowering accession-1 + 100°C	-	-	22	12	50	36	>100	93
Yellow flowering accession-2 + 50°C	-	-	37	21	75	54	>100	>100
Yellow flowering accession-2 + 75°C	-	-	15	9	44	32	92	79
Yellow flowering accession-2 + 100°C	-	-	29	15	50	41	>100	>100
Orange flowering accession-3 + 50°C	-	-	40	30	92	65	>100	>100
Orange flowering accession-3 + 75°C	-	-	17	10	60	35	>100	85
Orange flowering accession-3 +100°C	-	-	35	17	73	47	>100	>100

B - Bacterial count **Y/M** - Yeast / Mould count

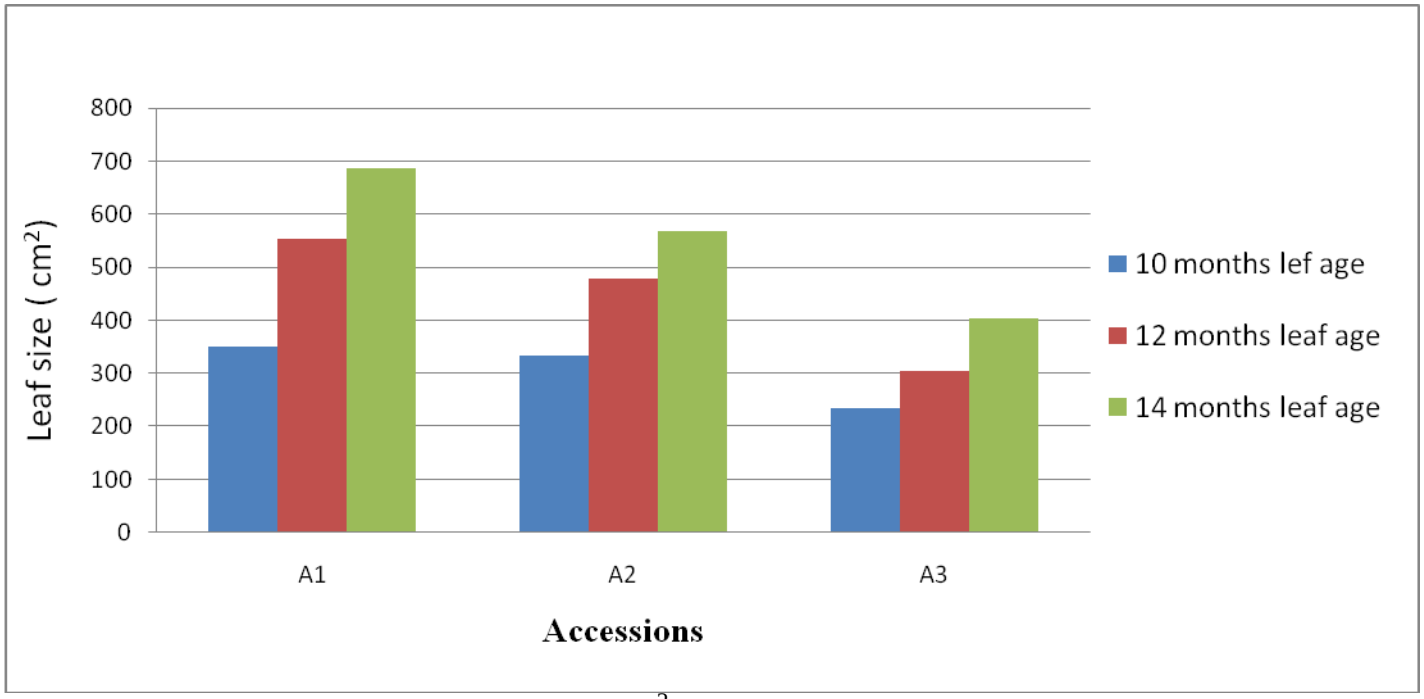

Fig: 1 Influence of leaf age on leaf size (cm^2) in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

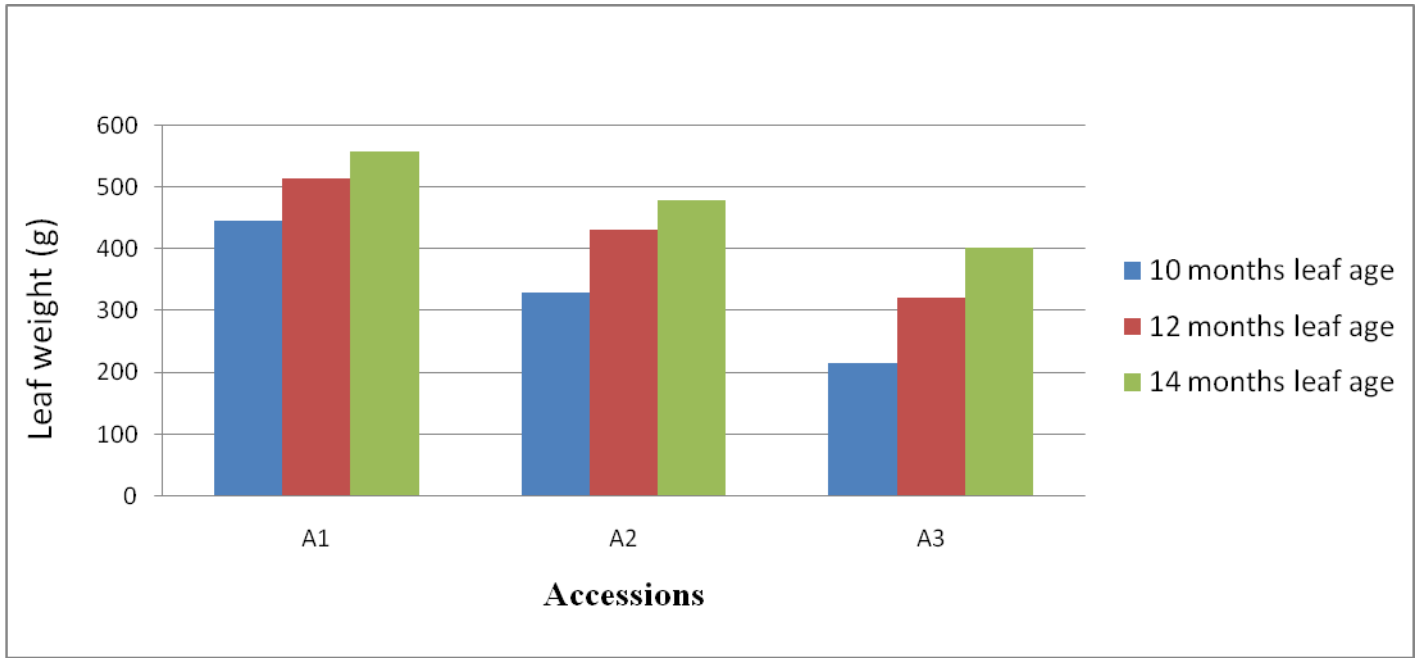

Fig: 2 Influence of leaf age on leaf weight (g) in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

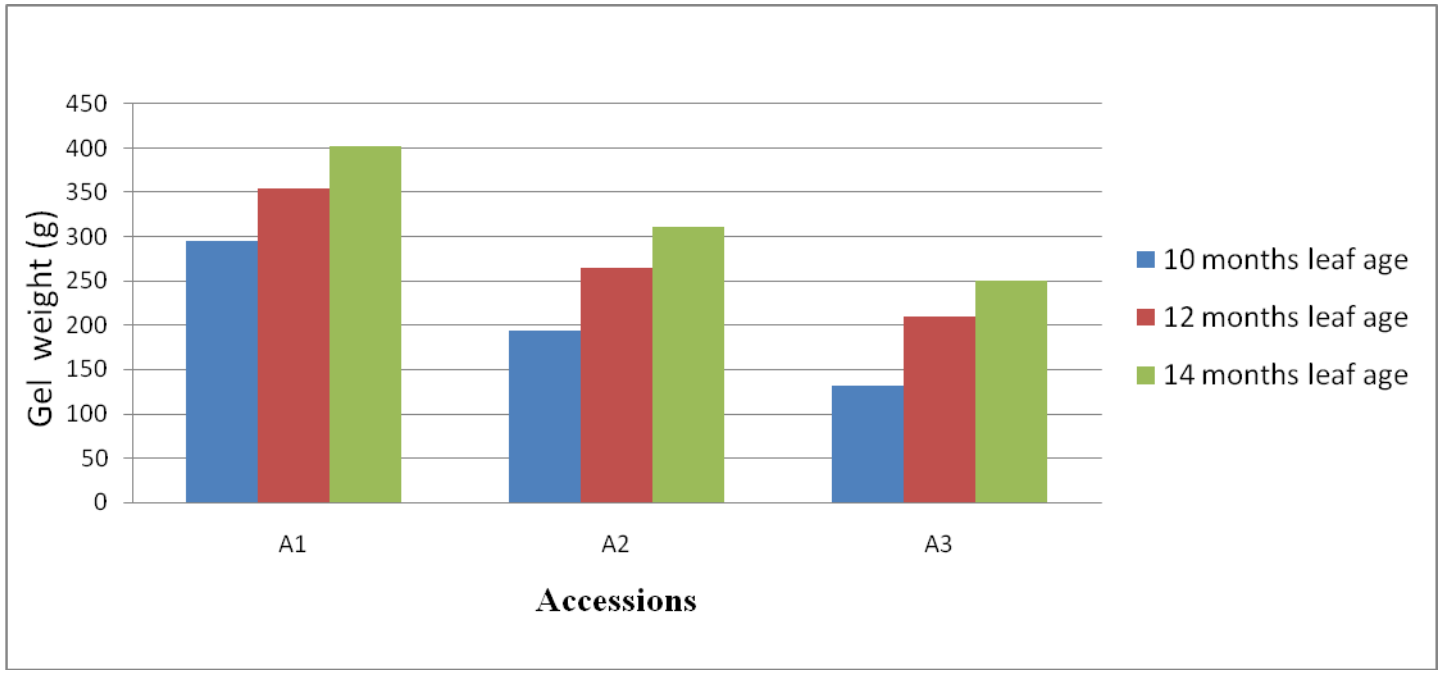

Fig: 3 Influence of leaf age on gel weight (g) in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

