DEVELOPMENT OF AN *Aloe vera* (*Aloe barbadensis* Miller) INCORPORATED READY-TO-SERVE BEVERAGE

RESEARCH REPORT PRODUCED BY

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To the

National Science Foundation

in fulfillment of the requirement

for the research agreement made

Under the Technology Grant Number TG/2012/Tech-D/11

between

National Science Foundation

and

Postgraduate Institute of Science, University of Peradeniya, Peradeniya

For the period of

April, 2013 to April, 2014

Certified by:

A. N. N 31/07/14

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DEVELOPMENT OF AN *Aloe vera* (*Aloe barbadensis* Miller) INCORPORATED READY-TO-SERVE BEVERAGE

Aloe vera L. or *Aloe vera Burm.f.*, has been well known as a miracle plant due to its remarkable therapeutic properties. Aloe gel possesses over 200 nutrients including acemannan, the major bioactive mucopolysaccharide, which contributes to many health beneficial effects including mainly anti-diabetic effect. Aloe gel has been extensively used as a functional ingredient in food and beverage production. The objectives of this study were to determine the best anti-diabetic process formulation of aloe based ready-to-serve beverage with crushed aloe gel particles and assess its shelf-life. To get the best mouthfeel of the product and to preserve acemannan, a pectinase enzyme treatment and two different pasteurization conditions; high temperature short time (HTST) and low temperature long time (LTLT) were tried out. Effect of possible combinations of above thermal treatments was tested by spectrophotometry, based on acemannan preservation. Proportions of crushed aloe gel particles, sucralose and acidulates were determined by conducting ranking tests based on sensory qualities. The shelf-life of the product was assessed based on physico-chemical, microbiological and sensory qualities at 35±2 °C. The absorbances intended for acemannan were significantly ($p<0.05$) higher in HTST pasturization followed by pectinase enzyme treatment. The beverage developed by incorporating aloe gel, sucralose, citric acid, ascorbic acid, nature identical flavors and permitted preservatives was the most acceptable formula based on sensory qualities. pH, titratable acidity, turbidity, total soluble solid and acemannan content of the beverage 2.82±0.05, 0.52±0.02% w/v (as citric acid), 16.3±0.9 NTU, 0.0±0.0 °Brix and 2427±115 mg dm^{-3}, respectively. The shelf-life was nine months at ambient temperature (27±2 °C). Hence, the developed formula is satisfactory, assessment of the effectiveness of the anti-diabetic activity by *in vivo* studies is important.

Keywords: *Aloe vera* gel, ready-to-serve beverage and anti-diabetic formula

Acknowledgments

First and foremost, we express our gratitude to Postgraduate Institute of Science, University of Peradeniya for granting permission to facilitate this research to be conducted at the University.

We are gratefully express our gratitude to National Science Foundation for the financial support to carry out this research. We offer our sincere appreciation and special thanks to the Director, Head of the Technology Division and supporting staff of National Science Foundation providing us all the assistance to carry out this research successfully.

We must acknowledge technical staff of Department of Chemistry, Faculty of Science and Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya for the technical assistance.

Furthermore, we are gratefully indebted to Postgraduate Institute of Science, University of Peradeniya for providing Analytical Chemistry laboratory facilities.

Our thanks and sincerer appreciations also go to our colleagues who gave us the support and encouragement to complete this research successfully.

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List of abbreviations and symbols

cfu- Colony form units

cm- Centimeter

Da- Dalton

dm- Decimeter

FDA- Food and drug administration

kg- Kilogram

mg-Miligram

min- Minutes

NTU- Nephlometric turbidity units

p- Probability

RTS- Ready-to-serve

SD- Standard deviation

v/v- Volume/volume

w/v- Weight/volume

w/w- Weight/weight

INTRODUCTION

Type 2 diabetes mellitus (T2DM), a chronic metabolic disease results in hyperglycemia or high sugar levels in blood. Nowadays, T2DM is reported to be more prevalent in Asian countries. According to reports published by the Diabetes Association of Sri Lanka, about 8.3% of the world population comprising 366 million people was affected by diabetes in 2011. This number will further rise up to about 9.9% by 2030. In 2007, more than 110 million people in Asia were diagnosed with diabetes, probably due to obesity and overweight caused by sedentary lifestyles of these individuals, nutrition transition and economic development of the Asian Region (Chan *et al*., 2009). Diabetes shortens the life expectancy of a population by 5-10 years as reported by the Diabetes Association of Sri Lanka. A cross–sectional study conducted between 2005 and 2006 has revealed that 11.5% of the Sri Lankan population was affected by pre-diabetes and also about 21.8% experienced dysglycaemia (Katulanda *et al*., 2008). Furthermore, one in five adults in Sri Lanka is affected with either diabetes or pre-diabetes and one third of those with diabetes are undiagnosed (Katulanda *et al*., 2008).

Changing the lifestyles by adapting to better eating habits and increasing physical activities can reduce the potential of development of T2DM by about 80 % (Diabetes Association of Sri Lanka). Nowadays, as people tend to follow nutritional guidelines on diabetes mellitus, they would rather use dietary supplements than changing lifestyles in an attempt to either prevent or control diabetes. Plants or their extracts have been used in traditional medicine to treat diabetes. Those herbal preparations have shown to possess constituents with hypoglycemic activities. Furthermore, these kinds of plants can be used to make herbal preparations, which can replace western medicinal drugs used for treating T2DM. Such preparation can possibly reduce the amount of money spent on expensive anti-diabetic drugs. Moreover, these preparations may possibly have some potential in the export market while improving the working capacity of those afflicted in Sri Lanka with diabetes, thereby contributing to national development.

Aloe vera (*Aloe barbadansis* Miller), a tropical and subtropical succulent plant known as '*komarika*' in Sinhala, is known as a magical plant or a panacea and has been proven to possess anti-diabetic properties as revealed by both animal and human clinical studies (Davis *et al*., 1989; Mohamed *et al*., 2009; Rajasekaran *et al*., 2006; Reynolds and Dweck1999). Furthermore, administration of *Aloe vera* juice has reduced the blood sugar in human as revealed by clinical trials (Agarwal, 1985; Bunyapraphatsara *et al*., 1996; Ghannam *et al*., 1986; Yongchaiyudha *et al*., 1996).

Aloe gel is the major part of the leaf by volume, which possesses anti-diabetic activities and other therapeutic effects (Ni *et al*., 2004). Aloe gel consists of primarily water (98.5 - 99.5 % of fresh matter) and polysaccharides which contributes to more than 60 % of the remaining solid (Femenia *et al*., 1999). Of these polysaccharides, 20-25% is mucopolysaccharides (MPS), which are especial long chain polysaccharides possessing the biologically activity. Among these mucopolysaccharides, acemannan and glucomannan are known to impart anti-diabetic effects (Chearskul *et al*., 2007; Martino *et al*., 2005). These anti-diabetic effects are imparted as these mucopolysaccahrides are not enzymatically digested in the digestive tract, thus getting into the blood stream through an especial mechanism called endocytosis, which preserves their chain structure. These health beneficial properties such as reduce inflammation and blood glucose, free radical scavenging activity, anti-microbial effects and immune modulating effects of mucopolysaccahrides depends on their molecular size.

Aloe gel is used as a food ingredient in many food products such as health drinks (Gautam and Awasthi, 2007; Eshun and He, 2004; Hamman, 2008; Ramachandra and Rao, 2008). The principle objective of this study was to develop an *Aloe vera* incorporated ready- to-serve beverage to be used as a measure for treating T2DM. Specific objectives of this study were,

- Determination of the effect of different processing steps used in preparation the beverage on mucopolysaccharides.
- Determination of the effect of processing parameters on sensory qualities of the product based on appearance, taste, flavor, aroma and mouthfeel.
- Determination of the effect of processing parameters on physico-chemical properties of the product based on turbidity, total soluble solids, pH and titratable acidity.
- Determination of the effectiveness of the measures taken for preservation on the microbiological qualities of the product based on total plate count, yeast and mould count and total coliform count.
- Determination of the shelf-life of the beverage in glass bottles based on microbiological, physico-chemical (including mucopolysaccharides) and sensory properties of the product.

Though aloe gel has been used to produce many foods products, these products have not been tested for their effectiveness on treating diabetes. In this context, a clinical trials needs to be carried out on diabetic patients. Scaling up of the process and clinical trials are equally important for future commercialization of the developed product.

CHAPTER 01

Literature review

1.1 Diabetes mellitus

T2DM (adult-onset/ non insulin-dependent) is a chronic metabolic disease, which is caused by the inability of the body to properly metabolize glucose due to insufficient insulin production by the pancreas. This condition results in hyperglycemia or high glucose levels in blood. T2DM is diagnosed mainly in persons over 40 years of age. T2DM is reported to be more prevalent in Asian countries than in European countries. According to reports published by the Diabetes Association of Sri Lanka, about 8.3% of the world population comprising 366 million people was affected by diabetes in 2011. This number will further rise up to about 9.9% by 2030. In 2007, more than 110 million people in Asia were diagnosed with diabetes, probably due to obesity and overweight caused by sedentary lifestyles of these individuals, nutrition transition and economic development of the Asian Region (Chan *et al.*, 2009).

Diabetes was reported to be the primary cause of death of about 4.6 million people worldwide in 2011 and 48% of them was below 60 years of age. Diabetes shortens the life expectancy of a population by 5-10 years as reported by the Diabetes Association of Sri Lanka. Figure 1.1 shows the prevalence of T2DM among different age groups of Sri Lankans in 2010. A cross–sectional study conducted between 2005 and 2006 has revealed that 11.5% of the Sri Lankan population was affected by pre-diabetes and also about 21.8% experienced dysglycaemia (Katulanda *et al.*, 2008). Furthermore, one in five adults in Sri Lanka is affected with either diabetes or pre-diabetes and one third of those with diabetes are undiagnosed (Katulanda *et al.*, 2008).

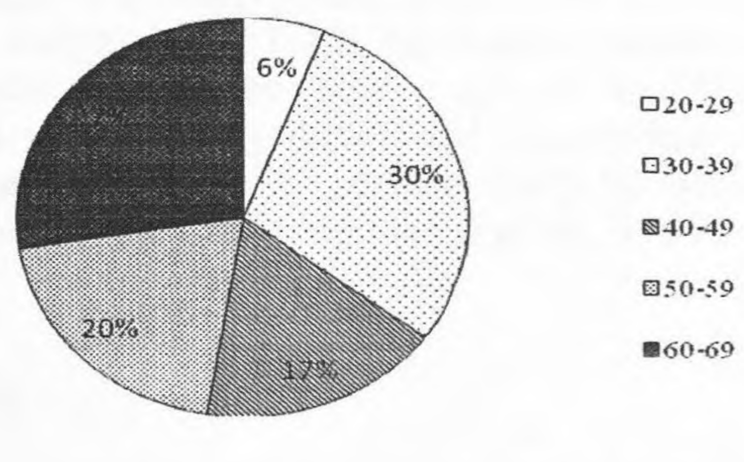


Figure 1.1. Percentage prevelance of diabetes in Sri Lanka among different age groups
Source: Hanwella and Silva, 2010

1.1.1 Diabetes control and prevention

Changing the lifestyles by adapting to better eating habits, stress management and increasing physical activities can reduce the potential of development of T2DM by about 80% (Diabetes Association of Sri Lanka). Nowadays, as people tend to follow nutritional guidelines on diabetes mellitus, they would rather use dietary supplements than changing lifestyles in an attempt to either prevent or control diabetes. A great deal of information and many treatments are available for controlling diabetes and associated complications. Plants or their extracts have been used in traditional medicine to treat diabetes. Those herbal preparations have shown to possess constituents with hypoglycemic activities. These constituents stimulate or regenerate cells in organs such as pancreas contributing to control T2DM. Furthermore, this kind of plants can be used to make herbal preparations, which can replace western medicinal drugs used for treating T2DM. Such preparation can possibly reduce the amount of money spent on expensive anti-diabetic drugs. Moreover, these preparations may possibly have some potential in the export market while improving the working capacity of those afflicted in Sri Lanka with diabetes, thereby contributing to national development.

1.2 A*loe vera* plant

Aloe vera (L.) *Burm. f.* (*Aloe barbadansis* Miller), a tropical and subtropical succulent plant known as '*komarika*' in Sinhala, belongs to sub-family 'Aloaceae' of family 'Xantorrhoeaceae' (Gradlay and Reynolds, 1986). The plant is native to North Africa and now it is cultivated worldwide including mainly America, Europe and Asia (Ni *et al*., 2004). The plant is widespread on the Northern coast (in the Jaffna peninsula and Mannar), Western coast (at Kalpitiya) and Southern coast (near Hambantota) of Sri Lanka (Jansz *et al*., 1995). It is known as a magical plant or a panacea and has been proven to possess anti-diabetic properties as revealed by both animal and human clinical studies (Davis *et al*., 1989; Mohamed *et al*., 2009; Rajasekaran *et al*., 2006; Reynolds and Dweck, 1999).

Aloe vera has rosettes of very thick fleshy lance-shaped green leaves about 15-20 inches in length with jagged edges and sharp points. Each plant usually has 12-16 leaves. When mature, each leaf may weigh up to 1-1.5 kg (Lachenmeier *et al*., 2005). The average height is about one meter and mature plants can grow as tall as two meters. The plants survive for hundreds of years. Fully developed mature leaves are harvested to obtain aloe juice (Maiti and Chandra, 2002).The plant is ready for harvesting in one year, although the best yield is obtained in the third year (Jansz *et al*., 1995).

1.2.1 Composition of *Aloe vera*

Three main structural components of *Aloe vera* inner pulp, shown in Figure 1.2 are distinctive from each other in terms of morphology and sugar composition (Hamman, 2008). The components are,

- Cell wall
- Degenerated organelles and
- Viscous liquid contained within cells.

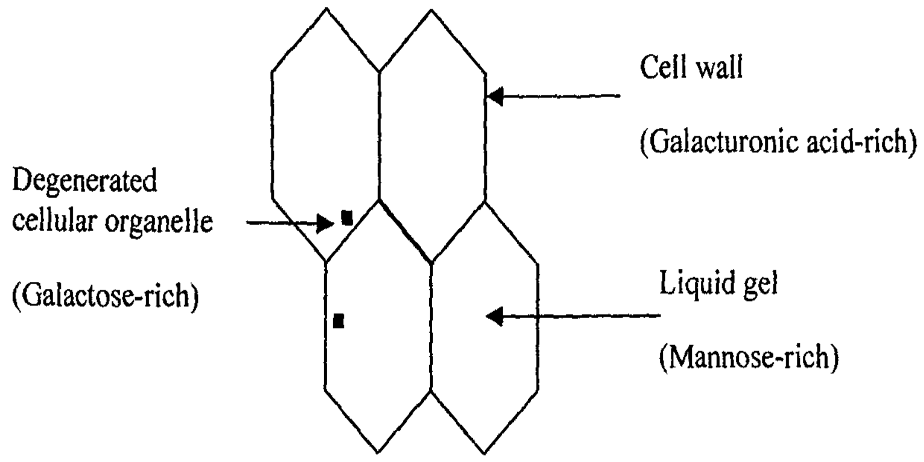


Figure 1.2: Structural components of *Aloe vera* inner pulp

Outer green rind of *Aloe vera* leaf is consisted of thick epidermis cells covered with cuticles and inner fillet is consisted of mesophyll (chorenchyma cells and parenchyma cells). Inner part of the leaf including cell walls and organelles is called as parenchyma tissue or pulp. The parenchyma cells contain the clear transparent mucilaginous pulp which is referred to as the aloe gel or mucilage (Anderson, 2007; Femenia *et al.*, 1999).

Active ingredients of aloe leaves may vary depending on the season and climatic and soil conditions (Vazquez *et al.*, 1996). Pericyclic cells, in just beneath of the outer green rind contains latex called aloe juice or aloe sap, which contains bitter compounds (Anderson, 2007; Femenia *et al.*, 1999). Heterogeneous composition of *Aloe vera* pulp may contribute many pharmacological and therapeutic activities.

1.2.1.1 Exudate compounds

The yellow exudates presents within pericyclic cells in just beneath of the skin of the leaf and contains cromone (aloesin and aloeresin) and anthrone (aloin and aloe-emodin) compounds such as hydroxyanthracene derivatives, aloins A and B, aloectic acid and emodin. There are considerable amounts of 1,8 dihydroxyanthraquinone derivatives (aloe emodin) and their glucosides (aloin/barbaloin) in the bitter yellow sap of the outer rind of *Aloe vera* plant (Vazquez *et al.*, 1996; Chang *et al.*, 2006). These two compounds have been reported to contribute up to 30% of the aloe plant's dried leaf exudates (Chang *et al.*, 2006; Schauss, 1990). Aloin and aloe emodin have toxicological properties and laxative effects at higher concentrations (Chang *et al.*, 2006).

