EFFECTS OF THERMAL AND NON-THERMAL PROCESSING ON QUALITY ATTRIBUTES OF CHOKANAN MANGO JUICE (*MANGIFERA INDICA* L.)

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ABSTRACT

The increasing demand for high quality fruit juice, along with safety standards have spurred the development of non-thermal processing such as sonication and ultraviolet-c (UV-C) light treatment. In this study, freshly squeezed Chokanan mango juice was subjected to thermal treatment (at 90 °C for 30 and 60 seconds), sonication (for 15, 30 and 60 minutes at 25 °C, 40 kHz frequency) and UV-C treatment (for 15, 30 and 60 minutes at 25 °C, 254 nm). In addition, combination of sonication (for 15 and 30 minutes at 25 °C, 40 kHz frequency) and UV-C treatment (for 15 and 30 minutes at 25 °C, 254 nm) in a hurdle concept was also conducted. The effects of thermal and non-thermal treatments on various quality parameters (microbial inactivation, physicochemical properties, colour, clarity, browning index, total carotenoid and ascorbic acid content, antioxidant activities, sensory attributes) were evaluated and compared with untreated juice (control).

After thermal and non-thermal treatments, no significant changes were observed in pH, total soluble solids and titratable acidity. However, significant differences in colour, browning index and ascorbic acid content of juice were observed after treatments (thermal and non-thermal). Non-thermal treatments showed significant improvement in selected quality parameters. Overall, sonication and UV-C treatment (as a stand-alone and combined treatment) exhibited significant enhancement in clarity, antioxidant activities, and extractability of carotenoids, polyphenols, and flavonoids, when compared to the control. In addition, significant reduction in microbial load was observed in non-thermal and thermal treatments. Although thermal treatment was

effective in completely inactivating microbial growth in juice, significant quality loss was observed.

The individual phenolic compounds in juice were identified and quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The results showed better retention of individual phenolic compounds in non-thermal treated juice, when compared to the control and thermally treated juice. Sensory attributes (colour, odour, taste, and overall acceptability) were evaluated by 90 panellists using a hedonic scale, and results showed that non-thermal treated juice was preferred more than thermally treated juice. The sensory evaluation verified that the combination of sonication and UV-C was the most acceptable treatment of the selected non-thermal treatments.

The shelf life of the thermally treated juice stored at 4 °C was extended for at least five weeks longer than control. With regards to sonication and UV-C treatment (as a stand-alone), the shelf life of juice stored at 4 °C was extended for at least four weeks longer than control. Besides that, combined treatment prolonged the shelf life of juice stored at 4 °C for at least five weeks longer than control. The results obtained support the use of non-thermal treatments (ultrasound and UV-C) for better retention of quality along with safety standards in Chokanan mango juice processing. Combination of ultrasound and UV-C therefore, is a promising alternative to thermal treatment.

ABSTRAK

Permintaan tinggi untuk jus buah-buahan yang berkualiti tinggi berserta dengan piawaian keselamatan telah merangsang perkembangan proses bukan terma seperti sonikasi dan rawatan cahaya ultraviolet-c (UV-C). Dalam kajian ini, jus mangga Chokanan yang baru diperah telah diberikan rawatan terma (pada 90 °C untuk 30 dan 60 saat), sonikasi (untuk 15, 30 dan 60 minit pada 25 °C, 40 kHz frekuensi) dan rawatan UV-C (untuk 15, 30 dan 60 minit pada 25 °C, 254 nm). Di samping itu, gabungan sonikasi (untuk 15 dan 30 minit pada 25 °C, 40 kHz frekuensi) dan rawatan UV-C (untuk 15 dan 30 minit pada 25 °C, 254 nm) sebagai konsep ‘halangan’ juga telah dilaksanakan. Kesan rawatan terma dan bukan terma terhadap ciri-ciri kualiti jus (inaktivasi mikroba, ciri fizikokimia, warna, kejernihan, indeks pemerangan, kandungan karotenoid dan asid askorbik, activiti antioksidan, penilaian deria, dan komponen bioaktif) telah dikaji dan dibandingkan dengan jus yang tidak dirawat (sampel kawalan).

Selepas rawatan terma dan bukan terma, tiada perubahan ketara didapati bagi pH, jumlah pepejal larut dan juga keasidan bagi jus tersebut. Walaubagaimanapun, perubahan ketara bagi warna, indeks pemerangan, dan kandungan asid askorbik diperhatikan selepas rawatan (terma dan bukan terma). Rawatan bukan terma menunjukkan peningkatan yang ketara bagi ciri-ciri kualiti yang telah dipilih. Secara keseluruhannya, sonikasi dan rawatan UV-C (sebagai rawatan tunggal dan gabungan) telah menunjukkan peningkatan yang ketara dalam kejernihan, activiti antioksidan, dan pengekstrakan karotenoid, polifenol, dan flavonoid, berbanding dengan sampel kawalan. Selain itu, pengurangan yang ketara dalam kiraan mikroorganisma diperhatikan selepas rawatan terma dan bukan terma. Walaupun rawatan terma didapati

berkesan sepenuhnya dalam mengaktifkan pertumbuhan mikroorganisma dalam jus, namun rawatan ini didapati menjejaskan kualiti jus.

Analisis kromatografi cecair gandingan spektrometri jisim (LC-MS/MS) telah digunakan untuk mengenal pasti and menentukan kuantiti sebatian fenolik individu dalam jus. Keputusan telah menunjukkan bahawa rawatan bukan terma mengekalkan sebatian fenolik individu dalam jus dengan lebih berkesan, jika dibandingkan dengan sampel kawalan dan jus yang dikenakan rawatan terma. Penilaian deria (warna, bau, rasa, dan tahap penerimaan) telah dinilai oleh 90 ahli panel dengan menggunakan skala hedonik, dan keputusan telah menunjukkan bahawa jus yang dikenakan rawatan bukan terma lebih digemari daripada jus yang dikenakan rawatan terma. Penilaian deria telah mengesahkan bahawa gabungan sonikasi dan UV-C adalah rawatan yang paling diterima berbanding rawatan bukan terma yang lain.

Jangka hayat bagi jus yang dikenakan rawatan terma dan disimpan pada suhu 4 °C telah dilanjutkan sekurang-kurangnya lima minggu lebih lama daripada sampel kawalan. Untuk sonikasi dan rawatan UV-C (sebagai rawatan tunggal), jangka hayat jus yang disimpan pada suhu 4 °C telah dilanjutkan sekurang-kurangnya empat minggu lebih lama daripada sampel kawalan. Selain itu, gabungan sonikasi dan UV-C melanjutkan jangka hayat jus yang disimpan pada suhu 4 °C sekurang-kurangnya lima minggu lebih lama daripada sampel kawalan. Keputusan yang diperolehi menyokong penggunaan rawatan bukan terma (sonikasi dan UV-C) untuk mengekalkan kualiti jus mangga Chokanan dengan lebih berkesan berserta dengan piawaian keselamatan. Sehubungan dengan itu, gabungan sonikasi dan UV-C merupakan alternatif yang baik kepada rawatan terma.

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LIST OF ABBREVIATIONS

ΔE	Colour differences
µg	Microgram
µl	Microliters
AAE	Ascorbic acid equivalent
ABTS	2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
ANOVA	Analysis of variance
APC	Aerobic plate count
CC	Coliform count
CE	Catechin equivalent
CFU	Colony-forming units
DCPIP	2,6-dichlorophenol-indophenol
DPCD	Dense phase carbon dioxide
DPPH	1,1-di-phenyl-2-picrylhydrazyl
FAMA	Federal Agricultural Marketing Authority
FAOSTAT	Statistics Division of the Food and Agriculture Organisation of the United Nations
FDA	Food and Drug Administration
GAE	Gallic acid equivalent
Ha	Hectares
HACCP	Hazard analysis critical control point
HHP	High hydrostatic pressure
HMF	5-hydroxymethyl furfural
HSD	Honestly significant difference
HTST	High temperature short time
HUS	Haemolytic uremic syndrome
IFST	Institute of Food Science and Technology
IR	Ionizing radiation
kGy	Kilogray
kHz	Kilohertz
LCMS/MS	Liquid Chromatography Tandem Mass Spectrometry
LTLT	Low temperature long time
ml	Mililiters

MPa	Megapascal
MT	Metric tonnes
NEBI	Non-enzymatic browning index
nm	Nanometers
PEF	Pulsed electric field
ROS	Reactive oxygen species
RPA	Reducing power assay
RT	Room temperature
SD	Standard deviation
SDW	Sterile distilled water
TA	Titratable acidity
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TFC	Total flavonoid content
TPC	Total polyphenol content
TSS	Total soluble solids
UPLC	Ultra High Performance Liquid Chromatography
UV-C	Ultraviolet-c
V	Volt
W	Watt
YMC	Yeast and mould count

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CHAPTER 1

GENERAL INRODUCTION

Mango (*Mangifera indica* L.) is a tropical fruit grown in 85 countries, ranking fifth in global production among other major fruit crops including bananas, citruses, grapes and apples. According to the Statistics Division of the Food and Agriculture Organisation of the United Nations, FAOSTAT (2014), about 72% of worldwide mango production is concentrated mainly in Asia, thus contributing approximately 30.2 million metric tonnes to the international market. In Malaysia, commercialization of domestic mango cultivars, especially Chokanan has reached worldwide market as they are exported to Singapore, Brunei and Hong Kong. The increasing demand for this cultivar is due to its vibrant colour, exotic flavour, distinctive taste, pleasant aroma and nutritional properties (Arauz, 2000; Agri-food Business Development Centre, 2010).

There is a large stock of Chokanan mango yearly as it has two more harvests in June and August apart from the main harvest in May. This is due to its ability to yield off-season flowering without chemical initiation, in contrast of most mango varieties (Spreer *et al.,* 2009). Thus, the market for value-added mango products such as juice, puree, and nectar has progressively grown due to its perishable nature (Loelillet, 1994). According to a study conducted by Rivera and Cabornida (2008), fruit juices have the highest acceptability among other beverages, generally due to their natural taste, as well as to the nutritional value associated with them. Consumption of mango juice has been linked to the prevention of cardiovascular diseases and cancer, owing to its antioxidant properties (Block *et al.,* 1992; Liu, 2003).

The number of outbreaks and cases of illness caused by consumption of contaminated juices, especially unpasteurized juices has increased over the last decade. According to Centre for Disease Control and Prevention (1996), one of the current foodborne disease outbreaks have been linked to pathogens such as *Escherichia coli*, where the emphasis was on unpasteurized juices. Currently, conventional thermal treatment is the preferred technology to inactivate microorganisms and enzymes causing spoilage, thus prolonging the shelf life of juice. Due to the relatively high temperatures generally needed to inactivate food-poisoning and spoilage microorganisms, thermal treatment can adversely affect the quality of food products, by reducing their nutritional value and altering sensory attributes, such as colour and flavour (Rawson *et al.,* 2011). In addition, some studies on thermally treated fruit juices such as orange (Cortes *et al.,* 2008), strawberry (Aguilo-Aguayo *et al.,* 2009) and watermelon (Zhang *et al.,* 2011) reported significant loss of quality and degradation of bioactive compounds such as ascorbic acid.

The growing interest for fresh-like products has promoted the effort for developing innovative non-thermal food preservation methods. Non–thermal processing techniques have been explored for their efficacy to extend shelf life and enhance safety of fresh juice while preserving organoleptic and nutritional qualities (Morris *et al.,* 2007). These technologies include sonication and short-wave UV-C light treatment, which are emerging technologies that achieve the U.S. Food and Drug Administration (FDA) condition of a 5 log reduction of food borne pathogens in fruit juices (Salleh-Mack and Roberts, 2007; FDA, 2000).

Propagation of high power ultrasound at low frequencies (20–100 kHz) in liquid causes cavitation (formation and collapse of bubbles). Consequently, these 'tiny hotspots' provide energy to disrupt microbial cell membrane and alter the properties of food (O'Donnell *et al.,* 2010). Several studies using ultrasonic treatment on fruit juice reported minimal effect on the degradation of quality parameters and improved functionalities such as in orange (Tiwari *et al.,* 2008), blackberry (Tiwari *et al.,* 2009a), kasturi lime (Bhat *et al.,* 2011a), apple (Abid *et al.,* 2013) and carrot juice (Jabbar *et al.,* 2014).

The UV-C light (peak emission at 254 nm) exhibits germicidal effect by preventing the reproduction of microorganisms, and eventually may result in cell death (Guerrero-Beltran and Barbosa-Canovas, 2004). Several studies using short-wave UV-C light treatment on fruit juices reported minimal changes in nutritional and quality attributes, and significant microbial inactivation, such as in starfruit (Bhat *et al.,* 2011b), watermelon (Zhang *et al.,* 2011), and orange juice (Pala and Toklucu, 2013).

Sonication and UV-C treatment are simple, reliable, and cost-effective with improved efficiency (O'Donnell *et al.,* 2010; Pala and Toklucu, 2013). These technologies have different mode of microbial inactivation, therefore being potential choices for a hurdle concept. The hurdle technology is a combination of preservation techniques at lower individual intensities that may have an additive or, even, a synergistic effect on microbial destruction, with minimal impact on the quality of the food product (Leistner, 2000). Some studies have demonstrated that fruit juices were successfully preserved by combining non-thermal technologies (Noci *et al.,* 2008, Walkling-Ribeiro *et al.,* 2008).

In this study, Chokanan mango juice will be subjected to thermal and non-thermal processing (ultrasonic and UV-C) as a stand-alone. In addition, the combination of ultrasonic and UV-C treatment will also be studied. Generally, the impact on product quality has received less attention than microbial stability and safety aspects with regards to thermal and non-thermal technologies (as a stand-alone or combination). Hence, a comprehensive approach is needed to understand the effects of processing procedure on the overall quality of the final product. This information is necessary to improve the progress of positive implementation of novel processing methods in the juice industry.

In order to establish a complete quality profile of thermal and non-thermal treated Chokanan mango juice, various quality parameters will be analysed including microbial inactivation, physicochemical properties (pH, total soluble solids and titratable acidity), colour, clarity, browning index, hydroxymethyl furfural content, total carotenoid and ascorbic acid content, antioxidant activities, sensory attributes. Besides that, individual phenolic compounds in Chokanan mango juice will be identified and quantified to provide a better understanding on the effects of processing on specific bioactive compounds. The final part of this study will be focusing on the shelf-life analysis of treated and non-treated Chokanan mango juice during storage at 4 °C.

Hence, this study aims to answer the following questions:

1) Does thermal treatment affect the quality attributes and shelf-life of Chokanan mango juice?

2) Does ultrasonic treatment (as a stand-alone) affect the quality attributes and shelf-life of Chokanan mango juice?

3) Does UV-C treatment (as a stand-alone) affect the quality attributes and shelf-life of Chokanan mango juice?

4) Does the combination of ultrasonic and UV-C treatment affect the quality attributes and shelf-life of Chokanan mango juice?

Correspondingly, the objectives of this study are:

1) To evaluate the effects of thermal treatment on the quality attributes and shelf-life of Chokanan mango juice.

2) To evaluate the effects of ultrasonic treatment (as a stand-alone) on the quality attributes and shelf-life of Chokanan mango juice.

3) To evaluate the effects of UV-C treatment (as a stand-alone) on the quality attributes and shelf-life of Chokanan mango juice.

4) To evaluate the effects of combined treatment (ultrasonic and UV-C) on the quality attributes and shelf-life of Chokanan mango juice.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION TO MANGO (*Mangifera indica* L.)

In the global market, the production of mango (*Mangifera indica* L.) ranks fifth among other major fruit crops, including bananas, citruses, grapes and apples (FAOSTAT, 2014). Mango has become an important fruit crop in tropical and subtropical regions, predominantly in Asia due to its wide range of adaptability (Nakasone and Paull, 1998). This tropical fruit has been commonly known as the 'king of fruits'. Mango belongs to the genus *Mangifera* in the family Anacardiaceae (cashew family). There are numerous species in the genus that bear edible fruits, especially *M. indica.* Mostly, other edible *Mangifera* species are referred to as wild mangoes and found in India, Sri Lanka, Bangladesh, Thailand, Vietnam, Myanmar, Laos, Southern China, Indonesia, Malaysia, Singapore, Brunei, the Philippines, Papua New Guinea, and the Solomon and Caroline Islands (Bompard and Schnell, 1997; International Tropical Fruits Network, 2008).

Mango was originated from Southeast Asia (Indo-Burmese region) and has been cultivated for at least 4,000 years. Its cultivation has spread to Malaysia, Eastern Africa, and Eastern Asia (Mitra and Baldwin, 1997). In Malaysia, mangoes are mainly found in Peninsular Malaysia, where the production is focused in Perlis, Kedah, Perak, Negeri Sembilan and Malacca (Department of Agriculture, 2009). According to Jedele *et al.* (2003), mango plays a key role in the global trade as it constitutes approximately 50% of all tropical fruits produced worldwide. Therefore it is a valuable and economically important tropical fruit.

2.1.1 Botanical description and cultivars

The diversity of the family Anacardiaceae consists of almost 73 genera and 600 to 850 species. The genus *Mangifera* comprise of 69 species which is native to tropical Asia. The nomenclature of *M. indica* is as follows (Singh, 1960; Bompard and Schnell, 1997):

Kingdom Plantae (Plants)

Subkingdom Tracheobionta (Vascular plants)

Superdivision Spermatophyta (Seed plants)

Division Magnoliophyta (Flowering plants)

Class Magnoliopsida (Dicotyledons)

Subclass Rosidae

Order Sapindales

Family Anacardiaceae (Cashew family)

Genus *Mangifera* L.

Species *Mangifera indica* L.

A mango fruit is classed as a drupe, which is fleshy with a single seed bounded in a fibrous endocarp (Figure 2.1). There is a great variation in shape, size, weight, colour, and quality of fruit depending on its cultivar. The shape varies between nearly round, oval, ovoid-oblong or elongated. While, fruit length ranges from 2.5 to more than 30 centimetres. Fruit weight varies from less than 50 grams to over 2 kilograms (Mukherjee and Litz, 2009). Mesocarp is the edible part of the fruit (Figure 2.1) with variable thickness and usually is sweet. When ripe, the pulp colour differs from yellow to orange. In addition, the peel colour is dark green when developing on the tree and turns lighter green to yellow or red as it ripens (Bally, 2006).

Figure 2.1: Longitudinal section of a mango fruit

(Source: Armstrong, 2004)

There are approximately 1000 different mango cultivars throughout the world, however only 800 cultivars have been named. The large number of cultivars is due to different climates, geological characteristic, harvest period, and marketing season of each mango growing country. Hence, each country usually has its own major cultivars for commercial use (Pandey, 1986; Nakasone and Paull, 1998).

Mango cultivars could be categorized into two groups, which is Indian or Indo-Chinese. These groups have distinct features such as peel coloration, seed type, sensory characteristics, and resistance to fruit diseases, especially anthracnose. Most of the Indian varieties have seeds with one embryo (monoembryonic), whereas Indo-Chinese varieties have seeds with multiple embryos (polyembryonic). Furthermore, Indian varieties have more intense peel colouration and less resistance to anthracnose when compared to Indo-Chinese varieties (Crane *et al.*, 1997).

In Malaysia, there are about 216 clones or cultivars of mango, but only a few cultivars are recommended for commercial planting. These cultivars include Chokanan, Harumanis, Sala, Mas Muda, Siam Panjang, and Maha 65 (Agri-food Business Development Centre, 2010).

2.1.2 Economic importance

According to FAOSTAT (2014), about 72% of global production of mango is concentrated mainly in Asia, thus contributing approximately 30.2 million metric tonnes (MT) to the international market. India being the leading producer of mango has about 15.3 million MT from a total cultivated area of 2.3 million hectares (Ha) in 2012. Apart from India, other countries such as China, Thailand, Pakistan, Mexico, Indonesia, Brazil and the Philippines are also among the top mango producers. Mangoes are grown in 94 countries, resulting in an estimated worldwide production of about 42.1 million MT in 2012. Between 2009 and 2012, there was a 21% upturn in production of mango globally, highlighting its commercial value in the international commodity market. Currently, the total area cultivation for mango worldwide is about 5.2 million Ha (FAOSTAT, 2014).

Mexico ranks first in mango export contributing about 0.29 million MT (valued at US$ 715 per tonne) to the global market, followed by India (0.23 million MT) and Thailand (0.15 million MT) in 2011. Moreover, the world's largest mango importing country is the United States of America (USA), which is estimated 0.37 million MT (valued at US$ 901 per tonne). The European Union (EU) including Netherlands, Germany and United Kingdom, and Saudi Arabia are among the top mango importers. The mango

market in USA has progressively grown in response to increasing demand (FAOSTAT, 2014).

In Malaysia, commercialization of domestic mango cultivars, especially Chokanan has reached the worldwide market as they are exported to Singapore, Brunei and Hong Kong (Agri-food Business Development Centre, 2010). Other potential markets that can be developed are USA, EU, United Arab Emirates, China, Japan and Netherlands. FAOSTAT (2014) reported that the production of mango in Malaysia has increased approximately 11% from 67.7 thousand MT in 2011 to 75.1 thousand MT in 2012. Similarly, the total area of cultivation has increased approximately 5.5% from 14.5 thousand Ha in 2011 to 15.3 thousand Ha in 2012.

2.2 CHOKANAN MANGO

Mangifera indica L. cv. Chokanan (also called MA224), is mostly found in Malaysia and Thailand. It is one of the most popular cultivar grown in Malaysia for local and export market. The production of this cultivar is focused in Perlis, Kedah, Perak, Negeri Sembilan, and Malacca. Chokanan mango is also known as ‘honey mango’ in the market due to its succulent sweet taste. The increasing demand for this cultivar is due to its vibrant colour, exotic flavour, distinctive taste, pleasant aroma and nutritional properties (Arauz, 2000; Agri-food Business Development Centre, 2010).

The average weight of the fruit is 300 gram, which is considered as medium-sized. It has an oblong shape (bigger at the top and narrow at the bottom), golden yellow skin and yellowish orange pulp. The best stage for fresh consumption is when the fruit is ripe, where the peel colour is fully yellow (Figure 2.2). At this stage the pulp is firm, slightly fibrous, and has high total soluble solids (14 to 17 °Brix), with a pleasant aroma (Agri-food Business Development Centre, 2010).

Chokanan mango bears fruit continuously even during rainy season. Another desirable characteristic of this cultivar is that it can tolerate adverse weather conditions, thus the flowers develop into quality eating fruits even without the need for spraying fungicide and insecticide. Moreover, it has a longer shelf life as it is resistant to fruit fly attack due to its thick peel. This dwarf mango can be used for high density planting due to its fairly free flowering habit (The Philippine Star, 2005; Department of Agriculture, 2009).

According to Spreer *et al.* (2009), there is a large stock of Chokanan mango every year as it has two more harvests in June and August apart from the main harvest in May. This is due to its ability to yield off-season flowering without applying chemicals (potassium nitrate) for initiation, in contrast of most mango varieties. Hence, this characteristic allows the fruit to be processed into products including juice, nectars, puree, pickles, and canned slices that are globally accepted (Loelillet, 1994).

Figure 2.2: Ripe Chokanan mango

2.3 COMMERCIAL VALUE OF MANGO AND MANGO JUICE

Mango is filled with minerals, organic acids and vitamins, depending on the various cultivars and maturity indices. Ripe mango pulp is a good source of beta-carotene (pro-vitamin A) and vitamin B1, B2, as well as a fair source of vitamin C. In addition, the fruit is a rich source of carbohydrates and dietary fiber. Mango pulp has low levels of saturated fat, cholesterol and sodium. Organic acids predominantly citric and malic acid contribute to the fruit acidity (Singh *et al*., 2013; USDA, 2014).

Several studies have reported phytochemical compounds in mango, especially polyphenolics, including ellagic acid, gallic acid, quercetin, isoquercetin, catechin, epicatechin, chlorogenic acid, mangiferin, and kaempferol (Schieber *et al.*, 2000; Berardini *et al.*, 2005; Masibo and He, 2008; Poovarodom *et al.*, 2010). Some of these bioactive compounds have been linked to the prevention of cardiovascular diseases and

cancer, owing to its antioxidant properties (Block *et al.*, 1992; Liu, 2003). Furthermore, mango has also been reported to exhibit anti-inflammatory (Garrido *et al*., 2004) and anti-allergic (Rivera *et al.,* 2006) properties.

The market for value-added mango products such as juice has progressively grown due to its perishable nature and limited shelf-life (Liu *et al*., 2014). According to a study conducted by Rivera and Cabornida (2008), fruit juices have the highest acceptability among other beverages, generally due to their natural taste, as well as to the nutritional value associated with them. Therefore, consumption of mango juice could provide substantial dietary source for consumers.

Generally, consumers are not aware about the differences between juices, nectars and fruit drink. Products labelled as 'fruit juice' must contain 100% juice obtained from the fruit. These products contain no preservatives, sweeteners and artificial colouring. This form of juice may or may not contain the fruit pulp and is often categorised as 'not from concentrate'. However, if the fruit juice is concentrated for transportation and then reconstituted using the same amount of water, then it is categorised as 'made from concentrate' With regards to products labelled as 'fruit nectar', they contain lesser volume of fruit juice (30 to 99%) and may include preservatives, sweeteners and artificial colouring. Products labelled 'fruit drink' are similar to nectars, except they contain less than 29% fruit juice (Food Standard Agency, 2007; Neves *et al.,* 2011).

Business Insights (2010), reported that the global market for juices valued about US$ 79 billion in 2009 and is estimated to reach a value of US$ 93 billion in 2014. Fruit beverages are the largest juice category accounting for 23% share in 2009, as shown in

Figure 2.3. Moreover, the market for 100% fruit juice (not from concentrate) is expected to outperform all the other categories of juices to reach the highest market value of US$ 27 billion (accounting for 29% share) in 2014. The key driver for the growth of fruit juice market is the increase in awareness among consumers on preventive healthcare and wellness benefits.

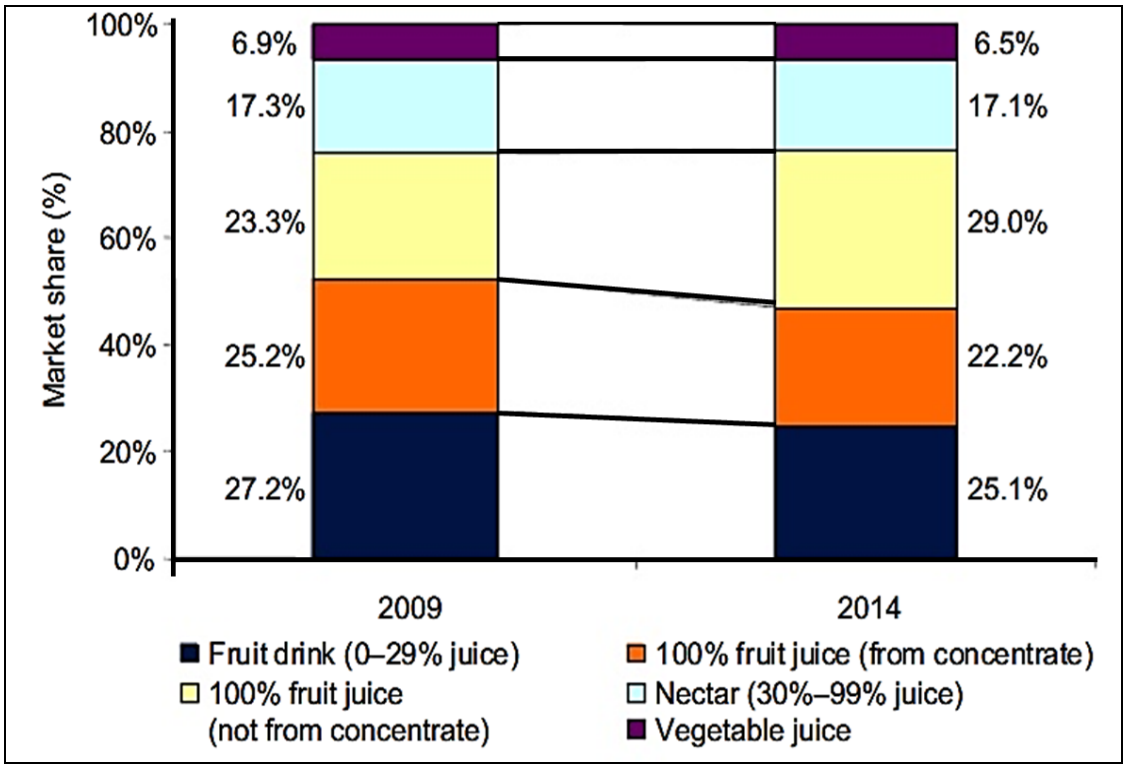


Figure 2.3: Juices market value (%) by category, from 2009 to 2014

(Source: Business Insights, 2010)

2.4 ANTIOXIDANTS AND OXIDATIVE STRESS

Oxygen is essential for living organisms as its oxidative mechanism is important for cell survival in the body. However, some oxygen molecules may not be completely reduced in the body, thus forming free radicals and other reactive oxygen species (ROS) such as peroxyl radical ($ROO^{\cdot}$), hydroxyl radical ($^{\cdot}OH$), singlet oxygen ($^1O_2$) and superoxide ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO) (Nordberg and Arner, 2001). Generally, these compounds are formed in the human body as a result of environmental factors such as radiation, tobacco smoke, chemical additives in processed foods and industrial pollution, although they can occur naturally during metabolism (Mendoza Perez and Fregoso Aguilar, 2013).

When the accumulation of free radicals and other ROS exceeds the antioxidant defence of cells, they cause damaging effects known as oxidative stress. Oxidative stress can induce damage to biological molecules such as proteins, lipids and DNA, due to the properties of free radicals and ROS that are potentially toxic, mutagenic and carcinogenic. Recently, oxidative stress has been linked to accelerated aging process as well as development of a variety of diseases such as atherosclerosis, diabetes, asthma, cancer, immunodepression, heart disease and kidney damage (Nordberg and Arner, 2001; Shahidi and Naczk, 2004; Romero *et al.*, 2013).

Antioxidants are synthetic or natural substances that protect against the harmful effects of free radical and ROS by different mechanisms such a scavenging hydroxyl radicals, chelation of metal ions, and converting primary products of oxidation to nonradical forms. As a whole, antioxidant prevents lipid oxidation, DNA mutation and formation of protein cross-linkages, thus lowering the risks of physiological and pathological abnormalities. They are normally present in the body or in foods at low concentration compared with the biomolecules that they should protect (Shahidi and Naczk, 2004; Alothman *et al.*, 2009; Romero *et al.*, 2013).

Antioxidants are classified into two groups, namely endogenous and exogenous. Endogenous antioxidants are naturally produced in cells, such as superoxide dismutase, superoxide reductase, glutathione and catalase. While, exogenous antioxidants are obtained through dietary sources and supplements (Romero *et al.*, 2013). Alternatively, antioxidants are categorized as hydrophilic (water soluble) and hydrophobic (lipid soluble). Hydrophilic antioxidants function in the blood plasma and cell cytoplasm by reacting with oxidants. On the other hand, hydrophobic antioxidants protect cell membranes against lipid peroxidation (Mendoza Perez and Fregoso Aguilar, 2013).

2.5 PHYTOCHEMICALS

Several epidemiological studies have shown that consumption of fruits and vegetables play a vital role in the maintenance of human health, mainly due to the presence of various phytochemicals. Phytochemicals are biologically active secondary metabolites in plants that are non-essential nutrients but possess a protective role against disease in the human body. These compounds are commonly referred to as nutraceutical (Kalt, 2001; Oms-Oliu *et al.,* 2012). It is well known that plant metabolism is divided into two groups, namely primary and secondary. The primary metabolism is essential for cell maintenance (metabolism of lipids, proteins, carbohydrates, and nucleic acids). Alternatively, the secondary metabolism results in several biosynthetic pathways generating substances that is restricted to determined groups of organism (Giada, 2013).

Plant derived phytochemicals are linked to various health-promoting properties such as protection against several chronic human diseases such as cancer, cardiovascular diseases and diabetes. The positive effects of most phytochemicals are attributed to their antioxidant activity, although there are other mechanisms such as increased activity of enzymes that detoxify carcinogens, alteration of estrogen metabolism and maintenance of DNA repair (Oms-Oliu *et al.,* 2012; Tiwari and Cummins, 2013).

Antioxidant phytochemicals are widely known for their inhibitory effects against propagation of free radicals (Bae and Suh, 2007). The major group of such phytochemicals includes polyphenols, carotenoids, and the traditional antioxidant vitamins such as ascorbic acid (vitamin C) and alpha-tocopherol (vitamin E) (Lako *et al.,* 2007).

2.5.1 Phenolic compounds

Secondary metabolites that are widely distributed in plants are largely comprised of phenolic compounds. These compounds are derived from phenylalanine and tyrosine. Phenolic compounds are mostly involved in plant defence against pathogenic attack as well as protective agents against ultraviolet radiation. In addition, they contribute to pigmentation and function as structural materials for plant stability. Besides that, these compounds are involved in sensorial attributes (colour, aroma, astringency and taste) and pollination of plants (Shahidi and Naczk, 2004; Manach *et al.*, 2004; Giada, 2013).

They can be characterised as substances possessing one or more hydroxyl groups attached to an aromatic ring (benzene). Another common characteristic of these compounds is that they are presented generally bound to other molecules, such as sugars (glycosyl residue) and proteins. However, they exist in their free form in plant tissues. Phenolic compounds can be classified into several groups including simple phenols, phenolic acids, flavonoids, stilbenes, tannins, lignins and lignans (Manach *et al.,* 2004; Giada, 2013).

Classification of phenolic compounds can be done in numerous ways because they are chemically organized in various heterogeneous structures. Initially, these compounds were classified according to ‘common’ and ‘less common’ groups. An alternative classification was suggested by Harborne and Simmonds (1964), based on the number of carbon in the molecules, as shown in Table 2.1. In addition, phenolic compounds were categorized into three specific groups, namely: (1) widely distributed phenols (simple phenols, hydroquinone and pyrocatechols); (2) less widely distributed phenols

(phenolic acids and flavonoids); and (3) phenolic constituents present as polymers (tannin and lignin) (Manach *et al.,* 2004; Giada, 2013).

Last but not least, these compounds were classified according to ‘soluble’ and ‘insoluble’ groups. Phenolic compounds that are not bounded to membrane components in plants, for example simple phenols and flavonoids are categorized as ‘soluble’. While, compounds such as condensed tannins and phenolic acids that are bound to cell wall components (polysaccharide and protein), thus forming insoluble complexes are categorized as ‘insoluble’ (Giada, 2013).

Phenolic compounds found in plants are gaining interest among researchers as a natural antioxidant. Their antioxidant activity is related to their chemical structures, particularly the reaction of aromatic ring with free radicals. Some phenolic compounds act as reducing agents, while some possess hydrogen donating ability and metal ion chelation (Ashokkumar *et al.,* 2008). In addition, these compounds possess pharmacological properties, thus allowing them to be used with therapeutic purposes (Giada, 2013).

Table 2.1: Main classes of phenolic compounds according to their carbon chain

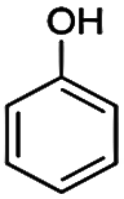
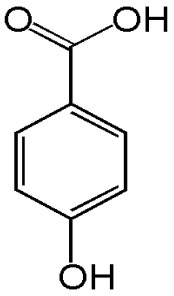
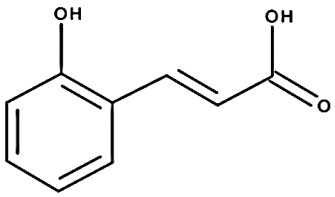
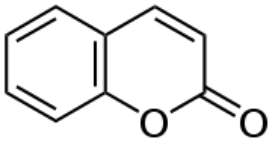
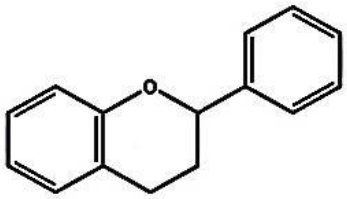
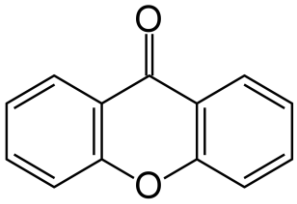
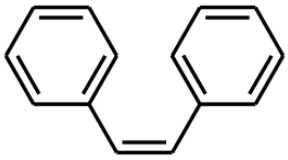
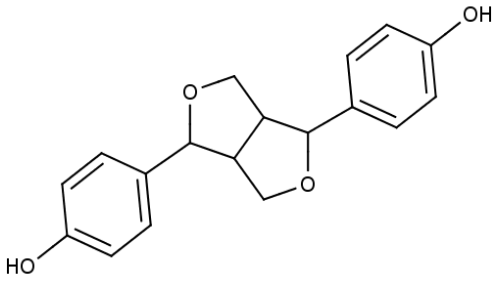
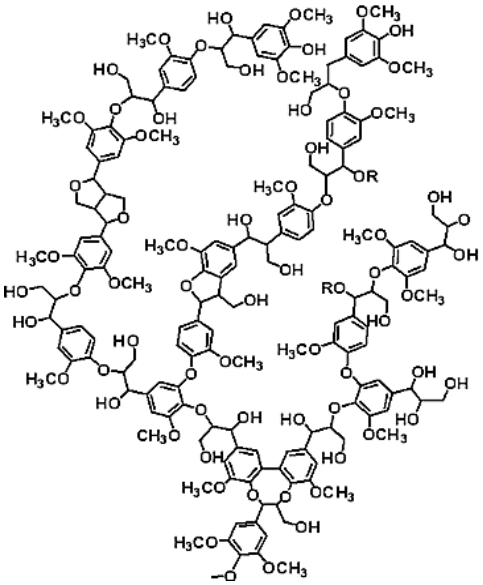
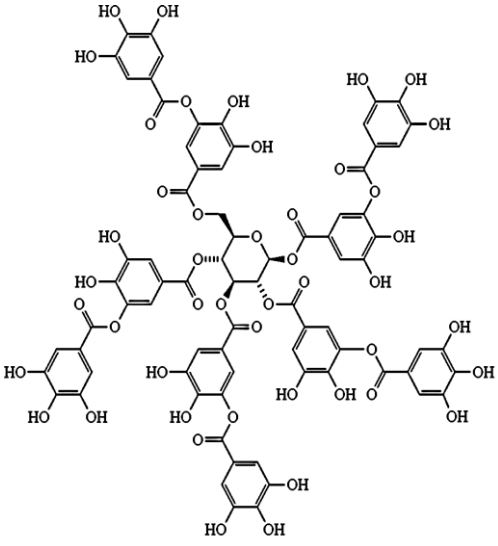
Basic skeleton (carbon)	Class	Basic structure
C_6	Simple phenols	
C_6-C_1	Phenolic acids	
C_6-C_3	Hydroxycinnamic acids	
	Coumarins, isocaumarins	
C_6-C_3-C_6 (C_{15})	Flavonoids (flavans, flavanones, flavones, isoflavones, anthocyanidin.	
C_6-C_1-C_6	Xanthones	
C_6-C_2-C_6	Stilbenes	

Table 2.1, continued

Basic skeleton (carbon)	Class	Basic structure
Oligomers $(C_6\text{-}C_3)_2$	Lignans	
Polymers $(C_6\text{-}C_3)_n$	Lignin	
Oligomers and polymers	Condensed, hydrolysable and complex tannins	

(Source: Adapted from Manach *et al.*, 2004; Giada, 2013)

2.5.1.1 Phenolic acids

Phenolic acids are phenols that possess one carboxylic acid group, and have been related to colour, nutritional and antioxidant properties of foods (Robbins, 2003). Phenolic acids can be classified into two groups, namely derivatives of benzoic acid (hydroxybenzoic acid) and derivatives of cinnamic acid (hydroxycinnamic acid). Hydroxybenzoic acids are the simplest phenolic acids found in nature with seven carbon atoms (C_6-C_1). They are found in plants as free and esterified. On the other hand, hydroxycinnamic acids are more common in vegetables with nine carbon atoms (C_6-C_3). They are commonly found in plants as esters of quinic acid, shikimic acid, and tartaric acid (Yang *et al.*, 2001; Manach *et al*., 2004; Giada 2013). The general formula and examples of hydroxybenzoic and hydroxycinnamic acid are shown in Figure 2.4 (a) and (b), respectively.

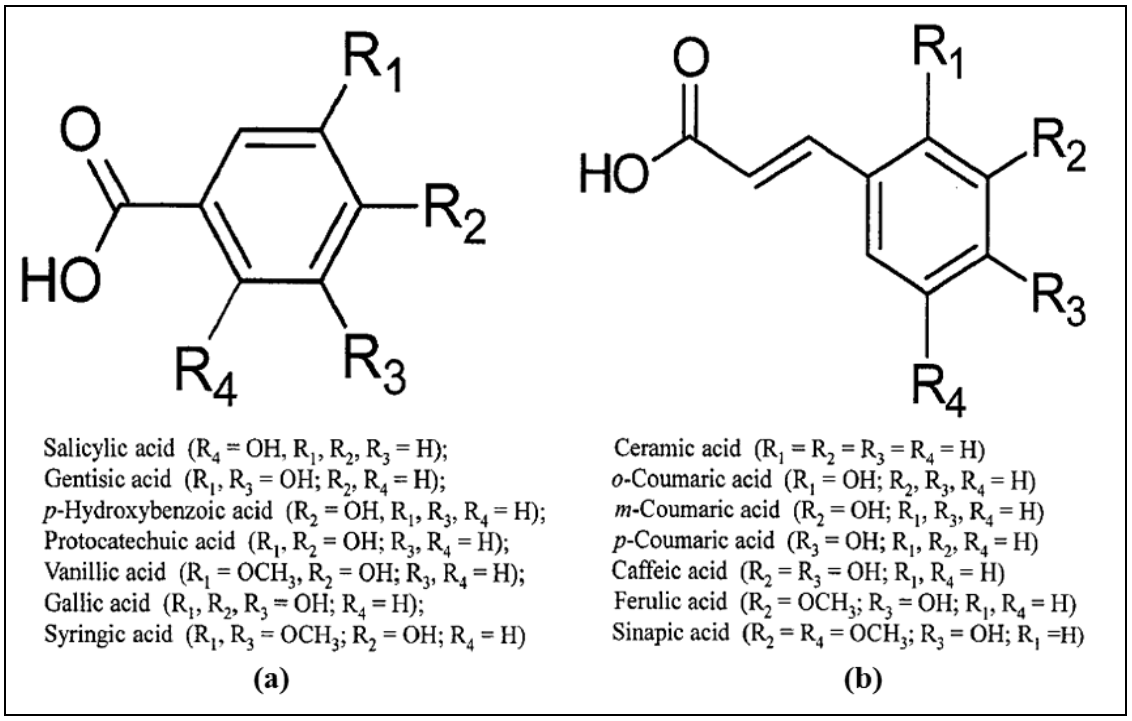


Figure 2.4: The general formula and examples of (a) hydroxycinnamic acid and (b) hydroxybenzoic acid

(Source: Giada 2013)

The main hydroxybenzoic acids include protocatechuic acid, vanillic acid, syringic acid, gentisic acid, salicylic acid, *p*-hydroxybenzoic acid and gallic acid. With regards to hydroxycinnamic acids, *p*-coumaric, ferulic, caffeic and sinapic acids are the most common in nature. Phenolic acids and their esters, especially hydroxybenzoic acid, hydroxycinnamic acid, caffeic acid and chlorogenic acid, are well known for their high antioxidant properties. The antioxidant activity is related to their chemical structures, particularly the number of hydroxyl group. Hence, hydroxylated cinnamic acids are more effective than hydroxylated benzoic acids (Manach *et al*., 2004; Giada, 2013).

2.5.1.2 Flavonoids

Flavonoids are the most common and widely distributed group of plant phenolics. These compounds share a common skeleton with diphenylpyrenes (C_6-C_3-C_6), thus having 2-phenyl-ring (A and B) and an oxygenated heterocycle (C), as shown in Figure 2.5. Flavonoids are divided into six groups depending on variations of heterocyclic C, which are flavones, flavonols, flavanones, flavanols, anthocyanidin or anthocyanins, and isoflavones. Some members of certain classes of flavonoids (for instance, flavonones) are colourless, while others (for instance, anthocyanins) are coloured, such as flower pigments (Shahidi and Naczk, 2004; Romero *et al.*, 2013). The basic chemical structures and examples of the main classes of flavonoids are presented in Table 2.2.

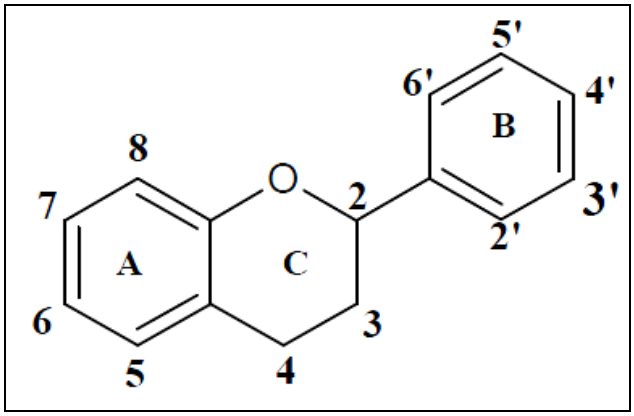


Figure 2.5: Basic structure of flavonoids

(Source: Shahidi and Naczk, 2004)

Table 2.2: Chemical structures and examples of the main classes of flavonoids

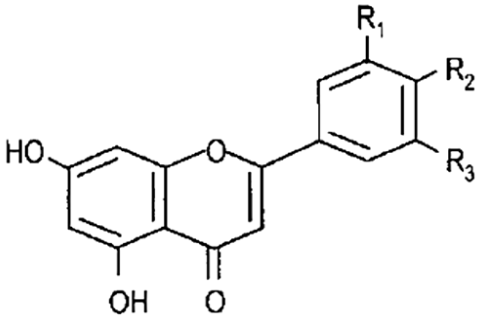
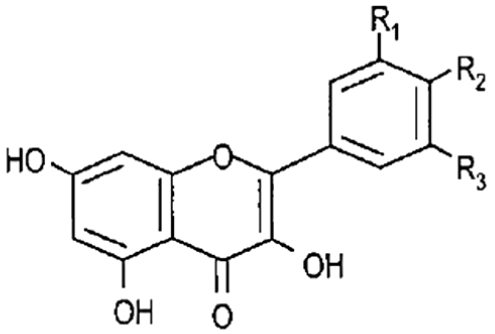
Class	Basic structure and examples
Flavones	 *R_1 = H; R_2 = OH : Apigenin* *R_1 = R_2 = OH : Luteolin*
Flavonols	 *R_2 = OH; R_1 = R_3 = H : Kaempferol* *R_1 = R_2 = OH; R_3 = H : Quercetin* *R_1 = R_2 = R_3 = OH : Myricetin*

Table 2.2, continued

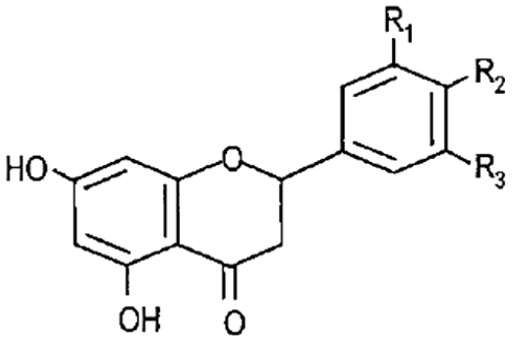
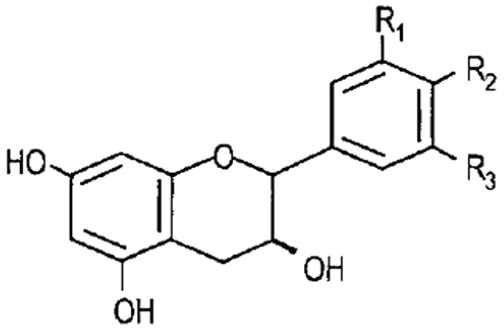
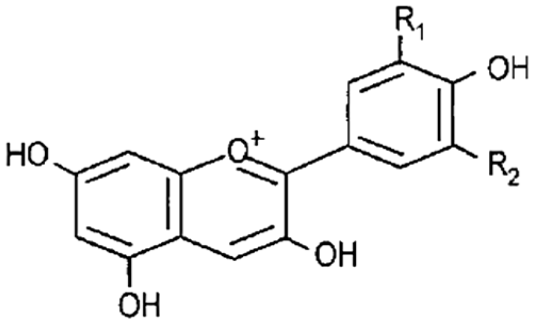
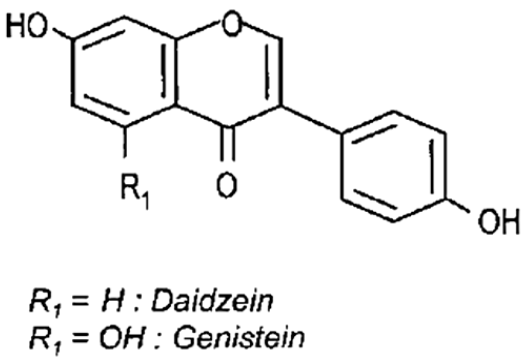
Class	Basic structure and examples
Flavanones	 *R_1 = H; R_2 = OH : Naringenin* *R_1 = R_2 = OH : Eriodictyol* *R_1 = OH; R_2 = OCH_3 : Hesperetin*
Flavanols	 *R_1 = R_2 = OH; R_3 = H : Catechins* *R_1 = R_2 = R_3 = OH : Gallocatechin*
Anthocyanidin / anthocyanins	 *R_1 = R_2 = H : Pelargonidin* *R_1 = OH; R_2 = H : Cyanidin* *R_1 = R_2 = OH : Delphinidin* *R_1 = OCH_3 ; R_2 = OH : Petunidin* *R_1 = R_2 = OCH_3 : Malvidin*

Table 2.2, continued

Class	Basic structure and examples
Isoflavones	 $R_1 = H$: Daidzein $R_1 = OH$: Genistein

(Source: Adapted from Manach *et al.*, 2004)

The antioxidant capacities of flavonoids depend on their chemical structures, especially their redox properties of their hydroxyl phenolic group. The antioxidant action of flavonoids is mainly attributed to their free radical quenching ability, metal ion chelation and capability to block the catalytic actions of free radicals (Romero *et al.*, 2013). In addition, numerous epidemiological studies support that consumption of foods rich in the flavonoids may reduce the risk of developing cancer (Middleton *et al.*, 2000; Manach *et al.*, 2004).

2.5.1.3 Tannins

Tannins are phenolic compounds with intermediate to high molecular weight and can be classified into two groups, namely hydrolysable and non-hydrolysable or condensed tannin. Hydrolysable tannins include gallotannins and elagitannins. They are readily hydrolysed by acids, bases or enzymes. In addition, these compounds may be oxidatively condensed to form polymers of high molecular weight. On the other hand, condensed tannins include dimers, oligomers and polymers of flavanols. They are also referred to as proanthocyanidins. These compounds are not readily hydrolysed by acid

treatment. In addition, they are responsible for the astringency in vegetables. The antioxidant capacities of tannins are related to their chemical structures, particularly the degree of polymerization (Shahidi and Naczk, 2004; Giada, 2013).

Since condensed and hydrolysable tannins are not absorbed by the mucosa, they are referred as insoluble antioxidants that exhibit high antioxidant capacities in the gastrointestinal tract, thus protecting lipids and proteins from oxidative damage during digestion. Besides that, tannins protect plants against microorganism attracts by inactivating aggressive enzymes (Romero *et al.*, 2013).

2.5.2 Carotenoids

Carotenoids are widely distributed natural pigments that contribute to the yellow, orange and red colours in plant based food. However in green plants, the colour of carotenoids is masked by chlorophylls. The colours of carotenoids depend on their conjugated double bonds and various functional groups (Khoo *et al.*, 2011). Carotenoids are synthesized in plants as accessory pigments for harvesting light and preventing photo-oxidative damage. In general, these compounds are symmetrical, linear tetraterpenoids, consisting of eight 5-carbon isoprenoid residues joined in two, 20 carbon units. Thus, all carotenoids have a basic carbon skeleton, C_{40}, as shown in Figure 2.6. The basic skeleton can be modified by changes in hydrogenation levels, isomerization, cyclization, rearrangement and addition of oxygen containing functional groups (Rodriguez-Amaya, 1997; Dutta *et al.*, 2005).

Carotenoids are classified as hydrocarbon and oxygenated carotenoids based on their chemical structure, as shown in Figure 2.7. Hydrocarbon carotenoids consisting of

carbon and hydrogen are termed carotenes. Alternatively, oxygenated carotenoids consisting of carbon, hydrogen and at least one oxygen molecule are termed xanthophylls. The unique feature of carotenoids is their polyene chain or known as extensive conjugated double bond system (Rodriguez-Amaya, 1997; Rodriguez-Amaya, 2001). This feature is responsible for their light absorbing properties and strong colouring capability. In order for carotenoids to impart colour, at least seven conjugated double bonds are required (Britton, 1995; Rodriguez-Amaya, 1997).