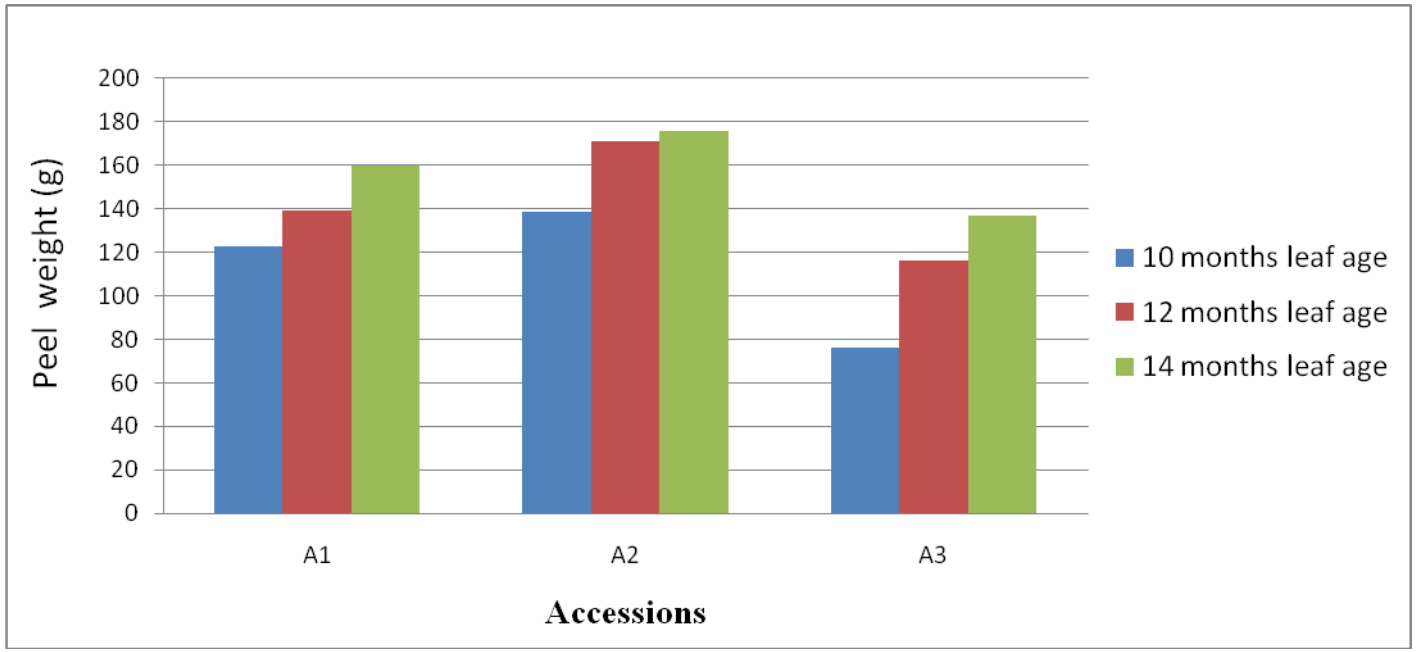

Fig: 4 Influence of leaf age on peel weight (g) in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

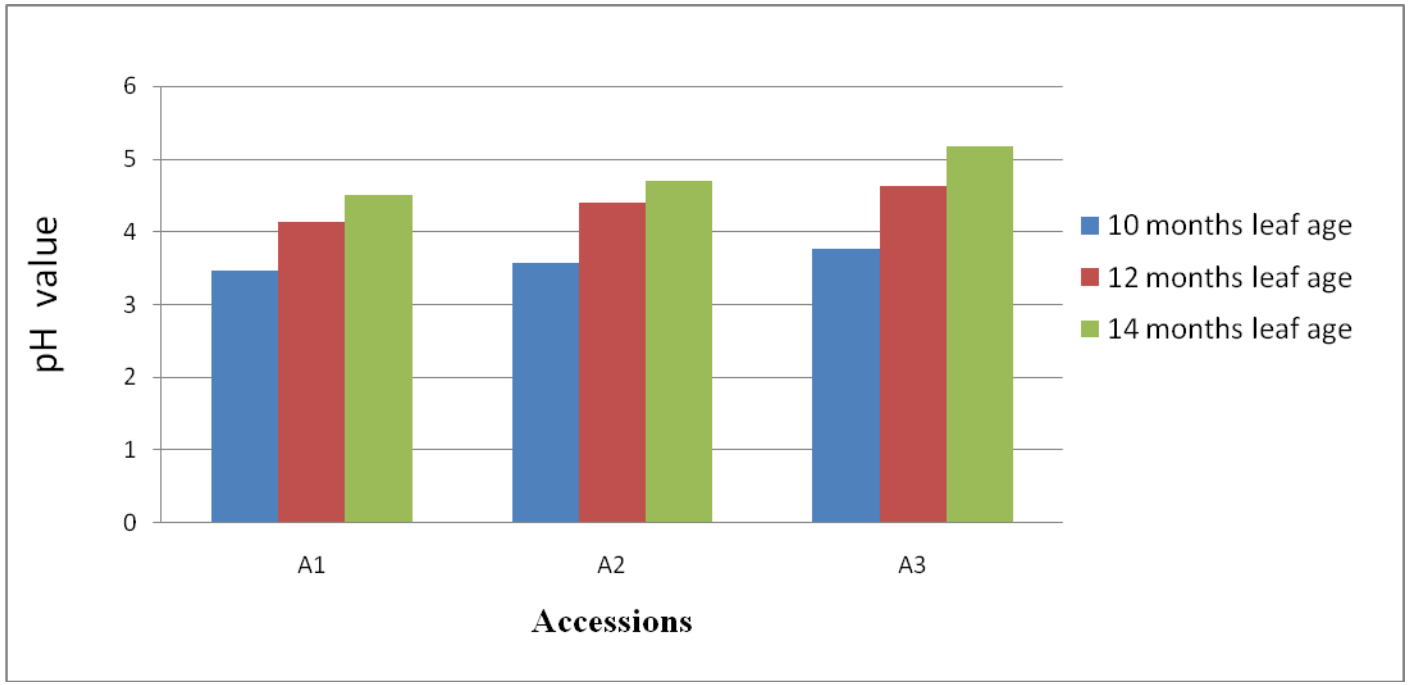

Fig: 5 Influence of leaf age on gel pH in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

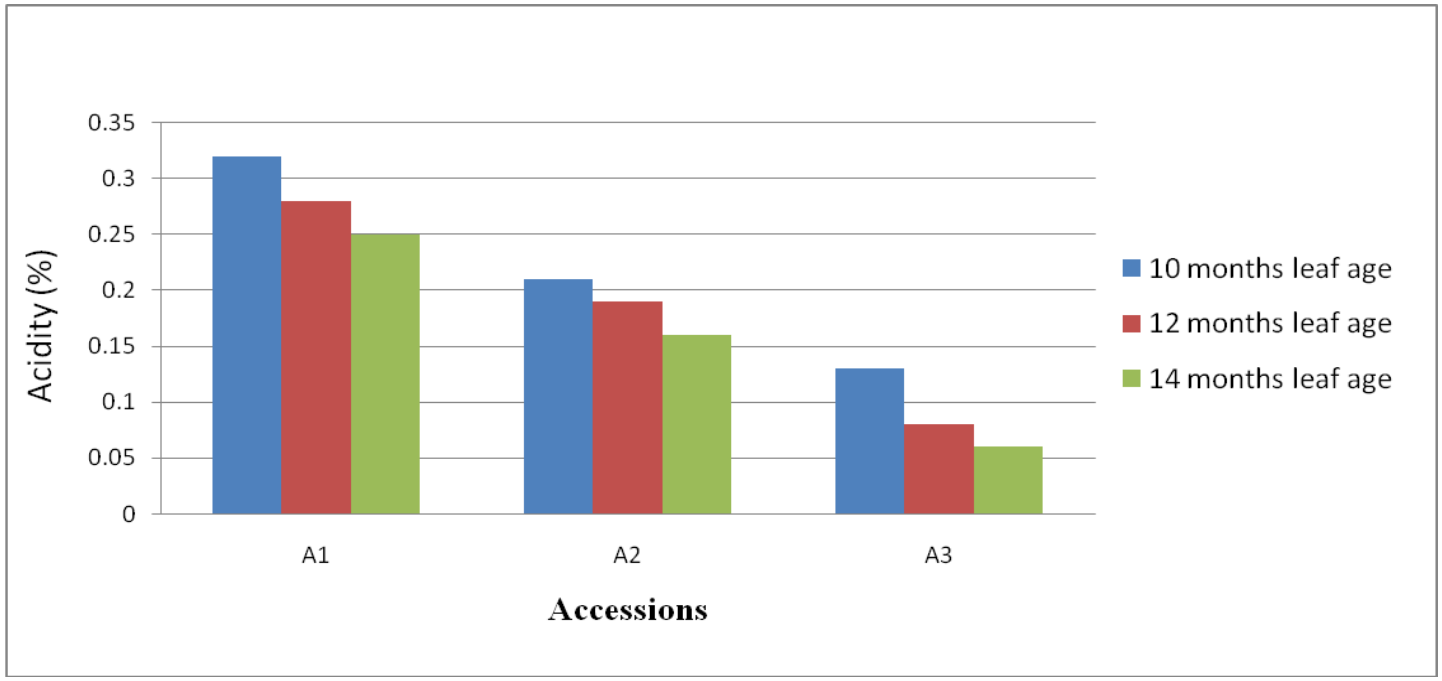

Fig: 6 Influence of leaf age on gel acidity (%) in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

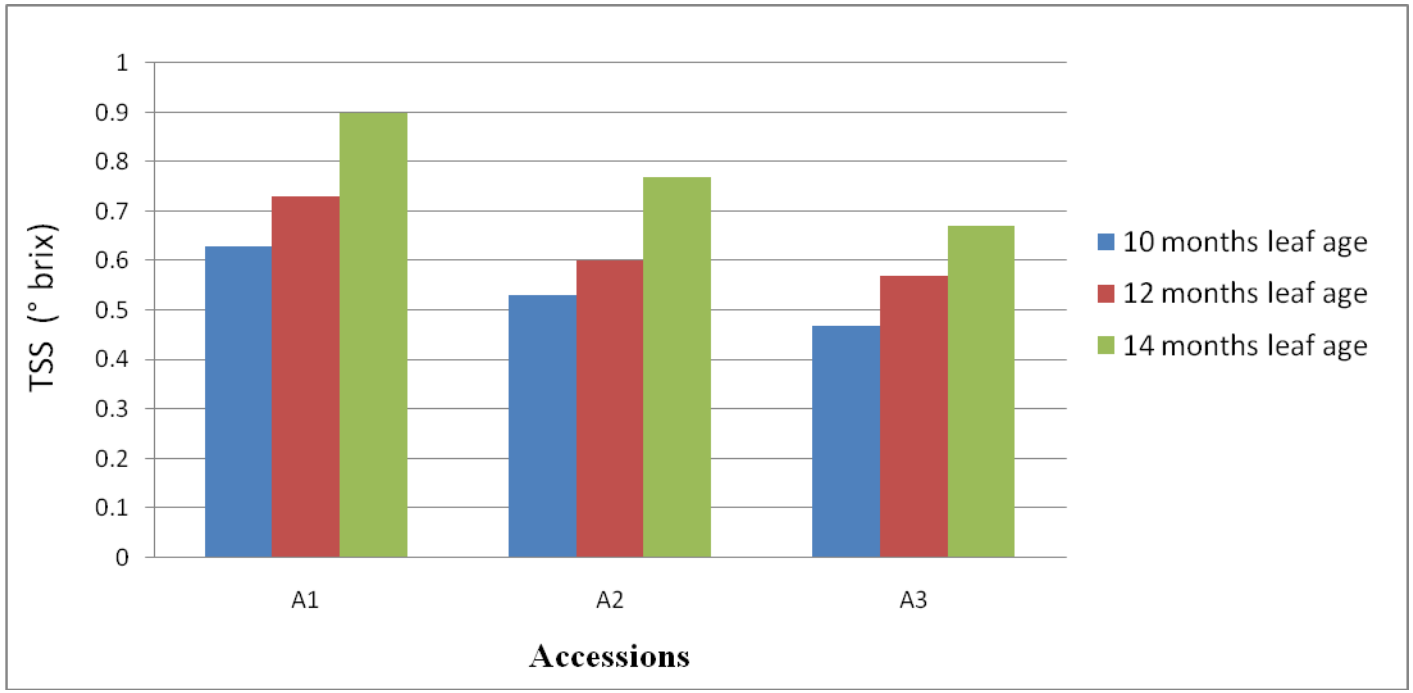

Fig: 7 Influence of leaf age on gel TSS (° brix) in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