The dried exudates is named as aloes and commercially known as 'Curacao aloe' (Mcanalley and Bill, 1989). Different types of aloin in aloes have been named after their origin (Jansz *et al.*, 1995), which include,

- Socotrine aloes: Socaloin
- Barbardos aloes: Cape aloe/ Curacao aloe: Barbaloin
- Natal aloes: Nataloin

- Zanzibar aloes: Zanaloin

Nataloin (10- (1-deoxy- glucopyranosyl) aloe-emodinanthrone (I), containing homonataloin (10- (10deoxyglucopyaranosyl) 1,7-dihydroxy-3 methyl-8-methoxyanthrone) (II) is the major type of aloin (Jansz *et al.*, 1995). Dry aloe juice (aloes) is classified into three chemical groups as class I; containing barbaloin, class II; containing homonataloin and class III; containing neither. Class I has isobarbaloin, aloinosides, aloenin and aloesin in addition to barbaloin (Jansz *et al.*, 1995). Also, a considerable amount of xylose-containing polysaccharides presents in the skin of the aloe leaf (Hamman, 2008).

Aloin

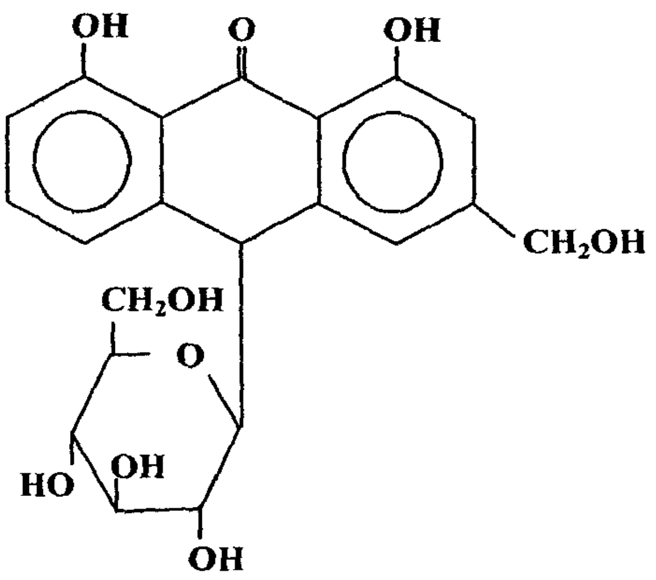
Aloin, a water soluble compound (8300 mg dm^{-3} at 20 °C) is identical to barbaloin is an anthrone (Figure 1.4). Aloin is the common ingredient in the laxative products due to its laxative effect. In the gastrointestinal tract, aloin is deglucosidated by glucosidases and the resulting iso-emodin possesses the actual laxative effect (Gen *et al.*, 2007).

Aloin content of Sri Lankan aloes varies from 3 to 20% (Jansz *et al.*, 1995). The aloin content of Jaffna aloes is reported to be higher (16.3%) than that of Mannar aloes (9%), while low quantities are reported in plants grown in Colombo (Jansz *et al.*, 1995).

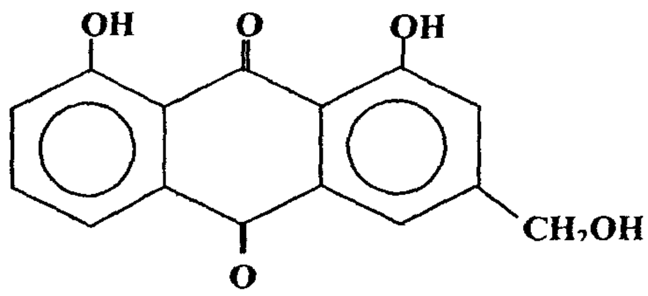
Normally aloe is yellow in color and bitter in taste. Color of Jaffna and Mannar aloes are black and yellow-brown respectively (Jansz *et al.*, 1995). Browning reactions occur with resin formation, via phenolic oxidation (Jansz *et al.* 1995). Heat process is favorable for eliminating barbaloin with increasing temperature (the most rapid decline at 90 °C) and time (Chang *et al.*, 2006). Aloin has anti-inflammatory and cathartic effects (Chang *et al.*, 2006). Also, phenolic compounds present in the rind have been reported to be responsible for antioxidant activity, including aloin (Hu *et al.*, 2005).

Aloe emodin

Aloe emodin is 1,8-dihydroxy-3-hydroxymethylanthraquinone, the anthraquinone of barbaloin (Figure 1.3). Aloin compounds are readily oxidized into aloe-emodin. Aloe emodin presents in *Aloe vera* is a type of anti-cancer agent against nuroectodermal tumours (Pecere *et al.*, 2000). It inhibits nuroectodermal tumor cell growth in human tissue cultures and animals.



(a)



(b)

Figure 1.3: Chemical structures of (a): Aloin/ $C_{21}H_{22}O_9$ and (b): Aloe-emodin/ $C_{15}H_{10}O_5$,

(a): (10S)-10-Glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9(10H)-anthracenone

(b): 1,8-Dihydroxy-3-(hydroxymethyl)-9,10-anthracenedinone

Aloesin

Aloesin is a C-glucosylated 5-methylchromone, a strong competitive inhibitor of tyrosinase activity, while 2"-O-feruloylaloesin, one of its derivatives, is a non competitive inhibitor (Farrow *et al.*, 2003). Aloesin is capable of 50% inhibition of tyrosinase activity at the concentration of 0.2 mM (Farrow *et al.*, 2003). Aloesin has antioxidant activity as well (Farrow *et al.*, 2003).

Other compounds in exudates

Other than aforementioned compounds, there are several compounds in yellow exudates (Figure 1.4). Chrysophanic acid in yellow exudates is employed as an application in certain ailments such as eczema, mentagra, herpes and psoriasis. Moreover, p-coumaric acid is reported to possess antioxidant properties and lowers stomach cancer by reducing the formation of carcinogenic nitrosamines. (Ferguson *et al.*, 2005).

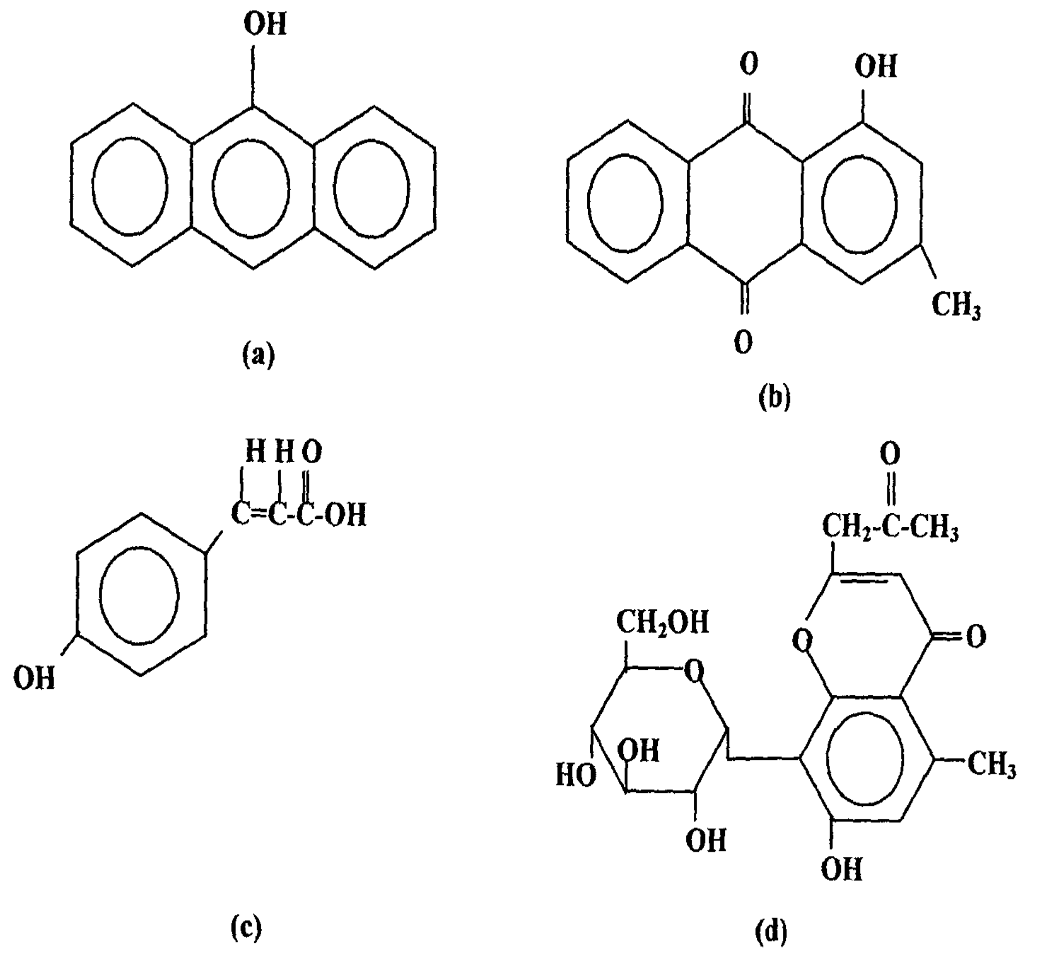


Figure 1.4: Schematic chemical structures of (a)- Anthranol, (b)- Chrysophanic acid (c)- p-Caumaric acid and (d)- Aloesin

1.2.1.2 Gel compounds

Aloe gel is the major part of the leaf by volume, which possesses anti-diabetic activities and other therapeutic effects (Ni *et al.*, 2004). Aloe gel is translucent with slight vegetable like odor, having 3.8-4.8 pH value. Aloe gel consists of primarily water (98.5-99.5% of fresh matter) and polysaccharides which contributes to more than 60% of the remaining solid (Femenia *et al.*, 1999). Of these polysaccharides, 20-25% is mucopolysaccharides (MPS), which are especial long chain polysaccharides possessing the biologically activity. Among these mucopolysaccharides, acemannan are known to impart anti-diabetic effects (Chearskul *et al.*, 2007; Martino *et al.*, 2005). Other than acemannan, main polysaccharides are pectin, cellulose, hemicelluloses, glucomannan, and mannose derivatives and considerable amounts of arabinan, arabinorhamnogalactan, galactan, polyuronides, aloeride galactogalacturan, glucogalactomannan, galactoglucoarabinomannan, arabinorhanogalactan and glucouronic acid-containing polysaccharides (Hamman, 2008; Ni *et al.*, 2004).

Mucopolysaccharides (MPS)

Mucopolysaccharide is any of a group of polysaccharides containing an amino sugar and uronic acid: a constituent of mucoprotiens, glycoprotiens and blood group substances. *Aloe vera* mucopolysaccharide is a long chain sugar molecule composed of individual mannose and glucose sugar molecules connected together. Subsequently, aloe polysaccharide is called as glucomannan. Furthermore, it is called as polymannan or polymannose, in the presence of far more numerous mannose rather than glucose. Although solutions of some short polysaccharides are clear and appear just like water, solutions of mucopolysaccharides are difficult to prepare and they make a matrix, mucus likes structure appearing cloudiness.

The aloe mucopolysaccharides possessing varying molecular sizes, which determine their health beneficial properties, are,

- Small (50-600 molecules/under 50,000 Da): Reduce inflammation and blood glucose
- Medium (up to 1500 molecules/ 50,000-150,000 Da): Free radical scavenging activity
- Large (up to 5000 molecules/ 150,000-1000,000 Da): Anti-microbial effects and healing properties
- Very large (up to 9000 molecules/ >1000,000 Da): Immune modulating effects.

Thus, acemannan and glucomannan are the special mucopolysaccharides present in aloe gel.

Acemannan

Chemical properties

Acemannan, which comprises mannose, glucose and galactose in a 31:1:1 ratio (Figure 1.5) is the main biologically active substance presents in aloe gel (Cowsert, 2010; Femenia *et al.*, 1999; Hamman, 2008). Acemannan is made up of mannoacetate as monomer, linking by β-1,4- glycosidic linkages, which are important in terms of the therapeutic effects as humans lack the ability to enzymatically break down these bonds (Hamman, 2008).

Medicinal properties

The toxicology studies of acemannan have been studied in both *in vivo* and *in vitro* systems (Carpenter *et al.*, 1998). *In vitro*, acemannan has been proven that it is not mutagenic or blastogenic. Furthermore, acemannan has been proven to be non toxic and does not impart adverse clinical effects in the studies of *in vivo* systems.

Acemannan possesses a unique combination of immunomodulatory and anti-viral properties (Carpenter *et al.*, 1998). It may exert its therapeutic effect by two main mechanisms. One is the altering glycosylation (addition of carbohydrate molecules to a protein molecule), such as inhibition of glucosidase I or the incorporation of the acetylated mannan derivative into glycoprotein (Carpenter *et al.*, 1998). The other possible mechanism is enhancement of the antigenicity of the virus or the tumour, or the enhancement of immunocompetency of the host (Carpenter *et al.*, 1998). Furthermore, acemannan stimulates synthesis and release of insulin (Cowsert, 2010).

Acemannan compound is an immunomodulant by the mechanisms of stimulation of macrophages to produce nitric oxide and cytokines, enhancement of phagocytosis by macrophages, increasing the number and activity of monocytes and macrophages and activate compliment via the alternative pathway (Cowsert, 2010). Acemannan has the diminishing ability of varying health problems such as asthma, artheritis, leukemia *etc.* (Carpenter *et al.*, 1998). Acemannan also is used in the treatment of psoriasis, which is a red and irritant skin condition, by inhibition of oxygen consumption of cells, reducing the size in the intracellular spaces, inducing mitochondrial damage and retardation of cell division (Cowsert, 2010). Moreover, acemannan has the ability to remove scales, crusts and irregularities on skin (Carpenter *et al.*, 1998). The animal and human clinical studies on cholesterol have been proven that acemannan can reduce the serum cholesterol level considerably (Carpenter *et al.*, 1998).

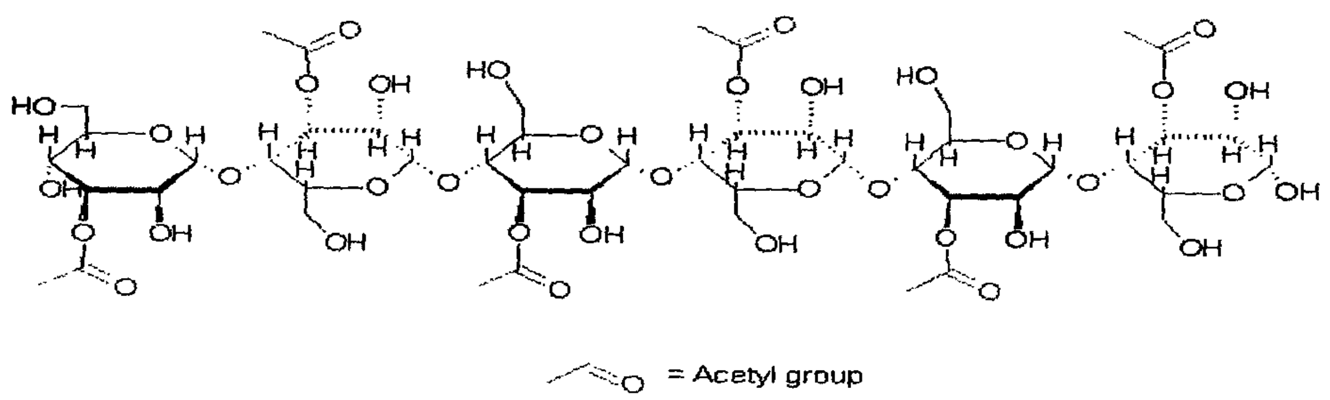


Figure 1.5: Chemical structure of acemannan

Mechanism of acemannan intake to the blood

Enterons, epithelial cells lining the digestive tract produce glucokelics, sticky substances which have mannose-specific and non-specific receptors for active uptake of mannose from the gut and transportation into the blood stream. Furthermore, majority of the aloe polysaccharides are bound to non-specific receptors, which provides a marvelous protective action along the digestive tract of the body. Also, specific receptors facilitate the complete migration of aloe polysaccharides to epithelial cells through cell wall and this special mechanism is called as endocytosis/cynocytosis, which protects their chain structure. Aloe polysaccharides are transported to blood stream through a system called as linkphalics system. Acemannan is digested by probiotics. Animal studies have shown that, acemannan is detected in blood within 90 min after the oral administration. The majority of acemannan is distributed to liver, followed by spleen, brain, kidney, heart, pancreas and thymus (Danhof, Audio). The permeability or bioavailability of acemannan has accounted as one. About 12-14% of small and 5% of very large, long chain aloe polysaccharides are absorbed into the blood stream. Such a very small amount of aloe mucopolysaccharides has absolutely remarkable health beneficial activities (Danhof, Audio).

In human subjects, beneficial actions are readily apparent with the ingestion of about 56.7 g of aloe gel twice daily (Danhof, Audio). Furthermore, 25 cm^3 of pure aloe gel juice 100% per day before three main meals for a few months is suitable as the effective natural remedy for diabetes (Bassetti and Sala, 2005). Moreover, mucopolysaccharides between 0.6-1.2 g/day are recommended for a healthy adult, while amount of 1.2-3.0 g/day is recommended for an unhealthy person.