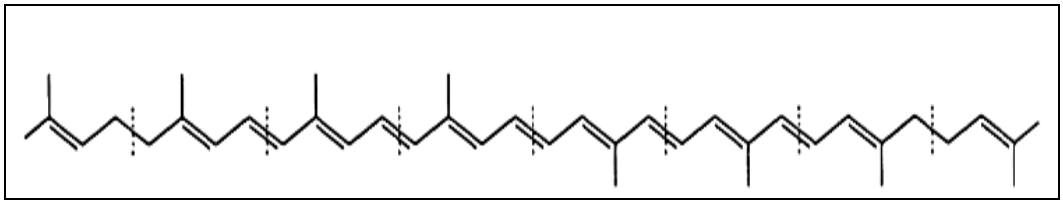


Figure 2.6: Basic carbon skeleton of carotenoids.

Broken lines (--) indicate formal division into isoprenoid units.

(Source: Rodriguez-Amaya, 1997)

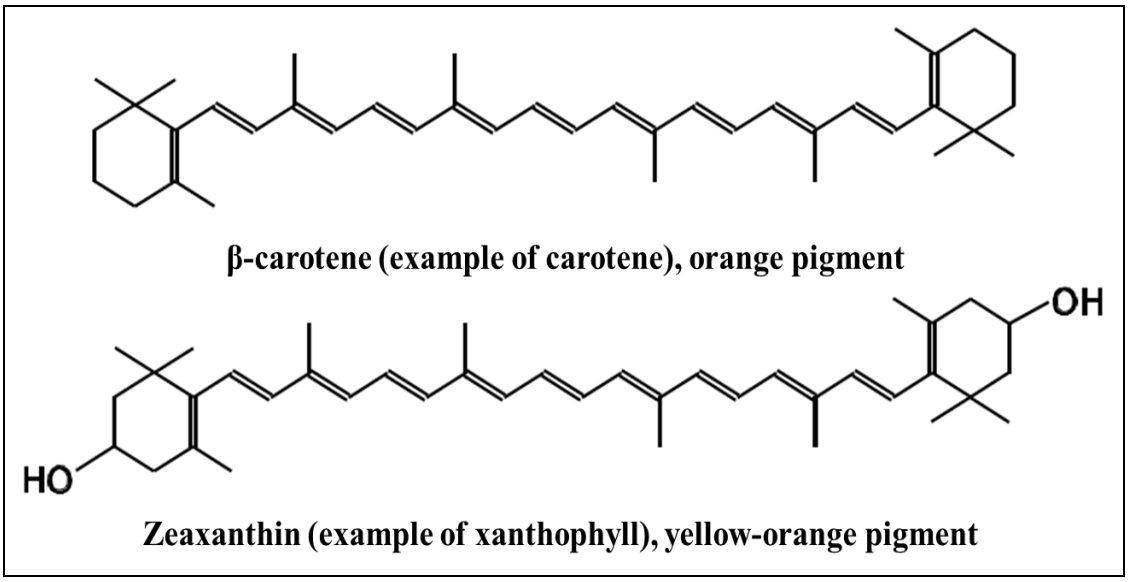


Figure 2.7: Chemical structure of hydrocarbon carotenoid (carotene) and oxygenated carotenoid (xanthophyll)

(Source: Adapted from Rodriguez-Amaya, 1997)

Carotenoids possess health promoting properties such as reduced risk of cardiovascular disease (Krinsky, 1990) and cancer (Ziegler, 1991). In addition, carotenoids have been linked to the enhancement of the immune system. Indeed, these compounds are well known for their provitamin A activity, which is involved in vision, synthesis of glycoprotein, and development of bones (Dutta *et al.*, 2005) The polyene chain of carotenoids provides a reactive electron system, thus contributing to their antioxidant capacity in quenching singlet oxygen and deactivating free radicals (Britton, 1995; Khoo *et al.*, 2011).

However, carotenoids are highly susceptible to degradation due to the instability of their conjugated double bond system, resulting in oxidation and geometric isomerization (conversion of *trans*-isomers to *cis*-isomers). External agents such as heat, light exposure, acids promote geometric isomerization of carotenoids. Most carotenoids occur in nature predominantly in *trans*-forms. Hence, geometric isomerization results in some loss of colour and provitamin activity. In addition, oxidation of carotenoids is stimulated by metals, heat, light exposure, enzymes and peroxides, resulting in formation of initial products known as epoxides (Rodriguez-Amaya, 1997; Dutta *et al.*, 2005).

2.5.3 Vitamin C

Vitamins are biologically active and possess no energetic value. These compounds are necessary for humans in very small quantities and should be supplied mainly by diet as humans are unable to synthesize most of the vitamins. Vitamin C is an antioxidant water-soluble vitamin that is vital for numerous biological functions. This substance is essential for the biosynthesis of collagen proteins and neurotransmitters, development of

teeth, bone and cartilage, and promotes resistance to infection. Vitamin C is a 6-carbon ketolactone that is found mainly in foods of plant origin in two chemically interchangeable forms known as ascorbic acid (reduced form) and dihydroascorbic acid (oxidated form), as shown in Figure 2.8 (Nordberg and Arner, 2001; Romero *et al.*, 2013).

Vitamin C exhibits antioxidant properties due to its electron donating ability, thus reducing the damage by free radicals and other ROS such as superoxide. In addition, these compound captures free radicals, preventing the chain reaction in oxidative stress (Mendoza Perez and Fregoso Aguilar, 2013). Besides that, it was found that vitamin C has been linked with protection against several types of cancers (Block, 1991).

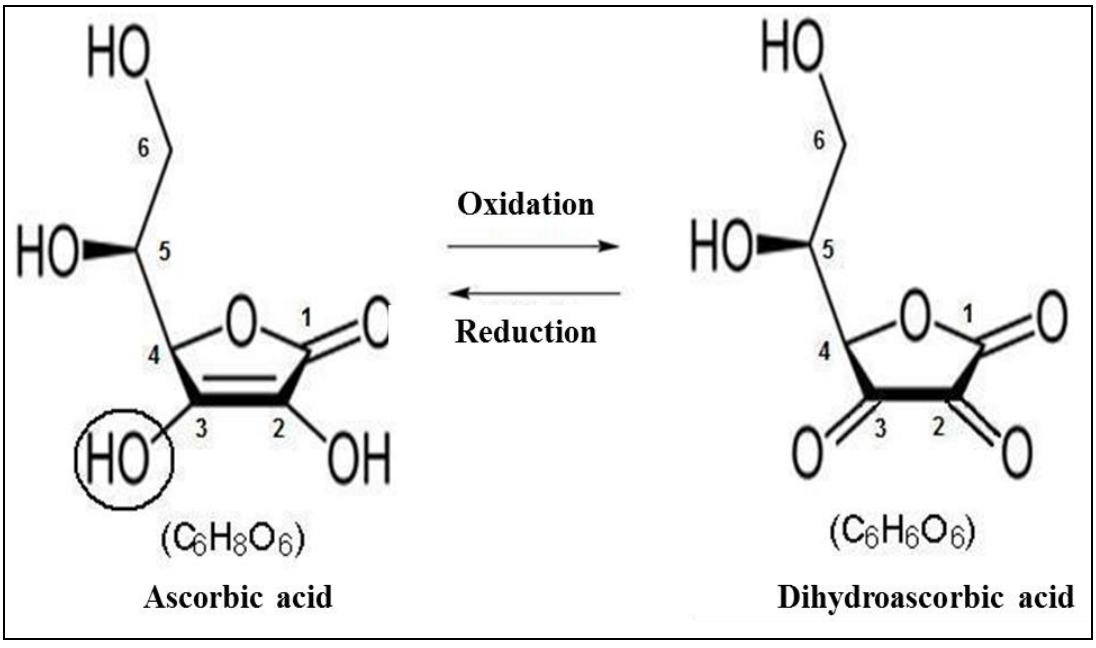


Figure 2.8: The redox reaction of vitamin C

(Source: Romero *et al.*, 2013)

2.6 MEASUREMENT OF ANTIOXIDANT ACTIVITY

Plant based food contain numerous compounds that contribute to their antioxidant activity. Since most natural antioxidants are multifunctional, thus more than one method is necessary to measure antioxidant activities according to their ability to scavenge specific radicals, to chelate metal ions and to inhibit lipid peroxidation. Colorimetric methods have been widely used to determine the presence of antioxidants, mostly in food extract (Martinez *et al.*, 2012).

The first example of antioxidant assay is the Folin-Ciocalteu assay. This assay is based on the detection of phenolic compounds by reduction of Folin-Ciocalteu reagent, which contains tungsten and molybdenum oxides. This results in the formation of a blue coloured chromogen under basic conditions, and can be characterized by an absorption band at 745 to 750 nm (Waterhouse, 2002). The method is simple, sensitive, and precise. However, the drawback of this assay is that it lacks specificity and detects all phenolic groups found in extracts including extractable proteins (Shahidi and Naczk, 2004). On the other hand, aluminium chloride colorimetric assay is used for the determination of flavonoids. In this assay, aluminium chloride form acid labile complexes with the ortho- dihydroxyl groups in the aromatic ring of flavonoids, and can be measured at 510 nm (Mabry *et al.*, 1970).

The radical scavenging activity can be determined by 1,1-di-phenyl-2-picrylhydrazyl (DPPH) assay. This assay measures the hydrogen donating capacity of the antioxidant to the stable free radical $DPPH^{\bullet}$, resulting in the formation of diphenylpicrylhydrazine. The $DPPH^{\bullet}$ radical (Figure 2.9a) bears a deep violet colour, and characterised by an absorption band at 517 nm. This reaction causes the decolourization of $DPPH^{\bullet}$ solution from violet to yellow, thus indicating the radical scavenging potential of the sample extract (Shon *et al*., 2003; Alam *et al.*, 2013). This method is simple, fast, and does not require any special preparations. However, $DPPH^{\bullet}$ radical is decolourized by other reducing agents, which also contributes to inaccurate interpretations of antioxidant activity. Besides that, another disadvantage of this assay is that it is strongly influenced by solvent system and pH (Prior *et al.*, 2005).

Similarly, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay measures the ability of antioxidants to scavenge the long-life radical cation $ABTS^{\bullet+}$. The $ABTS^{\bullet+}$ radical cation (Figure 2.9b) is a blue-green chromophore, and characterised by an absorption band at 734 nm. Antioxidants reduce $ABTS^{\bullet+}$ to ABTS, resulting in the decolourization of $ABTS^{\bullet+}$ solution. Hence, the degree of discolouration indicates the radical scavenging potential of the sample extract (Martínez *et al.*, 2012; Alam *et al.*, 2013). This method is simple, rapid, and can be used over a wide range of pH. However, one of the drawbacks of this assay is that it requires special preparation of $ABTS^{\bullet+}$ solution, unlike DPPH assay (Prior *et al.*, 2005).

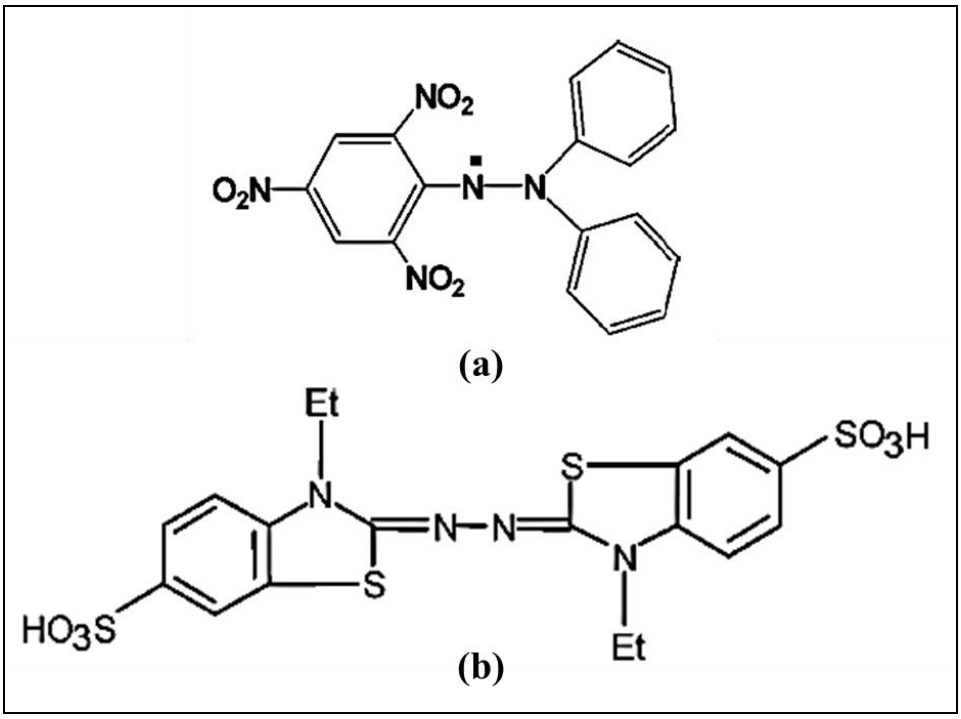


Figure 2.9: Structure of free radical (a) DPPH and (b) ABTS

(Source: Prior *et al.*, 2005)

Another example of antioxidant assay is reducing power assay. The reductive ability of a compound (Fe^{3+} to Fe^{2+} transformation) may serve as an important indicator of its potential antioxidant activity. The presence of antioxidants in the sample extract may cause the reduction of the ferricyanide complex to ferrocyanide complex in this assay. The Perl's Prussian blue coloured ferrous complex formed can be measured at 700 nm. The increase in the absorbance of the reaction mixture indicates an increase in antioxidant activity (Rama Prabha and Vasantha, 2011).

The phosphomolybdenum method or also known as total antioxidant capacity assay is used for the quantitative determination of antioxidant capacity. This assay is evaluated by the reduction of Mo (VI) to Mo (V) in an acidic medium. This results in the formation of green coloured Mo (V) phosphate complex that can be measure at 695 nm. This method is simple and cheap when compared to other antioxidant assays (Prieto *et al.,* 1999).

2.7 FRUIT JUICE DETERIORATION AND SPOILAGE

Freshly squeezed fruit juices are gaining popularity among consumers due to their fresh-like characteristics. However, inappropriate handling and storage conditions may cause fruit juice deterioration and spoilage. Natural fruit juices are susceptible to spoilage, mainly due to their intrinsic properties such as pH, water activity, redox potential, and nutrients (Odumeru, 2012).

Fruit juice deterioration is mostly caused by enzymatic, chemical and microbial reactions. Enzymes in fruit juices such as polyphenol oxidase and peroxidase may react with oxygen, thus contributing to juice browning and off-flavour (Bates *et al.*, 2001). In addition, chemical reactions such as Maillard reaction cause non-enzymatic browning of juices. In Maillard reaction, the carbonyl group of reducing sugars reacts with free amino group of amino acids to form intermediates. Subsequently, these intermediates react further to produce brown coloured pigments, which induce undesirable colour changes in juices (Bharate and Bharate, 2012).

The causal agents of microbiological spoilage of fruit juices can be bacteria, as well as yeast and moulds. However, yeast and moulds are the main spoilage agents due to the low pH of fruit juices (Raybaudi-Massilia *et al.*, 2009). The number of outbreaks and cases of illness caused by consumption of contaminated juices, especially unpasteurized juices are increasing over the last decade. According to Centre for Disease Control and Prevention (1996), one of the current foodborne disease outbreaks have been linked to pathogens, such as *Escherichia coli* O157:H7, where the emphasis was on unpasteurized juices. In addition, bacterial pathogens including *Campylobacter* spp., *Salmonella* spp. and *Listeria monocytogenes* are top cases of bacterial foodborne infections reported. Usually, foodborne illness resulting from severe infections such as salmonellosis and haemolytic uremic syndrome (HUS) require hospitalization. Infections by *E. coli* O157:H7 causes HUS, which results in kidney failure, abdominal cramps, and eventually death. While, salmonellosis is caused by *Salmonella* spp. resulting in diarrhoea, nausea, fever and abdominal pain (Odumeru, 2012).

Certain common moulds causing spoilage in fruit products include *Penicillium* spp., *Aspergillus* spp., *Eurotium* spp., *Alternaria* spp., *Cladosporium* spp., *Paecilomyces* spp., and *Botrytis* spp. Besides that, yeast pathogens including *Saccharomyces* spp., *Zygosaccharomyces* spp., *Candida* spp., and *Debaryomyces* spp. are reported to be involved in fruit product spoilage (Bates *et al.*, 2001; Raybaudi-Massilia *et al.*, 2009). The most commonly encountered spoilage microorganisms in fruit juices and beverages are summarised in Table 2.3.

Table 2.3: Spoilage microorganisms in fruit juices and beverages

Causal agents	Type of microorganism	Food products
E. coli O157:H7	Bacteria	Apple cider, apple and orange juice
L. monocytogenes	Bacteria	Apple cider and juice
Salmonella ser. Enteritidis	Bacteria	Orange, grapefruit, and lemonade juice
Salmonella ser. Typhimurium	Bacteria	Apple cider and orange juice
Clostridium spp.	Bacteria	Carrot and tomato juice
Saccharomyces cerevisiae	Yeast	Orange juice
Torulaspora delbruckii	Yeast	Orange juice and soft drinks
Paecilomyces fulva	Mould	Apple concentrate, grape and apple juice
Byssochlamys fulva	Mould	Apple, blueberry, and grape juice, fruit punch and soft drinks

(Source: Adapted from Bates *et al.,* 2001; Raybaudi-Massilia *et al.,* 2009)

Spoilage of fruit juice and related products as a result of microbial growth may contribute to physical and chemical changes in food products. These alterations include unacceptable flavour and odour, changes in colour and turbidity, gas production, and formation of slime (Jay and Anderson, 2001). Usually, growth of microorganism to high numbers is necessary before spoilage becomes noticeable. Hence, it is important to control the growth of spoilage organisms in order to inhibit microbial spoilage (Odumeru, 2012).

2.8 FRUIT JUICE PROCESSING AND QUALITY

The increase in outbreaks and cases of illness related to consumption of unpasteurized juices have urged the development of a more effective food safety control program, known as hazard analysis critical control point (HACCP) program. HACCP is a systematic approach to identify, assess and control microbiological, chemical, and physical hazards of public health concern (Odumeru, 2012). Currently, there are a number of fruit juice preservation technologies for controlling microbial growth and survival. These preservation methods must be evaluated to avoid significant organoleptic changes in food products. Hence, the main objective of fruit juice processing is to prevent microbiological spoilage while assuring safety and maintaining quality characteristics. Fruit juice processing technologies can be divided into two groups, namely thermal and non-thermal processing (Bates *et al.*, 2001).

2.8.1 Thermal processing

Conventional thermal treatment is the preferred technology to inactivate microorganisms and enzymes causing spoilage, thus prolonging the shelf life of juice. Traditional thermal processing depends on the generation of heat outside the product to be heated and its transfer into the product via convection and conduction mechanisms (Pereira and Vicente, 2010). Pasteurization is an example of thermal treatment that is commonly practiced in the food industry. There are two types of pasteurization methods, which include low temperature long time (LTLT) and high temperature short time (HTST). LTLT or also known as batch pasteurization was initially established for the preservation of milk, whereby it is conducted at 63 °C for 30 min. The most

common pasteurization method for fruit juice is high temperature short time (HTST), or known as flash pasteurization (David *et al.*, 1996).

According to Nagy *et al.* (1993), HTST treatment for fruit juices range from 90 °C to 95 °C for 15 to 60 seconds to assure at least 5 log reduction in microbial count. This method is faster and efficient than LTST. However, the short holding time of HTST may require special equipment, thus it is less cost-effective than LTST (David *et al.*, 1996; Osaili, 2012).

The time and temperature variables for pasteurization of juice depend on the type of juice, initial microbial count, pH, water activity, and thermal inactivation kinetics of microorganisms present in juice. Hence, pasteurization conditions should be selected appropriately to avoid over-processing. Besides that, under-processing may not completely inactivate microorganism growth, thus resulting in juice spoilage (Rawson *et al*., 2011; Osaili, 2012).

Heat causes adverse effects on microbes by damaging organic molecules (nucleic acids and proteins) required for the proper functioning of cells, thus inactivating microbial growth. Due to the relatively high temperatures generally needed to inactivate food-poisoning and spoilage microorganisms, conventional pasteurisation can adversely affect the quality of food products, by reducing their nutritional value or altering sensory attributes, such as colour and flavour (Rawson *et al.*, 2011). Some studies on thermally treated fruit juices such as orange (Cortes *et al.*, 2008), strawberry (Aguilo-Aguayo *et al.*, 2009) and watermelon (Zhang *et al.*, 2011) reported significant loss of quality and degradation of bioactive compounds such as ascorbic acid. In addition,

Rattanathanalerk *et al.* (2005) reported significant colour degradation in thermal treated pineapple juice (at 85 and 95 °C for 60 seconds). Similarly, Pala and Toklucu (2013) observed significant loss of phenolic compounds in orange juice subjected to HTST treatment (at 90 °C for 2 minutes).

2.8.2 Non-thermal processing

The growing interest for fresh-like products has promoted the effort for developing innovative non-thermal food preservation methods. Non–thermal processing techniques have been explored for their efficacy to extend shelf life and enhance the safety of fresh juice while preserving organoleptic and nutritional qualities. In addition, these preservation methods are considered to be more energy efficient and provide better retention of quality when compared to conventional thermal processing. Some of the non-thermal processing methods extensively studied for juice preservation include high hydrostatic pressure, pulsed electric field, ionizing radiation, dense phase carbon dioxide, ozone, ultrasound, and ultraviolet light irradiation (Morris *et al.*, 2007). These treatments are classified as ‘non-thermal’ as they eliminate the use of high temperature to achieve microbial inactivation, thus avoiding the adverse effects of heat on physical, nutritional, and sensory properties of foods. However, some of these treatments, such as high pressure and pulsed electric field may involve heat due to generation of internal energy (Pereira and Vicente, 2010).

2.8.2.1 High hydrostatic pressure (HHP)

HHP or also known as ultra-high pressure is a cold pasteurization technique that entails the transmission of pressure ranging from 100 to 1000 MPa to food. However, pressures between 300 and 700 MPa are commonly applied for food processing, from a millisecond pulse to over 20 minutes, in a batch or semi-continuous system. Food products (as solid, liquid or packages) are loaded into steel vessels filled with hydraulic fluid (normally water). Then, pressurization takes place via direct or indirect compression. For every 100 MPa of pressure applies, there is an increase in temperature (3 °C) of the product (Morris *et al.,* 2007; Lopez-Gomez *et al.,* 2009). Since the pressure is transmitted uniformly throughout the product and reaches isostatic equilibrium, it is important to highlight that this process is not influenced by the size and shape of product, unlike thermal processing (Oms-Oliu *et al.,* 2012b).

HHP inactivates microbial cells by breaking non-covalent bonds and damaging cell membrane. In addition, this process causes protein denaturation, thus interrupting cellular functions, and eventually resulting in cell death (Morris *et al.,* 2007; Lopez-Gomez *et al.,* 2009). Effective microbial inactivation was reported in HHP processed apple juice (at 250 MPa, 35 °C for 15 minutes) and pomegranate juice (at 400 to 600 MPa, 25 to 50 °C, for 5 and 10 minutes) (Dede *et al.,* 2007; Ferrari *et al.,* 2010).

Besides that, there is minimal alteration in nutritional and sensory quality of HHP processed food due to its limited effects on the covalent bonds (Oey *et al.,* 2008; Ferrari *et al.,* 2010). Indeed, better retention of ascorbic acid and antioxidant capacity was reported in HHP treated juices when compared to conventional thermal processing, such

as observed in Valencia and Navel orange (Bull *et al.,* 2004; Polydera *et al.,* 2005) and apple juice (Dede *et al.,* 2007).

2.8.2.2 Pulsed electric field (PEF)

PEF treatment applies short pulses (1 to 100 microseconds) with high voltage (10 to 50 kV/cm) to liquid products in a continuous system. A simple PEF system consists of a high voltage power supply, a pulse generator, treatment chamber and a switch to discharge energy to electrodes. In addition, there is a cooling system to balance moderate temperature rise during treatment. Some of the limitations of PEF is that it cannot be used on products that have variable electrical conductivity as well as products that contain or could form air bubbles. Besides that, PEF has limited effects on microbial spores (Ohlsson and Bengtsson, 2002; Morris *et al.,* 2007; Lopez-Gomez *et al.,* 2009). The effectiveness of PEF processing is dependent on variables such as pulse width, electric field strength, flow rate, treatment temperature and time of exposure (Ohlsson and Bengtsson, 2002).

PEF creates pores in the cell membrane of microbial cells. This electroporation process causes leakage of cellular contents, and eventually resulting in microbial cell disruption. Indeed, a longer pulse width and higher intensity pulse fields were more effective in inactivating microbial growth (Morris *et al.,* 2007; Rawson *et al.,* 2011). The reduction in microbial loads greater than 5 log was observed in PEF treated orange juice (40 kV/cm) (McNamee *et al.,* 2010).

Some studies reported that juices treated with lower intensity pulse fields and shorter pulse width exhibited higher retention of ascorbic acid, such as observed in orange (Elez-Martinez and Martin-Belloso, 2007) and tomato juice (Odriozola-Serrano *et al.,* 2008). In addition, enhancement of antioxidant capacity was observed in PEF processed juices due to increased extraction yield of secondary metabolites and generation of free radicals (Schilling *et al.,* 2008; Rawson *et al.,* 2011).

2.8.2.3 Ionizing radiation (IR)

IR involves the exposure of food products to ionizing radiation sources including high-energy electrons, X-rays (machine generated) and gamma rays (from radioisotopic source cobalt-60 or cesium-137). The World Health Organization has declared that IR exposure of any food commodity, up to 10 kGy does not produce any toxicological hazard s or nutritional or microbiological problems in food (Lopez-Gomez *et al.,* 2009; Sorour *et al.,* 2014). The IR plant consists of a conveyer belt, an irradiation chamber containing the radiation source, with separate loading and unloading areas (Ohlsson and Bengtsson, 2002).

IR damages microbial DNA, therefore terminating reproduction and cellular functions, and eventually resulting in microbial inhibition. Besides that, IR indirectly promotes the formation of radiolytic products such as free radicals (H^+ and OH^-) which inactivates spoilage microorganism in juices (Sorour *et al.,* 2014). Alighourchi *et al.* (2008) reported significant reduction in microbial growth in pomegranate juice exposed to 2 kGy dose of gamma rays. In addition, low doses of IR cause minimal modification of nutritional and organoleptic (flavour and colour) qualities of food (Morris *et al.,* 2007).

However, doses of IR above 10 kGy induce reduction in total phenolics of irradiated product (Villavicencio *et al.,* 2000).

2.8.2.4 Dense phase carbon dioxide (DPCD)

DPCD or also known as supercritical carbon dioxide is a cold pasteurization technique that involves the application of pressurized carbon dioxide (below 50 MPa) to food. DPCD is a continuous system for pasteurizing liquid food products. The effectiveness of DPCD processing is dependent on pressure and temperature. Several studies have reported microbial inactivation by DPCD due to lowering of cytoplasmic pH, modification of cell membrane permeability, enzyme inactivation and oxygen elimination (Damar and Balaban, 2006; Morris *et al.,* 2007). Garcia-Gonzalez *et al*. (2007) reported microcidal action of DPCD in carrot (6 MPa, 45 °C, for 360 minutes), orange (6 MPa, 45 °C, for 240 minutes) and peach juice (6 MPa, 45 °C, 180 min).

Meanwhile, DPCD treated food products ranked higher for sensory analysis (aroma, flavour and overall acceptability) when compared with thermal treatment, as reported by Damar and Balaban (2006) and Del Pozo-Insfran *et al.* (2006). There were no changes in antioxidant capacity and ascorbic acid content of muscadine grape juice after subjected to DPCD at 4.5 MPa (Del Pozo-Insfran *et al.*, 2006).

2.8.2.5 Ozone

Ozone is a triatomic allotrope of oxygen and is characterised by its high bactericidal and virucidal properties. The Food and Drug Administration (FDA) approved ozone as a direct additive to food due to its wide antimicrobial spectrum for food preservation. Ozone can be generated by reaction of free oxygen radicals (by ultraviolet radiation or electric discharge method) with diatomic oxygen. Ozone processing can be conducted on solid foods by either washing with ozonated water or by gaseous treatment (Rawson *et al.,* 2011; Sorour *et al.,* 2014).

Ozone inactivates microbial cells by oxidation, thus making it an environmental friendly antimicrobial agent. In addition, ozone causes disruption of cell membrane, thus leading to microbial cell lysis. The strong biocidal action of ozone in fruit juice processing was reported in apple and orange juice (Steenstrup and Floros, 2004; Patil *et al.,* 2009). However, ozone treatment causes significant difference in colour of apple cider (Choi and Nielsen, 2005).

2.8.3 Ultrasonic treatment

Ultrasound is sound waves with frequencies (>20 kHz) above the threshold for human hearing. Normally, ultrasound equipment uses frequencies ranging from 20 kHz to 10 MHz. The ultrasonic spectrum can be divided into two zones, namely power and diagnostic ultrasound (Figure 2.10). Power ultrasound (or high-intensity ultrasound) has a frequency range from 20 kHz to 1 MHz. Diagnostic ultrasound (or low-intensity ultrasound) has a frequency range from 5 MHz to 10 MHz (Soria and Villamiel, 2010; Kentish and Ashokkumar, 2011).

Power ultrasound (10–1000 W/cm^2) is used to alter food properties, either physically or chemically, such as disrupt cells, inactivate enzymes, generate emulsions and tenderize meat. On the other hand, diagnostic ultrasound (0.1–1 W/cm^2) is used mostly for medical imaging and non-destructive assessment (Demirdoven and Baysal, 2008; Soria and Villamiel, 2010; Kentish and Ashokkumar, 2011). Ultrasonic treatment or commonly known as sonication is simple and reliable, thus providing reduced processing time and improved efficiency (O'Donnell *et al.,* 2010).

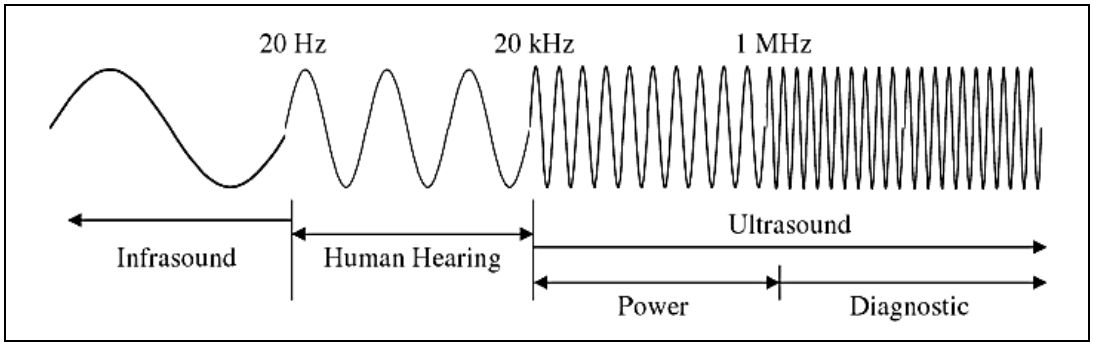


Figure 2.10: The sound spectrum

(Source: Kentish and Ashokkumar, 2011)

2.8.3.1 Ultrasonic processing equipment

Most of the ultrasonic applications used in food processing utilize liquid-liquid or liquid-solid systems. This could be attributed to the properties of ultrasonic waves which are easily transmitted in liquid. Ultrasonic processing equipment includes ultrasonic bath and probe system (Carcel *et al.,* 2012). A power ultrasound system consists of three parts, namely generator, transducer and coupler. The generator is an oscillator that transforms electrical energy into ultrasound energy at ultrasonic frequencies via a transducer. The coupler aids in transferring the ultrasonic vibrations to the substance being treated (usually liquid) (Demirdoven and Baysal, 2008; Carcel *et al.,* 2012).

There are two main types of transducers, which are magnetostrictive and piezoelectric. Magnetostrictive transducers are high-strength metallic alloys that change dimension in response to the application of an electromagnetic field. In contrast, piezoelectric transducers are ceramic materials that produce piezoelectric vibrations in response to the application of an alternate electromagnetic field. The latter is most commonly used as it functions efficiently in a wider range of ultrasound frequencies (Leadley and Williams, 2006; Carcel *et al.*, 2012).

The effects of ultrasound in liquid media depends on several variables including treatment medium (viscosity, surface tension, concentration of the dissolved gas, and the presence of solid particles), treatment parameters (pressure and temperature), ultrasound generator performance (frequency, power input), size of the treatment vessel (Demirdoven and Baysal, 2008).

2.8.3.2 Mechanism of action and effects of ultrasound

The mechanism of action for sonication is generally explained in three different approaches, which are cavitation, localized heating and formation of free radicals. When high power ultrasound at low frequencies (20–100 kHz) propagates in liquid, cavitation (formation and collapse of bubbles) occurs (Figure 2.11). As a result, there is elevation of localized pressure (up to 500 MPa) and temperature (up to 5000 °C). These ‘tiny hotspots’ provide the energy to alter the properties of food product either physically or chemically. Accordingly, these cavitation bubbles induce microstreaming and shear stress, resulting in the disintegration of the microbial cells. Besides that, cavitation causes intracellular micromechanical shock that disrupts the functional components of cell, thus inactivating enzymes (O’Donnell *et al.,* 2010; Gogate, 2011; Abid *et al.,*

2013). Another mode of action of microbial inactivation is the formation free radicals due to sonolysis of water. Therefore, hydrogen peroxide, OH^- and H^+ ions which have bactericidal effects are produced (O'Donnell *et al.*, 2010; Soria and Villamiel, 2010).

The cavitation phenomenon is affected by the frequency and amplitude of ultrasound waves. At lower frequencies (20 kHz), the bubbles produced are larger in size and when they collapse higher energies are produced. Additionally, higher intensity cavitation can be produced by using higher amplitude ultrasound (Leadley and Williams, 2006).

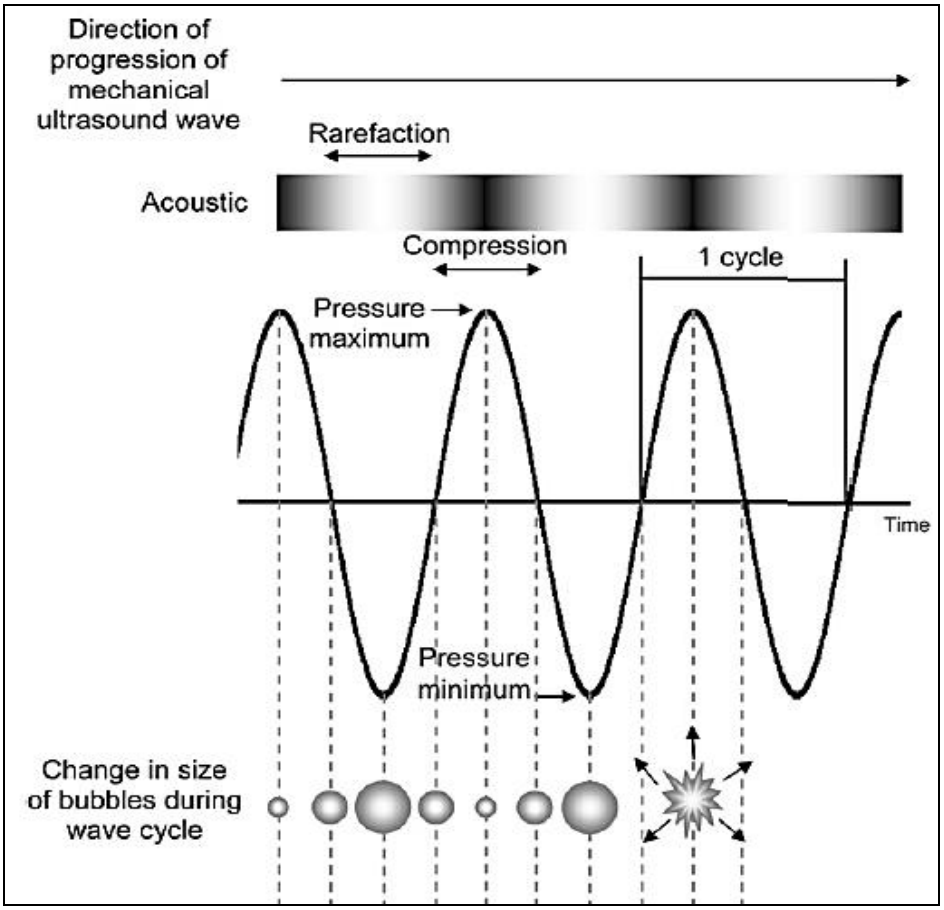


Figure 2.11: Ultrasonic cavitation

(Source: Soria and Villamiel, 2010)

2.8.3.3 Application of ultrasound in juice processing

Sonication is a potential technology to achieve the U.S. FDA condition of a 5 log reduction of food borne pathogens in fruit juices (Salleh-Mack and Roberts, 2007). Previous studies have reported that sonication is effective in reducing the microbial count of foodborne pathogens, such as in orange (500 kHz, 240 W power, for 15 minutes), apple (25 kHz for 30, 60 and 90 minutes) and carrot juice (20 kHz, 750W power, for 2 minutes) (Valero *et al.*, 2007; Abid *et al.*, 2013; Jabbar *et al.*, 2014). This could be explained by the collapse of cavitation-induced micro bubbles resulting in a localised decontamination effect (Mason, 1991).

Several studies using ultrasonic treatment on fruit juice reported minimal effect on the degradation of quality parameters, and improved functionalities such as in orange (Tiwari *et al.*, 2008), blackberry (Tiwari *et al.*, 2009a), kasturi lime (Bhat *et al.*, 2011a), apple (Abid *et al.*, 2013) and carrot juice (Jabbar *et al.*, 2014). Besides that, Rawson *et al.* (2011) reported that sonication provides better retention of bioactive compounds. In addition, Tiwari *et al.* (2009b) reported that ultrasonic processing (25 kHz for 2 min) improves the cloud value and stability of orange juice during storage. However, significant colour degradation was observed in guava and orange juice subjected to sonication (Cheng *et al.*, 2007; Tiwari *et al.*, 2009b).

2.8.4 Ultraviolet-c (UV-C) light treatment

UV light is a part of the electromagnetic spectrum with wavelengths between 100 to 400 nm. The UV light spectrum is divided into four regions, namely UV-A, UV-B, UV-C and vacuum-UV, as shown in Figure 2.12. UV-A (315 to 400 nm) is responsible for changes in human skin or tanning, whereas UV-B (280 to 315 nm) is responsible for skin burning and may lead to skin cancer. UV-C (200 to 280 nm) exhibits germicidal properties as it inactivates bacterial and viral microorganisms. While, vacuum-UV (100 to 200 nm) is absorbed by almost all substances, but it can only be transmitted in vacuum (Gomez-Lopez *et al.,* 2012; Ribeiro *et al.,* 2012).

The application of UV light with germicidal effects has been used in air disinfection, liquid sterilization, as well as inhibition of microorganisms on the surface of milk, fruit juice, cider, eggs and other fresh products. The advantages of UV-C treatment include cost effective, simple, and low maintenance (Falguera *et al.,* 2011; Guerrero-Beltran and Barbosa-Canovas, 2004).

Although UV-C radiation technology is considered as an effective method for food preservation, consumers' misconception about this process have delayed many of its potential applications in the food industry. Actually, UV-C radiation is a physical treatment which does not cause chemical residues. Hence, consumption of UV-C treated food products is not harmful to humans (Environmental Protection Agency, 1999).

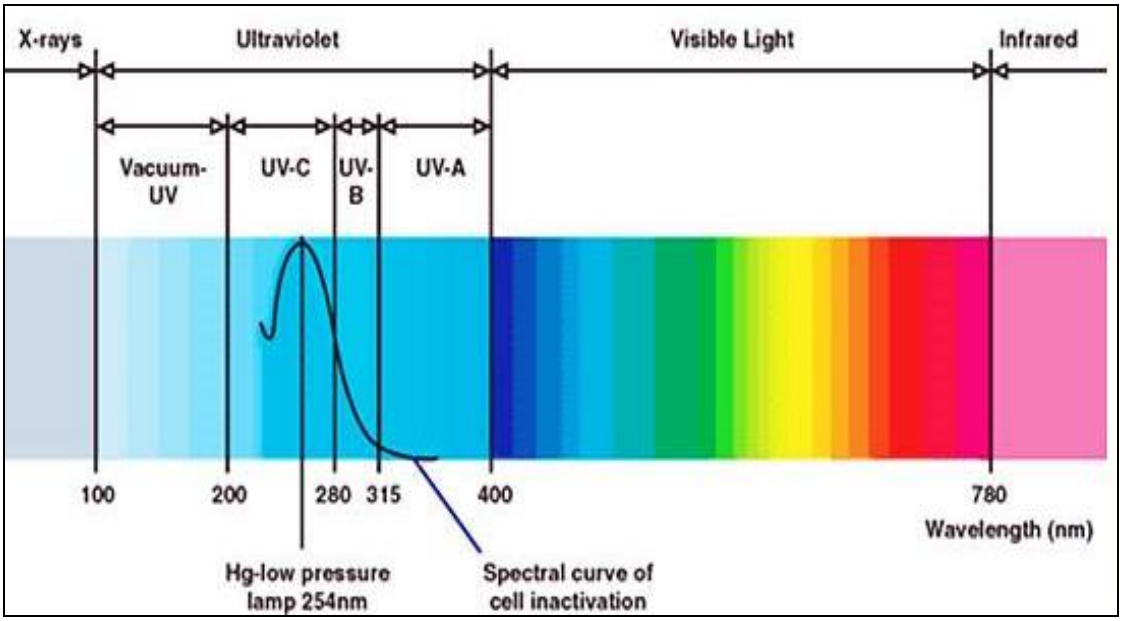


Figure 2.12: The electromagnetic spectrum

(Source: Aquafine, 2014)

2.8.4.1 UV-C processing equipment

The examples of UV light sources include continuous UV low-pressure and medium-pressure mercury lamps, excimer and broadband pulse lamps. Generally, low pressure mercury lamps are used in food processing and are approved by the FDA (Koutchma *et al.*, 2009).

One of the limitations of the application of UV in juice processing is associated to the high absorbance coefficients of juice. According to Koutchma *et al.* (2004), UV-C penetration largely depends on the presence of dissolved organic solutes (suspended solids) and coloured compounds which act as a barrier, thus exhibiting UV-C attenuation effects. Hence, an appropriate UV reactor should be designed to reduce the interference of UV absorbance and improve microbial inactivation efficiency. The reactor design should include a narrow laminar flow or conditions with high turbulence,

where juices are mixed resulting in all parts being exposed to UV light source (Koutchma *et al.,* 2004).

The effectiveness of UV-C processing is dependent on variables such as the absorbance of medium, amount of solid particles and suspended materials, flow rate of fluid, thickness of fluid, exposure time, and UV dose delivered (Guerrero-Beltran and Barbosa-Canovas, 2004; Koutchma *et al.,* 2004).

2.8.4.2 Mechanism of action and effects of UV-C

The UV-C light, with a peak emission at the wavelength of 254 nm exhibits germicidal effect on bacteria, yeasts, moulds, and viruses. This could be explained by the characteristic of microbial DNA to absorb UV-C light photons, thus generating cross links between neighbouring cytosine and thymine (pyrimidine) bases in the same DNA strand (Tran and Farid, 2004; Guerrero-Beltran and Barbosa-Canovas, 2004). Therefore, these pyrimidine dimers prevent DNA transcription and translation. Subsequently, this condition prevents microorganisms from reproducing, and eventually resulting in cell death (Guerrero-Beltran and Barbosa-Canovas, 2004). However, yeast and mould are more resistant to UV-C. This could be due to the difference in thickness of the cell wall and size of microorganism, thus influencing the passage of UV-C light. In addition, lesser pyrimidine bases on the DNA strand of yeast and mould contributes to less probability of cross link formation, thus higher resistance to UV-C (Miller *et al.,* 1999).

2.8.4.3 Application of UV-C in juice processing

The U.S. FDA criterion of a 5 log reduction of chosen pathogen in fruit juices can be achieved by UV-C radiation (FDA, 2000). Previous studies have reported that UV-C treatment is effective in reducing the microbial count of foodborne pathogens, such as in apple (Walkling-Ribeiro *et al.,* 2008), starfruit (Bhat *et al.*, 2011b), and orange juices (Tran and Farid, 2004; Pala and Toklucu, 2013).

Several studies using short-wave ultraviolet-c (UV-C) light treatment on fruit juices reported better retention of nutritional and quality attributes, such as in starfruit (Bhat *et al.,* 2011b), watermelon (Zhang *et al.,* 2011), pomegranate (Pala and Toklucu, 2011) and orange juice (Pala and Toklucu, 2013). Besides that, Bhat *et al.* (2011b) reported that UV-C processing (for 30 and 60 minutes) induces significant increase in polyphenol and flavonoid content of starfruit juice. However, there is an increasing trend in browning degree and colour changes corresponding to increased UV-C treatment time, as previously reported by Bhat *et al.* (2011b) and Zhang *et al.* (2011).

2.8.5 Combination of treatments or hurdle concept

The hurdle concept is based on the combination of different preservation methods or factors (‘hurdles’) to produce minimally processed food with fresh-like characteristics, safe, nutritious, stable, and economical. These hurdles can be classified into three groups, namely physical, physicochemical and microbially derived hurdles, as summarised in Table 2.4 (Leistner and Gorris, 1994; 1995). Generally, by placing a number of sublethal stress (hurdles) on a microbial cell, the organism makes an effort to overcome the hostile environment, thus leading to metabolic exhaustion, and eventually death (Leistner and Gorris, 1995; Leistner, 2000).

According to the hurdle technology, combination of two or more novel non-thermal processing techniques at lower individual intensities may have an additive or, even, a synergistic on microbial destruction, with minimal impact on the quality of the food product (Leistner, 2000). Non-thermal technologies have different mode of microbial inactivation, therefore being potential choices for a hurdle concept. Some studies have demonstrated that fruit juices were successfully preserved by combining non-thermal technologies (Noci *et al.*, 2008, Walkling-Ribeiro *et al.*, 2008).

Table 2.4: Potential hurdles used in food preservation

Type of hurdle	Examples
Physical	a) Electromagnetic energy (microwave, radio frequency, pulsed magnetic fields, high electric fields); b) UV radiation, IR, sonication and HHP; c) High temperature (blanching, pasteurisation and sterilisation); d) Low temperature (chilling and freezing); e) Modified-atmosphere packaging
Physicochemical	a) Low water activity, pH and redox potential; b) Maillard reaction products, organic acids, oxygen, ozone, phenols, phosphates, salt and ascorbic acid
Microbially derived	a) Antibiotics (ampicillin and tetracycline); b) Bacteriocins, competitive flora and protective culture

(Source: Leistner and Gorris, 1994; 1995)

CHAPTER 3

MATERIALS AND METHODS

3.1 PLANT MATERIAL

Mature-green Chokanan mango fruits of uniform size and free from external defects were harvested 13 weeks after anthesis from a mango farm in Tobiar (Kedah, Malaysia) which is located about 428 kilometres from the Postharvest Biotechnology Laboratory, University of Malaya. The fruits were rinsed with running water, air dried and left to ripen at room temperature (25 ± 1 °C).

3.2 EXTRACTION OF MANGO JUICE

Ripe mangoes (stage 5) were selected based on visual assessment of the peel colour according to the maturity indices standard specified by the Federal Agricultural Marketing Authority (FAMA) Malaysia, as shown in Figure 3.1 (Department of Agriculture, 2009). Each mango was peeled and the seed was discarded. Mango pulp were macerated using a domestic juice extractor (Philips Juice Extractor HR 2820, Holland), and then centrifuged (Beckman J2-MI Centrifuge, California) at 12000 rpm for 10 minutes at 4 °C. The supernatant was filtered using a steel sieve with an approximate diameter of 2 mm to obtain the juice and remove any remaining fibre. The filtered juice samples were stored in sterile glass bottles prior to deployment into experiment.

1 2 3

4 5 6

Maturity index	Peel colour	Ripeness stage
1	Green and faded	Immature
2	Light green and glossy	Mature
3	More green than yellow	Partially-ripe
4	More yellow than green	Firm-ripe
5	Fully yellow	Soft-ripe
6	Yellow with orange blush	Over-ripe

Figure 3.1: Maturity indices of Chokanan mango according to the standard specified by FAMA Malaysia

(Department of Agriculture, 2009)

3.3 THERMAL AND NON-THERMAL TREATMENTS

3.3.1 Thermal treatment

Freshly squeezed Chokanan mango juice (20 ml) in a glass boiling tubes (10.5 cm length, 2 cm inner diameter, 0.2 cm wall thickness) was pasteurized in a covered water bath (Memmert, Germany) (Figure 3.2) with continuous shaking at 90 ± 1 °C for 30 seconds (mild heat pasteurization) and 90 ± 1 °C for 60 seconds (high heat pasteurization). These conditions were selected because thermal treatment for fruit

juices range from 90 °C to 95 °C for 15 to 60 seconds to assure at least 5 log reduction in microbial count (Nagy *et al.,* 1993). The schematic diagram of the thermal system is shown in Figure 3.3. Temperature of the juice at the center of the tube was regularly monitored using a thermometer. After the juice samples reached the target temperature, the treatment time was measured. The temperature versus time curve of juice during high heat pasteurization is shown in Figure 3.4. After thermal treatment, the juice samples were immediately cooled to room temperature (RT), 25 ± 1 °C by immersing in an ice-water bath. All treatments were carried out in triplicates

Figure 3.2: Covered water bath used for thermal treatment

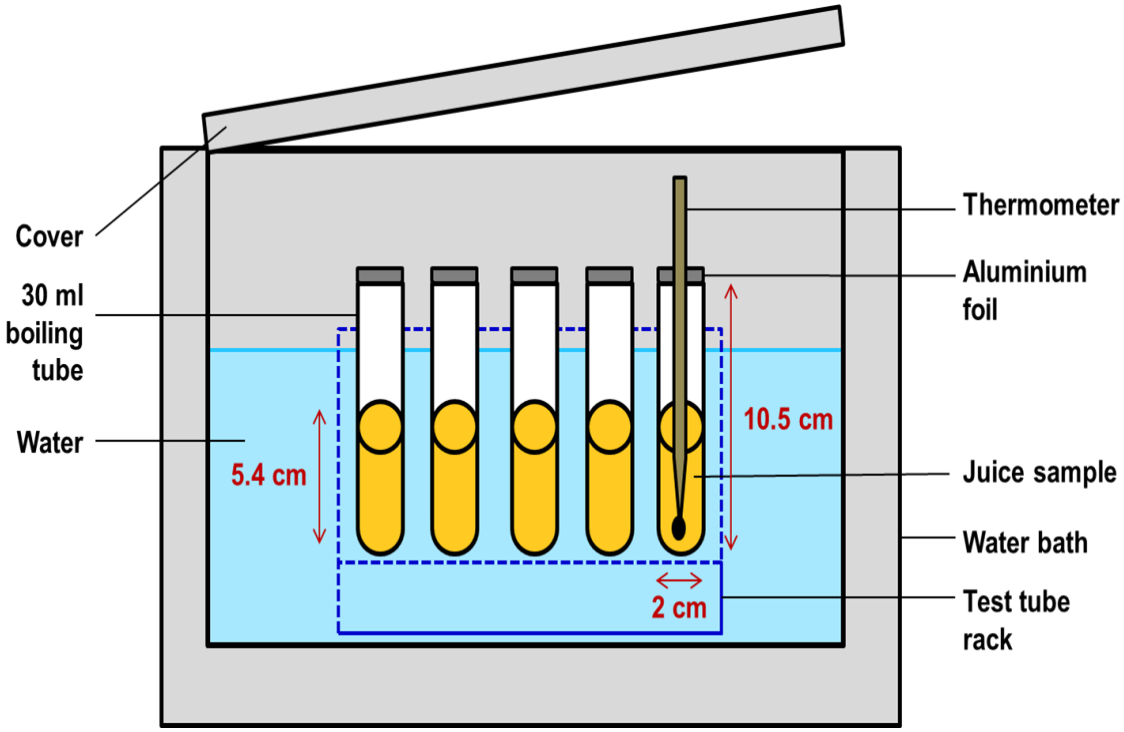


Figure 3.3: Schematic diagram of thermal treatment

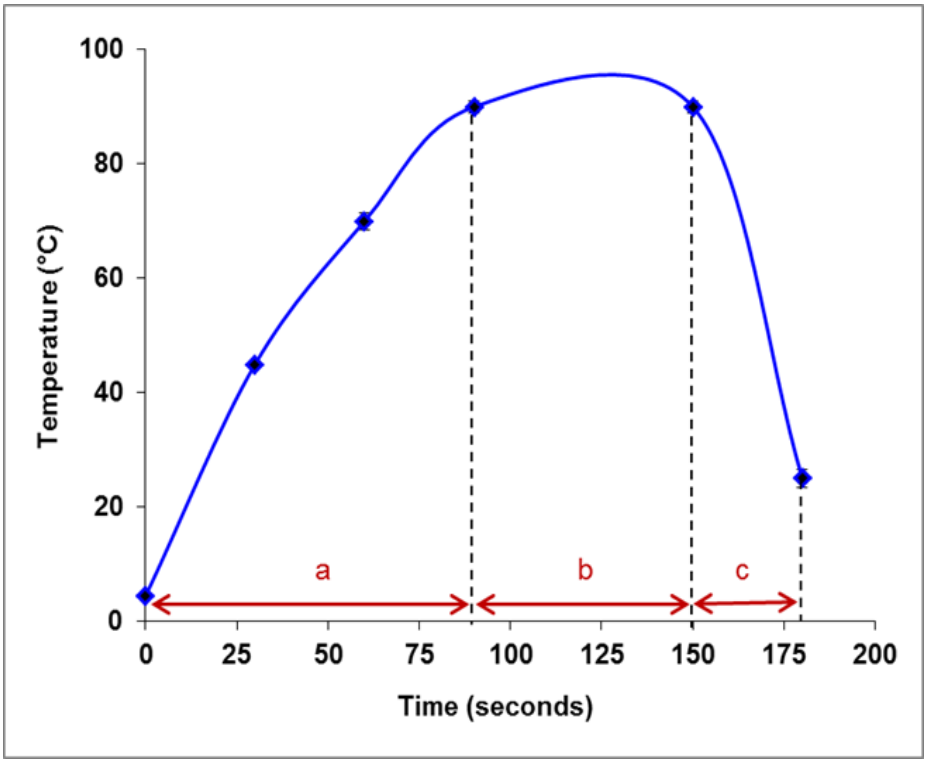


Figure 3.4: Temperature versus time curve of juice during high heat pasteurization. (a) come up time, (b) holding time, (c) cooling time.