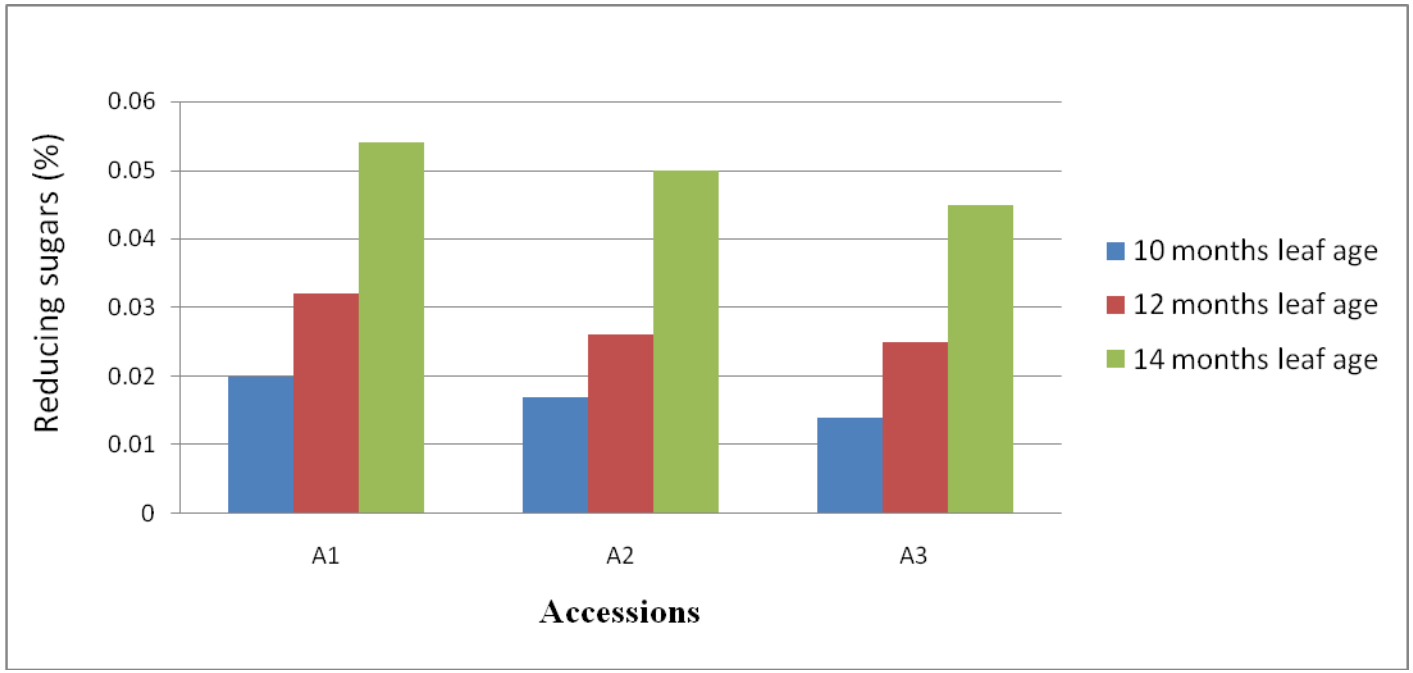

Fig: 8 Influence of leaf age on reducing sugars (%) of gel in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

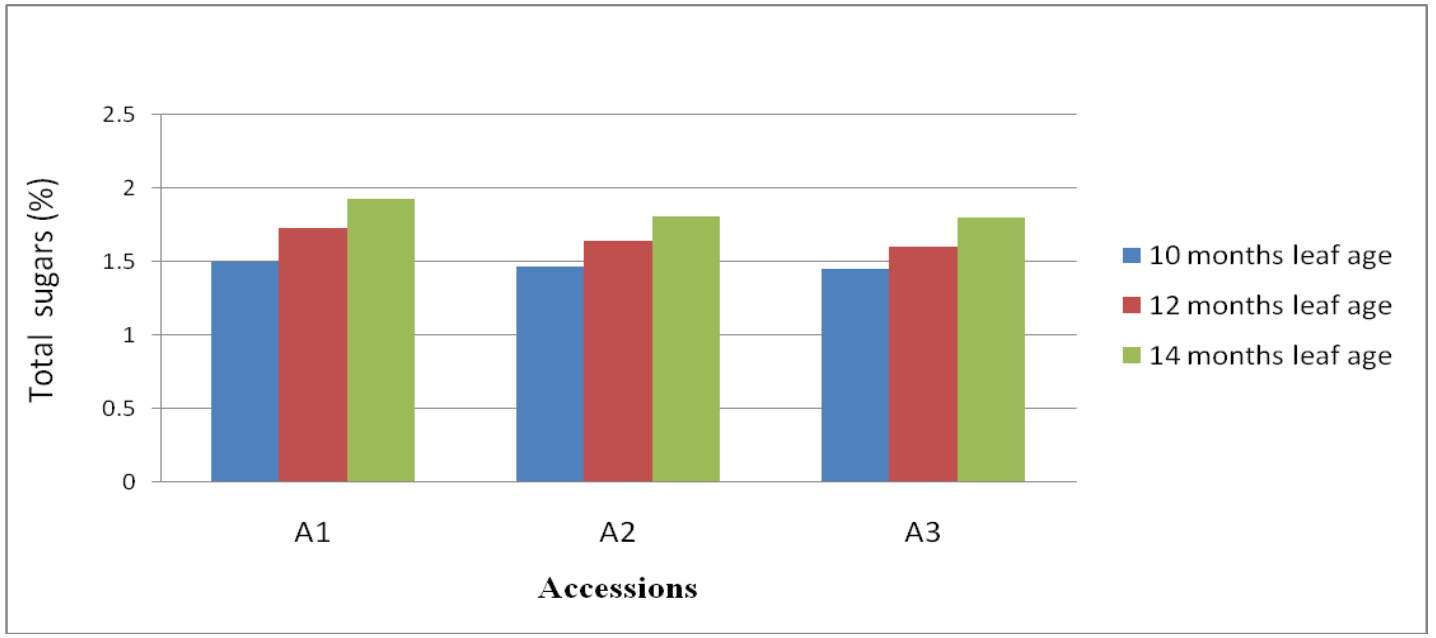

Fig: 10 Influence of leaf age on total sugars (%) of gel in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

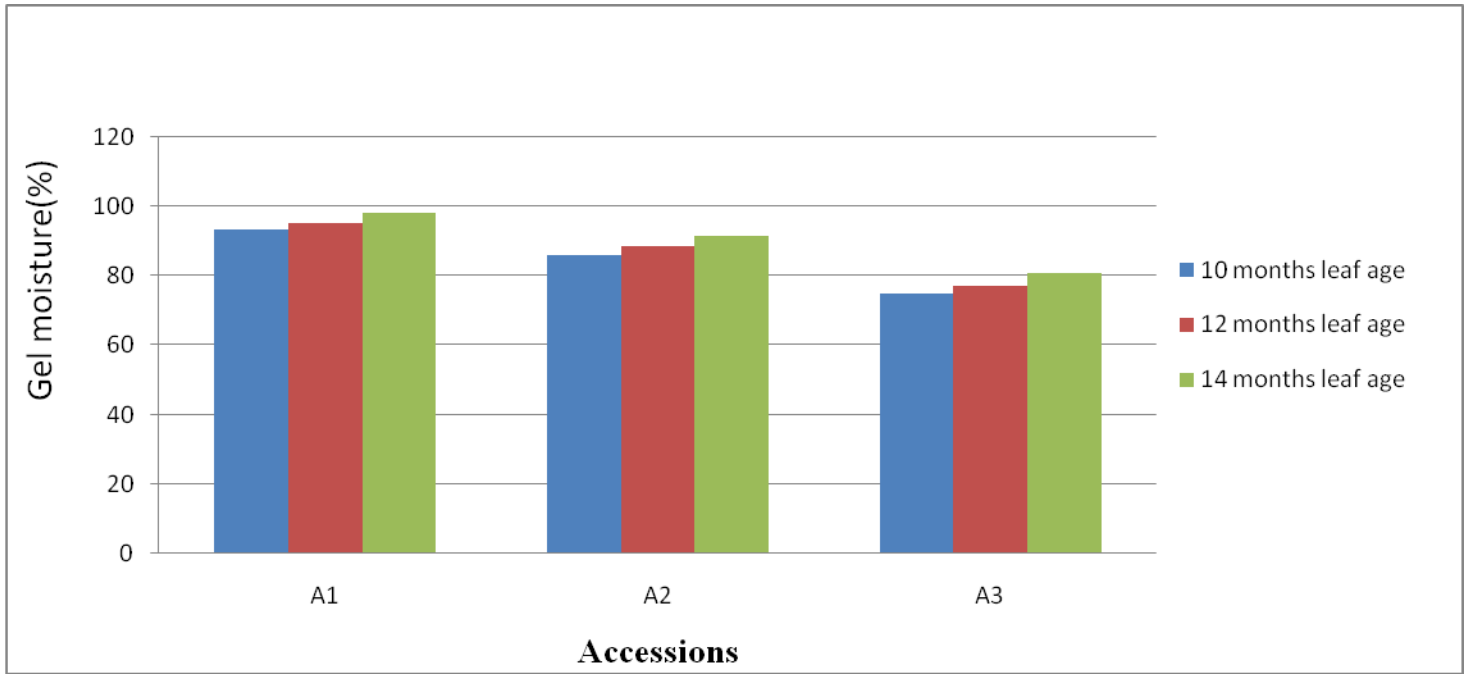

Fig: 11 Influence of leaf age on gel moisture (%) in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