Glucomannan

Glucomannan comprises 1,4-b-linked D-glucosyl and D-mannosyl residues (Figure 1.6). Acetylated glucomannan molecules are responsible for thick and mucilage like properties of raw aloe gel (Hamman, 2008). Glucomannan has been studied for its potential for treating obesity, diabetics and high cholesterol. In treating mechanism on obesity, glucomannan discourages overeating by feeling of fullness in the stomach due to its fiber swelling ability. Glucomannan is good for diabetics due to slower absorption of carbohydrate from food and increasing sensitivity of body tissues to insulin. Glucomannan lowers the total cholesterol, low density lipoproteins and triglycerides. Also, it can lower the cholesterol level by preventing re-absorption of bile acids, as cholesterol is used to produce more bile acids.

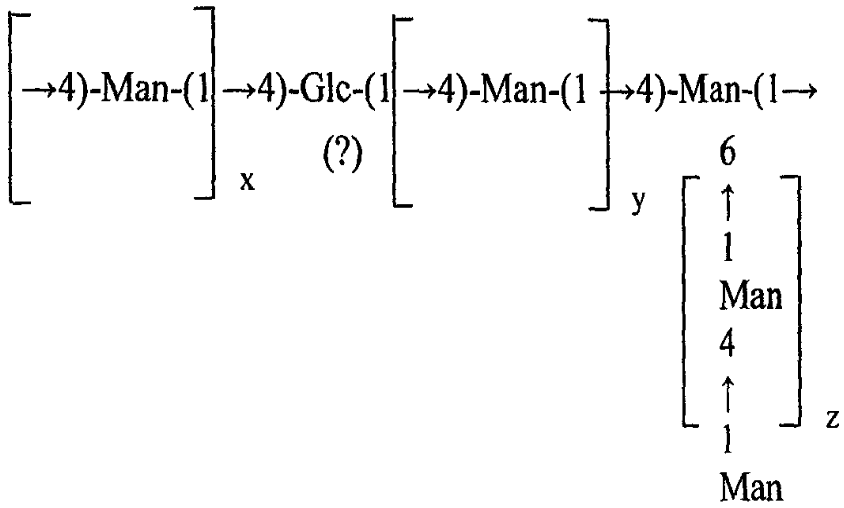


Figure 1.6: Average repeating unit of aloe glucomannan. (x + y + z) = 20 mannosyl residues. (?): Location of the glucosyl residue (in the main or side chain) is uncertain. Source: Mandal and Das, 1980.

Pectic substance

Pectic substance refers to closely related polysaccharides including pectin, pectic acid and arabinogalactan (Hamman, 2008). Aloe pectin is extracted from the inner gel or outer rind cell wall fibers (Ni *et al*, 2002). Pectin consists of an α-1,4 linked polygalacturonic acid backbone intervened by rhamnose residues and modified with neutral sugar side chains and non-sugar components such as methyl and acetyl groups. It has been suggested that the insertion of rhamnose residues in the backbone cause a T-shaped kink in the backbone chain (Ni *et al*, 1999). An increase of rhamnose contents leads to flexibility of molecules (Ni *et al*, 1999). Trans 1-4 linkage is resulted due to axial position of hydroxyl groups of D-galactouronic acid at 1 and 4 carbon atoms, and results the increment of stiffness (Ni *et al*, 1999). Aloe pectin possesses a rare sugar, 3-OMe-rhamnose, which has not been found in other pectin (Ni *et al.*, 2002). Another unique property of aloe pectin is its high molecular weight; >1.times.10.sup.6 Da and high intrinsic viscosity; >550 ml/g (Ni *et al.*, 2002).

Pectin is more stable at acidic pH, approximately 3–4 (Ni *et al*, 1999; Ni *et al.*, 2002). Aloe pectins has been proven to be at least two times higher than in other pectins, mainly citrus, apple, sugar beet, and sunflower (Ni *et al*, 1999). As high as 50%, w/w of pectin with an average galactouronic acid content >70% (w/w) could be extracted from *Aloe vera*. Low methoxy pectin presents in aloe gel has, >90 % galactouronic acid content and <10 % degree of methylation (Ni *et al*, 1999; Ni *et al.*, 2002). Aloe low methoxy pectin is capable of gel formation in the presence of calcium ions and this gelation is more proficient at neutral pH, around 7.0 (Ni *et al*, 1999; Ni *et al.*, 2002). Also, aloe pectin is capable of monovalent cation based gel formation at low temperatures, at ~4 ^{o}C (Ni *et al*, 1999; Ni *et al.*, 2002). Aloe pectins are clear solutions, and can be extracted as off white powder (Ni *et al*, 1999; Ni *et al.*, 2002).

Other compounds in aloe gel

Other than polysaccharides, aloe gel contains over 200 nutrients including 18 amino acids, lipids, sterols (lupeol, campesterol, and β-sitosterol), tannins, enzymes (about 92), vitamins, minerals, phenolic compounds, organic acids and oxalates. Among the vitamins, vitamin A, C, E, B_1, B_2, B_3, B_5, B_6 and B_{12} are reported to be prominent. The main minerals present in aloe gel are calcium and potassium and also considerable amounts of boron, chloride, chromium, copper, iron, magnesium, phosphorous, aluminum, barium and zinc. The plant produces six anti-septic agents such as lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulfur (Maiti and Chandra, 2002). There are anti-inflammatory fatty acids such as campesterol and β-sitosterol, which are plant sterols (Maiti and Chandra, 2002). In addition, *Aloe vera* contains substances possessing antibiotic properties (Schauss, 1990).

1.2.2 Anti-diabetes properties of *Aloe vera* leaf gel

1.2.2.1 Pre-clinical evidences of anti-diabetes properties of *Aloe vera*

In-vivo evidences of *Aloe vera* have been reported to contribute remarkable health beneficial properties including anti-diabetic property on both animal studies (Can *et al.*, 2004; Kim *et al.*, 2009; Mohamed *et al.*, 2009). *Aloe vera* gel administered to T2DM rats has increased hypoglycemic, anti-athirogenic and hyperinsulinemic effects (Okyar *et al.*, 2001; Helal *et al.*, 2003; Gupta *et al.*, 2011). Studies done using rats revealed restoration of blood glycoprotein components by reducing the glycosylation process and inhibiting glycosidase upon oral administration of *Aloe vera* leaf gel extract (Carpenter *et al.*, 1998; Rajasekaran and Sathishekar, 2007).

Oral administration of fresh *Aloe vera* gel to alloxan induced diabetic rats has been reported that there is a significant decrement of blood glucose level and significant increment of both liver glycogen and serum insulin levels (Helal *et al.*, 2003; Gupta *et al.*, 2011). A similar effect was achieved on mice, made diabetes with alloxan treatment (Ajabnoor, 1990). Another, oral administration of *Aloe vera* gel extract has reported the significant decrement of serum glucose, total cholesterol and triglycerides in alloxan induced diabetic rats (Mohamed, 2011). Furthermore, oral supplementation of *Aloe vera* leaf gel extract has significantly restored the glycoprotein components near normalcy and reduced the blood glucose and HbA1C while increasing the hemoglobin in streptozotocin induced diabetes rats (Rajasekaran and Sathishekar, 2007; Rajasekaran *et al.*, 2005).

Other than the research evidences, veterinary medicinal applications prove the safety of oral administration of *Aloe vera* gel in animals such as cattle, sheep, goats, fish, snakes, lizards and birds (Urch, 1999). Acemanann imuno-stimulant ™ is an orally consumable pulp extract of *Aloe vera,* which has been licensed for the treatment of fibroscarcoma; a form of cancer in cats and dogs (Ni *et al.*, 2004; Cowsert, 2010). Moreover, oral *Aloe vera* gel supplementations have been used for horses (www.aloeveraforhorses.com; www.aloequine.com).

1.2.2.2 Clinical evidences of anti-diabetes properties of *Aloe vera*

Aloe gel is used in folk medicine of many African and Asian nations (Jansz *et al.*, 1995). According to a study done in India, aloe gel used as oral supplement has reduced the blood glucose level of more than 90% of patients used for the study (Agarwal, 1985). Also, that research has reported the remarkable effects in lipid metabolism (decrease total cholesterol and triglycerides and increase high density lipoprotein), carbohydrate metabolism (decrease fasting and postprandial blood glucose levels in diabetes patients) and angina pectoris (decrease frequency of angina attacks) (Agrawal, 1985). Administration of fresh aloe gel to T2DM patients in a small trial resulted in a sustained lowering of blood glucose levels (Ghannam *et al.*, 1986).

Furthermore, administration of *Aloe vera* juice has reduced the blood sugar and triglycerides levels in human as revealed by clinical trials conducted in Thailand (Yongchaiyudha *et al.*, 1996). In this trial conducted with placebo run, blood glucose (from 250.36±7.65 to 141.92±4.12% mg) and triglyceride (from 220.31±11.40 to 122.72±5.46% mg) levels have fallen during 42 days of daily oral supplementation of fresh *Aloe vera* gel at the dosage of one tablespoon twice a day (Yongchaiyudha *et al.*, 1996). Furthermore, blood glucose levels of the *Aloe vera* treatment group have reduced from 288.14±8.45 to 148.03±4.61% mg of a 42 days clinical assessment of one tablespoon of aloe gel and 5 mg of glibenclamide oral supplementation with placebo run (Bunyapraphatsara *et al.*, 1996). Also, triglyceride levels have fallen significantly in the fresh *Aloe vera* gel and glibenclamide group after four weeks (Bunyapraphatsara *et al.*, 1996). Daily oral administration of 10 cm^3 of *Aloe vera* gel juice against 20 cm^3 of placebo for 12 weeks for hyperlipidemia patients has been reported the considerable decrement of blood cholesterol, low density lipoprotein and triglycerides (Nasiff *et al*, 1993). The clinical use of *Aloe vera* as an anti-diabetic drug succeeded when applied to diabetic women, dropping the blood glucose level significantly from an average of 250 to 141 mg/dl in comparison those who received placebo (Jackie-Hart, 1996). Oral administration of two table spoons (0.05 g) of *Aloe vera* high molecular fractions three times daily for 12 weeks has been significantly reduced the fasting blood glucose level by 32% compared to the before administration (Yagi *et al.*, 2009). Aloe gel powder has been exhibited significant anti-diabetic effects without hepato and pheprotoxicity (Yagi *et al.*, 2009).

1.2.3 Food applications of *Aloe vera* leaf gel

Aloe gel is used as a vegetable, a functional food and as an ingredient in many other food products such as health drinks, yoghurt, milk, ice cream, frozen dairy dessert, backed goods, pudding and confectionary in the forms of tablets, powder and juice

(Goutam and Awasthi, 2007; Hamman, 2008; Ramachandra and Rao, 2008 and Schauss, 1990). Furthermore, aloe gel has been used as an edible coating material to maintain sweet cherry quality and to preserve functional properties of table grapes (Romero *et al.*, 2006; Serrano *et al.*, 2006 and Valuerde *et al.*, 2005). Among the aloe based food products. *Aloe vera* ready-to-serve beverages are the most common.

1.2.4 Toxicity of *Aloe vera*

Although, aloin had been used as a laxative product, after 2003, it was banned thereafter by FDA, in USA as an over-the-counter medication due unfavorable health effects of aloin. However FDA approved aloe gel without aloin (below the permissible level i.e <5 mg dm^{-3} preferably and <0.1 mg dm^{-3} most preferably) for aloe based food commodities (Moore *et al.*, 1995). Thus, *Aloe vera* juice should not contain high amounts of aloin compounds and they should be eliminated while the processing according to the FDA decision. Also, recent studies have reported that aloin and aloe-emodin compounds have phototoxic effects at high doses (Cosmetic ingredients review expert panel, 2007). Although the phototoxicity aloin compounds in aloe plants have been discovered, clinical studies of preparations derived from *Aloe vera* plants demonstrated no phototoxicity confirming that the concentrations of aloin compounds in such preparations are too low (Cosmetic ingredients review expert panel, 2007). Aloe gel powder containing less than 10 mg dm^{-3} of aloin has been exhibited significant anti-diabetic effects without hepato and nephrotoxicity (Yagi *et al.*, 2009). Furthermore, aloe derived ingredients have not been reported to be toxic in acute oral studies in mice and rats (Cosmetic ingredients review expert panel, 2007). Deaths or abnormalities have not been found in a safety evaluation of a test of acute toxicity of aloe gel extract at 150 mg/kg body weight (Tanaka *et al.*, 2012). Oral administration of aloe gel to albino rats has not been reported to be acute and sub chronic toxicity (Charles, 1981; Matsuda *et al.*, 2008). Thus, *Aloe vera* gel extracts has been known as a safe functional food ingredient (Tanaka *et al.*, 2012). Furthermore, aloe gel without anthrone compounds has been reported as nontoxic material (Bergfield, 2007).

1.2.5 Processing of aloe gel

1.2.5.1 Thermal influence on acemannan of aloe gel

Aloe mucopolysaccharide has been reported to exhibit a maximum stability at 70 °C decreasing the stability either at higher or lower temperatures (Chang *et al.*, 2006; Ramachandra and Rao, 2008). Subsequently, thermal treatments affect the bioactivity of mucopolysaccharide/acemannan in aloe gel and care must be taken to preserve them during the process of aloe gel.

Most of the organisms that cause the decay of therapeutic properties of the gel are aerobic and heat is necessary to destroy them, in order to inhibit decay. The spoilage microflora of aloe gel is limited to acid tolerant bacteria, yeast and mold (He *et al.*, 2005) Microbiological test must indicate the absence of *Staphylococcus aureus*, *E. coli*, *Bacillus spp.* and *Salmonella* (He *et al.*, 2005). Whereas, application of the heat causes adverse effects by destroying substantial portion of the active ingredients (Billy and Coates, 1993). Furthermore, heat destroys mucopolysaccharides, enzymes and proteins (Billy and

Coates, 1993). In order to preserve biological active compounds, suitable heat conditions should be applied.

Sterilization and pasteurization are the widely used heat treatments in beverage processing. Although all microorganisms are killed by sterilization process, pasteurization process helps to reduce the number of viable pathogens. In the aloe gel processing it is more imperative to reduce heat to preserve the biological active components. Consequently pasteurization is adequate in aloe gel processing rather than sterilization.

1.2.5.2 Enzymatic treatment

Pectinase enzymatic treatment is done prior to processing; detrimental to biological active compounds mainly acemannan (Ramachandra and Rao, 2008). Pectic polysaccharides are degraded at higher temperatures due to the reaction of β-disposal provoked by heating, although degradation of enzymes of pectic polysaccharides increases at >50 °C (Garcia *et al.*, 2010). The optimum pH for the pectinase activity is 3.5-4.5 (Arampath and Vincent, 1997).

CHAPTER 02

Methodology

2.1 Sampling of *Aloe vera* leaves

Mature *Aloe vera* leaves were obtained from the plantation of Natures' Beauty Creations Ltd., Horana, Kaluthara, Sri Lanka. The leaves were washed thoroughly under running tap water and disinfected by immersing them in 70% v/v propylalcohol for 1-2 min. A transverse cut was made at about one inch above the stem of the leaf using a sharp stainless steel knife. Cut leaves were allowed to stand vertically for 15 min to drain off the yellow exudates, and washed under running tap water. The washed leaves were manually filleted to obtain aloe gel by trimming off serrated margins and about two inches off the top part of the leaf (Ramachandra and Rao, 2008).

2.1.1 Elimination of aloin from aloe gel

Aloin was eliminated according with a water treatment method by swirling of aloe gel slices (~1 cm^3) in water (patented). Aloe gel slices were agitated in water at room temperature (27±2 ^oC). The agitation of aloe gel in water was done at three washing steps and water was removed in each step by filtering out with a filter (Patent- pending; Application number LK/P/1/17228).

2.1.2 Determination of aloin of aloe gel

The sampling was carried out by protecting from light. Aloe gel was blended and fibrous materials were removed by filtering through a piece of muslin cloth. Ten cm^3 of blended aloe gel and commercial aloin standard solution (4.0×10^{-4} mol dm^{-3}) were separately refluxed in a boiling water bath (90-97 ^oC) with 1 cm^3 of 60% w/v $FeCl_3$ and 6 cm^3 of concentrated HCl. Aloin was analyzed quantitatively by visible spectrophotometry at 512 nm.

2.2 Determination of the effect of different processing steps used in preparation the beverage on mucopolysaccharides

2.2.1 Effect of time-temperature combinations

Aloe vera leaves were thoroughly washed under running tap water and filleted manually to separate aloe gel as shown in Figure 2.1 (Ramachandra and Rao, 2008). Blended aloe gel (5 cm^3/ tube) was put into boiling tubes after filtering and homogenization. Seven sets of those tubes (18/ set) were incubated in a water bath at 40-90 ^oC and boiling temperature (~97 ^oC), respectively for 30 min and triplicate was removed in each 5 min interval from each set. The removed samples were rapidly cooled by dipping in ice crystals.

