THE EFFECT OF THERMAL TREATMENT ON QUALITY ATTRIBUTES OF POMEGRANATE (*Punica granatum* L.) JUICE

A THESIS SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF
MASTER OF TECHNOLOGY
IN
FOOD TECHNOLOGY AND BIOCHEMICAL ENGINEERING
2014-16

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I hereby declare that the thesis contains survey and original research work by the undersigned candidate, as part of her Master of Technology in Food Technology and Biochemical Engineering studies.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

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I hereby recommend the thesis entitled, **"The Effect of Thermal Treatment on Quality Attributes of Pomegranate (*Punica granatum* L.) Juice"** prepared by Prama Pal, (M4FTB1605) student of M.tech 2nd year, under the guidance and supervision of Dr. Uma Ghosh has been evaluated thus found satisfactory. It is therefore accepted and submitted as a dissertation for the partial fulfilment for the requirement for awarding the degree of Masters of Technology in Food Technology and Biochemical Engineering.

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This is to certify that Miss Prama Pal (M4FTB1605) has carried out her research work entitled **"The Effect of Thermal Treatment on Quality Attributes of Pomegranate (*Punica granatum* L.) Juice"** under the direct supervision of Dr. Uma Ghosh, at Department of Food Technology and Biochemical Engineering, Jadavpur University. We are satisfied that she has carried out this work independently with proper care and confidence. We hereby recommend that this dissertation be accepted in the partial fulfilment of the requirements of the degree of Maters of Technology in Food Technology and Biochemical Engineering.

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Acknowledgement

This Thesis entitled **"The Effect of Thermal Treatment on Quality Attributes of Pomegranate (*Punica granatum* L.) Juice"** is by far the most significant scientific accomplishment in my life and it would be impossible without people who supported me and believed in me.

To begin with, I express my deepest regards, unbound gratitude with sincerest thanks to my guide Dr. Uma Ghosh, Dept. of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata, without her efficient and untiring guidance, my work on this practical would have remained incomplete. She has been very kind and affectionate and allowed me to exercise thoughtful and intelligent freedom to proceed with this project work and finally produce this thesis.

I would like to thank Prof. Runu Chakraborty (HOD, Dept of Food Technology and Biochemical Engineering) for extending his constant encouragements and guidance which helped me to do the work and complete it within schedule time.

I am also thankful to research scholars and my lab mates, Ranu Paul, Ipsita Banerjee, Modhuleena Mandal, Tapasi Polley, Priyanka Ghosh, Atreyi Sarkar, Soumik Banerjee for their valuable help and cooperation.

I am also thankful to library staffs, laboratory staffs, all Research scholars, non-teaching staff and sub-staff of this department for their cooperation.

I must not forget to express my deep sense of gratitude to my family, for being very supportive over the years.

Contents

Abstract

Pomegranate (*Punica granatum* L.) is one of the most important fruit crops in India. Pomegranate juice has recently received great attention for their health benefits, mainly due to their high polyphenol content and the related antioxidant capacity. Due to their antioxidant properties, phenolic compounds including anthocyanins are thought to have preventive roles in a number of chronic diseases such as cardiovascular disease and cancers. An attractive red colour is the most important quality criteria for fruit juices containing anthocyanin, including pomegranate juice. The primary colour deterioration in fruit juices containing anthocyanins occurs as a result of the degradation of monomeric anthocyanins, polymerisation of anthocyanins and the subsequent formation of brown colour. These colour changes strongly affect consumer behaviour and result in a loss of marketability of processed pomegranate products. The loss in antioxidant property, total phenolic content and also total monomeric anthocyanin of pomegranate juice produced from arils were investigated over the temperature range of 65-90^{o}C. Heat treatment for 60 minutes at 65 ^{o}C resulted in 12.82%, 9% and 2.1% losses in monomeric anthocyanin, phenolic content and antioxidant content respectively which was the least as compared to other temperatures. Further the degradation kinetic studies for monomeric anthocyanin, total phenolic content and total antioxidant content in pomegranate juice were observed during thermal treatment at 65-90^{o}C. The present data shows that degradation of thses compounds follows first order reaction kinetics. This was also being extrapolated in Arrhenius equation for which activation energy was obtained. The computed values of the activation energies were 52.39 kJ/mol for anthocyanin degradation, 26.27 kJ/mol for the antioxidant degradation and 26.32 kJ/mol for the total phenol degradation.

1. Introduction

The pomegranate (*Punica granatum* L.) is an ancient fruit; it has been widely consumed in various cultures for thousands of years. The use of pomegranate fruit dates back to Biblical times and reports of its therapeutic qualities have echoed throughout the millennia. (1)

The present scientific name of pomegranate, Punica granatum, is derived from the name Pomum (apple) granatus (grainy), or seeded apple.(2) Pomegranate belongs to the family Punicaceae. It is native from the area of Iran to the Himalayas in northern India, and has been cultivated and naturalized over the entire Mediterranean region since ancient times. Pomegranate is widely cultivated throughout Iran, India, Mediterranean countries, and the drier parts of Southeast Asia, Malaysia, the East Indies, and tropical Africa and, to some extent, in the United States (drier parts of California and Arizona), China, Japan, and Russia.(3)

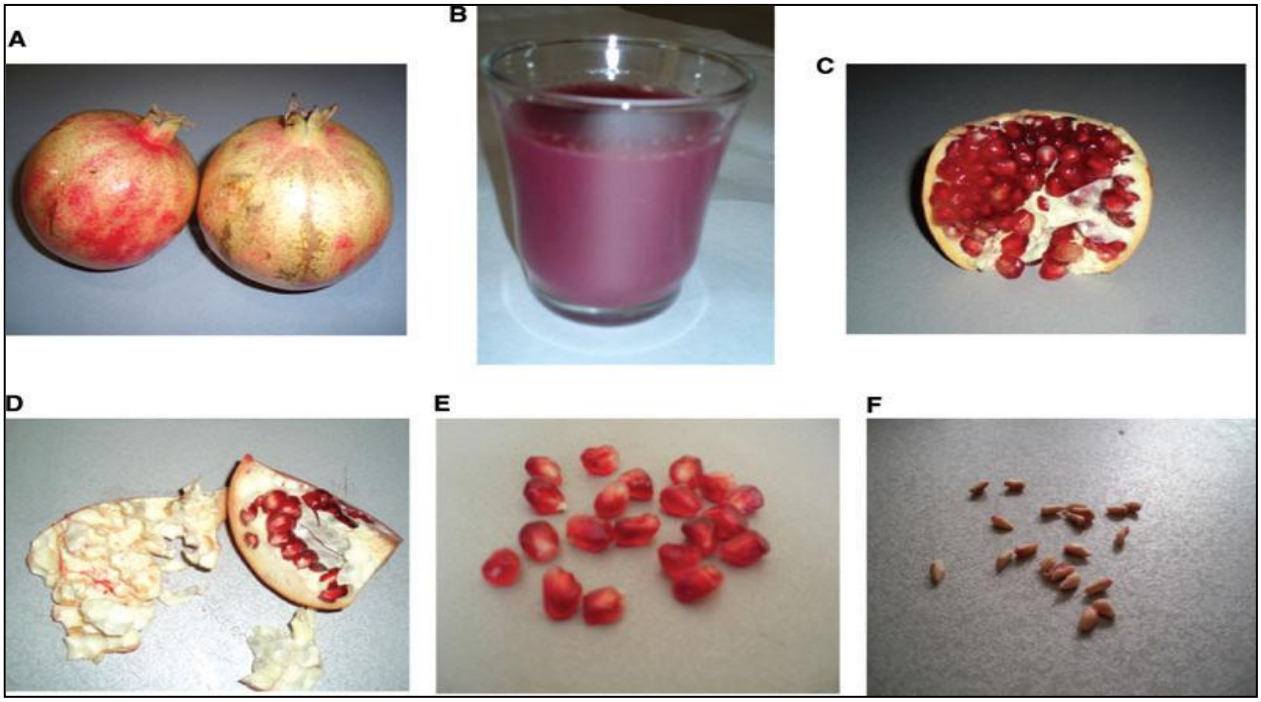
India is one of the largest producers of pomegranate in the world. There is no exact data available on area and production in the world due to the rapid increase in the production and expansion, although it is estimated that around 1.5 million tonnes of pomegranate fruits are produced in the world annually.(4) In terms of productivity, Spain ranks first (18.5 t/ha) followed by the USA (18.3 t/ha) while Iran ranks first for exports (60,000 t/year) followed by India (35,176 t). In India, during 2013-14, pomegranate was cultivated over 1.31 lakh ha with an annual production of 13.46 lakh tonnes and productivity of 10.27 tonnes/ ha.(5) At present, Maharashtra is the leading state in acreage covering about 68.7 per cent of the area under pomegranate. Similarly around 70.2 per cent of total production comes from Maharashtra. The other important states next to Maharashtra with respect to pomegranate cultivation are Karnataka, Gujarat and Andhra Pradesh. (6)

Pomegranate is an economically important plant and has been used by mankind since the dawn of civilization. There is a vast amount of evidence that reveals the multifaceted use of pomegranate in different cultures and mythology. Besides using pomegranate as raw fruit, it has been used as herbal healer since ancient times. Pomegranate has found a place as the

most important medicine even in ancient literature by Hippocrates, Pliny, Soranus and Dioscorides. Due to its immense potential for health benefits, pomegranate has achieved the title of "superfood". (7) The edible parts of pomegranate fruits are consumed fresh or used for the preparation of fresh juice, canned beverages, jelly, jam, and paste and also for flavoring and coloring beverage products. In addition, it is widely used in therapeutic formulas, cosmetics, and food seasonings.(8) There has been a virtual explosion of interest in the pomegranate as a medicinal and nutritional product because of its multifunctionality and its great benefit in the human diet as it contains several groups of substances that are useful in disease risk reduction. As a result, the field of pomegranate research has experienced tremendous growth.

1.1 Chemical Composition of Pomegranates:

The pomegranate fruit has valuable compounds in different parts of the fruit. These can be divided into several anatomical origins: peel, seeds, and arils. Another important product obtained from pomegranate fruit is the juice that can be obtained from arils or from whole fruit.



Different parts of the pomegranate fruit (A). B: pomegranate juice; C: section of pomegranate; D: pomegranate peel; E: pomegranate arils; F: pomegranate seeds.(1)

The chemical composition of the fruits differs depending on the cultivar, growing region, climate, maturity, cultivation practice, and storage conditions.(9) Significant variations in organic acids, phenolic compounds, sugars, water-soluble vitamins, and minerals of pomegranates have been reported over the years by various.(10) About 50% of the total fruit weight corresponds to the peel, which is an important source of bioactive compounds such as phenolics, flavonoids, ellagitannins (ETs), and proanthocyanidin compounds, minerals, mainly potassium, nitrogen, calcium, phosphorus, magnesium, and sodium, and complex polysaccharides. The edible part of the pomegranate fruit (50%) consists of 40% arils and 10% seeds. Arils contain 85% water, 10% total sugars, mainly fructose and glucose, and 1.5% pectin, organic acid such as ascorbic acid, citric acid, and malic acid, and bioactive compounds such as phenolics and flavonoids, principally anthocyanins.(11) The seeds are a rich source of total lipids; pomegranate seed oil comprises 12% to 20% of total seed weight. The oil is characterized by a high content of polyunsaturated (n-3) fatty acids such as linolenic, linoleic, and other lipids such as punicic acid, oleic acid, stearic acid, and palmitic acid. The seeds also contain protein, crude fibers, vitamins, minerals, pectin, sugars, polyphenols, isoflavones (mainly genistein), and the phytoestrogen coumestrol.(12) Nowadays, it is widely accepted that the beneficial health effects of fruits and vegetables in the prevention of disease are due to the bioactive compounds they contain. The presence of significant amounts of bioactive compounds, such as phenolic acids, flavonoids, and tannins in pomegranate fruits assures them considerable nutritional value.(13)