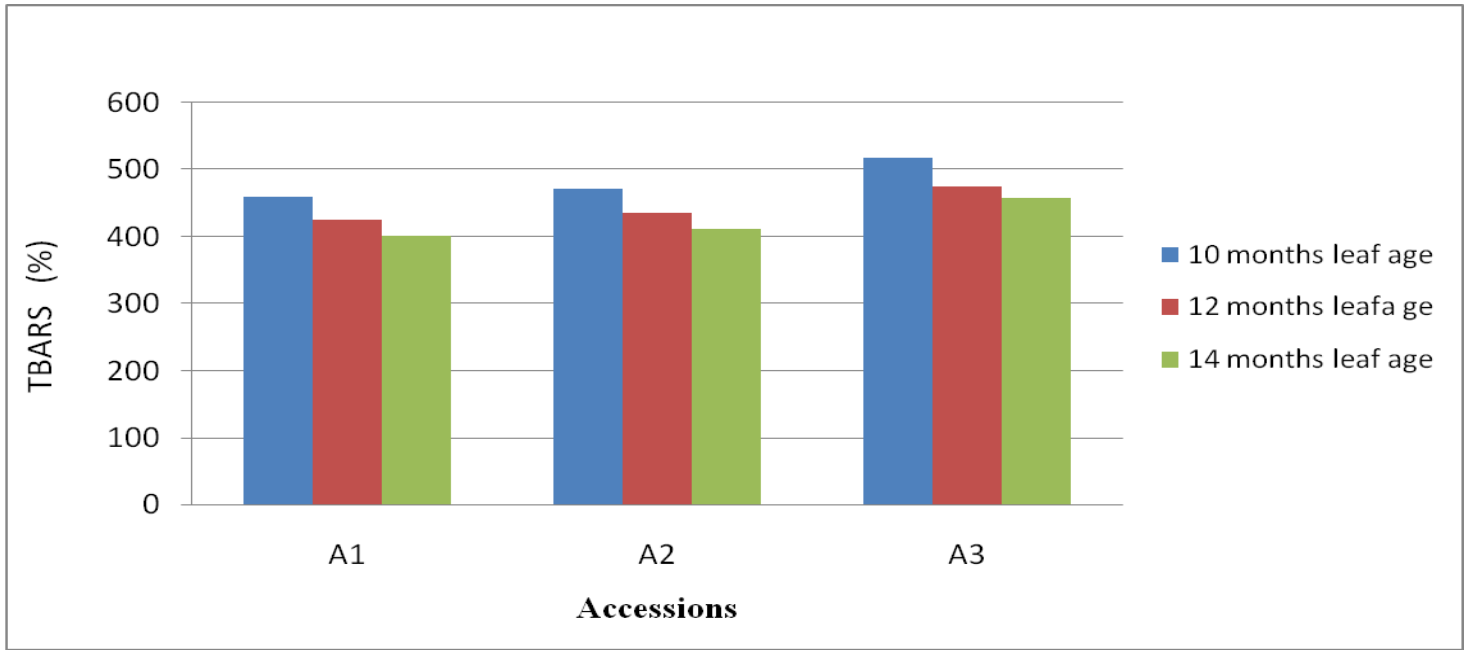

Fig: 12 Influence of leaf age on gel thiobarbituric acid reactive substances (% TBARS) in different accessions of Aloe.

Accessions

A_1- Yellow flowering accession-1

A_2- Yellow flowering accession-2

A_3- Orange flowering accession-3

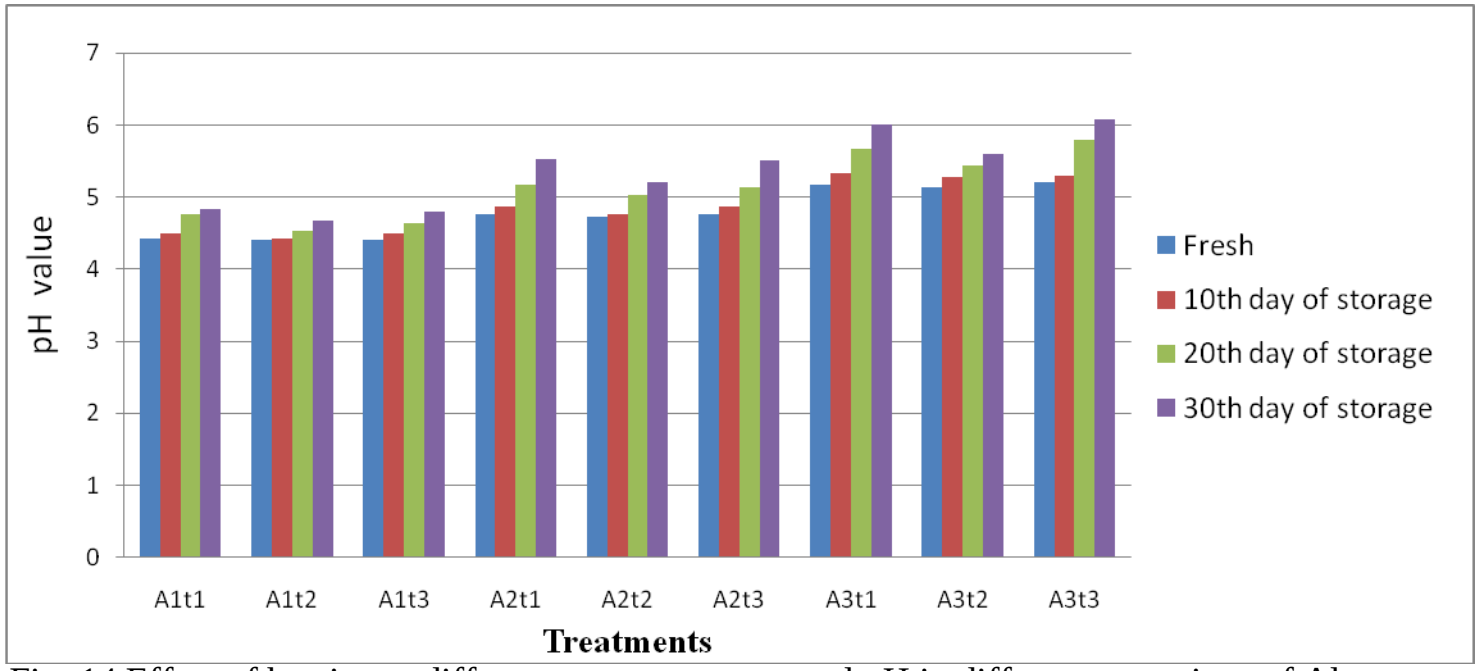

Fig: 14 Effect of heating at different temperatures on gel pH in different accessions of Aloe.

Accessions	**Temperatures**
A_1- Yellow flowering accession-1	t_1- 50°C
A_2- Yellow flowering accession-2	t_2- 75°C
A_3- Orange flowering accession-3	t_3- 100°C

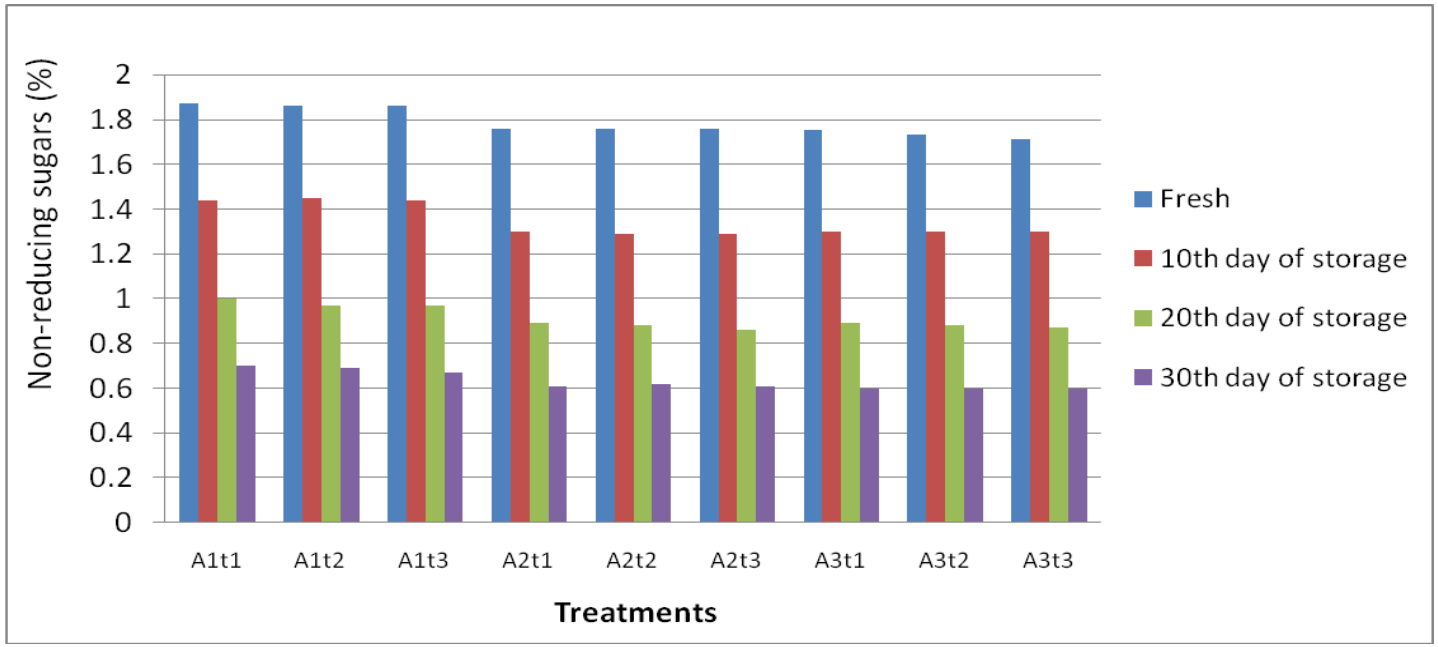

Fig: 18 Effect of heating at different temperatures on non-reducing sugars (%) of gel in different accessions of Aloe.

Accessions	**Temperatures**
A_1- Yellow flowering accession-1	t_1- 50°C
A_2- Yellow flowering accession-2	t_2- 75°C
A_3- Orange flowering accession-3	t_3- 100°C

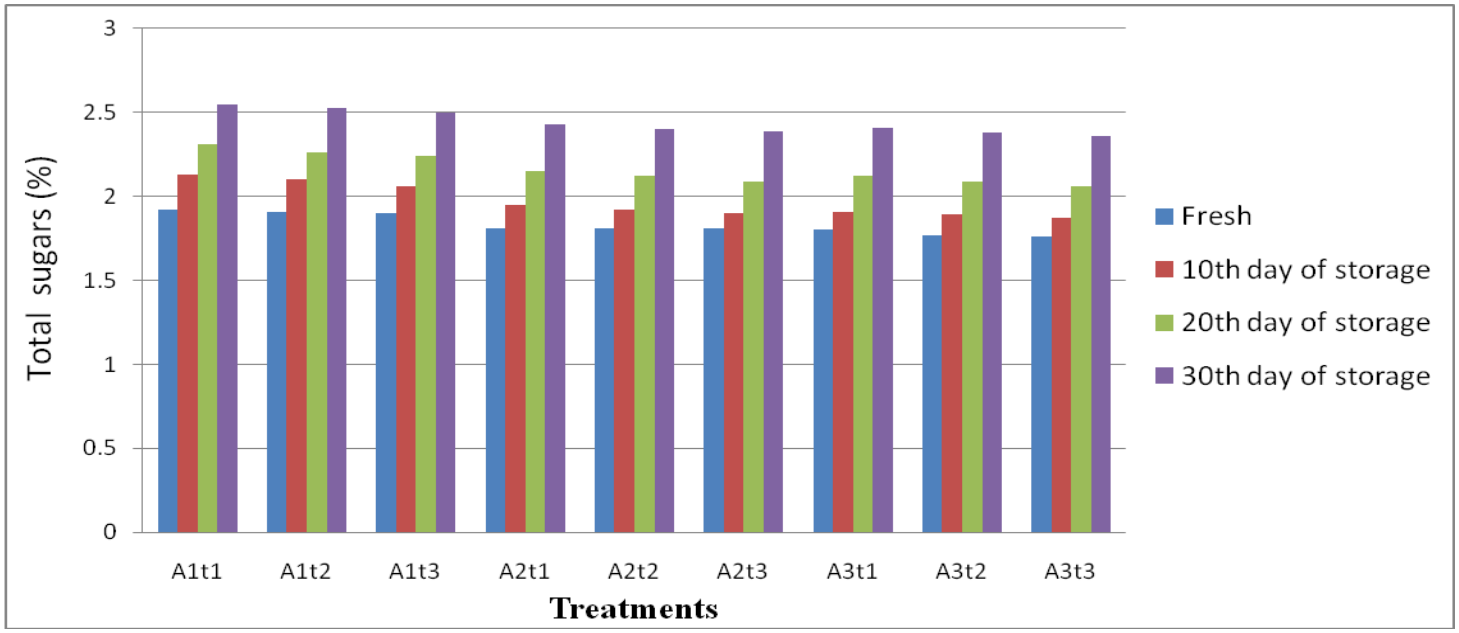

Fig: 19 Effect of heating at different temperatures on total sugars (%) of gel in different accessions of Aloe.