Total carbohydrates (Dubios *et al.*, 1956; Krishnavent *et al.*, 1984) and acemannan (Eberendu and McAnalley, 1996), of aloe gel were quantified in triplicate, using UV/Vis spectrophotometry and glucose and acemannan standard, respectively, as standards. Subsequently, total soluble solids (TSS) were measured with a digital refractometer (Model; PR-1). Data were analyzed in a randomized completely block design (RCBD) (time as the blocking factor) and complete randomized block design (CRD), to determine the effect of temperature and the effect of time-temperature combination on total carbohydrate, acemannan and TSS, respectively, using analysis of variance (1994; SAS Institute, Cary, NC, USA). Means were compared at $p \leq 0.05$ according to the Duncan's multiple range test.

2.2.2 Effect of pectinase enzyme treatment and pasteurization

A part of blended aloe gel was subjected to pectinase enzyme treatment by incubating in a shaking water bath. A half of each with and without enzyme treated samples was subjected to low temperature long time (LTLT). The rest was subjected to high temperature short time (HTST). The heated samples were cooled by dipping in ice crystals. The experimental treatments were arranged as a two factor factorial with HTST or LTLT pasteurization as the levels of factor one, and the presence or absence of pectinase enzyme treatment as the levels of factor two, in a CRD. Acemannan and total carbohydrate were determined in triplicate as mentioned in 2.2.1. Data were subjected to analysis of variance using the SAS package (1994; SAS Institute, Cary, NC, USA). Means were compared at $p \leq 0.05$ according to the Duncan's multiple range test.

2.3. Development of the anti-diabetic formula of *Aloe vera* ready-to-serve beverage

Aloe vera leaves were washed thoroughly under running tap water and aloe gel was separated as described in section 2.1. Aloe gel was cut into small slices (Figure 2.2) and aloin was removed. Aloe gel slices, after removing aloin, were crushed into small pieces using a stainless steel fruit crusher (Figure 2.3). The crushed gel (pH=4.15±0.14) was treated with pectinase enzyme and the samples were shaken in a shaking water bath.

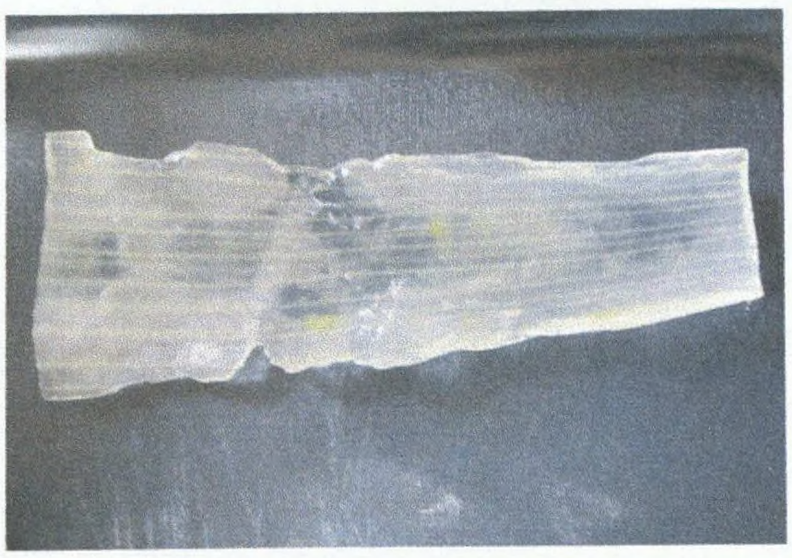


Figure 2.1: ***Aloe vera*** **fillet**

Figure 2.2: ***Aloe vera*** **cubes prepared for aloin elimination by agitation with water**

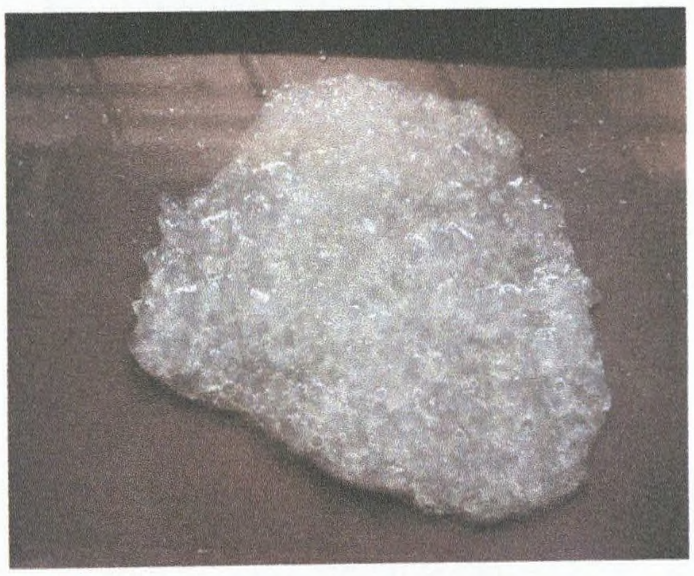


Figure 2.3: Crushed ***Aloe vera*** **gel for the enzyme treatment**

2.3.1 Effect of processing parameters on sensory qualities

Selection of sucralose amount in the beverage

Four different *Aloe vera* RTS beverage samples were prepared with different sucralose amounts and other food grade ingredients (Figure 2.4) followed by the pectinase enzyme treatment and HTST pasteurization with some modifications of the developed normal formula of the normal formula *Aloe vera* ready-to-serve beverage Wijesinghe *et al.*, 2012).

The preference for sweetness and overall taste of *Aloe vera* RTS beverage was tested using a ranking test and 32 untrained panelists in the sensory laboratory of the Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya.

Selected two different *Aloe vera* RTS beverage samples from the above samples with sucralose, citric acid, orange flavor and other ingredients were further evaluated for the preference for sweetness, overall flavor and after taste using a ranking test and 32 untrained panelists. Sensory data were subjected to Friedman's analysis, by taking the least significant difference at $p \leq 0.05$, using the SAS package (1994; SAS Institute, Cary, NC, USA).

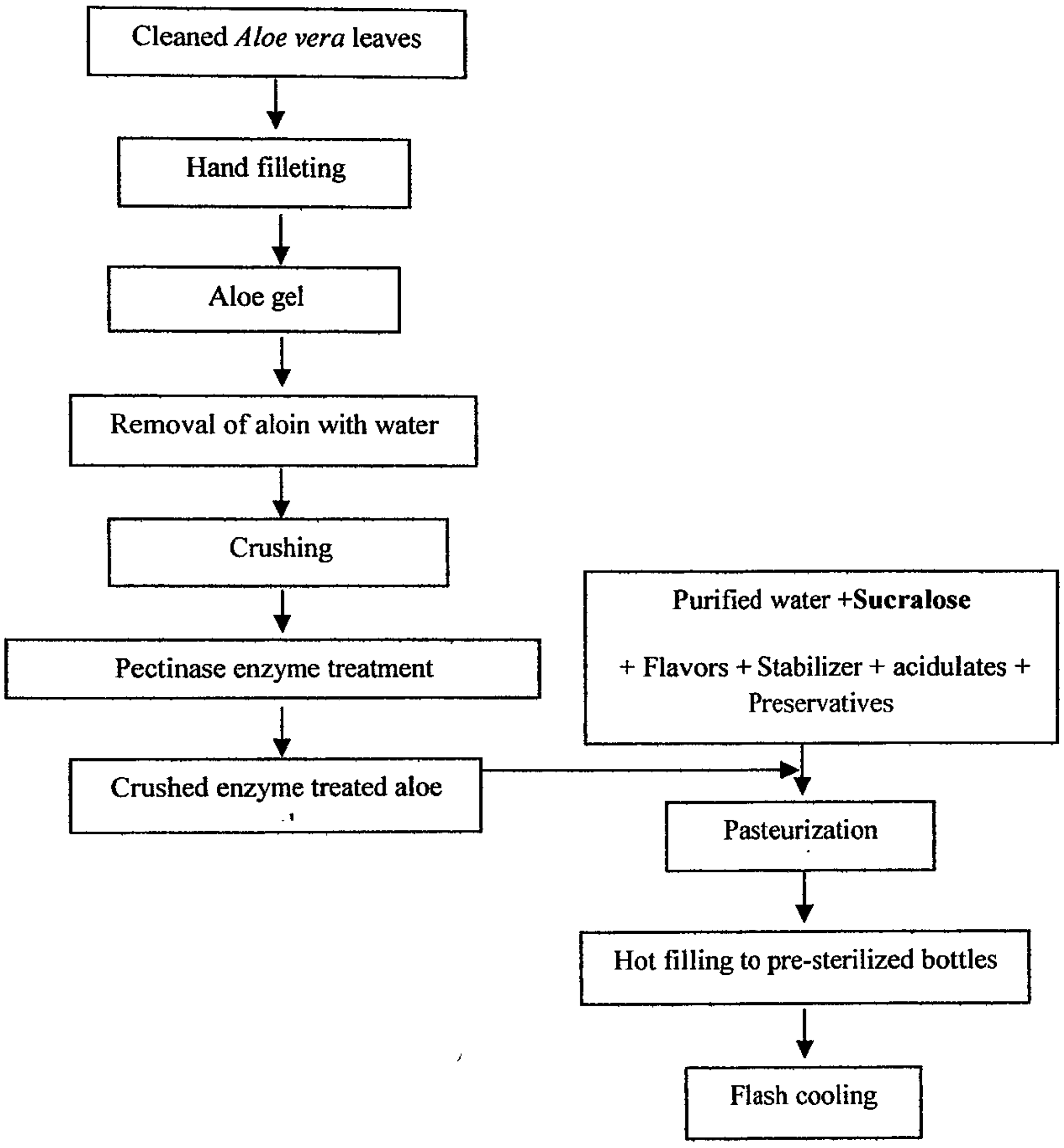


Figure 2.4: Process flow diagram for the preparation of the normal formula of *Aloe vera* RTS beverage

2.3.2 Sample preparation

Pectinase enzyme treated aloin free aloe gel, sucralose, citric acid, ascorbic acid, orange flavor (LA 03932, France) and permitted preservatives (Sri Lanka standards 729, 2010) were used to prepare the RTS beverage. The final formulation was pasteurized under HTST.

2.3.3 Effect of processing parameters on beverage quality and its shelf-life evaluation in glass bottles

The shelf-life of the beverage in glass bottles was tested in triplicate, arranged in a CRD. The pasteurized samples were hot filled into pre-sterilized glass bottles and screw capped. Packed bottles were stored at 35±2 °C in an incubator.

2.3.3.1 Physico-chemical properties

Physico-chemical properties of the samples were measured in triplicate, in every two week. Total soluble solids (TSS), turbidity and pH of the beverage were measured using a digital refractometer (Model; PR-1), turbidy meter (2100 P, USA) and pH meter (ORION 2 STAR, Singapoure), respectively. Titratable acidity was determined by titrimetry with 0.1 mol dm^{-3} NaOH and expressed as percentage citric acid (AOAC, 1995).

2.3.3.2 Microbiological properties

Microbiological properties of the samples were tested in triplicate along the storage period, using 0.1% w/v peptone as the diluents. Total coliform count was determined using MacConkey broth as the medium, by most probable number method (Sri Lanka standards 516 Part 3, 1982). Total plate count was determined using plate count agar as the medium, by pour plate method (SLS 516, 1991). Yeast-mould count was determined using yeast extract-dextrose-chloramphenicol agar as the medium, by pour plate method (SLS 516, 1991).

2.3.3.3 Sensory properties

A sensory test was conducted to evaluate consumer preference based on aroma, overall appearance, mouthfeel, overall flavor and overall acceptability after the determination of the microbiology properties. Thirty untrained panelists and nine-point hedonic scale (1- Dislike extremely and 9- Like extremely) were used for the sensory test.

The data was subjected to analysis of variance using the SAS package (1994; SAS Institute, Cary, NC, USA). Means were compared at $p \leq 0.05$ according to the Duncan's multiple range test. Sensory data was subjected to Friedman's analysis, by taking the least significant difference at $p \leq 0.05$, using the SAS package.

CHAPTER 03

Results and discussion

3.1 Elimination of aloin from aloe gel

Aloe vera leaves (292.30±69.20 g) dripped out 1.02±0.23 g (0.34±0.29% w/w from the total wet weight of the leaf) of yellow coloured exudates, after standing 15 min and considerable amounts were not found to be removed thereafter. Aloe gel was found to contain 17.24±1.53 ppm of aloin. Considerably low amount of aloin; 7.29±1.18 ppm was found to be remained in the washed aloe gel. Before, incorporating into the beverage, 10% w/v aloe gel was found to contain 0.73±0.12 mg dm^{-3} of aloin followed by the water treatment method.

3.2 Determination of the effects of thermal treatments on major bioactive constituents of aloe gel

3.2.1Effect of time-temperature combinations

The amount of total carbohydrates of aloe gel was significantly ($p \leq 0.05$) affected by temperature. The amounts of total carbohydrates were significantly ($p \leq 0.05$) reduced in the aloe gel samples incubated at 40 °C (25.33±2.97 mg dm^{-3}), 50 °C (35.67±3.35 mg dm^{-3}), 60 °C (31.51±2.69 mg dm^{-3}) and 70 °C (35.76±2.41 mg dm^{-3}), while it was non-significantly ($p \leq 0.05$) reduced in the samples incubated at 80 °C (53.84±1.23 mg dm^{-3}) and 90 °C (48.30±1.28 mg dm^{-3}) (Figure 3.1- a). However, at 97 °C, a non-significant ($p \leq 0.05$) higher amount of total carbohydrates of aloe gel (55.15±2.15 mg dm^{-3}) could be obtained than in the control (54.90±1.00 mg dm^{-3}).

The amounts of acemannan and TSS of aloe gel were not significant ($p \leq 0.05$) with the temperature. However, a non-significant ($p \leq 0.05$) higher amount of acemannan (24078±3687 mg dm-3) was obtained in the aloe gel sample incubated at 70 °C (Figure 3.1-b). Similarly, Chang *et al.*, (2006) and Ramachandra and Rao, (2008) reported that the maximum stability of acemannan is at 70 °C. This may be due to the structural modification such as deacetylation and loss of galactose rich side-chains from the mannose backbone to form new hydrogen bonds between mannose and long chains of acemannan, as reported by Femenia *et al.*, (2003) and Rodriguez-Gonzalee *et al.*, (2011). Furthermore, Femenia *et al.*, (2003) reported that heating promotes changes in average molecular weights of aloe polysaccharides of dehydrated aloe gel at 70 °C and 80 °C than of fresh aloe gel.

Significant ($p \leq 0.05$) lower amounts of acemannan were obtained in the aloe gel samples incubated at 90 °C and 97 °C than of others. The inverse correlation between amounts of total carbohydrates and acemannan revealed that the lower amounts of acemannan with higher amounts of total carbohydrates of the aloe gel samples incubated at higher temperatures, 90 °C and 97 °C.

TSS refers to the measurement of soluble dry substances or approximate measurement of sugar content in liquid. Sucrose is completely decomposed at 185 °C after 30 min as reported by Simkovic *et al.*, (2003). Such a high temperature was not used in this experiment and this may be the reason for non-significant ($p \leq 0.05$) thermal effect on TSS contents of aloe gel (Figure 3.1-c).