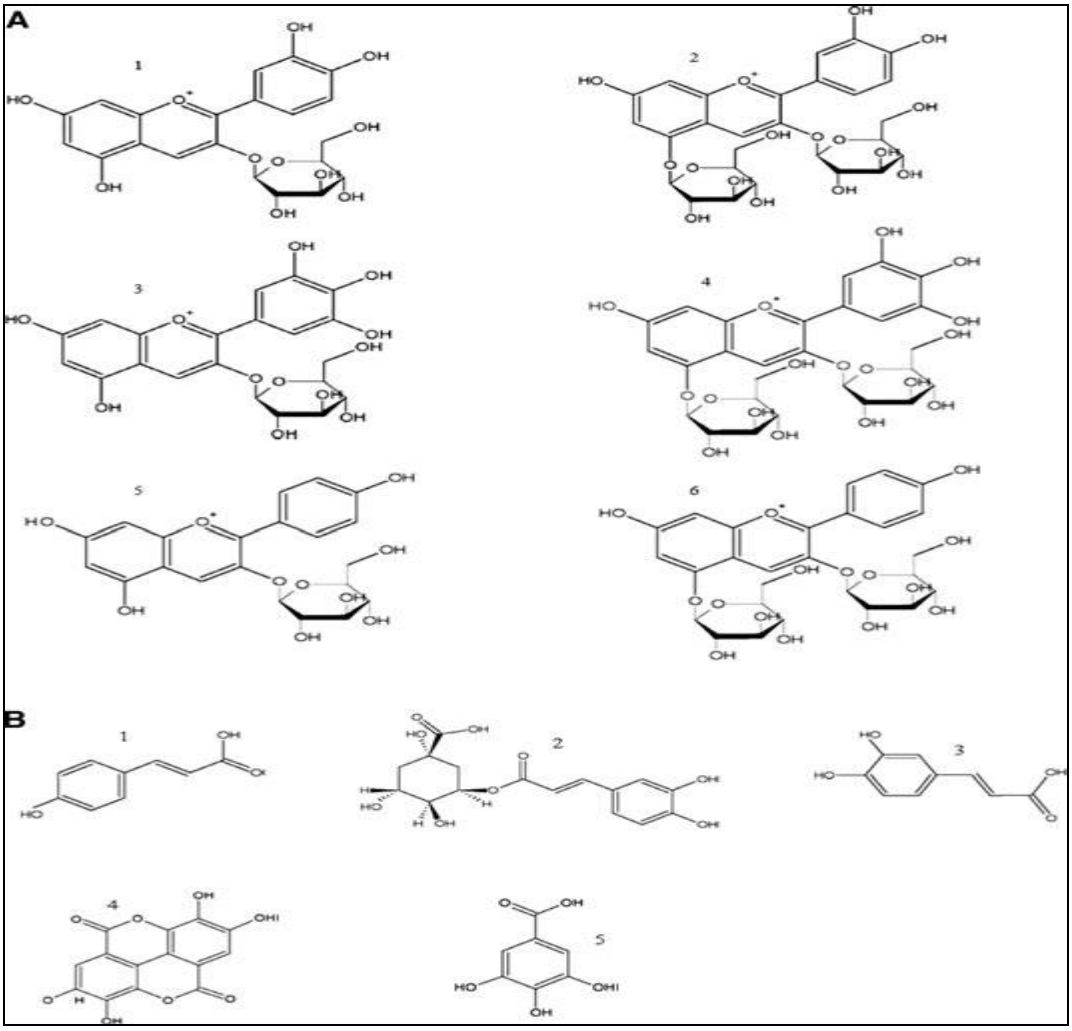
Constituent	
Moisture	72.6-86.4%
Protein	0.05-1.6%
Fat	0.01-0.9%
Mineral elements	0.36-0.73%
Fibre	3.4-5.0%
Carbohydrates	15.4-19.6%
Calcium	3.0-12.0 mg
Phosphorus	8.0-37.0 mg
Iron	0.3-1.2 mg
Sodium	3.0 mg
Magnesium	9.0 mg
Ascorbic acid (Vitamin C)	4.0-14.0 mg
Thiamine (Vitamin B_1)	0.01 mg
Riboflavine (Vitamin B_2)	0.012-0.03 mg
Niacine	0.18-0.3 mg

*Values per 100 g of edible portions

Chemical Composition of Pomegranate(1)

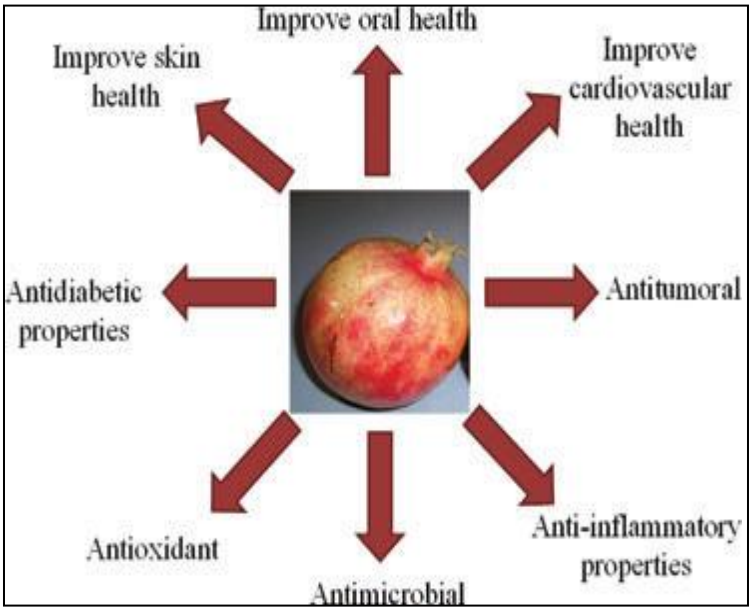
1.2 Phenolic compounds

Pomegranate juice is primarily considered an important source of phenolic compounds. Natural polyphenols can range from simple molecules (phenolic acids, phenylpropanoids, flavonoids) to highly polymerized compounds (lignins, melanins, tannins), with flavonoids representing the most common and widely distributed subgroup. Anthocyanins are the largest and most important group of flavonoids present in pomegranate arils, which are used to obtain the juice. These pigments give the fruit and juice its red color. (14) There is a great variety of anthocyanins present in pomegranate juice, principally cyanidin-3-*O*-glucoside, cyanidin-3,5-di-*O*-glucoside, delphinidin-3-*O*-glucoside, delphinidin-3,5-di-*O*glucoside, pelargonidin-3-*O*-glucoside, and pelargonidin-3,5-di-*O*-glucoside.(15) The main differences between them are the number of hydroxylated groups, the nature and the number of bonded sugars to their structure, the aliphatic or aromatic carboxylates bonded to the sugar in the molecule, and the position of these bonds. The phenolic acids present in pomegranate juice can be divided into 2 groups: (1)hydroxybenzoic acids, mainly gallic acid and ellagic acid and (2) hydroxycinnamic acids, principally caffeic acid, chlorogenic acid, and *p*-coumaric acid.(16) The soluble polyphenols content varies from 0.2 to 1.0 g/100 g, being anthocyanins one of the most important together with lignans, gallagyl-type tannins, ellagic acid derivatives, and other hydrolysable tannins; mainly punicalin, pedunculagin, and punicalagin. They differ from proanthocyanidins in their chemical structures.(17)



(A)Principal anthocyanins present in pomegranate juice. 1: cyanidin-3-*O*-glucoside; 2: cyanidin-3,5-di-*O*-glucoside; 3: delphinidin-3-*O*glucoside; 4: delphinidin-3,5-di-*O*-glucoside; 5: pelargonidin-3-*O*-glucoside; 6: pelargonidin-3,5-di-*O*-glucoside. (B) Principal phenolic acids present in pomegranate juice: 1: *p*-coumaric acid; 2: chlorogenic acid; 3: caffeic acid; 4: EA; 5: gallic acid.(1)

1.3 Functional Properties of pomegranate fruit



Principal functional and medicinal effects of pomegranate(1)

1.3.1 Antioxidant Properties

The determination of the antioxidant capacity of pomegranate components and their derivatives is being given greater importance by researchers and those involved in the agro-food industry for use as natural additives to replace synthetic antioxidants, whose use is increasingly restricted due to the secondary effects they may produce. All these activities may be related to the diverse phenolic compounds present in pomegranate, including punicalagin isomers, Ellagic Acid derivatives, and anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides, and 3,5-diglucosides). These compounds are known for their properties to scavenge free radicals and chelate metal cations and to inhibit lipid oxidation.(18) Punicalagin originating from the peels is one of the major phytochemicals contributing to the total antioxidant capacity of pomegranate juice.(19) The antioxidant and sensory qualities of pomegranates depend on several factors, such as cultivar and climatic conditions during fruit maturation and ripening and the part of the fruit used. Pomegranate juice possessed a 3-fold higher antioxidant activity than that of red wine or green tea, and 2-, 6-, and 8-fold higher levels than those detected in grape/cranberry, grapefruit, and orange juices, respectively.(20)

1.3.2 Cardiovascular Health

Oxidation of low-density lipoprotein (LDL) is thought to contribute to atherosclerosis and cardiovascular disease. *In vitro*, animal, and human trials have examined the effects of various pomegranate constituents on the prevention and attenuation of atherosclerosis and LDL oxidation.(10) The principal mechanisms of action of pomegranate juice is antiatherogenic and may include the following: increased serum antioxidant capacity, decreased plasma lipids and lipid peroxidation, decreased oxidized- LDL uptake by macrophages, decreased intima media thickness, decreased atherosclerotic lesion areas, enhanced biological actions of nitric oxide, decreased inflammation, decreased angiotensin converting enzyme activity, and decreased systolic blood pressure, thereby causing an overall favorable effect on the progression of atherosclerosis and the subsequent potential development of coronary heart disease.(21) High blood pressure or hypertension is one of the most prevalent cardiovascular risk factors and the single greatest contributor to cardiovascular disease worldwide. Aviram and others (2004) reported that, after 1 y of pomegranate juice consumption, systolic blood pressure was reduced by 21%, an effect the researchers believed to be related to the particularly potent antioxidant properties of pomegranate polyphenols.

1.3.3 Protection against cancer

Several studies have since been conducted to evaluate the efficacy of pomegranate fruit and derivates endowed with a very high antioxidant activity as an anti-proliferative, anti-invasive, and pro-apoptotic agent in various cancer cell lines.

1.3.4 Prostatic cancer. A phase II clinical trial was performed by Pantuck et al. University of California (US), and recruited a total of 48 patients that undergone to surgery or radiotherapy, having a rising Prostate Specific Antigen (PSA), a marker of prostate cell disorder, Men whose PSA levels double in a short period of time are at increased risk of death from prostate cancer. The patients were treated over a period of a year with pomegranate juice daily, and the results showed that pomegranate significantly slowed the PSA rate of growth, compared to historical values of other patients.(22)

1.3.5 Breast cancer. Adams et al. from the University of California, have shown that urolithins (ellagic acid metabolites) were active in inhibiting breast cancer cell proliferation, testosterone dependent. Besides, Punic acid, an omega-5 fatty acid of aril seeds, demonstrated similar inhibition of cell proliferation in different breast cancer cell lines.(23)

1.3.6 Antidiabetic Properties

One of the ways to control diabetes mellitus is through the diet and it is here that pomegranate fruits and derivates can play a part due to the hypoglycemic activity of flowers, seeds, and juice of pomegranate through different mechanisms, including the inhibition of glucose absorption in the gut or of its uptake by peripheral tissues. Pomegranate compounds associated with antidiabetic effects include oleanolic, ursolic, and gallic acids.(24) Li and others (2005) suggest that pomegranate flower extract improves postprandial hyperglycemia in type 2 diabetes and obesity, at least in part, by inhibiting intestinal α-glucosidase activity.(25)

1.3.7 **Improving Skin Health**

Prolonged exposure to ultraviolet (UV) radiation has been identified as a cause of serious adverse effects to human skin, including oxidative stress, premature skin aging, sunburn, immune-suppression, and skin cancer. Pomegranate juice can protect against UVA mediated cellular damage that occurs primarily through the release of reactive oxygen species and is responsible for immune-suppression, photo-dermatoses, photo-aging, and photo-carcinogenesis due to its extract is an effective agent for ameliorating UVA-mediated damages by modulating cellular pathways and merits further evaluation as a photo-chemopreventive agent. The protective effects of pomegranate polyphenolics against UVA- and UVB-induced cell death of human skin fibroblasts may be attributed to reduced generation of intracellular ROS(Reactive oxygen species) and increased intracellular antioxidant capacity. (26)

1.3.8 Improving Oral Health

Pomegranate contains agents, especially polyphenolic flavonoids, which exert actions that could be considered conducive to good oral health, particularly in relation to gingivitis

development.(27) The antioxidant activity of polyphenols explain their preventive effect against diseases of the oral cavity, where polyphenols come into direct contact with tissues before being absorbed and metabolized and are activated into aglycones by human and bacterial enzymes. Mouth-rinsing with pomegranate extracts lowered saliva activities of aspartate aminotransferase, an indicator of cell injury that shows high values with periodontal disease. Additionally, rinsing the mouth for 1 min with a mouthwash containing pomegranate extract effectively reduced the amount of microorganisms cultured from dental plaque.(28)

1.3.9. **Antimicrobial Properties**

The antimicrobial activity of some of the common pomegranate cultivars has been widely studied. Several *in vitro* assays demonstrate its bactericidal activity against several highly pathogenic and sometimes antibiotic-resistant organisms.(29) Pomegranate extracts inhibit or delay *Staphylococcus aureus* growth and subsequent enterotoxin production at 0.01%, 0.05%, and 1% v/v concentrations. At a low extract concentration (0.01% v/v), bacterial growth was delayed, and at a higher concentration (1% v/v), such growth was eliminated. At a concentration of 0.05% (v/v) of extract, *Staphylococcus* enterotoxin production was inhibited. In general, the extent of the inhibitory effects of the pomegranate extracts could be attributed to the phenolic, anthocyanin, and tannin contents of fruits.(30)

1.4 Fruit Juice processing

Although India is the largest producer of fruits in the world, the production per capital is only about 100 g per day. However, it is estimated that more than 20–22% of the total production of fruits is lost due to spoilage at various postharvest stages. Thus, the per capita availability of fruits is further reduced to around 80 g per day, which is almost half the requirement for a balance diet. Also it is estimated that around 20–25% of total vegetables are lost due to poor postharvesting practices. Less than 2% of the total vegetables produced in the country are commercially processed as compared to 70% in Brazil and 65% in USA.Juice formation of fruits is a one of the way to preserve fruits. Traditionally, the shelflife stability of juices has been achieved by thermal processing. Low temperature long time (LTLT) and high temperature short time (HTST) treatments are the most commonly used techniques for juice pasteurization. However, thermal pasteurization tends to reduce the product quality and

freshness. Therefore, some non-thermal pasteurization methods have been proposed during the last couple of decades, including high hydrostatic pressure (HHP), high pressure homogenization (HPH), pulsed electric field (PEF), and ultrasound (US). These emerging techniques seem to have the potential to provide “fresh-like” and safe fruit juices with prolonged shelf-life. Apart from thermal pasteurization, some chemical preservatives are also widely used for the extension of the shelf-life of fruit juices and beverages.