Accessions	**Temperatures**
A_1- Yellow flowering accession-1	t_1- 50°C
A_2- Yellow flowering accession-2	t_2- 75°C
A_3- Orange flowering accession-3	t_3- 100°C

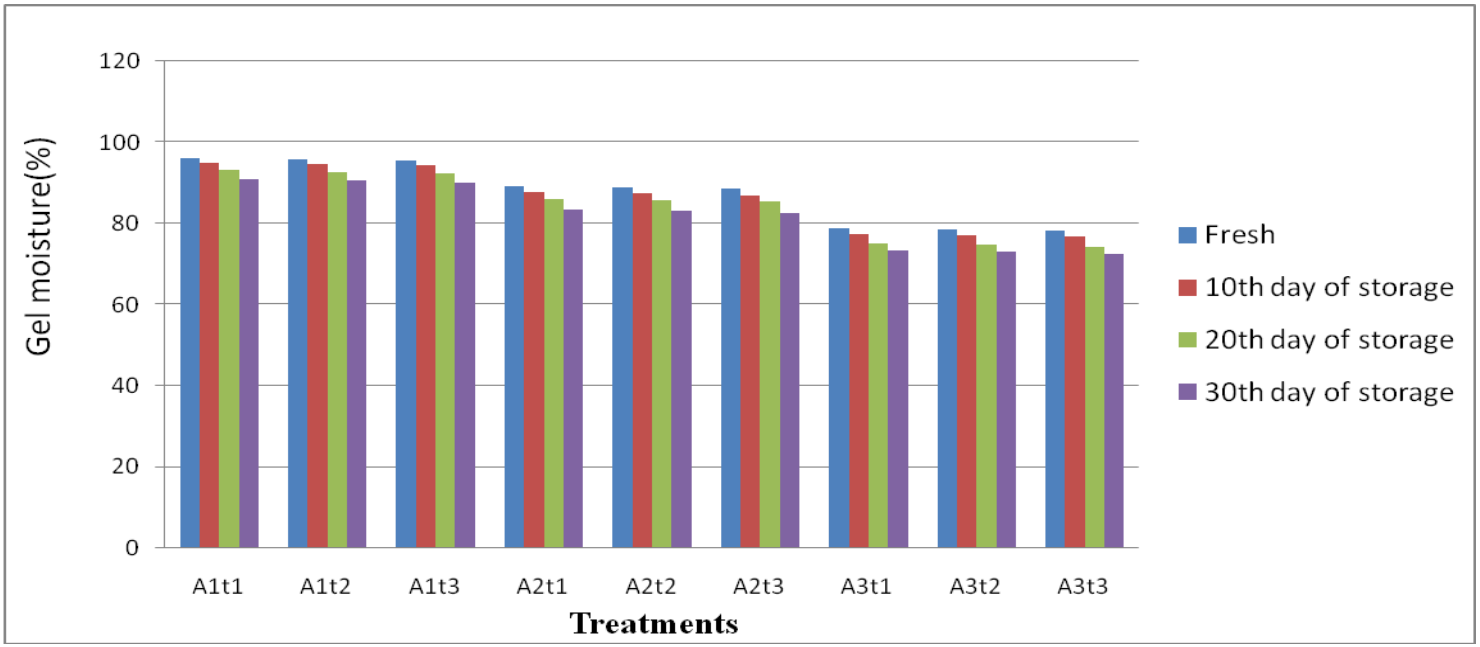

Fig: 20 Effect of heating at different temperatures on gel moisture (%) in different accessions of Aloe.

Accessions	**Temperatures**
A_1- Yellow flowering accession-1	t_1- 50°C
A_2- Yellow flowering accession-2	t_2- 75°C
A_3- Orange flowering accession-3	t_3- 100°C

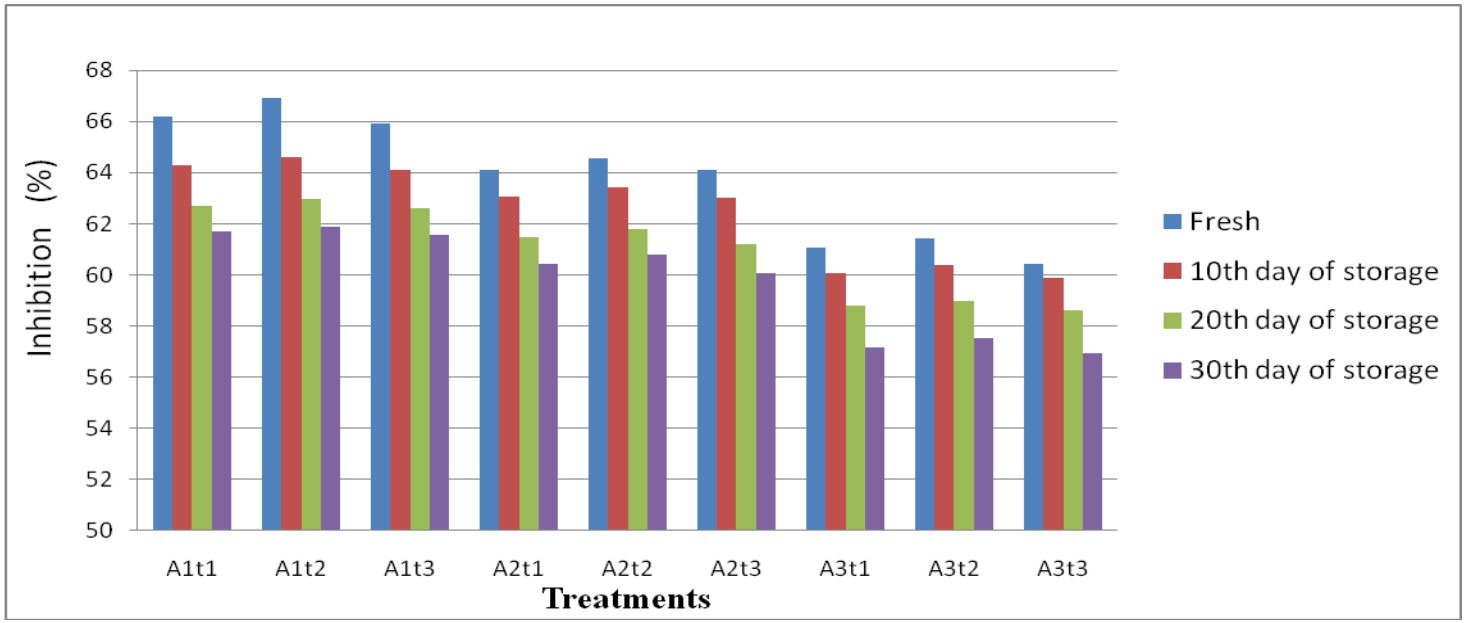

Fig: 22 Effect of heating at different temperatures on % inhibition in different accessions of Aloe.

Accessions	**Temperatures**
A_1- Yellow flowering accession-1	t_1- 50°C
A_2- Yellow flowering accession-2	t_2- 75°C
A_3- Orange flowering accession-3	t_3- 100°C