The following terms were used to describe juice samples in this study, as shown in Table 3.1.

Table 3.1: Chokanan mango juice samples subjected to thermal treatment

Treatment	Mango juice samples	
Control	Control	Freshly squeezed or no treatment
Thermal	MP	Mild heat pasteurization (90 ± 1°C for 30 s)
	HP	High heat pasteurization (90 ± 1°C for 60 s)

3.3.2 Ultrasonic treatment

The sonication of freshly squeezed Chokanan mango juice was performed at 40 kHz frequency, using an ultrasonic cleaning bath (Branson Model 3510 Ultrasonic Cleaner, CT, USA) (Figure 3.5). The ultrasonic cleaning bath is a rectangular container (290 x 150 x 150 mm) with the maximal tank capacity of 5.5 L. The 40 kHz transducers at the bottom transmit ultrasound waves of 130 W power from the bottom to above. The schematic diagram of the ultrasonic system is shown in Figure 3.6. Juice samples (100 ml) in 250 ml beaker were placed exactly at the center of the ultrasonic cleaning bath with the circulating water (flow rate 0.5 L/min) temperature being monitored at 25 ± 1 °C. The juice level in the beaker was 3.5 cm below the water surface in the ultrasonic bath. The height of the bottom surface of the beaker from the bottom surface of the tank (face of transducers) is 4.5 cm. The processing time was for 15, 30, and 60 minutes under dark condition. The actual power dissipated in the ultrasonic bath was 68-75 W, which was determined by calorimetric method (Sutkar and Gogate, 2009; Gogate *et al.*, 2011). Juice samples not sonicated were considered as control. All treatments were carried out in triplicates.

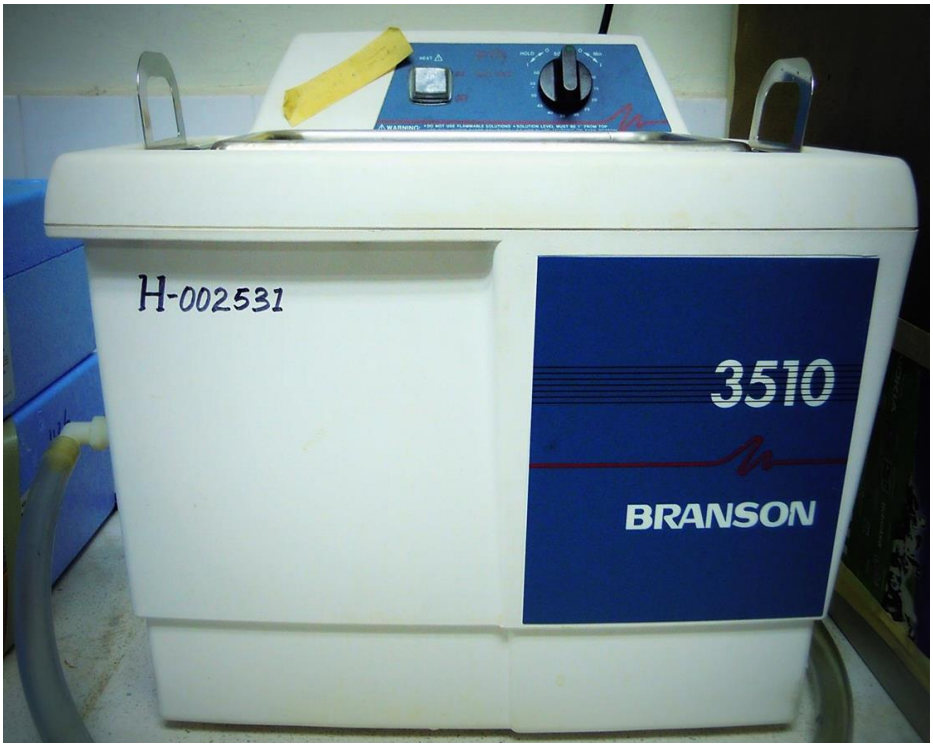


Figure 3.5: Ultrasonic cleaning bath used for sonication

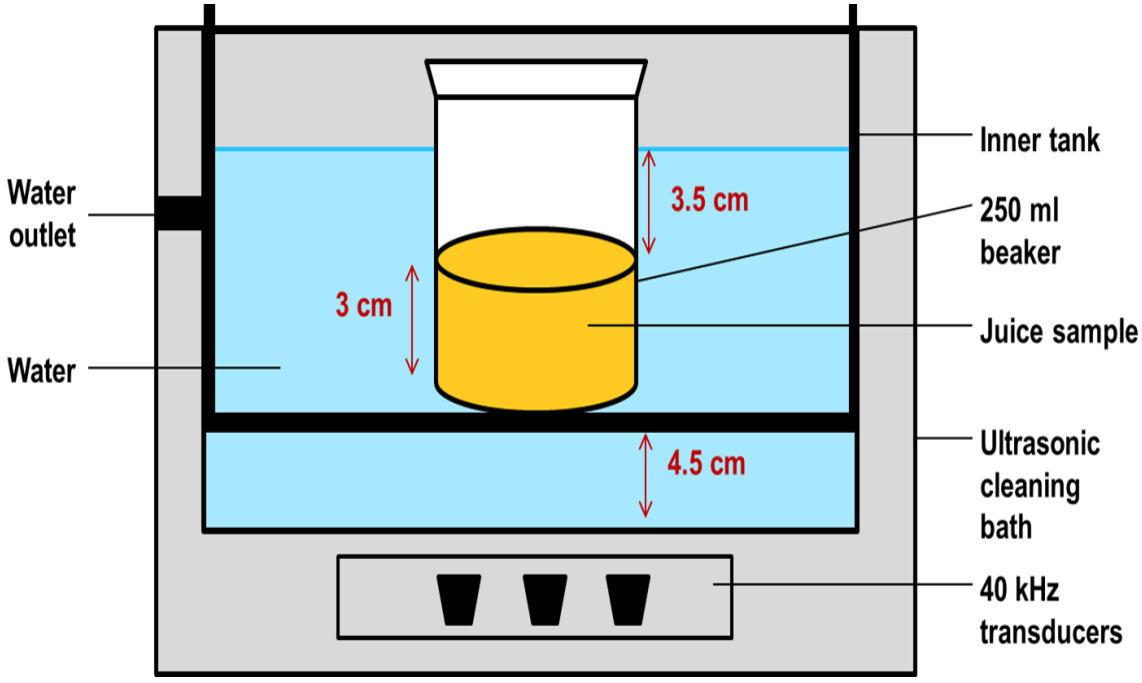


Figure 3.6: Schematic diagram of sonication

The following terms were used to describe juice samples in this study, as shown in Table 3.2.

Table 3.2: Chokanan mango juice samples subjected to ultrasonic treatment

Treatment	Mango juice samples	
Control	Control	Freshly squeezed or no treatment
Ultrasonic	S15	Sonication for 15 min
	S30	Sonication for 30 min
	S60	Sonication for 60 min

3.3.3 Ultraviolet-c (UV-C) light treatment

Juice samples were exposed to UV-C light under batch conditions. Freshly squeezed Chokanan mango juice (50 ml) was poured into sterile Petri dishes (11 cm diameter, 0.5 cm fluid depth) and then exposed to a germicidal fluorescent UV-C lamp (30 W, 89.3 cm length, 25.5 cm diameter, Sankyo Denki, Japan) in a laminar flow cabinet (Gelman Science Biological Safety Cabinet Class II, NSW, Australia) (Figure 3.7). The UV-C lamp has a peak emission at 254 nm. The distance between the juice surface and the UV-C lamp was 35 cm. The schematic diagram of the UV-C system is shown in Figure 3.8. The UV-C lamps were allowed to stabilize for 30 minutes prior to use. The duration of exposure was 15, 30, and 60 minutes, at 25 ± 1 °C under dark condition. The mean of UV radiation dose received by each juice sample is 3.525 J/m^2 (Keyser *et al.,* 2008). Juice samples not exposed to UV-C were considered as control. All treatments were carried out in triplicates.

Figure 3.7: UV-C lamp in a laminar flow cabinet

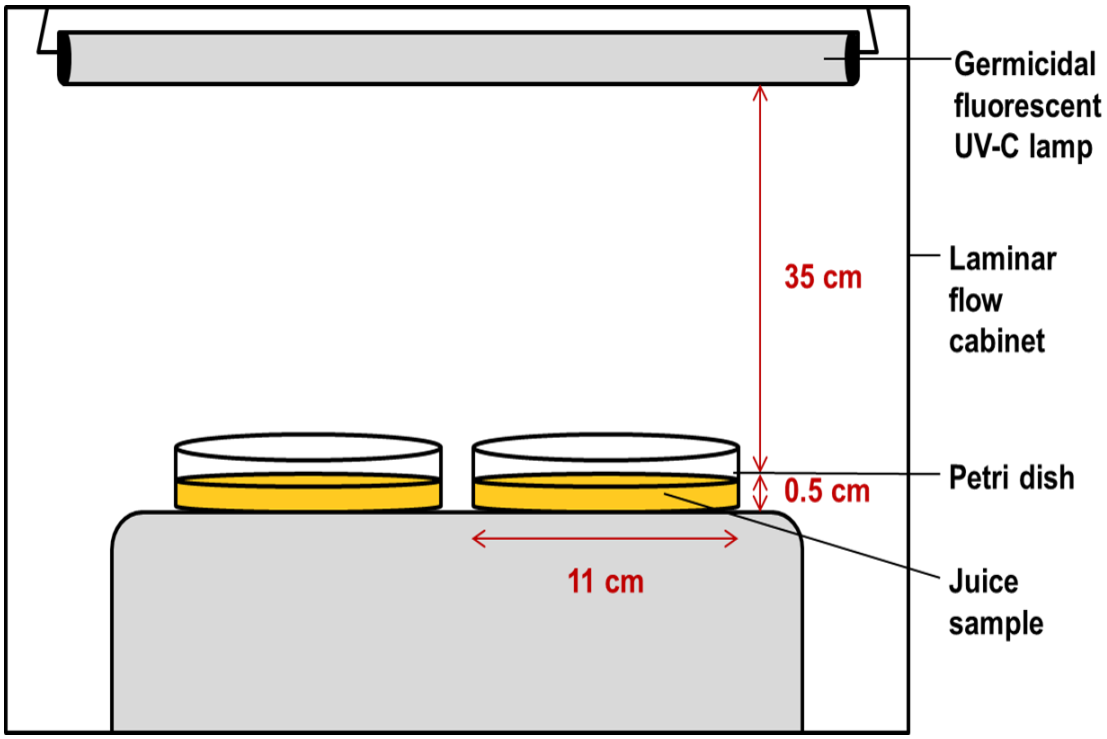


Figure 3.8: Schematic diagram of UV-C treatment

The following terms were used to describe juice samples in this study, as shown in Table 3.3.

Table 3.3: Chokanan mango juice samples subjected to UV-C treatment.

Treatment	Mango juice samples	
Control	Control	Freshly squeezed or no treatment
UV-C	U15	UV-C treatment for 15 min
	U30	UV-C treatment for 30 min
	U60	UV-C treatment for 60 min

3.3.4 Combined treatment (combination of ultrasonic and UV-C treatment in a hurdle concept)

The freshly squeezed Chokanan mango juice was treated by four paired combinations of selected non-thermal technologies. Ultrasonic treatment (for 15 and 30 minutes at 25 ± 1 °C, 40 kHz frequency) was employed as the first hurdle followed by UV-C treatment (for 15 and 30 minutes at 25 ± 1 °C), using the method described in Section 3.3.2 and Section 3.3.3. Selection of processing variables was based on preliminary studies on stand-alone treatments to achieve significant microbial reduction and quality retention.

The following terms were used to describe juice samples in this study, as shown in Table 3.4.

Table 3.4: Chokanan mango juice samples subjected to combined treatment.

Treatment	Mango juice samples	
Control	Control	Freshly squeezed or no treatment
Combination (Hurdle concept)	S15+U15	Combined sonication for 15 minutes and UV-C treatment for 15 minutes
	S30+U15	Combined sonication for 30 minutes and UV-C treatment for 15 minutes
	S15+U30	Combined sonication for 15 minutes and UV-C treatment for 30 minutes
	S30+U30	Combined sonication for 30 minutes and UV-C treatment for 30 minutes

Juice samples were aseptically filled into sterile glass bottles in a laminar flow cabinet (Gelman Science Biological Safety Cabinet Class II, NSW, Australia) and tightly capped, leaving minimum amount of headspace volume. Juice samples were stored at 4 ± 1 °C in a refrigerator (under dark condition) until further analysis. All analysis was carried out in triplicates.

3.4 MICROBIAL INACTIVATION ANALYSIS

3.4.1 Preparation of reagent

0.1% peptone water
0.1 g peptone (R & M Chemicals) was dissolved in 100 ml of SDW

3.4.2 Sample preparation

The microbial count of juice samples were determined using 3M™ Petrifilm™ plates (3M Center, MN, USA). Petrifilm plate methods are recognized as AOAC International Official Methods of Analysis (3M Food Safety, 2010). Serial dilution bottles were filled

with 9 ml of 0.1% peptone water and then autoclaved at 121 °C for 20 min. 1 ml of mango juice was added to the stock solution and mixed thoroughly, then serially diluted (10^{-1} to 10^{-3}). The final pH of the diluted samples was adjusted to pH 6.6 to 7.2 using 0.1 N sodium hydroxide solution for optimum growth.

3.4.3 Aerobic plate count (APC)

Petrifilm aerobic count plates containing an indicator dye (tetrazolium), dehydrated nutrients, and water-soluble gelling agent were used for the enumeration of aerobic bacteria. Plates have two films; top and bottom films. The top film was lifted and 1 ml of diluted juice sample was placed on the center of bottom film. The top film was released. A plastic spreader (3M Center, MN, USA) was used to distribute the inoculum evenly on the circular area. Plates were set aside for 1 minute to allow the gel to solidify, and then incubated at 35 ± 1 °C for 48 hours. The indicator dye colours the colonies red. All red colonies regardless of their size or colour intensity were counted.

3.4.4 Coliform count (CC)

Petrifilm coliform count plate containing an indicator dye (tetrazolium), violet red bile nutrients and water-soluble gelling agent was used for the enumeration of coliforms. Diluted juice sample (1 ml) was inoculated as described in Section 3.2.4.1.3. Then, plates were incubated at 35 ± 1 °C for 24 hours. Similar to aerobic bacteria, coliform colonies are coloured red. However, only red colonies associated with gas bubbles indicate confirmed coliforms.

3.4.5 Yeast and mould count (YMC)

Petrifilm yeast and mould count plate containing an indicator dye, nutrients supplemented with antibiotics, and water-soluble gelling agent was used for the enumeration of yeast and mould. Diluted juice sample (1 ml) was inoculated as described in Section 3.2.4.1.3. Then, plates were incubated at room temperature (25 ± 1 °C) for 3 to 5 days. The indicator dye colours yeast colonies blue-green with defined edges (small), whereas other variably coloured colonies with diffuse edges (large) are mould colonies.

3.4.6 Calculation

The aerobic bacteria, coliform, and total yeast and mould colonies were counted using a hand tally counter (Green Show's, Taiwan) under a light source. Colony-forming units (CFU) per millilitre of juice were calculated according to the equation:

CFU per ml = (number of colonies x dilution factor of plate) / aliquot plated

Results were expressed as log (CFU/mL).

3.5 PHYSICOCHEMICAL ANALYSIS

3.5.1 pH

The pH of juice samples was determined using a pH meter (Hanna Microprocessor pH 211, Italy). The pH meter was first calibrated using buffer standards of pH 7 and pH 4. The probe was placed in juice sample and the pH was measured at 25 ± 1 °C.

3.5.2 Total soluble solids (TSS)

TSS was determined using a digital refractometer (Atago PR-1 digital refractometer, Tokyo, Japan). Prior to usage, the refractometer was calibrated with sterile distilled water (SDW) (0 °Brix). Juice sample (1 ml) was placed on the prism of the refractometer and analysed at 25 ± 1 °C. Results were expressed in standard °Brix unit.

3.5.3 Titratable acidity (TA)

3.5.3.1 Preparation of reagents

0.1 N sodium hydroxide
2 g of sodium hydroxide (Merck) was dissolved in 500 ml of SDW. The solution was standardized before use.

50% ethanol
50 ml of ethanol (Merck) was added into 50 ml of SDW.

Phenolphthalein indicator
0.5 g of phenolphthalein (East Anglia Chemicals) was dissolved in 100 ml of 50% ethanol.

3.5.3.2 Determination of TA

Juice sample (5 ml) was added to 200 ml of boiled and cooled SDW in a 250 ml conical flask. This solution was titrated with standardized 0.1 N sodium hydroxide to a definite faint pink end point (colour should persist for ≥ 15 seconds) using phenolphthalein as an indicator. The volume of sodium hydroxide used for titration was converted to grams of citric acid per 100 ml of juice according to the method by Sadler and Murphy (2010). TA was calculated using the following equation:

$$\%TA = (V_1 \times 0.1\ N\ NaOH \times Eq.\ wt. \times 100) / (V_2 \times 1000)$$

where

V_1 = volume of titrant (ml)

Eq. wt. = equivalent weight of anhydrous citric acid (64 mg/mEq)

V_2 = volume of sample (ml)

3.6 COLOUR

The colour of juice samples were measured using a Chroma Meter (Minolta Chroma Meter CR-200, Minolta, Osaka, Japan) with CIE LAB colour scale (L^*, a^*, and b^* values). Prior to usage, the instrument was calibrated using white reference tile. The colour parameters L^* (lightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness) were evaluated. Colour differences (ΔE), in comparison to control (Caminiti *et al.*, 2011), were calculated using the following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{½}$$

According to Cserhalmi *et al.* (2006), ΔE can be classified as not noticeable ($0 < \Delta E < 0.5$), slightly noticeable ($0.5 < \Delta E < 1.5$), noticeable ($1.5 < \Delta E < 3.0$), well visible ($3.0 < \Delta E < 6.0$) and greatly visible ($6.0 < \Delta E < 12$).

3.7 CLARITY

Clarity of juice samples was determined by measuring transmittance at 660 nm using a spectrophotometer (UV-200-RS Spectrophotometer, MRC, Israel), as described by Glevitzky *et al.* (2008). SDW served as a blank. High percentage of transmittance at 660 nm corresponds to high clarity.

3.8 NON-ENZYMATIC BROWNING INDEX (NEBI) AND 5-HYDROXYMETHYL FURFURAL (HMF) CONTENT

3.8.1 Preparation of reagents

95% ethanol
95 ml of ethanol (Merck) was added into 5 ml of SDW.

12% trichloroacetic acid (TCA)
6 g of TCA (Fisher Scientific) was dissolved in 50 ml of SDW.

0.025 M thiobarbituric acid (TBA)
0.18 g of TBA (Acros Organics) was dissolved in 50 ml of SDW

HMF standard curve
Stock solution was prepared by dissolving 5 mg of HMF (Acros Organics) in 500 ml of 95% ethanol. The different concentrations of HMF standards were prepared according to Table 3.5 and the final volume was 2 ml.

Table 3.5: HMF standard preparation

Concentration (mg/l)	0	0.25	0.50	1.00	2.00	4.00	6.00	8.00
Volume of stock solution (ml)	0	0.05	0.1	0.2	0.4	0.8	1.2	1.6
Volume of 95% ethanol (ml)	2.00	1.95	1.9	1.8	1.6	1.2	0.8	0.4

3.8.2 Determination of NEBI and HMF

NEBI and HMF assay was carried out according to the method by Cohen *et al.* (1998) with slight modifications. 5 ml of 95% ethanol was added to 5 ml of juice sample. The mixture was centrifuged (Beckman J2-MI Centrifuge, California) at 5000 rpm for 10 minutes at 5 °C. The supernatant was collected and separated into two portions. The first portion was used to determine NEBI by measuring absorbance at 420 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (95% ethanol).

For determination of HMF content, the second portion or HMF standard solution (1 ml) was added with 1 ml of 12% (w/v) trichloroacetic acid (TCA) and 1 mL of 0.025 M thiobarbituric acid (TBA) in a screw cap vial, and mixed thoroughly. The vials were placed in a water bath at 40 °C for 50 minutes. After incubation, the vials will be cooled to room temperature (25 ± 1 °C) under running water. The absorbance of samples were measured at 443 nm against a prepared blank (replace sample extract with 95% ethanol). A standard curve of HMF ($y=0.0406x$, $r^2=0.9967$) was prepared ranging from 0 to 8 mg/l, and results were expressed as milligrams of HMF per litre juice sample.

3.9 TOTAL CAROTENOID CONTENT

The carotenoid extraction was performed with modifications according to Lee *et al.* (2001) with slight modifications. Juice sample (10 ml) was homogenized (Omni Mixer Homogenizer, CT, USA) for 30 seconds (speed 4) with 20 ml of extracting solvent. The extracting solvent consists of 10 ml of hexane (Baker Analysed), 5 ml of acetone (Systerm) and 5 ml of ethanol (Merck). The mixture was centrifuged (Beckman J2-MI Centrifuge, California) for 10 minutes at 6500 rpm at 5 °C. The top layer of hexane containing carotenoids (yellow) was collected and adjusted to 10 ml with hexane.

Total carotenoid content was determined according to Scott (2001). For an aliquot of hexane extract, absorbance was measured at 450 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (hexane). The total carotenoid content using β-carotene as a reference was calculated according to the following formula:

$$\textbf{Total carotenoid content} = (A \times V_1 \times C^{1\%}) / A^{1\%}$$

where

A = absorbance reading of the diluted sample

V_1 = dilution factor

$A^{1\%}$ = absorbance of a 1% solution (the extinction coefficient for β-carotene: 2592 AU)

$C^{1\%}$ = concentration of a 1% solution (10 mg/ml)

3.10 ASCORBIC ACID CONTENT

3.10.1 Preparation of reagents

3% Metaphosphoric acid (HPO_3)
6 g of HPO_3 stick (R & M Chemicals) was dissolved in 200 ml of SDW.

Ascorbic acid standard solution
10 mg of L-ascorbic acid (Baker analysed) was dissolved in 10 ml of 3% HPO_3 solution. Volume of solution was made up to 100 ml with 3% HPO_3 solution. This stock solution (5 ml) was further diluted with 5 ml of 3% HPO_3 solution.

Dye solution
50 mg of sodium salt of 2,6-dichloroindophenol-indophenol (Sigma) and 42 mg of sodium bicarbonate (BDH) were dissolved in 150 ml of hot SDW. After cooling, the volume of solution was made up to 200 ml with SDW and filtered through fluted Whatman No.1 filter paper. The solution was standardized before use.

3.10.2 Determination of ascorbic acid content

The ascorbic acid content was determined based on the 2,6-dichlorophenol-indophenol (DCPIP) visual titration method (Ranganna, 1977). The dye factor was obtained by titration of standard solution of ascorbic acid with dye solution to a pink end point (colour should persist for ≥ 15 seconds). Juice sample (10 ml) was made up to 100 mL with 3% HPO_3 solution, and then filtered through Whatman No. 1 filter paper. Then, 5 ml of the filtrate was titrated with standardized dye solution until the pink end point.

Results obtained were expressed as milligrams of ascorbic acid per 100 ml sample, using the following equation:

$$\textbf{mg of ascorbic acid per 100 ml} = (V_1 \times \text{dye factor} \times V_2 \times 100) / (S_1 \times S_2)$$

where

V_1 = titre (ml)

V_2 = volume made up (ml)

S_1 = aliquot of extract taken for estimation (ml)

S_2 = volume of sample taken for estimation (ml)

Dye factor = 0.5 / titre

3.11 ANTIOXIDANT ACTIVITY

3.11.1 Sample preparation

Juice samples were extracted according to the method by Xu *et al.* (2008) with slight modifications. Equal parts of juice sample and 80% methanol was added into a 50 ml Falcon tube to purify the sample. The tubes were placed in a shaking incubator (Shel lab Orbital Shaking Incubator S14, OR, USA) at 250 rpm for 30 minutes at 25 °C, and then centrifuged (Beckman J2-MI Centrifuge, California) at 6500 rpm for 15 minutes at 5 °C. The supernatant of the centrifuged sample was used for the analysis of antioxidant activity.

3.11.2 Total polyphenol content (TPC)

3.11.2.1 Preparation of reagents

20% sodium carbonate

2 g of anhydrous sodium carbonate (BDH) was dissolved in 10 ml of SDW.

Gallic acid standard curve

Stock solution was prepared by dissolving 20 mg of gallic acid (Sigma) in 100 ml of SDW. The different concentrations of gallic acid standards were prepared according to Table 3.6 and the final volume was 2 ml.

Table 3.6: Gallic acid standard preparation

Concentration (mg/100 ml)	0	20	40	60	80	100	120	140
Volume of stock solution (ml)	0	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Volume of SDW (ml)	2.0	1.8	1.6	1.4	1.2	1.0	0.8	0.6

3.11.2.2 Determination of TPC

Total polyphenol contents of juice samples were determined using Folin-Ciocalteu assay (Singleton *et al.,* 1965) modified to a microscale (Bae and Suh, 2007). Juice extract or gallic acid standard solution (10 µl) was added to 790 µl SDW and 50 µl Folin-Ciocalteu reagent (Sigma-Aldrich) in a 1.5 ml microcentrifuge tube, and mixed. After 1 minute, 150 µl of 20% sodium carbonate solution was added, and the solution was mixed by inverting the tubes. The mixture was allowed to stand at room temperature (25 ± 1 °C) for 120 minutes (in dark). Absorbance was measured at 750 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (replace juice extract with SDW). A standard curve of gallic acid ($y=0.00566x$, $r^2=0.9955$) was prepared ranging from 0 to 140 mg/100 ml, and results were expressed as milligrams of gallic acid equivalent (GAE) per 100 ml juice extract.

3.11.3 Total flavonoid content (TFC)

3.11.3.1 Preparation of reagents

5% sodium nitrite
2.5 g of anhydrous sodium carbonate (Systerm) was dissolved in 50 ml of SDW.

10% aluminium chloride
5 g of anhydrous aluminium chloride (Fisher Scientific) was dissolved in 50 ml of SDW.

1N sodium hydroxide
2 g of sodium hydroxide (Merck) was dissolved in 50 ml of SDW.

95% ethanol
95 ml of ethanol (Merck) was added into 5 ml of SDW.

Catechin standard curve
Stock solution was prepared by dissolving 10 mg of (+)-catechin (Sigma-Aldrich) in 10 ml of 95% ethanol. The different concentrations of catechin standards were prepared according to Table 3.7 and the final volume was 1.5 ml.

Table 3.7: Catechin standard preparation

Concentration (mg/100 ml)	0	5	10	20	40	60	80	100
Volume of stock solution (ml)	0	0.08	0.20	0.30	0.60	0.90	1.20	1.50
Volume of 95% ethanol (ml)	1.5	1.4	1.3	1.2	0.9	0.6	0.3	0

3.11.3.2 Determination of TFC

Total flavonoid content of juice samples were evaluated using aluminium chloride colorimetric method described by Sakanaka, Tachibana, and Okada (2005). Juice extract or catechin standard solution (250 µl) was added to 1.25 ml of SDW and 75 µl of a 5% sodium nitrite solution in a test tube, and mixed. After 5 minutes of incubation at room temperature (25 ± 1 °C), 150 µl of a 10% aluminium chloride solution was

added to the mixture. The mixture was allowed to stand for another 5 minutes and then, 500 µl of 1N sodium hydroxide was added. The mixture was made up to 2.5 ml with SDW and vortexed. Absorbance was measured at 510 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (replace juice extract with SDW). A standard curve of catechin ($y=0.0135x$, $r^2=0.9943$) was prepared ranging from 0 to 100 mg/100 ml, and results were reported as milligrams of catechin equivalent (CE) per 100 ml juice extract.

3.11.4 1,1-di-phenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

3.11.4.1 Preparation of reagents

80% methanol
80 ml of methanol (Systerm) was added into 20 ml of SDW.

80% methanolic 0.1 mM DPPH solution
3.94 mg of DPPH (Sigma) was dissolved in 100 ml of 80% methanol.

Ascorbic acid standard curve
Stock solution was prepared by dissolving 100 mg of L-ascorbic acid (Baker analysed) in 10 ml of SDW. A new stock solution (100 µg/ml) was prepared by diluting 50 µl of this existing stock solution with SDW to a final volume of 5 ml. The different concentrations of ascorbic acid standards were prepared according to Table 3.8 and the final volume was 2 ml.

Table 3.8: Ascorbic acid standard preparation for DPPH assay

Concentration (µg/ml)	0	0.5	1.0	2.0	40	6.0	8.0	10.0
Volume of new stock solution (ml)	0	0.01	0.02	0.04	0.08	0.12	0.16	0.20
Volume of SDW (ml)	2.00	1.99	1.98	1.96	1.92	1.88	1.84	1.80

3.11.4.2 Determination of DPPH assay

This assay is based on the measurement of scavenging ability of antioxidants towards the stable radical DPPH, as described by Oyaizu (1986) and Bae and Suh (2007). Juice extract or ascorbic acid standard solution (500 µl) was added to 1 ml of 80% methanolic 0.1 mM DPPH solution in a 2 ml amber microcentrifuge tube. The mixture was vortexed and incubated in the water bath (Memmert, Germany) at 37 ºC for 30 minutes (in dark). Absorbance was measured at 517 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (80% methanol) and a control (replace juice extract with 80% methanol). A standard curve of ascorbic acid ($y=10.145x$, $r^2=0.9907$) was prepared ranging from 0 to 10 µg/ml, and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract.

The radical scavenging activity was calculated accordingly:

$$\textbf{\% DPPH inhibition} = (A_{control} - A_{sample} / A_{control}) \times 100$$

where

$A_{control}$ is absorbance reading of control

A_{sample} is absorbance reading of the sample

3.11.5 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay

3.11.5.1 Preparation of reagents

7.4 mM $ABTS^+$ solution

20 mg of ABTS diammonium salt (Sigma) was dissolved in 5 ml of SDW.

2.6 mM potassium persulfate

3.51 g of potassium persulfate (Sigma-Aldrich) was dissolved in 5 ml of SDW.

Ascorbic acid standard curve

Stock solution was prepared by dissolving 100 mg of L-ascorbic acid (Baker analysed) in 10 ml of SDW. A new stock solution (100 µg/ml) was prepared by diluting 100 µl of this existing stock solution with SDW to a final volume of 10 ml. The different concentrations of ascorbic acid standards were prepared according to Table 3.9 and the final volume was 2 ml.

Table 3.9: Ascorbic acid standard preparation for ABTS assay

Concentration (µg/ml)	0	20	40	60	80	100
Volume of new stock solution (ml)	0	0.4	0.8	1.2	1.6	2.0
Volume of SDW (ml)	2.0	1.6	1.2	0.8	0.4	0

3.11.5.2 Determination of ABTS assay

The ABTS assay was conducted according to the method of Arnao, Cano, and Acosta (2001) with minor modifications. The ABTS radical cation ($ABTS^{\bullet+}$) stock solution was prepared by mixing equal parts of 7.4 mM $ABTS^{+}$ solution and 2.6 mM potassium persulfate solution and allowed to stand at room temperature (25 ± 1 °C) for 12 hours (in dark). This $ABTS^{\bullet+}$ stock solution was diluted by mixing 3.5 ml of stock solution with 30 ml methanol (Systerm) to obtain an absorbance of 0.880 ± 0.05 units at 734 nm (UV-200-RS Spectrophotometer, MRC, Israel). Juice extract or ascorbic acid standard solution (100 µl) was added to 1.9 ml of the diluted ABTS•+ solution in a screw cap vial and incubated at room temperature (25 ± 1 °C) for 2 hours (in dark). Absorbance was measured at 734 nm against a prepared blank (methanol) and a control (replace juice extract with methanol). A standard curve of ascorbic acid ($y=0.0038x$, $r^2=0.9958$) was prepared ranging from 0 to 100 µg/ml, and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract.

3.11.6 Reducing power assay (RPA)

3.11.6.1 Preparation of reagents

0.2 M phosphate buffer (pH 6.6)
Reagent A
4.82 g of sodium phosphate monobasic (Sigma) was dissolved in 250 ml of SDW.
Reagent B
4.04 g of sodium phosphate dibasic (Sigma) was dissolved in 250 ml of SDW.

For the phosphate buffer, 205.5 ml of Reagent A was added into 94.5 ml of Reagent B.

1% Potassium ferricyanide
0.05 g of potassium ferricyanide (Sigma) was dissolved in 5 ml of SDW.

10% Trichloroacetic acid (TCA)
0.05 g of TCA (Fisher Chemical) was dissolved in 50 ml of SDW.

0.1% Ferric chloride
0.01 g of ferric chloride (Sigma) was dissolved in 10 ml of SDW.

Ascorbic acid standard curve
Stock solution was prepared by dissolving 100 mg of L-ascorbic acid (Baker analysed) in 10 ml of SDW. The different concentrations of ascorbic acid standards were prepared according to Table 3.10 and the final volume was 2 ml.

Table 3.10: Ascorbic acid standard preparation for RPA

Concentration (µg/ml)	0	5	25	50	100	200	400	800
Volume of stock solution (ml)	0	0.001	0.005	0.010	0.020	0.040	0.080	0.160
Volume of SDW (ml)	2.000	1.999	1.995	1.990	1.980	1.960	1.920	1.840

3.11.6.2 Determination of RPA

A spectrophotometric method by Oyaizu (1986) with slight modifications was used for measuring the reducing power of juice samples. Juice extract or ascorbic acid standard solution (50 µl) was added to 200 µl of 0.2M phosphate buffer (pH 6.6) and 200 µl of

1% potassium ferricyanide in a 1.5 ml microcentrifuge tube. The mixture was incubated in the water bath (Memmert, Germany) for 20 minutes at 50 ºC (in dark). Next, 250 µl of 10% trichloroacetic acid was added to the mixture, and then centrifuged (UEC Micro 14/B, New York) at 1000 rpm for 5 minutes at room temperature (25 ± 1 °C). The supernatant of the centrifuged sample (500 µl) was mixed with 500 µl of SDW and 100 µl of 0.1% ferric chloride. The mixture was allowed to stand at room temperature (25 ± 1 °C) for 5 minutes. Absorbance was measured at 700 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (phosphate buffer) and a control (replace juice extract with phosphate buffer). A standard curve of ascorbic acid ($y=0.0014x$, $r^2=0.9906$) was prepared ranging from 0 to 800 µg/ml, and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract.

3.11.7 Total antioxidant capacity (TAC)

3.11.7.1 Preparation of reagents

0.6 M sulfuric acid
33.33 ml of concentrated (18 N) sulfuric acid (Systerm) was made up to 1L with SDW.

28 mM sodium phosphate
0.336 g of sodium phosphate (Sigma) was dissolved in 100 ml of SDW.

4 mM ammonium molybdate
0.494 g of ammonium molybdate (BDH) was dissolved in 100 ml of SDW.

Ascorbic acid standard curve
Stock solution was prepared by dissolving 100 mg of L-ascorbic acid (Baker analysed) in 10 ml of SDW. The different concentrations of ascorbic acid standards were prepared according to Table 3.11 and the final volume was 2 ml.

Table 3.11: Ascorbic acid standard preparation for TAC

Concentration (µg/ml)	0	5	25	50	100	200	400	800
Volume of stock solution (ml)	0	0.001	0.005	0.010	0.020	0.040	0.080	0.160
Volume of SDW (ml)	2.000	1.999	1.995	1.990	1.980	1.960	1.920	1.840

3.11.7.2 Determination of TAC

The antioxidant capacity of juice samples were determined using the phosphomolybdenum method described by Prieto *et al.* (1999). Juice extract or ascorbic acid standard solution (100 µl) was added to 1 ml of reagent solution in a 1.5 ml microcentrifuge tube. The reagent solution consists of equal volume of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were incubated in a water bath (Memmert, Germany) at 95°C for 90 minutes (in dark). After cooling to room temperature (25 ± 1 °C), absorbance was measured at 695 nm (UV-200-RS Spectrophotometer, MRC, Israel) against a prepared blank (replace juice extract with SDW). A standard curve of ascorbic acid ($y=0.0018x$, $r^2=0.9981$) was prepared ranging from 0 to 800 µg/ml, and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract.

3.12 LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LCMS/MS) ANALYSIS: IDENTIFICATION AND QUANTIFICATION OF PHENOLIC COMPOUNDS

3.12.1 Preparation of reagents

80% methanol

80 ml of HPLC grade methanol (Friendmann Schmidt) was added into 20 ml of SDW.

3.12.2 Sample preparation

Juice samples were extracted according to the method by Belajová and Suhaj (2004) with slight modifications. Juice sample (10 ml) was extracted with 10 ml of 80% HPLC grade methanol in a 50 ml Falcon tube. The tubes were placed in a shaking incubator (Shel lab Orbital Shaking Incubator S14, OR, USA) at 250 rpm for 30 minutes at 25 °C, and then centrifuged (Beckman J2-MI Centrifuge, California) at 6500 rpm for 20 minutes at 5 °C. The supernatant of the centrifuged sample was collected and filtered through a 0.22 µm nylon membrane filter before injected into the LCMS/MS instrument.

3.12.3 LCMS/MS conditions

Juice extract (20 µl) was injected into the LCMS/MS system (AB Sciex 3200QTrap LCMS/MS, MA, USA) coupled to Ultra High Performance Liquid Chromatography (UHPLC) system (Perkin Elmer Flexar FX15, MA, USA). The column used was Phenomenex Aqua C_{18}-50mm x 2.1mm x 5uM (CA, USA). Negative ionisation mode was used for scanning. Gradient elution was performed using water with 0.1% formic acid and 5mM ammonium formate (buffer A), and acetonitrile with 0.1% formic acid and 5mM ammonium formate (buffer B) at flow rate of 800 µl/min. The gradient run

program was as follows: 10% A and 90% B at 0.01 to 8 minutes, then hold for 3 minutes, and back to 10% A at 0.1min, and re-equilibrated for 5 minutes. Rapid screening was performed at 15 min run time.

Identification of phenolic compounds were based on accurate masses of parent and their fragment ions, according to ACD/Labs Advanced Chemometrics Mass Fragmentation predictive software, the National Institute of Standards and Technology Database (accessed at http://webbook.nist.gov/chemistry) and Mass Bank Spectral Database (accessed at http://www.massbank.jp). The phenolic compounds were quantified by the absorbance (intensity) of their corresponding peaks.

3.13 SENSORY ANALYSIS

Sensory evaluation of juice samples (Pala and Toklucu, 2013; Caminiti *et al.*, 2011) was conducted at the Postharvest Biotechnology Laboratory (University of Malaya) with four separated booths. The evaluation was carried out by 90 untrained panellists (randomly). Prior to the assessment, panellists were provided with a questionnaire to record their observations. The sample questionnaire is attached in Appendix 1.

Juice samples were refrigerated to equilibrate the temperature to 8 ± 2 °C °C for the sensory evaluation. Chilled juice samples (20 ml) were given to the panellists seated separately in booths to allow an unbiased evaluation of the sensory attributes. Juice samples were served in a randomized order in capped glass vials labelled with a three-digit code number. All samples were evaluated during the same session and distilled water was served for cleansing the palate between samples. The sensory evaluation was

carried out under white lights. Acceptance test were performed using a 1 to 9 hedonic scale for colour, odour, taste, and overall acceptability, with 1 corresponding to ‘‘dislike extremely”, 9 to ‘‘like extremely”, and 5 as rejection point.

3.14 SHELF LIFE STUDY

Juice samples were aseptically filled into sterile glass vials in a laminar flow cabinet (Gelman Science Biological Safety Cabinet Class II, NSW, Australia) and tightly capped, leaving minimum amount of headspace volume. The vials were stored at 4 ± 1 °C in a refrigerator (under dark condition) for five weeks and analysed at one week intervals. All analysis was carried out in triplicates.

3.14.1 APC

The APC of juice samples was determined as described in Section 3.5.1.

3.14.2 TSS

TSS of juice samples was determined as described in Section 3.5.2.

3.14.3 Visual observation

Juice samples were visually assessed for colour changes and photographs were taken.

3.15 STATISTICAL ANALYSIS

Data obtained were subjected to statistical analysis using SPSS 22.0 software (SPSS Inc., IBM). In this study, data were represented as mean values ± standard deviation (SD). The significant differences between mean values of juice samples were determined by analysis of variance (one way-ANOVA) using Tukey's HSD (Honestly Significant Difference) test at a significance level of $p < 0.05$. The relationship between variables was determined using Pearson's correlation test at a significance level of $p < 0.01$ and $p < 0.05$.

CHAPTER 4

RESULTS

4.1 MICROBIAL INACTIVATION ANALYSIS

The coliform, aerobic bacteria, yeast and mould counts in freshly squeezed Chokanan mango juice were 1.00 log CFU/ml, 2.74 log CFU/ml and 2.42 log CFU/ml, respectively, as shown in Table 4.1. After thermal treatment, significant reduction in microbial count ($p<0.05$) was observed in juice samples. Thermal treatment regardless of mild or high heat pasteurization reduced coliform, aerobic bacteria, yeast and mould count to below detection limits.

Table 4.1: Effects of thermal treatment on microbial inactivation analysis of Chokanan mango juice

Treatment	CC (log CFU/ml)	APC (log CFU/ml)	YMC (log CFU/ml)
Control	1.00 ± 0.02^{a}	2.74 ± 0.01^{a}	2.42 ± 0.02^{a}
MP	ND^{b}	ND^{b}	ND^{b}
HP	ND^{b}	ND^{b}	ND^{b}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

[2] ND, not detected; CFU, colony-forming unit.

The coliform, aerobic bacteria, yeast and mould counts in freshly squeezed Chokanan mango juice were 1.00 log CFU/ml, 2.74 log CFU/ml and 2.42 log CFU/ml, respectively, as shown in Table 4.2. After sonication, significant reduction in microbial count was observed in juice samples. The ultrasonic treatment reduced coliform counts to below detection limits. With regards to aerobic bacteria, the highest reduction in microbial count (26%) was observed in S60 sample when compared to other sonicated samples, S30 (17%) and S15 (8%). For yeast and mould count, the maximum percentage of inactivation was for S60 sample (14%), which is lower than aerobic bacteria inactivation (26%). However, complete inactivation of microbial growth in juice was not observed in this study for ultrasonic treatment for 15, 30 and 60 minutes.

Table 4.2: Effects of sonication on microbial inactivation analysis of Chokanan mango juice

Treatment	CC (log CFU/ml)	APC (log CFU/ml)	YMC (log CFU/ml)
Control	1.00 ± 0.02^{a}	2.74 ± 0.01^{a}	2.42 ± 0.02^{a}
S15	ND^{b}	2.53 ± 0.02^{b}	2.30 ± 0.04^{b}
S30	ND^{b}	2.28 ± 0.03^{c}	2.19 ± 0.01^{c}
S60	ND^{b}	2.02 ± 0.03^{d}	2.09 ± 0.03^{d}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) ($n=12$).

[2] ND, not detected; CFU, colony-forming unit.

The coliform, aerobic bacteria, yeast and mould counts in freshly squeezed Chokanan mango juice were 1.00 log CFU/ml, 2.74 log CFU/ml and 2.42 log CFU/ml, respectively, as shown in Table 4.3. After UV-C treatment, significant reduction in microbial count was observed in juice samples. UV-C treatment reduced coliform counts to below detection limits. For aerobic bacteria, the highest reduction in microbial load (45%) was exhibited in U60 sample when compared to other UV-C treated samples, U30 (30%) and U15 (18%). The percentage of inactivation of yeast and mould (10 to 32%) was lower than aerobic bacteria (18 to 45%) for UV-C treated samples, exhibiting maximum inactivation in U60 sample (32%). However, complete removal of microbial load in juice samples were not observed in this study for UV-C exposure for 15, 30 and 60 minutes.

Table 4.3: Effects of UV-C treatment on microbial inactivation analysis of Chokanan mango juice

Treatment	CC (log CFU/ml)	APC (log CFU/ml)	YMC (log CFU/ml)
Control	1.00 ± 0.02^{a}	2.74 ± 0.01^{a}	2.42 ± 0.02^{a}
U15	ND^{b}	2.25 ± 0.02^{b}	2.18 ± 0.01^{b}
U30	ND^{b}	1.92 ± 0.05^{c}	1.84 ± 0.02^{c}
U60	ND^{b}	1.52 ± 0.03^{d}	1.65 ± 0.04^{d}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

[2] ND, not detected; CFU, colony-forming unit.

The coliform, aerobic bacteria, yeast and mould counts in freshly squeezed Chokanan mango juice were 1.00 log CFU/ml, 2.74 log CFU/ml and 2.42 log CFU/ml, respectively, as shown in Table 4.4. After combined treatment, significant reduction in microbial count was observed in juice samples. Combined treatment reduced coliform count to below detection limits. For aerobic bacteria, 100% reduction in microbial count was exhibited in S30+U30 sample when compared to other combined treated samples, S15+U15 (46%), S30+U15 and S15+U30 (64%).). In this study, the percentage of inactivation of total yeast and mould (24 to 59%) was lower than aerobic bacteria (46 to 100%) for combined treated samples, exhibiting maximum inactivation in S30+U30 sample (59%). However, complete inactivation of yeast and mould in juice samples were not observed in this study for all combined treated samples.

Table 4.4: Effects of combined treatment on microbial inactivation analysis of Chokanan mango juice

Treatment	CC (log CFU/ml)	APC (log CFU/ml)	YMC (log CFU/ml)
Control	1.00 ± 0.02^{a}	2.74 ± 0.01^{a}	2.42 ± 0.02^{a}
S15 + U15	ND^{b}	1.48 ± 0.02^{c}	1.84 ± 0.05^{b}
S30 + U15	ND^{b}	1.00 ± 0.08^{d}	1.50 ± 0.03^{c}
S15 + U30	ND^{b}	1.00 ± 0.04^{d}	1.39 ± 0.03^{d}
S30 + U30	ND^{b}	ND^{b}	1.00 ± 0.05^{e}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

[2] ND, not detected; CFU, colony-forming unit.

4.2 PHYSICOCHEMICAL ANALYSIS

The pH, TSS, and TA of juice (Table 4.5) showed no significant changes after thermal treatment (mild and high heat pasteurization). Values for pH (4.58 to 4.62), TSS (14.6 to 14.7 °Brix) and TA (0.19 to 0.21%) of treated juice samples was within the range of standards desirable for freshly squeezed Chokanan mango juice.

Table 4.5: Effects of thermal treatment on physicochemical analysis of Chokanan mango juice

Treatment	pH	TSS (°Brix)	TA (%)
Control	4.62 ± 0.01[a]	14.7 ± 0.06[a]	0.20 ± 0.01[a]
MP	4.60 ± 0.02[a]	14.6 ± 0.06[a]	0.21 ± 0.01[a]
HP	4.58 ± 0.02[a]	14.6 ± 0.05[a]	0.19 ± 0.01[a]

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

The pH, TSS, and TA of juice (Table 4.6) showed no significant changes after ultrasonic treatments. Hence, the pH (4.59 to 4.62), TSS (14.5 to 14.7 °Brix) and TA (0.20 to 0.21%) of treated juice samples was within the range of standards desirable for freshly squeezed Chokanan mango juice.

Table 4.6: Effects of sonication on physicochemical analysis of Chokanan mango juice

Treatment	pH	TSS (°Brix)	TA (%)
Control	4.62 ± 0.01[a]	14.7 ± 0.06[a]	0.20 ± 0.01[a]
S15	4.59 ± 0.01[a]	14.6 ± 0.08[a]	0.21 ± 0.01[a]
S30	4.61 ± 0.01[a]	14.6 ± 0.05[a]	0.20 ± 0.01[a]
S60	4.59 ± 0.02[a]	14.5 ± 0.06[a]	0.21 ± 0.01[a]

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) ($n=12$).

The pH, TSS, and TA of juice (Table 4.7) showed no significant changes after UV-C treatment. Values for pH (4.59 to 4.62), TSS (14.5 to 14.7 °Brix) and TA (0.19 to 0.22%) of treated juice samples was within the range of standards desirable for freshly squeezed Chokanan mango juice.

Table 4.7: Effects of UV-C treatment on physicochemical analysis of Chokanan mango juice

Treatment	pH	TSS (°Brix)	TA (%)
Control	4.62 ± 0.01[a]	14.7 ± 0.06[a]	0.20 ± 0.01[a]
U15	4.60 ± 0.01[a]	14.7 ± 0.12[a]	0.22 ± 0.01[a]
U30	4.59 ± 0.01[a]	14.5 ± 0.11[a]	0.21 ± 0.01[a]
U60	4.61 ± 0.01[a]	14.5 ± 0.05[a]	0.19 ± 0.01[a]

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

The pH, TSS, and TA of juice (Table 4.8) showed no significant changes after combined treatment. Values for pH (4.59 to 4.63), TSS (14.5 to 14.7 °Brix) and TA (0.19 to 0.22%) of treated juice samples was within the range of standards desirable for freshly squeezed Chokanan mango juice.

Table 4.8: Effects of combined treatment on physicochemical analysis of Chokanan mango juice

Treatment	pH	TSS (°Brix)	TA (%)
Control	4.62 ± 0.01[a]	14.7 ± 0.06[a]	0.20 ± 0.01[a]
S15 + U15	4.59 ± 0.02[a]	14.6 ± 0.08[a]	0.22 ± 0.01[a]
S30 + U15	4.60 ± 0.01[a]	14.7 ± 0.06[a]	0.21 ± 0.02[a]
S15 + U30	4.61 ± 0.02[a]	14.5 ± 0.08[a]	0.21 ± 0.01[a]
S30 + U30	4.63 ± 0.01[a]	14.5 ± 0.08[a]	0.19 ± 0.01[a]

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

4.3 COLOUR

Significant differences in the colour of juice samples were observed after thermal treatment when compared to the control (Table 4.9). According to Cserhalmi *et al.* (2006), the differences in colour parameters (ΔE) can be classified as not noticeable ($0 < \Delta E < 0.5$), slightly noticeable ($0.5 < \Delta E < 1.5$), noticeable ($1.5 < \Delta E < 3.0$), well visible ($3.0 < \Delta E < 6.0$) and greatly visible ($6.0 < \Delta E < 12$). Both mild and high pasteurization induce 'well visible' colour changes in juice samples. In addition, an increase in lightness (L^*) (3%), and decrease in redness ($+a^*$) (35%) and yellowness ($+b^*$) (6%) were observed in juice samples after treatments.

Table 4.9: Effects of thermal treatment on colour analysis of Chokanan mango juice

Treatment	Colour parameters			
	L^*	a^*	b^*	ΔE
Control	70.28 ± 0.10^a	5.00 ± 0.03^a	54.45 ± 0.09^a	-
MP	72.40 ± 0.22^b	3.38 ± 0.15^b	51.44 ± 0.02^b	4.02 ± 0.07^a
HP	72.26 ± 0.11^b	3.27 ± 0.11^b	51.23 ± 0.15^b	4.16 ± 0.08^a

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

Significant differences in the colour of juice samples were recorded between the control and sonication, as shown in Table 4.10. According to the classification of Cserhalmi *et al.* (2006), the lowest variation from the control was observed in S15 and S30 samples, therefore falling within the 'slightly noticeable' colour change range ($0.5 < \Delta E < 1.5$). After ultrasonic treatment, an increase in lightness (L^*) (2%), and decrease in redness ($+a^*$) (3 to 7%) and yellowness ($+b^*$) (2 to 3%) were observed in juice samples.