1.4.1 Traditional thermal pasteurization

Thermal processing is the most widely used technology for pasteurization of fruit juices and beverages. Juice pasteurization is based on a 5-logreduction of the most resistant microorganisms of public health significance (USFDA 2001). The process could be accomplished by different time-temperature combinations.(31)

1.4.1.1 Low temperature long time (LTLT). Fruit juice has been traditionally pasteurized by batch heating at 63-65°C for relatively long time (D'Amico et al. 2006).(32) This method has been replaced by high temperature short time treatment due to the undesirable quality changes during this process.

1.4.1.2 High temperature short time (HTST).HTST treatment could minimise those undesirable quality changes made by batch heating due to the much less duration of heat treatment. Currently, HTST pasteurization is the most commonly used method for heat treatment of fruit juice. (33)

1.4.2 Physical methods (Non-thermal pasteurization)

Some non-thermal pasteurization methods have been proposed during the last couple of decades, including high hydrostatic pressure (HHP), high pressure homogenization (HPH), pulsed electric field (PEF), and ultrasound (US). These emerging techniques seem to have the potential to provide“fresh-like”and safefruit juices with prolonged shelf-life.

1.4.2.1 High hydrostatic pressure (HHP)

Generally, there are two principlesthat govern the behaviour of foods under pressure: the Le Chatelier-Braun principle and the Isostatic principle. The Le Chatelier-Braun principle indicates that any phenomenon (such as phase transition, change in molecular configuration, chemical reaction, etc.) accompanied by a decrease in volume is enhanced by the increase in applied pressure. The isostatic principle means that the distribution of pressure into the sample is uniform and instantaneous. Thus, the process time is independent of sample size and shape.(34) Compared with thermal processing, HHP has many advantages. It can provide safe product with reduced processing time. It can maintain maximum fresh-like flavor and taste in the product due to the lower processing temperatures. Moreover, it is environmentally friendly since it requires only electrical energy and no waste by-products generated.(35)

1.4.2.2 Pulsed electric field (PEF)

The principlesof PEF processing have been explained by several theories including the trans-membrane potential theory, electromechanical compression theory and the osmotic imbalance theory. One of the most accepted theories is associated with the electroporation of cell membranes. It is generally believed that electric fields induce structural changes in the membranes of microbial cells based on generation of pores of the cell membrane, leading consequently to microbial destruction and inactivation.(36)

Compared with thermal processing, PEF processing has many advantages. It can preserve the original sensory and nutritional characteristics of foods due to the very short processing time and low processing temperatures. Energy savings for PEF processing are also important compared with conventional thermal processing. Moreover, it is environmentally friendly with no waste generated.

1.4.2.3 Ultrasound (US)

The principle of ultrasonic processing could be explained as follows: Firstly, the ultrasonic transducers convert electrical energy to sound energy. Secondly, when the ultrasonic waves propagate in liquid, small bubbles will be formed and collapsed thousands of times per second. This rapid collapse of the bubbles (cavitation) results in high localized temperatures and pressure, causing breakdown of cell walls, disruption of cell membranes and damage of DNA (37,38)

1.4.2.4 High pressure homogenization (HPH)

High pressure homogenization (HPH) is considered to be one of the most promising nonthermal technologies proposed for preservation of fruit juice and beverages. The primary mechanisms of HPH has been identified as a combination of spatial pressure and velocity gradients, turbulence, impingement, cavitation and viscous shear, which leads to the microbial cell disruption and food constituent modification during the HPH process. HPH has shown its ability to increase the safety and shelf-life of fruit juices including orange juice (Lacroix et al. 2005; Tahiri et al. 2006; Welti-Chanes et al. 2009)(39,40), apple juice (Kumar et al. 2009; Pathanibul et al. 2009)(41) and apricot juice (Patrignani et al. 2009)(42)

1.4.2.5 Membrane filtration

Ultrafiltration (UF) and microfiltration (MF) are the most commonly used membrane filtration techniques for fruit juice processing. They have been used commercially for the clarification of fruit juices.

1.4.3 Chemical methods (natural antimicrobials)

Apart from physical methods, some chemical preservatives are widely used for the shelf-life extension of fruit juices and beverages. The most commonly used preservatives are potassium sorbate and sodium benzoate. However, consumer demand for natural origin, safe and environmental friendly food preservatives is increasing. Natural antimicrobials such as bacteriocins, lactoperoxidase, herb leaves and oils, spices, chitozan and organic acids have shown feasibility for use in some food products. Some of them have been considered as Generally Recognized As Safe (GRAS) additives in foods.(43)

1.4.3.1 Bacteriocins

Bacteriocins are series of antimicrobial peptides which are readily degraded by proteolytic enzymes in the human body. Among them, nisin is the most commonly used food preservative and the GRAS additives permitted by the Food Additive Status List . Apart from dairy, it has been used to preserve fruit and vegetable juices.(44)

1.4.3.2 Lactoperoxidase

Lactoperoxidase is an enzyme that is widely distributed in colostrum, raw milk and other body liquid. It is an oxidoreductase and catalyses the oxidation of thiocyanate with the consumption of H2O2, to produce intermediate products with antibacterial properties (Corbo et al. 2009).(45) These products have been indicated to be bactericidal for some spoilage and pathogenic microorganisms and yeasts. Not much information had beenfound on the application of lactoperoxidase in fruit juices. Until recently, it was used for the preservation of tomato juice and mango fruits.(46)

1.4.3.3 Herb, spice and flavor oils

Some herbs and spices contain essential oils, which are natural antimicrobials. The main elements of these antimicrobials are phenolic compounds, including caffeic, cinnamic, ferulic and gallic acids, oleuropein, thymol and eugenol .

1.4.3.4 Chitosan

Chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin [poly-β-(1 →4)-N-acetyl-D-glucosamine]. It is widely produced from crab, shrimp and crawfish, with different deacetylation grades and molecular weights which contribute to different functionalities.(47)

1.4.4 Combination of physical and chemical methods

It is proved that some individual non-thermal methods as well as natural antimicrobials are effective in inactivating microorganisms and atthe same time do not adversely affect the sensory and nutritional quality of the fruit juice and other products. Moreover, the combination of these techniques could provide synergistic effects on prolonging the fruit juice shelf-life and potentially as replacement for traditional pasteurization methods.

1.5 Quality issues

Quality can be regarded as a measure of the suitability of a fruit juice, fruit juice concentrate or fruit juice extract for an intended application. In general, what-ever the application, it will be the consistency in performance of the product, from batch to batch and season to season, that is the prime concern. In order to meet quality targets, therefore, it becomes critical that processing is carried out in the correct manner using fruit of an optimum level of maturity, and that the product is stored under suitable conditions to limit effects of degradation during a required shelf life.

An attractive red colour is the most important quality criteria for fruit juices containing anthocyanin, including Pomegranate juice. Anthocyanins are also responsible for the orange, red and blue colours of many fruits and vegetables. Unfortunately, anthocyanins are unstable and susceptible to degradation, leading to a brownish colour during processing and storage. The primary colour deterioration in fruit juices containing anthocyanins occurs as a result of the degradation of monomeric anthocyanins, polymerisation of anthocyanins and the subsequent formation of brown colour.(48) These colour changes strongly affect consumer behaviour and result in a loss of marketability of processed pomegranate products.

Various factors affect the stability of anthocyanins, including the temperature of processing and storage, the chemical nature of anthocyanins(acylation or glucosylation), pH, ascorbic acid, hydrogen peroxide, sugars, light and metals. The other important step in processing pomegranates is the heating of fruit juices at various stages. Heating right after pressing inhibits native polyphenol oxidase (PPO) enzymes that cause brown colour formation by oxidising polyphenols.(49) PPO also degrades monomeric anthocyanins indirectly by forming O-quinones from polyphenols during enzymatic browning that react with and degrade monomeric anthocyanins. Pasteurisation is the process of heating before packaging to inactivate pathogenic or spoilage microorganisms. Heat applied during juice processing causes the degradation of monomeric anthocyanins, polymerization of anthocyanins, and the formation of brown colour in red fruit products.(48)

2. Review of Literature

This chapter deals with the background and review of literary information required to explain the work and to discuss the findings.

1. **Ferial A. Ismail et al.** studied **The Physico-Chemical Properties of Pomegranate Juice (*Punica granatum* L.) Extracted From Two Egyptian Varieties** to evaluate and compare the physico-chemical properties of fresh juice obtained from two pomegranate cultivars (Wardey and Manfalouty). The obtained results established that the two cultivars are different in all measured parameters for physical properties of the pomegranate cultivars. With respect to the effect of extraction methods of tested juices, blender had a higher juice yield (72.60 % and 73.21 %) compared to mechanical press (53.08 % and 45.02 %) in two pomegranate cultivars (Manfalouty & Wardey varieties, respectively). Slight differences were detected in pH and total acidity values of juice obtained from whole fruit compared to juice obtained from seeds. The highest amount of total sugars was observed for Manfalouty (pressing extraction), while Wardey variety had the highest content of ascorbic acid in pressing extraction. The antioxidant activity, tannins, polyphenols and pectin contents were higher in juices extracted from whole pomegranates than in experimental juices obtained from the arils alone.(50)
2. **Graca Miguel et al.** studied **The Effect of Two Methods of Pomegranate (*Punica granatum* L.) Juice Extraction on Quality During Storage at 4◦C** and reported that the first method consisted of separation of the seeds from fruits and centrifugation whereas the second method consisted of squeezing fruit halves with an electric lemon squeezer. During a period of 72 hours of cold storage at 4◦C, the juices were evaluated for the presence of sugars, organic acids, and anthocyanins. Delphinidin 3-glucoside was identified to be the major anthocyanin present at the level of 45–69 mg/L. Among the organic acids, oxalic and tartaric acids dominated. The major sugars detected in pomegranate juice were glucose and sucrose. No significant differences in the content of sugars, organic acids, or anthocyanins in juices obtained through application of the two different extraction methods were detected, with the exception of the drastic decrease of cyanidin 3, 5-diglucoside level in juice obtained by seed

centrifugation. The pH did not show differences between treatments. Titrable acidity and the level of sugars expressed as ◦Brix decreased after 32 and 15 hours after extraction, respectively, when juice was obtained by centrifuging the seeds.(51)

3. **Xueqing Zhao et al.** studied the **Characterization and evaluation of major anthocyanins in pomegranate (*Punica granatum* L.) peel of different cultivars and their development phases** and quantitatively analyzed three red pomegranate cultivars of different coloration in China (Lvbaoshi, Hongbaoshi, Moshiliu) during ripening for individual anthocyanin pigment content. cyanidin 3-glucoside, Pelargonidin 3-glucoside, and cyanidin 3, 5-diglucoside were the first three major anthocyanins in Hongbaoshi and followed a similar changing pattern with ripening time. Large amounts of cyanidin 3-glucoside and Delphinidin 3-glucoside were found in dark red Moshiliu cultivar, exhibiting a similar pattern over time. The main concentration of cyanidin 3-glucoside, Pelargonidin 3-glucoside, and cyanidin 3, 5-diglucoside levels in Hongbaoshi presented significant differences during ripening period ($p<0.05$) compared with the Lvbaoshi cultivar. The Anthocyanin profile was linked closely to the level of pigmentation. Data derived from study of the ratio between diglucosides and monoglucosides led to characterize the Anthocyanins in three different red pomegranate fruits.(52)
4. **Neslihan Alper et al.** studied the **Influence of processing and pasteurization on color values and total phenolic compounds of pomegranate juice** and investigated the influences of processing and pasteurization on color values and total phenolic compounds of pomegranate juices. Pomegranate juices were prepared by different clarification methods (conventional fining, conventional fining together with polyvinylpolypyrrolidone [PVPP], ultrafiltration). Unclarified juice was also prepared as control. Clarification methods, heat treatments and methods and heat treatments together significantly ($P< 0.05$) affected the color values of pomegranate juices. Moreover, conventional fining together with PVPP treatment was found to be the most suitable method to eliminate phenolic compounds.(53)
5. **Anand P. Kulkarni et al.** studied the **Chemical changes and antioxidant activity in pomegranate arils during fruit development** and observed the major chemical changes and significance of antioxidant activity during development of pomegranates. Pomegranate arils showed a considerable ($P\leq0.05$) increase in total soluble solids, total sugar and reducing sugar contents up to 100 days of fruit maturity, followed by a steady-state in their rate of buildup.