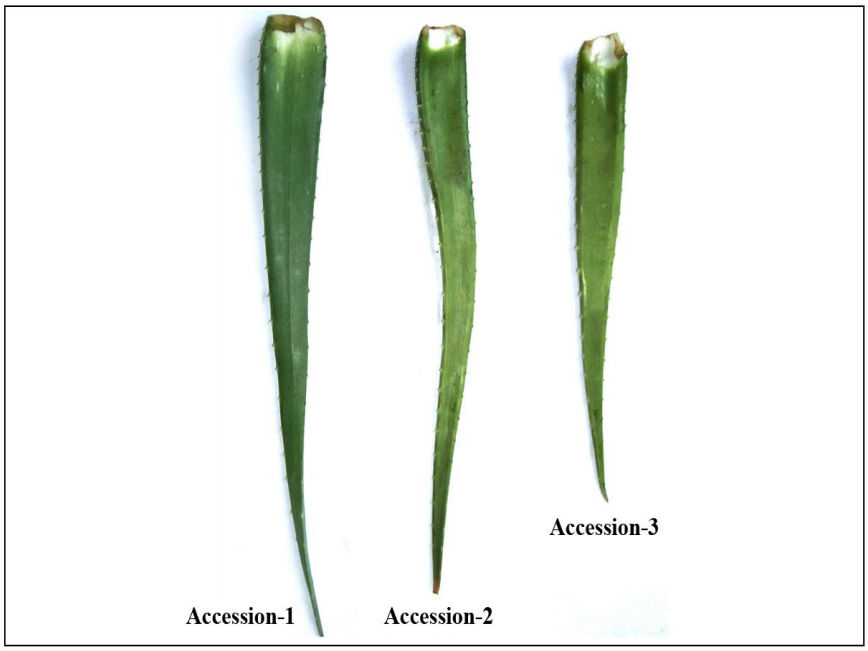

Plate8: 10 months aged leaves

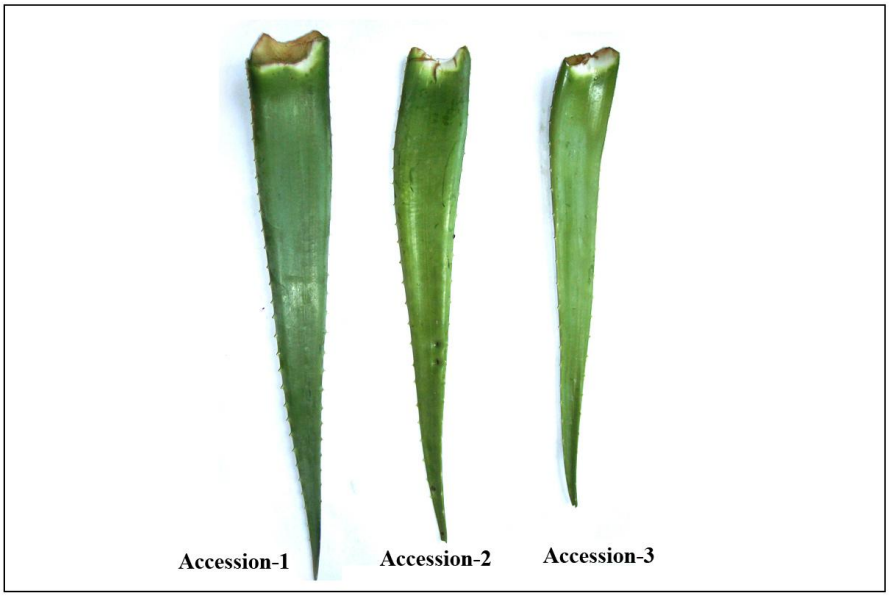

Plate6: 14 months aged leaves

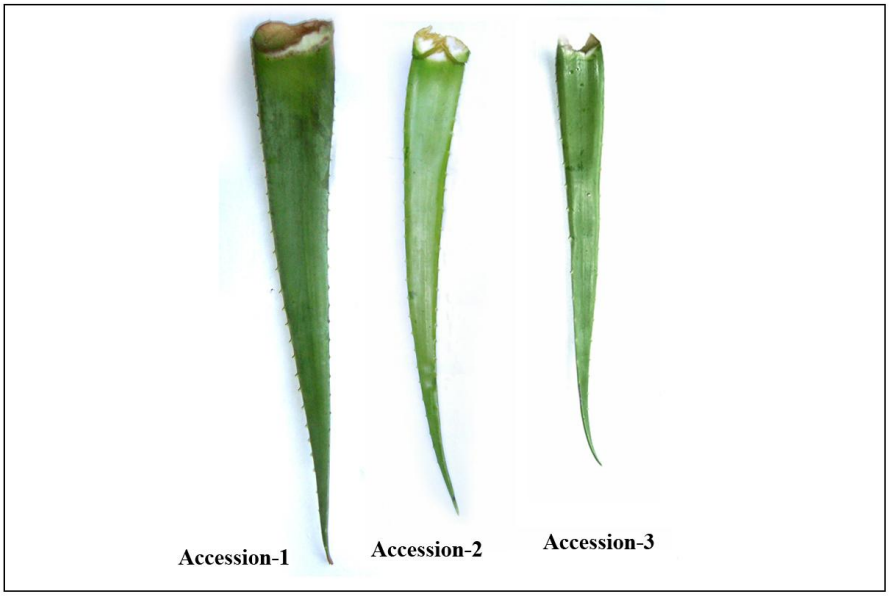

Plate7: 12 months aged leaves

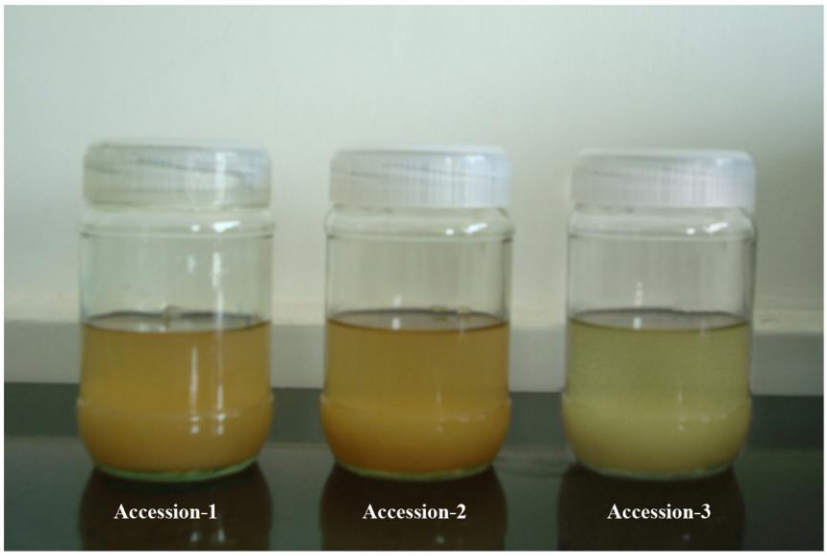

Plate5: Aloe gel

Plate4: Orange flowering accession-3

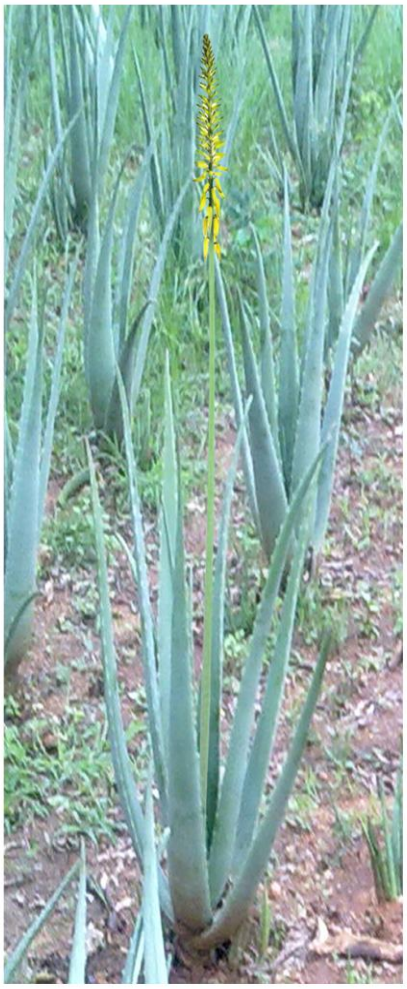

Plate2: Yellow flowering accession-1

Plate3: Yellow flowering accession-2

DISCUSSION

CHAPTER V

DISCUSSION

Aloe is nature's wonderful and valuable gift to mankind. Aloe plant is also called as miracle plant. It is widely used as a folk medicine. The two products viz., gel and yellow latex obtained from Aloe leaves have been medicinally used for centuries. Processing of gel from Aloe leaf pulp has become a big industry worldwide due to its usage in food industry. The plant is commonly found in the non-agricultural fields and forest areas. Presently, the farmers are coming forward for its commercial cultivation. The present study was undertaken to investigate the influence of leaf age and heating of the gel on the quality of different accessions of Aloe. Various treatments were subjected to chemical analysis periodically during storage and evaluated for their quality and microbial count. The data obtained on different quality characters are discussed here under.

In the present study, out of the three accessions of Aloe, yellow flowering accession-1 exhibited the optimum physico-chemical composition. In respect of different maturity ages of Aloe leaf (10 months, 12 months and 14 months), 14 months aged leaf recorded the optimum physico-chemical composition. With regard to different gel heating temperatures (50°C, 75°C and 100°C), Aloe gel heated at 75°C recorded the maximum storage stability.

Physico-chemical composition of Aloe leaf:

Aloe is one of the most popular naturally occurring plants with excellent therapeutic uses. The active principle of Aloe is a mixture of glycosides called "aloin". The chief constituent of 'aloin', is barbaloin which is a glucoside of aloe

emodin. The leaves of Aloe contain protein (3.2 g), fibre (15.2 g), iron (9.4 g) and also good amount of vitamin B_{12}, vitamin C, E and carotene, a precursor of vitamin A (Saroj *et al*., 2004).

A good knowledge of the physico-chemical composition of the leaves contributes to its importance in its usage in the preparation of commercial products. Chandegara and Varshney (2005) reported that Aloe gel contains 99.8 per cent moisture, 0.2 per cent fiber, 4.6 pH, total sugar content of 1.91%, reducing sugars of 0.026% and TSS of 0.93° brix in case of an optimum aged Aloe leaf.

Experiment I : Studies on gel recovery and quality components of Aloe leaf in different accessions at different ages.

Physical parameters:

Significant differences were observed in leaf size due to age, accessions and their interactions. As the age of the leaf increased, the size of the leaf also increased and the maximum was noticed with the oldest leaf among the three *i.e.,* 14 months aged leaf (553.45 cm^2). It is a fact that the growth of the plant parts increases with the increase of age and reaches the highest at certain age which is considered as an optimum age. In the present investigation, 14 months age of the leaf may be optimum among the three age groups which recorded the maximum leaf size.

Yellow flowering accession-1, which is the most vigorously growing Aloe accession has recorded significantly maximum leaf size (530.11 cm^2) compared to the other accessions *viz.,* yellow flowering accession-2 and orange flowering accession-3 (460.22 and 314.33 cm^2 respectively). The yellow flowering accession-1 is robust and putting forth big sized leaves among the three as evident from the data (Fig. 1 and plate 6). A combination of the above two factors *viz.,* yellow flowering accession-1

and highest age of the leaf (14 months) has ultimately resulted in the production of large sized leaves (686.67 cm^2).

Leaf weight was also significantly influenced by the leaf age as the 14 months aged leaf recorded the highest leaf weight (480.22 g) followed by 12 months age (422.78 g) and the lowest in 10 months age (331.33 g). Among the three accessions, yellow flowering accession-1 has recorded the highest leaf weight (506.67 g) followed by yellow flowering accession-2 (413.78 g) and the lowest in orange flowering accession-3 (313.89 g).

There was no significant difference in the peel weight according to the age. The highest peel weight (158.22 g) was recorded with the 14 months aged leaf (33% of the total leaf weight) followed by 12 months aged leaf, recording peel weight of 142.33 g (34% of leaf weight) and the lowest (112.56 g) with 10 months aged leaf (34% of leaf weight). Though there were significant differences among the peel weights due to leaf age, the difference was very meager if we consider the ratio of leaf weight and peel weight. However, there were significant differences among the accessions regarding the per cent of peel weight in overall leaf weight (28%, 40% and 35% in yellow flowering accession-1, yellow flowering accession-2 and orange flowering accession-3 respectively).

Yellow flowering accession-1, owing to the highest leaf weight and lowest peel weight of 72% per cent, it has recorded the highest gel weight (350.78 g). Yellow flowering accession-2 has recorded the lowest per cent of gel weight (256.56 g) *i.e.,* 60 % of the total leaf weight. The reason being the highest peel weight of 161.89g (40% in overall leaf weight). Similarly orange flowering accession-3 has recorded

more per cent of gel weight i.e., 65% of the overall leaf weight owing to its lower peel weight (35% of leaf weight) compared to yellow flowering accession-2. It clearly indicates that though the leaf weight is more, the per cent of recovery of gel is less in yellow flowering accession-2 as there is wastage through peel compared to orange flowering accession-3.

The treatment combination of yellow flowering accession-1 with 14 months aged leaf has recorded in highest per cent of gel (72% of leaf weight) recording 402.67 g. Irrespective of the leaf age, yellow flowering accession-1 has recorded highest gel weights (350.78 g) followed by yellow flowering accession-2 (256.56 g) and orange flowering accession-3 (197.56 g).