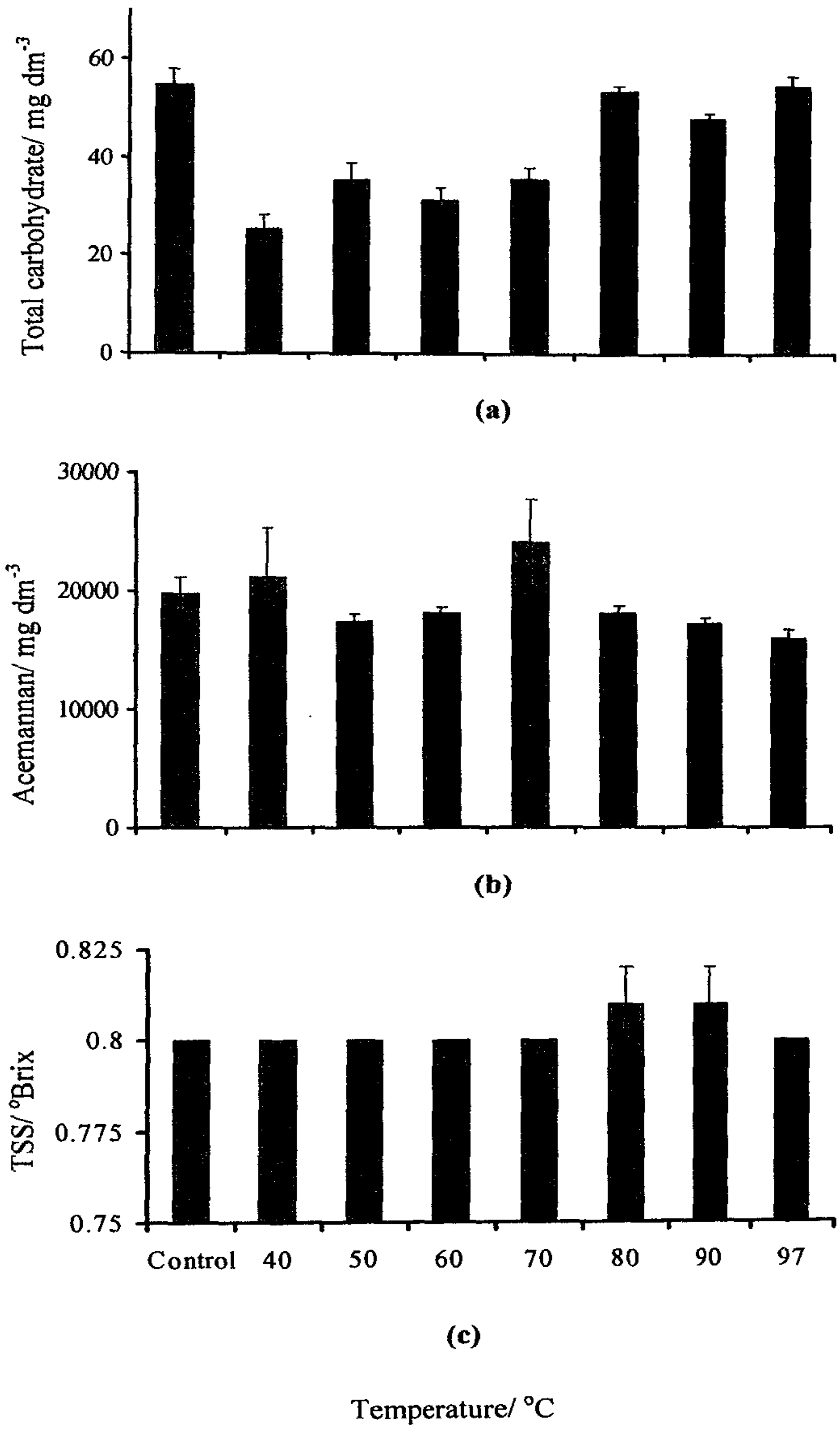


Figure 3.1: Effect of temperature on (a) total carbohydrate, (b) acemannan and (c) TSS contents of aloe gel

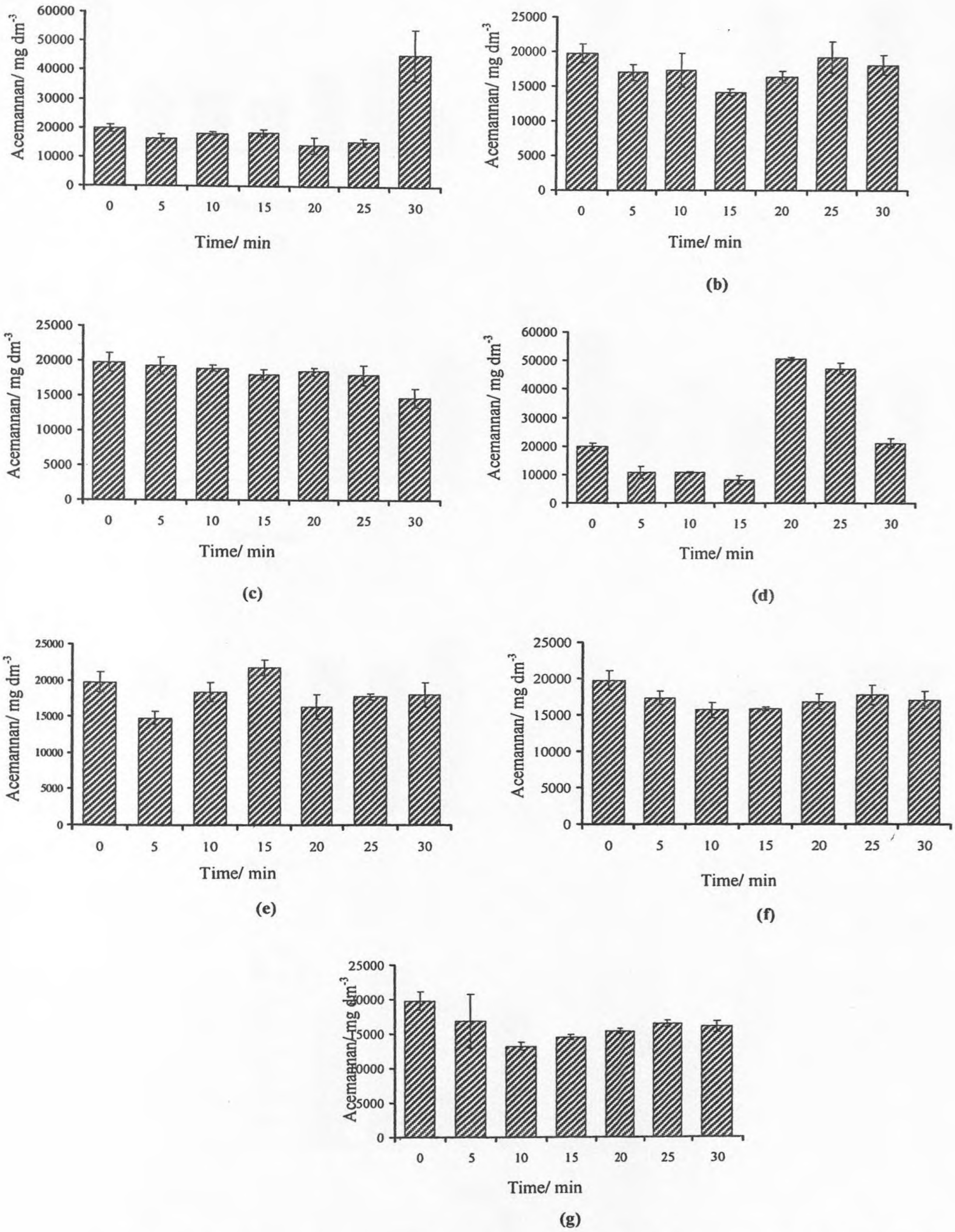


Figure 3.2: Effect of time-temperature combinations on acemannan content of aloe gel

(a) 40 °C, (b) 50 °C, (c) 60 °C, (d) 70 °C, (e) 80 °C, (f) 90 °C and (g) 97 °C

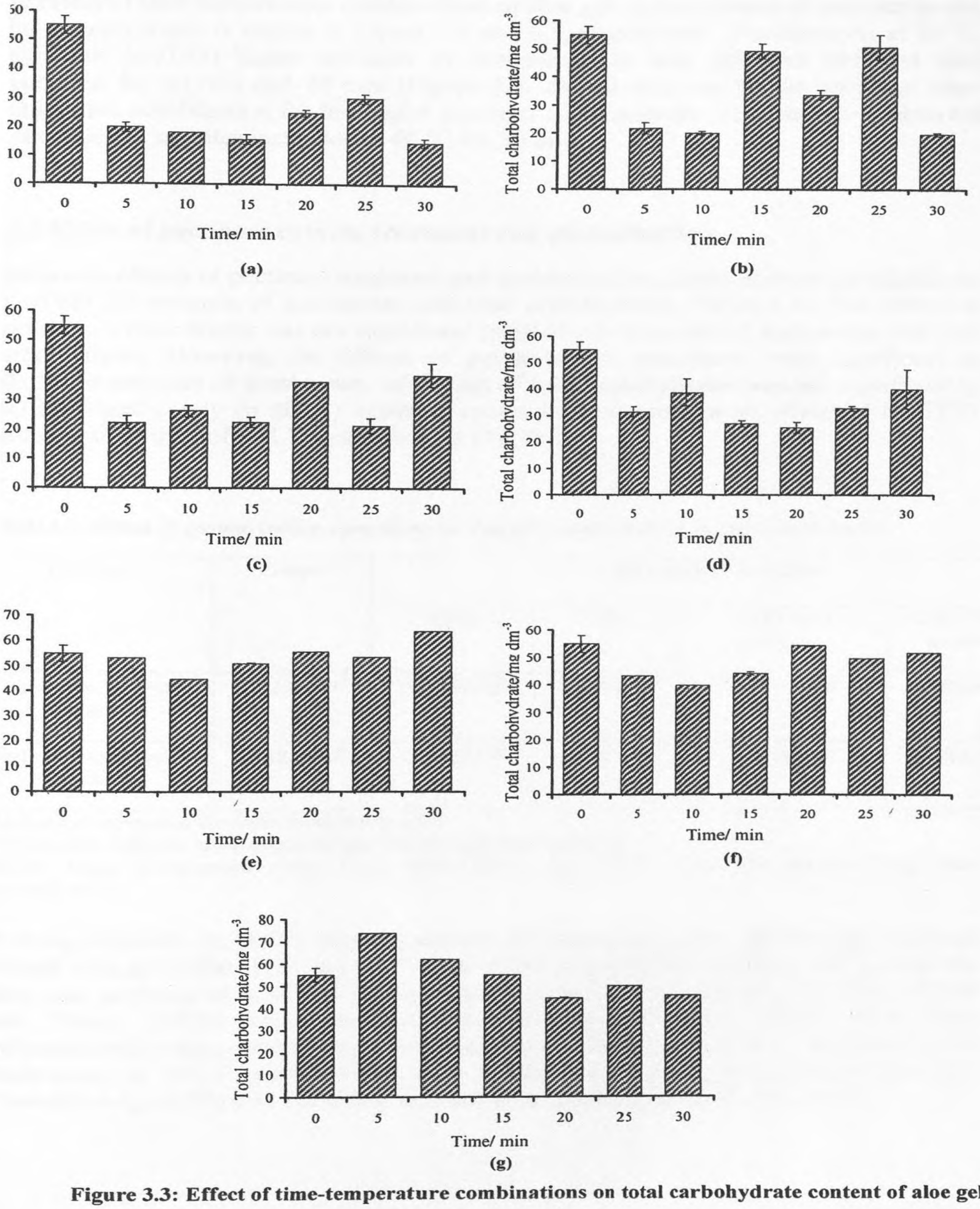


Figure 3.3: Effect of time-temperature combinations on total carbohydrate content of aloe gel

(a) 40 °C, (b) 50 °C, (c) 60 °C, (d) 70 °C, (e) 80 °C, (f) 90 °C and (g) 97 °C

The effect of time-temperature combinations of aloe gel on its contents of acemannan and total carbohydrates is shown in Figure 3.2 and 3.3, respectively. Furthermore, at 70 °C, significant ($p \leq 0.05$) higher amounts of acemannan in aloe gel were obtained after incubation for 20 min and 25 min (Figure 3.2- d) and this may be the optimum time-temperature combination for the higher amounts of acemannan. The same was observed in the aloe gel samples incubated at 40 °C for 30 min.

3.2.2 Effect of pectinase enzyme treatment and pasteurization

Interaction effects of pectinase treatment and pasteurization condition were not significant ($p \leq 0.05$) for amounts of acemannan and total carbohydrates (Table 3.1). The effects of pectinase, a main factor was not significant ($p \leq 0.05$) for amounts of acemannan and total carbohydrates. However, the effects of pasteurization conditions were significant ($p \leq 0.05$) for amounts of acemannan, while that of total carbohydrates was not significant ($p \leq 0.05$). Significantly ($p \leq 0.05$) higher amounts of acemannan were observed in HTST pasteurization than of LTLT pasteurization (Table 3.1).

Table 3.1: Effect of pasteurization conditions on aloe gel content and its major constituents

Attribute	Control	Pasteurization Conditions			
		HTST	LTLT	HTST with pectinase	LTLT with pectinase
Acemannan content [S]/ mg dm^{-3}	11925±693[a]	11304±605[a]	7468±3223[b]	8575±2464[a]	6385±624[b]
Total carbohydrates [NS]/ mg dm^{-3}	20.12±3.47[a]	13.98±2.64[b]	15.87±1.37[ab]	12.75±2.67[a]	15.71±3.29[ab]

Each value represents the mean of triplicate ± SD
Values with different superscripts are significantly different ($p \leq 0.05$)
HTST: High Temperature Short Time pasteurization and LTLT: Low Temperature Long Time pasteurization

Non-significantly ($p \leq 0.05$) higher amounts of acemannan were obtained in pectinase treated aloe gel (6990±1201 mg dm^{-3}) than of the control (6803±186 mg dm^{-3}), when the data was analyzed in a RCBD (pasteurization as the blocking factor). Similarly, Mulik and Phale, (2009) have reported that pectinase treatment yields 45% more polysaccharides than obtained by thermal treatments without pectinase. The decrement of acemannan in LTLT pasteurization may be due to the long time thermal exposure. Correlation ($p \leq 0.05$) was not found between total carbohydrate and acemannan.

3.3 Development of the anti-diabetic formula of *Aloe vera* ready-to-serve beverage

3.3.1 Effect of processing parameters on sensory qualities

Selection of sucralose amount in the beverage

Sucralose is stable in the presence of heat and wide pH range and suitable for thermally processed food and beverages (Quinlan and Jenner, 1990). Sucralose provides similar taste to sucrose as reported by Damodaran *et al.*, (2007) and the same was experienced in preliminary studies of this study. Furthermore, sucralose is safe for diabetes as reported by Grotz *et al.*, (2003).

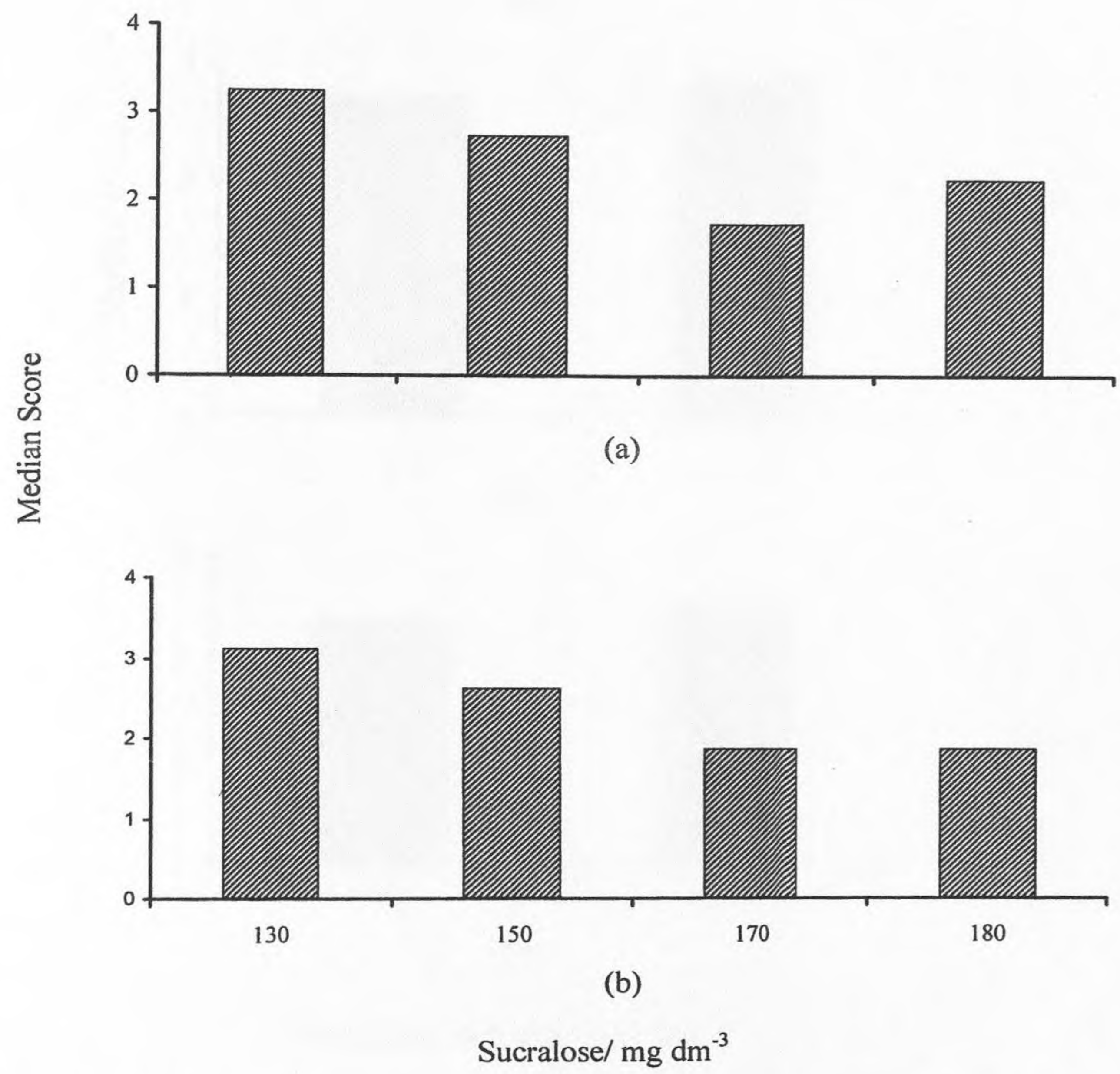


Figure 3.4: (a) Sweetness and (b) overall flavor of *Aloe vera* RTS as affected amount of sucralose