The maximum anthocyanin pigment content (138 mg/100 g) was observed in 100 day-old fruit. Significant ($P \leq 0.05$) decreases, of 76.2% and 71.1% in the concentration of ascorbic acid and total phenolics, respectively, were observed from 20 to 100 days of fruit development. After an initial rapid decrease (by 66.9%) in total protein content, pomegranate arils showed a significant ($P \leq 0.05$) increase (by 58.7%) during the late-developmental stages (80–120 days). The high antioxidant activity (71.2%) of arils recorded in 20 day-old fruit decreased significantly (by 13%) up to 60 days, associated with a decrease in ascorbic acid and total phenolics by 68.4% and 63.9% respectively. An increase in antioxidant activity by 10.6% in the late-developmental stage was due to increase of anthocyanins. A decrease in anthocyanin pigment concentration (9.3%) from 100 days onwards, as well as a significant decrease in acidity was found to be the major chemical factor for increased incidence of internal browning in over-ripe fruits.(54)

6. **Olaniyi A. Fawole et al.** studied the **Changes in physical properties, chemical and elemental composition and antioxidant capacity of pomegranate fruit at five maturity stages** and investigated physico-chemical properties such as fruit mass, size, juiciness, colour, total soluble solids (TSS), pH, titratable acidity (TA), individual organic acids and sugars, and phenolic composition. Mineral element concentrations were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) while total antioxidant capacity was measured by ferric ion reducing power (FRAP). Results showed that major compositional changes in fruit are developmentally regulated. Significant increases in total soluble solids (TSS), sugars (glucose and fructose) and anthocyanins composition, coupled with significant decline in titratable acidity (TA), organic acids and total phenolics occurred with advancing maturity. Principal component analysis (PCA) showed that fruit at advanced maturity stages (132 and 139 DAFB) were characterized by intense peel and aril pigmentation and better juice quality. PCA results also showed that peel and aril colour attributes and indices of sugar/acid ratio (TSS/TA and BrimA index) could be useful measures of fruit maturity and ripeness and therefore might be suitable for the development of reliable fruit maturity index to assess fruit optimal maturity.(55)
7. **Massimiliano Rinaldi et al.** studied **The effect of fruit processing and enzymatic treatments on pomegranate juice composition, antioxidant activity and polyphenols content** and considered the two important steps of pomegranate juice production, the

separation of arils from peels (cleaning) and the enzymatic clarification of the juice and reported the characterization (pH, acidity, total soluble solids, protein, polyphenols and antioxidant activity) of pomegranate juices obtained in different cleaning and clarification conditions. Results showed that the cleaning methods largely have an effect on the juice composition and its organoleptic properties, and an important positive correlation was found among peels content before pressing, total polyphenols, antioxidant capacity and perceived bitterness of the juice. Juice with endocarp and mesocarp content of 2 and 10 g/100 g and only mesocarp of 10 g/100 g showed a TEAC value of 14.1 and 13.1 mmol/L, respectively, significantly higher compared to juice with no peels (9.5 mmol/L). Treatment with pectolytic enzymes resulted in a better extraction of all the fruit components, as polyphenols (about 2-fold) and a decrease of turbidity.(56)

8. **Hamidreza Alighourchi et al**. studied the **Some physicochemical characteristics and degradation kinetic of anthocyanin of reconstituted pomegranate juice during storage** and observed the variation of anthocyanins content, chromatic parameters (L*, a*, b*, hue angle, chroma, and DE), pH, titratable acidity, and soluble solid content of reconstituted pomegranate juices at one month intervals during storage period at 4, 20, and 37 ^{o}C for 210 days. The main anthocyanins reported by HPLC–UV were cyanidin, delphinidin, and pelargonidin, 3-glucoside, and their 3,5-diglucoside. Juice color was investigated using Hunterlab colorimeter. The anthocyanin content and chromatic parameters of pomegranate juice changed significantly with increasing storage temperature and time ($p < 0.05$), and the degradation of pigments followed first-order reaction kinetics. The total anthocyanin content significantly correlated with the some chromatic parameters of the pomegranate juice. The soluble solid content and pH increased significantly during storage at all temperatures.(57)
9. **Yasuko noda et al.** studied **Antioxidant Activities of Pomegranate Fruit Extract and Its Anthocyanidins: Delphinidin, Cyanidin, and Pelargonidin** Antioxidant activities of freeze-dried preparations of a 70% acetone extract of pomegranate (Punica granatum L.) and its three major anthocyanidins (delphinidin, cyanidin, and pelargonidin) were evaluated. Free radical scavenging activities were examined using an ESR technique with spin trapping; DMPO for hydroxyl (âOH) and superoxide (O2 â-) radicals; and [(MGD)2Fe^{+2}] for nitric oxide (NO). Inhibitory effects on lipid peroxidation were estimated by the levels of malonaldehyde and 4-hydroxyalkenals in rat brain homogenates. Pomegranate extract exhibited scavenging activity

against âOH and O2 â-. Anthocyanidins inhibited a Fenton reagent âOH generating system possibly by chelating with ferrous ion. Anthocyanidins scavenged O2 â- in a dose-dependent manner. The ID50 values of delphinidin, cyanidin, and pelargonidin were 2.4, 22, and 456 *i*M, respectively. In contrast, anthocyanidins did not effectively scavenge NO. Anthocyanidins inhibited H_2O_2-induced lipid peroxidation in the rat brain homogenates. The ID50 values of delphinidin, cyanidin, and pelargonidin for them were 0.7, 3.5, and 85 *i*M, respectively. These findings suggest that the above anthocyanidins contribute to the antioxidant activity of pomegranate fruits.(58)

10. **Filiz Tezcan et al.** studied **Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices** and analysed the antioxidant activities, along with the organic acid and sugar contents of pomegranate juices available in the Turkish markets. Total phenolics (TPs), free radical scavenging capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric reducing capacity of seven commercial PJs were investigated. Organic acid and sugar contents of juices were evaluated by capillary zone electrophoresis. The results showed that commercial pomegranate juices had noticeably high total phenolic contents and antioxidant capacity. Fructose (F) and glucose (G) were found as the major sugars. The major acids were citric and malic. From the F/G ratio, organic acid profiles, TPs, and antioxidant capacity values, a possible adulteration was detected in one of the juices.(11)
11. **Ozge Turfan et al.** studied the **Anthocyanin and colour changes during processing of pomegranate (Punica granatum L., cv. Hicaznar) juice from sacs and whole fruit** and observed the effects of clarification and pasteurisation on anthocyanins and the colour of pomegranate juice produced from sacs and whole fruits. Clarification caused a loss of 4% of anthocyanins in juice from sacs and a loss of 19% in juice from whole fruit. After pasteurisation, there was an 8–14% and 13–9% loss of anthocyanins from unclarified and clarified juice from sacs and juice from whole fruit samples, respectively. Polymeric colour was very high even in unclarified samples (25–29%). Compared to juice from sacs, higher polymeric colour was formed in juice from whole fruit. HPLC analyses of pomegranate juice revealed that cyanidin-3,5-diglucoside was the major anthocyanins, followed by cyanidin-3-glucoside and delphinidin-3-glucoside. Cyanidin-3,5-diglucoside showed higher stability to clarification and pasteurisation than cyanidin-3-glucoside in both pomegranate juice samples.

Cold clarification with only gelatin is recommended for pomegranate juice. To prevent excessive anthocyanin loss and the formation of brown colouring, pomegranate juice should be subjected to minimal heating.(59)

12. **Jungmin lee et al.** conducted a collaborative study for the **Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, andWines by the pH Differential Method** which is a rapid and simple spectrophotometric method based on the anthocyanin structural transformation that occurs with a change in pH (colored at pH 1.0 and colorless at pH 4.5). Eleven collaborators representing commercial laboratories, academic institutions, and government laboratories participated. Seven Youden pair materials representing fruit juices, beverages, natural colorants, and wines were tested. The repeatability relative standard deviation (RSDr) varied from 1.06 to 4.16%. The reproducibility relative standard deviation (RSDR) ranged from 2.69 to 10.12%. The HorRat values were ≤1.33 for all materials. The Study Director recommends that the method be adopted Official First Action.(60)
13. **J. Fernando Ayala-Zavala et al.** studied the **Effect of storage temperatures on antioxidant capacity and aroma compounds in strawberry fruit.** The antioxidant capacity (measured as oxygen radical absorbance capacity, ORAC), total anthocyanins, total phenolics, aroma compounds, and postharvest quality of strawberry fruit kept at 0^{o}C, 5^{o}C, and 10^{o}C were investigated. Strawberry fruit stored at 10^{o}C or 5^{o}C showed higher antioxidant capacity, total phenolics, and anthocyanins than those stored at 0^{o}C. However, the postharvest life based on overall quality was longer at 0^{o}C than at 5^{o}C or 10^{o}C. The production of aroma compounds was markedly influenced by storage time and temperature. Individual aroma compounds were affected differently. For example, ethyl hexanoate, hexyl acetate, methyl acetate, and butyl acetate increased, while 3-hexenyl acetate and methyl hexanoate decreased during storage. In general, strawberries stored at 10^{o}C or 5^{o}C produced higher levels of these volatiles than those stored at 0^{o}C. In conclusion, strawberries stored at 0^{o}C retained an acceptable overall quality for the longest storage duration; however, berries stored at temperatures higher than 0^{o}C showed higher content of aroma compounds and antioxidant capacity during the post-harvest period.(61)
14. **Changjiang Guo et al.** studied the **Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay.** The antioxidant activities of peel, pulp

and seed fractions of 28 fruits commonly consumed in China were determined using the ferric reducing/antioxidant power assay (FRAP assay). The contribution of vitamin C to the antioxidant activity of fruit pulps was also calculated. The results showed that hawthorn pulp had the highest FRAP value among all fruit pulps and followed by date, guava, kiwifruit, purple mulberry, strawberry, white pomegranate, lukan and honey tangerine pulps and etc. Most of fruit peel and seed fractions were stronger than the pulp fractions in antioxidant activity based on their FRAP values. The contribution of vitamin C to the FRAP value of fruit pulps varied greatly from fruit to fruit as calculated. We concluded that peel and seed fractions of some fruits, such as pomegranate peel, grape seed, hawthorn peel, longan and lychee seeds possessed relatively high antioxidant activity and might be rich sources of natural antioxidants.(62)

15. **Marisa Rattanathanalerk et al.** studied the **Effect of thermal processing on the quality loss of pineapple juice.** Three indexes, namely colorimetric Hunter parameters (L, a, b and DE), hydroxymethylfurfural (HMF) and brown pigment formation, were monitored to determine the quality loss of pineapple juice at temperatures ranging from 55 to 95 ^{o}C. The changes in a and b values followed first order kinetics while DE fitted well to a combined model which described both non-enzymatic browning reaction and destruction of carotenoid pigment. For browning indexes, HMF and brown pigment formation increased linearly with heating time and could be explained using zero order reaction kinetics. The results suggested that processing temperature had significant effect on the color change of pineapple juice. The dependence of the rate constant on temperature was represented by an Arrhenius equation.(63)
16. **Ramesh C. Khanal et al.** studied **the Effect of heating on the stability of grape and blueberry pomace procyanidins and total anthocyanins.** Freeze dried blueberry pomace and grape pomace were heated in a forced air oven at 40, 60, 105, and 125^{o}C for 72, 48, 16, and 8 h respectively, to study the stability of procyanidins and total anthocyanins. Heating decreased procyanidin concentrations significantly ($p < 0.05$) in both blueberry and grape pomace, except when heated at 40^{o}C for 72 h. Reduction occurred when heated at 60^{o}C or above with no further reduction when heating temperature increased from 105 to 125^{o}C. Heating also affected total anthocyanin contents in both grape and blueberry pomace with no significant ($p > 0.05$) loss when heated at 40^{o}C. Total anthocyanin loss was highest at 125^{o}C

for both blueberry (52%) and grape pomace (70%). Results suggested that while heating at lower temperatures for up to 3 days may not be detrimental, heating at higher temperatures for more than 8 h results in considerable loss of both the compounds.(64)

17. **Pavlina D. Drogoudi et al.** studied **the physical and chemical characteristics of pomegranates.** Twenty pomegranate (*Punica granatum* L.) accessions were collected from different regions in northern Greece and evaluated under uniform conditions for leaf dimensions, frost resistance, and fruit physical and chemical characteristics, such as the juice antioxidant activity ascorbate equivalent antioxidant activity (AEAC)], using the radical DPPH, ascorbic acid, total phenolic and total anthocyanin contents. Considerable variation in the characteristics studied was found and valuable pomological traits were exhibited. Cluster analysis produced cases of homonymy between some accessions. Principal component analyses showed that the component explaining the greatest variability positively correlated with percent juice, fruit chroma, AEAC, total anthocyanins, and soluble solids content (SSC), but negatively correlated with fruit fresh weight (FW), fruit and seed hue angle (less red color), total acidity, and leaf dimensions. Fruit size was positively correlated with acidity, while acidity was negatively correlated with SSC. Skin thickness and FW were correlated with fruit hue angle and chroma, suggesting that red color may better develop in thick-skinned and/or small-sized pomegranates. Total anthocyanin content was negatively correlated with FW and fruit hue angle. AEAC was positively correlated with total anthocyanin and ascorbic acid contents, the latter one constituted a 15% contribution to AEAC. The associations found among physical and chemical traits suggest that consumers interested in a healthier produce should be directed to small and red pomegranates.(65)
18. **A. hager et al.** studied the **Processing and Storage Effects on Monomeric Anthocyanins, Percent Polymeric Color, and Antioxidant Capacity of Processed Black Raspberry Products** and evaluated the effects of processing and 6 months of storage on total monomeric anthocyanins, percent polymeric color, and antioxidant capacity of black raspberries that were individually quick-frozen (IQF), canned-in-syrup, canned-in-water, pureed, and juiced (clarified and nonclarified). Total monomeric anthocyanins, percent polymeric color, and oxygen radical absorbing capacity (ORAC) assay using fluorescein as fluorescent probe ($ORAC_{FL}$) were determined 1 day postprocessing and after 1, 3, and 6 months of storage. Thermal processing resulted in marked losses in total anthocyanins ranging from37% in puree

to 69% to 73% in nonclarified and clarified juices, respectively, but only the juices showed substantial losses (38% to 41%) in $ORAC_{FL}$. Storage at 25 ◦C of all thermally processed products resulted in dramatic losses in total anthocyanins ranging from 49% in canned-in-syrup to 75% in clarified juices. This coincided with marked increases in percent polymeric color values of these products over the 6-mo storage. $ORAC_{FL}$ values showed little change during storage, indicating that the formation of polymers compensated for the loss of antioxidant capacity due to anthocyanin degradation. Total anthocyanins and $ORAC_{FL}$ of IQF berries were well retained during long-term storage at –20 ◦C.(66)