Table 4.10: Effects of sonication on colour analysis of Chokanan mango juice

Treatment	**Colour parameters**			
	L^*	a^*	b^*	ΔE
Control	70.28 ± 0.10^{a}	5.00 ± 0.03^{a}	54.45 ± 0.09^{a}	-
S15	70.75 ± 0.20^{b}	4.69 ± 0.13^{b}	53.30 ± 0.05^{b}	1.28 ± 0.07^{a}
S30	71.20 ± 0.22^{b}	4.83 ± 0.05^{ab}	53.29 ± 0.06^{b}	1.49 ± 0.10^{a}
S60	70.82 ± 0.08^{b}	4.63 ± 0.08^{b}	52.78 ± 0.03^{c}	1.79 ± 0.02^{b}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) ($n=12$).

Significant differences in the colour of juice samples were observed after UV-C treatments when compared to the control (Table 4.11). According to the classification of Cserhalmi *et al.* (2006), the least variation from the control was observed in U15 sample, therefore falling within the 'slightly noticeable' range ($0.5 < \Delta E < 1.5$). In addition, a significant increase in ΔE value corresponding to increased UV-C treatment time (from 15 to 60 minutes) was observed. After UV-C exposure, an increase in lightness (L^*) (1 to 2 %), and decrease in redness ($+a^*$) (9%) and yellowness ($+b^*$) (2 to 4%) were observed in juice samples.

Table 4.11: Effects of UV-C treatment on colour analysis of Chokanan mango juice

Treatment	**Colour parameters**			
	L^*	a^*	b^*	ΔE
Control	70.28 ± 0.10^{a}	5.00 ± 0.03^{a}	54.45 ± 0.09^{a}	-
U15	70.83 ± 0.12^{b}	4.82 ± 0.06^{ab}	53.60 ± 0.15^{b}	1.03 ± 0.15^{a}
U30	71.05 ± 0.09^{b}	4.79 ± 0.03^{ab}	52.89 ± 0.10^{c}	1.75 ± 0.10^{b}
U60	71.42 ± 0.08^{c}	4.53 ± 0.15^{b}	52.50 ± 0.02^{d}	2.31 ± 0.07^{c}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

Significant differences in the colour of juice samples were observed between control and combined treatments, as shown in Table 4.12. According to the classification of Cserhalmi *et al.* (2006), combined treated samples S15+U15, S30+U15, S15+U30 showed lowest variation from the control, therefore falling within the 'slightly noticeable' range ($0.5 < \Delta E < 1.5$). In addition, an increase in lightness (L^*) (1 to 2 %), and decrease in redness ($+a^*$) (11%) and yellowness ($+b^*$) (1 to 3%) were observed after combined treatments. Indeed, a significant increase in ΔE corresponding to increased ultrasonic treatment time (from 15 to 30 minutes) was observed, regardless of the UV-C treatment.

Table 4.12: Effects of combined treatment on colour analysis of Chokanan mango juice

Treatment	**Colour parameters**			
	L^*	a^*	b^*	ΔE
Control	70.28 ± 0.10^{a}	5.00 ± 0.03^{a}	54.45 ± 0.09^{a}	-
S15 + U15	70.55 ± 0.22^{b}	4.72 ± 0.05^{ab}	53.85 ± 0.03^{b}	0.72 ± 0.02^{a}
S30 + U15	70.78 ± 0.12^{b}	4.89 ± 0.02^{ab}	53.55 ± 0.08^{bc}	1.04 ± 0.06^{b}
S15 + U30	70.76 ± 0.12^{b}	4.64 ± 0.13^{b}	53.88 ± 0.05^{b}	0.83 ± 0.12^{a}
S30 + U30	71.68 ± 0.13^{c}	4.46 ± 0.14^{b}	52.94 ± 0.10^{d}	2.17 ± 0.10^{c}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

4.4 CLARITY, NEBI AND HMF CONTENT

Clarity indicates the degree of turbidity or darkening of fruit juices. A significant decrease in clarity of juice (Table 4.13) was observed after thermal treatment. The least clarity (7.3) was observed in high heat pasteurized samples, when compared to the control (25.5), thus indicating the highest percentage of turbidity. Hence, thermal treatment causes 71% reduction in clarity of juice samples.

A significant increase in NEBI (Table 4.13) was observed in juice processed by thermal. It was clearly exhibited that higher browning index was observed in juice sample subjected to high heat pasteurisation, when compared to mild heat pasteurisation. Accordingly, HMF content also increased in thermally treated samples (0.97 to 1.12 mg/l) when compared to the control (0.60 mg/l), as shown in Table 4.13.

Table 4.13: Effects of thermal treatment on clarity, NEBI and HMF content of Chokanan mango juice

Treatment	Clarity (Transmittance at 660 nm)	NEBI (Absorbance at 420 nm)	HMF (mg/l)
Control	25.50 ± 0.22^{a}	0.06 ± 0.00^{a}	0.60 ± 0.03^{a}
MP	7.60 ± 0.53^{b}	0.10 ± 0.01^{b}	0.97 ± 0.08^{b}
HP	7.30 ± 0.24^{b}	0.13 ± 0.01^{c}	1.12 ± 0.05^{b}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) ($n=12$).

There was a significant improvement in clarity of juice samples (Table 4.14) subjected to ultrasonic treatment except S60, when compared to the control. The highest increase (7%) in clarity was observed in S30 sample.

A significant increase in NEBI (Table 4.14) was observed in juice processed by sonication. However, minimum increase in NEBI (0.08) was observed in sonicated juice samples, S15 and S30, when compared to the control (0.06). According to Table 4.14, no significant changes in HMF content was exhibited in juice subjected to ultrasonic treatment except S60, when compared to the control.

Table 4.14: Effects of sonication on clarity, NEBI and HMF content of Chokanan mango juice

Treatment	Clarity (Transmittance at 660 nm)	NEBI (Absorbance at 420 nm)	HMF (mg/l)
Control	25.50 ± 0.22^{a}	0.06 ± 0.00^{a}	0.60 ± 0.03^{a}
S15	27.10 ± 0.20^{b}	0.08 ± 0.01^{bc}	0.64 ± 0.02^{a}
S30	27.30 ± 0.35^{b}	0.08 ± 0.00^{b}	0.63 ± 0.06^{a}
S60	24.10 ± 0.22^{c}	0.09 ± 0.00^{c}	0.89 ± 0.05^{b}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

No significant changes in clarity of juice samples were observed after UV-C treatment (U15 and U30), as shown in Table 4.15. In contrast, the clarity of U60 sample significantly decreased (5%).

There was a significant increase in NEBI of juice (Table 4.15) processed by UV-C, except U15. However, minimum increase in NEBI was observed in UV-C treated samples, U30 (0.09) and U60 (0.10), when compared to the control (0.06). Besides that, there was a significant increase in browning index corresponding to increased UV-C exposure time (from 15 to 60 minutes). According to Table 4.15, no significant changes in HMF content was observed in UV-C treated samples except U30 and U60, when compared to the control.

Table 4.15: Effects of UV-C treatment on clarity, NEBI and HMF content of Chokanan mango juice

Treatment	Clarity (Transmittance at 660 nm)	NEBI (Absorbance at 420 nm)	HMF (mg/l)
Control	25.50 ± 0.22^{a}	0.06 ± 0.00^{a}	0.60 ± 0.03^{a}
U15	25.40 ± 0.28^{a}	0.07 ± 0.01^{a}	0.77 ± 0.08^{ab}
U30	25.17 ± 0.35^{a}	0.09 ± 0.00^{b}	0.86 ± 0.02^{b}
U60	24.25 ± 0.20^{b}	0.10 ± 0.01^{b}	1.08 ± 0.05^{c}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

Significant improvement in clarity was observed in all combined treated samples, as shown in Table 4.16. The highest increase in clarity (12%) was observed in S30+U15 sample. In addition, there was a significant increase in browning index corresponding to increased sonication time (from 15 to 60 minutes).

No significant variation in NEBI and HMF content were observed in combined treated samples, S15+U15, S30+U15, S15+U30 except S30+U30, when compared to the control (Table 4.16).

Table 4.16: Effects of combined treatment on clarity, NEBI and HMF content of Chokanan mango juice

Treatment	Clarity (Transmittance at 660 nm)	NEBI (Absorbance at 420 nm)	HMF (mg/l)
Control	25.50 ± 0.22^{a}	0.06 ± 0.00^{a}	0.60 ± 0.03^{a}
S15 + U15	27.40 ± 0.22^{b}	0.07 ± 0.00^{ab}	0.67 ± 0.02^{a}
S30 + U15	28.55 ± 0.44^{c}	0.07 ± 0.01^{ab}	0.71 ± 0.05^{a}
S15 + U30	27.64 ± 0.32^{b}	0.07 ± 0.01^{ab}	0.69 ± 0.02^{a}
S30 + U30	26.02 ± 0.35^{d}	0.11 ± 0.00^{b}	0.98 ± 0.07^{b}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

4.5 TOTAL CAROTENOID AND ASCORBIC ACID CONTENT

Significant decrease in carotenoids was exhibited in juice samples subjected to thermal treatment, as shown in Table 4.17. Mild heat pasteurized samples showed 56.13 µg/100 ml and high heat pasteurized samples showed 48.92 µg/100 ml of carotenoid content, respectively, in comparison with control (82.03 µg/100 ml).

Significant decrease in the ascorbic acid content was observed in juice processed by thermal treatment when compared to control (Table 4.17). The highest degradation (65%) of ascorbic acid was recorded in high heat pasteurized sample, when compared to the control.

Table 4.17: Effects of thermal treatment on total carotenoid and ascorbic acid content of Chokanan mango juice

Treatment	Total carotenoid content (µg/100 ml)	Ascorbic acid content (mg/100 ml)
Control	82.03 ± 1.29^{a}	8.91 ± 0.26^{a}
MP	56.13 ± 2.23^{b}	3.62 ± 0.25^{b}
HP	48.92 ± 1.32^{c}	3.10 ± 0.31^{b}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

Significant increase in carotenoid content was observed in juice samples subjected to sonication (S15 and S30), as shown in Table 4.18. The highest enhancement in extractability of carotenoids (9%) was observed in S15 sample when compared to the control. However, degradation of carotenoids was exhibited in S60 sample.

After sonication, significant reduction in the ascorbic acid content was observed in juice samples when compared to control (Table 4.18) In addition, the least reduction in ascorbic acid after sonication was about 13 to 15%, which is in S15 (7.74 mg/100 ml) and S30 (7.52 mg/100 ml) juice samples, when compared to the control (8.91 mg/100 ml).

Table 4.18: Effects of sonication on total carotenoid and ascorbic acid content of Chokanan mango juice

Treatment	Total carotenoid content (µg/100 ml)	Ascorbic acid content (mg/100 ml)
Control	82.03 ± 1.29^{a}	8.91 ± 0.26^{a}
S15	89.53 ± 1.82^{b}	7.74 ± 0.26^{b}
S30	85.06 ± 1.75^{b}	7.52 ± 0.20^{b}
S60	78.16 ± 2.10^{a}	6.43 ± 0.15^{c}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) ($n=12$).

Significant increase in carotenoid content was observed juice samples subjected to UV-C exposure (U15 and U30), as shown in Table 4.19. The highest enhancement in extractability of carotenoids (6%) was recorded in U15 sample when compared to the control. After 60 minutes of UV-C treatment, no significant changes in carotenoid content were observed.

After UV-C treatment, significant reduction in the ascorbic acid content was observed in juice samples when compared to control (Table 4.19). The minimum degradation of ascorbic acid for UV-C treated samples was about 12 to 15%, which is in U15 (7.85 mg/100 ml) and U30 (7.55 mg/100 ml), when compared to the control (8.91 mg/100 ml).

Table 4.19: Effects of UV-C treatment on total carotenoid and ascorbic acid content of Chokanan mango juice

Treatment	Total carotenoid content (µg/100 ml)	Ascorbic acid content (mg/100 ml)
Control	82.03 ± 1.29^{a}	8.91 ± 0.26^{a}
U15	87.10 ± 1.14^{b}	7.85 ± 0.12^{b}
U30	84.97 ± 1.35^{b}	7.55 ± 0.30^{b}
U60	80.16 ± 1.80^{a}	6.87 ± 0.13^{c}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

Significant enhancement (8 to 15%) in carotenoid content was observed in juice subjected to combined treatment, as shown in Table 4.20. The highest increase in extractability of carotenoids (15%) was observed in S30+U30 sample when compared to the control, from 82.03 µg/100 ml to 94.70 µg/100 ml.

There was significant reduction in ascorbic acid content in juice processed by combined method when compared to control (Table 4.20). The least degradation of ascorbic acid after combined treatment was about 11% in S15+U15 sample.

Table 4.20: Effects of combined treatment on total carotenoid and ascorbic acid content of Chokanan mango juice

Treatment	Total carotenoid content (µg/100 ml)	Ascorbic acid content (mg/100 ml)
Control	82.03 ± 1.29^{a}	8.91 ± 0.26^{a}
S15 + U15	90.60 ± 1.52^{b}	7.92 ± 0.17^{b}
S30 + U15	93.65 ± 2.10^{c}	7.72 ± 0.12^{b}
S15 + U30	88.29 ± 1.43^{b}	7.88 ± 0.29^{b}
S30 + U30	94.70 ± 1.83^{c}	6.33 ± 0.26^{c}

[1] Values followed by different letters within the same column are significantly different ($p<0.05$) (n=12).

4.6 ANTIOXIDANT ACTIVITY

The effects of thermal treatment on antioxidant activity in juice extracts are shown in Figure 4.1 (a) to (f). After thermal treatment, a significant reduction in total polyphenols (15 to 38%) and total flavonoids (18 to 25%) were observed. The maximum loss of polyphenols and flavonoids was exhibited by high heat pasteurized samples, when compared to mild heat pasteurized samples.

With regards to antioxidant assays, significant reduction was observed in DPPH and ABTS radical scavenging activity after thermal treatment. Likewise, significant decrease in reducing ability (345 to 355 µg AAE/ml) and total antioxidant capacity (997 to 1012 µg AAE/ml) were observed in thermal pasteurized samples when compared to the control (360.71 µg AAE/ml; 1022 µg AAE/ml), respectively.

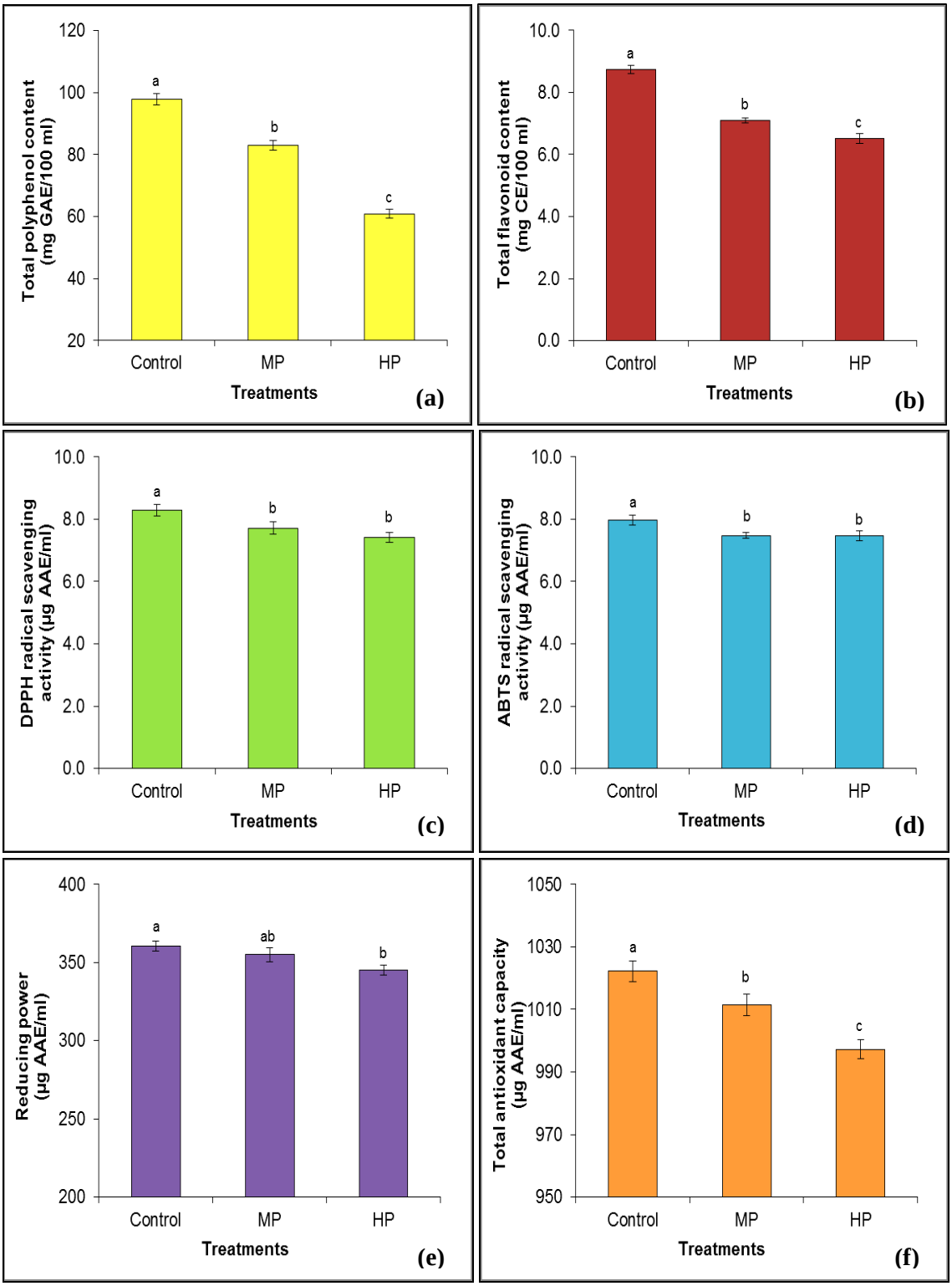


Figure 4.1: Effects of thermal treatment on antioxidant activity (a-f) of Chokanan mango juice

[1] Values followed by different letters are significantly different ($p<0.05$) (n=12).

[2] GAE, gallic acid equivalent; CE, catechin equivalent; AAE, ascorbic acid equivalent.

The effects of ultrasonic treatment on antioxidant activity in juice extracts are shown in Figure 4.2 (a) to (f). A significant increase in the extractability of polyphenols was observed in all sonicated juice samples when compared to control. The highest increase (35%) was from 97.8 mg GAE/100 ml to 132.2 mg GAE/100 ml in S30 sample. Similarly, significant enhancement (37%) in extractability of flavonoids was observed in all sonicated juice samples, being the highest in S30 (11.9 mg CE/100 ml) when compared to the control (8.7 mg CE/100 ml).

With regards to antioxidant assays, significant increase in DPPH and ABTS radical scavenging activity were observed in sonicated juice samples, when compared to the control. The highest DPPH radical scavenging activity was 9.0 µg AAE/ml in S15 sample, compared to the control (8.3 µg AAE/ml). While, the highest ABTS radical scavenging activity was 8.6 µg AAE/ml in S15 and S30 sample, compared to the control (8.0 µg AAE/ml). Besides that, significant increase in reducing power and total antioxidant capacity were exhibited by sonicated juice samples, being the highest in S30 sample.

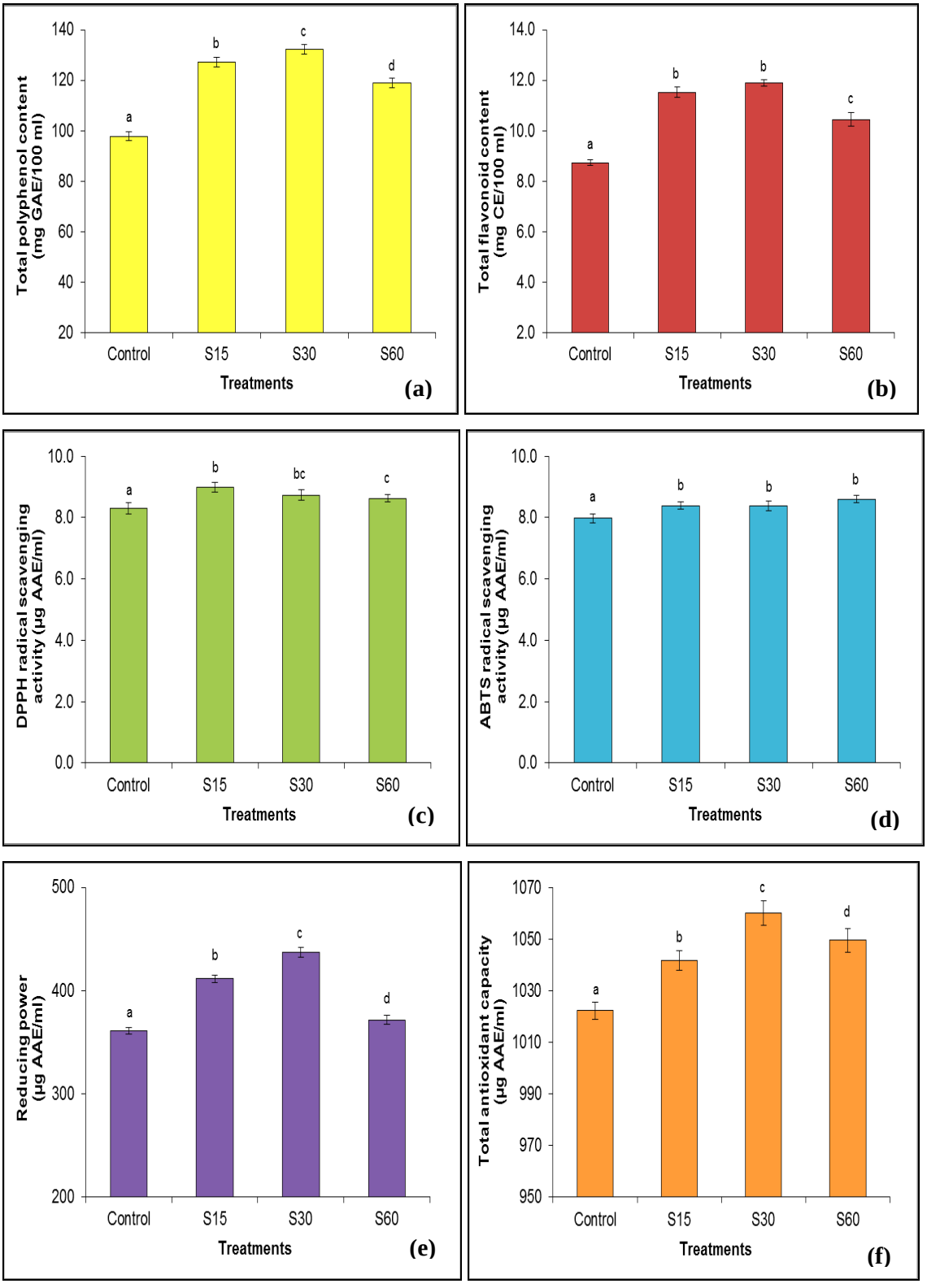


Figure 4.2: Effects of sonication on antioxidant activity (a-f) of Chokanan mango juice

[1] Values followed by different letters are significantly different ($p<0.05$) (n=12).

[2] GAE, gallic acid equivalent; CE, catechin equivalent; AAE, ascorbic acid equivalent.

The effects of UV-C treatment on antioxidant activity in juice extracts are shown in Figure 4.3 (a) to (f). Significant enhancement in extraction yield of polyphenols was observed in all UV-C treated juice samples when compared to the control. The highest increase (31%) was from 97.8 mg GAE/100 ml to 128.1 mg GAE/100 ml in control and U30 sample, respectively. In addition, significant increase (5%) in extractability of flavonoids was observed in UV-C treated juice sample, U15 (9.1 mg CE/100 ml) when compared to the control (8.7 mg CE/100 ml). However, degradation of flavonoids was observed in U60 sample.

With regards to antioxidant assays, significant increase in DPPH radical scavenging activity was exhibited in UV-C treated juice samples, except U60 when compared to control. The maximum increase in DPPH activity was 9.0 µg AAE/ml in U30 sample, compared to the control (8.3 µg AAE/ml). Likewise, significant increase in ABTS radical scavenging activity was observed in all juice samples after UV-C exposure. In terms of reducing power and total antioxidant capacity, a significant increase was observed in UV-C treated samples (U15 and U30) in comparison with control.

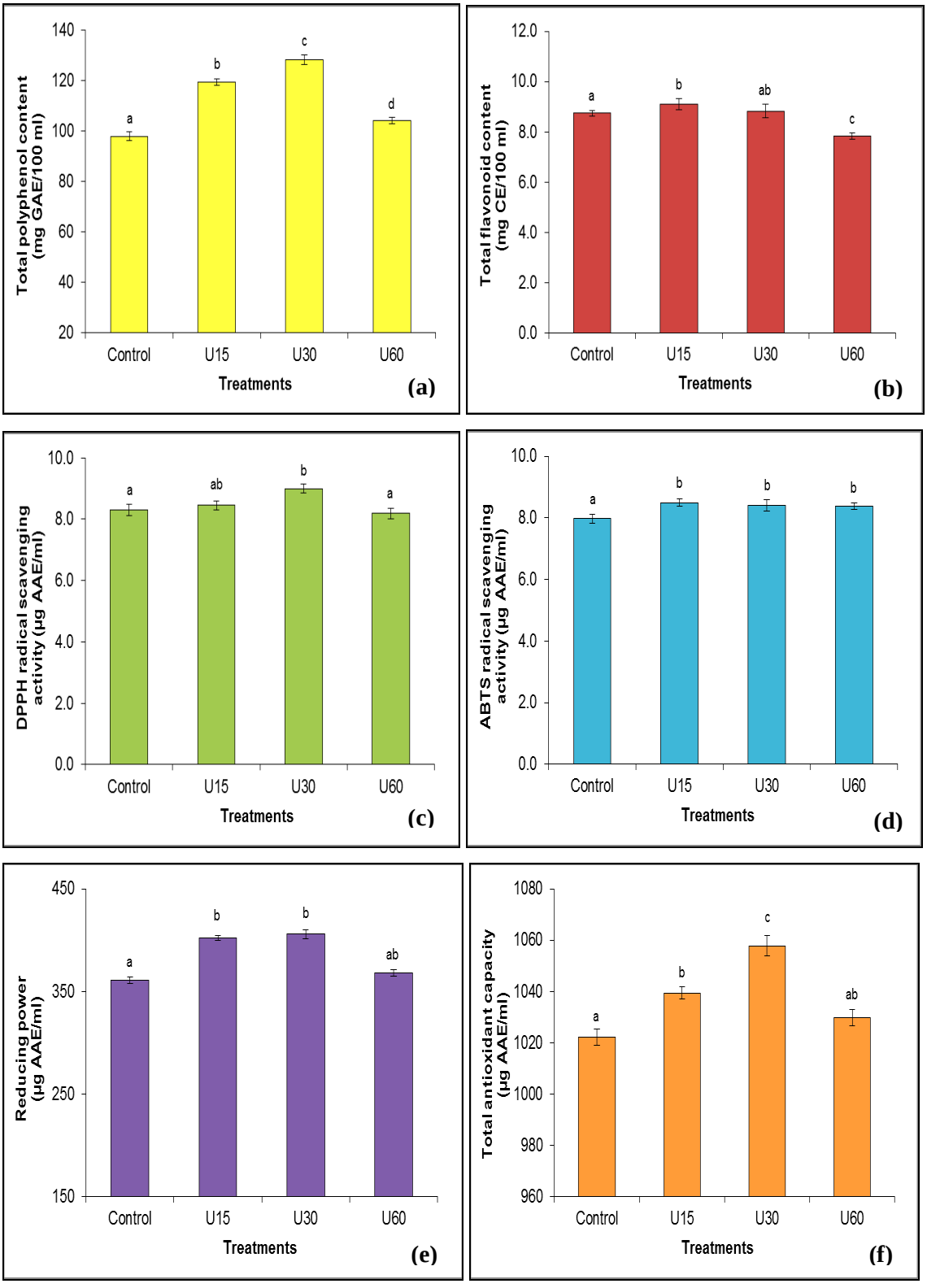


Figure 4.3: Effects of UV-C treatment on antioxidant activity (a-f) of Chokanan mango juice

[1] Values followed by different letters are significantly different ($p<0.05$) (n=12).

[2] GAE, gallic acid equivalent; CE, catechin equivalent; AAE, ascorbic acid equivalent.

The effects of combined treatment on antioxidant activity in juice extracts are shown in Figure 4.4 (a) to (f). A significant increase in extraction of polyphenols (23 to 37%) and flavonoids (29 to 35%) was exhibited in all combined treated juice samples when compared to control. The highest increase (37%) was from 97.8 mg GAE/100 ml to 134.0 mg GAE/100 ml in S30+U15 sample for phenolic compounds. With regards to total flavonoids, the highest increase (35%) was in S30+U30 sample (11.8 mg CE/100 ml), when compared to control (8.7 mg CE/100 ml).

With regards to antioxidant assays, significant increase in DPPH radical scavenging activity was observed in combined treated samples (S15+U15, S30+U15, S15+U30), when compared to control. The highest DPPH activity was 91.4% (9.0 µg AAE/ml) in S30+U15 sample, compared to the control, 84.1% (8.3 µg AAE/ml). Accordingly, there was a significant increase in ABTS radical scavenging activity in combined treated samples (S30+U15, S15+U30, S30+U30), when compared to control. Besides that, a significant increase in reducing capacity was recorded by combined treated samples. The maximum increase (27%) was from 360.7 µg AAE/ml to 458.7 µg AAE/ml in S30+U30 sample. For total antioxidant capacity, an increased value was observed in all combine treated samples, being the highest in S30+U15 (1066 µg AAE/ml), when compared to the control (1022 µg AAE/ml).

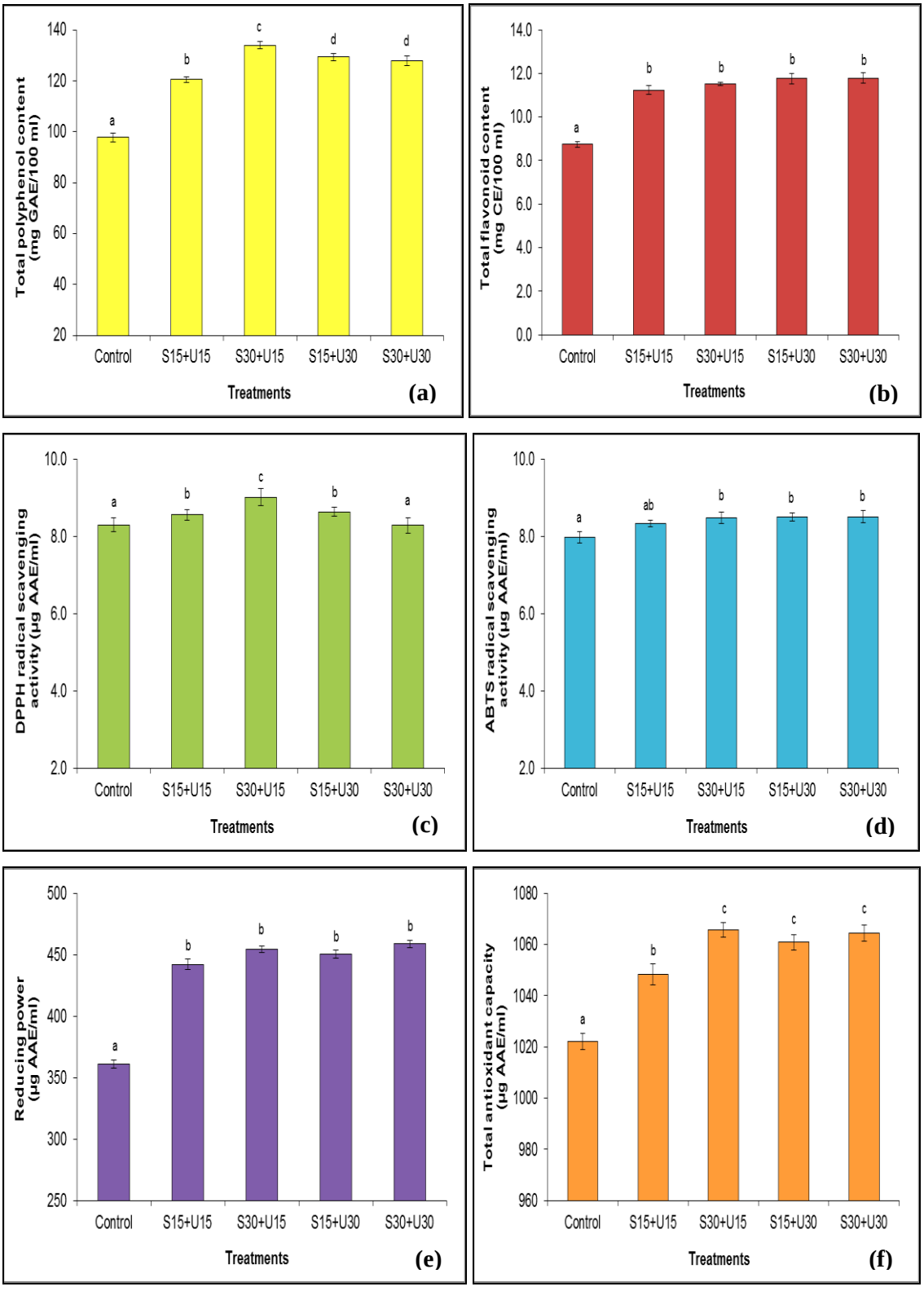


Figure 4.4: Effects of combined treatment on antioxidant activity (a-f) of Chokanan mango juice

[1] Values followed by different letters are significantly different ($p<0.05$) (n=12).

[2] GAE, gallic acid equivalent; CE, catechin equivalent; AAE, ascorbic acid equivalent.

The correlation between total polyphenols, total flavonoids and antioxidant assays (DPPH, ABTS, RPA, and TAC) are shown in Table 4.21. Total polyphenols and total flavonoids exhibited a strong positive correlation ($p<0.01$) with the antioxidant assays studied. Polyphenol content strongly correlated with TAC (R^2=0.962) and DPPH (R^2=0.916), while flavonoid content strongly correlated with RPA (R^2=0.877) and TAC (R^2=0.866). Besides that, there was a strong correlation between antioxidant assays, DPPH with ABTS (R^2=0.818), and RPA with TAC (R^2=0.877). Overall, TAC results correlated well with those obtained by other assays ($p<0.01$).

Table 4.21: Pearson's correlation coefficients between TPC, TFC and antioxidant activity measured by different assays (DPPH, ABTS, TAC and RPA)

	TPC	**TFC**	**DPPH**	**ABTS**	**TAC**	**RPA**
TPC	1					
TFC	0.865**	1				
DPPH	0.916**	0.730**	1			
ABTS	0.904**	0.741**	0.818**	1		
TAC	0.962**	0.866**	0.826**	0.867**	1	
RPA	0.829**	0.877**	0.646*	0.657*	0.877**	1

[1] *Correlation is significant at the 0.05 level.

[2] **Correlation is significant at the 0.01 level.

4.7 LCMS/MS ANALYSIS: IDENTIFICATION AND QUANTIFICATION OF PHENOLIC COMPOUNDS

The chromatogram of phenolic compounds in Chokanan mango juice analysed using LCMS/MS (negative ionisation mode) is shown in Figure 4.5. There were 12 peaks of phenolic compounds identified according to their accurate masses of parent and fragment ions. Among them, nine individual phenolic compounds were identified. These key phenolic compounds include phenolic acids (caffeoyl glucose, quinic acid, monogalloyl glucose, ellagic acid, gallic acid), flavonoids (kaempferol and quercetin), xanthanoid (mangiferin), and tannin (tannic acid). The retention time, mass spectrometric data, classification, molecular formula and structures of these compounds are summarised in Table 4.22.

Nine individual phenolic compounds in juice samples were quantified, as shown in Table 4.23. The phenolic compounds were quantified by their corresponding peak area. The most abundant phenolic compound in Chokanan mango juice was identified as caffeoyl glucose and tannic acid with 7.35 and 7.45 mg/100 ml, respectively, following with quinic acid (5.56 mg/100 ml), monogalloyl glucose (4.28 mg/100 ml), gallic acid (3.93 mg/100 ml), mangiferin (3.23 mg/100 ml), and ellagic acid (3.21 mg/100 ml). Besides that, there was low concentration of kaempferol (1.39 mg/100 ml) and quercetin (1.76 mg/100 ml) detected in juice samples.

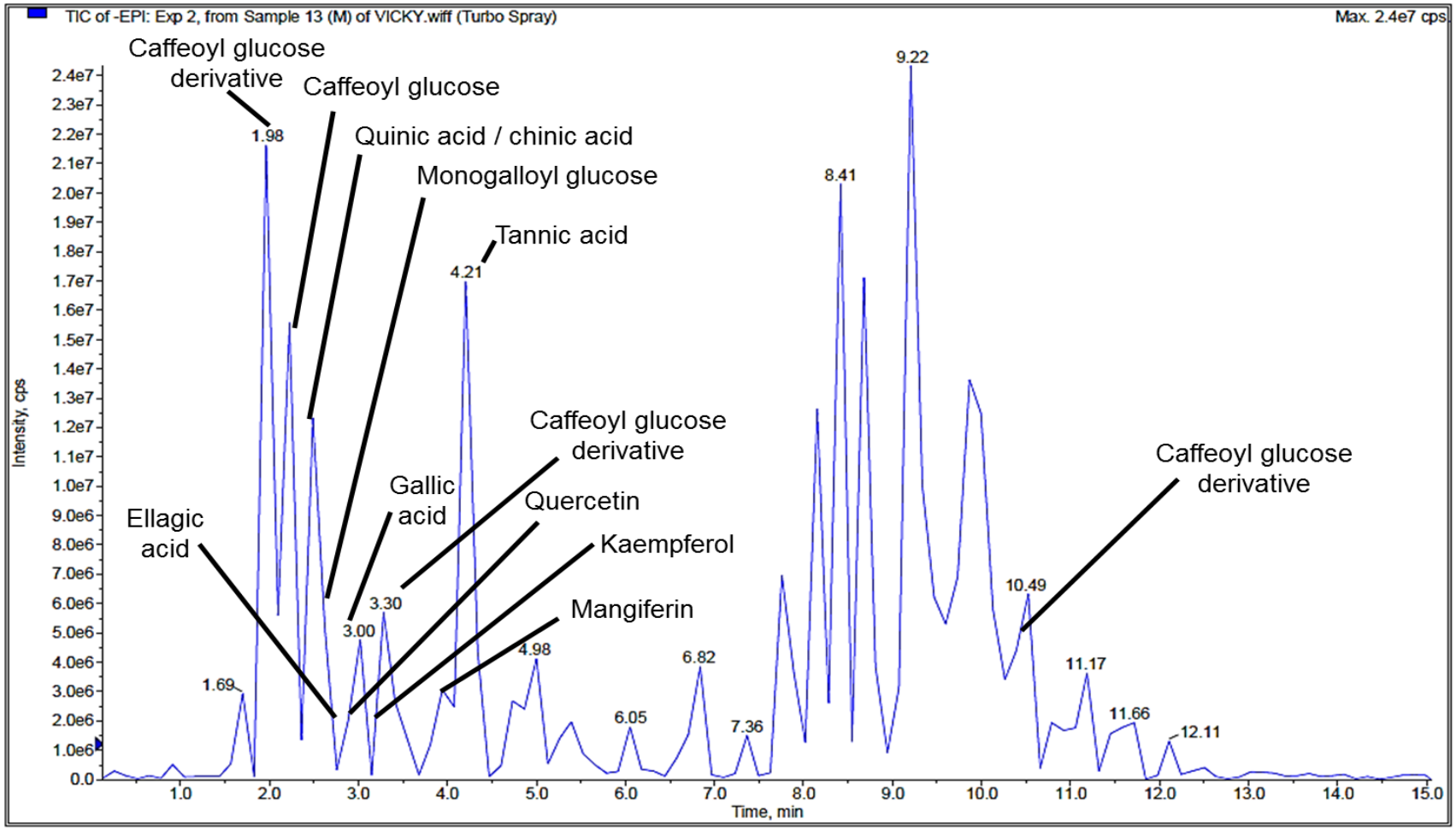


Figure 4.5: LCMS/MS profile of phenolic compounds in Chokanan mango juice

Table 4.22: Retention time and mass spectrometric data of phenolic compounds in Chokanan mango juice determined by LCMS/MS analysis

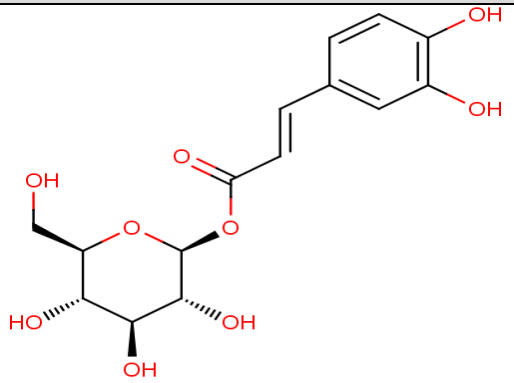
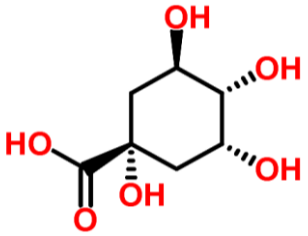
Retention time (min)	$[M-H]^-$ (m/z)	MS/MS (m/z)	Molecular formula	Assigned identity	Class	Chemical structure
2.23	341.43	179, 85, 59	$C_{15}H_{18}O_9$	Caffeoyl glucose	Phenolic acid	
2.49	191.11	111, 87, 85	$C_7H_{12}O_6$	Quinic acid / chinic acid	Phenolic acid	

Table 4.22, continued

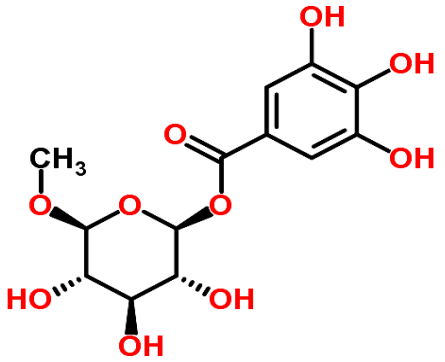
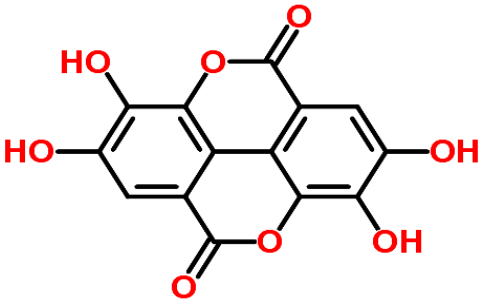
Retention time (min)	[M-H]$^-$ (m/z)	MS/MS (m/z)	Molecular formula	Assigned identity	Class	Chemical structure
2.62	331.31	169, 125, 107	$C_{13}H_{16}O_{10}$	Monogalloyl glucose	Phenolic acid	
2.79	301.19	257, 230, 185	$C_{14}H_6O_8$	Ellagic acid	Phenolic acid	

Table 4.22, continued

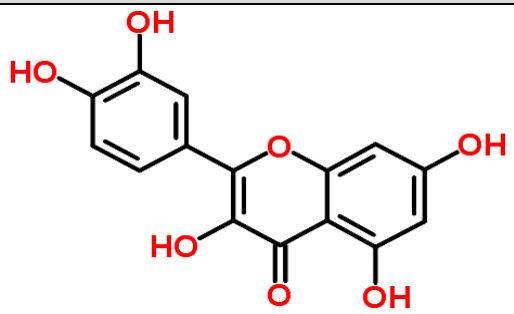
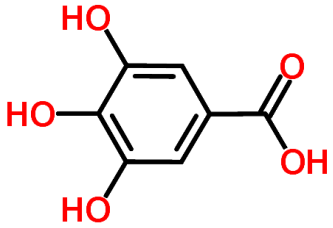
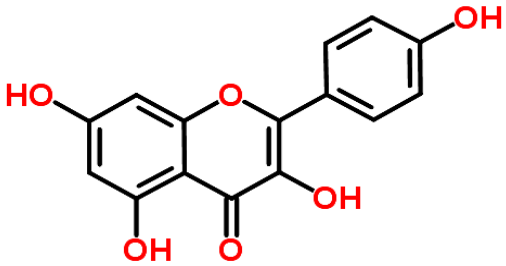
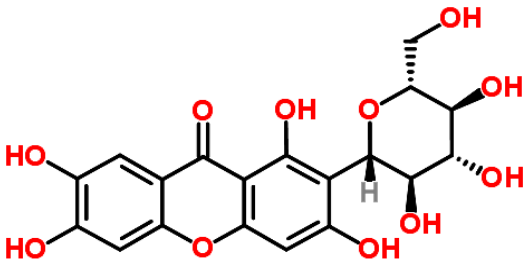
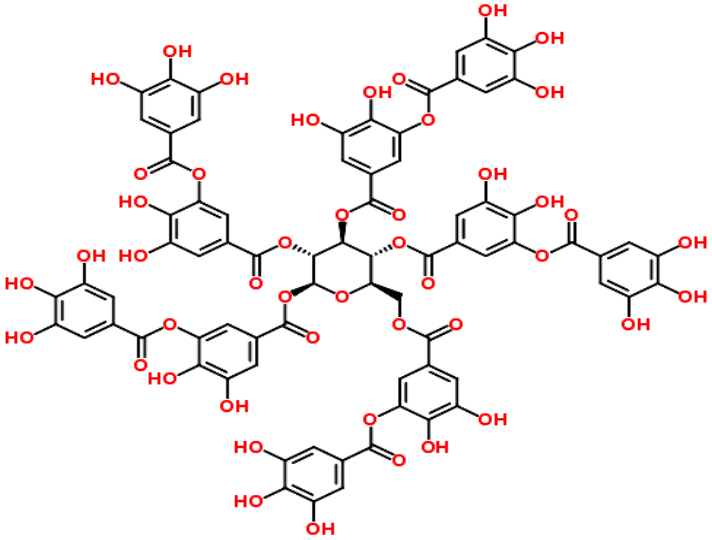
Retention time (min)	$[M-H]^-$ (m/z)	MS/MS (m/z)	Molecular formula	Assigned identity	Class	Chemical structure
2.97	301.23	230, 152, 136	$C_{15}H_{10}O_7$	Quercetin	Flavonoid	
3.02	169.11	125, 67, 69	$C_7H_6O_5$	Gallic acid	Phenolic acid	
3.28	285.15	211, 187, 158	$C_{15}H_{10}O_6$	Kaempferol	Flavonoid	

Table 4.22, continued

Retention time (min)	[M-H]$^-$ (m/z)	MS/MS (m/z)	Molecular formula	Assigned identity	Class	Chemical structure
3.94	421.34	301, 271, 258	$C_{19}H_{18}O_{11}$	Mangiferin	Xanthonoid	
4.21	183.11	140, 124, 65	$C_{76}H_{52}O_{46}$	Tannic acid / gallotannin	Tannin	

After thermal treatment, there were no significant differences in the concentration of caffeoyl glucose, quinic acid, monogalloyl glucose, ellagic acid, kaempferol, and mangiferin, as shown in Table 4.23. However, there was significant reduction in concentration of quercetin and gallic acid in thermally treated juices.

Table 4.23: Effects of thermal treatment on phenolic compounds in Chokanan mango juice

Relative concentration of phenolic compounds (mg/100 ml)	**Treatment**		
	Control	**MP**	**HP**
Caffeoyl glucose	7.35 ± 0.06^{a}	7.31 ± 0.09^{a}	7.33 ± 0.05^{a}
Quinic acid / chinic acid	5.56 ± 0.12^{a}	5.50 ± 0.11^{a}	5.35 ± 0.11^{a}
Monogalloyl glucose	4.28 ± 0.15^{a}	4.20 ± 0.09^{a}	4.22 ± 0.12^{a}
Ellagic acid	3.21 ± 0.05^{a}	3.11 ± 0.05^{a}	3.10 ± 0.05^{a}
Quercetin	1.76 ± 0.02^{a}	1.28 ± 0.02^{b}	1.00 ± 0.03^{c}
Gallic acid	3.93 ± 0.12^{a}	3.48 ± 0.10^{b}	3.32 ± 0.10^{b}
Kaempferol	1.39 ± 0.06^{a}	1.25 ± 0.08^{a}	1.28 ± 0.04^{a}
Mangiferin	3.23 ± 0.14^{a}	3.25 ± 0.12^{a}	3.12 ± 0.14^{a}
Tannic acid / gallotannin	7.24 ± 0.21^{a}	4.96 ± 0.22^{b}	5.02 ± 0.22^{b}

[1] Values followed by different letters within the same row are significantly different ($p<0.05$) (n=9).

After sonication, there were no significant differences in the concentration of caffeoyl glucose and monogalloyl glucose, as shown in Table 4.24. However, there were significant enhancement in extractability of other phenolic compounds including quinic acid, ellagic acid, quercetin, gallic acid, kaempferol, mangiferin and tannic acid in sonicated juice samples, being the highest in S15 and S30.

Table 4.24: Effects of sonication on phenolic compounds in Chokanan mango juice

Relative concentration of phenolic compounds (mg/100 ml)	**Treatment**			
	Control	**S15**	**S30**	**S60**
Caffeoyl glucose	7.35 ± 0.06^{a}	7.54 ± 0.10^{a}	7.47 ± 0.08^{a}	7.46 ± 0.06^{a}
Quinic acid / chinic acid	5.56 ± 0.12^{a}	6.15 ± 0.09^{b}	6.21 ± 0.06^{b}	6.54 ± 0.03^{c}
Monogalloyl glucose	4.28 ± 0.15^{a}	4.49 ± 0.09^{a}	4.41 ± 0.09^{a}	4.30 ± 0.11^{a}
Ellagic acid	3.21 ± 0.05^{a}	3.47 ± 0.04^{b}	3.68 ± 0.06^{c}	3.25 ± 0.07^{a}
Quercetin	1.76 ± 0.02^{a}	1.90 ± 0.05^{b}	1.99 ± 0.03^{b}	1.96 ± 0.03^{b}
Gallic acid	3.93 ± 0.12^{a}	4.15 ± 0.11^{b}	4.49 ± 0.08^{b}	4.19 ± 0.05^{b}
Kaempferol	1.39 ± 0.06^{a}	1.63 ± 0.02^{b}	1.68 ± 0.05^{b}	1.61 ± 0.03^{b}
Mangiferin	3.23 ± 0.14^{a}	4.00 ± 0.10^{b}	3.85 ± 0.11^{b}	3.25 ± 0.08^{a}
Tannic acid / gallotannin	7.24 ± 0.21^{a}	8.53 ± 0.19^{b}	8.05 ± 0.18^{c}	7.88 ± 0.21^{c}

[1] Values followed by different letters within the same row are significantly different ($p<0.05$) ($n=9$).

After UV-C treatment, there were no significant differences in the concentration of caffeoyl glucose and monogalloyl glucose, as shown in Table 4.25. However, there were significant enhancement in extractability of other phenolic compounds including quinic acid, ellagic acid, quercetin, gallic acid, kaempferol, mangiferin and tannic acid in UV-C treated juice samples, being the highest in U30 and U60.

Table 4.25: Effects of UV-C treatment on phenolic compounds in Chokanan mango juice

Relative concentration of phenolic compounds (mg/100 ml)	Treatment			
	Control	**U15**	**U30**	**U60**
Caffeoyl glucose	7.35 ± 0.06^{a}	7.46 ± 0.08^{a}	7.25 ± 0.08^{a}	7.37 ± 0.06^{a}
Quinic acid / chinic acid	5.56 ± 0.12^{a}	6.15 ± 0.07^{b}	6.47 ± 0.06^{c}	6.53 ± 0.07^{b}
Monogalloyl glucose	4.28 ± 0.15^{a}	4.43 ± 0.12^{a}	4.38 ± 0.10^{a}	4.32 ± 0.11^{a}
Ellagic acid	3.21 ± 0.05^{a}	4.10 ± 0.05^{b}	5.93 ± 0.05^{c}	4.08 ± 0.06^{b}
Quercetin	1.76 ± 0.02^{a}	1.82 ± 0.05^{a}	2.00 ± 0.02^{b}	1.88 ± 0.02^{ab}
Gallic acid	3.93 ± 0.12^{a}	4.17 ± 0.10^{a}	4.38 ± 0.08^{b}	4.18 ± 0.06^{a}
Kaempferol	1.39 ± 0.06^{a}	1.72 ± 0.05^{b}	1.63 ± 0.05^{b}	1.62 ± 0.03^{b}
Mangiferin	3.23 ± 0.14^{a}	3.93 ± 0.08^{b}	3.83 ± 0.12^{b}	3.56 ± 0.10^{ab}
Tannic acid / gallotannin	7.24 ± 0.21^{a}	7.93 ± 0.20^{b}	8.12 ± 0.23^{b}	8.00 ± 0.17^{b}

[1] Values followed by different letters within the same row are significantly different ($p<0.05$) (n=9).

After combined treatment, there were no significant differences in the concentration of caffeoyl glucose and monogalloyl glucose, as shown in Table 4.26. However, there were significant enhancement in extractability of other phenolic compounds including quinic acid, ellagic acid, quercetin, gallic acid, kaempferol, mangiferin and tannic acid in combined treated juice samples, being the highest in S30+U15 and S15+U30.