Ranking scale; The most preferred sample represents a rank value of 1; the samples with the next prefer rank values of 2 & 3 and the sample with the least preferred represents a rank value of 4

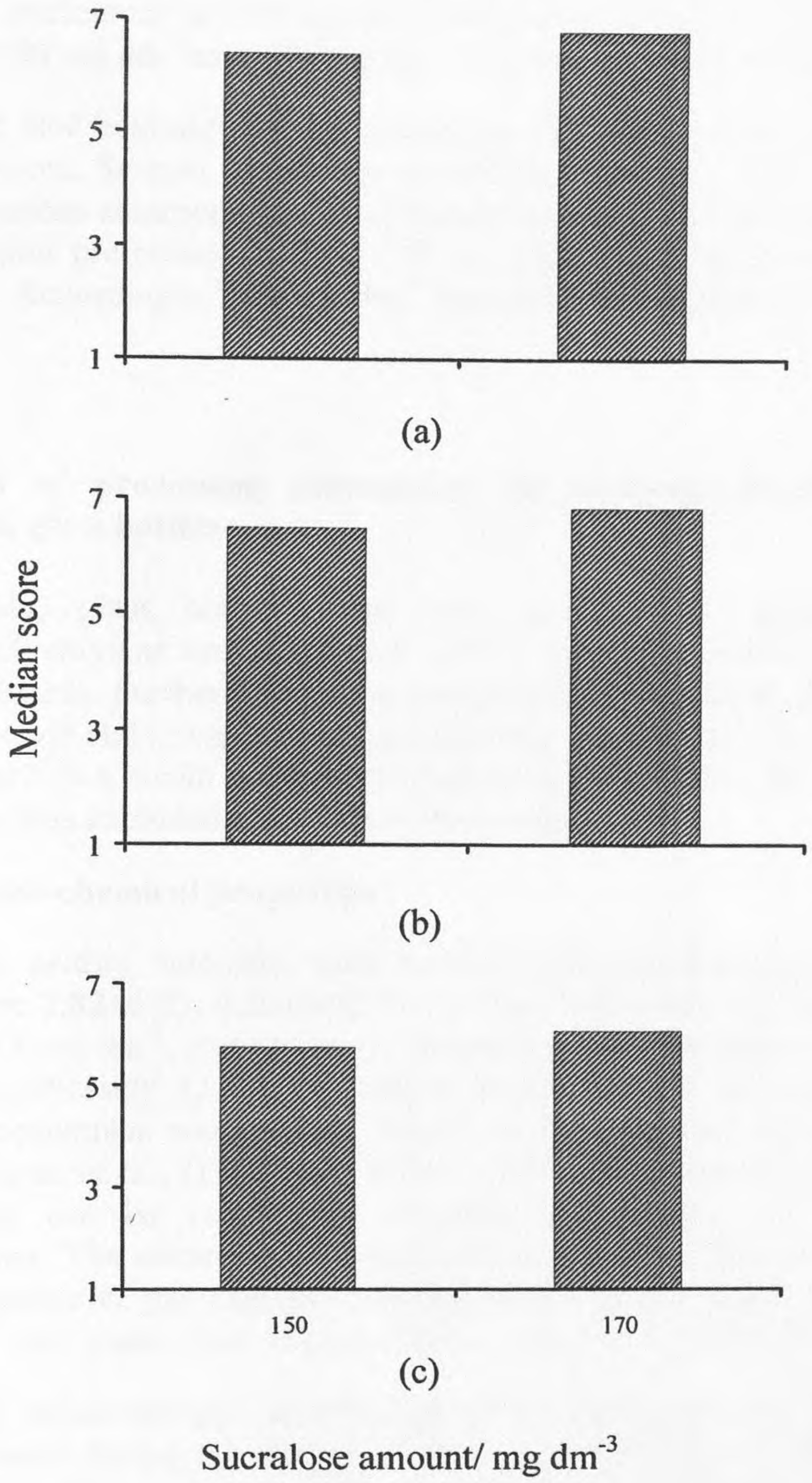


Figure 3.5: (a) Sweetness, (b) after taste and (c) overall flavor of *Aloe vera* RTS as affected amount of sucralose

Ranking scale; The most preferred sample represents a rank value of 1; the samples with the next prefer rank values of 2 & 3 and the sample with the least preferred represents a rank value of 4.
Values with different letters are significantly different at $p \leq 0.05$

The selected sugar amount in the normal formula of the *Aloe vera* RTS beverage was 9% w/v (Wijesinghe *et al.*, 2012). At 9% sweetness equivalency of sucrose, representative sweetness of many diet beverages the level has been reported to be about 170 mg dm^{-3}, as described by Wiet and Beyts, (1992). Similarly, sensory evaluation scores for preference of sweetness and overall taste of *Aloe vera* RTS beverage revealed the significant ($p \leq 0.05$) most preference in 170 mg dm^{-3} sucralose amount and it was non-significant ($p \leq 0.05$) with 150 mg dm^{-3} and 180 mg dm^{-3} sucralose amounts in this study (Figure 3.5).

Value added aloe beverage sample could be obtained with nature identical orange and pineapple flavors. Sensory scores for sweetness, after taste and overall flavor of the 150 mg dm^{-3} sucralose incorporated aloe RTS beverage revealed the non-significant ($p \leq 0.05$) lower consumer preference than of 170 mg dm^{-3} sucralose incorporated RTS beverage (Figure 3.5). Accordingly, 150 mg dm^{-3} sucralose was selected to be incorporated in the beverage.

3.3.2 Effect of processing parameters on beverage quality and its shelf-life evaluation in glass bottles

In this study, glass bottles were used as glass is more cost effective than crystalline polyethylene terephthalate (C-PET). Also, glass bottles can be re-used without any health hazards. Furthermore, upon prolonged storage and at elevated temperatures C-PET may liberate endocrine disrupting chemicals (Sax, 2010). Besides developed product in this research is a health formula (anti-diabetic). Therefore, the use of PET bottles for storage study was excluded due to all of these reasons.

3.3.2.1 Physico-chemical properties

pH, titratable acidity, turbidity, total soluble solid and acemannan content of the RTS beverage were 2.82±0.05, 0.52±0.02 % w/v (as citric acid), 16.3±0.9 NTU, 0.0±0.0 °Brix and 2427±115 mg dm^{-3}, respectively. Titratable acidity, turbidity and acemannan content were non-significantly ($p > 0.05$) varied throughout the storage period (Figure 3.6). However, acemannan content was found to be decreased along the storage period. Similarly, Yaron *et al.*, (1992) and Yaron, (1993) have reported that the degradation of aloe mannan can be caused by elevated temperature, pH change and bacterial contaminations. The decrement of acemannan content in this study may be due to the storage temperature, pH changes and bacterial contamination. This is proved by the increment of total plate count of the product along the storage period (Figure 3.7).

Total soluble solids contents zero throughout the storage period. pH was significantly ($p \leq 0.05$) decreased during the storage (Figure 3.6). Furthermore, significant ($p \leq 0.05$) pH decrement was observed after 20th week during the storage (Figure 3.6). Moreover, converse correlation ($p > 0.05$) was found between pH and acemannan content of the aloe beverage.

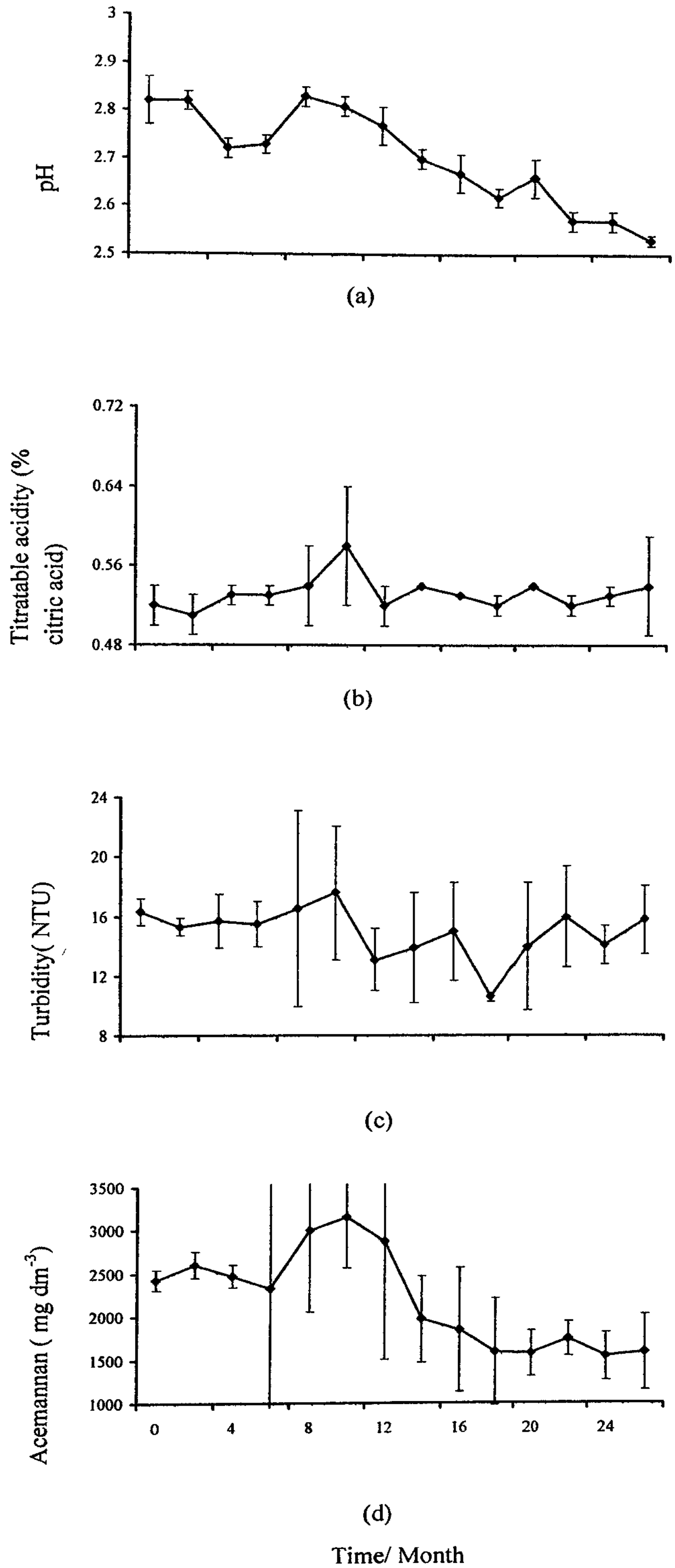


Figure 3.6: Changes of physico-chemical properties of *Aloe vera* RTS beverage on storage (a): pH, (b): Titrattable acidity, (c): turbidity and (d): acemannan

Each value repents the mean of triplicate

3.3.2.2 Microbiological properties

Coliform and yeast and mould were not found to be present in the beverage throughout the storage period. Significant ($p \leq 0.05$) increment of total plate count (34.33±20.12 cfu/cm^3) was found in sixth month. However, it was less than the Sri Lankan standards (50 cfu/cm^3) (Figure 3.7).

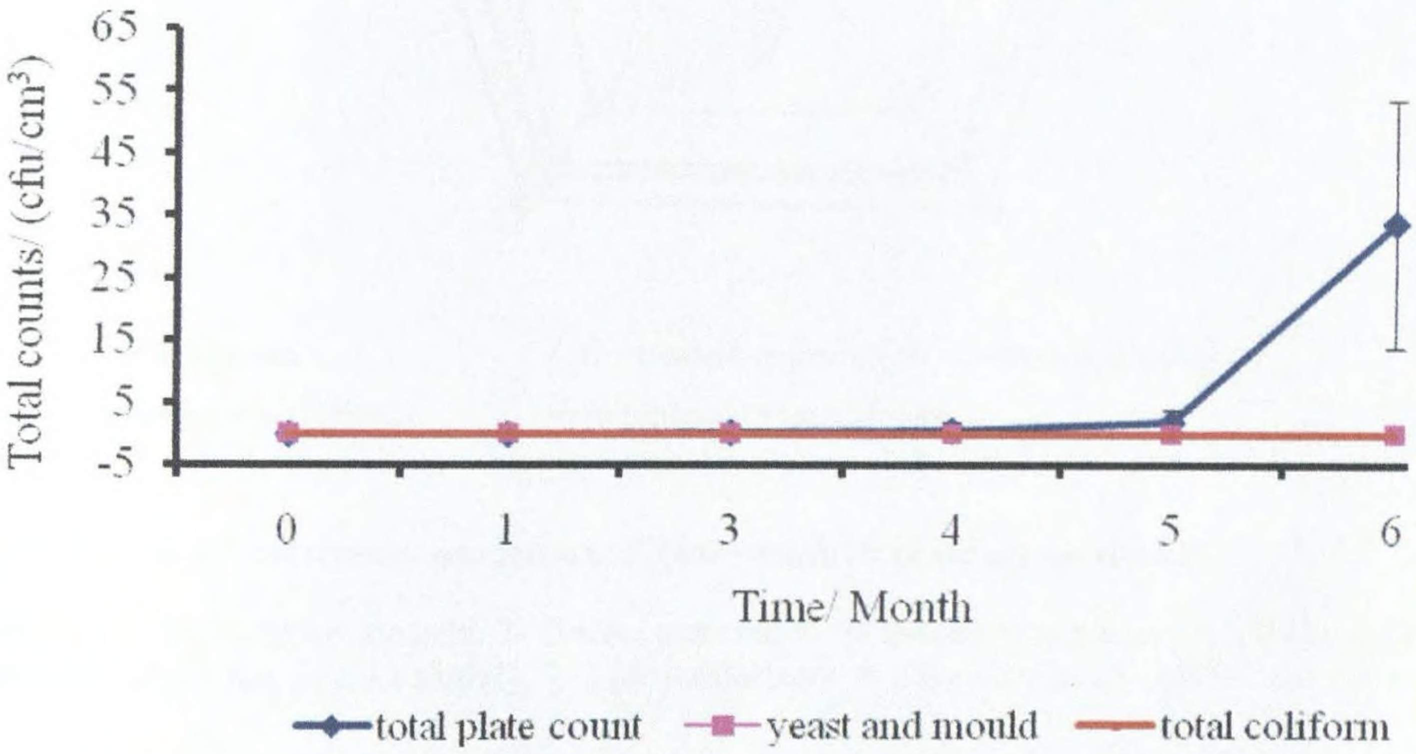


Figure 3.7: Changes of microbiological properties of *Aloe vera* RTS beverage on storage

Each value repents the mean of triplicate

3.3.2.3 Sensory properties

Sensory score for the preference for aroma (median hedonic score- 7.66) of the beverage was significantly ($p \leq 0.05$) higher than for the scores for overall appearance, mouthfeel, overall flavor and overall acceptability, at the initial of the storage study (median hedonic scores- 6.34, 5.76, 5.62 and 6.00, respectively). Sensory score for the preference for aroma, overall appearance and mouthfeel of the beverage were not significantly ($p > 0.05$) changed during the storage period. However, during the storage period, there were significant ($p \leq 0.05$) effects for the preference on overall flavor and overall acceptability of the beverage (Figure 3.8). The shelf-life of the beverage was ~9 months based on physico-chemical, microbiological and sensory properties at ambient temperature (27±2 °C).