19. **G.H. Laleh et al.** studied **The Effect of Light, Temperature, pH and Species on Stability of Anthocyanin Pigments in Four *Berberis* Species.** The anthocyanin pigment was extracted from the four different *Berberis* plant (*B. khorasanica, B.nintegerrima, B. orthobotrys,* and *B. vulgaris*) using the soaking and wetting in Ethanol (1% acidified). The extracted anthocyanin pigments then were exposed to number of environmental conditions, which could destabilize the anthocyanin molecules. These environmental conditions were included three different pHs (0, 1.5 and 3), various temperatures (5ºC, 15ºC, 25ºC and 35ºC) and presence or absence of light. The results of the study showed that increasing in pH, temperature or exposure to light is able to spoil the anthocyanin molecule. Another factor affecting the tolerance of anthocyanin towards the environmental condition is the role of different species. Among the various *Berberis* species anthocyanin pigment in *B. khorasanica*, showed the greatest resistance to destruction by environmental conditions followed by *B. vulgaris, B. orthobotrys, and B. integerrima.*(67)
20. **Mustafa Ozgen et al.** studied **the Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey.** These properties included total phenolics (TP), total monomeric anthocyanins (TMA), soluble solids (TSS), titratable acidity (TA), individual sugars and organic acids. Antioxidant capacities of arils were determined by both the ferric reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) assays. The antioxidant capacities averaged 5.60 and 7.35 mmol TE/l by the TEAC and FRAP methods. Variability among cultivars was greatest for TMA content (CV 132%); individuals ranged from 6.1 to 219 mg cy3-Gluc l^{-1}. TP means averaged 1507 mg GAE/l. Levels of FRAP, TEAC, TP, and TMA were strongly correlated (r = 0.82– 0.96). The major

sugars were fructose (6.4 g/100 ml) and glucose (6.8 g/100 ml), the major acids were citric (1.78 g/100 ml) and malic (0.12 g/100 ml).(68)

21. **Gelareh Mousavinejad et al.** studied **the Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars.** Important health-promoting compounds, including six types of anthocyanins, phytoestrogenic flavonoids and ellagic acid were determined individually in pomegranate juices (Punica granatum L.) of eight Iranian cultivars by high performance liquid chromatography coupled to UV–vis detector (HPLC–UV) using individual calculation from the peak area based on standard curves of each component. Total phenolics and antioxidant activities were determined by Folin–Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods, respectively, and compared among the cultivars. The predominant anthocyanins were delphinidin 3,5-diglucoside (372–5301 mg/l) followed by cyanidin 3,5-diglucoside (242–2361 mg/l), delphinidin 3-glucoside (49–1042 mg/l) and pelargonidin 3,5-diglucoside (7–90 mg/l), respectively. The highest level of total tannins was found in Sweet Alak cultivar (3 mg/l). Saveh Black Leather showed the highest level of ellagic acid (160 mg/l). Antioxidant activity varied among the cultivars (18–42 Trolox equivalents antioxidant capacity) and was directly related to the total phenolics in each type of juice.(69)
22. **Wei-Dong Wang et al.** studied the **Degradation kinetics of anthocyanins in blackberry juice and concentrate.** Thermal and storage stabilities of anthocyanins in blackberry (Rubus fruticosus L.) juice and concentrate were studied over the temperature range 60–90 ^{o}C and 5–37 ^{o}C. Results indicate that the thermal degradation of anthocyanins followed first-order reaction kinetics. The temperature-dependent degradation was adequately modeled on the Arrhenius equation. The activation energy value for the degradation of blackberry anthocyanins during heating was 58.95 kJ/mol for the 8.90^{o}Brix blackberry juice. During storage, antocyanins in the 65.0^{o}Brix blackberry juice concentrate degraded more rapidly than that in 8.90^{o}Brix blackberry juice, with the activation energies of 65.06 kJ/mol and 75.5 kJ/mol, respectively.(70)
23. **Mehmet Karaaslan et al.** studied **Drying kinetics and thermal degradation of phenolic compounds and anthocyanins in pomegranate arils dried under vacuum conditions.** The arils were divided into two groups, and half of the samples were pretreated by dipping into 80 °C hot water for 2 min. The drying process was conducted in the vacuum drier at the

temperatures of 55, 65 and 75 °C. The fastest drying was completed at 75 °C after pretreatment of the samples. The highest anthocyanin–phenolic compound contents and antioxidant capacity were detected in the arils dried at 55 °C. Seven thin-layer drying models were used to predict drying curves, and Arrhenius and Eyring–Polanyi models were employed to predict phytonutrient degradation kinetics. Activation energy for drying was 24.26 kJ mol^{-1} for pretreated samples and 31.54 kJ mol^{-1} for untreated samples. Effective moisture diffusivities were ranged from 1.43×10^{-9} to $6.03 \times 9\ 10^{-9}$ $m^2\ s^{-1}$.(71)

24. **Bianca Moldovan et al.** studied the **Degradation Kinetics of Anthocyanins from European Cranberrybush (*Viburnum opulus L.*) Fruit Extracts.** The stability of anthocyanins from *Viburnum opulus* fruits, in aqueous and ethanolic extracts, stored under darkness for 7 days at different temperatures (2 °C, 37 °C and 75 °C) and pH values (pH = 3 and 7), was studied here. The lowest stability was showed by the anthocyanins from the water extract stored at 75 °C and pH = 7, with half-life and constant rate values of 1.98 h and 0.3488 h^{-1}, respectively. The results showed a good correlation between the total anthocyanin content (determined using the pH differential method) and the time of storage, with determination coefficients varying from R^2 = 0.9298 to R^2 = 0.9971. Results indicate that the storage degradation of anthocyanins followed first-order reaction kinetics under all investigated conditions.(72)
25. **Aysegul Kırca et al.** studied **Thermal and storage stabilities of anthocyanins in blood orange juice and concentrate** over the temperature range 70–90 ^oC and 5–37 ^oC. Analysis of kinetic data suggested a first-order reaction for the degradation of blood orange anthocyanins with the half-lives of 6.3 to 1.5, 3.4 to 0.7 and 2.0 to 0.4 h for 11.2, 45 and 69^{o}Brix samples between 70 and 90 _C, respectively. At 5, 20 and 37 ^oC, the half-lives were between 55.7 and 2.1, and 115.7 and 3.1 days for 45 and 69 ^{o}Brix samples, respectively. Activation energies for solid content of 11.2–69 ^{o}Brix ranged from 73.2 to 89.5 kJ mol^{-1}.(73)
26. **Salah A. Al-Maiman et al.** studied **Changes in physical and chemical properties during pomegranate (Punica granatum L.) fruit maturation.** Physicochemical studies of pomegranate fruits (Punica granatum) variety Taifi, including total seed juice extracted from unripe, half-ripe and full-ripe stages are reported. Edible portion of pomegranate (57.51% of total fruit wt.) comprised 63.58% of juice and 36.21% of seeds. Fresh juice contained 84.57% moisture, 14.1% sugar, 1.05% protein and 0.33% ash. Total proteins, ascorbic acid, fat and

phenolic compounds in seeds were 4.06, 0.23, 0.15, 2.92%, respectively. The pH of the fruit increased with the advance in maturity, whereas ripe fruits were significantly less acidic than green unripe and half-ripe fruits. Ripe fruits had a higher percentage of glucose (53.5%) than fructose (46.6%). Polyphenols were lower in full-ripe fruits than unripe. The amounts of K, Na, Mg and Ca were highest among other minerals in the fruit. Cu, Zn and Ca contents were higher in seeds, whereas K, Na and Fe were higher in juices.(74)

27. **Jungmin Lee et al.** studied the **Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods** and established a relationship between the two analytical methods. Seven juice samples containing a range of different individual anthocyanins were analyzed by pH differential and HPLC (two different columns and mobile phase conditions). In general, total anthocyanins were greater when expressed as malvidin-glucoside than as cyanidin-glucoside, despite the method used. This paper demonstrates the high correlation ($R \geq 0.925$, $p \leq 0.05$) between the pH differential method and HPLC (both systems) when determining the amount of anthocyanins found in samples. For laboratories that do not have the capability for HPLC analysis, the pH differential is a simple and economical method to determine total anthocyanins.(75)
28. **Ranu Paul et al**. studied the **Effect of thermal treatment on ascorbic acid content of pomegranate juice** and studied The loss in antioxidant property in response to vitamin C and total phenolic content over the temperature range of 70-90°C. The degradation kinetics of ascorbic acid in pomegranate juice during thermal treatment of 70-90°C was also studied. The loss of ascorbic acid followed the first order kinetic model. The temperature dependence of this degradation reaction can be described well by Arrhenius equation. The activation energy for this reaction was 81.67 kJ/mol but the retention of ascorbic acid and total phenolic content of the juice treated at 70°C for 90 min was more than 69 and 90%, respectively as that of the fresh pomegranate juice.(76)

3. Materials and Methods

3.1 Sample:

In the present study fresh pomegranates are used as a sample. It is collected from local grocery shop. Reagents used here are all of analytical grade.

3.2 Juice extraction and preparation of sample

Pomegranate Fruit Samples were obtained. The fruits were washed with water and wiped completely dry. To obtain juice, two extraction methods were applied. The first method consisted of manually peeling the fruits, separating the seeds, and extracting the juice by an Electric juice centrifuge. In the second method, fruits were cut in two halves and the juice was immediately extracted using a hand operated juice extractor/mechanical press. The obtained raw juice from each extraction was filtered through muslin cloth. The juices were immediately stored at 4°C in the dark until analysis.

3.3 Chemical analysis

Physico-chemical characteristics of the pomegranate juice were evaluated in fresh and heat treated pomegranate juice.

3.3.1 Estimation of Antioxidant Activity

In this study antioxidant content was measured using FRAP(Ferric Reducing Ability of Plasma). FRAP assay depends upon the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. Fe(II)-TPTZ has an intensive blue color and can be monitored at 593 nm. In the FRAP assay, excess FeIII is used, and the rate-limiting factor of Fe(II)-TPTZ, and hence color, formation is the reducing ability of the sample. The procedure described by Benzie and Strain was followed.(77) The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants.

Reagetns required- For FRAP assay FRAP reagent was prepared and this reagent requires the following chemicals

a) Acetate buffer 300 mM pH 3.6. b) 10 mM TPTZ (2, 4, 6- tripyridyl-s- triazine) in 40 mM HCl c) 20 mM FeCl3. 6 H2O solution. The working FRAP reagent was prepared freshly by mixing a, b and c in the ratio of 10:1:1. Reagent was kept at 37°C.

♦ Procedure- The antioxidant activity was estimated according to the method described by Benzie and Strain(1996) with a little modification.300µl distilled water was added to 100µl sample, mixed, then 3 ml FRAP reagent was added after 1 minute interval, mixed thoroughly. Absorbance was taken at 593 nm after 4 minute incubation at ambient temperature against distilled water. FeSO4.7H2O was used as standard. The regression equation was used to express the FRAP values in mM Fe(II) per liter of the samples.

3.3.2 Estimation of Total Phenolic Content

Total phenolics contents were determined according to the Folin-Ciocalteu method described by Singleton et al.(1999).(78)

♦ Reagents required- 80% ethanol, Folin-Ciocalteu reagent, 10% sodium carbonate solution.

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Briefly, 1 mL of pomegranate juice was exactly diluted to 10 mL with distilled water, then 0.25ml of the diluted juice was mixed with 0.5 mL of Foline-Ciocalteu reagent(0.25ml FC+0.25ml Distilled water), then alkalinized with 1 mL of 20 g/100 g sodium carbonate solution. The mixture was allowed to stand for 120 min at room temperature, then the absorbance was measured by a UV/vis spectrophotometer at 765 nm. Total phenolics were calculated against a calibration curve obtained with gallic acid and results were reported as gallic acid equivalents (mg/L).

3.3.3 Estimation of Titratable Acidity & Total Soluble Solids (TSS %) Content

The acidic character of a juice contributes to its flavour type and is taken into consideration when assessing the value of the juice for inclusion into new beverage product formulations.

♦ Reagents required- 0.1(N) NaOH Solution, Phenolpthalein indicator

Titrable acidity was calculated as percentage of citric acid by titrating 5mL of the pomegranate juice with 0.1 (N) NaOH using phenolphthalein indicators expressed as % citric acid. (79)

The level of soluble solids was measured as Brix degrees by a refractometer. The refractometer was calibrated using distilled water and measurement was done with the temperature compensated mode.

3.3.4 Estimation of Reducing Sugar

Reducing sugar is estimated by Dinitrosalisylic acid method (DNS).(80) It is a simple and sensitive method. The dinitrosalicylic acid reagent, in a form consisting only of dinitrosalicylic acid dissolved in strong alkali, has been used with apparent success for molecular weight measurement of starch breakdown products. This method depends upon the assumption that all higher oligosaccharides of the homologous series starting with maltose would produce equivalent amounts of color with the reagent. The principal virtue of the dinitrosalicylicacid test for reducing sugar lies in its great convenience compared to most other sugar tests, particularly when large numbers of tests must be carried out.

♦ Reagents required- DNS reagent (for 500mlreagent-5gm NaOH, 8gm DNS, and 200gm Na-K tartarate were used and volume make up with distilled water), glucose standard solution (0.1%concentration).

♦ Procedure-3ml DNS reagent was added to 1 ml crude sample solution in test tubes. It was cotton plugged to avoid evaporation. The mixture was heated in a boiling water bath for 3-5 minutes to develop the red brown color. After cooling to room temperature the color intensities were measured in a spectrophotometer at 540 nm. The amount of reducing sugar was estimated as glucose equivalents from the standard curve.

3.3.5 Estimation of Ascorbic acid

The outstanding property of ascorbic acid is its strong reducing power, and most methods for its determination are based on this property. Tillmans, Hirsch, and Hirsch introduced the use of 2,6-dichlorophenol indophenol as oxidizing agent for the titration of ascorbic acid. This method has been extended and developed by Bessey and King, Farmer and Abt, and others.