Table 4.26: Effects of combined treatment on phenolic compounds in Chokanan mango juice

Relative concentration of phenolic compounds (mg/100 ml)	**Treatment**				
	Control	**S15 + U15**	**S30 + U15**	**S15 + U30**	**S30 + U30**
Caffeoyl glucose	7.35 ± 0.06[a]	7.47 ± 0.04[a]	7.53 ± 0.10[a]	7.52 ± 0.05[a]	7.37 ± 0.02[a]
Quinic acid / chinic acid	5.56 ± 0.12[a]	6.90 ± 0.08[b]	6.82 ± 0.11[b]	6.97 ± 0.06[b]	6.42 ± 0.03[c]
Monogalloyl glucose	4.28 ± 0.15[a]	4.35 ± 0.13[a]	4.40 ± 0.06[a]	4.42 ± 0.10[a]	4.30 ± 0.12[a]
Ellagic acid	3.21 ± 0.05[a]	6.96 ± 0.04[b]	5.73 ± 0.05[c]	5.65 ± 0.06[c]	5.97 ± 0.06[d]
Quercetin	1.76 ± 0.02[a]	3.76 ± 0.04[b]	3.78 ± 0.06[b]	2.68 ± 0.05[c]	2.23 ± 0.02[c]
Gallic acid	3.93 ± 0.12[a]	4.59 ± 0.12[b]	5.15 ± 0.09[c]	4.75 ± 0.08[bc]	4.56 ± 0.11[b]
Kaempferol	1.39 ± 0.06[a]	3.75 ± 0.04[b]	2.78 ± 0.04[c]	1.69 ± 0.05[ac]	2.40 ± 0.06[c]
Mangiferin	3.23 ± 0.14[a]	5.14 ± 0.14[b]	5.33 ± 0.14[b]	5.83 ± 0.09[c]	5.78 ± 0.09[c]
Tannic acid / gallotannin	7.24 ± 0.21[a]	9.13 ± 0.19[b]	9.21 ± 0.19[b]	9.18 ± 0.15[b]	9.01 ± 0.21[b]

[1] Values followed by different letters within the same row are significantly different ($p<0.05$) (n=9).

4.8 SENSORY ANALYSIS

The degree of liking juice samples were investigated using a consumer acceptability test. As shown in Figure 4.6, control was positioned in the 'like moderately' point of the hedonic scale with the highest score for colour, odour, taste and overall acceptability. Thermally treated juices, regardless of mild or high heat pasteurization exhibited the lowest score, falling within the 'dislike moderately' scale for odour and 'dislike slightly' scale for colour, taste and overall acceptability. In addition, thermally treated juice samples, MP and HP were below the rejection point, as score 5 is the rejection limit.

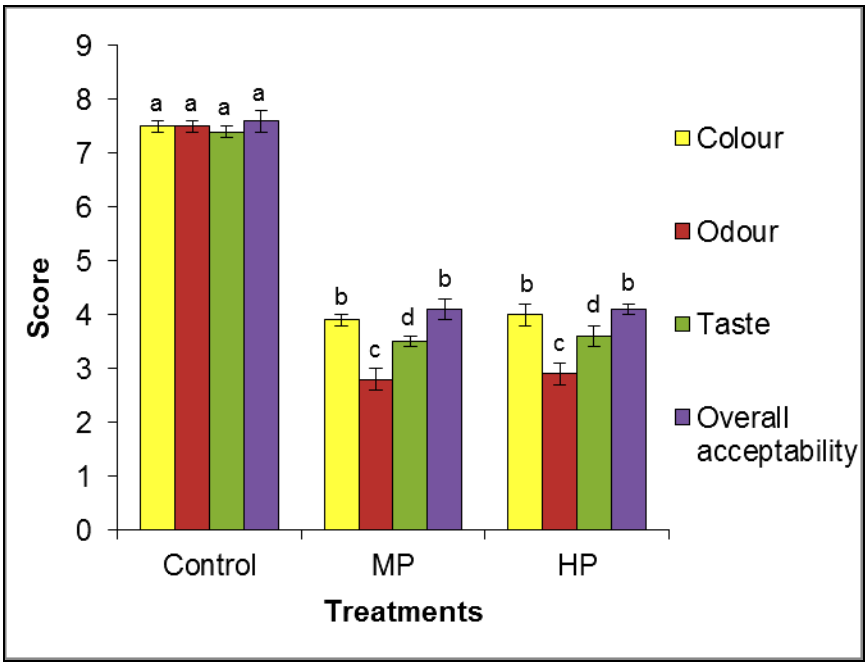


Figure 4.6: Effects of thermal treatment on sensory analysis of Chokanan mango juice

[1] Mean values followed by different letters are significantly different ($p<0.05$) (n=90).

[2] Score: 1= dislike extremely; 2= dislike very much; 3= dislike moderately; 4= dislike slightly, 5= neither like nor dislike, 6= like slightly; 7= like moderately; 8= like very much; 9= like extremely.

As shown in Figure 4.7, control was positioned in the 'like moderately' point of the hedonic scale with the highest score for colour, odour, taste and overall acceptability. Sonicated juice samples, regardless of treatment time exhibited lowest variation from control, thus falling within the 'like slightly' scale for colour and odour. With regards to taste and overall acceptability, there was no significant difference between control and S15 sample. Besides that, there was a significant decrease in overall acceptability corresponding to increased sonication time (from 15 to 60 minutes).

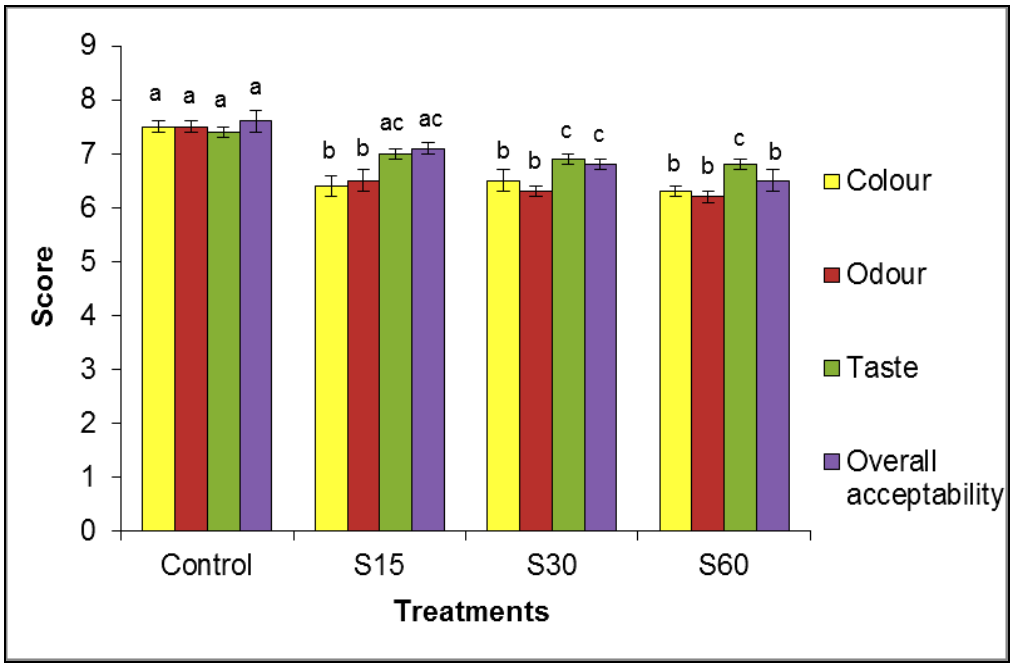


Figure 4.7: Effects of sonication on sensory analysis of Chokanan mango juice

[1] Mean values followed by different letters are significantly different ($p<0.05$) (n=90).

[2] Score: 1= dislike extremely; 2= dislike very much; 3= dislike moderately; 4= dislike slightly, 5= neither like nor dislike, 6= like slightly; 7= like moderately; 8= like very much; 9= like extremely.

As shown in Figure 4.8, control was positioned in the 'like moderately' point of the hedonic scale with the highest score for colour, odour, taste and overall acceptability. UV-C treated juice samples, U15 and U30 exhibited lowest variation from control, thus falling within the 'like slightly' scale for colour, odour and taste. With regards to overall acceptability, there was no significant difference between control and U15 sample. Besides that, there was a significant decrease in overall acceptability corresponding to increased UV-C treatment time (from 15 to 60 minutes).

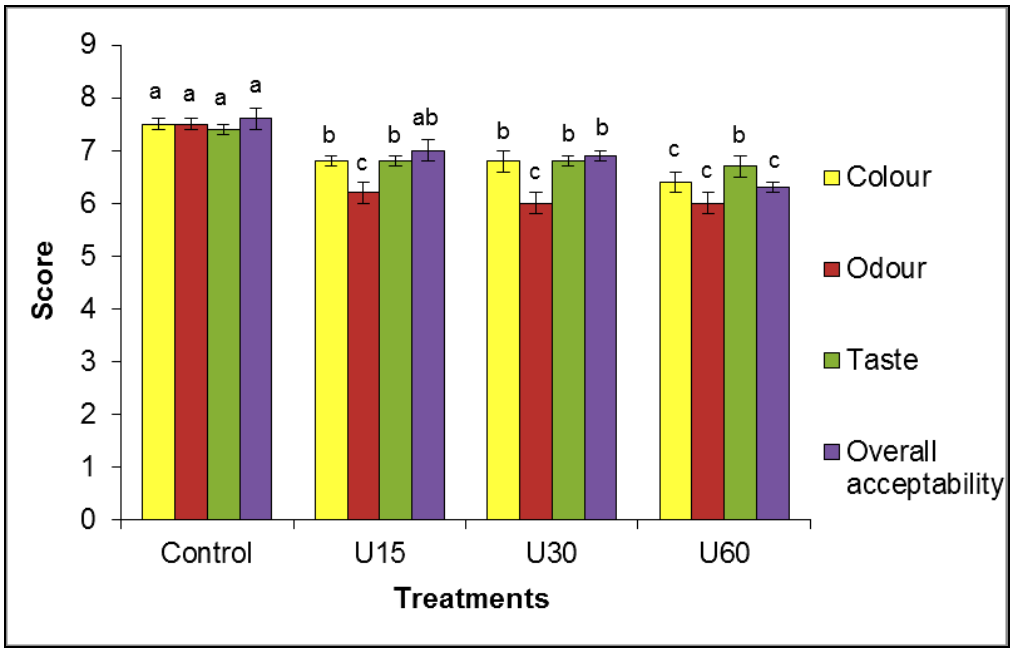


Figure 4.8: Effects of UV-C treatment on sensory analysis of Chokanan mango juice

[1] Mean values followed by different letters are significantly different ($p<0.05$) (n=90).

[2] Score: 1= dislike extremely; 2= dislike very much; 3= dislike moderately; 4= dislike slightly, 5= neither like nor dislike, 6= like slightly; 7= like moderately; 8= like very much; 9= like extremely.

As shown in Figure 4.9, control was positioned in the 'like moderately' point of the hedonic scale with the highest score for colour, odour, taste and overall acceptability. Combined treated juice samples (S15+U15, S30+U15, S15+U30), exhibited least difference in score when compared to the control, thus falling within the 'like slightly' scale for colour and odour. With regards to taste and overall acceptability, there were no significant difference between control and combined treated juice samples, except S30+U30.

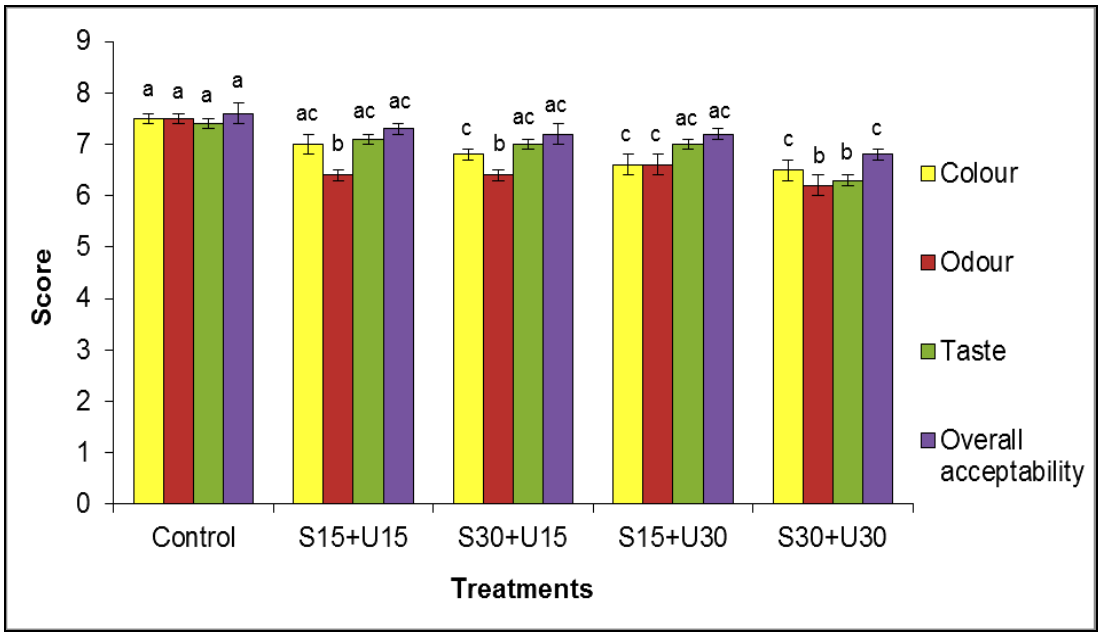


Figure 4.9: Effects of combined treatment on sensory analysis of Chokanan mango juice

[1] Mean values followed by different letters are significantly different ($p<0.05$) (n=90).

[2] Score: 1= dislike extremely; 2= dislike very much; 3= dislike moderately; 4= dislike slightly, 5= neither like nor dislike, 6= like slightly; 7= like moderately; 8= like very much; 9= like extremely.

4.9 SHELF LIFE STUDY

Changes in APC and TSS of thermally treated juices stored at refrigeration (4 °C) for five weeks are shown in Figure 4.10 (a) and (b). The acceptable maximum microbial load for aerobic bacteria in fruit juices is about 4 log CFU/ml. The APC of control (untreated juice) increased from 2.74 to 7.44 log CFU/ml after five weeks of storage. Thermally treated juice, regardless of mild or high heat pasteurization had almost no microorganism growth during five weeks storage, thus remaining below microbial load limit. While, TSS of control decreased from 14.7 to 11.9 °Brix after five weeks storage. No significant variation in TSS was observed in thermally treated juice samples, MP and HP until four weeks of storage. On the fifth week of storage, there was a significant decrease in TSS of thermally treated juices.

The visual observation of juice samples during storage is shown in Table 4.27. There were significant colour changes in control throughout five weeks of storage. Besides that, browning of juice was observed in control during the third week of storage. In contrast, the colour of thermally treated juice remained constant during five weeks of storage.

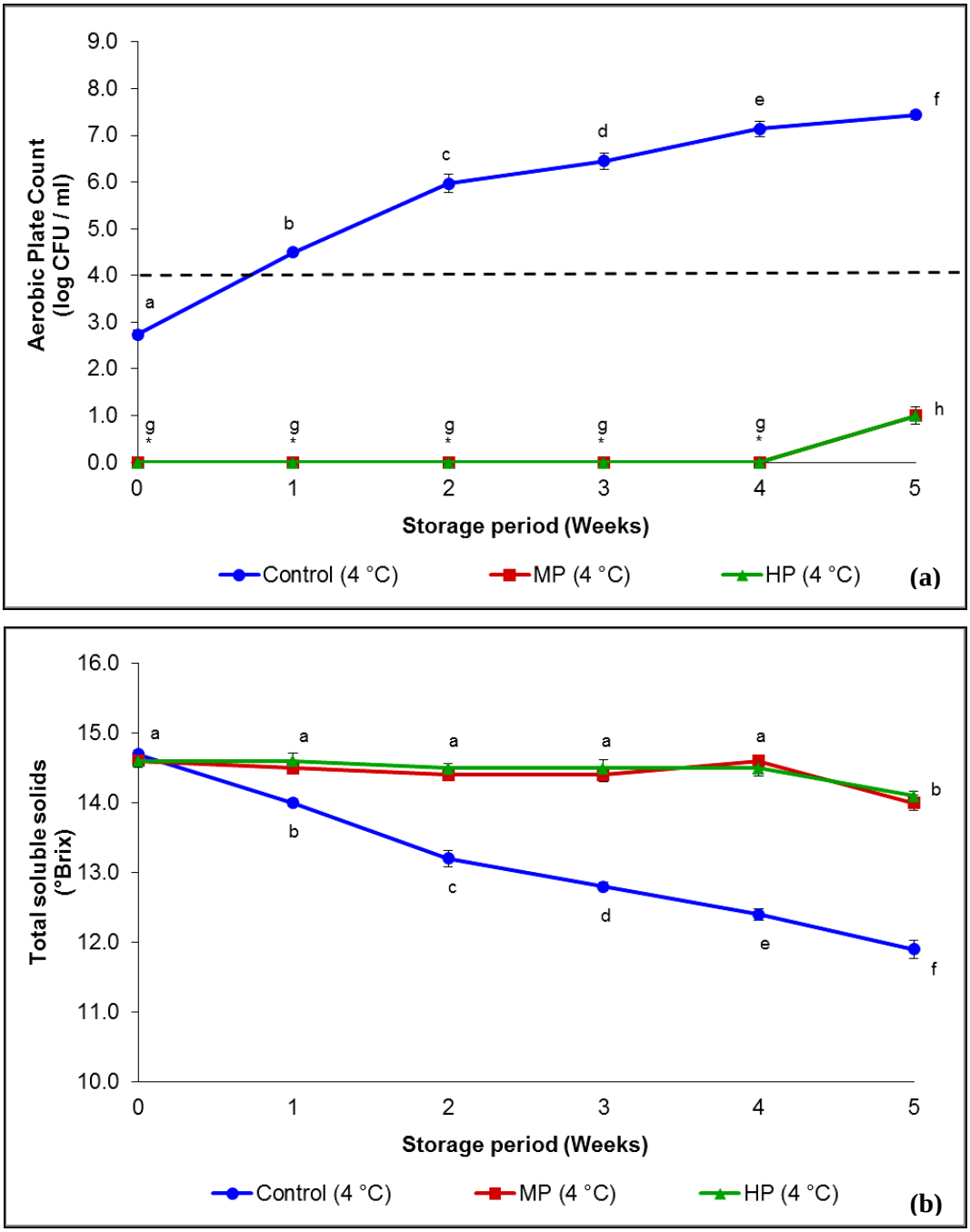


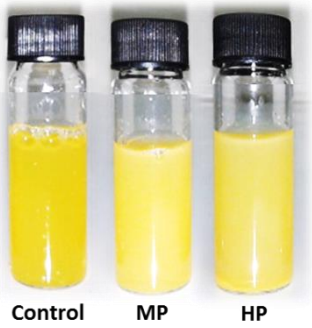
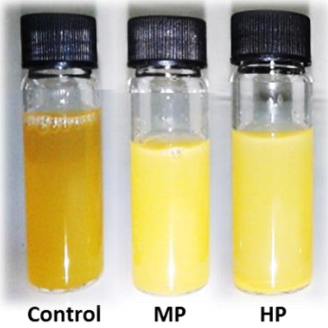
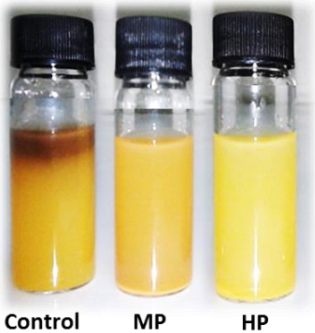
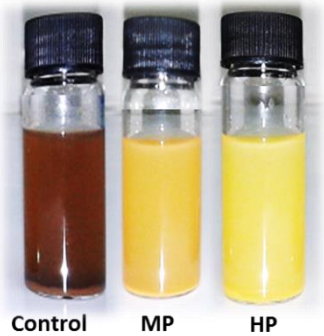
Figure 4.10: Effects of thermal treatment on (a) APC and (b) TSS of Chokanan mango juice during storage at 4 °C

[1] Values followed by different letters are significantly different ($p<0.05$) (n=9).

[2] The dashed line (---) indicates the limit of microbial shelf-life

[3] CFU, colony-forming unit; * not detected.

Table 4.27: Effects of thermal treatment on visual observation of Chokanan mango juice during storage at 4 °C

Storage	Weeks of storage			
	0	1	3	5
4 °C				

Changes in APC and TSS of sonicated juices stored at refrigeration (4 °C) for five weeks are shown in Figure 4.11 (a) and (b). The APC of control (untreated juice) increased from 2.74 to 7.44 log CFU/ml after five weeks of storage. There was a significant increase in microbial count in sonicated juice samples during five weeks storage. There was less microbial growth in S30 and S60 samples, thus remaining below microbial load limit for four and five weeks of storage, respectively. While, TSS of control decreased from 14.7 to 11.9 °Brix after five weeks storage. No significant variation in TSS was observed in sonicated juice samples, until three weeks of storage. After three weeks of storage, there was significant reduction in TSS of sonicated juices from 14.7 to 13.6 °Brix.

The visual observation of juice samples during storage is shown in Table 4.28. There were significant colour changes in control throughout five weeks of storage. Besides that, browning of juice was observed in control during the third week of storage. In contrast, the colour of sonicated juice (S15, S30, and S60) remained constant until four weeks of storage. On the fifth week of storage, browning of sonicated juice was observed, especially in S30 and S60 samples.

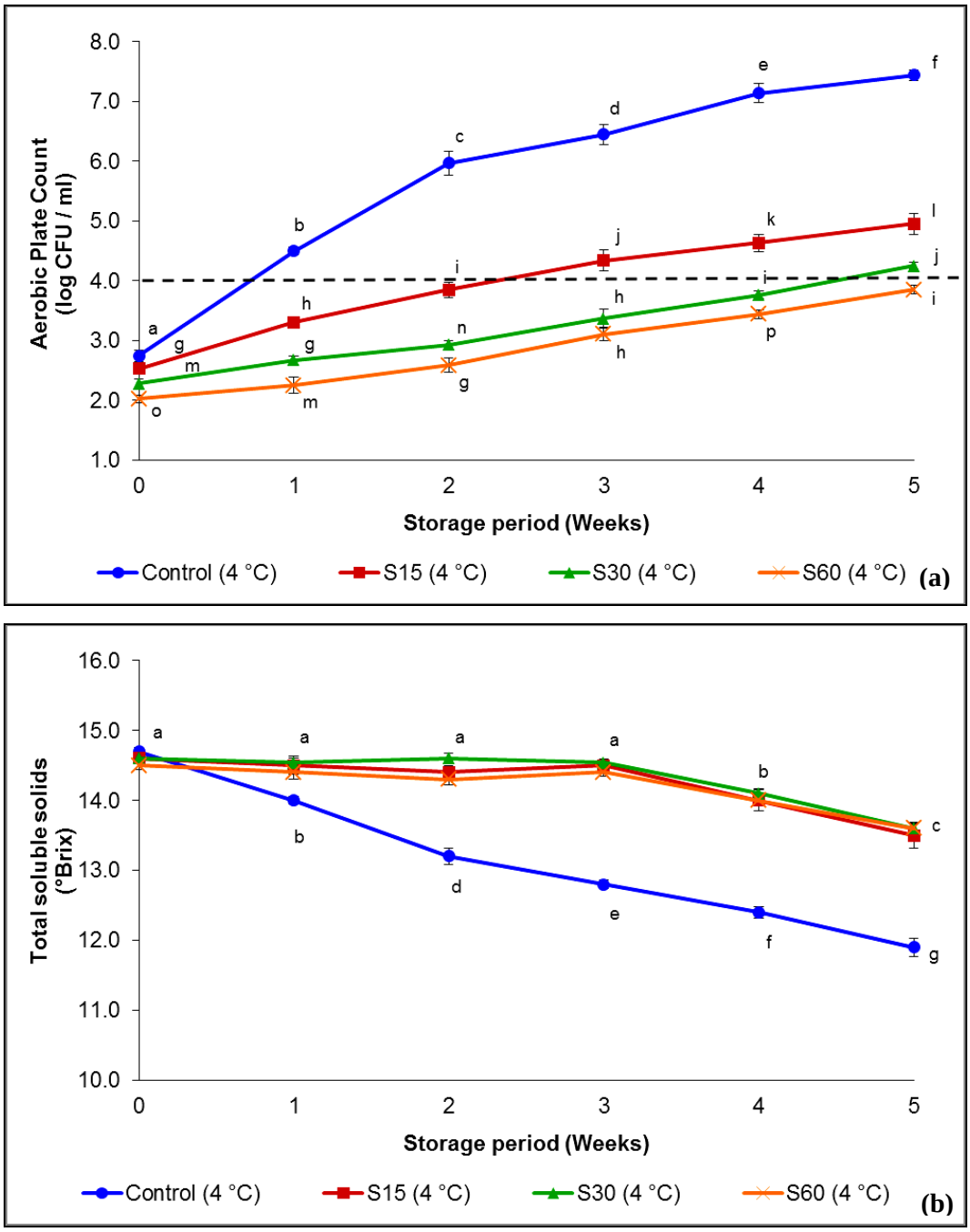


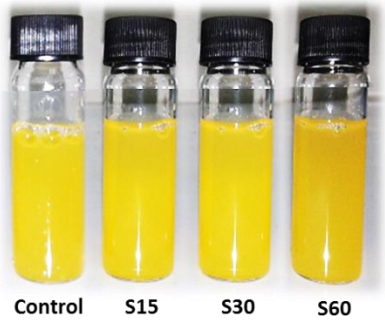
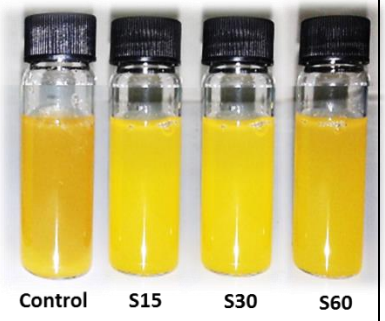
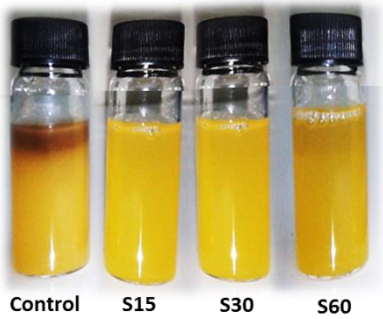
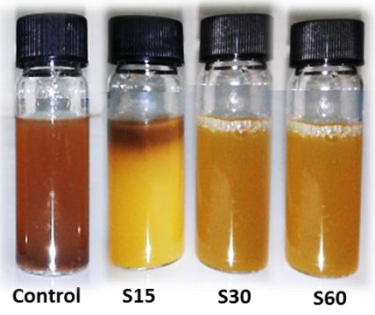
Figure 4.11: Effects of sonication on (a) APC and (b) TSS of Chokanan mango juice during storage at 4 °C

[1] Values followed by different letters are significantly different ($p<0.05$) (n=9).

[2] The dashed line (---) indicates the limit of microbial shelf-life

[3] CFU, colony-forming unit; * not detected.

Table 4.28: Effects of sonication on visual observation of Chokanan mango juice during storage at 4 °C

Storage	Weeks of storage			
	0	1	3	5
4 °C				

Changes in APC and TSS of UV-C treated juices stored at refrigeration (4 °C) for five weeks are shown in Figure 4.12 (a) and (b). The APC of control (untreated juice) increased from 2.74 to 7.44 log CFU/ml after five weeks of storage. There was a significant increase in microbial count in UV-C treated juice samples during five weeks storage. There was less microbial growth in U30 and U60 samples, thus remaining below microbial load limit for four and five weeks of storage, respectively. While, TSS of control decreased from 14.7 to 11.9 °Brix after five weeks storage. No significant variation in TSS was observed in all UV-C treated juice samples, until three weeks of storage. After three weeks of storage, there was significant reduction in TSS of UV-C treated juices from 14.7 to 13.7 °Brix.

The visual observation of juice samples during storage is shown in Table 4.29. There were significant colour changes in control throughout five weeks of storage. Besides that, browning of juice was observed in control during the third week of storage. In contrast, the colour of UV-C treated juices (U15, U30, and U60) remained constant until four weeks of storage. On the fifth week of storage, browning of UV-C treated juices was observed, especially in U60 samples.

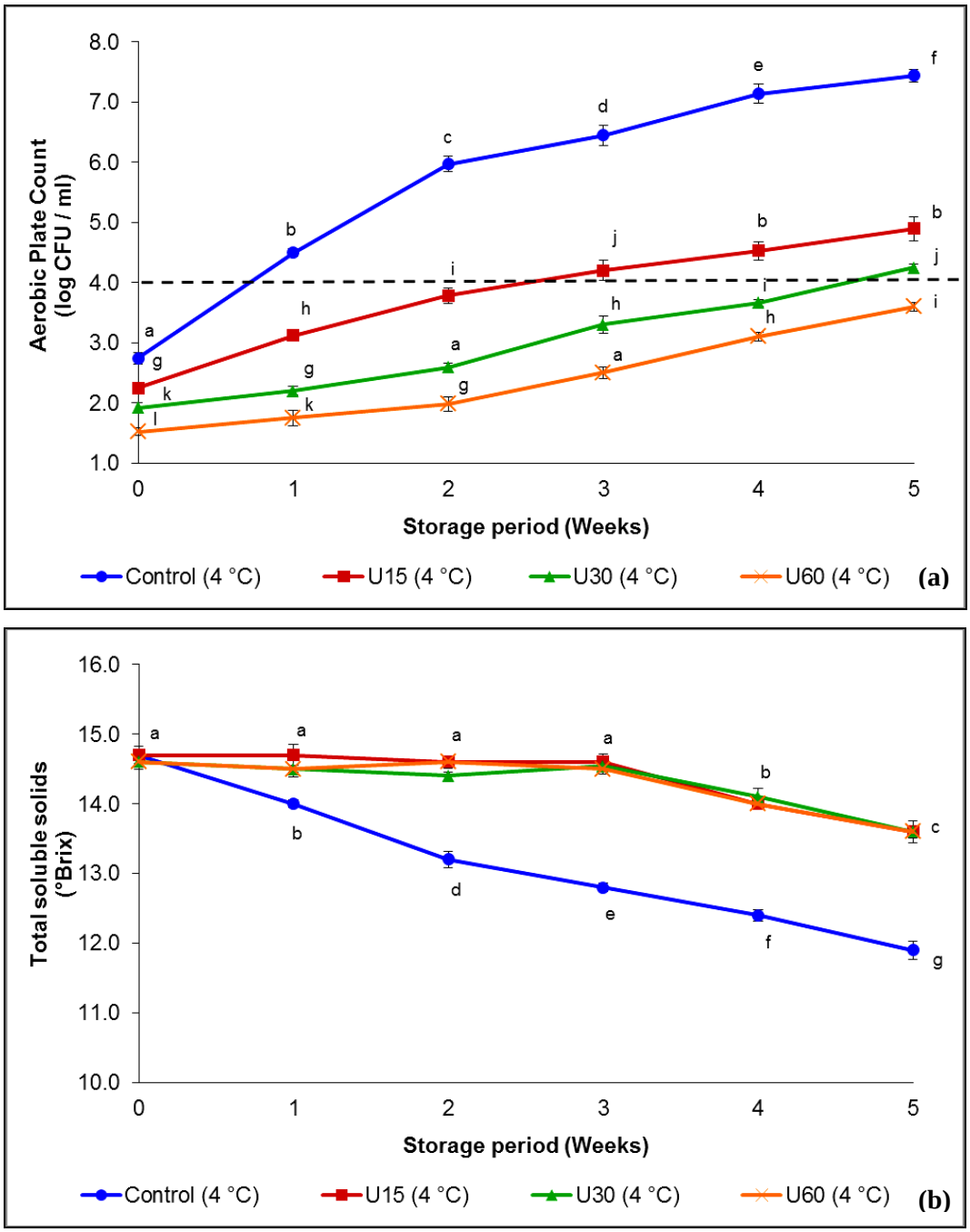


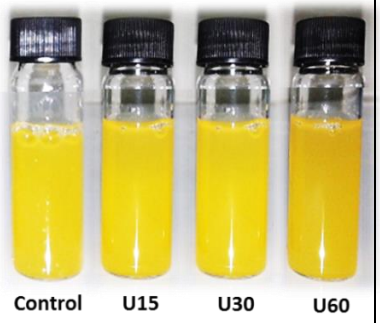
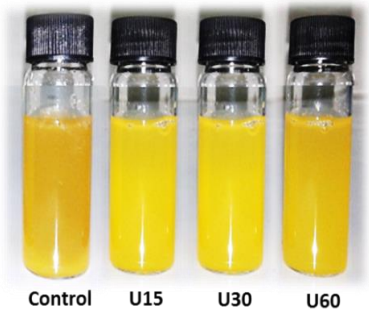
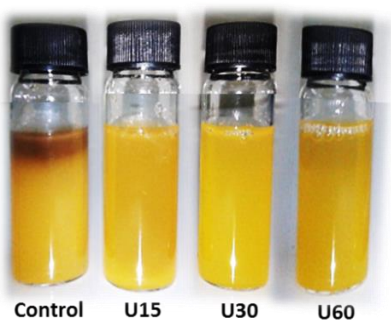
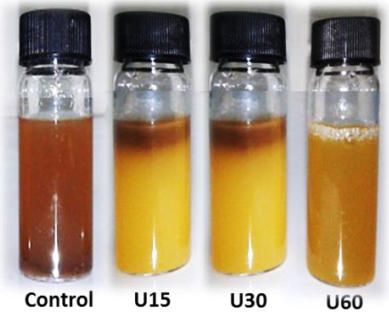
Figure 4.12: Effects of UV-C treatment on (a) APC and (b) TSS of Chokanan mango juice during storage at 4 °C

[1] Values followed by different letters are significantly different ($p<0.05$) (n=9).

[2] The dashed line (---) indicates the limit of microbial shelf-life

[3] CFU, colony-forming unit; * not detected.

Table 4.29: Effects of UV-C treatment on visual observation of Chokanan mango juice during storage at 4 °C

Storage	Weeks of storage			
	0	1	3	5
4 °C				

Changes in APC and TSS of combined treated juices stored at refrigeration (4 °C) for five weeks are shown in Figure 4.13 (a) and (b). The APC of control (untreated juice) increased from 2.74 to 7.44 log CFU/ml after five weeks of storage. All combined treated juice samples had no significant increase in microbial growth during three weeks storage, thus remaining below microbial load limit for five weeks of storage. While, TSS of control decreased from 14.7 to 11.9 °Brix after five weeks storage. No significant variation in TSS was observed in all combined treated juice samples, until four weeks of storage. After four weeks of storage, there was significant reduction in TSS of combined treated juices from 14.7 to 14.2 °Brix.

The visual observation of juice samples during storage is shown in Table 4.30. There were significant colour changes in control throughout five weeks of storage. Besides that, browning of juice was observed in control during the third week of storage. In contrast, there was no significant variation in colour of all combined treated juices (S15+U15, S30+U15, S15+U30, S30+U30) during five weeks of storage.

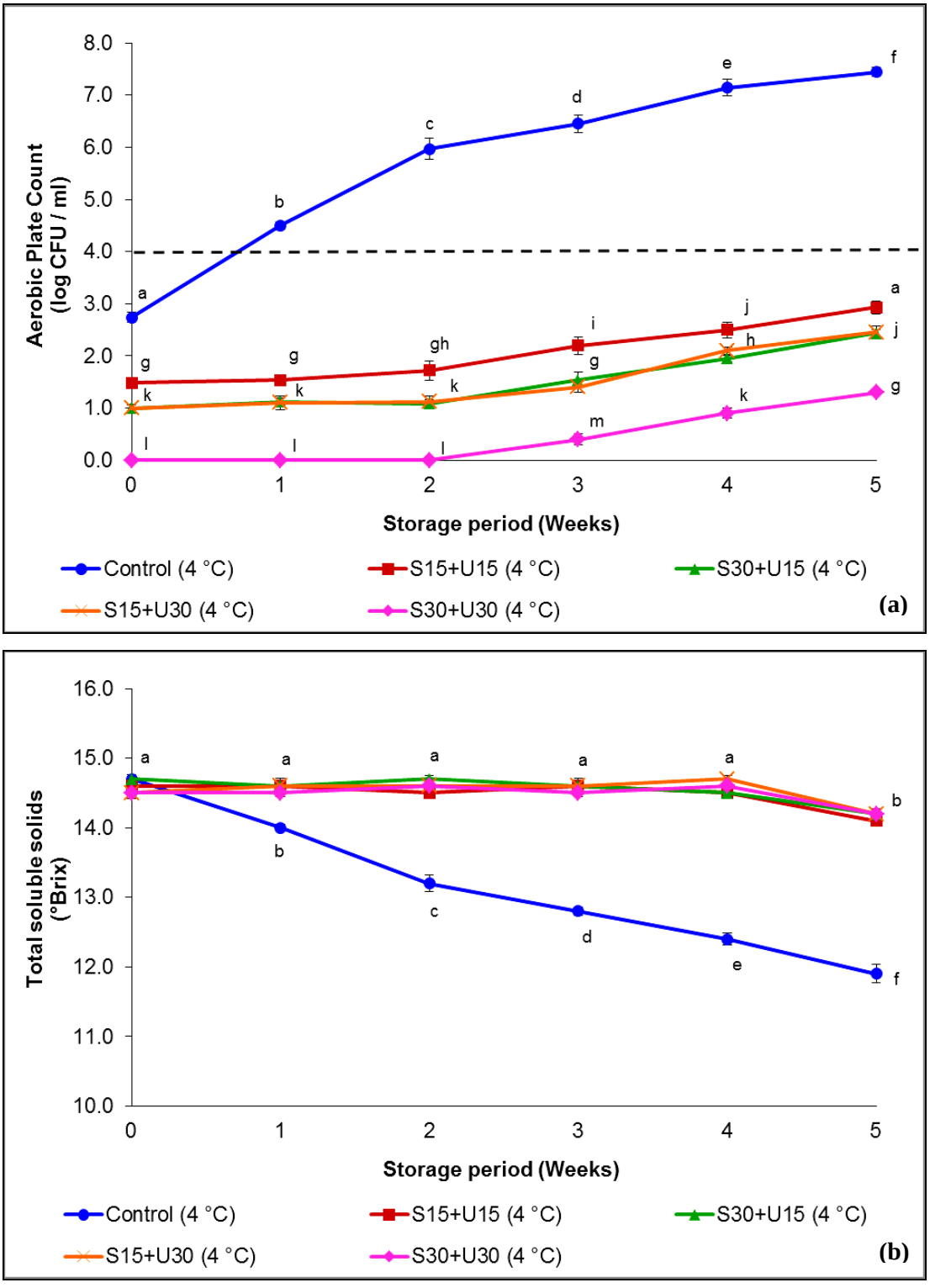


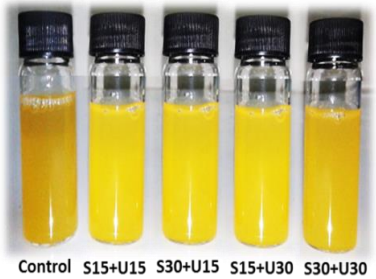
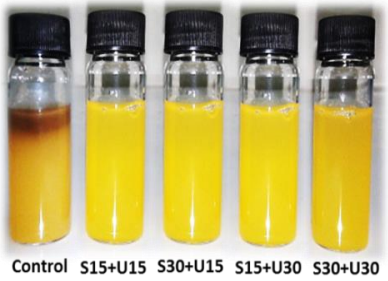
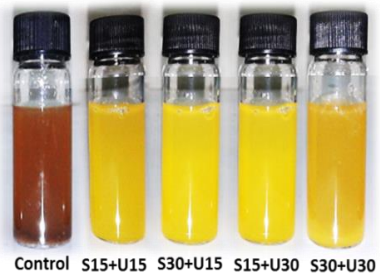
Figure 4.13: Effects of combined treatment on (a) APC and (b) TSS of Chokanan mango juice during storage at 4 °C

[1] Values followed by different letters are significantly different ($p<0.05$) (n=9).

[2] The dashed line (---) indicates the limit of microbial shelf-life

[3] CFU, colony-forming unit; * not detected.

Table 4.30: Effects of combined treatment on visual observation of Chokanan mango juice during storage at 4 °C

Storage	Weeks of storage			
	0	1	3	5
4 °C				

CHAPTER 5

DISCUSSION

Thermal treatment regardless of mild or high heat pasteurisation completely inactivated aerobic bacteria, coliform, yeast and mould in the juice samples. This is consistent with the study conducted by Noci *et al.* (2008) on heat treated apple juice, where the microbial count was reduced to below detection limit. High temperature causes adverse effects on microbes by damaging organic molecules (nucleic acids and proteins) required for the proper functioning of cells, thus causing cytolytic effects. In addition, heat disrupts the integrity of the cellular membrane, eventually inactivating microbial growth. Correspondingly, 100% reduction of microbial load was observed in thermally pasteurised orange and carrot juice blend, as reported by Rivas *et al.* (2006).

After non-thermal treatments (sonication, UV-C and combined method), significant reduction in microbial load was observed in juice samples. Besides that, non-thermal treatments reduced coliform counts to below detection limits. This could be explained by the collapse of cavitation-induced micro bubbles resulting in a localised decontamination effect (Mason, 1991). In addition, formation of free radicals (OH^- and H^+) and hydrogen peroxide due to sonolysis of water, inactivates microbial cells (O'Donnell *et al.*, 2010). Previous studies have reported that sonication is effective in reducing the microbial count of foodborne pathogens, such as in orange and carrot juice (Valero *et al.*, 2007; Jabbar *et al.*, 2014).

Moreover, the characteristic of microbial DNA allows absorption of UV-C light photons, thus generating cross links between neighbouring cytosine and thymine (pyrimidine) bases in the same DNA strand (Tran and Farid, 2004; Guerrero-Beltran and Barbosa-Canovas, 2004). Therefore, these pyrimidine dimers prevent DNA transcription and translation, eventually, inactivating microbial growth. Similarly, microbial inactivation effect of UV-C was reported in apple and orange juices (Walkling-Ribeiro *et al.*, 2008; Pala and Toklucu, 2013).

However, complete inactivation of microbial growth in juice could not be observed for non-thermal treatments (ultrasonic and UV-C) for 15, 30 and 60 minutes as a stand-alone treatment. According to Koutchma *et al.* (2004), UV-C penetration largely depends on the presence of dissolved organic solutes (suspended solids) and coloured compounds which act as a barrier, thus exhibiting UV-C attenuation effects. Hence, combination of non-thermal processing methods in a hurdle sequence can speed up the rate of sterilization of food products (Vercet *et al*., 2001; Walkling-Ribeiro *et al.*, 2008).

Combination of ultrasonic and UV-C treatment (S30+U30 sample) completely inactivated aerobic bacteria in juice samples. This could be explained by the formation of hydroxyl radicals during UV photon generation, and also by bubble implosion during sonication, thus resulting in the disintegration of the microbial cells (Zafra-Rojas *et al.*, 2013; Pala and Toklucu, 2013). As a hurdle, ultrasound and UV-C improves the rate of sterilization of the juice samples which may be due to their synergistic effects.

For yeast and mould count, the percentage of inactivation was lower than aerobic bacteria. Therefore, yeast and mould may display higher resistance to sonication and UV-C beam when compared to bacteria due to the difference in thickness of the cell wall and size of microbial cells (Pala and Toklucu, 2013). In addition, lesser pyrimidine bases on the DNA strand of yeast and mould contributes to less probability of cross link formation, thus less susceptible to UV-C (Miller *et al.,* 1999).

There were no significant changes in the physicochemical properties of juice after thermal, ultrasonic, UV-C and combined treatment. Similarly, thermally treated Valencia and Navel orange (Bull *et al.,* 2004) and pomegranate juice (Pala and Toklucu, 2011) exhibited no significant variations in pH, TSS and TA. Results obtained are in agreement with previous studies that reported no significant alteration in pH, TSS and TA of juice processed by either ultrasound or UV-C, such as in sonicated apple and carrot juice, as well as UV-C treated apple, pomegranate, and orange juice (Walkling-Ribeiro *et al.,* 2008; Pala and Toklucu, 2011; Pala and Toklucu, 2013; Abid *et al.,* 2013; Jabbar *et al.,* 2014).

Significant differences in colour of juice samples were observed between control and treatments (thermal and non-thermal). In comparison to thermal treatment, the lowest variation from the control was observed in juice samples subjected to sonication (S15 and S30), UV-C (U15), and combined treatment (S15+U15, S30+U15, S15+U30). This is consistent with the study conducted by Lee and Coates (2003) on heat treated Valencia orange juice, where significant variations in colour were observed resulting in a lighter colour (increase in L^* value) and more saturated juice. In addition, significant colour degradation in thermally treated pineapple juice was reported by

Rattanathanalerk *et al.* (2005). This is in agreement with the study conducted by Abid *et al.* (2013) on ultrasonic treated apple juice, where significant colour changes were observed. Similarly, colour degradation in sonicated orange juice was reported by Tiwari *et al.* (2008). This could be explained by accumulation of unstable particles in the juice that are partially precipitated (Genovese *et al.,* 1997). Besides that, Caminiti *et al.* (2012) reported significant differences in colour of orange and carrot juice blend, after UV-C treatment.

An increase in lightness (L^*), and decrease in redness ($+a^*$) and yellowness ($+b^*$) were observed after all treatments. The increase in L^* values could be attributed to the brightening effect of juice due to cavitational collapse of bubbles during sonication and UV-C photodegradation of coloured compounds (Tiwari *et al.,* 2008; Bhat *et al.,* 2011b). Since the colour of mango juice is mostly contributed by natural pigments such as carotenoids, thus accelerated carotenoid isomerization caused by high temperature (Chen *et al.,* 1995) and sonication (Mason, 1991) may result in the loss of yellow colour (decreased $+b^*$ value). Besides that, the increase in L^* value makes the juice more transparent due to the destruction of coloured compounds formed previously. While the decrease in ($+a^*$) and ($+b^*$) might be attributed to the development of browning components (Ibarz *et al.,* 2005).

In addition, an increase in ΔE corresponding to increased UV-C exposure from 15 to 60 minutes (as a stand-alone treatment) was observed, as previously reported by Bhat *et al.* (2011b) and Zhang *et al.* (2011). However, in a hurdle sequence, an increase in ΔE corresponding to increased ultrasonic treatment time (from 15 to 30 minutes) was observed, regardless of the UV-C treatment. Thus, sonication could be responsible for

the colour degradation observed in this hurdle system, as previously suggested by Cheng *et al.* (2007) in guava juice treatment.

The clarity of juice significantly decreased after thermal treatment, thus indicating an increase in turbidity. This is consistent with the study conducted by Rivas *et al.* (2006) on heat pasteurized orange and carrot juice blend, where diminishing clarity was observed resulting in a more saturated juice. This may be explained by the 'swelling' of particles and the diffusion of water between the cellulose chains during heat pasteurization, thus increasing viscosity of the juice. Furthermore, high temperature greatly ruptures cell structure during treatment, and allows pectin to leak out contributing to higher concentration of colloidal pectin in juice. Hence, increased viscosity and pectin content causes decrease in the clarity of juice subjected to thermal treatment (Shamsudin *et al.,* 2013; Aguilo-Aguayo *et al.,* 2009).

In contrast, significant improvement in clarity was observed in sonicated (S15 and S30) and all combined treated samples. Indeed, there is an increasing trend in clarity corresponding to increased sonication time (from 15 to 30 minutes) in a hurdle system. This could be explained by the mechanical stress derived from cavitational collapse of bubbles, which causes the breakdown of large macromolecules and particles in the juice (Demirdoven and Baysal, 2008). In addition, the application of low-power ultrasound, such as ultrasonic cleaning bath will decrease the viscosity and pectin content of juice, thus improving the clarity of juice (Abid *et al.,* 2013). However, high power ultrasound may bring undesirable changes in rheology of juice (Chemat *et al.,* 2011). Thus, ultrasound processing variables have to be evaluated appropriately to avoid negative impacts on fruit juice quality.

No significant changes in clarity of juice samples were exhibited after UV-C treatment (U15 and U30). This could be explained by the properties of UV-C treatment, wherein no significant modification of viscosity and pectin content of juice were induced. According to Shamsudin *et al.* (2013), UV-C processed Yankee pineapple juice showed no changes in particle size, thus coagulation of colloidal materials were absent, resulting in unchanged viscosity of juice. In contrast, U60 sample exhibited significant reduction in clarity. The longer treatment time may cause reduced activity of pectin methylesterase (PME) and polygalacturonase (PG), therefore affecting rheology of juice (Aguilo-Aguayo *et al.*, 2009).

NEBI is the browning of juice due to Maillard reactions, subsequently causing colour changes and loss of nutrients (Caminiti *et al.*, 2011). In this study, significant increase was found in the NEBI of juice processed by thermal, ultrasonic and UV-C. It was clearly exhibited that high heat pasteurisation accelerated formation of brown pigments, consequently darkening the juice when compared to mild heat pasteurisation. Hence, these results are consistent with the study conducted by Aguilo-Aguayo *et al.* (2009) on high heat pasteurised strawberry juice, where significant browning were observed. Correspondingly, Bull *et al.* (2004) also reported increase in browning index of thermally treated Valencia and Navel orange juice.

In addition, browning of sonicated orange juice was reported by Valero *et al.* (2007). This could be due to the cavitation phenomenon as a result of sonication, which triggers Maillard reaction causing accumulation of brown pigments (Tiwari *et al.*, 2009b). Correspondingly, Caminiti *et al.* (2011) observed a significant increase in NEBI of UV-C treated apple and cranberry juice blend. There is an increasing trend in browning

index corresponding to increased UV-C exposure time (from 15 to 60 minutes), as previously reported by Zhang *et al.* (2011). This could be due to photodegradation reactions that promote Maillard reaction between amino acids and reducing sugars causing enhancement of browning degree (Ibarz *et al.*, 2005). The results of NEBI agrees with colour analysis, whereby a decrease in (+*b**) and increase in *L** values were observed, indicating the shift of colour from yellow towards light brown.

However, combined treated juice samples, S15+U15, S30+U15, S15+U30 showed no significant variation in NEBI, when compared to the control. This is consistent with the study conducted by Caminiti *et al.* (2011) on apple and cranberry juice blend subjected to combined non-thermal processing methods, where no significant browning was promoted.

HMF is one of the chemical substances (intermediate) produced during browning process, thus serving as a good indicator of Maillard reaction (Rattanathanalerk *et al.*, 2005). Significant increase in HMF content was observed in thermally treated samples. This is in agreement with the study conducted by Aguilo-Aguayo *et al.* (2009) on heat pasteurized strawberry juice, where a significant increase in HMF content was observed. The results of HMF content agrees with NEBI, as increased NEBI can be attributed to enhancement in the development of HMF.

In contrast, non-thermal treated juice samples (except S60, U60 and S30+U30) showed no significant changes in HMF content, when compared with the control. Nevertheless, it is important to highlight that the maximum concentration of HMF allowed by the Association of the Industry of Juices and Nectars from Fruits and Vegetables is ≤ 5 mg/l

(AIJN, 1996). Hence, the concentration of HMF in all treated juice samples (thermal and non-thermal) was low and remained below the limit allowed.

Thermally treated juice exhibited significant degradation of carotenoids. In a previous study conducted by Lee and Coates (2003), it was observed that high temperature induces geometric isomerization (trans-form to cis-form) of carotenoids, resulting in significant loss of carotenoids in thermally pasteurized orange juice. In addition, the instability of the polyene chain (extensive conjugated double bond system) of carotenoids, contribute to their susceptibility to oxidation, thus forming initial products known as epoxides (Rodriguez-Amaya, 1997). Similar results were reported by Goh *et al.* (2012) on degradation of carotenoids in heat treated pineapple juice.

After non-thermal treatments, significant enhancement in extractability of carotenoids was observed in juice samples (except S60 and U60). Thus, carotenoids are still stable after 15 and 30 minutes of sonication or UV-C exposure. This could be attributed to the alteration of carotenoid-binding protein, consequently increasing the availability of free carotenoids. In addition, the formation of cavitation-induced shock waves and UV photons may cause inactivation of enzymes responsible for the degradation of carotenoids, thus improving carotenoid extraction yield (Oms-Oliu *et al.*, 2012a). Similarly, Jabbar *et al.* (2014) reported significant improvements in total carotenoids of carrot juice subjected to sonication. However, for S60 samples, degradation of carotenoids was observed. This could be attributed to the carotenoid isomerization due to the high shearing effect after 60 minutes of sonication.

It was found that combined treated sample (S30+U30 and S30+U15) exhibited the highest increase in extractability of carotenoids. These phenomena were due to the sonochemical and UV photochemical reaction as a result of ultrasonic and UV-C treatment, respectively (Demirdoven and Baysal, 2008; Oms-Oliu *et al.,* 2012a). According to Rodriguez-Amaya (1997), there is an increase in certain individual carotenoids owing to better stability. This may be explained by the enhancement of antioxidants due to UV-C exposure and sonication, thus providing better retention of carotenoids. The increased availability of free carotenoids might benefit health conscious consumers as these compounds possess health promoting properties such as reduced risk of cardiovascular disease (Krinsky, 1990) and cancer (Ziegler, 1991).