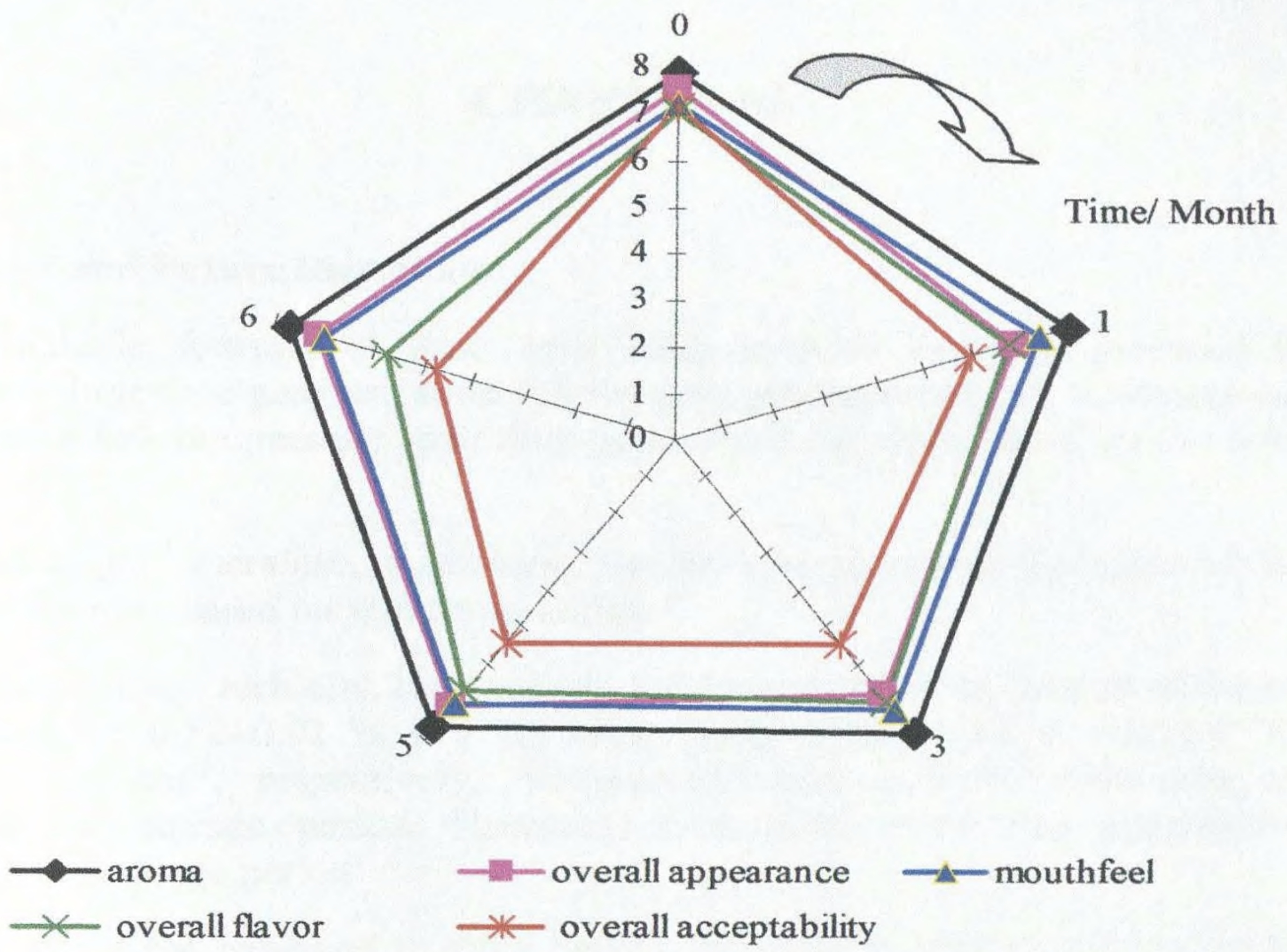


Figure 3.8: Changes of sensory properties of *Aloe vera* RTS beverage on storage

Hedonic scale : 1- Dislike extremely, 2- Dislike very much, 3- Dislike moderately, 4- Dislike slightly, 5- Neither like nor dislike, 6- Like slightly, 7- Like moderately, 8- Like very much and 9- Like extremely

CHAPTER 04

Conclusions and Future Directions

The anti-diabetic formula of *Aloe vera* ready-to-serve beverage prepared by high temperature short time pasteurization followed by pectinase enzyme treatment was more preferred than low temperature long time pasteurized beverage, based on the acemannan content.

Crushed aloe gel, sucralose, acidulates, flavors and preservatives imparted the most acceptable formula based on sensory qualities.

pH, titratable acidity, turbidity, total soluble solid and acemannan content of the beverage were 2.82±0.05, 0.52±0.02 % w/v (as citric acid), 16.3±0.9 NTU, 0.0±0.0 °Brix and 2427±115 mg dm^{-3}, respectively. Yeast-mould and coliform were not observed throughout the storage period. However, total plate count was considerably low throughout the storage period.

The shelf-life of the beverage in glass bottles was nine months at ambient temperature (27±2 °C).

Aloin content of the beverage was very low (0.73±0.12 mg dm^{-3}) and safe for oral consumption (aloin content should be < 5 mg dm^{-3} for oral consumption).

Though the product development has been completed, the process needs to be scaled up. Moreover, the product needs to be assessed for its potential for treating type 2 diabetes mellitus. In this context, a clinical trial needs to be carried out on diabetic patients. Scaling up of the process and clinical trials are equally important for future commercialization of the developed product.

References

Agrawal, O. P. (1985). Prevention of atheromatous heart disease. *Angiology* **36**, 485-492.

Ajabnoor, M.A. (1990). Effect of aloes on blood glucose levels in normal and alloxan diabetic mice. *Journal of ethno pharmacology* **28**, 215-220.

Anderson, F.A. (2007). Final reports on the safety assessment of *Aloe andongensis* extract, *Aloe andongensis* leaf juice, *Aloe arborescens* leaf extract, *Aloe arborescens* leaf juice, *Aloe arborescens* leaf protoplasts, *Aloe barbadensis* flower extract, *Aloe barbadensis* leaf, *Aloe barbadensis* leaf extract, *Aloe barbadensis* leaf juice, *Aloe barbadensis* leaf polysachcharides, *Aloe barbadensis* leaf water, *Aloe ferox* leaf extract, *Aloe ferox* leaf juice and *Aloe ferox* leaf juice extract (Cosmetic ingredient review expert panel, Washington, DC). *International Journal of Toxicology* **26** (2), 1-50.

Arampath, P.C. and Vincent, J. (1997). Production of clarified, concentrated juice from mango variety kaew. *Tropical Agricultural Research* 9, 49-61.

Bassetti, A. and Sala, S. (2005). The great aloe book. First edition. Practical applications of aloe. pp: 121.

Bergfield, W.F. (2007). Final report of the cosmetic ingredient review. *International Journal of Toxicology* 26 (2), 1–50.

Billy, C. and Coates. Method of processing stabilized *Aloe vera* gel obtained from the whole *Aloe vera* leaf. United States patent number 5,356,811, 1993.

Bunyapraphatsara, N., Yongchaiyudha, S., Rungpitarangsi, V., Chokechaijaroenporn, O. (1996). Antidiabetic activity of *Aloe vera* L juice. II. Clinical trial in diabetes mellitus patients in combination with glibenclamide. *Phytomedicine* **3**, 245-248.

Can, A., Akev, N., Ozsoy, N., Bolkent, S., Arda, B.P., Yanardag, R. and Okyar, A. (2004). Effect of Aloe vera leaf gel and pulp extracts on the liver in type 2 diabetes rat models. *Biological and pharmaceutical bulletin* **27**(5), 694.

Carpenter, Robert H., McDaniel, Harley R., McAnalley and Bill H. Uses of aloe products in the treatment of chronic respiratory diseases. United states patent number 5,786,342,1998.

Chan, J.C.N., Malik, V., Gia, W., Kadowaki, T., Yagnic, C.S., Yoon K.H. and Hu F.B. (2009). Diabetes in Asia. *American medical association.* 301 (20), 2129-2140.

Chang, X.L., Wang, C., Feng, Y. and Liu, Z. (2006). Effects of heat treatments of the stabilities of polysaccharides substances and barbaloin in gel juice from *Aloe vera* Miller. *Journal of Food Engineering* **75**(2), 245-251.

Charles, B. (1981). Acute oral toxicity study. Dawson Research Corporation. DRC 2765.

Chearskul, S., Sangurai, S., Nitiyanant, W., Kriengsinyos, W., Kooptiwut, S. and Harindhanavudhi, T. (2007). Glycemic and Lipid Responses to Glucomannan in Thais with Type 2 Diabetes Mellitus. *Journal of the Medical Association of Thailand* **90** (10), 2150-2157.

Cosmetic ingredient review expert panel. (2007). Final reports on the safety assessment of *Aloe andongensis* extract, *Aloe andongensis* leaf juice, *Aloe arborescens* leaf extract, *Aloe arborescens* leaf juice, *Aloe arborescens* leaf protoplasts, *Aloe barbadensis* flower extract, *Aloe barbadensis* leaf, *Aloe barbadensis* leaf extract, *Aloe barbadensis* leaf juice, *Aloe barbadensis* leaf polysachcharides, *Aloe barbadensis* leaf water, *Aloe ferox* leaf extract, *Aloe ferox* leaf juice and *Aloe ferox* leaf juice extract. *International Journal of Toxicology* **26** (2), 1-50.

Cowsert, L.M. (2010). Biological activities of acemannan. 2-9.

Damodaran, S., Parkin, K.L. and Fennema, O.R. (2007). Fennema's Food Chemistry. 4th edition. CRC press, New York. p. 119-721.

Danhof, I. The therapeutic component in *Aloe vera.* Toll free: 800-760-3530. International: 503-526-9130. www.healthbreakthroughs.net/mps-gold-100.php.

Davis, R.H. (1989). Aloe vera and gibberelllin anti-inflammatory activity in diabetes. *Journal of the American Podiatric Medical Association* 79 (1), 1-4.

Diabetes association of Sri Lanka, national diabetes centre, no. 50, Sarana Mawatha, Rajagiriya, Sri Lanka. http://www.diabetessrilanka.org/about_us.php. Online 28/01/2012. 13.35 pm.

Dubios, M.m Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Analytical Chemistry 26 p. 350.

Eberendu, A.N.R. and McAnalley, B.H. Colorimetric assay for bioactive polysaccharides. United States patent number 5 512 488. 1996.

Eshun, K. and He, Q. (2004) Aloe vera: A valuable ingredient for the food, pharmaceutical and cosmetic industries: a review. *Critical reviews in Food Science and Nutrition* 44, 91–96.

Farrow, Jia, Qi and Thomas, M. 7-Hydroxy chromones as potent antioxidants. Australian patent number 239350, 2003.

Femenia, A., Garcia-Pascual, P., Simal, S. and Russello, C. (2003). Effect of heat treatment and dehydration on bioactive polysaccharide acemannan and cell wall polymers from *Aloe barbadensis* Miller. *Carbohydrate Polymers* 51(4), 397-405.

Femenia, A., Sanchez, E.S., Simal, S. and Rossello, C. (1999). Compositional features of polysaccharides from *Aloe vera* (*Aloe barbadensis* Miller) plant tissues. *Carbohydrate polymers* 39, 109-117.

Ferguson, L.R., Shuotun, Z. and Harris, P.J. (2005). Antioxidant and antigenotoxic effects of plant cell wall hydroxycinnamic acids in cultured HT-29". *Molecular Nutrition & Food Research* **49** (6), 585–693.

Gautum, S. and Awasthi, P. (2007). Nutrient composition and physico-chemical characteristics of aloe vera (*Aloe barbadensis*) powder. *Jounal of food science and technology* 44 (2), 224-225.

Gen, R., Mark, K., Dornbrack, Andrea, Beer and Kurt. Method for the removal of aloin, emodin and/or iso-emodin from *Aloe vera* by treatment with an oxidase. United States patent number 20070275107, 2007.

Ghannnam, N., Kingston, M., Al-Meshaal, I.A., Tariq, M., Parman, N.S. and Woodhouse, N. (1986). The anti-diabetic activity of aloes: preliminary clinical and experimental observations. *Hormone research* **24**, 288-294.

Gridlay, D. and Reynolds, T. (1986). The *Aloe vera* phenomenon. A review of the properties and modern uses of the leaf parenchyma gel. *Journal of Enthnopharmacol* **16**, 117-151.

Grotz, V.L., Henry, R.R., McGill, J.B., Prince, M.J., Sharmoon, H., Trout, J.R. and Pi-Sunyer, F.X. (2003). Lack of effect of sucralose on glucose homeostasis in subjects with type 2 diabetes. *Journal of the American Diabetic Association* **103**(12), 1607-1612.

Gupta, A., Sethi, J., Sood, S., Dahiya, K., Singh, G. and Gupta, R. (2011). Evaluation of hypoglycemic and anti-atherogenic effect of aloe vera in diabetes mellitus. *Pharmacie globale* **2** (8), 1-4.

Hamman, J.H. (2008). Composition and application of *Aloe vera* leaf gel. *Molecules* 13, 1599-1616.

Hanwella, R. and Silva, V.D. (2010). Diabetes and impaired fasting glucose in Sri Lankan patients with schizophrenia. *Sri lankan journal of psychiatry* 1 (2), 2010.

He Q., Eshun K. and Tian Z. (2005). Quality and safety assurance in the process of *Aloe vera* gel juice. School of food science and technology. Southern Yangtze university. Wuxi. P.R. China. pp 1-12.

Helal, E.G.E., Hasan, M. H. A. Mustafa, A. M. and Al-Kamel, A. (2003). Effect of aloe vera extract on some physiological parameters in diabetic albino rats. *The Egyptian journal of hospital medicine* **12**, 53-61.

http://www.aloeforhorses.com/stories.htm vailable online 30th June at 12.30 pm.

http://www.aloequine.com/ available online 30th June 2012 at 12.06 pm.

Hu, Q., Hu, Y., Xu, J. (2005). Free radical-scavenging activity of *Aloe vera* (*Aloe barbadensis Miller*) extracts by supercritical carbon dioxide extraction. *Food Chemistry* **91**, 85-90.

Janasz, E.R., Wijeyaratne, R., Silva, V. and Ratnayake, D. (1995). Sri Lankan medicinal plants of economic value. Aloe. pp 5-11

Katulanda, P., Constantine, G.R., Mahesh, J.C., Sheriff, R., Senevirathne, R.D.A., Wijerathne, S., Wijesuriya, M., MacCarthy, M.I., Adler, A.I., Matthews, D.R. (2008). Prevalence and projections of diabetes and pre-diabetes of Sri Lanka- Sri Lanka diabetes, cardiovascular study (SLDCS). *Diabetic Medicine* **25**, 1062-1069.

Kim, K., Kim, H., Kwon, J., Lee, S., Kong, H., Im, S., Lee, Y.H., Lee, Y.R., Oh, S.T., Jo, T.H., Park, Y.I., Lee, C.K. and Kim, K. (2009). Hypoglycemic and hypolipidamic effects of processed *Aloe vera* gel in a mouse modal of non-insulin-dependent diabetes mellitus. *Phytomedicine* **16** (9), 856-863.

Krishnavent, S., Balasubramanium, T. and Sadasivam, S. (1984). Food Chemistry 15 p. 229.

Lachenmeier, K., Kuepper, U., Mussoff, F., Madea, B., Reusch, H. and Lachenmeier, D.W. (2005). Quality control of *Aloe vera* beverages. *Electric Journal of Environmental, Agricultural and Food Chemistry* **4** (4), 1033-1042.

Maiti, S. and Chandra, R. (2002). Cultivation of *Aloe vera*. National research centre for medicinal and aromatic plants. pp 1-6.

Martino, F., Martino, E., Morrone, F., Carnevali, E., Forcone, R. and Niglio, T. (2005). Effect of dietary supplementation of glucomannan on plasma total cholesterol and low density lipoprotein cholesterol in hypercholesterolemic children. *Nutrition, metabolism and cardiovascular diseases* 15 (3), 174-180.

Matsuda, Y., Yokahira, M., Suzuki, S., Hosokava, K., Yamakava, K., Zeng, Y., Ninomia, F., Saoo, K., Kuno, T. and Imaida, K. (2008). One-year chronic toxicity study of *Aloe babadensis* Miller var. natalensis Berger in wistar hannover rats. A pilot study. *Journal of Food Chemistry Toxicology* **46** (2), 733-739.

McAnalley and Bill, H. Process for preparation of aloe products. United States patent number 4,851,224, 1989.

Microbiological test methods. (1982). Sri Lanka standards 516: part 3. Sri Lanka standards institution. pp 4-14.

Microbiological test methods. (1991). Sri Lanka standards 516: Part 1. First revision. Sri Lanka standards institution. pp 4-9.

Mohamed, A.E., Abdel-Aziz, A.F., El-Sherbiny, E.M., Morsi, R.M. (2009). Anti-diabetic effect of *Aloe vera* juice and evaluation of thyroid function in female diabetic rats. *Bioscience research* 6 (1), 28-34.

Mohamed, A.E., Abdel-Aziz, A.F., El-Sherbiny, E.M., Morsi, R.M. (2009). Anti-diabetic effect of *Aloe vera* juice and evaluation of thyroid function in female diabetic rats. *Bioscience research* **6** (1), 28-34.

Mohamed, E.A.K. (2011). Antidiabetic, antihypercholestermic and antioxidative effect of *Aloe vera* gel extract on alloxan induced diabetic rats. *Australian journal of basic and applied sciences* **5** (11), 1321-1327.

Moore, Eric D., McAnalley and Bill, H. Drink containing mucilagenious polysaccharides and its preparation. United States patent number 5,443,830, 1995.

Mulik, M. and Phale, M. (2009). Extraction, purification and identification of aloe gel from *Aloe vera* (*Aloe barbadensis* Miller). 5(3). Divakar Chemicals ltd., 203-204, Sant Bhavan, Sharma Industrial Estate, Walbhat Road, Goregaon – East, Mumbai - 400 063, (INDIA).

Nassiff, H.A., Fajardo, F., Velez, F. (1993). Effecto del aloe sobre la hiperlipidemiaen pacientes refractariosa la dieta. *Rev Cuba Med Gen Integr* **9**, 43-51.