The intensely colored titrant, 2,6-dichloroindophenol, or DCP, is quite specific in its ability to oxidize only vitamin C. DCP is dark blue in neutral and basic solutions and red in acidic solutions. The compounds involved in this redox reaction are shown in the half-reactions.

Ox: vitamin Cred → vitamin Cox + 2 e− + 2 H^+ (1)

Red: DCPox + 2 e− + 2 H+ → DCPred (2)

Overall: vitamin Cred + DCPox → vitamin Cox + DCPred (3)

So, vitamin C is oxidized by DCP in a 2 e–/2 H^+ transfer.

Redox titration with DCP provides a quantitative measure of the vitamin C content in a sample. The solution stays colorless until all the ascorbic acid has been oxidized. After this point, further addition of DCP will turn the solution pink. The amount of vitamin C is found using its quantitative relationship to the standardized DCP titrant. (81)

3.3.6 Total monomeric anthocyanins determination:

The total anthocyanins content in the extract from fruits was estimated by the pH differential method.(60)

The pH differential method has been used extensively by food technologists and horticulturists to assess the quality of fresh and processed fruits and vegetables. The method can be used for the determination of total monomeric anthocyanin content, based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5. The concept of determining the amount of anthocyanin present in a material by measuring the change in absorbance at 2 different pH values (3.4 and 2.0) was first introduced by Sondheimer and Kertesz in 1948. Since then, researchers have proposed using the pH values of 1.0 and 4.5.(82,83) Monomeric anthocyanins undergo a reversible structural transformation as a function of pH (colored oxonium form at pH 1.0 and colorless hemiketal form at pH 4.5; Figure). Thus, the difference in absorbance at the λvis-max (ca 520 nm) of the pigment is proportional to the concentration of pigment.

Degraded anthocyanins in the polymeric form are resistant to color change with change in pH. Therefore, polymerized anthocyanin pigments are not measured by this method because they absorb both at pH 4.5 and 1.0

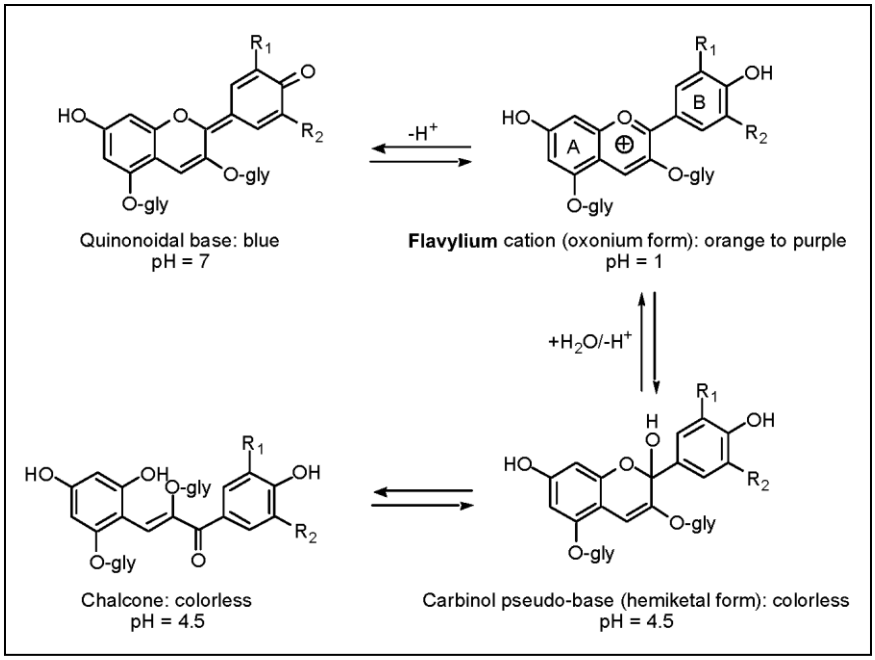

Figure shows the spectra of huckleberry anthocyanins in buffers at pH 1.0 and 4.5. (60)

The pH of juice samples was brought to 1.0 with potassium chloride and 4.5 with sodium acetate buffers. The dilutions were then allowed to equilibrate for 15 min at room temperature. Prior to absorbance measurements, the solutions were filtered through a 0.45 lm PVDF filter to remove the haze. The absorbance of equilibrated solutions at 512 nm (kmax) for Anthocyanin content and 700 nm for haze correction was measured on a UV–VIS double beam spectrophotometer with 1 cm path length disposable cuvettes. All absorbance measurements were carried out at room temperature against distilled water as a blank. Pigment content was calculated as cyanidin-3-glucoside (Cy-3-glu) equivalents with a molecular weight of 449.2 and an extinction coefficient of 26 900 L cm^{-1} mol^{-1}. The difference in absorbance values at pH 1.0 and 4.5 was directly proportional to ACN concentration. All Anthocyanin measurements were replicated three times.

Anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, was calculated as follows:

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) = {(A x MW x DF x 100)/ MA}

where A= (*A*520nm – *A*700nm)pH1.0 – (*A*520nm – *A*700nm)pH 4.5; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established; MA= 26 900 molar extinction coefficient, in L $mol^{-1}cm^{-1}$, for cyd-3-glu; and 10^3 = factor for conversion from g to mg.

3.4 Determination of Thermal Kinetics Parameters:

Thermal degradation kinetics of total anthocyanin, total phenol content and total antioxidant was studied by isothermal heating at 60, 65, 70, 75, 80, 85 and 90°C respectively. 10 mL samples were taken in sealed glass tubes and heated by placing them in a hot water bath.

At regular time interval of 15 min, the tubes were taken out and rapidly cooled by plunging them into ice water and analyzed for total anthocyanin, total phenol content and total antioxidant respectively.

3.5 Degradation studies:

The order of the reaction in thermal degradation of anthocyanins, total phenol and total antioxidant content was predicted using the model:

$$\frac{dC}{dT} = -k\,(C)^n \qquad (1)$$

where k is the rate constant, n is the reaction order, C is the concentration of total anthocyanins, or total phenol or total antioxidant content and t is the reaction time.(70)

The order of reaction was determined by graphical analysis, where exponent n in eq 1 was set to zero, half, one, and two to compare the coefficients of determination among zero-, half-, first-, and second-order reactions, respectively. The integrated forms of zero-, half-, first-, and second-order kinetic models are given in eqs 2 to 5. (84)

zero-order : $C_t = C_0 - kt$ (2)

half-order: $2\sqrt{C_t} - \sqrt{C_0} = kt$ (3)

first-order: $\ln \frac{Ct}{C0} = -kt$ (4)

second order: $\frac{1}{Ct} - \frac{1}{C0} = kt$ (5)

` Using the experimental data of anthocyanin, total phenol and antioxidant content, the coefficient of determination was observed to be minimum for n = 1, predicting a first-order reaction. According to the activated complex theory for chemical reaction rates, for first order, the Arrhenius equation relates the reaction rate constants to the absolute temperature.

$$\ln k = \ln A_0 + \frac{Ea}{RT} \quad (6)$$

where Ea is the activation energy (kJ/mol), A_0 is a pre-exponential factor/ frequency factor (1/s), T is the absolute temperature (K), and R is the gas constant (8.314 J/mol . K). The reaction rate constant k and the activation energy, Ea were determined graphically from a plot of $\ln(C_t/C_0)$ versus time and ln k versus 1/T, respectively. (84)

The half-life ($t_{1/2}$) was calculated as

$$t_{1/2} = \frac{\ln 2}{K} \quad (7)$$

4. Results and Discussions

4.1 Physical and Chemical characteristics of fresh pomegranate juice:

Table 1 shows the results of some physical and chemical analyses of fresh pomegranate juice. The values agree well with the values reported in the literature for pomegranate juice samples.

The Total soluble solids(TSS) content in fresh pomegranate juice was 14.5 ^{o}Brix. Similar results were reported by Ferial A. Ismail et al. for fresh juice obtained from two pomegranate cultivars (Wardey and Manfalouty).(50)

Citric acid were detected as the main organic acids, sometimes followed closely by malic acid in the commercial pomegranate juices, as reported earlier by Melgarejo et al.(90) As seen from the Table 1, citric acid concentrations of pomegranate juice come out to be 0.66 g/100 mL. Citric acid levels of 40 Spanish pomegranate cultivars were reported by Melgarejo et al. as between 0.142–2.317 g/100mL; by Poyrazog˘lu et al. (2002), for 13 pomegranates from four different region of Turkey, as between 0.033–0.896 g/100 ml.(11)

The amount of reducing sugar detected in fresh pomegranate juice was 6.54gm/100mL. Individual sugar contents reported in the literature are 5.66–6.45 g /100 g for Glucose for 40 Spanish cultivars (Melgarejo et al., 2000) and 5.80–7.62 g/100 ml for six pomegranate arils from Turkey (Ozgen et al., 2008). The main carbohydrates detected in the pomegranate juices were glucose and fructose.(90,91)

The total phenolic content exhibited by pomegranate juice was 2.75mg/100mL. Gil et al. reported the total phenols of pomegranate juice from fresh arils as 2.117 mg/100ml and for a commercial pomegranate juice as 2.56mg/100mL.(92)

Antioxidant activity of pomegranate juice was found out to be 0.0415mmol Fe^{+2}/ml. PJ contains high amounts of hydrolyzable tannins and anthocyanines. However, the results of extensive work of Gil et al. on antioxidant activity of PJs recommended that punicalagin originating from the peels is one of the major phytochemicals contributing to the total antioxidant capacity of pomegranate juice, whilst anthocyanins also play only a major role in this activity.(92) Filiz Tezcan et al. reported 0.0484 mmol Fe^{+2}/ml of antioxidant in fresh pomegranate juice from arils.(11)

Ascorbic acid is another important component of pomegranate juice. 0.201mg/100ml of ascorbic acid was detected in the pomegranate juice in this study (Table 1). Ranu Paul et al. reported 0.198mg/ml of ascorbic acid in fresh pomegranate juice.(76)

The total monomeric Anthocyanin content of fresh pomegranate juice detected was 26. 47mg/100ml as determined by pH differential method (Table 1). Ferial A. Ismail et al. reported 33.01–33.34 mg/100 ml of anthocynin from Wardey cultivar and 25.85 – 26.01 mg/100ml of anthocyanin in Manfalouty cultivar.(50) The Anthocyanin content and composition of pomegranates strongly depend on the cultivar and the stage of maturity. Ozkan, Turkyılmaz, and Guzel (2009) determined the total anthocyanin content of nine registered Turkish pomegranate varieties by HPLC and found large differences in anthocyanin contents, ranging from 28 to 447 mg Anthocyanin/mL juice.(93)

Table 1. The properties of fresh pomegranate juice:

TSS (^{o}B)	14.5±0.66
Total Phenolic Content (gallic acid; mg/ml)	2.75±0.42
Ascorbic Acid (mg/100mL)	0.201±0.15
Reducing Sugar (mg/100mL)	6.54±0.31
Total Acidity (Citric acid; g/100mL)	0.665±0.26
Antioxidant Activity (mmol Fe^{+2}/ml)	0.0415±0.35
Total anthocyanin Content (mg/ml) (Expressed as cyanidin-3-glucoside)	26.47±0.16

*Data are mean of 3 replicate observations
*Significance of variance is adjusted at 5% level

4.2 Thermal treatment and total monomeric anthocyanin content:

The total anthocyanin contents in the pomegranate juice were determined before and after heat treatments are summarized in Table 2. Results showed that loss of monomeric anthocyanin was 12.82% at 65^{o}C after 60 minutes of heat treatment. Ozge Turfan et al. reported there was an 8% loss in the total Anthocyanin content of juice from sacs and a 13% loss in juice from whole sacs after pasteurisation. Lee et al. (2002) reported a substantial loss of anthocyanins (76%) in pasteurised blueberry juices. The increase in colour density is the result of the polymeric colour that forms from the degradation of anthocyanins. A plausible explanation for the degradation of anthocyanins during the pasteurisation of pomegranate samples is a low-temperature, long-time treatment (LTLT). During the LTLT treatment, enzymes that degrade anthocyanin, including polyphenol oxidase, may be activated.(95)

Table 2. Effect of heat treatment on total monomeric anthocyanin concentration (Expressed as cyanidin-3-glucoside; mg/ml) in pomegranate juice:

Time (mins)	Temperature (65^{o}C)	Temperature (70^{o}C)	Temperature (75^{o}C)	Temperature (80^{o}C)	Temperature (85^{o}C)	Temperature (90^{o}C)
0	26.35±0.50	25.66±0.32	25.20±0.35	24.56±0.24	24.35±0.27	24.06±0.62
15	25.85±0.61	24.67±0.45	24.62±0.38	23.36±0.50	23.03±0.23	22.96±0.39
30	24.73±0.36	23.96±0.28	23.62±0.42	22.72±0.18	22.39±0.65	21.76±0.28
45	23.95±0.40	23.35±0.34	22.72±0.33	21.65±0.25	21.26±0.43	20.78±0.24
60	22.97±0.42	22.73±0.23	21.82±0.63	20.86±0.36	20.54±0.45	20.19±0.45

*Data are mean of 3 replicate observations
*Significance of variance is adjusted at 5% level