Ascorbic acid is an important antioxidant that has been linked with protection against several types of cancers (Block, 1991). There was significant reduction in ascorbic acid content in juice processed by thermal and non-thermal. Similarly, significant loss of ascorbic acid was observed in thermal processed orange juice, owing to its thermolabile characteristic (Pala and Toklucu, 2013). Heating affects the degradation of ascorbic acid in an aerobic pathway due to its heat-sensitive characteristic in the presence of oxygen (Oms-Oliu *et al.,* 2012a). Correspondingly, Goh *et al.* (2012) observed lower ascorbic acid content in thermally treated pineapple juice than control.

Formation of hydroxyl radicals during UV photon generation, and also by bubble implosion during sonication could be responsible for the decreased content of ascorbic acid (Hart and Henglein, 1985; Bhat *et al.,* 2011). Similarly, Tiwari *et al.* (2009b) observed lower ascorbic acid content in ultrasonic treated orange juice. The degradation of ascorbic acid could be attributed to the thermolysis of ascorbic acid in bubbles

(cavities) as a result of the sonication. Subsequently, Maillard reaction is triggered (Tiwari *et al.,* 2009b). Besides that, significant decrease of the ascorbic acid content was recorded in sonicated strawberry juice, where the largest reduction was below 15% (Tiwari *et al.,* 2009c).

In addition, the oxidative degradation as a result of enzyme activities such as ascorbate oxidase and peroxidase, and the presence of oxygen and light, mainly contribute to the detrimental effects on ascorbic acid (Davey *et al.,* 2000). Significant decrease in ascorbic acid content was observed in UV-C treated starfruit juice, where the least reduction was 10% (Bhat *et al.,* 2011b). Similarly, Tran and Farid (2004) also reported loss of ascorbic acid (12%) in orange juice after UV-C treatment. In comparison to thermal treatment, juice samples subjected to non-thermal treatments showed least degradation of ascorbic acid from the control. Hence, it is important to emphasize that non-thermal treatments provide better retention of ascorbic acid when compared to thermal treatment due to the absence of heat supply.

Fruit juices contain numerous compounds that contribute to their antioxidant activity. Therefore, more than one method was used to measure antioxidant properties according to their ability to scavenge specific radicals, to chelate metal ions and to inhibit lipid peroxidation (Martínez *et al.,* 2012). In this study, polyphenol and flavonoid content of juice were analysed, along with four antioxidant assays (DPPH, ABTS, RPA and TAC). The Folin-Ciocalteu method is based on the detection of phenolic compounds by reduction of the reagent, which contains tungsten and molybdenum oxides (Waterhouse, 2002). While, flavonoids are naturally-occurring polyphenolic compounds that form acid labile complexes in aluminium chloride colorimetric method (Mabry *et al.,* 1970).

The DPPH radical scavenging assay measures the hydrogen donating capacity of the antioxidant to the stable free radical DPPH, resulting in the formation of diphenylpicrylhydrazine (Shon *et al*., 2003). Similarly, ABTS assay measures the ability of antioxidants to scavenge the long-life radical cation $ABTS^{+}$ (Martínez *et al.*, 2012). In addition, the reductive ability of a compound (Fe^{3+} to Fe^{2+} transformation) may also serve as an important indicator of its potential antioxidant activity (Rama Prabha and Vasantha, 2011). On the other hand, TAC was evaluated by the reduction of Mo (VI) to Mo (V) in a phosphomolybdenum method (Prieto *et al.*, 1999).

After thermal treatment, a significant reduction in total polyphenols and total flavonoids were observed. Likewise, significant decrease in antioxidant activities was observed in juice subjected to thermal treatment. This is in agreement with the study conducted by Pala and Toklucu (2013) on heat treated orange juice, where significant loss of phenolic compounds was observed. Nevertheless, there are previous reports suggesting that thermal pasteurization can either increase or decrease the extractability of antioxidants due to treatment time. For instance, Caminiti *et al.* (2012) reported that thermal treatment did not induce significant changes in antioxidant capacity of orange and carrot juice blend.

Non-thermal treatments caused significant enhancement of extractability of total polyphenols and total flavonoids. Similarly, significant increase in antioxidant activities was observed in juice subjected to non-thermal treatments. It was found that combined treated sample (S30+U15) showed the highest increase in extractable polyphenols and flavonoids. These observations are in agreement with a study by Abid *et al.* (2013), reporting a significant increase in extractability of phenolic compounds and flavonoids

in sonicated apple juice. This may be explained by the reaction of aromatic ring of polyphenols with hydroxyl radicals generated sonochemically, therefore improving the antioxidant activity (Ashokkumar *et al.,* 2008).

Besides that, the exposure of UV-C light stimulates the formation of free radicals, which may trigger stress responses such as accumulation of phytoalexins. As a result, there is an increase in the level of flavonoids related to a defence mechanism (Alothman *et al.,* 2009). However, for U60 samples, degradation of flavonoids was observed. This could be attributed to the longer UV-C exposure time, which induces too much stress thus, suppressing flavonoid content. Nevertheless, Alothman *et al.* (2009) reported that UV-C treatment can either increase or decrease the extractability of antioxidants due to some variables, including dose delivered, exposure time, and raw materials used. Thus, UV-C processing variables have to be evaluated appropriately to avoid negative impacts on fruit juice quality.

Previous studies have reported that non-thermal processing methods increase extractability of antioxidants, such as observed in sonicated kasturi lime juice and UV-C treated starfruit juice (Bhat *et al.,* 2011a; Bhat *et al.,* 2011b). Therefore, combined treated samples with enhanced antioxidant capacity may allow additional quenching of active oxygen species, thus being an advantage to health conscious consumers, such as prevention of the degenerative processes (Block *et al.,* 1992).

Total polyphenols and total flavonoids exhibited a strong positive correlation with the antioxidant assays studied, thus indicating that phenolic compounds (including flavonoids) are the major contributor of antioxidant activity, in terms of reducing

capacity and radical scavenging ability. The correlation between phenolic content and antioxidant activity has been previously reported (Gorinstein *et al.,* 2004; Rama Prabha and Vasantha, 2011). Since TAC results correlated well with those obtained by other assays, therefore it serves as a good estimate of antioxidant capacity in juice samples.

Phenolic compounds are secondary metabolites found in plants, which are gaining interest among researchers as a natural antioxidant. Their antioxidant activity is related to their chemical structures, particularly the reaction of aromatic ring with free radicals (Ashokkumar *et al.,* 2008). In this study, rapid screening for phenolic compounds in Chokanan mango juice was analysed using LCMS/MS (negative ionisation mode), based on their molecular weights and characteristic fragmentation patterns. LCMS/MS is a highly sensitive and selective technique for fast and rapid screening of a wide range of compounds in many applications (Agilent Technologies, 2001). The recovery of phenolic compounds from juice is related to the polarity of solvent used for extraction, mainly because it affects the solubility of polyphenols in the solvent system (Naczk and Shahidi (2006). Previous studies suggested the use of methanol to extract phenolic compounds, as it has been shown to exhibit high antioxidant activities (Bhat *et al.,* 2011b; Xu *et al.,* 2008).

Chokanan mango juice is a good source of phenolic compounds. Among them, nine individual phenolic compounds were identified, including phenolic acids (caffeoyl glucose, quinic acid, monogalloyl glucose, ellagic acid, gallic acid), flavonoids (kaempferol and quercetin), xanthanoid (mangiferin), and tannin (tannic acid). Phenolic acids are phenols that possess one carboxylic acid group, and have been related to color, nutritional and antioxidant properties of foods (Robbins, 2003). There are two classes of

phenolic acids, namely derivatives of cinnamic acid and derivatives of benzoic acid. The hydroxycinnamic acid is commonly found in plants as esters of quinic acid, shikimic acid, and tartaric acid. Caffeoyl glucose is an example of hydroxycinnamic acid (ester of caffeic acid). While, hydroxybenzoic acid is found in plants as free and esterified. Monogalloyl glucose, ellagic acid and gallic acid are examples of hydroxybenzoic acids (Manach *et al.,* 2004; Masibo and He, 2008). Similarly, ellagic acid and gallic acid were previously reported in mango by El-Ansari *et al.* (1969).

Flavonoids are the most common and widely distributed group of plant phenolics. The key representatives of flavonols include kaempferol and quercetin. Numerous epidemiological studies support that consumption of foods rich in the flavonoids may reduce the risk of developing cancer (Middleton *et al.,* 2000; Manach *et al.,* 2004). Mangiferin is an example of xanthone, and has been found to exhibit antioxidant, anticancer, antimicrobial, antiallergenic, and anti-inflammatory effects (Masibo and He, 2008). Gallotannin (tannic acid) and mangiferin were also previously reported in mango by El-Ansari *et al.* (1969). Tannic acid is an example of hydrolysable tannin, and therefore it is converted into gallic acid when oxidized. Gallic acid is well known for its antioxidant, anti-inflammatory, and antimutagenic properties (Madsen and Bertelsen, 1995; Masibo and He, 2008).

After thermal treatment, there was no significant difference in the concentration of six individual phenolic compounds (caffeoyl glucose, quinic acid, monogalloyl glucose, ellagic acid, kaempferol, and mangiferin. Similarly, Pala and Toklucu (2011) reported no significant variation in phenolic compounds of thermally treated pomegranate juice. This is in agreement with the study conducted by Sentandreu *et al.* (2007), where

negligible effects of heat treatment on phenolic compounds were observed. However, concentration of quercetin and gallic acid decreased in thermally treated juices. This could be due to incomplete inactivation of polyphenol oxidase by heat, thus causing further loss of phenolic substances (Huang *et al.,* 2013).

After non-thermal treatments, there were no significant differences in the concentration of caffeoyl glucose and monogalloyl glucose. However, there was a significant enhancement in extractability of six individual phenolic compounds (quinic acid, ellagic acid, quercetin, gallic acid, kaempferol, mangiferin and tannic acid) in non-thermal treated juice samples. This could be attributed to the properties of UV-C and ultrasound, wherein the polyphenol oxidase enzyme is inactivated, therefore providing better retention of phenolic compounds (Bhat *et al.,* 2011b; Oms-Oliu *et al.,* 2012a). In addition, the active removal of occluded oxygen from the juice by sonication was suggested to contribute to the enhancement of phenolic compounds (Masuzawa *et al.,* 2000). The increased activity of phenylalanine ammonia lyase enzyme by UV-C exposure was suggested to contribute to the activation of the phenolic biosynthesis pathway, which leads to enhancement of phenolic compounds (Alothman *et al.,* 2009). Similarly, sonication and UV-C treatment increase extractability of phenolic compounds, such as observed in sonicated purple cactus pear juice and UV-C treated starfruit juice (Zafra-Rojas *et al.,* 2013; Bhat *et al.,* 2011b).

With regards to sensory analysis, significant decrease in hedonic scores was observed in juice samples subjected to thermal treatment, regardless of mild or high heat pasteurization. Panellist rated thermally treated juices as ‘dislike moderately’ for odour and ‘dislike slightly’ for colour and taste. Evaluation of the overall acceptability

revealed that juice samples processed by thermal were least preferred (below the rejection score). Correspondingly, Sentandreu *et al.* (2005) reported a significant decrease in the fresh taste of citrus juices heated above 70 °C. Likewise, significant lower scores for sensory attributes (flavour and aroma) were observed for thermal processed orange juice, as reported by Pala and Toklucu (2013).

After non-thermal treatments, juice samples (S15, S30, U15, U30, S15+U15, S30+U15, S15+U30) exhibited lowest variation in hedonic scores, when compared to the control. Panellist rated non-thermal treated juices as 'like slightly' for colour, odour and taste. Correspondingly, Caminiti *et al.* (2011) reported significant lower scores for sensory attributes (odour and flavour) of ultrasonic treated apple and cranberry juice blend. While, significant lower hedonic scores for flavour and aroma of UV-C processed orange juice was reported by Pala and Toklucu (2013).

However, there is a significant decrease in overall acceptability corresponding to increased ultrasonic or UV-C treatment time (from 15 to 30 minutes). This could be attributed to the formation of free radicals induced by sonication, thus causing off-flavour of ultrasonic treated juices. In addition, cavitation-induced colour changes and development of browning compound due to UV-C photodegradation may cause a decrease in hedonic scores (Vercet *et al.*, 1998; Caminiti *et al.*, 2011; Bhat *et al.*, 2011b; Zhang *et al.*, 2011).

Nevertheless, it is important to highlight that non-thermal treated juice was preferred more than thermally treated juice. Hence, the sensory evaluation verified that combined treatment (S15+U15, S30+U15, S15+U30) was the most acceptable treatment of the

selected non-thermal treatments. Previous studies have reported promising retention of sensory quality of fruit juices by combined non-thermal processing methods (Caminiti *et al.,* 2011; Caminiti *et al.,* 2012).

Shelf life of juice samples were evaluated according to the Institute of Food Science and Technology, IFST (1999). The acceptable maximum microbial load of aerobic bacteria in fruit juices is about 4 log CFU/ml. The rate of microbial growth observed in control (untreated juice) was higher than thermal and non-thermal treated juice samples during storage. This is mainly due to naturally occurring microorganisms in untreated juice that can grow under refrigeration (4 °C), therefore causing juice spoilage during storage (Vegara *et al.,* 2013). The result of APC agrees with visual observations, whereby an increase in microbial count may result in the browning of juice, thus indicating the shift of colour from yellow towards light brown.

Thermally treated juices had almost no microorganism growth during storage, thus remaining below the microbial load limit. After sonication (S30 and S60) or UV-C treatment (U30 and U60), there was less microbial growth, thus remaining below the microbial load limit for four and five weeks of storage, respectively. In addition, all combined treated juice samples had no significant increase in microbial growth during three weeks storage, thus remaining below the microbial load limit for five weeks of storage.

TSS of control decreased significantly during storage at 4 °C. For juices subjected to thermal and combined treatment, TSS remained constant until four weeks of storage, but started to decrease on the fifth week of storage. With regards to sonication and UV-

C treatment (as a stand-alone), TSS of juice samples remained constant until three weeks of storage, but started to decrease on the fourth week of storage.According to Rivas *et al.* (2006), there is a reduction in TSS due to the presence of microorganisms causing juice spoilage via sugar fermentation. Correspondingly, Bull *et al.* (2004) reported no significant differences in TSS of thermally treated Valencia and Navel orange juice during storage at 4 °C.

Control (untreated juice) has a shelf life for less than one week, in terms of APC and TSS. Indeed, it is important to highlight that thermally treated juice has a shelf life for more than five weeks. Hence, the shelf life of thermally treated juice stored at 4 °C was extended for at least five weeks longer than control. The results obtained are in agreement with a previous study reporting prolonged shelf life in thermally pasteurised orange and carrot juice blend (Rivas *et al.,* 2006).

The shelf life of ultrasonic treated juices, S15 (2 to 3 weeks), S30 (4 weeks), S60 (5 weeks) were longer than control (less than 1 week). Hence, the shelf life of sonicated juice stored at 4 °C was extended for at least four weeks longer than control. Similarly, Valero *et al*. (2007) reported that sonication extended the shelf life of orange juice.

For UV-C treatment, the shelf life of juices, U15 (2 to 3 weeks), U30 (4 weeks), U60 (5 weeks) were longer than control (less than 1 week). Hence, the shelf life of UV-C treated juice stored at 4 °C was extended for at least four weeks longer than control. The results obtained are in agreement with previous studies reporting extended shelf life in UV-C treated orange juice (Tran and Farid, 2004; Pala and Toklucu, 2013).

With regards to combined treatment, all juice samples (S15+U15, S30+U15, S15+U30, S30+U30) have a shelf life for more than five weeks. Hence, the shelf life of combined treated juice stored at 4 °C was extended for at least five weeks longer than control. Likewise, Walkling-Ribeiro *et al.* (2008) reported that combination of non-thermal processing methods can prolong the shelf life of juice.

CHAPTER 6

GENERAL DISCUSSION

The market for value-added mango products such as juice, puree and nectar has progressively grown due to the perishable nature and limited shelf-life of mangoes (Liu *et al.,* 2014). In Malaysia, the Chokanan mango is one of the most popular cultivars that bears fruit continuously even during rainy season. However, juice extraction from Chokanan mangoes is a recent development and therefore, various questions have arisen from the processing method to the quality standard. Furthermore, research conducted on the Chokanan mango is scarce, despite its abundance in Malaysia. The research conducted in this project is the first to report on the Chokanan mango juice.

More recently it was reported that fruit juices have the highest acceptability among other beverages, generally due to their natural taste, as well as the nutritional values associated with them. Besides that, the global market for juices is estimated to reach a value of US$ 93 billion in 2014 (Business Insights, 2010). However, the number of outbreaks and cases of illness caused by consumption of contaminated fruit juices, especially unpasteurized juices, has increased over the last decade (Odumeru, 2012).

Conventional thermal pasteurization is the preferred technology used to achieve microbial inactivation, and extend the shelf life of juices. Lately however, consumers' demand for a new preservation technology that retains freshness and at the same time ensures food safety, has resulted in growing interest for non-thermal processing methods, such as sonication and UV-C treatment. The advantages of sonication and UV-C treatment are that they are simple, cost-effective, require low maintenance, and

have improved efficiency (O'Donnell *et al.*, 2010; Pala and Toklucu, 2013). These technologies have different modes of microbial inactivation, thus offering the possibility of combining them, applying the concept of hurdle technology (Leistner and Gorris, 1995; Leistner, 2000).

Processing technology may have a possible effect on the physicochemical properties, antioxidant capacity and sensory attributes of the processed food product. Generally, the impact on product quality has received less attention than microbial stability and safety aspects with regards to thermal and non-thermal technologies (as a stand-alone or combination). This is mainly due to the importance given in extending the shelf-life of food products during processing. Therefore, it is essential for food processors to understand the effects of different processing procedures on the overall quality of the final product. This study was undertaken to address several issues pertaining to the development of a novel processing procedure as a feasible and effective alternative to the conventional method.

In this study, Chokanan mango juice was subjected to thermal and non-thermal processing. The thermal processing methods included mild and high heat pasteurization while non-thermal processing methods included ultrasonic and UV-C treatment as stand-alone treatments and in combination. The study was carried out using freshly squeezed Chokanan mango juice (without the addition of any preservatives or additives) to determine the stability of natural, unadulterated fruit juice. A complete quality profile of thermal and non-thermal treated Chokanan mango juice was established by analysing various quality parameters, including physicochemical, nutritional, microbiological and sensorial. The results obtained were published as four research papers in *Ultrasonics*

Sonochemistry, *Food and Bioproducts Processing*, *Food Science and Technology International*, and *Journal of Food Science* (see Publications).

Results from this study showed that thermal treatment regardless of mild or high heat pasteurization was effective in completely inactivating microbial growth in the juice. However, significant loss in quality was observed after thermal treatment. Thermally treated juice exhibited significant reduction in clarity, colour, and ascorbic acid while increasing extractability of carotenoids, phenolic compounds, and flavonoids as compared to freshly squeezed juice. In addition, thermal treatment results in a significant decrease in the antioxidant activities of juice. As for consumers' acceptance, thermally pasteurized juice was the least preferred and rated below rejection level. The shelf life of thermally treated juice stored at 4 °C was extended for at least five weeks longer than control. Thus, thermal treatment is a recommended food processing technology to extend the shelf life of the Chokanan mango juice. However, process optimisation of thermal processing should be considered to minimise the negative effect on the quality of Chokanan mango juice for the satisfaction of consumers.

With regards to non-thermal treatments, significant reduction in microbial load was observed in the juice samples subjected to sonication and UV-C treatment (as stand-alone treatments and in combination). Combined treatment showed complete inactivation of coliforms and aerobic bacteria, along with a significant reduction in yeast and mould count. Non-thermal treated juice samples exhibited higher percentage of retention in most of the quality parameters. Significant improvement in clarity was observed in juice samples subjected to ultrasonic treatment for 15 and 30 minutes (as stand-alone treatments and in combination) when compared to freshly squeezed juice.

A notable discovery in this study was that non-thermal treatments caused significant enhancement of the extractability of carotenoids, phenolic compounds, and flavonoids from the juice. Similarly, significant increases in antioxidant activities were observed in juice subjected to non-thermal treatments. It was found that combined treated sample (S30+U15) showed the highest increase in extractable polyphenols and flavonoids. The enhanced antioxidant capacity of non-thermal treated samples will be an added advantage to health conscious consumers, as it plays a role in prevention of the degenerative processes.

Sensory attributes (colour, odour, taste, and overall acceptability) were evaluated by 90 panellists using a hedonic scale. Control was positioned in the 'like moderately' point of the hedonic scale with the highest score. Non-thermal treated juice exhibited the least difference in score when compared to the control, thus falling within the 'like slightly' point of the scale. It was clearly observed that non-thermal treated juice was preferred more than thermally treated juice. Hence, the sensory evaluation verified that combined treatment (S15+U15, S30+U15, S15+U30) was the most acceptable treatment of the selected non-thermal treatments.

In this study, rapid screening for phenolic compounds in Chokanan mango juice was analysed using LCMS/MS (negative ionisation mode). Results from this study showed that Chokanan mango juice is a good source of phenolic compounds. Among them, nine individual phenolic compounds were identified, including phenolic acids (caffeoyl glucose, quinic acid, monogalloyl glucose, ellagic acid, gallic acid), flavonoids (kaempferol and quercetin), xanthanoid (mangiferin), and tannin (tannic acid). After ultrasonic, UV-C and combined treatment, there were significant enhancement in the

extractability of the six individual phenolic compounds (quinic acid, ellagic acid, quercetin, gallic acid, kaempferol, mangiferin and tannic acid). Therefore, the results obtained provide a better understanding on the effects of processing on specific bioactive compounds, especially phenolic compounds.

With regards to sonication and UV-C treatment (as stand-alone treatments), the shelf life of juice stored at 4 °C was extended for at least four weeks longer than control. Combined treatment prolonged the shelf life of juice stored at 4 °C for at least five weeks longer than control. This is a clear indication that the combination of ultrasound and UV-C in a hurdle concept may have an additive or, even, a synergistic effect on microbial destruction (Leistner, 2000), thus extending the shelf-life of juice samples.

As a conclusion, all the results collectively point towards the potential of sonication and UV-C treatment (as stand-alone treatments and in combination) to be used in Chokanan mango juice processing for safer and high quality juice with an extended shelf-life. Although thermal treatment was effective in completely inactivating microbial growth in juice, significant quality loss was observed. In addition, the combination of ultrasound and UV-C is a promising hurdle for better retention of quality of Chokanan mango juice and a feasible alternative food processing technology. This thesis was a comprehensive study on the effects of ultrasonic and UV-C treatment (as stand-alone treatments and in combination) in Chokanan mango juice processing; therefore, the results might further encourage the positive implementation of these novel technologies on a pilot scale in food processing industries. Significantly, these studies are the first reported studies of UV-C and sonication treatment used in tandem as a hurdle sequence for safer and high quality juice with an extended shelf-life. The positive results

observed are likely to increase the interest in this sequence as an alternative hurdle sequence.

Generally, all the objectives proposed earlier in this study have been achieved. With the information and knowledge obtained from this study, further research work is needed to develop models to optimise some of the critical processing variables during sonication, UV-C treatment and the hurdle sequence. Additional research work needs to be initiated towards evaluating enzyme activities in juice samples subjected to sonication and UV-C treatments (as stand-alone treatments and in combination). Besides that, future research studies are needed to evaluate the effects of processing methods on mineral elements, flavour and aroma compounds in juice. Moreover, storage study of thermal and non-thermal treated juice is necessary to further investigate the changes in juice quality.

LITERATURE CITED

3M Food Safety (2010). 3M Petrifilm interpretation guide. Available online: <http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/>

Abid M, Jabbar S, Wu T, Hashim MM, Hu B, Lei S, Zhang X & Zeng X (2013). Effect of ultrasound on different quality parameters of apple juice. Ultrasonics Sonochemistry 20: 1182–1187.

Agilent Technologies (2001). Basics of LC/MS. Agilent: USA. Available online: <http://www.chem.agilent.com>

Agri-food Business Development Centre (2010). Ministry of Malaysia Agriculture and Agro-Bases Industry (MOA): Mango. Available online: <http://www.agribdc.gov.my/mango>

Aguilo-Aguayo I, Oms-Oliu G, Soliva-Fortuny R & Martin-Belloso O (2009). Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments. LWT- Food Science and Technology 42: 813–818.

Alam MR, Bristi NJ & Rafiquzzaman M (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal 21: 143–152.

Alighourchi H, Barzegar M & Abbasi S (2008). Effect of gamma irradiation on the stability of anthocyanins and shelf-life of various pomegranate juices. Food Chemistry 110: 1036−1040.

Alothman M, Bhat R & Karim AA (2009). Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends in Food Science and Technology 20: 201-212.

Aquafine (2014). UV technology. Available online: <http://www.aquafineuv.com.aspx>

Arauz L (2000). Mango anthracnose: Economic impact and current options for integrated management. Plant Disease 84: 600−611.

Armstrong WP (2004). The sumac family (Anacardiaceae). Available online: <http://waynesword.palomar.edu/ecoph43.htm.>

Arnao MB, Cano A & Acosta M (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chemistry 73: 239-244.

Ashokkumar M, Sunartio D, Kentish S, Mawson R, Simons L, Vilkhu K & Versteeg C (2008). Modification of food ingredients by ultrasound to improve

functionality: A preliminary study on a model system. Innovative Food Science and Emerging Technologies 9: 155–160.

Association of the Industry of Juices and Nectars from Fruits and Vegetables, AIJN (1996). Association of the industry of juices and nectars of the European Economic Community code of practice for evaluation of fruit and vegetable juices. AIJN: Brussels.

Bae SH & Suh HJ (2007). Antioxidant activities of five different mulberry cultivars in Korea. LWT- Food Science and Technology 40: 955-962.

Bally ISE (2006). Species profiles for Pacific Island agroforestry: *Mangifera indica* (Mango). Available online: <http://www.traditionaltree.org>

Bates RP, Morris JR & Crandall PG (2001). Principles and practices of small- and medium-scale fruit juice processing: Food and Agriculture Organization (FAO) Agricultural Services Bulletin Issue 146. FAO: Rome, Italy.

Belajova, E & Suhaj M (2004). Determination of phenolic constituents in citrus juices: Method of high performance liquid chromatography. Food Chemistry 86(3): 339-343.

Berardini N, Knodler MA, Schieber A & Carle R (2005). Utilization of mango peels as a source of pectin and polyphenolics. Innovative Food Science and Emerging Technologies 6: 442–452.

Bharate SS & Bharate SB (2012). Non-enzymatic browning in citrus juice: Chemical markers, their detection and ways to improve product quality. Journal of Food Science and Technology *(in press)*: 1-18. DOI: 10.1007/s13197-012-0718-8.

Bhat R, Ameran SB, Voon HC, Karim AA & Tze LM (2011b). Quality attributes of starfruit (*Averrhoa carambola* L.) juice treated with ultraviolet radiation. Food Chemistry 127: 641–644.

Bhat R, Kamaruddin NS, Min-Tze L & Karim AA (2011a). Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. Ultrasonics Sonochemistry 18: 1295-1300.

Block G (1991). Vitamin C and cancer prevention: The epidemiologic evidence. The American Journal of Clinical Nutrition 53: 270-282.

Block G, Patterson B & Subar A (1992). Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. Nutrition and Cancer 18: 1–29.

Bompard J M & Schnell RJ (1997). Taxonomy and systematics. In: RE Litz (Ed.), The mango: Botany, production and uses. CAB International: Wallingford, UK, pp.21–47.

Britton G (1995). Structure and properties of carotenoids in relation to function. Federation of American Societies for Experimental Biology Journal 9: 1551-1558.

Bull M, Zerdin M, Howe E, Goicoechea D, Paramanandhan P & Stockman R (2004). The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice. Innovative Food Science and Emerging Technologies 5: 135-149.

Business Insights (2010). Innovations in fruit and vegetable juices: Emerging opportunities in premiumization, sustainability and positive health. Available online: <http://www.scribd.com/doc/50026681/Innovations-in-Fruit-and-Vegetable-Juices>

Butz P & Tauscher B (2002). Emerging technologies: Chemical aspects. Food Research International 35: 279-284.

Caminiti IM, Noci F, Morgan DJ, Cronin DA & Lyng JG (2012). The effect of pulsed electric fields, ultraviolet light or high intensity light pulses in combination with manothermosonication on selected physico-chemical and sensory attributes of an orange and carrot juice blend. Food and Bioproducts Processing 90: 442–448.

Caminiti IM, Noci F, Munoz A, Whyte P, Morgan DJ, Cronin DA & Lyng JG (2011). Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. Food Chemistry 124: 1387-1392.

Carcel JA, Garcia-Perez JV, Benedito J & Mulet A (2012). Food process innovation through new technologies: Use of ultrasound. Journal of Food Engineering 110: 200–207.

Centers for Disease Control and Prevention, CDC (1996). Outbreak of *Escherichia coli* O157:H7 infections associated with drinking unpasteurised commercial apple juice: British Columbia, California, Colorado, and Washington. Morbidity and Mortality Weekly Report 45: 975.

Chemat F, Zill-e-Huma & Khan MK (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrasonics Sonochemistry 18: 813–835.

Chen HE, Peng HY & Chen BH (1995). Changes of carotenoids, color and vitamin A contents during processing of carrot juice. Journal of Agricultural and Food Chemistry 43: 1912–1918.

Cheng LH, Soh CY, Liew SC & Teh FF (2007). Effects of sonication and carbonation on guava juice quality. Food Chemistry 104: 1396–1401.

Choi LH & Nielsen SS (2005). The effects of thermal and non-thermal processing methods on apple cider quality and consumer acceptability. Journal of Food Quality 28(1): 13–29.

Cohen E, Birk Y, Mannheim CH & Saguy IS (1998). A rapid method to monitor quality of apple juice during thermal processing. Lebensmittel-Wissenschaft & Technologie 31: 612–616.

Cortes C, Esteve M & Frigola A (2008). Color of orange juice treated by high intensity pulsed electric fields during refrigerated storage and comparison with pasteurized juice. Food Control 19: 151-158.

Crane JH, Bally ISE, Mosqueda-Vazquez RV & Tomer E (1997). Crop Production. In: Litz RE (Ed.), The mango: Botany, production and uses. CAB International: Wallingford, U.K, pp.203-256.

Cserhalmi Z, Sass-Kiss A, Toth-Markus M & Lechner N (2006). Study of pulsed electric field treated citrus juices. Innovative Food Science & Emerging Technologies 7: 49–54.

Damar S & Balaban MO (2006). Review of dense phase CO2 technology: Microbial and enzyme inactivation, and effects on food quality. Journal of Food Science 71: R1–R11.

Davey MW, Van Montagu M, Inze D, Sanmartin M, Kanellis A, Smimoff N, Benzie LJJ, Strain JJ, Favell D & Fletcher J (2000). Plant L-ascorbic: Chemistry, function, metabolism, bioavailable and effects of processing. Journal of the Science of Food and Agriculture 80: 825-860.

David JRD (1996). Principles of thermal processing and optimization. In: JRD David, RH Graves and VR Carlson (Eds.), Aseptic processing and packaging of food: A food industry perspective. CRC Press LLC: Florida, pp.14.

Dede S, Alpas H & Bayindirli A (2007). High hydrostatic pressure treatment and storage of carrot and tomato juices: Antioxidant activity and microbial safety. Journal of the Science of Food and Agriculture 87: 773–782.

Del Pozo-Insfran D, Balaban MO & Talcott ST (2006). Microbial stability, phytochemical retention, and organoleptic attributes of dense phase CO2 processed muscadine grape juice. Journal of Agricultural and Food Chemistry 54: 5468–5473.

Demirdoven A & Baysal T (2008). The use of ultrasound and combined technologies in food preservation, Food Reviews International 25: 1-11

.

Department of Agriculture, DOA (2009). Pakej teknologi mangga. Available online: <http://www. doa.gov.my>

Dutta D, Chaudhuri UR & Chakraborty R (2005). Structure, health benefits, antioxidant property and processing and storage of carotenoids. African Journal of Biotechnology 4(13): 1510-1520.

El Ansari MA, Reddy KK, Sastry KNS & Nayudamma Y (1969). Polyphenolic components of mango (Magnifera indica). Leather Science 16: 13-14.

Elez-Martinez P & Martin-Belloso O (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. Food Chemistry 102: 201-209.

Environmental Protection Agency, EPA (1999). Wastewater technology fact sheet: Ultraviolet disinfection EPA 832-F-99-064. EPA: USA.

Falguera V, Pagan J, Garza S, Garvin A & Ibarz A (2011). Ultraviolet processing of liquid food (A review Part 2): Effects on microorganisms and on food components and properties. Food Research International 44: 1580–1588.

Ferrari G, Maresca P & Ciccarone R (2010). The application of high hydrostatic pressure for the stabilization of functional foods: Pomegranate juice. Journal of Food Engineering 100: 245−253.

Food and Agriculture Organisation of the United Nation Statistics Division, FAOSTAT (2014) Food and Agricultural commodities production. Available online: <http://faostat.fao.org/site>

Food and Drug Administration, FDA (2000). Irradiation in the production, processing and handling of food. Federal Register-Rules and Regulations 65: 71056-71058.

Food Standard Agency (2007). The Fruit Juices and Fruit Nectars Regulations 2003 (as amended). Available online: <http://www.food.gov.uk>

Garcia-Gonzalez L, Geeraerd AH, Spilimbergo S, Elst K, Van Ginneken L, Debevere J, Van Impe JF & Devlieghere F (2007). High pressure carbon dioxide inactivation of microorganisms in foods: The past, the present and the future. International Journal of Food Microbiology 117(1): 1−28.

Garrido G, Gonzalez D, Lemus Y, Garcia D, Lodeiro L, Quintero D, Delporte C, Nunez-Selles AJ & Delgado R (2004). *In vivo* and *in vitro* anti-inflammatory activity of *Mangifera indica* L. extract (Vimang). Pharmacological Research 50: 143-149.

Genovese DB, Elustondo MP & Lozano JE (1997). Color and cloud stabilization in cloudy apple juice by steam heating during crushing. Journal of Food Science 62: 1171-1175.

Giada MLR (2013). Food phenolic compounds: Main classes, sources and their antioxidant power. In: JA Morales-Gonzalez (Ed.), Oxidative stress and chronic degenerative diseases: A role for antioxidants. InTech: Croatia, pp.87-106.

Glevitzky M, Pop M, Brusturean GA, Bogdan I, Calisevici M & Perju, D (2008). Efficient use of antioxidants to preserve fruit juice. Revista de Chimie (Bucureºti) 59: 1291-1295.

Gogate PR (2011) Hydrodynamic cavitation for food and water processing. Food and Bioprocess Technology 4: 996-1011.

Gogate PR, Sutkar VS & Pandit AB (2011). Sonochemical reactors: Important design and scale up considerations with a special emphasis on heterogeneous systems. Chemical Engineering Journal 166: 1066-1082.

Goh SG, Noranizan M, Leong CM, Sew CC & Sobhi B (2012). Effect of thermal and ultraviolet treatments on the stability of antioxidant compounds in single strength pineapple juice throughout refrigerated storage. International Food Research Journal 19: 1131-1136.

Gomez-Lopez VM, Koutchma T & Linden K (2012). Ultraviolet and pulsed light processing of fluid foods. In: PJ Cullen, BK Tiwari, P Vasilis and Valdramidis (Eds.), Novel thermal and non-thermal technologies for fluid foods. Academic Press: USA, pp.185-219.

Gorinstein S, Zachwieja Z, Katrich E, Pawelzikc E, Haruenkitd R, Trakhtenberge S & Martin-Belloso O (2004). Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid, Lebensmittel-Wissenschaft & Technologie 37: 337-343.

Guerrero-Beltran JA & Barbosa-Canovas GV (2004). Advantages and limitations on processing foods by UV light. Food Science and Technology International 17: 137-147.

Harborne JB & Simmonds NW (1964). The natural distribution of the phenolic aglycones. In: JB Harborne (Ed.), Biochemistry of phenolic compounds. Academic Press: London, pp.77-128.

Hart EJ &Henglein A (1985). Free radical and free atom reactions in the sonolysis of aqueous iodide and formate solutions. The Journal of Physical Chemistry 89: 4342-4347.

Huang W, Bi X, Zhang X, Liao X, Hu X & Wu J (2013). Comparative study of enzymes, phenolics, carotenoids and color of apricot nectars treated by high hydrostatic pressure and high temperature short time. Innovative Food Science and Emerging Technologies 18: 74–82.

Ibarz A, Pagan J, Panades R & Garza S (2005). Photochemical destruction of colour compound in fruit juices. Journal of Food Engineering 69: 155–160.

Institute of Food Science and Technology, IFST (1999). Development and use of microbiological criteria for foods. Institute of Food Science and Technology: London.

International Tropical Fruits Network (2008). Mango. Available online: <http://www.itfnet.org/index_archive.jsp>

Jabbar S, Abid M, Hu B, Wu T, Hashim MM, Lei S, Zhu X & Zeng X (2014). Quality of carrot juice as influenced by blanching and sonication treatments. LWT - Food Science and Technology 55(1): 16-21.

Jay S & Anderson J (2001). Fruit juice and related products. In: Spoilage of processed foods: Causes and diagnosis. AIFST Inc.: NSW, pp.189.

Jedele S, Hau AM & Oppen MV (2003). An analysis of the world market for mangos and its importance for developing countries. Conference on International Agricultural Research for Development, 8-10 October, Gottingen.

Kalt W (2001). Health functional phytochemicals of fruit. Horticultural Reviews 27: 269-315.

Kentish S & Ashokkumar M (2011). The physical and chemical effects of ultrasound. In: H Feng, GV Barobosa-Canovas, J Weiss (Eds.), Ultrasound technologies for food and bioprocessing. Springer: New York, USA, pp.1-13.

Keyser M, Muller IA, Cilliers FP, Nel W & Gouws PA (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. Innovative Food Science and Emerging Technologies 9: 348–354.

Khoo HE, Prasad KN, Kong KW, Jiang Y & Ismail A (2011). Carotenoids and their isomers: color pigments in fruits and vegetables. Molecules 16: 1710–1738.

Klopotek Y, Otto K & Bohm V (2005). Processing strawberries to different products alters contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. Journal of Agricultural and Food Chemistry 53: 5640–5646.

Koutchma T, Forney LJ & Moraru CL (2009). Ultraviolet light in food technology: Principles and application. CRC Press: New York.

Koutchma T, Keller S, Chirtel S & Parisi B (2004). Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. Innovative Food Science and Emerging Technologies 5: 179–189.

Krinsky NI (1990). Carotenoids in medicine. In: NI Krinsky, MM Mathews-Roth, RF Taylor (Eds.), Carotenoids: Chemistry and biology. Plenum Press: New York, pp.279-291.

Lako J, Trenerry VC, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S & Premier R (2007). Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetable and other readily available foods. Food Chemistry 101: 1727-1741.

Leadley CE & Williams A (2006). Pulsed electric field processing, power ultrasound and other emerging technologies. In: JG Brennan (Ed.), Food processing handbook. Wiley-VCH: Germany, pp.214-236.

Lee HS & Coates GA (2003). Effect of thermal pasteurization on Valencia orange juice color and pigments. LWT- Food Science and Technology 36: 153–156.

Lee HS, Castle WS & Coates GA (2001). High-performance liquid chromatography for the characterization of carotenoids in the new sweet orange (Earlygold) grown in Florida, USA. Journal of Chromatography A 913: 371 –377.

Leistner L (2000). Basic aspects of food preservation by hurdle technology. International Journal of Food Microbiology 55: 181–186.

Leistner L & Gorris LGM (1994). Food preservation by combined process (EUR 15776 EN). Commission of the European Community: Brussels, Belgium.

Leistner L & Gorris LGM (1995). Food preservation by hurdle technology. Trends in Food Science and Technology 6: 41-46.

Liu F, Wang Y, Li R, Bi X & Liao X (2014). Effects of high hydrostatic pressure and high temperature short time on antioxidant activity, antioxidant compounds and colour of mango nectars. Innovative Food Science and Emerging Technologies 21: 35-43.

Liu RH (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. The American Journal of Clinical Nutrition 78(3): 517–520.

Loelillet D (1994). The European mango market: A promising tropical fruit. Fruit 49: 332–334.

Lopez-Gomez A, Fernandez PS, Palop A, Periago PM, Martinez-Lopez A, Marin-Iniesta F & Barbosa-Canovas GV (2009). Food safety engineering: An emergent perspective. Food Engineering Reviews 1:84–104.

Mabry TJ, Markham KR & Thomas MB (1970). The systematic identification of flavonoids. Springer-Verlag: New York.

Madsen HL & Bertelsen G (1995). Spices as antioxidants. Trends in Food Science & Technology 6: 271-277.

Manach C, Scalbert A, Morand C, Remesy C & Jimenez L (2004). Polyphenols: Food sources and bioavailability. The American Journal of Clinical Nutrition 79: 727–747.

Martinez R, Torres P, Meneses MA, Figueroa JG, Perez-Alvarez JA & Viuda-Martos M (2012). Chemical, technological and *in vitro* antioxidant properties of mango, guava, pineapple and passion fruit dietary fibre concentrate. Food Chemistry 135: 1520–1526.

Masibo M & He Q (2008). Major mango polyphenols and their potential significance to human health. Comprehensive Review of Food Science and Food Safety 7: 309–319.

Mason TJ (1991). Practical sonochemistry: User's guide to application in chemistry and chemical engineering. Ellis Horwood: UK.

Masuzawa N, Ohdaira E & Ide M (2000). Effects of ultrasonic irradiation on phenolic compounds in wine, Japanese Journal of Applied Physics 39: 2978-2979.

McNamee C, Noci F, Cronin DA, Lyng JG, Morgan DJ & Scannell AGM (2010). PEF based hurdle strategy to control *Pichia fermentans*, *Listeria innocua* and *Escherichia coli* k12 in orange juice. International Journal of Food Microbiology 138(1-2): 13-18.

Mendoza Perez JA & Fregoso Aguilar TA (2013). Chemistry of natural antioxidants and studies performed with different plants collected in mexico. In: JA Morales-Gonzalez (Ed.), Oxidative stress and chronic degenerative diseases: A role for antioxidants. InTech: Croatia, pp.59-85.

Middleton E Jr, Kandaswami C & Theoharides TC (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews 52: 673-751.

Miller R, Jeffrey W, Mitchell D & Elasri M (1999). Bacteria responses to ultraviolet light. American Society for Microbiology 65: 535-541.

Mitra SK & Baldwin EA (1997). Mango. In: SK Mitra (Ed.), Postharvest physiology storage of tropical and subtropical fruit. CAB International: New York, pp.85-122.

Morris C, Brody AL & Wicker L (2007). Non-thermal food processing/preservation Technologies: A review with packaging implications. Packaging Technology and Science 20: 275–286.

Mukherjee SK & Litz RE (2009). Introduction: Botany and importance. In: RE Litz (Ed.), The mango: Botany, producion and uses, 2nd edition. CAB International: UK, pp.2.

Naczk M & Shahidi F (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. Journal of Pharmaceutical and Biomedical Analysis 41: 1523-1542.

Nagy S, Chen CS & Shaw PE (1993). Fruit juice processing technology. Agscience: Florida.

Nakasone HY & Paull RE (1998). Tropical fruits. CAB International: Oxon, UK, pp.208-238.

Neves MF, Trombin VG, Milan P, Lopes FF & Wageningen RK (2011). The orange juice business: A Brazilian perspective. Academic Publishers: The Netherlands.

Noci F, Riener J, Walkling-Ribeiro M, Cronin DA, Morgan DJ & Lyng JG (2008). Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple juice. Journal of Food Engineering 85: 141–146.

Nordberg J & Arner ES (2001). Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. Free Radical Biology and Medicine 31: 1287-1312.

O'Donnell CP, Tiwari BK, Bourke P & Cullen PJ (2010). Effect of ultrasonic processing on food enzymes of industrial importance. Trends in Food Science and Technology 21: 358-367.

Odriozola-Serrano I, Soliva-Fortuny R & Martín-Belloso O (2008). Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. Innovative Food Science and Emerging Technologies 9: 272-279.

Odumeru JA (2012). Microbial safety of food and food products. In: BK Simpson, LML Nollet, F Toldra, S Benjakul, G Paliyath and YH Hui (Eds.), Food biochemistry and food processing, 2nd edition. John Wiley & Sons: UK. pp.787-797.

Oey I, Lille M, Loey AV & Hendrickx M (2008). Effect of high pressure processing on colour, texture and flavour of fruit and vegetable-based food products: A review. Trends in Food Science and Technology 19: 320−328.

Ohlsson T & Bengtsson N (2002). Minimal processing of foods with non-thermal methods. In: T Ohlsson and N Bengtsson (Eds.), Minimal processing technologies in the food industry. Woodhead Publishing Limited and CRC Press LLC: USA. pp.34-57.

Oms-Oliu G, Odriozola-Serrano I & Martín-Belloso O (2012a). The effects of non-thermal technologies on phytochemicals. In: V Rao (Ed.), Phytochemicals: A global perspective of their role in nutrition and health. InTech: Croatia, pp.108-126.

Oms-Oliu G, Odriozola-Serrano I, Soliva-Fortuny R, Elez-Martinez P & Martín-Belloso O (2012b). Stability of health-related compounds in plant foods through the application of non-thermal processes. Trends in Food Science and Technology 23(2): 111-123.

Osaili TM (2012). Developments in the thermal processing of food. In: R Bhat, AK Alias, G Paliyath (Eds.), Progress in food preservation, 1st edition. John Wiley & Sons: UK. pp.211-230.

Oyaizu M (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition 103: 413-419.

Pala CU & Toklucu AK (2011). Effect of UV-C light on anthocyanin content and other quality parameters of pomegranate juice. Journal of Food Composition and Analysis 24: 790-795.

Pala CU & Toklucu AK (2013). Microbial, physicochemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage. LWT - Food Science and Technology 50: 426-431.

Pandey SN (1986). Mango cultivars: Nomenclature and registration. International Symposium on Taxonomy of Cultivated Plants, ISHS Acta Hort 182: 1.

Patil S, Bourke P, Frias JM, Tiwari BK & Cullen PJ (2009). Inactivation of *Escherichia coli* in orange juice using ozone. Innovative Food Science and Emerging Technologies 10(4): 551–557.

Pereira RN & Vicente AA (2010). Environmental impact of novel thermal and nonthermal technologies in food processing. Food Research International 43: 1936-1943.

Polydera AC, Stoforos NG & Taoukis PS (2005). Effect of high hydrostatic pressure treatment on post processing antioxidant activity of fresh Navel orange juice. Food Chemistry 91: 495–503.

Poovarodom S, Haruenkit R, Vearasilp S, Namiesnik J, Cvikrova M, Martincova O, Ezra A, Suhaj M, Ruamsuke P & Gorinstein S (2010). Comparative characterisation of durian, mango and avocado. International Journal of Food Science and Technology 45: 921-929.

Prieto P, Pineda M & Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry 269: 337–341.

Prior RL, Wu X & Schaich K (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry 53: 4290-4302.

Rama Prabha M & Vasantha K (2011). Antioxidant, cytotoxicity and polyphenolic content of Calotropis procera (Ait.) R. Br. Flowers. Journal of Applied Pharmaceutical Science 1: 136-140.

Ranganna S (1977). Chapter 5: Ascorbic acid. In: S Ranganna (Ed.), Manual of analysis of fruit and vegetable products. Tata McGrawHill Publishing Company: New Dehli, pp.94-96.

Rattanathanalerk M, Chiewchan N & Srichumpoung W (2005). Effect of thermal processing on the quality loss of pineapple juice. Journal of Food Engineering 66: 259-265.

Rawson A, Patras A, Tiwari BK, Noci F, Koutchma T & Brunton N (2011). Effect of thermal and non-thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. Food Research International 44: 1875-1887.

Raybaudi-Massilia RM, Mosqueda-Melgar J, Soliva-Fortuny R & Martin-Belloso O (2009). Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. Comprehensive Reviews In Food Science And Food Safety 8: 157-180.

Ribeiro C, Canada J & Alvarenga B (2012). Prospects of UV radiation for application in postharvest technology. Emirates Journal of Food and Agriculture 24(6): 586-597.

Rivas A, Rodrigo D, Martinez A, Barbosa-Canovas GV & Rodrigo M (2006). Effect of PEF and heat pasteurization on the physical-chemical characteristics of blended orange and carrot juice. LWT- Food Science and Technology 39: 1163-1170.

Rivera AP & Cabornida IMB (2008). Development of ready-to-drink green mango juice. University of Southern Mindanao R&D Journal 16: 71-77.

Rivera DG, Balmaseda IH, Leon AA, Hernandez BC, Montiel LM, Garrido GG, Cuzzocrea S & Hernandez RD (2006). Anti-allergic properties of *Mangifera indica* L. extract (Vimang) and contribution of its glucosylxanthone mangiferin. Journal of Pharmacology and Pharmacotherapeutics 58: 385-392.

Robbins RJ (2003). Phenolic acids in foods: An overview of analytical methodology. Journal of Agricultural and Food Chemistry 51(10): 2866-2887.

Rodríguez-Amaya DB (1997). Carotenoids and food preparation: The retention of provitamin a carotenoids in prepared, processed and stored foods. OMNI: Washington DC.

Rodriguez-Amaya DB (2001). A guide to carotenoid analysis. ILSI press: Washington DC, USA.

Romero AC, Hernandez EGO, Ceron TF & Chavez AA (2013). The exogenous antioxidants. In: JA Morales-Gonzalez (Ed.), Oxidative stress and chronic degenerative diseases: A role for antioxidants. InTech: Croatia, pp. 33-55.

Sadler GD & Murphy PA (2010). pH and titratable acidity. In: SS Nielsen (Ed.), Food Analysis. Springer: New York, pp.231-233.

Sakanaka S, Tachibana Y & Okada Y (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chemistry 89: 569-575.

Salleh-Mack SZ & Roberts JS (2007). Ultrasound pasteurization: The effects of temperature, soluble solids, organic acids and pH on the inactivation of Escherichia coli ATCC 25922. Ultrasonics Sonochemistry 14: 323−329.

Schieber A, Ullrich W & Carle R (2000). Characterization of polyphenols in mango puree concentrate by HPLC with diode array and mass spectrometric detection. Innovative Food Science & Emerging Technologies 1: 161–166.

Schilling S, Toepfl S, Ludwig M, Dietrich H, Knorr D, Neidhart S, Schieber A & Carle R (2008). Comparative study of juice production by pulsed electric field treatment and enzymatic maceration of apple mash. European Food Research and Technology 226: 1389-1398.

Scott KJ. (2001). Detection and measurement of carotenoids by UV/VIS spectrophotometry. In: RE Wrolstad, TE Acree, EA Decker, MH Penner, DA Reid, SJ Schwartz, CF Shoemaker, D Smith and P Sporns (Eds.), Current protocols in food analytical chemistry. John Wiley and Sons Inc: New York, pp.F2.2.1-F2.2.10.

Sentandreu E, Carbonell L, Carbonell JV & Izquierdo L (2005). Effects of heat treatment conditions on fresh taste and on pectinmethylesterase activity of chilled Mandarin and orange juices. Food Science and Technology International 11: 217-222.

Sentandreu E, Navarro JL & Sendra JM (2007). Effect of technological processes and storage on flavonoids content and total, cumulative fast-kinetics and cumulative slow-kinetics antiradical activities of citrus juices. European Food Research and Technology 225(5–6): 905–912.

Shahidi F & Naczk M (2004). Phenolics in food and nutraceuticals: Sources, applications and health effects. CRC Press: Boca Raton.

Shamsudin R, Ling CS, Adzahan NM & Wan Daud WR (2013). Rheological properties of ultraviolet-irradiated and thermally pasteurized Yankee pineapple juice. Journal of Food Engineering 116: 548–553.

Shon MY, Kim TH & Sung NJ (2003). Antioxidants and free radical scavenging activity of *Phellinus baumii* (Phellinus of Hymenochaetaceae) extracts. Food Chemistry 82: 593-597.

Singh LB (1960). Pollination. In: LB Singh (Ed.), The mango: Botany, cultivation, and utilization. Interscience Publishers: New York, pp.42-43.

Singh Z, Singh RK, Sane VA & Nath P (2013). Mango-postharvest biology and biotechnology. Critical Reviews in Plant Sciences 32(4): 217–236.

Singleton VL, Joseph A & Rossi JRJA (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144-158.

Soria AC & Villamiel M (2010). Effect of ultrasound on the technological properties and bioactivity of food: A review. Trends in Food Science and Technology 21: 323-331.

Sorour H, Tanaka F & Uchino T (2014). Impact of non-thermal processing on the microbial and bioactive content of foods. Global Journal of Biology, Agriculture and Health Sciences 3(1): 153-167.

Spreer W, Ongprasert S, Hegele M, Wunsche JN & Muller J (2009). Yield and fruit development in mango (*Mangifera indica* L. cv. Chok Anan) under different irrigation regimes. Agricultural Water Management 96: 574-584.

Steenstrup DL & Floros JD (2004). Inactivation of *E. coli* O157:H7 in apple cider by ozone at various temperatures and concentrations. Journal of Food Processing Preservation 28: 103-116.