Ni, Y., Turner, D., Yates, K.M. and Tizard, I. (2004). Isolation and characterization of structural components of *Aloe vera* Miller. *Journal of food engineering* 75, 245-251.

Ni, Yawei, Yates and Kenneth, M. In-situ gel formation of pectin. United States patent number 20020119941, 2002.

Ni, Yawei-Yates, Kenneth, M., Zarzycki and Ryszard. Aloe pectins. United States patent number 5,929,051, 1999.

Okyar, A., Can, A., Akey, N., Buktir, G. and Sutlupinar, N. (2001). Effect of *Aloe vera* on blood glucose leves in type 1 and type 2 diabetes rat models. *Phytotherapy Research* **15** (2), 157-161.

Pecere, T., Gazzola, M.V., Mucignat, C., Parolin, C., Vecchia, F.D., Cavaggioni, A., Basso, G., Diaspro, A., Salvato, B., Carli, M. and Palu, G. (2000). Aloe-emodin is a new type of anti-cancer agent with selective activity against neuroectodermal tumors. *Cancer Research* **60**, 2800-2804.

Quinlan, M.F. and Jenner, M.R. (1990). Analysis and stability of the sweetener sucralose in beverages. *Journal of Food Science* **55**, 224.

Rajasekaran, S. and sathishekar, D. (2007). Therapeutic evaluation of aloe vera leaf gel extract on glycoprotein components in rats with streptozotocin diabetes. *Journal of pharmacology and toxicology* **2** (4), 380-385.

Rajasekaran, S., Ravi, K., Sivagnanam, K. and Subramannian, S. (2006). Beneficial effects of aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. *Clinical and Experimental Pharmacology and Physiology* **33**, 232-237.

Rajasekaran, S., Sivagnanam, K. and Subramanian, S. (2005). Antioxidant effect of *Aloe vera* gel extract in streptozotocin-induced diabetes in rats. *Pharmacol rep*. **57**(1), 90-96.

Ramachandra, C.T. and Rao, P.S. (2008). Processing of *Aloe vera* leaf gel. *American journal of Agricultural and Biological Science* **3** (2), 502-510.

Reynolds, T. and Dweck, A.C. (1999). *Aloe vera* gel: a review update. *Journal of ethno pharmacology* **68**, 3-37.

Rodriguez-Gonzalez, V.M., femenia, A., Gonzalez-Laredo. R.F., Rocha-Guzman, N.E., Gallegas-Infante, J.A., Candelas-Cadillo, M.G., Ramirez-Baca, P., Simal, S. and Rossello, C. (2011). Effects of pasteurization on bioactive polysaccharide acemannan and cell wall polymers from *Aloe barbadansis* Miller. *Carbohydrate Polymers* **88**(4), 1675-1683.

Romero, D.M., Alburquerque, N., Valverde, J.M., Guillen, F., Castillo, S., Valero, D. and Serrano, M. (2006). Post-harvest sweet cherry quality and safety maintenance by *Aloe vera* treatment: A new edible coating. *Journal of post-harvest biology and technology* **39** (1), 93-100.

Sax, L. (2010). Polyethylene terephthalate may yield endocrine disruptors. *Environmental health perspectives* **118** (4), 445-448.

Schasuss, A.J. (1990). *Aloe vera.* American institute for biosocial research. Available at http://www.desertharvest.com/physicians/documents/DH154.pdf. (March 05 2011 at 1.00 p.m.).

Serrano, Valverde, J.M., Guillen, F., Castillo, S., Martínez-Romero, D. and Valero, D. (2006). Use of *Aloe vera* gel coating preserves functional properties of table grapes. *Journal of agriculture food chemistry* **54** (11), 3882-3886.

Simkovic, I., Surina, I. and Vrican, M. (2003). Primary reactions of sucrose thermal degradation. *Journal of Analytical and Applied Pyrolysis* **70**(2), 493-504.

Tanaka, M., Yamada, M., Toida, T. and Iwatsuki, K. (2012). Safety evaluation of superficial carbon dioxide extract of *Aloe vera* gel. *Journal of food science* **77** (1), 2-9.
The whole *Aloe vera* leaf United States patent number 5,356,811, 1985.

Urch, D. (1999). *Aloe vera* Nature's gift. *Aloe vera* in veterinary practice. pp: 171-223.

Valverde, J. M., Valero, D., Martinez-Romero, D., Guillen, F., Castillo, S. and Serrano, M. (2005). Novel edible coating based on *Aloe vera* gel to maintain table grape quality and safety. *Journal of agricultural and food chemistry* **53**, 7807-7813.

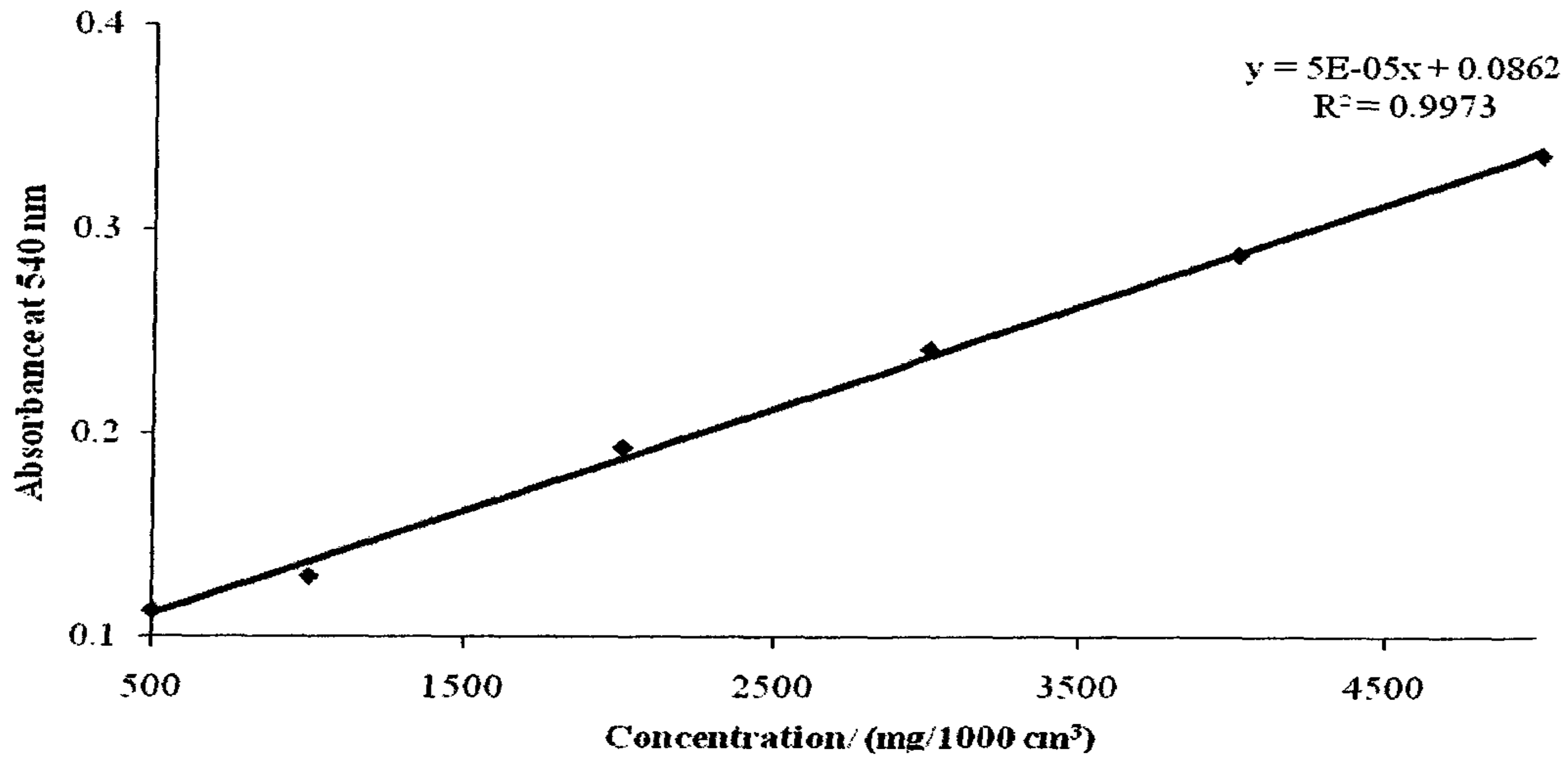
Vazquez, B., Avila, G., Segura, D. and Escalante, B. (1996). Anti-inflammatory activity of extracts from *Aloe vera* gel. *Journal of Ethno Pharmacology* **55**, 69-75.

Wiet, S.G. and Beyts, P.K. (1992). Sensory characteristics of sucralose and other high intensity sweeteners. *Journal of Food Science* **57** (4), 1014-1018.

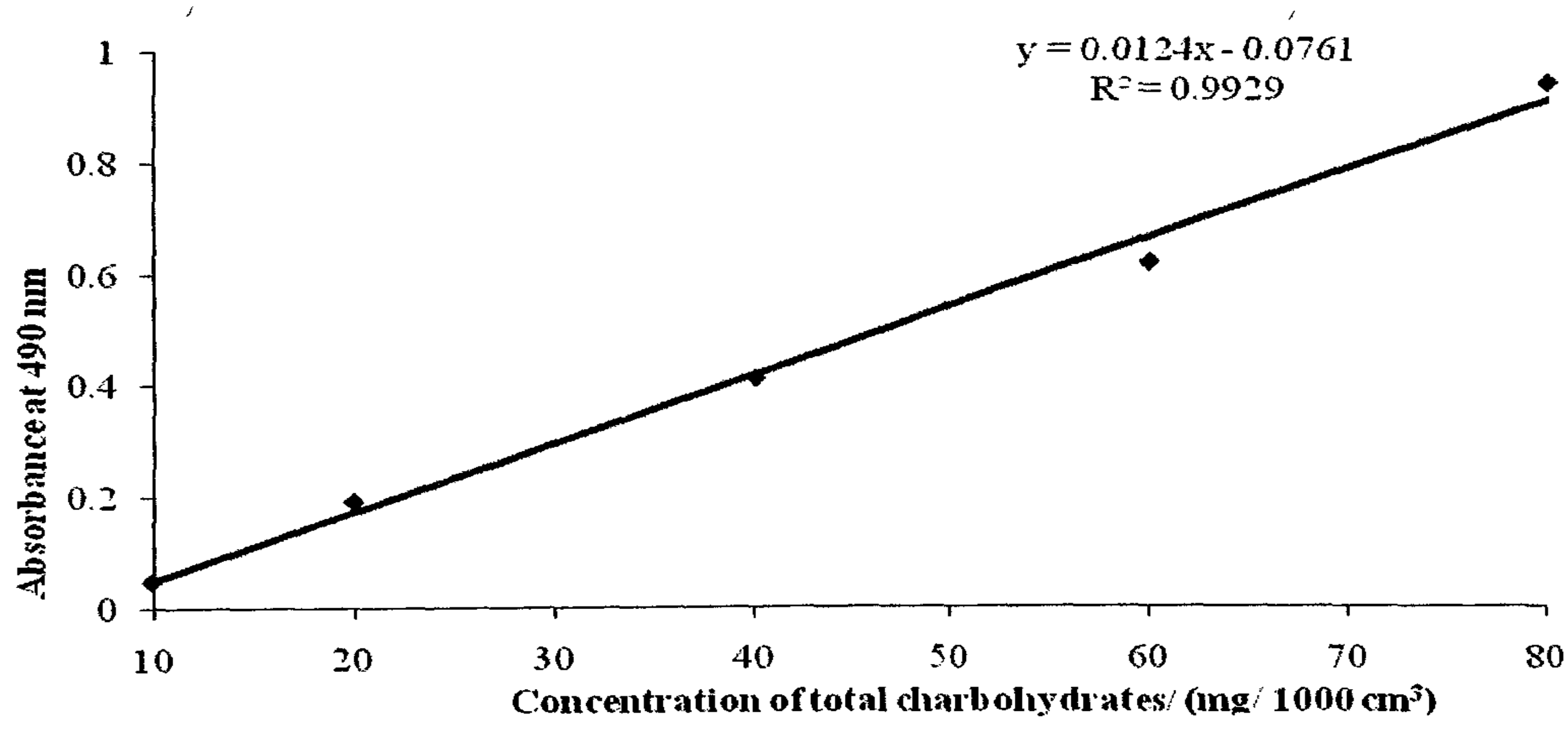
Wijesinghe, M.A.R.N., Navaratne, A.N. and Illeperuma, D.C.K. (2012). Development of a ready-to-serve beverage from *Aloe vera* (L.) *Burm. f.* with maximum elimination of aloin and minimum processing steps. *Proceedings of the Peradeniya University Research Session* **17**, 97.

Yagi, A., Hegazy, S., Kabbash, A. and Wahab, E.A. (2009). Possible hypoglycemic effect of Aloe vera L. high molecular weight fraction on the type 2 diabetes mellitus patients. *Saudi pharmaceutical journal* 17(3), 209-215.

Yongchaiyudha, S., Rungpitarangsi, V., Buyapraphatsara, N. and Chokechaijaroenporn, O. (1996). Anti-diabetic activity of *Aloe vera* L. juice clinical trial in new cases of diabetes mellitus. *Phytomedicine* 3, 241-243.



Calibration curve for acemannan



Calibration curve for total carbohydrates

Questioner for sweetness and overall taste of the *Aloe vera* ready-to-serve beverage

Name: ....

Date: ....

You have been given four samples of a beverage. Rank them for preference for their sweetness and overall taste. Assign the most preferred sample a rank value of 1; the samples with the next prefer rank values of 2 & 3 and the sample with the least preferred a rank value of 4. No ties allowed.

Code	Rank assigned	
	Sweetness	Overall taste
....	....	....
....	....	....
....	....	....
....	....	....

Comments...

Questioner for sensory attributes of the aloe beverage with three different concentrations of sucralose

Name ....

Date ....

You have been given three samples of beverages. Indicate how much you like or dislike each sample for aroma and overall acceptability by checking the appropriate word phrase under the sample code number for each sensory attribute.

Overall flavor			**After taste**		
Code.......	**Code.......**	**Code.......**	**Code.......**	**Code.......**	**Code.......**
–Like Extremely	–Like Extremely	–Like Extremely	–Like Extremely	–Like Extremely	–Like Extremely
–Like Very Much	–Like Very Much	–Like Very Much	–Like Very Much	–Like Very Much	–Like Very Much
–Like Moderately	–Like Moderately	–Like Moderately	–Like Moderately	–Like Moderately	–Like Moderately
–Like Slightly	–Like Slightly	–Like Slightly	–Like Slightly	–Like Slightly	–Like Slightly
–Neither Like Nor Dislike	–Neither Like Nor Dislike	–Neither Like Nor Dislike	–Neither Like Nor Dislike	–Neither Like Nor Dislike	–Neither Like Nor Dislike
–Dislike Slightly	–Dislike Slightly	–Dislike Slightly	–Dislike Slightly	–Dislike Slightly	–Dislike Slightly
–Dislike Moderately	–Dislike Moderately	–Dislike Moderately	–Dislike Moderately	–Dislike Moderately	–Dislike Moderately
–Dislike Very Much	–Dislike Very Much	–Dislike Very Much	–Dislike Very Much	–Dislike Very Much	–Dislike Very Much
–Dislike Extremely	–Dislike Extremely	–Dislike Extremely	–Dislike Extremely	–Dislike Extremely	–Dislike Extremely
Comments	Comments	Comments	Comments	Comments	Comments

Questioner for sensory attributes of the final product

Name ....

Date ....

You have been given a sample of a beverage. Indicate how much you like or dislike the sample for aroma, overall appearance, mouthfeel, overall flavor and overall acceptability by checking the appropriate word phrase under each sensory attribute.

Aroma	**Overall appearance**	**Mouthfeel**	**Overall flavor**	**Overall acceptability**
-Like Extremely	-Like Extremely	-Like Extremely	-Like Extremely	-Like Extremely
-Like Very Much	-Like Very Much	-Like Very Much	-Like Very Much	-Like Very Much
-Like Moderately	-Like Moderately	-Like Moderately	-Like Moderately	-Like Moderately
-Like Slightly	-Like Slightly	-Like Slightly	-Like Slightly	-Like Slightly
-Neither Like Nor Dislike	-Neither Like Nor Dislike	-Neither Like Nor Dislike	-Neither Like Nor Dislike	-Neither Like Nor Dislike
-Dislike Slightly	-Dislike Slightly	-Dislike Slightly	-Dislike Slightly	-Dislike Slightly
-Dislike Moderately	-Dislike Moderately	-Dislike Moderately	-Dislike Moderately	-Dislike Moderately
-Dislike Very Much	-Dislike Very Much	-Dislike Very Much	-Dislike Very Much	-Dislike Very Much
-Dislike Extremely	-Dislike Extremely	-Dislike Extremely	-Dislike Extremely	-Dislike Extremely
Comments	Comments	Comments	Comments	Comments