4.3 Kinetic study on anthocyanin degradation during heat treatment:

The bright colour of pomegranate fruit and juice is due to anthocyanins, so their stability through juice processing is of major importance. The effect of heat treatment on the concentration of the total anthocyanin contents in pomegranate juice for different time periods have been studied and the results showed that degradation of anthocyanin increased with the increase of temperature. The order of the thermal degradation was estimated by examining the coefficient of determination (R^2) from the graph and is shown in Table 3. On the basis of the mean R^2, the thermal degradation of anthocyanins tended to follow first-order kinetics (R^2= 0.99). Previous studies showed that thermal degradation of anthocyanins followed a first-order reaction (Cemeroglu et al., 1994; Garzon & Wrolstad, 2002).(85,86) The temperature-dependent rate constants, k values, from first order over 65-90℃ as calculated from a plot of $\ln(Ct/C_0)$ versus treatment time (Figure 1) were increased from 2.1×10^{-3}(for 65℃) to 3.2×10^{-3} min^{-1}(for 90℃) and the half-life decreased from 0.330×10^{-3} min to 0.216×10^{-3} min as the temperature increased from 65-90℃. Other authors have also reported that k values increase with increasing temperature and $t_{1/2}$ values decrease (Cemeroglu et al., 1994; Kirca et al., 2007; Kirca & Cemeroglu, 2003; Wang & Xu, 2007).(85,87,70) The values of the first-order model are given in Table 4. Effect of the heat treatment temperature on the anthocyanins degradation rate constants(k) is shown in Fig. 2. Temperature-dependent rate constant obeyed the Arrhenius relationship (Eq. (6)). Activation energy Ea (kJ/mol) were calculated as a product of gas constant R and the slope of the graph obtained by plotting lnk versus 1/T. The computed values of the activation energy was 52.39 kJ/mol for the juice samples.(Table 9). Mehmet Karaaslan et al. reported that $t_{1/2}$ values for anthocyanins degradation were 0.413×10^{-3}, 0.193×10^{-3} and 0.121×10^{-3} in pomegranate arils at 55, 65 and 75 ℃, respectively and the calculated activation energy (Ea) was 54.50 kJ/mol.(71) High activation energy implies that the degradation of anthocyanins in pomegranate juice are more susceptible to temperature elevations than the degradation of antioxidant and polyphenols(57)

Table 3. Estimation of the Order of Anthocyanin Degradation by Examining Coefficients of Determination (R^2) from Plots of Zero-, Half-, First-, and Second-Order Reactions:

Temperature(^oC)	Zero Order	Half Order	First Order	Second Order
65	0.9898	0.9888	0.995	0.9848
70	0.9887	0.989	0.993	0.9839
75	0.9865	0.9855	0.996	0.9804
80	0.9890	0.9795	0.991	0.9840
85	0.9878	0.9742	0.993	0.9828
90	0.9875	0.9798	0.992	0.9745

Kinetics of anthocyanins degradation during heat treatment:

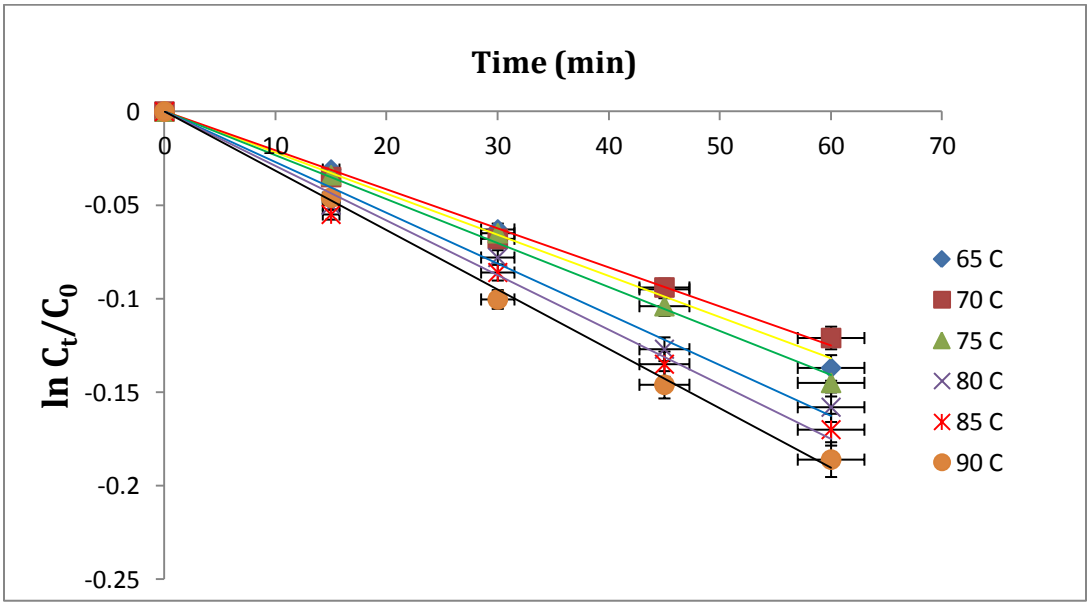

Fig 1: First-order plot for the degradation of total monomeric anthocyanin content of Pomegranate juice during heating over the temperature range of 65 to 90℃ for 0 to 60 min. Data are the mean of duplicate samples

Arrhenius plot of the anthocyanin loss rate of pomegranate juice:

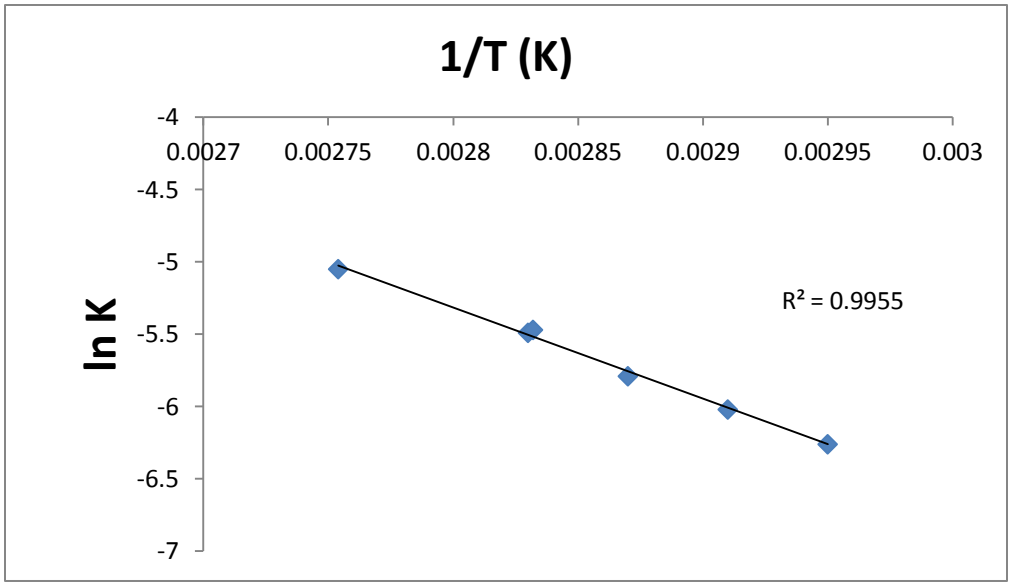

Fig 2. Arrhenius plot of total monomeric anthocyanin content of Pomegranate juice during heating over the temperature range of 65 to 90℃ for 0 to 60 min

Table 4 . Kinetic parameters for Anthocyanin degradation in pomegranate juice:

Temperature(^{o}C)	k (min^{-1})	R^2	$t_{1/2}$ (min)
65	2.1×10^{-3}	0.995	0.330×10^{-3}
70	2.2×10^{-3}	0.993	0.315×10^{-3}
75	2.4×10^{-3}	0.996	0.288×10^{-3}
80	2.7×10^{-3}	0.991	0.256×10^{-3}
85	2.9×10^{-3}	0.993	0.239×10^{-3}
90	3.2×10^{-3}	0.992	0.216×10^{-3}

4.4 Thermal treatment and total Phenolic content:

The total polyphenol contents in the pomegranate juice were determined before and after heat treatments are summarized in Table 5. The loss in phenolic content was calculated to be 9% at 65°C after 60 minutes of heat treatment. It is clear from the data that total phenolic content in pomegranate juice was decreased as the temperature and time of heat treatment increases. Ranu Paul et al. reported that there was a 9.76% loss in the phenolic contents after 60minutes of heat treatment at 70℃. The present results are in conformity with the previous studies where total phenolic content of some vegetables was found decreased with the increase in temperature and time of thermal processing.(94)

Table 5 . Effect of heat treatment on total phenolic content (gallic acid; mg/ml):

Time (mins)	Temperature (65°C)	Temperature (70°C)	Temperature (75°C)	Temperature (80°C)	Temperature (85°C)	Temperature (90°C)
0	2.809±0.63	2.771±0.34	2.727±0.18	2.676±0.27	2.654±0.43	2.613±0.66
15	2.726±0.26	2.703±0.20	2.650±0.44	2.613±0.45	2.568±0.26	2.523±0.43
30	2.653±0.42	2.638±0.33	2.581±0.26	2.524±0.43	2.491±0.35	2.466±0.24
45	2.592±0.35	2.563±0.45	2.506±0.53	2.469±0.36	2.418±0.46	2.358±0.58
60	2.556±0.25	2.521±0.36	2.451±0.32	2.395±0.24	2.348±0.56	2.295±0.33

*Data are mean of 3 replicate observations
*Significance of variance is adjusted at 5% level

4.5 Kinetic study on Total Polyphenol degradation during heat treatment:

The plots of $\ln(Ct/C_0)$ versus t for thermal degradation of phenolic content is shown in Fig 3. The degradation of total phenol content in pomegranate juice over 65-90℃ was also fitted to first-order reaction kinetics. The coefficients of determination (R^2) values were more than 0.99 for all cases. (Table 6), indicating a good data fit to the first-order kinetic model, which is in accordance with previous studies.(71) The k and $t_{1/2}$ values further confirmed the influence of temperature. The k values of total phenol degradation increased with temperature from 1.4×10^{-3}(for 65℃) to 2.1×10^{-3} (for 90℃) and the half-life decreased from 0.495×10^{-3} min to 0.277×10^{-3} min as the temperature increased from 65-90℃. Temperature-dependent rate constant obeyed the Arrhenius relationship. The computed value of the activation energy was 26.32 kJ/mol for the total phenol degradation obtained by plotting ln k versus 1/T and shown in Fig 4(Table 9). Mehmet Karaaslan et al. reported that $t_{1/2}$ values for phenolicdegradation were 0.68×10^{-3}, 0.55×10^{-3} and 0.428×10^{-3} in pomegranate arils at 55, 65 and 75℃, respectively and the calculated activation energy (Ea) was 34.23 kJ/mol.(71)

Kinetics of Total polyphenol degradation during heat treatment:

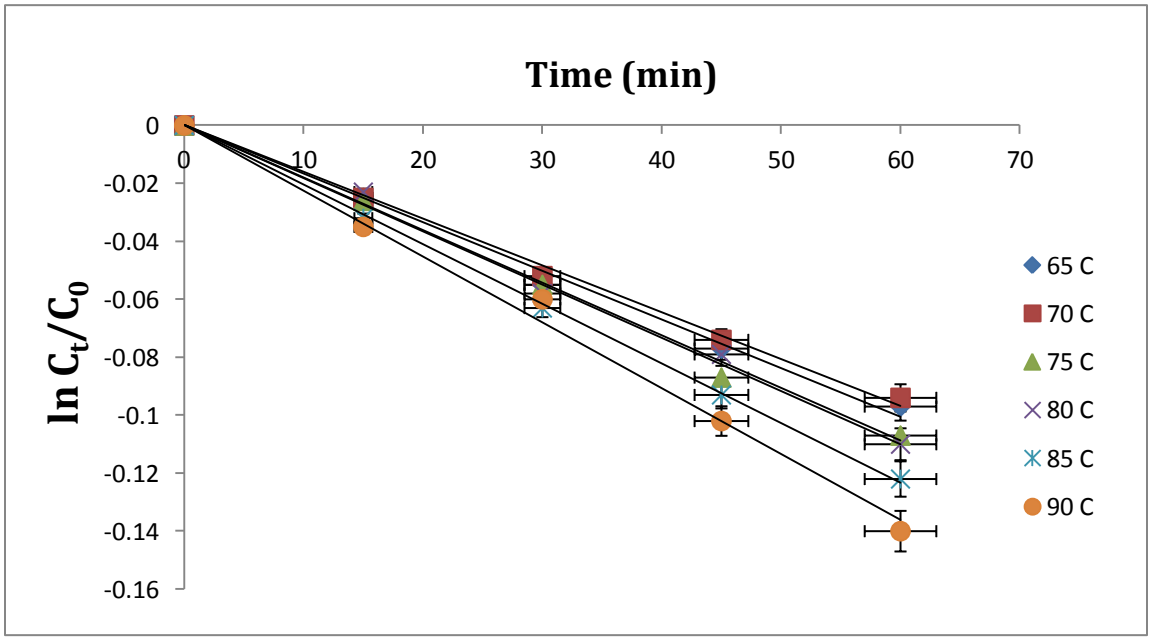

Fig 3. First-order plot for the degradation of total phenol content of Pomegranate juice during heating over the temperature range of 65 to 90℃ for 0 to 60 min. Data are the mean of duplicate samples

Table 6. Kinetic parameters for total phenol degradation in pomegranate juice:

Temperature(^{o}C)	k (min^{-1})	R^2	$t_{1/2}$ (min)
65	1.4×10^{-3}	0.993	0.495×10^{-3}
70	1.6×10^{-3}	0.995	0.433×10^{-3}
75	1.8×10^{-3}	0.995	0.385×10^{-3}
80	2.1×10^{-3}	0.994	0.330×10^{-3}
85	2.3×10^{-3}	0.999	0.301×10^{-3}
90	2.5×10^{-3}	0.993	0.277×10^{-3}

Arrhenius plot of the total phenol loss rate of pomegranate juice :