Sutkar VS & Gogate PR (2009). Design aspects of sonochemical reactors: techniques for understanding cavitational activity distribution and effect of operating parameters. Chemical Engineering Journal 55: 26–36.

The Philippine Star - Agriculture/Environment (2005). Chokanan Thai honey mango showing promise. Available online: <http://www.dizonexoticfruittrees.com.htm>

Tiwari BK, Muthukumarappan K, O'Donnell CP & Cullen PJ (2008). Color degradation and quality parameters of sonicated orange juice using response surface methodology. LWT- Food Science and Technology 41: 1876–1883.

Tiwari BK, O'Donnell CP & Cullen PJ (2009a). Effect of sonication on retention of anthocyanins in blackberry juice, Journal of Food Engineering 93: 166–171.

Tiwari BK, O'Donnell CP, Muthukumarappan K & Culllen PJ (2009b). Ascorbic acid degradation kinetics of sonicated orange juice during storage and comparison with thermally pasteurised juice. LWT- Food Science and Technology 42: 700-704.

Tiwari BK, O'Donnell CP, Patras A, Brunton N & Culllen PJ (2009c). Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage. European Food Research and Technology 228: 717-724.

Tiwari U & Cummins E (2013). Factors influencing levels of phytochemicals in selected fruit and vegetables during pre- and post-harvest food processing operations. Food Research International 50 (2): 497-506.

Tran MTT & Farid M (2004). Ultraviolet treatment of orange juice. Innovative Food Science and Emerging Technologies 5: 495-502.

U.S. Department of Agriculture, Agricultural Research Service (2014). USDA National Nutrient Database for Standard Reference: Release 26. Available online: <http://www.ars.usda.gov/ba/bhnrc/ndl>

Valero M, Recrosio N, Saura D, Munoz N, Martic N & Lizama V (2007). Effects of ultrasonic treatments in orange juice processing, Journal of Food Engineering 80: 509-516.

Vegara S, Marti N, Mena P, Saura D & Valero M (2013). Effect of pasteurization process and storage on color and shelf-life of pomegranate juices. LWT - Food Science and Technology 54: 592-596.

Vercet A, Burgos J & Lopez-Buesa P (2001) Manothermosonication of foods and food-resembling systems: Effect on nutrient content and nonenzymatic browning. Journal of Agricultural and Food Chemistry 49: 483-489.

Vercet A, Lopez P & Burgos J (1998). Free radical production by manothermosonication. Ultrasonics 36(1–5): 615–618.

Villavicencio ALCH, Mancini-Filho J, Delincee H & Greiner R (2000). Effect of irradiation on anti-nutrients (total phenolics, tannins and phytate) in Brazilian beans. Radiation Physics and Chemistry 57: 289-293

.

Walkling-Ribeiro M, Noci F, Cronin DA, Riener J, Lyng JG & Morgan DJ (2008). Reduction of Staphylococcus aureus and quality changes in apple juice processed by ultraviolet irradiation, pre-heating and pulsed electric fields. Journal of Food Engineering 89: 267–273.

Waterhouse A L (2002). Determination of total phenolics. In: RE Wrolstad, TE Acree, EA Decker, MH Penner, DA Reid, SJ Schwartz, CF Shoemaker, D Smith, P Sporns (Eds.), Current protocols in food analytical chemistry. John Wiley and Sons Inc: New York, pp.I1.1.1-I1.1.8.

Xu G, Liu D, Chen J, Ye X, Maa Y & Shi J (2008). Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chemistry 106: 545–551.

Yang CS, Landau JM, Huang MT & Newmark HL (2001). Inhibition of carcinogenesis by dietary polyphenolic compounds. Annual Review of Nutrition 21: 381-406.

Zafra-Rojas QY, Cruz-Cansino N, Ramírez-Moreno E, Delgado-Olivares L, Villanueva-Sánchez J & Alanís-García E (2013). Effects of ultrasound treatment in purple cactus pear (Opuntia ficus-indica) juice. Ultrasonics Sonochemistry 20: 1283–1288.

Zhang C, Trierweiler B, Li W, Butz P, Xu Y, Rufer CE, Maa Y & Zhao X (2011). Comparison of thermal, ultraviolet-c, and high pressure treatments on quality parameters of watermelon juice. Food Chemistry 126: 254–260.

Ziegler RG (1991). Vegetables, fruits, and carotenoids and the risk of cancer. The American Journal of Clinical Nutrition 52: 251-259.

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Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice

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ABSTRACT

Ultrasonic treatment is an emerging food processing technology that has growing interest among health-conscious consumers. Freshly squeezed Chokanan mango juice was thermally treated (at 90 °C for 30 and 60 s) and sonicated (for 15, 30 and 60 min at 25 °C, 40 kHz frequency, 130 W) to compare the effect on microbial inactivation, physicochemical properties, antioxidant activities and other quality parameters. After sonication and thermal treatment, no significant changes occurred in pH, total soluble solids and titratable acidity. Sonication for 15 and 30 min showed significant improvement in selected quality parameters except color and ascorbic acid content, when compared to freshly squeezed juice (control). A significant increase in extractability of carotenoids (4–9%) and polyphenols (30–35%) was observed for juice subjected to ultrasonic treatment for 15 and 30 min, when compared to the control. In addition, enhancement of radical scavenging activity and reducing power was observed in all sonicated juice samples regardless of treatment time. Thermal and ultrasonic treatment exhibited significant reduction in microbial count of the juice. The results obtained support the use of sonication to improve the quality of Chokanan mango juice along with safety standard as an alternative to thermal treatment.

1. Introduction

Mango (*Mangifera indica* L.) is a tropical fruit that originated from Southeast Asia and has been cultivated for at least 4000 years. Worldwide mango production has increased about 9% from 35.5 million metric tons in 2008 to 38.7 million metric tons in 2010, highlighting the economic importance of mango in the international commodity market [1]. Chokanan mango is one of the most popular cultivar grown in Malaysia for local and export market. According to Spreer et al. [2], there is a large stock of Chokanan mango every year as it has three harvests in May, June and August. This is due to its ability to yield off-season flowering without applying chemicals for initiation. Hence, this characteristic allows the fruit to be processed into products including juice, nectars, puree, pickles, and canned slices that are globally accepted [3]. The increasing demand for this cultivar is due to its vibrant colors, exotic flavors, distinctive taste and nutritional properties [4]. Therefore, consumption of mango juice has been linked to the prevention of cardiovascular diseases and cancer [5,6].

The popularity of natural fruit juices has urged studies on juice processing. Thermal pasteurization is the preferred technology used to achieve microbial inactivation, and extend the shelf life of juices. However, there have been reports that show significant loss of quality in thermally treated juices such as orange, strawberry, and watermelon [7]. The growing interest for fresh-like products has promoted the effort for developing innovative non-thermal food preservation methods. Sonication is a potential technology to achieve the U.S. Food and Drug Administration (FDA) condition of a 5 log reduction of food borne pathogens in fruit juices [8].

Several studies using ultrasonic treatment on fruit juice reported promising results of significant microbial inactivation, minimal effect on the degradation of quality parameters, and improved functionalities [9]. When high power ultrasound at low frequencies (20–100 kHz) propagates in liquid, cavitation (formation and collapse of bubbles) occurs. As a result, elevation of localized pressure and temperature ('tiny hotspots') provide the energy to alter the properties of food product either physically or chemically. Sonication is simple and reliable, thus providing reduced processing time and improved efficiency [10,11].

The purpose of this study was to compare the effect of ultrasonic and thermal treatment on the quality parameters of

Abbreviations: TSS, total soluble solids; TA, titratable acidity; NEBI, non-enzymatic browning index; DCPIP, 2,6-dichlorophenol-indophenol; CFU, colony-forming units; GAE, gallic acid equivalent; AAE, ascorbic acid equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl.

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Chokanan mango juice such as physicochemical properties (pH, total soluble solids and titratable acidity), color, clarity, browning index, carotenoid content, ascorbic acid content, antioxidant activities and microbial inactivation. It is expected that the information gained from this study will increase the awareness of using ultrasonic treatment for preserving the quality of Chokanan mango juice.

2. Materials and methods

2.1. Chemicals

Gallic acid, L-ascorbic acid, DCPIP sodium salt, potassium ferricyanide, ferric chloride, Folin–Ciocalteu reagent, sodium hydroxide, trichloroacetic acid and DPPH were purchased from Sigma (MO, USA). Metaphosphoric acid and peptone water were purchased from R & M Chemicals (Essex, UK). Sodium bicarbonate and sodium carbonate were purchased from BDH (Poole, UK). All chemical solvents used were analytical reagent grade and purchased from Sigma (MO, USA).

2.2. Plant material

Mature-green Chokanan mango fruits of uniform size and free from external defects were harvested 13 weeks after anthesis from a mango farm in Tobiar (Kedah, Malaysia) which is located about 428 km from the Postharvest Biotechnology Laboratory, University of Malaya. The fruits were rinsed with running water, air dried and left to ripen at room temperature (25 ± 1 °C).

2.3. Extraction of mango juice

Ripe mangoes were selected based on visual assessment of the peel color according to the maturity indices standard specified by the Federal Agricultural Marketing Authority (FAMA) Malaysia [12]. Each mango was peeled and the seed was discarded. Mango pulp were macerated using a domestic juice extractor (Philips Juice Extractor HR 2820, Holland), and then centrifuged (Beckman J2-MI Centrifuge, California) at 12,000 rpm for 10 min at 4 °C. The supernatant was filtered using a steel sieve with an approximate diameter of 2 mm to obtain the juice and remove any remaining fibre. The filtered juice samples were stored in sterile glass bottles prior to deployment into experiment.

2.4. Thermal and ultrasonic treatments

Freshly squeezed mango juice stored in glass bottles were pasteurized in a covered water bath (Memmert, Germany) at 90 ± 1 °C for 30 s (mild heat pasteurization) and 90 ± 1 °C for 60 s (high heat pasteurization). These conditions were selected because thermal treatment for fruit juices range from 90 to 95 °C for 15 to 60 s to assure at least 5 log reduction in microbial count [13]. After the thermal treatment, the juice samples were immediately cooled by immersing in an ice-water bath.

The sonication of freshly squeezed mango juice was performed at 40 kHz frequency, using an ultrasonic cleaning bath (Branson Model 3510 Ultrasonic Cleaner, CT, USA). The ultrasonic cleaning bath is a rectangular container (290 × 150 × 150 mm) with the maximal tank capacity of 5.5 L. The 40 kHz transducers at the bottom transmit ultrasound waves of 130 W power from bottom to above. Juice samples in 250 ml beaker were placed exactly at the centre of the ultrasonic cleaning bath with the circulating water (flow rate 0.5 L/min) temperature being monitored at 25 ± 1 °C. The juice level in the beaker was 3.5 cm below the water surface in the ultrasonic bath. The height of the bottom surface of the beaker from the bottom surface of the tank (face of transducers) is 4.5 cm. The processing time was for 15, 30, and 60 min under dark condition. The actual power dissipated in the ultrasonic bath was 68–75 W, which was determined by calorimetric method [14,15].

The juice samples were bottled directly after the treatment. Once filled, the glass bottles were tightly capped and stored at −15 °C until analysis. The following terms were used to describe the different treatments in this study: control (freshly squeezed or no treatment); MP (mild heat pasteurization); HP (high heat pasteurization); S15 (sonication for 15 min); S30 (sonication for 30 min); S60 (sonication for 60 min). All treatments and analysis were carried out in triplicates.

2.5. Microbial inactivation analysis

The microbial count of juice samples were determined using Petrifilm plates (3 M Center, MN, USA) for aerobic bacteria, coliform, yeast and mould. Serial dilution bottles were filled with 0.1% peptone water and then autoclaved. Mango juice was appropriately diluted (10^{-1}–10^{-3}) and the pH was adjusted for optimum growth. Then, 1 ml diluted sample was placed on the Petrifilm plates. The coliform and aerobic Petrifilms were incubated at 35 ± 1 °C for 24 and 48 h, respectively. The yeast and mould Petrifilms were incubated at 25 ± 1 °C for 3–5 days. The aerobic plate count, coliform count, and total yeast and mould count in samples were calculated as colony-forming units (CFU) per millilitre of juice according to the equation:

$$\text{CFU per ml} = (\text{number of colonies} \times \text{dilution factor of plate})/\text{aliquot plated}$$

The results will be expressed as log (CFU/ml).

2.6. Physicochemical analysis (pH, total soluble solids and titratable acidity)

The pH of mango juice was determined using a pH meter (Hanna Microprocessor pH 211, Italy) at 25 ± 1 °C. Total soluble solids (TSS) were determined using a digital refractometer (Atago PR-1 digital refractometer, Tokyo, Japan) at 25 ± 1 °C and results were expressed in standard °Brix unit.

For determination of titratable acidity, diluted mango juice was titrated with standardized 0.1 N sodium hydroxide to a definite faint pink end point (color should persist for ⩾15 s) using phenolphthalein as an indicator. The volume of sodium hydroxide used for titration was converted to grams of citric acid per 100 ml of juice and the titratable acidity (%TA) was calculated according to the method of Sadler and Murphy [16].

2.7. Color, clarity and non-enzymatic browning index (NEBI)

The color of juice samples were measured using a colorimeter (Minolta Chroma Meter CR-200, Minolta, Osaka, Japan). The color parameters L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were evaluated. Color differences (ΔE), in comparison to control [17], were calculated using the following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The transmittance at 660 nm was measured using a spectrophotometer (UV-200-RS Spectrophotometer, MRC, Israel) to determine clarity [18,19] against a blank (distilled water). High percentage of transmittance at 660 nm corresponds to high clarity. The NEBI assay was carried out according to the method by Cohen et al. [20].

2.8. Total carotenoid content

The carotenoid extraction was performed according to Lee et al. [21], while the total carotenoid content [22] using β-carotene as a reference was calculated using the following formula:

$$\text{Total carotenoid content} = (A \times V_1 \times C^{1\%})/A^{1\%}$$

where A is absorbance reading of the diluted sample, V_1 is dilution factor, $A^{1\%}$ is absorbance of a 1% solution (the extinction coefficient for β-carotene; 2592 AU), and $C^{1\%}$ is concentration of a 1% solution (10 mg/ml).

2.9. Ascorbic acid content

The ascorbic acid content in samples was determined based on the 2,6-dichlorophenol-indophenol (DCPIP) visual titration method [23]. Mango juice was diluted with 3% metaphosphoric acid and filtered. Then, the filtrate was titrated with standardized dye solution (2,6-dichloroindophenol-indophenol and sodium bicarbonate) to a pink end point (color should persist for $\geqslant$15 s). The results obtained were expressed as milligrams of ascorbic acid per 100 ml sample.

2.10. Antioxidant activity

2.10.1. Preparation of extract

The extraction method was performed according to Xu et al. [24] with slight modifications. Equal parts of mango juice were added to 80% methanol to purify the sample. The mixture was placed in a shaking incubator (Shellab Orbital Shaking Incubator S14, OR, USA) at 250 rpm for 30 min at room temperature, and then centrifuged. The supernatant was used for the analysis of antioxidant activity.

2.10.2. Total polyphenol content

Total polyphenol content of juice samples were determined using Folin–Ciocalteu assay [25] modified to a microscale [26]. A standard curve of gallic acid ($y = 0.00566x$, $r^2 = 0.9955$) was prepared and results were reported as milligrams of gallic acid equivalent (GAE) per 100 ml juice extract.

2.10.3. DPPH radical scavenging assay

The DPPH assay is based on the method described by Oyaizu [27] and Bae and Suh [26]. A standard curve of ascorbic acid ($y = 10.145x$, $r^2 = 0.9907$) was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract. The radical scavenging activity was calculated accordingly:

$$\%\text{DPPH inhibition} = (A_{control} - A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is absorbance reading of control and A_{sample} is absorbance reading of the sample.

2.10.4. Reducing power assay

A spectrophotometric method Oyaizu [27] was used for measuring the reducing power of juice samples. A standard curve of ascorbic acid ($y = 0.0014x$, $r^2 = 0.9906$) was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract.

2.11. Statistical analysis

The data obtained were subjected to statistical analysis using SPSS 19.0 software (SPSS Inc., IBM). In this study, data was represented as mean values ± standard deviation (SD). The significant differences between mean values of juice samples were determined by analysis of variance (one way-ANOVA) using Tukey's HSD (Honestly Significant Difference) test at a significance level of $p < 0.05$.

3. Results and discussion

3.1. Microbial inactivation analysis (aerobic plate count, coliform count, total yeast and mould count)

The Petrifilm plate methods are recognized as AOAC International Official Methods of Analysis. The Petrifilm aerobic count and coliform count plates were used for the enumeration of aerobic bacteria and coliform colonies, respectively. In both plates an indicator dye colors the colonies red. However, only red colonies associated with gas bubbles indicate confirmed coliforms. For the total yeast and mould count plate, an indicator dye colors yeast colonies blue-green with defined edges, whereas other colonies with diffuse edges are mould colonies [28]. In this study, juice samples after sonication and thermal treatment showed a significant reduction of microbial count (Table 1). The aerobic bacteria, coliform, and yeast and mould counts in freshly squeezed Chokanan mango juice were 2.74 log CFU/ml, 0.99 log CFU/ml, and 2.42 log CFU/ml, respectively. Thermal treatment regardless of mild or high pasteurization inactivated 100% aerobic bacteria, coliform, yeast and mould. The results obtained are in agreement with thermally pasteurized blended orange and carrot juice, where 100% reduction of microbial load was observed [29]. Heat disrupts the integrity of the cellular membrane and damages nucleic acids, thus causing cytolytic effects.

The ultrasonic treatment reduced coliform counts to below detection limits. Besides that, for aerobic plate count, S60 sample recorded the highest reduction of microbial count (26%) when compared to other sonicated samples, S30 (17%) and S15 (8%). This could be explained by the collapse of cavitation-induced micro bubbles resulting in a localized decontamination effect [30]. For yeast and mould count, the maximum percentage of inactivation was for S60 sample (14%), which is lower than aerobic bacteria inactivation (26%). Thus, yeast and mould may display higher resistance to sonication when compared to bacteria due to the difference in thickness of cell wall. In addition, formation of free radicals (OH^- and H^+) and hydrogen peroxide due to sonolysis of water, inactivates microbial cells [11]. Previous studies have reported that sonication is effective in reducing the microbial count of foodborne pathogen, such as in orange juice [31]. However, complete inactivation of microbial growth in juice was not observed in this study for ultrasonic treatment for 15, 30 and 60 min. According Vercet et al. [32] the combination of ultrasound with mild heat can speed up the rate of sterilization of food products.

3.2. Physicochemical analysis (pH, TSS and TA)

In this study, the pH, TSS, and TA of juice (Table 2) showed no significant changes after thermal treatment (mild and high pasteurization) and sonication ($p < 0.05$). Hence, the pH (4.58–4.62), TSS (14.5–14.7 °Brix) and TA (0.19–0.21%) of treated juice samples still lies within the range of standards desirable for freshly squeezed Chokanan mango juice. Observations on non-significant deviations of pH, TSS and TA are in agreement with thermally treated Valencia and Navel orange juice [33]. Correspondingly, orange juice showed no significant changes in pH, TSS, and TA after ultrasonic treatment [34]. In addition, Bhat et al. [35] reported no significant variations in pH and TSS in sonicated kasturi lime juice.

3.3. Color, clarity and NEBI

Significant differences in color were recorded between the control and both treatments (sonication and thermal), as shown

Table 1
Effects of thermal treatment and sonication on microbial inactivation analysis of Chokanan mango juice.

Treatment	Coliform count (log CFU/ml)	Aerobic plate count (log CFU/ml)	Yeast and mould count (log CFU/ml)
Control	1.00 ± 0.02[a]	2.74 ± 0.01[a]	2.42 ± 0.02[a]
MP	ND[b]	ND[b]	ND[b]
HP	ND[b]	ND[b]	ND[b]
S15	ND[b]	2.53 ± 0.02[c]	2.30 ± 0.04[c]
S30	ND[b]	2.28 ± 0.03[d]	2.19 ± 0.01[d]
S60	ND[b]	2.02 ± 0.03[e]	2.09 ± 0.03[e]

Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 6$).
ND, not detected; CFU, colony-forming unit.
Control (freshly squeezed or no treatment); MP (mild heat pasteurization); HP (high heat pasteurization); S15 (sonication for 15 min); S30 (sonication for 30 min); S60 (sonication for 60 min).

Table 2
Effects of thermal treatment and sonication on physicochemical analysis (pH, total soluble solids, titratable acidity) of Chokanan mango juice.

Treatment	pH	Total soluble solids (°Brix)	Titratable acidity (%)
Control	4.62 ± 0.02[a]	14.7 ± 0.06[a]	0.20 ± 0.02[a]
MP	4.60 ± 0.01[a]	14.6 ± 0.06[a]	0.21 ± 0.01[a]
HP	4.58 ± 0.02[a]	14.6 ± 0.05[a]	0.19 ± 0.01[a]
S15	4.59 ± 0.01[a]	14.6 ± 0.08[a]	0.21 ± 0.01[a]
S30	4.61 ± 0.01[a]	14.6 ± 0.05[a]	0.20 ± 0.02[a]
S60	4.59 ± 0.01[a]	14.5 ± 0.05[a]	0.21 ± 0.01[a]

Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
Control (freshly squeezed or no treatment); MP (mild heat pasteurization); HP (high heat pasteurization); S15 (sonication for 15 min); S30 (sonication for 30 min); S60 (sonication for 60 min).

in Table 3. According to the classification of Cserhalmi et al. [36], S15 and S30 samples showed the lowest variation from the control, therefore falling within the 'slightly noticeable' color change range ($0.5 < \Delta E < 1.5$). In contrast, thermal treatments induce 'well visible' ($3.0 < \Delta E < 6.0$) color changes in juice samples. This is consistent with the study conducted by Lee and Coates [37] on heat treated Valencia orange juice, where significant variations in color were observed resulting in a lighter color (increase in L^* value) and more saturated juice. After sonication, an increase in lightness (L^*), and decrease in redness ($+a^*$) and yellowness ($+b^*$) were observed. Likewise, color degradation in sonicated orange juice was reported by Tiwari et al. [34]. This could be explained by accumulation of unstable particles in the juice that are partially precipitated [38]. In addition, the color of mango juice is mostly contributed by natural pigments such as carotenoids, thus accelerated carotenoid isomerization caused by high temperature [39] and sonication [30] may result in the loss of yellow color (decreased $+b^*$ value). Besides that, the increase in L^* value makes the juice more transparent due to the destruction of colored compounds formed previously. While the decrease in ($+a^*$) and ($+b^*$) might be attributed to the enhancement of browning degree [40].

Table 3
Effects of thermal treatment and sonication on color analysis of Chokanan mango juice.

Treatment	Color parameters			
	L^*	a^*	b^*	ΔE
Control	70.28 ± 0.05[a]	5.00 ± 0.03[a]	54.45 ± 0.04[a]	–
MP	72.40 ± 0.06[b]	3.38 ± 0.15[b]	51.44 ± 0.02[b]	4.02 ± 0.07[a]
HP	72.26 ± 0.11[b]	3.27 ± 0.11[b]	51.23 ± 0.15[b]	4.16 ± 0.08[a]
S15	70.75 ± 0.17[c]	4.69 ± 0.13[c]	53.30 ± 0.05[c]	1.28 ± 0.07[b]
S30	71.20 ± 0.08[c]	4.83 ± 0.05[c]	53.22 ± 0.06[c]	1.50 ± 0.15[b]
S60	70.82 ± 0.05[c]	4.63 ± 0.08[c]	52.78 ± 0.03[d]	1.79 ± 0.02[c]

Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
Control (freshly squeezed or no treatment); MP (mild heat pasteurization); HP (high heat pasteurization); S15 (sonication for 15 min); S30 (sonication for 30 min); S60 (sonication for 60 min).

The clarity indicates the degree of turbidity or darkening of fruit juices. The clarity of juice (Table 4) showed significant decrease after thermal treatment. HP samples recorded the least clarity which is (7.3), when compared to the control (25.5), thus indicating the highest percentage of turbidity. Rivas et al. [29] reported similar results on heat pasteurized orange and carrot blend juice. However, there was a significant improvement in clarity of juice samples subjected to ultrasonic treatment except S60, when compared to the control. The highest increase (7%) in clarity was recorded by S30 sample. This could be explained by the mechanical stress derived from cavitational collapse of bubbles, which causes the breakdown of large macromolecules and particles in the juice [9]. In addition, the application of low-power ultrasound, such as ultrasonic cleaning bath will decrease the viscosity and pectin content of juice, thus improving the clarity of juice. However, high power ultrasound may bring undesirable changes in rheology of juice [41]. Thus, ultrasound processing variables have to be evaluated appropriately to avoid negative impacts on fruit juice quality.

The NEBI is the browning of juice as a result of Maillard reactions causing color changes, and nutrient losses [17]. In this study, significant increase was found in the NEBI (Table 4) of juice processed by sonication and thermal. It was clearly exhibited that juice sample subjected to high pasteurization accelerated formation of brown pigments, consequently darkening the juice when compared to mild pasteurization. Hence, these results are consistent with the studies conducted by Aguilo-Aguayo et al. [42] on high pasteurized strawberry juice causing significant browning. Bull et al. [33] also reported increase in browning index of thermal treated Valencia and Navel orange juice. However, the sonicated samples S15 and S30 showed minimum increase (0.08) from the control (0.06), when compared to thermal treated juice samples. Correspondingly, Valero et al. [31] observed slight increase in NEBI of sonicated orange juice. This could be due to the cavitation phenomenon as a result of sonication, which triggers Maillard reaction causing enhancement of browning degree [43]. The results of NEBI agrees with color analysis, whereby a decrease in ($+b^*$) and increase in L^* values were observed, indicating the shift of color from yellow towards light brown.

3.4. Total carotenoid content

Thermal treated juice exhibited significant decrease in carotenoids ($p < 0.05$) as shown in Table 4. MP sample showed 56.13 μg/100 ml and HP sample showed 48.92 μg/100 ml of carotenoid content, respectively, in comparison with control (82.03 μg/100 ml). In a previous study conducted by Lee and Coates [37], it was observed that thermal treatment induces geometric isomerization of carotenoids, resulting in significant loss

Table 4
Effects of thermal treatment and sonication on clarity, non-enzymatic browning index, total carotenoid and ascorbic acid content of Chokanan mango juice.

Treatment	Clarity (transmittance at 660 nm)	NEBI (absorbance at 420 nm)	Total carotenoid content (μg/100 ml)	Ascorbic acid content (mg/100 ml)
Control	25.50 ± 0.22[a]	0.06 ± 0.00[a]	82.03 ± 1.29[a]	8.91 ± 0.51[a]
MP	7.60 ± 0.53[b]	0.10 ± 0.00[b]	56.13 ± 2.23[b]	3.62 ± 0.50[b]
HP	7.30 ± 0.14[b]	0.13 ± 0.01[c]	48.92 ± 1.32[c]	3.10 ± 0.62[b]
S15	27.10 ± 0.10[c]	0.08 ± 0.01[d]	89.53 ± 1.82[d]	7.74 ± 0.51[c]
S30	27.30 ± 0.15[c]	0.08 ± 0.00[d]	85.06 ± 1.75[d]	7.52 ± 0.20[c]
S60	24.10 ± 0.12[d]	0.09 ± 0.00[d]	78.16 ± 2.10[e]	6.43 ± 0.30[d]

Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
NEBI, non-enzymatic browning index.
Control (freshly squeezed or no treatment); MP (mild heat pasteurization); HP (high heat pasteurization); S15 (sonication for 15 min); S30 (sonication for 30 min); S60 (sonication for 60 min).

of carotenoids in orange juice. In addition, heat causes instability of the conjugated double bond system of carotenoids, resulting in oxidation [44]. Similar results were reported by Cortes et al. [45] on degradation of carotenoids in heat treated orange juice. However, juice samples subjected to sonication (S15 and S30) showed significant increase in carotenoid content. S15 sample showed the highest enhancement in extractability of carotenoids (9%) when compared to the control. Thus, carotenoids are still stable after 15 and 30 min sonication. This may be explained by the inactivation of enzymes responsible for degradation of carotenoids due to cavitation-induced shock waves and sonochemical reaction [9]. The increase in extractable carotenoids might benefit health conscious consumers as these compounds possess health promoting properties such as reduced risk of cardiovascular disease [46] and cancer [47]. However, for S60 samples, degradation of carotenoids was observed. This could be attributed to the carotenoid isomerization due to high shearing effect after 60 min of sonication.

3.5. Ascorbic acid content

Ascorbic acid (vitamin C) is an important antioxidant that has been linked with protection against several types of cancers [48]. In this study, significant decrease in the ascorbic acid content was observed in juice processed by sonication and thermal treatment when compared to control (Table 4). For thermally treated juice, HP sample recorded the highest degradation (65%) of ascorbic acid when compared to the control. The loss of ascorbic acid might be due to its heat-sensitive characteristic. Likewise, Tiwari et al. [43] observed lower ascorbic acid content in thermally treated orange juice than control and sonicated juices. In addition, the least reduction in ascorbic acid after sonication was about 13%, which is from 8.91 mg/100 ml in S15 sample to 6.43 mg/100 ml in control. The degradation of ascorbic acid could be explained by the formation of free radical by sonochemical reaction, associating with oxidative process [49]. Results on the loss of ascorbic acid agree with the observation of sonicated orange juice. This is mainly attributed to the thermolysis of ascorbic acid in bubbles (cavities) as a result of the sonication. Subsequently, Maillard reaction is triggered [43]. Besides that, significant decrease of ascorbic acid content was recorded in sonicated strawberry juice, where the largest reduction was below 15% [50].

3.6. Antioxidant activity (total polyphenol content, DPPH radical scavenging and reducing power assay)

The results on the effects of sonication and thermal treatment on antioxidant activity in juice extracts are shown in Table 5. The Folin–Ciocalteu method is based on the detection of phenolic compounds by reduction of reagent, which contains tungsten and molybdenum oxides [51]. In this study, a significant decrease in total polyphenol content was observed in heat treated juices, being lowest for HP sample (37.8% reduction). In contrast, a significant increase in the total phenolics was observed in all sonicated juice when compared to control. The highest increase was from 97.83 mg GAE/100 ml to 132.2 mg GAE/100 ml in S30 sample. Likewise, Bhat et al. [35] observed an increase in phenolic compounds of sonicated kasturi lime juice. This may be explained by the reaction of aromatic ring of polyphenols with hydroxyl radicals generated sonochemically, therefore improving the antioxidant activity [52]. In addition, the active removal of occluded oxygen from the juice by sonication was suggested to contribute to the enhancement of phenolic compounds [53]. The correlation between phenolic content and antioxidant activity has been previously reported [54].

The DPPH radical scavenging assay measures the hydrogen donating capacity of the antioxidant to the stable free radical DPPH, which forms diphenylpicrylhydrazine [55]. In this study, juice samples subjected to thermal treatment showed no significant changes in percentage of DPPH inhibition. However, sonicated juice samples showed significant increase in the percentage of DPPH inhibition compared to control, except S60. The percentage inhibition of DPPH was 91.15% (8.98 μg AAE/ml) for S15 sample, compared to the control, 84.10% (8.29 μg AAE/ml). Cavitation induced by sonication can increase extraction of antioxidant compounds and is in agreement with Bhat et al. [35], where sonication induces antioxidant capacity of kasturi lime juice.

The reductive ability of a compound (Fe^{3+}–Fe^{2+} transformation) may serve as an important indicator of its potential antioxidant activity. The presence of antioxidants in the sample extract may cause the reduction of the ferricyanide complex to ferrocyanide complex in this assay [56]. With regard to reducing power, thermal treatment showed non-significant decrease when compared to the control. The reducing power was 355.00 and 345.00 μg AAE/ml in MP and HP samples, respectively compared to control (360.71 μg AAE/ml). In contrast, all sonicated juice samples showed increase in reducing capacity. The maximum increase (21%) was from 360.71 to 437.14 μg AAE/ml in S30 sample. According to Oyaizu [27], when the concentration of juice extract increases, an increase in reducing power was also observed. This explanation is applicable in this study as well.

4. Conclusions

In comparison with conventional thermal treatment, sonication of Chokanan mango juice showed higher percentage of retention in most of the quality parameters. The juice samples subjected to ultrasonic treatment for 15 and 30 min exhibit significant improvement in clarity, carotenoid content, phenolic compounds and antioxidants, when compared to freshly squeezed juice. In addition, sonication showed significant reduction in microbial load. Thermal treatment was more effective in completely inactivating microbial growth in the juice, but significant quality loss

Table 5
Effects of thermal treatment and sonication on antioxidant activity of Chokanan mango juice.

Treatment	Total polyphenol content (mg GAE/100 ml)	DPPH radical scavenging activity (μg AAE/ml)	Reducing power (μg AAE/ml)
Control	97.83 ± 1.76[a]	8.29 ± 0.18[a]	360.71 ± 3.20[a]
MP	82.91 ± 1.64[b]	7.98 ± 0.20[a]	355.00 ± 7.46[a]
HP	60.87 ± 1.42[c]	8.09 ± 0.15[a]	345.10 ± 6.10[a]
S15	127.16 ± 2.03[d]	8.98 ± 0.15[b]	411.43 ± 6.78[b]
S30	132.20 ± 1.96[e]	8.73 ± 0.18[b,c]	437.14 ± 4.76[c]
S60	118.85 ± 1.95[f]	8.62 ± 0.12[c]	371.43 ± 7.46[d]

Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
GAE, gallic acid equivalent; AAE, ascorbic acid equivalent.
Control (freshly squeezed or no treatment); MP (mild heat pasteurization); HP (high heat pasteurization); S15 (sonication for 15 min); S30 (sonication for 30 min); S60 (sonication for 60 min).

was observed. Thus, ultrasonic treatment is a good alternative to thermal treatment. Combination of sonication with other thermal or non-thermal food processing technologies to produce improved quality juice may provide more awareness for positive implementation of this novel technology on a pilot scale. Further research work is needed to develop models to optimize the processing variables during sonication treatments.

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References

[1] Food and Agriculture Organisation of the United Nation Statistics Division (FAOSTAT), Food and Agricultural commodities production, 2012. Available from: <http://faostat.fao.org/site> (accessed 25.5.2012).
[2] W. Spreer, S. Ongprasert, M. Hegele, J.N. Wunsche, J. Muller, Yield and fruit development in mango (*Mangifera indica* L. cv. Chok Anan) under different irrigation regimes, Agric. Water Manage. 96 (2009) 574–584.
[3] D. Loelillet, The European mango market: a promising tropical fruit, Fruit 49 (1994) 332–334.
[4] L. Arauz, Mango anthracnose: economic impact and current options for integrated management, Plant Dis. 84 (2000) 600–611.
[5] G. Block, B. Patterson, A. Subar, Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence, Nutr. Cancer 18 (1992) 1–29.
[6] R.H. Liu, Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals, Am. J. Clin. Nutr. 78 (2003) 517–520.
[7] A. Rawson, A. Patras, B.K. Tiwari, F. Noci, T. Koutchma, N. Brunton, Effect of thermal and non-thermal processing technologies on the bioactive content of exotic fruits and their products: review of recent advances, Food Res. Int. 44 (2011) 1875–1887.
[8] S.Z. Salleh-Mack, J.S. Roberts, Ultrasound pasteurization: the effects of temperature, soluble solids, organic acids and pH on the inactivation of *Escherichia coli* ATCC 25922, Ultrason. Sonochem. 14 (2007) 323–329.
[9] A. Demirdöven, T. Baysal, The use of ultrasound and combined technologies in food preservation, Food Rev. Int. 25 (2008) 1–11.
[10] P.R. Gogate, Hydrodynamic cavitation for food and water processing, Food Bioprocess Technol. 4 (2011) 996–1011.
[11] C.P. O'Donnell, B.K. Tiwari, P. Bourke, P.J. Cullen, Effect of ultrasonic processing on food enzymes of industrial importance, Trends Food Sci. Technol. 21 (2010) 358–367.
[12] Department of Agriculture, Mango technology package, 2009. Available from: <http://www.doa.gov.my> (accessed 10.4.2012).
[13] S. Nagy, C.S. Chen, P.E. Shaw, Fruit Juice Processing Technology, Ag Science, Florida, 1993.
[14] V.S. Sutkar, P.R. Gogate, Design aspects of sonochemical reactors: techniques for understanding cavitational activity distribution and effect of operating parameters, Chem. Eng. J. 155 (2009) 26–36.
[15] P.R. Gogate, V.S. Sutkar, A.B. Pandit, Sonochemical reactors: important design and scale up considerations with a special emphasis on heterogeneous systems, Chem. Eng. J. 166 (2011) 1066–1082.
[16] G.D. Sadler, P.A. Murphy, pH and titratable acidity, in: S.S. Nielsen (Ed.), Food Analysis, fourth ed., Springer, New York, 2010, pp. 231–233.
[17] I.M. Caminiti, F. Noci, A. Munoz, P. Whyte, D.J. Morgan, D.A. Cronin, J.G. Lyng, Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend, Food Chem. 124 (2011) 1387–1392.
[18] M. Glevitzky, M. Pop, G.A. Brusturean, I. Bogdan, M. Calisevici, D. Perju, Efficient use of antioxidants to preserve fruit juice, Rev. Chim. (Bucure ti) 59 (2008) 1291–1295.
[19] J.J.P. Krop, W. Pilnik, Effect of pectic acid and bivalent cation on cloud loss of citrus juice, Lebensm. Wiss. Technol. 7 (1974) 62–63.
[20] E. Cohen, Y. Birk, C.H. Mannheim, I.S. Saguy, A rapid method to monitor quality of apple juice during thermal processing, Lebensm. Wiss. Technol. 31 (1998) 612–616.
[21] H.S. Lee, W.S. Castle, G.A. Coates, High-performance liquid chromatography for the characterization of carotenoids in the new sweet orange (Earlygold) grown in Florida, USA, J. Chromatogr. A. 913 (2001) 371–377.
[22] K.J. Scott, Detection and measurement of carotenoids by UV/VIS spectrophotometry, in: R.E. Wrolstad, T.E. Acree, E.A. Decker, M.H. Penner, D.A. Reid, S.J. Schwartz, C.F. Shoemaker, D. Smith, P. Sporns (Eds.), Current Protocols in Food Analytical Chemistry, John Wiley and Sons Inc., New York, 2001, pp. F2.2.1–F2.2.10.
[23] S. Ranganna, Chapter 5: ascorbic acid, in: S. Ranganna (Ed.), Manual of Analysis of Fruit and Vegetable Products, Tata McGrawHill Publishing Company, New Dehli, 1977, pp. 94–96.
[24] G. Xu, D. Liu, J. Chen, X. Ye, Y. Maa, J. Shi, Juice components and antioxidant capacity of citrus varieties cultivated in China, Food Chem. 106 (2008) 545–551.
[25] V.L. Singleton, A. Joseph, J.R.J.A. Rossi, Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Vitic. 16 (1965) 144–158.
[26] S.H. Bae, H.J. Suh, Antioxidant activities of five different mulberry cultivars in Korea, LWT – Food Sci. Technol. 40 (2007) 955–962.
[27] M. Oyaizu, Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutr. 103 (1986) 413–419.
[28] 3M Food Safety, 3M Petrifilm interpretation guide, 2010. Available from: <http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/> (accessed 2.10.2012).
[29] A. Rivas, D. Rodrigo, A. Martinez, G.V. Barbosa-Canovas, M. Rodrigo, Effect of PEF and heat pasteurization on the physical-chemical characteristics of blended orange and carrot juice, LWT – Food Sci. Technol. 39 (2006) 1163–1170.
[30] T.J. Mason, Practical sonochemistry: user's guide to application in chemistry and chemical engineering, Ellis Horwood, UK, 1991.
[31] M. Valero, N. Recrosio, D. Saura, N. Munoz, N. Martıc, V. Lizama, Effects of ultrasonic treatments in orange juice processing, J. Food Eng. 80 (2007) 509–516.
[32] A. Vercet, J. Burgos, P. Lopez-Buesa, Manothermosonication of foods and food-resembling systems: effect on nutrient content and nonenzymatic browning, J. Agric. Food Chem. 49 (2001) 483–489.
[33] M. Bull, M. Zerdin, E. Howe, D. Goicoechea, P. Paramanandhan, R. Stockman, The effect of high pressure processing on the microbial, physical and chemical properties of Valencia and Navel orange juice, Innov. Food Sci. Emerg. Technol. 5 (2004) 135–149.
[34] B.K. Tiwari, K. Muthukumarappan, C.P. O'Donnell, P.J. Cullen, Color degradation and quality parameters of sonicated orange juice using response surface methodology, LWT – Food Sci. Technol. 41 (2008) 1876–1883.
[35] R. Bhat, N.S. Kamaruddin, L. Min-Tze, A.A. Karim, Sonication improves kasturi lime (*Citrus microcarpa*) juice quality, Ultrason. Sonochem. 18 (2011) 1295–1300.
[36] Z. Cserhalmi, A. Sass-Kiss, M. Tóth-Markus, N. Lechner, Study of pulsed electric field treated citrus juices, Innov. Food Sci. Emerg. Technol. 7 (2006) 49–54.
[37] H.S. Lee, G.A. Coates, Effect of thermal pasteurization on Valencia orange juice color and pigments, LWT – Food Sci. Technol. 36 (2003) 153–156.
[38] D.B. Genovese, M.P. Elustondo, J.E. Lozano, Color and cloud stabilization in cloudy apple juice by steam heating during crushing, J. Food Sci. 62 (1997) 1171–1175.
[39] H.E. Chen, H.Y. Peng, B.H. Chen, Changes of carotenoids, color and vitamin A contents during processing of carrot juice, J. Agric. Food Chem. 43 (1995) 1912–1918.
[40] A. Ibarz, J. Pagan, R. Panades, S. Garza, Photochemical destruction of color compound in fruit juices, J. Food Eng. 69 (2005) 155–160.
[41] F. Chemat, Zill-e-Huma, M.K. Khan, Applications of ultrasound in food technology: processing, preservation and extraction, Ultrason. Sonochem. 18 (2011) 813–835.

[42] I. Aguiló-Aguayo, G. Oms-Oliu, R. Soliva-Fortuny, O. Martín-Belloso, Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments, LWT – Food Sci. Technol. 42 (2009) 813–818.
[43] B.K. Tiwari, C.P. O'Donnell, K. Muthukumarappan, P.J. Culllen, Ascorbic acid degradation kinetics of sonicated orange juice during storage and comparison with thermally pasteurized juice, LWT – Food Sci. Technol. 42 (2009) 700–704.
[44] D.B. Rodríguez-Amaya, Carotenoids and Food Preparation: The Retention of Provitamin a Carotenoids in Prepared, Processed and Stored Foods, OMNI, Washington, DC, 1997.
[45] C. Cortés, F. Torregrosa, M.J. Esteve, A. Frígola, Carotenoid profile modification during refrigerated storage in untreated and pasteurized orange juice and orange juice treated with high-intensity pulsed electric fields, J. Agric. Food Chem. 54 (2006) 6247–6254.
[46] N.I. Krinsky, Carotenoids in medicine, in: N.I. Krinsky, M.M. Mathews-Roth, R.F. Taylor (Eds.), Carotenoids: Chemistry and Biology, Plenum Press, New York, 1990, pp. 279–291.
[47] R.G. Ziegler, Vegetables, fruits, and carotenoids and the risk of cancer, Am. J. Clin. Nutr. 52 (1991) 251S–259S.
[48] G. Block, Vitamin C and cancer prevention: the epidemiologic evidence, Am. J. Clin. Nutr. 53 (1991) 270–282.
[49] E.J. Hart, A. Henglein, Free radical and free atom reactions in the sonolysis of aqueous iodide and formate solutions, J. Phys. Chem. 89 (1985) 4342–4347.
[50] B.K. Tiwari, C.P. O'Donnell, A. Patras, N. Brunton, P.J. Culllen, Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage, Eur. Food Res. Technol. 228 (2009) 717–724.
[51] A.L. Waterhouse, Determination of total phenolics, in: R.E. Wrolstad, T.E. Acree, E.A. Decker, M.H. Penner, D.A. Reid, S.J. Schwartz, C.F. Shoemaker, D. Smith, P. Sporns (Eds.), Current Protocols in Food Analytical Chemistry, John Wiley and Sons Inc., New York, 2002, pp. I1.1.1–I1.1.8.
[52] M. Ashokkumar, D. Sunartio, S. Kentish, R. Mawson, L. Simons, K. Vilkhu, C. Versteeg, Modification of food ingredients by ultrasound to improve functionality: a preliminary study on a model system, Innov. Food Sci. Emerg. Technol. 9 (2008) 155–160.
[53] N. Masuzawa, E. Ohdaira, M. Ide, Effects of ultrasonic irradiation on phenolic compounds in wine, Jpn. J. Appl. Phys. 39 (2000) 2978–2979.
[54] S. Gorinstein, Z. Zachwieja, E. Katrich, E. Pawelzikc, R. Haruenkitd, S. Trakhtenberge, O. Martin-Belloso, Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid, Lebensm. Wiss. Technol. 37 (2004) 337–343.
[55] M.Y. Shon, T.H. Kim, N.J. Sung, Antioxidants and free radical scavenging activity of *Phellinus baumii* (Phellinus of Hymenochaetaceae) extracts, Food Chem. 82 (2003) 593–597.
[56] M. Rama Prabha, K. Vasantha, Antioxidant, cytotoxicity and polyphenolic content of *Calotropis procera* (Ait.) R. Br. flowers, J. Appl. Pharm. Sci. 1 (2011) 136–140.

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Comparison of UV-C treatment and thermal pasteurization on quality of Chokanan mango (*Mangifera indica* L.) juice

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A B S T R A C T

Ultraviolet-c treatment is an emerging food processing technology for health-conscious consumers. Freshly squeezed Chokanan mango juice was exposed to UV-C light (for 15, 30 and 60 min at 25 °C) and thermally pasteurized (at 90 °C for 60 s) to compare the effect on microbial inactivation, physicochemical properties, antioxidant activities and other quality parameters. In addition, shelf life study of juice samples stored at 4 ± 1 °C was conducted for 5 weeks. After UV-C treatment and thermal pasteurization, no significant changes occurred in physicochemical properties. A significant increase in extractability of carotenoids (6%), polyphenols (31%), and flavonoids (3%) were observed in juice exposed to UV-C light for 15 and 30 min, when compared to freshly squeezed juice. Besides that, enhancement of antioxidant activity was observed after UV-C treatment. Thermal pasteurization and UV-C treatment exhibited significant reduction in microbial load and prolonged shelf-life of juice. The results obtained support the use of UV-C light to improve the quality of Chokanan mango juice along with safety standards as an alternative to thermal pasteurization.

Keywords: Ultraviolet treatment; Thermal pasteurization; Chokanan mango; Juice; Microbial count; Antioxidant activity

1. Introduction

Mango (*Mangifera indica* L.) is a tropical fruit that has a worldwide production of about 38.9 million metric tonnes in 2011. Between 2009 and 2011, there was a 12% upturn in production of mango globally, highlighting its commercial value in the international commodity market (FAOSTAT, 2013). Chokanan mango is one of the most popular cultivar in Malaysia that yields off-season flowering without chemical initiation. Hence, there is a large stock annually, enabling the fruit to be processed into products including juice, nectar, and puree (Spreer et al., 2009). Studies have reported several polyphenolic compounds in mango, including ellagic acid, gallic acid, quercetin, catechin, epicatechin, chlorogenic acid, mangiferin, and kaempferol (Berardini et al., 2005; Masibo and He, 2008). Some of these bioactive compounds are good antioxidants which have been related to the prevention of cardiovascular diseases and cancer (Block et al., 1992; Liu, 2003) thus making it a substantial dietary source.

Juice processing application are gaining wide acceptance predominantly to meet consumers' demand for safer and fresher food. Conventional thermal pasteurization is the preferred technology to inactivate microorganisms and enzymes causing spoilage, thus prolonging the shelf life of juice. However, some studies on thermally treated fruit juices such as strawberry, orange, and watermelon reported significant loss of nutritional quality and degradation of bioactive compounds such as ascorbic acid (Rawson et al., 2011). The increasing

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demand for fresh-like fruit juice has spurred the development of non-thermal processing methods. Several studies using short-wave ultraviolet-c (UV-C) light treatment on fruit juices reported minimal changes in nutritional and quality attributes, and significant microbial inactivation, such as in starfruit, watermelon, and orange juice (Bhat et al., 2011; Zhang et al., 2011; Pala and Toklucu, 2013).

The U.S. Food and Drug Administration (FDA) criterion of a 5 log reduction of chosen pathogen in fruit juices can be achieved by UV-C irradiation (FDA, 2000). The UV-C light, with a peak emission at the wavelength of 254 nm exhibits germicidal effect on bacteria, yeasts, moulds, and viruses. The absorption of UV-C ray prevents these microorganisms from reproducing, and eventually may cause cell death. Moreover, advantages of UV-C treatment include cost effective, simple, and low maintenance (Guerrero-Beltrán and Barbosa-Cánovas, 2004).

The purpose of this study was to compare the effect of UV-C treatment and thermal pasteurization on the quality parameters of Chokanan mango juice such as microbial inactivation, physicochemical properties (pH, total soluble solids and titratable acidity), colour, clarity, browning index, hydroxymethyl furfural content, carotenoid content, ascorbic acid content, and antioxidant activities. In addition, shelf life study of juice samples stored at 4 ± 1 °C was conducted for 5 weeks. The information obtained from this study could serve to improve the progress of utilizing UV-C treatment in the industry for maintaining the quality of Chokanan mango juice.

2. Materials and methods

2.1. Extraction of mango juice

Ripe Chokanan mango fruits of uniform size and free from external defects were purchased from a local market (Selangor, Malaysia) located about 30 km from the Postharvest Biotechnology Laboratory, University of Malaya. Overall, nine different batches of fruits were used for this study. The fruits were rinsed with running water and air dried at room temperature (25 ± 1 °C). Each mango was peeled and the seed was discarded. Mango pulp were macerated using a domestic juice extractor (Philips Juice Extractor HR 2820, Holland) and then centrifuged (Beckman J2-MI Centrifuge, California) at 12,000 rpm for 10 min at 4 °C. The supernatant was filtered using a steel sieve with an approximate diameter of 2 mm to obtain the juice and remove any remaining fibre. The filtered juice samples were stored in sterile glass bottles prior to deployment into experiment.

2.2. UV-C treatment and thermal pasteurization

Juice samples were exposed to UV-C light under batch conditions. Freshly squeezed mango juice was poured into sterile Petri dishes (11 cm diameter, 0.5 cm fluid depth) and then exposed to a germicidal fluorescent UV-C lamp (30 W, 89.3 cm length, 25.5 cm diameter, Sankyo Denki, Japan) in a laminar flow cabinet (Gelman Science Biological Safety Cabinet Class II, NSW, Australia). The UV-C lamp has a peak emission at 254 nm. The distance between the juice surface and the UV-C lamp was 35 cm. The UV-C lamp was allowed to stabilize for 30 min prior to use. The duration of exposure was 15, 30, and 60 min, at 25 ± 1 °C. The mean of UV radiation dose received by each juice sample is 3.525 J/m^2 (digital radiometer) (Keyser et al., 2008). Juice samples not exposed to UV-C were treated as control.

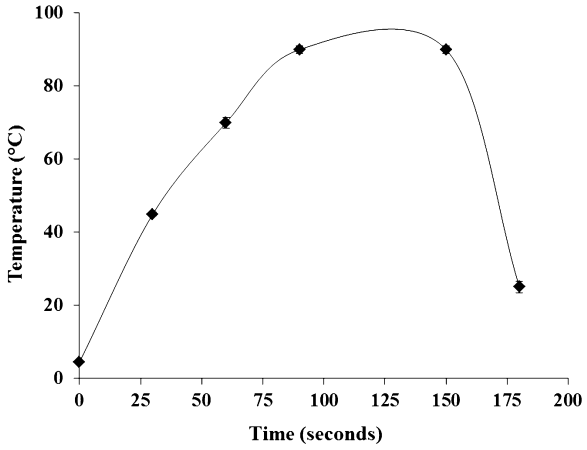

Fig. 1 – Temperature versus time curve of Chokanan mango juice during pasteurization.

Freshly squeezed mango juice in glass boiling tubes (10.5 cm length, 2 cm inner diameter, 0.2 cm wall thickness) were pasteurized in a covered water bath (Memmert, Germany) with continuous shaking at 90 ± 1 °C for 60 s (Nagy et al., 1993). Temperature of the juice at the centre of the tube was regularly monitored using a thermometer. After the juice samples reached the target temperature, the treatment time was measured. The temperature versus time curve of the juice during pasteurization is shown in Fig. 1. After thermal pasteurization, the juice samples were immediately cooled to room temperature (25 ± 1 °C) by immersing in an ice-water bath.

Juice samples were bottled directly after both treatments. Once filled, the glass bottles were tightly capped and stored at −15 °C until further analysis. The following terms were used to describe the different treatments in this study: control (freshly squeezed or no treatment); U15 (UV-C treatment for 15 min); U30 (UV-C treatment for 30 min); U60 (UV-C treatment for 60 min); TP (thermal pasteurization). All treatments and analysis were carried out in triplicates.