Physico-chemical parameters:

Extracted gel of Aloe had TSS of 0.90°brix, pH of 4.5, acidity of 0.25, total sugars of 1.93 per cent, reducing sugars of 0.054 per cent and non-reducing sugars of 1.87 per cent. Similar results on the composition of Aloe leaf were reported by Chandegara and Varshney (2005). However, the slight variation in the physico-chemical composition of leaves of different accessions might be due to varietal characters.

The gel pH was significantly influenced by the leaf age as the highest gel pH (4.79) was recorded with 14 months aged leaf followed by 12 months aged leaf (4.39) and the lowest in 10 months aged leaf (3.60). This could be due to the per cent acidity of Aloe gel decreased with increase in leaf age. The pH increased with the decrease of the acidity. So, 14 months aged leaf recorded the maximum pH. Among the three accessions, orange flowering accession-3 has recorded the highest gel pH (4.52),

followed by yellow flowering accession-2 (4.22) and the lowest in yellow flowering accession-1 (4.03).

A combination of orange flowering accession-3 with 14 months aged leaf has resulted in highest gel pH (5.17). Whereas yellow flowering accession-2 with 14 months leaf age recorded the gel pH of 4.7. The lowest gel pH (3.47) was recorded by yellow flowering accession-1 with 10 months leaf age.

Significant differences were observed in gel acidity due to maturity age, accessions and their interactions. As the age of the leaf increased, the gel acidity has decreased. The maximum gel acidity (0.22%) was noticed with 10 months aged leaf followed by 12 months and 14 months aged leaf (0.19 and 0.16 respectively). It is a fact that as the acidity decreased, the pH of gel increased with increased age of the leaf. In respect of three accessions, the highest acidity was recorded by yellow flowering accession-1 (0.28%) followed by yellow flowering accession-2 and orange flowering accession-3 (0.19% and 0.09% respectively). A combination of the above two factors *viz.*, yellow flowering accession-1 with 10 months leaf age has recorded the maximum gel acidity (0.32%). There was an inverse relationship between the gel pH and gel acidity. Irrespective of the maturity age, yellow flowering accession-1 has recorded the highest gel acidity and lowest gel pH when compared to yellow flowering accession-2 and orange flowering accession-3.

The highest gel TSS was observed with 14 months aged leaf (0.78) followed by 12 months and 10 months aged leaf (0.63 and 0.54 respectively), clearly indicating increased TSS with increased age of leaf. As the age of leaf increased there is an upsurge in the hydrolysis of the polysaccharides into soluble solids (Li Jin Ting Hu

ZhengHai Gaopeng, 2007). Similarly, Koukournaras *et al*. (2007) reported that leaf age had a significant effect on the levels of soluble solids in rocket leaves (*Eruca sativa* Mill). Among the three accessions, yellow flowering accession-1 has recorded the maximum TSS content (0.76) followed by yellow flowering accession-2 and orange flowering accession-3 (0.63 and 0.57 respectively).

The treatment combination of yellow flowering accession-1 with 14 months aged leaf has recorded highest TSS (0.90). Irrespective of the age of leaf, yellow flowering accession-1 has recorded the highest TSS (0.76) when compared to yellow flowering accession-2 (0.63) and orange flowering accession-3 (0.57).

Significant differences were also observed in reducing sugars content due to age of leaf, accessions and their interactions. The highest per cent of reducing sugars (0.050%) were recorded by 14 months aged leaf followed by 12 months (0.028%) and 10 months aged leaf (0.017%). Reducing sugars content has increased with increase in leaf age. This could be due to the inversion of non-reducing sugars to reducing sugars caused by acids present in the Aloe gel. Enzymes could also contribute to this conversion to some extent. The rate of inversion was rapid initially, which may be due to availability of more substrate for inversion at initial stages. These results are in line with the observations made by Lu Zhi Zhu JunLing Yan Zhiwen (2008) in compound juice made of Aloe and Apple; Gajanana (2002) in amla juice.

The highest non-reducing sugars (1.79%) were recorded by 14 months aged leaf followed by 12 months and 10 months aged leaf (1.63% and 1.45% respectively). Among the accessions, yellow flowering accession-1 has recorded the maximum (1.68%) non-reducing sugars followed by yellow flowering accession-2 and orange

flowering accession-3 (1.61 and 1.58% respectively). In combination, yellow flowering accession-1 with 14 months aged leaf has recorded the maximum non-reducing sugars (1.87%).

The total sugars were significantly influenced by the leaf age as the highest total sugars were recorded with 14 months aged leaf (1.84%) followed by 12 months and 10 months aged leaf (1.66% and 1.47% respectively). Total sugars were increased with increase in leaf age. The increase in total sugars of Aloe leaf might be due to significant increase of reducing sugars and slight decrease of non-reducing sugars with increase in leaf age of Aloe.

Jiang Mei HanYongBin Du Hong Yan Ji Qin Gu ZhenXin, 2004 h reported higher content of total sugars and reducing sugars in Aloe whole leaves when compared to Aloe gel.

With regard to three accessions, maximum total sugars were recorded with yellow flowering accession-1 (1.72%) followed by yellow flowering accession-2 and orange flowering accession-3 (1.64% and 1.61% respectively). The treatment combination of yellow flowering accession-1 with 14 months aged leaf has recorded the maximum total sugars (1.93%).

Among the three age groups, 14 months aged leaf has recorded the highest moisture content (90.23%) followed by 12 months and 10 months aged leaf (87.0 and 84.91% respectively). Aloe gel moisture content increased with increase in age of the leaf. It might be due to the fact that as the age of the leaf increased the size of the leaf and gel content of the leaf increased as reported in 14 months aged leaf (553.45 cm^2 and 321.56 g respectively). The increased gel content of Aloe leaf might have

resulted in high % of moisture content of Aloe. Among the three accessions, yellow flowering accession-1 has recorded the maximum moisture content (95.67%) followed by yellow flowering accession-2 (88.73%) and orange flowering accession-3 (77.74%). The treatment combination of yellow flowering accession-1 with 14 months aged leaf has recorded the highest moisture content (98.23%). An increase in moisture content with regard to the increase in age of the leaf was noticed and this is attributed to the fact that there will be biological degradation of the soluble solids and sugars in Aloe. Similar findings were reported by Roy *et al*. (2007) and Smita Gautam and Pratima Awasthi (2007). Irrespective of the age groups of leaves, yellow flowering accession-1 has recorded the highest moisture content when compared to yellow flowering accession-2 and orange flowering accession-3.

Antioxidant activity:

There is an inverse relationship between the per cent Thiobarbituric acid reactive substances and per cent inhibition of peroxidation (Amruta Pritam and Purushottam Kale, 2007). If the per cent inhibition of peroxidation is high, antioxidant activity is also high. The results indicated that the highest antioxidant activity (64.08 %) was noticed by 14 months aged leaf followed by 12 months (47.0%) and 10 months aged leaf (42.10%). Measurement of bioactivity such as antioxidant capacity becomes more useful for assessing the healthiness of foods than measurement of specific micronutrients (Van Beckel and Jongen, 1997). All the Aloe extracts showed significant antioxidant activity. But the extract from the aged leaves exhibited strongest radical scavenging activity. Hence, growth stages plays a vital role in the composition and antioxidant activity of Aloe where 14 months aged leaf showed

highest antioxidant activity compared to 12 months and 10 months aged leaves. Similar results were also reported by Hu Yun Xu Juan Hu Qiu Hui (2003) in Aloe. Among the three accessions, yellow flowering accession-1 has recorded the highest antioxidant activity (53.17%) followed by yellow flowering accession-2 (51.30%) and orange flowering accession-3 (48.71%). A combination of yellow flowering accession-1 with 14 months aged leaf has recorded the highest antioxidant activity (66.47%). Irrespective of the ages, when compared to yellow flowering accession-2 and orange flowering accession-3, yellow flowering accession-1 has recorded the highest antioxidant activity.

Experiment II: Effect of heating at different temperatures on gel quality and microbial count in different accessions of Aloe.

The 14 months aged leaf in three accessions which recorded best results were utilized for 2nd experiment Gel extracted from each accession is heated at three temperatures *i.e.*, 50°C, 75°C and 100°C for 15 minutes and was stored at room temperature. The data on all the parameters were recorded at 10 days interval up to 30th day of storage.

Physico-chemical parameters:

There was not much difference in Aloe gel pH and Aloe acidity due to heating temperatures. Among the three accessions, the highest pH (5.17, 5.30, 5.63 and 5.89) was recorded by orange flowering accession-3 followed by yellow flowering accession-2 (4.76, 4.83, 5.11 and 5.41) and yellow flowering accession-1 (4.41, 4.48, 4.64 and 4.77) at day1, 10th, 20th and 30th day of storage respectively. Among the three temperatures, the highest pH was recorded by heating at 50°C (4.79, 4.90, 5.20 and 5.46) and 100°C temperature (4.79, 4.89, 5.19 and 5.46) followed by 75°C (4.76, 4.82,

5.0 and 5.16). There was a significant increase in pH with increase in storage period in all the accessions. It might be due to the fact that, acidity of Aloe gel decreases with the extension of storage period irrespective of preservatives and storage temperature. Similar observations were reported by Hemalatha *et al*. (2005) in Aloe and Gajanana (2002) in amla juice. A combination of orange flowering accession-3 heated at 100°C has recorded the maximum pH (5.20, 5.30, 5.80 and 6.07) at all storage intervals. But the optimum pH of Aloe is maintained by yellow flowering accession-1 heated at 75°C (4.40, 4.43, 4.53 and 4.67 respectively) at day1, 10^{th}, 20^{th} and 30^{th} day of storage.

The highest gel acidity (0.24, 0.24, 0.22 and 0.22%) was recorded by yellow flowering accession-1 followed by yellow flowering accession-2 (0.16, 0.16, 0.15 and 0.14%) and orange flowering accession-3 (0.07, 0.07, 0.07 and 0.06% respectively) at day1, 10^{th}, 20^{th} and 30^{th} day of storage. Among the temperatures, 75°C has recorded the maximum gel acidity (0.16, 0.16, 0.15 and 0.15%) at all storage intervals. The results indicated that there was a corresponding decrease in acidity content during the storage period irrespective of preservatives and storage temperature. Similar observations were reported in Aloe by Hemalatha *et al.* (2005). This might be due to the increased pH during storage. The result showed that there was an inverse relationship between the pH and acidity in Aloe gel. In combination, the maximum gel acidity (0.24, 0.24, 0.23 and 0.23%) was recorded by yellow flowering accession-1 heated at 75°C at all storage intervals.

Similarly, Miranda *et al*. (2009) reported that a drying temperature of 80°C and 90°C resulted in significant variation and/ loss of the physico-chemical and nutritional

properties of the gel. But, there was a minor alteration in the physico-chemical and nutritional properties of Aloe gel produced at drying temperature of 60-70°C, which resulted in the production of high quality gel.