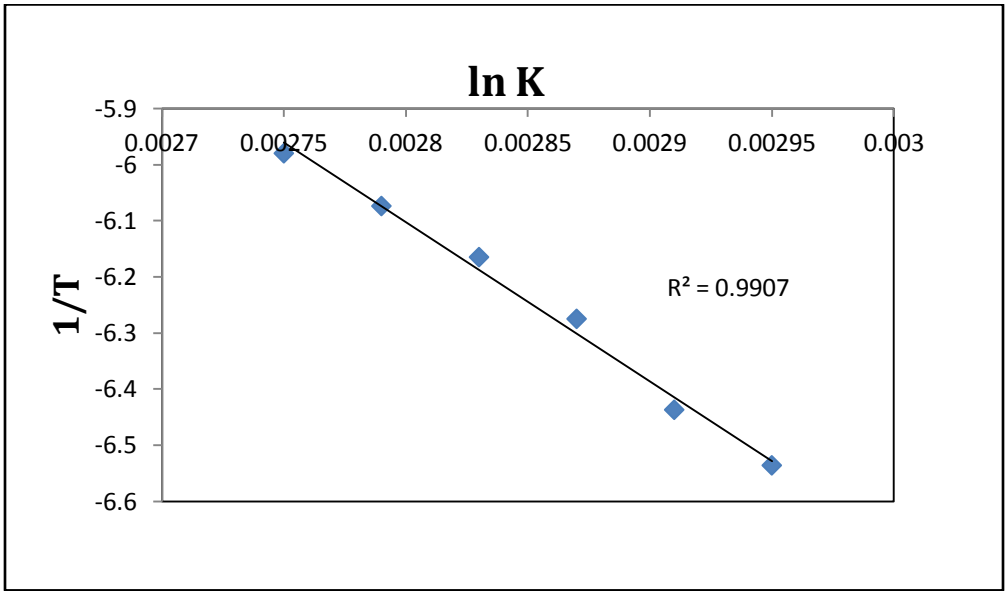

Fig 4. Arrhenius plot of total phenol content of Pomegranate juice during heating over the temperature range of 65 to 90℃ for 0 to 60 min

4.6 Thermal treatment and total Antioxidant content:

The total antioxidant contents in the pomegranate juice were determined before and after heat treatments are summarized in Table 7. The loss in antioxidant content was calculated to be 2.1% at 65°C after 60 minutes of heat treatment. As the total phenolic content is a measure of the antioxidant activity, it can be conferred that antioxidant activity of the juice decreases with the increase in temperature. It is clear from the data that the antioxidant activity in pomegranate juice was decreased as the temperature and time of heat treatment increases. The reduction in antioxidant activities during heat treatment may be associated with apparent decrease in quantity of polyphenols in the fruit juice (Gil et al., 2000).(92)

Table 7. Effect of heat treatment on antioxidant capacity (mmol Fe^{+2}/ml):

Time (mins)	Temperature (65^oC)	Temperature (70^oC)	Temperature (75^oC)	Temperature (80^oC)	Temperature (85^oC)	Temperature (90^oC)
0	0.0412±0.24	0.0409±0.40	0.0404±0.42	0.0401±0.50	0.0397±0.63	0.0396±0.39
15	0.0407±0.28	0.0402±0.43	0.0395±0.24	0.0390±0.38	0.0389±0.14	0.0392±0.26
30	0.0402±0.63	0.0396±0.28	0.0389±0.61	0.0383±0.23	0.0380±0.26	0.0378±0.48
45	0.0395±0.47	0.0392±0.33	0.0382±0.38	0.0376±0.61	0.0372±0.35	0.0369±0.22
60	0.0391±0.23	0.0386±0.27	0.0378±0.65	0.0368±0.36	0.0365±0.20	0.0362±0.68

*Data are mean of 3 replicate observations
*Significance of variance is adjusted at 5% level

4.7 Kinetic study on Antioxidant degradation during heat treatment:

The plots of ln(Ct/C0) versus t for thermal degradation of antioxidant content is shown in Fig 5. The degradation of antioxidant in pomegranate juice over 65-90℃ was also fitted to first-order reaction kinetics. The coefficients of determination (R^2) values were more than 0.99 for all cases. (Table 8), indicating a good data fit to the first-order kinetic model, which is in accordance with previous studies.(92) Similar results were also determined by the authors studied anthocyanin, total phenol, antioxidant, ascorbic acid and for other bioactive compounds' degradation kinetics for various food materials (Erenturk et al., 2005; Verbeyst et al., 2010;). (88,89) The k and $t_{1/2}$ values further confirmed the influence of temperature. The k values of antioxidant degradation increased with temperature from 0.9×10^{-3}(for 65℃) to 1.7×10^{-3} (for 90℃) and the half-life decreased from 0.770×10^{-3} min to 0.407×10^{-3} min as the temperature increased from 65-90℃. Temperature-dependent rate constant obeyed the Arrhenius relationship. The computed values of the activation energies were 26.27 kJ/mol for the antioxidant degradation obtained by plotting ln k versus 1/T and shown in Fig 6.(Table 9)

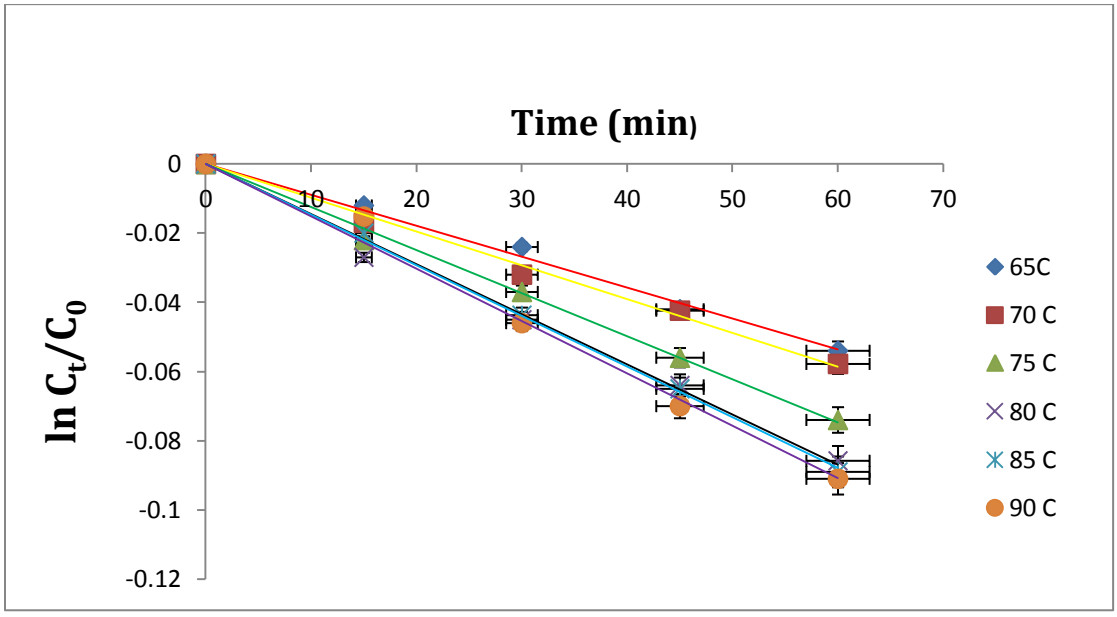

Fig 5. First-order plot for the degradation of total antioxidant content of Pomegranate juice during heating over the temperature range of 65 to 90℃ for 0 to 60 min. Data are the mean of duplicate samples

Table 8. Kinetic parameters for Antioxidant degradation in pomegranate juice :

Temperature(^{o}C)	k (min^{-1})	R^2	$t_{1/2}$ (min)
65	0.9×10^{-3}	0.993	0.770×10^{-3}
70	1.2×10^{-3}	0.992	0.577×10^{-3}
75	1.3×10^{-3}	0.996	0.533×10^{-3}
80	1.4×10^{-3}	0.992	0.495×10^{-3}
85	1.5×10^{-3}	0.998	0.462×10^{-3}
90	1.7×10^{-3}	0.989	0.407×10^{-3}

Arrhenius plot of the antioxidant loss rate of pomegranate juice :

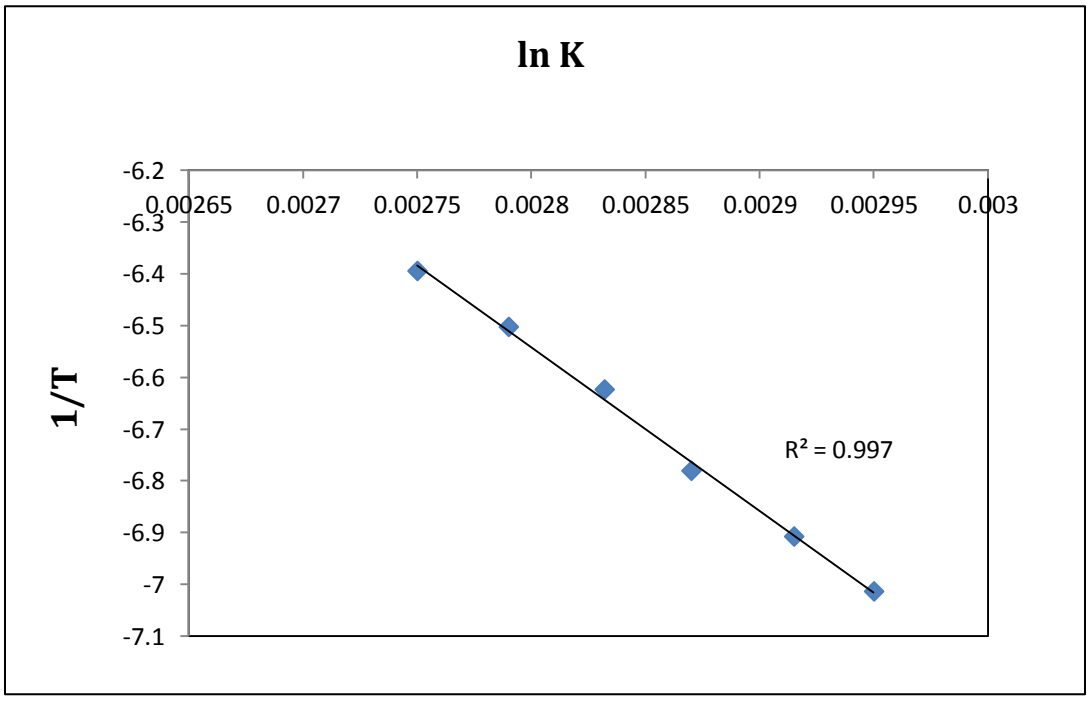

Fig 6. Arrhenius plot of total antioxidant content of Pomegranate juice during heating over the temperature range of 65 to 90℃ for 0 to 60 minutes.

Table 9. Arrhenius parameters for Total Anthocyanin, Total Antioxidant and Total Polyphenol degradation in pomegranate juice:

Parameters	$A_0(Min^{-1})$	$E_a(kJ/mol)$	R^2
Total Anthocyanin	0.124	52.39	0.995
Total Antioxidant	0.230	26.27	0.997
Total Polyphenol	0.185	26.32	0.990

5.Conclusion

In the present study we have observed how thermal treatment affects the major quality attributes of pomegranate juice. Pomegranate fruit and pomegranate juices have recently received great attention for their health benefits, mainly due to their high polyphenols content and the related antioxidant capacity. Unfortunately, these compounds are unstable and susceptible to degradation, leading to quality loss of the juice. The study revealed that the loss of anthocyanin content as well as phenolic content and antioxidant activity increases with time and temperature of processing. The results from the present study provide detailed information regarding the changes in kinetic stability of anthocyanin, phenolic content and antioxidant activity in pomegranate juice during heat treatment. Results showed that loss of monomeric anthocyanin was 12.82% at 65^{o}C after 60 minutes of heat treatment, which is least among all other temperatures applied (70°C, 75°C, 80°C, 85°C and 90°C). The loss in phenolic content was calculated to be 9% at 65°C after 60 minutes of heat treatment, which is least among all other temperatures applied (70°C, 75°C, 80°C, 85°C and 90°C). Also the loss in antioxidant content was calculated to be 2.1% at 65°C after 60 minutes of heat treatment, which is least among all other temperatures applied (70°C, 75°C, 80°C, 85°C and 90°C). It is clear from the data that total anthocyanin, total phenolic content and antioxidant content in pomegranate juice was decreased as the temperature and time of heat treatment increases. The results from the present study provide detailed information regarding the changes in kinetic stability of pomegranate anthocyanin as well as polyphenols and antioxidants in juice during heating. The present data shows that degradation of thses compounds follows first order reaction kinetics. Variation of degradation rate constants with temperature obeyed the Arrhenius relationship. Such information would be helpful for establishing appropriate processing and storage protocols to reduce the degradation of anthocyanins as well as total phenolic content, the natural antioxidants in pomegranate juice as well as in other juices also.

6. Future Scope

In the present study we observed the effects of thermal treatment on quality attributes of pomegranate juice.

- In future, further studies can be conducted to monitor the effects of non-thermal processing on different analytical parameters of pomegranate juice.

- Further studies can be carried out to examine the effect of storage and preservation on the juice characteristics

- Statistical optimization can be performed for maximum retention of the quality characteristics of pomegranate juice.

- As the juice is enriched in polyphenols, antioxidants, vitamins, minerals, it can be employed in developing neutraceuticals and food supplements.

7. Publications

1. Prama Pal and Uma Ghosh, "Effect of Thermal Treatment on Quality Attributes of Pomegranate (*Punica granatum* L.) Juice." Oral Presentation. National Seminar on Research Trends in Medicine and Biology: The Issues of Health, Ecology and Management, 20th March, 2016. (First Position Awarded)

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