Significant differences were observed in gel TSS due to accessions, temperatures and their interactions. Yellow flowering accession-1 has recorded the highest gel TSS (0.90, 0.97, 1.04 and 1.20) followed by yellow flowering accession-2 (0.77, 0.81, 0.89 and 1.07) and orange flowering accession-3 (0.62, 0.67. 0.74 and 0.97) at day1, 10^{th}, 20^{th} and 30^{th} day of storage. Among the temperatures, heating of gel at 75°C has recorded the maximum TSS (0.81, 0.89, 0.97 and 1.16) followed by 50°C (0.76, 0.80, 0.88 and 1.08) and 100°C (0.72, 0.76, 0.83 and 1.0 respectively) at all storage intervals. The results indicated that the TSS content increased with the advancement of storage in all accessions at all the temperatures. It might be due to increase in soluble solids content and total soluble sugars caused by hydrolysis of polysaccharides like starch, cellulose and pectin substances into simpler substances. The moisture content also decreased during the storage. There was an inverse relationship between moisture and total soluble solids. This indicated that during storage, there was change in the Aloe gel composition. Similar results were observed in amla juice during storage (Gajanana, 2002).

The maximum reducing sugars (0.050, 0.652, 1.288 and 1.838%), non-reducing sugars (1.86, 1.44, 0.98 and 0.69) and total sugars (1.91, 2.09, 2.27 and 2.53%) were recorded by yellow flowering accession-1 at day 1, 10^{th}, 20^{th} and 30^{th} day of storage respectively followed by yellow flowering accession-2 and orange flowering accession-3. Among the three temperatures, Aloe gel heated at 50°C has recorded the

maximum reducing (0.049, 0.647, 1.264 and 1.827%), non-reducing (1.79, 1.35, 0.93 and 0.64%) and total sugars (1.84, 2.0, 2.19 and 2.46% at all storage intervals) followed by 75°C and 100°C temperatures. A combination of above two factors viz., yellow flowering accession-1 heated at 50°C has recorded the maximum reducing (0.053, 0.687, 1.310, and 1.853%), non-reducing (1.87, 1.44, 1.0, 0.70%) and total sugar content (1.92, 2.13, 2.31 and 2.55% at all storage intervals). The reducing sugars and total sugars were found to increase significantly throughout the storage period but there was a corresponding decline in non-reducing sugars. The reduction in non-reducing sugars might be due to the inversion of non-reducing sugars to reducing sugars which caused by acids present in the Aloe gel. Similarly, hydrolysis of polysaccharides during storage might have resulted in increase of soluble sugars in Aloe gel. These results are similar with the observations made by Lu zhi zhu JunLing Yan Zhiwen (2008) in compound juice made of Aloe and apple and Gajanana (2002) in amla juice. Increase in reducing sugars and total sugars and decrease in non-reducing sugars during storage is a general phenomenon as noticed by Khambalkar *et al.* (2007) in Aloe.

Significant differences were observed in moisture of gel due to accessions, heating temperatures and their interactions. The highest moisture content (95.78, 94.73, 92.80 and 90.63%) was recorded by yellow flowering accession-1 followed by yellow flowering accession-2 (88.97, 87.42, 85.81 and 83.07%) and orange flowering accession-3 (78.57, 77.19, 74.74 and 73.02%) at day1, 10^{th}, 20^{th} and 30^{th} day of storage. Among the temperatures, Aloe gel heated at 50°C has recorded the highest moisture (88.07, 86.82, 84.78 and 82.68%) followed by 75°C (87.77, 86.46, 84.47 and

82.27%) and 100°C (87.48, 86.07, 84.11 and 81.78% respectively) at all storage intervals. The results indicated that there was a slight decrease in moisture content during the storage of Aloe gel. It might be due to the usage of the moisture for the chemical and biological degradation of the samples. Heating of the gel may also be one of the reasons for decrease of moisture content in Aloe gel. Similar results were obtained in Aloe leaf juice (Khambalkar, 2007) and compound juice made of Aloe and apple (Lu Zhi Zhu Jun Ling Yan ZhiWen, 2008).

Antioxidant activity:

There was an inverse relationship between the per cent TBARS and per cent inhibition of peroxidation in Aloe (Amruta Pritam and Purushottam Kale, 2007). Measurement of bioactivity such as antioxidant capacity becomes more useful for assessing the healthiness of foods than measurement of specific micronutrients (Van Beckel and Jongen, 1997). All the Aloe extracts showed significant antioxidant activity. Yellow flowering accession-1 heated at 75°C temperature recorded maximum antioxidant activity followed by the same accession heated at 50°C and 100°C. Miranda *et al*. (2009) reported that the antioxidant capacity of the gel was decreased at drying temperatures of 80°C and 90°C but Aloe gel produced at drying temperature of 60-70°C, resulted in the production of high quality gel. Irrespective of the temperatures, yellow flowering accession-1 has recorded the highest antioxidant activity when compared to yellow flowering accession-2 and orange flowering accession-3.

Microbial count:

Aloe gel and leaf itself has an antimicrobial activity. The results obtained showed that there was a slight increase in microbial load in all the treatments at all heating temperatures during the storage period. For fresh juice, on day 1 no microbial count was observed. The Aloe gel can be stored for 20 days at room temperature without deterioration in quality. Similar observations were reported by Hemalatha *et al*. (2005) in Aloe.

At 30^{th} day of storage of Aloe gel, significant microbial load was observed in all the treatments. But, Aloe gel heated at 75°C has more storage stability. Chang XiuLian (2006) reported that the polysaccharide from Aloe exhibited a maximal stability at temperature of 70°C where as the stability decreased either at higher or lower temperatures. The result also indicated that the microbial count was increased with increase in pH and decrease in acidity. The increased microbial count might be due to the decreased acidity and increased to sugars during the storage period. So, the significant microbial count was noticed with increased sugars which provide favorable conditions for growth of microbes during storage period. Similar results were recorded by Hemalatha *et al*. (2005) in Aloe.

Experiment 1:

parameters	Change with regard to increase of leaf age
1. Leaf size	Increased
2. Leaf weight	Increased
3. Gel weight	Increased
4. Peel weight	Increased
5. pH	Increased
6. Acidity	Decreased
7. TSS	Increased
8. Reducing sugars	Increased
9. Non-reducing sugars	Increased
10. Total sugars	Increased
11. Moisture	Increased
12. Antioxidant activity	Increased

Experiment 2:

parameters	Change with regard to increase of storage
1. pH	Increased
2. Acidity	Decreased
3. TSS	Increased
4. Reducing sugars	Increased
5. Non-reducing sugars	Decreased
6. Total sugars	Increased
7. Moisture	Decreased
8. Antioxidant activity	Decreased
9. Microbial count	Increased

Correlation between the physic-chemical parameters during the storage:

1. pH increased	Acidity decreased
2. TSS increased	Moisture decreased
3. Sugars increased	Acidity decreased
4. % TBARS increased	% Inhibition decreased
5. % TBARS increased	Antioxidant activity decreased
6. % Inhibition increased	Antioxidant activity increased
7. pH increased	Microbial count increased

SUMMARY

CHAPTER VI

SUMMARY

The present study entitled “Influence of leaf age on gel recovery and heating on quality, shelf life of Aloe (*Aloe barbadensis* Miller)” was conducted in the Laboratory located at Herbal garden, College of Horticulture, Rajendranagar, Hyderabad during the year 2010. The important salient findings of the investigation are summarized below.

- ✓ Among the different aged leaves, 14 months aged leaves showed optimum physico-chemical composition followed by 12 months and 10 months aged leaf.
- ✓ Regarding different accessions, yellow flowering accession-1 showed optimum physico-chemical composition and storage stability of gel followed by yellow flowering accession-2 and orange flowering accession-3.
- ✓ Similarly, with regard to different temperatures, heating at 75°C temperature showed optimum chemical composition and storage stability of gel followed by heating at 50°C and 100°C temperature.
- ✓ Out of the three ages of the Aloe leaf, 14 months aged leaf recorded the highest leaf size (553.45 g), leaf weight (480.22 g), gel weight (321.56 g) and peel weight (158.22 cm^2) followed by 12 months and 10 months leaf age.
- ✓ With regard to different accessions, yellow flowering accession-1 has recorded the highest leaf size (530.11 cm^2), leaf weight (506.67 g), gel weight (350.78 g)

and peel weight (141.78 g) followed by yellow flowering accession-2 and orange flowering accession-3.

- ✓ Among the different ages of Aloe leaf studied, the highest pH (4.79) was reported by 14 months aged leaf followed by 12 months and 10 months aged leaf.
- ✓ Among the different accessions, orange flowering accession-3 has recorded the highest pH (4.52) followed by yellow flowering accession-2 and yellow flowering accession-1.
- ✓ During the period of storage of gel, pH showed an increasing trend. The highest pH (6.07) was noticed in orange flowering accession-3 heated at 100°C followed by the same accession heated at 50°C and 75°C temperature.
- ✓ Similarly, the highest microbial count was recorded with orange flowering accession-3 heated at 100°C (> 100) followed by the same accession at 50°C and 75°C temperature.
- ✓ Highest TSS (0.76° B), moisture content (95.67%) and antioxidant activity (53.17%) were recorded with yellow flowering accession-1 followed by yellow flowering accession-2 and orange flowering accession-3.
- ✓ Among the different ages of Aloe leaf studied, 14 months aged leaf showed the maximum TSS (0.78° B), moisture content (90.23%) and antioxidant activity (64.08%) followed by 12 months and 10 months aged leaf.
- ✓ The results indicated that the gel TSS, moisture content and antioxidant activity have gradually decreased with increase in storage period up to 30^{th} day.

- ✓ Yellow flowering accession-1 heated at 75°C has showed maximum gel TSS (1.30° B) and antioxidant activity (61.90%) during the storage followed by the same accession at 50°C and 100°C. But the highest moisture content was recorded with yellow flowering accession-1 heated at 50°C (91.07%) followed by the same accession heated at 75°C and 100°C.
- ✓ Among the three maturity ages of the leaf studied, 10 months aged leaf recorded the maximum gel acidity (0.22%) followed by 12 months and 14 months aged leaf.
- ✓ Among the different accessions, yellow flowering accession-1 recorded maximum gel acidity (0.28%) followed by yellow flowering accession-2 and orange flowering accession-3.
- ✓ During the storage period, gel acidity showed decreasing trend. Highest gel acidity (0.23%) was noticed in case of yellow flowering accession-1 heated at 75°C followed by the same accession on heating at 50°C and 100°C temperature.
- ✓ Among the three maturity ages of Aloe leaf, 14 months aged leaf recorded the highest reducing sugars (0.050%), total sugars (1.84%) and non-reducing sugars (1.79%) followed by 12 months and 10 months aged leaf.
- ✓ In respect of different accessions, maximum reducing sugars (0.035%), total sugars (1.72%) and non-reducing sugars (1.68%) were recorded by yellow flowering accession-1 followed by yellow flowering accession-2 and orange flowering accession-3.

- ✓ The results indicated that there was a considerable increase in reducing sugars and total sugars during the storage period while the non-reducing sugars showed the decreasing trend with increase of storage period.
- ✓ Yellow flowering accession-1 heated at 50°C has recorded the highest reducing sugars (1.85%), total sugars (2.55%) and non-reducing sugars (0.70%) followed by the same accession on heating at 75°C and 100°C.

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