2.3. Microbial inactivation analysis

The 3M Petrifilm plate methods are recognized as AOAC International Official Methods of Analysis (3M Food Safety, 2010). Microbial count of juice samples were determined using Petrifilm plates (3M Center, MN, USA) for aerobic bacteria, coliform, yeast and mould according to Santhirasegaram et al. (2013) and were calculated as colony-forming units. The results were expressed as log (CFU/mL).

2.4. Physicochemical analysis (pH, total soluble solids and titratable acidity)

Mango juice pH was determined using a pH metre (Hanna Microprocessor pH 211, Italy) at 25 ± 1 °C. Total soluble solids (TSS) were determined using a digital refractometer (Atago PR-1 digital refractometer, Tokyo, Japan) at 25 ± 1 °C and results were expressed in standard °Brix unit.

For determination of titratable acidity, diluted mango juice was titrated with standardized 0.1 N sodium hydroxide to a definite faint pink end point using phenolphthalein as an indicator. The volume of sodium hydroxide used for titration was converted to grams of citric acid per 100 ml of juice according

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to the method of Sadler and Murphy (2010). The titratable acidity (%TA) was calculated using the following equation:

$$\%TA = \frac{V_1 \times 0.1\,N\,NaOH \times Eq.\ wt. \times 100}{V_2 \times 1000}$$

where V_1 is volume of titrant (ml), Eq. wt. is equivalent weight of anhydrous citric acid (64 mg/mEq), and V_2 is volume of sample (ml).

2.5. Colour, clarity, non-enzymatic browning index (NEBI), and 5-hydroxymethyl furfural (HMF) content

The colour of juice samples was measured using a Chroma Meter (Minolta Chroma Meter CR-200, Minolta, Osaka, Japan). The colour parameters L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were evaluated. Colour differences (ΔE), in comparison to control (Cserhalmi et al., 2006), were calculated using the following equation:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Transmittance at 660 nm was measured using a spectrophotometer (UV-200-RS Spectrophotometer, MRC, Israel) to determine clarity (Glevitzky et al., 2008) against a blank (distilled water). High percentage of transmittance at 660 nm corresponds to high clarity.

NEBI and HMF assay was carried out according to the method by Cohen et al. (1998). A standard curve of HMF ($y = 0.0406x$, $r^2 = 0.9967$) was prepared and results were expressed as milligrams of HMF per litre juice sample.

2.6. Total carotenoid content

Carotenoid was extracted according to Lee et al. (2001), while the total carotenoid content (Scott, 2001) using β-carotene as a reference was calculated using the following formula:

$$\text{Total carotenoid content} = \frac{A \times V_1 \times C^{1\%}}{A^{1\%}}$$

where A is absorbance reading of the diluted sample, V_1 is dilution factor, $A^{1\%}$ is absorbance of a 1% solution (the extinction coefficient for β-carotene; 2592 AU), and $C^{1\%}$ is concentration of a 1% solution (10 mg/ml).

2.7. Ascorbic acid content

The ascorbic acid content in samples was determined based on the 2,6-dichlorophenol-indophenol (DCPIP) visual titration method (Ranganna, 1977). Mango juice was diluted with 3% metaphosphoric acid and filtered. Then, the filtrate was titrated with standardized dye solution (2,6-dichloroindophenol-indophenol and sodium bicarbonate) to a pink end point. The results obtained were expressed as milligrams of ascorbic acid per 100 ml sample, using the following equation:

$$\text{Ascorbic acid content} = \frac{\text{titre} \times \text{dye factor} \times \text{volume made up} \times 100}{\text{aliquot of extract taken for estimation} \times \text{volume of sample taken for estimation}}$$

2.8. Antioxidant activity

2.8.1. Preparation of extract

Antioxidant was extracted according to Xu et al. (2008) with slight modifications. Equal parts of mango juice were added to 80% methanol to purify the sample. The mixture was placed in a shaking incubator (Shellab Orbital Shaking Incubator S14, OR, USA) at 250 rpm for 30 min at room temperature, and then centrifuged. The supernatant was used for the analysis of antioxidant activity.

2.8.2. Total polyphenol content

Total polyphenol content of juice samples were determined using Folin–Ciocalteu assay (Singleton et al., 1965) modified to a microscale (Bae and Suh, 2007). A standard curve of gallic acid ($y = 0.00566x$, $r^2 = 0.9955$) was prepared and results were reported as milligrams of gallic acid equivalent (GAE) per 100 ml juice extract.

2.8.3. Total flavonoid content

Flavonoid content of juice samples was determined using a colorimetric method described by Sakanaka et al. (2005). A standard curve of (+)-catechin ($y = 0.0135x$, $r^2 = 0.9943$) was prepared and results were reported as milligrams of catechin equivalent (CE) per 100 ml juice extract.

2.8.4. DPPH radical scavenging assay

DPPH assay was carried out as described by Oyaizu (1986) and Bae and Suh (2007). A standard curve of ascorbic acid ($y = 10.145x$, $r^2 = 0.9907$) was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract. The radical scavenging activity was calculated accordingly:

$$\%\text{DPPH inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is absorbance reading of control and A_{sample} is absorbance reading of the sample.

2.8.5. Reducing power assay

A spectrophotometric method by Oyaizu (1986) was used for measuring the reducing power of juice samples. A standard curve of ascorbic acid ($y = 0.0014x$, $r^2 = 0.9906$) was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) per ml juice extract.

2.9. Shelf life study

Control, UV-C treated and thermally pasteurized juices were aseptically filled into sterile glass bottles in a laminar flow cabinet (Gelman Science Biological Safety Cabinet Class II, NSW, Australia). The bottles were tightly capped, leaving minimum amount of headspace volume. Then, juice samples were stored at $4 \pm 1\,^{\circ}C$ in a refrigerator for five weeks. Juice samples were analyzed at one week intervals for aerobic bacteria, yeast and mould count, as described in Section 2.3.

2.10. Statistical analysis

Data obtained were subjected to statistical analysis using SPSS 19.0 software (SPSS Inc., IBM). In this study, data were represented as mean values ± standard deviation (SD) ($n = 9$). The significant differences between mean values of juice samples

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Table 1 – Effects of UV-C treatment and thermal pasteurization on microbial inactivation analysis of Chokanan mango juice.[f,g,h]

Treatment	Coliform count (log CFU/ml)	Aerobic plate count (log CFU/ml)	Yeast and mould count (log CFU/ml)
Control	1.00 ± 0.02^{a}	2.74 ± 0.01^{a}	2.42 ± 0.02^{a}
U15	ND[b]	2.25 ± 0.02^{b}	2.18 ± 0.01^{b}
U30	ND[b]	1.92 ± 0.05^{c}	1.84 ± 0.02^{c}
U60	ND[b]	1.52 ± 0.03^{d}	1.65 ± 0.04^{d}
TP	ND[b]	ND[e]	ND[e]

[f] Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[g] ND, not detected; CFU, colony-forming unit.
[h] Control (freshly squeezed or no treatment); U15 (UV-C treatment for 15 min); U30 (UV-C treatment for 30 min); U60 (UV-C treatment for 60 min); TP (thermal pasteurization).

Table 2 – Effects of UV-C treatment and thermal pasteurization on physicochemical analysis (pH, total soluble solids, titratable acidity) of Chokanan mango juice.[b,c]

Treatment	pH	Total soluble solids (°Brix)	Titratable acidity (%)
Control	4.62 ± 0.02^{a}	14.7 ± 0.06^{a}	0.20 ± 0.02^{a}
U15	4.60 ± 0.01^{a}	14.7 ± 0.06^{a}	0.22 ± 0.01^{a}
U30	4.59 ± 0.01^{a}	14.5 ± 0.08^{a}	0.21 ± 0.02^{a}
U60	4.61 ± 0.01^{a}	14.5 ± 0.05^{a}	0.19 ± 0.02^{a}
TP	4.60 ± 0.02^{a}	14.6 ± 0.05^{a}	0.19 ± 0.01^{a}

[b] Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[c] Control (freshly squeezed or no treatment); U15 (UV-C treatment for 15 min); U30 (UV-C treatment for 30 min); U60 (UV-C treatment for 60 min); TP (thermal pasteurization).

were determined by analysis of variance (one way-ANOVA) using Tukey's HSD (Honestly Significant Difference) test at a significance level of $p < 0.05$.

3. Results and discussion

3.1. *Microbial inactivation analysis (coliform count, aerobic plate count, total yeast and mould count)*

Aerobic bacteria, coliform, yeast and mould counts in freshly squeezed Chokanan mango juice were 2.74 log CFU/ml, 1.00 log CFU/ml, and 2.42 log CFU/ml, respectively. After UV-C treatment and thermal pasteurization, juice samples showed significant reduction of microbial count ($p < 0.05$), as shown in Table 1. UV-C treatment reduced coliform counts to below detection limits. For aerobic plate count, U60 sample exhibited the highest reduction of microbial load (45%) when compared to other UV-C treated samples, U30 (30%) and U15 (18%). This could be explained by the characteristic of microbial DNA to absorb UV-C light photons, thus generating cross links between neighbouring cytosine and thymine (pyrimidine) bases in the same DNA strand (Tran and Farid, 2004; Guerrero-Beltrán and Barbosa-Cánovas, 2004). Therefore, these pyrimidine dimers prevent DNA transcription and translation, eventually, inactivating microbial growth. The percentage of inactivation of yeast and mould (10–32%) was lower than aerobic bacteria (18–45%) for UV-C treated samples, exhibiting maximum inactivation for U60 sample (32%). This is a clear indication that yeast and mould are less susceptible to UV-C beam than bacteria. This could be due to the difference in thickness of cell wall and size of microorganism, thus influencing the passage of UV-C light. In addition, lesser pyrimidine bases on the DNA strand of yeast and mould contributes to less probability of cross link formation, thus higher resistance to UV-C (Miller et al., 1999). Similarly, microbial inactivation effect of UV-C was reported in apple and orange juices (Walkling-Ribeiro et al., 2008; Pala and Toklucu, 2013).

However, complete removal of microbial load in juice samples were not observed in this study for UV-C exposure for 15, 30 and 60 min. According to Koutchma et al. (2004), UV-C penetration largely depends on the presence of dissolved organic solutes (suspended solids) and coloured compounds which act as barrier, thus exhibiting UV-C attenuation effects. Combination of UV-C with other non-thermal processing method in a hurdle sequence can improve the rate of sterilization, as it has a synergistic effect (Walkling-Ribeiro et al., 2008). Results showed thermal pasteurization inactivated 100% aerobic bacteria, coliform, yeast and mould in juice sample. High temperature may damage organic molecules (nucleic acids and proteins) required for the proper functioning of microbial cells, thus causing cellular death. Correspondingly, microbial load were reduced to below detection limit in thermally pasteurized blended orange and carrot juice, as reported by Rivas et al. (2006).

3.2. *Physicochemical analysis (pH, TSS and TA)*

The pH, TSS, and TA of juice (Table 2) showed no significant changes after UV-C treatment and thermal pasteurization. Values for pH (4.59–4.62), TSS (14.5–14.7 °Brix) and TA (0.19–0.22%) of treated juice samples still lied within the range of standards desirable for freshly squeezed Chokanan mango juice. Results obtained are in agreement with previous studies that reported no significant alteration in pH, TSS and TA of juice processed by UV-C, such as apple, pomegranate, and orange juices (Walkling-Ribeiro et al., 2008; Pala and Toklucu, 2011, 2013). Similarly, thermally treated Valencia and Navel orange juice (Bull et al., 2004), and pomegranate juice (Pala and Toklucu, 2011) showed no changes in physicochemical properties.

3.3. *Colour, clarity, NEBI, and HMF content*

Significant differences in colour of juice samples were observed after both treatments (UV-C and thermal) when compared to the control (Table 3). According to Cserhalmi et al. (2006), the differences in colour parameters (ΔE) can be classified as not noticeable ($0 < \Delta E < 0.5$), slightly noticeable ($0.5 < \Delta E < 1.5$), noticeable ($1.5 < \Delta E < 3.0$), well visible ($3.0 < \Delta E < 6.0$) and greatly visible ($6.0 < \Delta E < 12$). The U15 sample showed the least variation from the control, therefore falling within the 'slightly noticeable' range. This is consistent with the study conducted by Caminiti et al. (2012) on UV-C treated orange and carrot juice blend, where 'slightly noticeable' colour changes were observed. In addition, an increase in ΔE corresponding to increased UV-C treatment time was observed, as previously reported by Bhat et al. (2011) and Zhang et al. (2011). With regard to thermal pasteurized samples, 'well visible' colour changes were observed. Similarly, significant colour degradation in thermal treated pineapple juice was reported by Rattanathanalerk et al. (2005).

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Table 3 – Effects of UV-C treatment and thermal pasteurization on colour analysis of Chokanan mango juice.[e,f]

Treatment	Colour parameters			
	L^*	a^*	b^*	ΔE
Control	70.28 ± 0.05[a]	5.00 ± 0.03[a]	54.45 ± 0.04[a]	–
U15	70.83 ± 0.12[b]	4.82 ± 0.06[b]	53.60 ± 0.15[b]	1.03 ± 0.15[a]
U30	71.05 ± 0.05[b]	4.79 ± 0.13[b]	52.89 ± 0.10[c]	1.75 ± 0.10[b]
U60	71.42 ± 0.06[b]	4.53 ± 0.08[c]	52.50 ± 0.02[c]	2.30 ± 0.05[c]
TP	72.26 ± 0.11[c]	3.27 ± 0.11[d]	51.23 ± 0.15[d]	4.16 ± 0.08[d]

[e] Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[f] Control (freshly squeezed or no treatment); U15 (UV-C treatment for 15 min); U30 (UV-C treatment for 30 min); U60 (UV-C treatment for 60 min); TP (thermal pasteurization).

After UV-C exposure and thermal pasteurization, an increase in lightness (L^*), and decrease in redness ($+a^*$) and yellowness ($+b^*$) were observed in juice samples. The increase in L^* value makes the juice brighter and more transparent due to impairment of coloured compound initially present in juice. Natural pigments such as carotenoids largely contribute to the yellow colour ($+b^*$ value) of Chokanan mango juice and heat causes accelerated carotenoid isomerization (Rodríguez-Amaya, 1997) which leads to loss of yellowness in juice (decreased $+b^*$ value). Moreover, decrease in ($+a^*$) and ($+b^*$) might be attributed to the development of browning components (Ibarz et al., 2005).

Clarity acts as an indicator of the level of turbidity in fruit juices. No significant changes in clarity of juice samples were exhibited after UV-C treatment (U15 and U30), as shown in Table 4. This could be explained by the properties of UV-C treatment, wherein no significant modification in viscosity and pectin content of juice was induced. According to Shamsudin et al. (2013), UV-C processed Yankee pineapple juice showed no changes in particle size, thus coagulation of colloidal materials were absent, resulting in unchanged viscosity of juice. In contrast, U60 sample exhibited significant decrease (5%) in clarity. The longer treatment time may cause reduced activity of pectin methylesterase (PME) and polygalacturonase (PG), therefore affecting rheology of juice (Aguiló-Aguayo et al., 2009). Thermal pasteurized samples showed the least clarity which is 7.30, when compared to the control (25.50), thus indicating highest percentage of turbidity. Hence, thermal treatment causes 71% reduction in clarity of juice samples. This is consistent with the study conducted by Rivas et al. (2006) on heat pasteurized orange and carrot blend juice, where diminishing clarity was observed resulting in a more saturated juice. This may be explained by the 'swelling' of particles and the diffusion of water between the cellulose chains during heat pasteurization, thus increasing viscosity of the juice. Furthermore, high temperature greatly ruptures cell structure during treatment, and allows pectin to leak out contributing to higher concentration of colloidal pectin in juice. Hence, increased viscosity and pectin content causes decrease in clarity of juice subjected to thermal treatment (Shamsudin et al., 2013; Aguiló-Aguayo et al., 2009).

NEBI is the browning of juice due to Maillard reactions, subsequently causing colour changes and loss of nutrients (Caminiti et al., 2011). There was a significant increase in NEBI of juice (Table 4) processed by UV-C and thermal, except U15. However, UV-C treated samples, U30 (0.09) and U60 (0.10) showed minimum increase from the control (0.06), when compared to thermal pasteurized samples (0.13). Correspondingly, Caminiti et al. (2011) observed non-significant increase of NEBI on UV-C treated apple and cranberry juice blend. There is an increasing trend in browning degree corresponding to UV-C exposure time, as previously reported by Zhang et al. (2011). This could be due to photodegradation reactions that promote Maillard reaction between amino acids and reducing sugars causing enhancement of browning degree (Ibarz et al., 2005). For thermal pasteurized samples, there is enhanced formation of browning compounds, subsequently darkening the juice and reaching the highest NEBI value. Similarly, significant browning was observed in heat treated Valencia and Navel orange juice (Bull et al., 2004). The results of NEBI agrees with colour analysis, whereby the shift of colour from yellow towards light brown can be attributed to the decrease in ($+b^*$) and increase in L^* values.

HMF is one of the chemical substances (intermediate) produced during browning process, thus serving as a good indicator of Maillard reaction (Rattanathanalerk et al., 2005).

Table 4 – Effects of UV-C treatment and thermal pasteurization on clarity, non-enzymatic browning index, HMF, total carotenoid and ascorbic acid content of Chokanan mango juice.[e,f,g]

Treatment	Clarity (transmittance at 660 nm)	NEBI (absorbance at 420 nm)	HMF (mg/l)	Total carotenoid content (μg/100 ml)	Ascorbic acid content (mg/100 ml)
Control	25.50 ± 0.22[a]	0.06 ± 0.00[a]	2.20 ± 0.03[a]	82.03 ± 1.29[a]	8.91 ± 0.51[a]
U15	25.40 ± 0.08[a]	0.07 ± 0.01[a]	2.39 ± 0.08[a]	87.10 ± 1.14[b]	7.85 ± 0.24[b]
U30	25.17 ± 0.15[a]	0.09 ± 0.00[b]	2.46 ± 0.02[a]	84.97 ± 1.35[b]	7.55 ± 0.30[b]
U60	24.25 ± 0.10[b]	0.10 ± 0.01[b]	2.68 ± 0.05[b]	80.16 ± 1.80[a]	6.87 ± 0.12[c]
TP	7.30 ± 0.14[c]	0.13 ± 0.01[c]	3.96 ± 0.05[c]	48.92 ± 1.32[c]	3.10 ± 0.62[d]

[e] Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[f] NEBI, non-enzymatic browning index; HMF, 5-hydroxymethyl furfural.
[g] Control (freshly squeezed or no treatment); U15 (UV-C treatment for 15 min); U30 (UV-C treatment for 30 min); U60 (UV-C treatment for 60 min); TP (thermal pasteurization).

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Table 4 shows that UV-C treated samples showed no significant changes in HMF content except U60, when compared with the control. In contrast, the maximum increase in HMF content was observed in thermal treated samples (3.96 mg/l) when compared with control (2.20 mg/l). This is consistent with the study conducted by Aguiló-Aguayo et al. (2009) on heat pasteurized strawberry juice, where significant increase in HMF content was observed. The results of HMF content agrees with NEBI, as increased NEBI can be attributed to enhancement in the development of HMF. Nevertheless, it is important to highlight that the maximum concentration of HMF allowed by the Association of the Industry of Juices and Nectars from Fruits and Vegetables is ≤5 mg/l (AIJN, 1996). Hence, the concentration of HMF in UV-C and thermal treated juices was low and remained below the limit allowed.

3.4. *Total carotenoid content*

Juice samples subjected to UV-C exposure (U15 and U30) exhibited significant increase ($p < 0.05$) in carotenoid content, as shown in Table 4. U15 sample showed the highest enhancement in extractability of carotenoids (6%) when compared to the control. This could be attributed to alteration of carotenoid-binding protein, consequently increasing the availability of free carotenoids. In addition, the formation of UV photons may cause inactivation of enzymes responsible for the loss of carotenoids, thus improving carotenoid extraction yield (Oms-Oliu et al., 2012). After 60 min of UV-C treatment, no significant changes in carotenoid content were observed. Therefore, carotenoids were still stable even after 60 min of UV-C exposure. According to Rodríguez-Amaya (1997), there is an increase in certain individual carotenoids owing to better stability. This may be explained by the enhancement of antioxidants due to UV-C exposure, thus providing better retention of carotenoids. Consequently, further degradation of carotenoids catalyzed by oxidative enzymes was prevented. Hence, the increase in extractable carotenoids is an advantage as these compounds possess health promoting properties such as reduced risk of cardiovascular disease (Krinsky, 1990). In contrast, thermal pasteurized juice samples showed significant decrease in carotenoid content (48.92 μg/100 ml) when compared to the control (82.03 μg/100 ml). Similarly, previous research studies have reported significant degradation of carotenoids in thermally treated Valencia orange and pineapple juices (Lee and Coates, 2003; Goh et al., 2012). High temperature promotes geometric isomerization of carotenoids from *trans*-form to *cis*-form. In addition, the instability of the polyene chain (extensive conjugated double bond system) of carotenoids, contribute to their susceptibility to oxidation, thus forming initial products known as epoxides (Rodríguez-Amaya, 1997).

3.5. *Ascorbic acid content*

After UV-C and thermal treatment, significant reduction in the ascorbic acid (vitamin C) content was observed in juice samples when compared to control (Table 4). The minimum degradation of ascorbic acid for UV-C treated samples was about 12–15%, which is in U15 (7.85 mg/100 ml) and U30 (7.55 mg/100 ml), when compared to the control (8.91 mg/100 ml). Likewise, a significant decrease in ascorbic acid content was observed in UV-C treated starfruit juice, where the least reduction was 10% (Bhat et al., 2011). Similarly, Tran and Farid (2004) also reported loss of ascorbic acid (12%) in orange juice after UV-C treatment. The depletion of ascorbic acid could be explained by the formation of free hydroxyl radicals by photochemical reaction, related to oxidative processes (Koutchma et al., 2009). In addition, oxidative degradation as a result of enzyme activities such as ascorbate oxidase and peroxidase, and the presence of oxygen and light, mainly contribute to detrimental effects on ascorbic acid (Davey et al., 2000). For thermal treated juice, a maximum loss (65%) in ascorbic acid was shown when compared to the control. Heating affects the degradation of ascorbic acid in an aerobic pathway due to its heat-sensitive characteristic in the presence of oxygen (Oms-Oliu et al., 2012). Correspondingly, Goh et al. (2012) observed lower ascorbic acid content in thermally treated pineapple juice than control and UV-C treated juices. Hence, this is a clear indication that UV-C treatment provides better retention of ascorbic acid when compared to thermal pasteurization due to the absence of heat. Therefore, better retention of ascorbic acid is an advantage as these compounds have been associated with protection against some types of cancers, due to its antioxidant properties (Block, 1991).

3.6. *Antioxidant activity (total polyphenol and flavonoid content, DPPH radical scavenging and reducing power assay)*

The effects of UV-C exposure and thermal pasteurization on antioxidant activity in juice extracts are shown in Table 5. The Folin–Ciocalteu method uses a reagent containing tungsten and molybdenum oxides to measure the content of phenolic compounds (Waterhouse, 2002). Significant enhancement in extraction yield of polyphenols was observed in all UV-C treated juice samples when compared to the control. The highest increase (31%) was from 97.83 mg GAE/100 ml to 128.12 mg GAE/100 ml in control and U30 sample, respectively. The

Table 5 – Effects of UV-C treatment and thermal pasteurization on antioxidant activity of Chokanan mango juice.[f,g,h]

Treatment	Total polyphenol content (mg GAE/100 ml)	Total flavonoid content (mg CE/100 ml)	DPPH radical scavenging activity (μg AAE/ml)	Reducing power (μg AAE/ml)
Control	97.83 ± 1.76[a]	8.74 ± 0.12[a]	8.29 ± 0.18[a]	360.71 ± 3.20[a]
U15	119.20 ± 1.25[b]	9.02 ± 0.22[b]	8.45 ± 0.15[ab]	402.12 ± 2.31[b]
U30	128.12 ± 2.01[c]	8.83 ± 0.26[a]	8.99 ± 0.14[b]	405.75 ± 4.05[b]
U60	104.15 ± 1.23[d]	7.83 ± 0.12[c]	8.18 ± 0.17[a]	358.22 ± 3.15[a]
TP	60.87 ± 1.42[e]	6.52 ± 0.15[d]	8.09 ± 0.15[a]	345.10 ± 6.10[a]

[f] Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[g] GAE, gallic acid equivalent; CE, catechin equivalent; AAE, ascorbic acid equivalent.
[h] Control (freshly squeezed or no treatment); U15 (UV-C treatment for 15 min); U30 (UV-C treatment for 30 min); U60 (UV-C treatment for 60 min); TP (thermal pasteurization).

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increased activity of enzyme phenylalanine ammonia lyase by UV-C exposure was suggested to contribute to the activation of phenolic biosynthesis pathway, which leads to enhancement of phenolic compounds (Alothman et al., 2009). In addition, UV-C induced inactivation of polyphenol oxidase may prevent further loss of polyphenols (Oms-Oliu et al., 2012). This is consistent with the study conducted by Bhat et al. (2011) on UV-C treated starfruit juice, where significant increase in polyphenol content was observed. In contrast, thermal pasteurized samples exhibited significant reduction (38%) in total polyphenol. Similarly, Pala and Toklucu (2013) reported significant loss of phenolic compounds in heat treated orange juice.

Flavonoids are naturally-occurring polyphenolic compounds that form acid labile complexes in aluminium chloride colorimetric method (Mabry et al., 1970). In this study, significant enhancement (3%) in extractability of flavonoids was observed in UV-C treated juice sample, U15 (9.02 mg CE/100 ml) when compared to the control (8.74 mg CE/100 ml). The exposure of UV-C light stimulates the formation of free radicals which may trigger stress responses such as accumulation of phytoalexins. As a result, there is an increase in the level of flavonoids related to a defence mechanism (Alothman et al., 2009). However, for U60 samples, degradation of flavonoids was observed. This could be attributed to the longer UV-C exposure time which induces too much stress thus, suppressing flavonoid content. Besides that, thermal treated samples exhibited significant reduction (25%) in total flavonoids.

DPPH radical scavenging assay measures the hydrogen donating capacity of the antioxidant to the stable free radical DPPH, resulting in the formation of diphenylpicrylhydrazine (Shon et al., 2003). Antioxidant activity may be indicated by the reductive ability of a compound (transformation of ferricyanide complex to ferrocyanide complex) as described by Rama Prabha and Vasantha (2011). In this study, UV-C treated juice samples, except U60 showed significant increase in the percentage of DPPH inhibition when compared to control. The highest percentage inhibition of DPPH was 91.2% (8.99 μg AAE/ml) for U30 sample, compared to the control, 84.1% (8.29 μg AAE/ml). In terms of reducing power, UV-C treated samples (U15 and U30) showed significant increase in comparison with control. The maximum increase in reducing capacity (12%) was from 360.71 μg AAE/ml to 405.75 μg AAE/ml in control and U30 sample, respectively. As explained previously, stress response induced by UV-C processing may increase extraction of antioxidant compounds and is in agreement with Bhat et al. (2011), where UV-C exposure induces antioxidant capacity of starfruit juice. In addition, enhanced antioxidant capacities may allow additional quenching of active oxygen species, thus being an advantage to health conscious consumers. Nevertheless, Alothman et al. (2009) reported that UV-C treatment can either increase or decrease the extractability of antioxidants due to some variables including dose delivered, exposure time, and raw materials used. Thus, UV-C processing variables have to be evaluated appropriately to avoid negative impacts on fruit juice quality. No significant changes were observed in percentage of DPPH inhibition after thermal treatment. Likewise, thermal pasteurized samples showed non-significant decrease in reducing ability (345.10 μg AAE/ml) when compared to the control (360.71 μg AAE/ml). This is consistent with the study conducted by Goh et al. (2012) on heat treated pineapple juice, where no significant changes in antioxidant activity were observed.

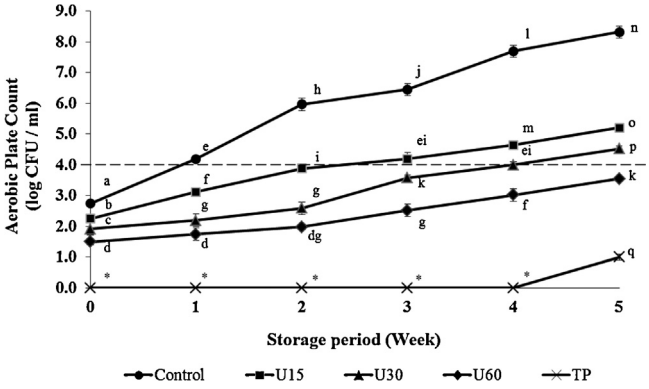

Fig. 2 – Changes in aerobic plate count of control, UV-C treated and thermally pasteurized Chokanan mango juice during storage at 4 °C.[r,s,t]
[r]Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[s]The dashed line (- - -) indicates the limit of microbial shelf-life; CFU, colony-forming unit; *not detected.
[t]Control (freshly squeezed or no treatment); U15 (UV-C treatment for 15 min); U30 (UV-C treatment for 30 min); U60 (UV-C treatment for 60 min); TP (thermal pasteurization).

3.7. *Shelf life study*

Changes in microbial counts (aerobic bacteria, yeast and mould) in juices stored at refrigeration temperature (4 ± 1 °C) for 5 weeks are shown in Figs. 2 and 3. Shelf life of juice samples was evaluated according to the Institute of Food Science and Technology, IFST (1999). The acceptable maximum microbial load including aerobic bacteria, and total yeast and mould in fruit juices are about 4 and 3 log CFU/ml, respectively. The aerobic plate counts (APC) in control (untreated juice) increased from 2.74 to 8.32 log CFU/ml after 5 weeks storage. While, yeast and mould counts (YMC) increased from 2.42 to 6.10 log CFU/ml after 5 weeks storage. The rate of microbial growth observed in control was higher than UV-C treated juice samples during storage. The shelf life of UV-C treated

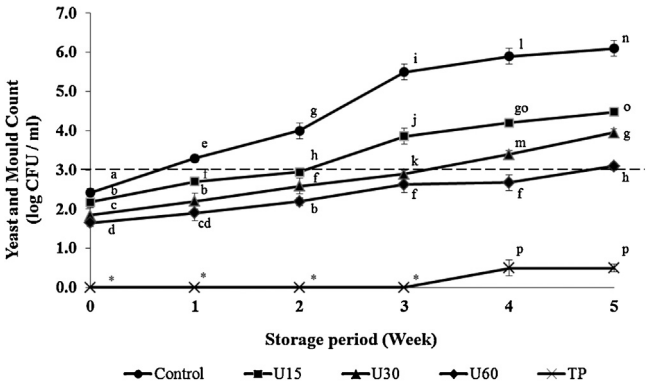

Fig. 3 – Changes in yeast and mould count of control, UV-C treated and thermally pasteurized Chokanan mango juice during storage at 4 °C.[q,r,s]
[q]Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[r]The dashed line (- - -) indicates the limit of microbial shelf-life; CFU, colony-forming unit; * not detected.
[s]Control (freshly squeezed or no treatment); U15 (UV-C treatment for 15 min); U30 (UV-C treatment for 30 min); U60 (UV-C treatment for 60 min); TP (thermal pasteurization).

juices, U15 (2 weeks), U30 (3–4 weeks), U60 (5 weeks) were longer than control (less than 1 week), in terms of microbial load (APC and YMC) limit. Hence, the shelf life of UV-C treated Chokanan mango juice stored at 4 ± 1 °C was extended for at least 4 weeks longer than freshly squeezed juice. Results obtained are in agreement with previous studies that reported prolonged shelf life in UV-C treated orange juice (Tran and Farid, 2004; Pala and Toklucu, 2013). Thermally pasteurized juice had almost no microorganism growth during 5 weeks storage, thus remaining below microbial load limit. This is a clear indication that thermally pasteurized juice has a shelf life for more than 5 weeks.

4. Conclusions

Chokanan mango juice samples subjected to UV-C exposure for 15 and 30 min exhibited significant improvement in antioxidant activities and extractability of carotenoids, phenolic compounds, and flavonoids, when compared to freshly squeezed juice. In addition, UV-C treatment showed significant reduction in microbial count. The shelf life of UV-C treated juice stored at 4 ± 1 °C was extended for at least 4 weeks longer than freshly squeezed juice. Thermal treatment was more effective in completely inactivating microbial growth in the juice, but detrimental effects on quality were observed. Hence, UV-C treatment with appropriate optimization of processing variables is a feasible alternative to thermal pasteurization. Further research work is needed for combining UV-C with other non-thermal food processing technologies, such as pulsed electric field and high pressure to guarantee improved quality juice together with safety standards. Consequently, this study provides more attention for positive implementation of UV-C treatment on a pilot scale.

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References

3M Food Safety, 3M Petrifilm interpretation guide, 2010. http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/ (accessed 07.02.13).

Aguiló-Aguayo, I., Oms-Oliu, G., Soliva-Fortuny, R., Martín-Belloso, O., 2009. Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments. LWT – Food Sci. Technol. 42, 813–818.

Alothman, M., Bhat, R., Karim, A.A., 2009. Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends Food Sci. Technol. 20, 201–212.

Association of the Industry of Juices and Nectars from Fruits and Vegetables (AIJN), 1996. Association of the industry of juices and nectars of the European Economic Community code of practice for evaluation of fruit and vegetable juices. AIJN, Brussels.

Bae, S.H., Suh, H.J., 2007. Antioxidant activities of five different mulberry cultivars in Korea. LWT – Food Sci. Technol. 40, 955–962.

Berardini, N., Knodler, M.A., Schieber, A., Carle, R., 2005. Utilization of mango peels as a source of pectin and polyphenolics. Innov. Food Sci. Emerg. Technol. 6, 442–452.

Bhat, R., Ameran, S.B., Voon, H.C., Karim, A.A., Tze, L.M., 2011. Quality attributes of starfruit (*Averrhoa carambola* L.) juice treated with ultraviolet radiation. Food Chem. 127, 641–644.

Block, G., 1991. Vitamin C and cancer prevention: the epidemiologic evidence. Am. J. Clin. Nutr. 53, 270–282.

Block, G., Patterson, B., Subar, A., 1992. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer. 18, 1–29.

Bull, M., Zerdin, M., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., 2004. The effect of high pressure processing on the microbial physical and chemical properties of Valencia and Navel orange juice. Innov. Food Sci. Emerg. Technol. 5, 135–149.

Caminiti, I.M., Noci, F., Munoz, A., Whyte, P., Morgan, D.J., Cronin, D.A., Lyng, J.G., 2011. Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. Food Chem. 124, 1387–1392.

Caminiti, I.M., Noci, F., Morgan, D.J., Cronin, D.A., Lyng, J.G., 2012. The effect of pulsed electric fields, ultraviolet light or high intensity light pulses in combination with manothermosonication on selected physico-chemical and sensory attributes of an orange and carrot juice blend. Food Bioprod. Process. 90, 442–448.

Cohen, E., Birk, Y., Mannheim, C.H., Saguy, I.S., 1998. A rapid method to monitor quality of apple juice during thermal processing. Lebensm. Wiss. Technol. 31, 612–616.

Cserhalmi, Z., Sass-Kiss, A., Tóth-Markus, M., Lechner, N., 2006. Study of pulsed electric field treated citrus juices. Innov. Food Sci. Emerg. Technol. 7, 49–54.

Davey, M.W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smimoff, N., Benzie, L.J.J., Strain, J.J., Favell, D., Fletcher, J., 2000. Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. J. Sci. Food Agric. 80, 825–860.

Food and Agriculture Organisation of the United Nation Statistics Division (FAOSTAT), Food and Agricultural commodities production, 2013. http://faostat.fao.org/site (accessed 23.03.13).

Food and Drug Administration (FDA), 2000. Irradiation in the production, processing and handling of food. Federal Register Rules and Regulations 65, 71056–71058.

Glevitzky, M., Pop, M., Brusturean, G.A., Bogdan, I., Calisevici, M., Perju, D., 2008. Efficient use of antioxidants to preserve fruit juice. Rev. Chim. 59, 1291–1295.

Goh, S.G., Noranizan, M., Leong, C.M., Sew, C.C., Sobhi, B., 2012. Effect of thermal and ultraviolet treatments on the stability of antioxidant compounds in single strength pineapple juice throughout refrigerated storage. Int. Food Res. J. 19, 1131–1136.

Guerrero-Beltrán, J.A., Barbosa-Cánovas, G.V., 2004. Advantages and limitations on processing foods by UV light. Food Sci. Technol. Int. 17, 137–147.

Ibarz, A., Pagan, J., Panades, R., Garza, S., 2005. Photochemical destruction of color compound in fruit juices. J. Food Eng. 69, 155–160.

Institute of Food Science and Technology, 1999. Development and Use of Microbiological Criteria for Foods. Institute of Food Science and Technology, London.

Keyser, M., Műller, I.A., Cilliers, F.P., Nel, W., Gouws, P.A., 2008. Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. Innov. Food Sci. Emerg. Technol. 9, 348–354.

Koutchma, T., Forney, L.J., Moraru, C.L., 2009. Ultraviolet Light in Food Technology: Principles and Application. CRC Press, New York.

Koutchma, T., Keller, S., Chirtel, S., Parisi, B., 2004. Ultraviolet disinfection of juice products in laminar and turbulent flow reactors. Innov. Food Sci. Emerg. Technol. 5, 179–189.

Krinsky, N.I., 1990. Carotenoids in medicine. In: Krinsky, N.I., Mathews-Roth, M.M., Taylor, R.F. (Eds.), Carotenoids: Chemistry and Biology. Plenum Press, New York, pp. 279–291.

Lee, H.S., Coates, G.A., 2003. Effect of thermal pasteurization on Valencia orange juice color and pigments. LWT – Food Sci. Technol. 36, 153–156.

Please cite this article in press as: Santhirasegaram, V., et al., Comparison of UV-C treatment and thermal pasteurization on quality of Chokanan mango (*Mangifera indica* L.) juice. Food Bioprod Process (2014), http://dx.doi.org/10.1016/j.fbp.2014.03.011

Lee, H.S., Castle, W.S., Coates, G.A., 2001. High-performance liquid chromatography for the characterization of carotenoids in the new sweet orange (Earlygold) grown in Florida, USA. J. Chromatogr. A 913, 371–377.
Liu, R.H., 2003. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am. J. Clin. Nutr. 78, 517–520.
Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer-Verlag, New York.
Masibo, M., He, Q., 2008. Major mango polyphenols and their potential significance to human health. Compr. Rev. Food Sci. Food Safety. 7, 309–319.
Miller, R., Jeffrey, W., Mitchell, D., Elasri, M., 1999. Bacteria responses to ultraviolet light. Am. Soc. Microbiol. 65, 535–541.
Nagy, S., Chen, C.S., Shaw, P.E., 1993. Fruit Juice Processing Technology. Agscience, Florida.
Oms-Oliu, G., Odriozola-Serrano, I., Martín-Belloso, O., 2012. The effects of non-thermal technologies on phytochemicals. In: Rao, V. (Ed.), Phytochemicals: A Global Perspective of Their Role in Nutrition and Health. InTech, Croatia.
Oyaizu, M., 1986. Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 103, 413–419.
Pala, C.U., Toklucu, A.K., 2011. Effect of UV-C light on anthocyanin content and other quality parameters of pomegranate juice. J. Food Comp. Anal. 24, 790–795.
Pala, C.U., Toklucu, A.K., 2013. Microbial, physicochemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage. LWT – Food Sci. Technol. 50, 426–431.
Rama Prabha, M., Vasantha, K., 2011. Antioxidant, cytotoxicity and polyphenolic content of *Calotropis procera* (Ait.) R. Br. Flowers. J. Appl. Pharm. Sci. 1, 136–140.
Ranganna, S., 1977. Chapter 5: ascorbic acid. In: Ranganna, S. (Ed.), Manual of Analysis of Fruit and Vegetable Products. Tata McGraw Hill Publishing Company, New Dehli, pp. 94–96.
Rattanathanalerk, M., Chiewchan, N., Srichumpoung, W., 2005. Effect of thermal processing on the quality loss of pineapple juice. J. Food Eng. 66, 259–265.
Rawson, A., Patras, A., Tiwari, B.K., Noci, F., Koutchma, T., Brunton, N., 2011. Effect of thermal and non-thermal processing technologies on the bioactive content of exotic fruits and their products: review of recent advances. Food Res. Int. 44, 1875–1887.
Rivas, A., Rodrigo, D., Martinez, A., Barbosa-Canovas, G.V., Rodrigo, M., 2006. Effect of PEF and heat pasteurization on the physical–chemical characteristics of blended orange and carrot juice. LWT – Food Sci. Technol. 39, 1163–1170.
Rodríguez-Amaya, D.B., 1997. Carotenoids and Food Preparation: The Retention of Provitamin A Carotenoids in Prepared, Processed and Stored Foods. OMNI, Washington, DC.
Sadler, G.D., Murphy, P.A., 2010. pH and titratable acidity. In: Nielsen, S.S. (Ed.), Food Analysis. Springer, New York, pp. 231–233.
Sakanaka, S., Tachibana, Y., Okada, Y., 2005. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem. 89, 569–575.
Santhirasegaram, V., Razali, Z., Somasundram, C., 2013. Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. Ultrason. Sonochem. 20, 1276–1282.
Scott, K.J., 2001. Detection and measurement of carotenoids by UV/VIS spectrophotometry. In: Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.A., Schwartz, S.J., Shoemaker, C.F., Smith, D., Sporns, P. (Eds.), Current Protocols in Food Analytical Chemistry. John Wiley and Sons Inc., New York, pp. F2.2.1–F2.2.10.
Shamsudin, R., Ling, C.S., Adzahan, N.M., Wan Daud, W.R., 2013. Rheological properties of ultraviolet-irradiated and thermally pasteurized Yankee pineapple juice. J. Food Eng. 116, 548–553.
Shon, M.Y., Kim, T.H., Sung, N.J., 2003. Antioxidants and free radical scavenging activity of *Phellinus baumii* (Phellinus of Hymenochaetaceae) extracts. Food Chem., 593–597.
Singleton, V.L., Joseph, A., Rossi, J.R.J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.
Spreer, W., Ongprasert, S., Hegele, M., Wunsche, J.N., Muller, J., 2009. Yield and fruit development in mango (*Mangifera indica* L. cv. Chok Anan) under different irrigation regimes. Agric. Water Manage. 96, 574–584.
Tran, M.T.T., Farid, M., 2004. Ultraviolet treatment of orange juice. Innov. Food Sci. Emerg. Technol. 5, 495–502.
Walkling-Ribeiro, M., Noci, F., Cronin, D.A., Riener, J., Lyng, J.G., Morgan, D.J., 2008. Reduction of *Staphylococcus aureus* and quality changes in apple juice processed by ultraviolet irradiation, pre-heating and pulsed electric fields. J. Food Eng. 89, 267–273.
Waterhouse, A.L., 2002. Determination of total phenolics. In: Wrolstad, R.E., Acree, T.E., Decker, E.A., Penner, M.H., Reid, D.A., Schwartz, S.J., Shoemaker, C.F., Smith, D., Sporns, P. (Eds.), Current Protocols in Food Analytical Chemistry. John Wiley and Sons Inc, New York, pp. I1.1.1–I1.1.8.
Xu, G., Liu, D., Chen, J., Ye, X., Maa, Y., Shi, J., 2008. Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chem. 106, 545–551.
Zhang, C., Trierweiler, B., Li, W., Butz, P., Xu, Y., Rüfer, C.E., Maa, Y., Zhao, X., 2011. Comparison of thermal, ultraviolet-c, and high pressure treatments on quality parameters of watermelon juice. Food Chem. 126, 254–260.

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Abstract

The growing demand for fresh-like food products has encouraged the development of hurdle technology of non-thermal processing. In this study, freshly squeezed Chokanan mango juice was treated by paired combinations of sonication (for 15 and 30 min at 25 °C, 40 kHz frequency) and UV-C treatment (for 15 and 30 min at 25 °C). Selected physicochemical properties, antioxidant activities, microbial inactivation and other quality parameters of combined treated juice were compared to conventional thermal treatment (at 90 °C for 60 s). After thermal and combined treatment, no significant changes occurred in physicochemical properties. A significant increase in extractability of carotenoids (15%), polyphenols (37%), flavonoids (35%) and enhancement in antioxidant capacity was observed after combined treatment. Thermal and combined treatment exhibited significant reduction in microbial load. Results obtained support the use of sonication and UV-C in a hurdle technology to improve the quality of Chokanan mango juice along with safety standards.

Keywords

Sonication, ultraviolet treatment, thermal treatment, Chokanan mango, juice, antioxidant activity

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INTRODUCTION

Mango (*Mangifera indica* L.) is a tropical fruit grown in 85 countries, ranking fifth in global production among other major fruit crops including bananas, citruses, grapes and apples. Mango production in Asia has increased about 15% from 2009 to 2011, contributing approximately 30.2 million metric tons to the international commodity market (FAOSTAT, 2013). In Malaysia, commercialisation of domestic mango cultivars especially Chokanan has reached the worldwide market as they are exported to Singapore, Brunei and Hong Kong. According to Spreer et al. (2009), there is a large stock of Chokanan mango yearly due to its ability to bear fruit even during rainy season. Thus, the market for value-added mango products such as juice, puree and nectar has progressively grown due to its perishable nature.

According to a study conducted by Rivera and Cabornida (2008), fruit juices have the highest acceptability among other beverages, generally due to their natural taste, as well as to the nutritional value associated with them. Consumption of mango juice has been linked to prevention of cancer, owing to its antioxidant properties (Block et al., 1992). Currently, thermal pasteurisation is the preferred technology used to achieve microbial inactivation and prolong the shelf life of juices. However, high temperature causes

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detrimental effects on nutritional quality of juices such as reported in cashew apple and pineapple juice (Rawson et al., 2011). Consumers' demand for a preservation technology that retains fresh-like quality and, at the same time, ensures food safety has resulted in growing interest for non–thermal-processing methods. Both sonication and short-wave ultraviolet-C (UV-C) light treatment are alternative novel technologies to achieve the U.S. Food and Drug Administration (FDA) condition of a 5 log reduction of food borne pathogens in fruit juices (FDA, 2000; Salleh-Mack and Roberts, 2007).

Propagation of high-power ultrasound at low frequencies (20–100 kHz) in liquid causes cavitation (formation and collapse of bubbles). Consequently, these 'tiny hotspots' provide energy to disrupt microbial cell membrane and alter the properties of food (O'Donnell et al., 2010). The UV-C light (peak emission at 254 nm) exhibits germicidal effect by preventing the reproduction of microorganisms and eventually may result in cell death (Guerrero-Beltrán and Barbosa-Cánovas, 2004). Sonication and UV-C treatment are simple, reliable and cost-effective with improved efficiency (O'Donnell et al., 2010; Pala and Toklucu, 2013). These technologies have different mode of microbial inactivation, therefore being potential choices for a hurdle concept. The hurdle technology is a combination of preservation techniques that may have a synergistic effect on microbial destruction, with minimal impact on the quality of the food product (Leistner, 2000).

The purpose of this study was to compare the effects of combined treatment (sonication and UV-C) and thermal treatment on the quality parameters of Chokanan mango juice such as microbial inactivation, physicochemical properties (pH, total soluble solids (TSS) and titratable acidity), colour, clarity, browning index, hydroxymethyl furfural content, carotenoid content, ascorbic acid content and antioxidant activities. The information obtained from this study could serve to improve the progress of utilising combined treatment of ultrasonic and UV-C for preserving the quality of Chokanan mango juice.

MATERIALS AND METHODS

Chemicals

Gallic acid, L-ascorbic acid, (+)-catechin, 5-hydroxymethyl furfural, 2,6-dichlorophenol-indophenol (DCPIP) sodium salt, potassium ferricyanide, ferric chloride, Folin-Ciocalteu reagent, sodium hydroxide, trichloroacetic acid, thiobarbituric acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (MO, USA). Metaphosphoric acid, aluminium chloride and peptone water were purchased from R&M Chemicals (Essex, UK). Sodium bicarbonate, sodium nitrite and sodium carbonate were purchased from BDH (Poole, UK). All chemical solvents used were analytical reagent grade and purchased from Sigma (MO, USA).

Extraction of mango juice

Ripe Chokanan mango fruits of uniform size and free from external defects were purchased from a local market (Selangor, Malaysia) located about 30 km from the Postharvest Biotechnology Laboratory, University of Malaya. The fruits were rinsed with running water and air-dried at room temperature (25 °C ± 1 °C). Each mango was peeled, and the seed was discarded. Mango pulp was macerated using a domestic juice extractor (Philips Juice Extractor HR 2820, Holland) and then centrifuged (Beckman J2-MI Centrifuge, California) at 12,000 rpm for 10 min at 4 °C. The supernatant was filtered using a steel sieve with an approximate diameter of 2 mm to obtain the juice and remove any remaining fibre. The filtered juice samples were stored in sterile glass bottles prior to deployment into experiment.

Experimental treatments

The freshly squeezed mango juice was treated by four paired combinations of selected non-thermal technologies. Sonication was employed as the first hurdle followed by UV-C treatment. Selection of processing variables was based on preliminary studies on standalone treatments to achieve significant microbial reduction and quality retention (Santhirasegaram et al., 2013, unpublished data).

The following terms were used to describe different treatments in this study:

1. Control (freshly squeezed or no treatment);
2. TT (thermal treatment);
3. S15 + U15 (combined sonication for 15 min and UV-C treatment for 15 min);
4. S30 + U15 (combined sonication for 30 min and UV-C treatment for 15 min);
5. S15 + U30 (combined sonication for 15 min and UV-C treatment for 30 min);
6. S30 + U30 (combined sonication for 30 min and UV-C treatment for 30 min).

All treatments and analysis were carried out in triplicates.

Thermal treatment

Mango juice (50 mL) stored in glass bottles was pasteurised in a covered water bath (Memmert,

Germany) at 90 °C ± 1 °C for 60 s. After thermal treatment, the juice samples were immediately cooled to room temperature (25 °C ± 1 °C) by immersing in an ice-water bath.

Sonication

The sonication of mango juice (50 mL) was performed using an ultrasonic cleaning bath (Branson Model 3510 Ultrasonic Cleaner, CT, USA) at 40 kHz frequency, as described by Santhirasegaram et al. (2013). The processing time was 15 and 30 min under dark condition. The actual power dissipated in the ultrasonic bath was 68–72 W, and the acoustic energy density was 1.36–1.44 W/cm^3, which was determined by calorimetric method (Sutkar and Gogate, 2009; Gogate et al., 2011).

UV-C treatment

Juice samples were exposed to UV-C light under batch conditions. Mango juice (50 mL) was poured into four sterile Petri dishes (11 cm diameter, 0.8-cm fluid depth) and then exposed to a germicidal fluorescent UV-C lamp (30 W, 89.3 cm length, 25.5 cm diameter, Sankyo Denki, Japan) in a laminar flow cabinet (Gelman Science Biological Safety Cabinet Class II, NSW, Australia). The UV-C lamp has a peak emission at 254 nm. The distance between the juice surface and the UV-C lamp was 35 cm. The UV-C lamps were allowed to stabilise for 30 min prior to use. The duration of exposure was 15 and 30 min at 25 °C ± 1 °C. The mean of UV radiation dose received by each juice sample is 3.525 J/m^2 (Keyser et al., 2008). Juice samples were bottled directly after treatment.

Physicochemical analysis (pH, TSS and titratable acidity)

Mango juice pH was determined using a pH metre (Hanna Microprocessor pH 211, Italy) at 25 °C ± 1 °C. TSSs were determined using a digital refractometre (Atago PR-1 digital refractometre, Tokyo, Japan) at 25 °C ± 1 °C, and results were expressed in standard °Brix unit.

For determination of titratable acidity, diluted mango juice was titrated with standardised 0.1 N sodium hydroxide to a definite faint pink end point using phenolphthalein as an indicator. The titratable acidity (%TA) was calculated (Sadler and Murphy, 2010) using the following equation

$$\%\,\mathrm{TA} = (V_1 \times 0.1\,\mathrm{N\,NaOH} \times \mathrm{Eq.wt.} \times 100)/(V_2 \times 1000)$$

where V_1 is volume of titrant (mL), Eq. wt. is equivalent weight of anhydrous citric acid (64 mg/mEq) and V_2 is volume of sample (mL).

Colour, clarity, non-enzymatic browning index and 5-hydroxymethyl furfural content

The colour of juice samples was measured using a Chroma Meter (Minolta Chroma Meter CR-200, Minolta, Osaka, Japan). The colour parameters L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) were evaluated. Colour differences (ΔE), in comparison to control (Caminiti et al., 2011), were calculated using the following equation

$$\Delta E = [(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2]^{1/2}$$

Transmittance at 660 nm was measured using a spectrophotometer (UV-200-RS Spectrophotometer, MRC, Israel) to determine clarity (Glevitzky et al., 2008) against a blank (distilled water). High percentage of transmittance at 660 nm corresponds to high clarity.

Non-enzymatic browning index (NEBI) and 5-hydroxymethyl furfural (HMF) assay were carried out according to the method by Cohen et al. (1998). A standard curve of HMF ($y = 0.0406x$, $r^2 = 0.9967$) was prepared, and results were expressed as milligrams of HMF per litre juice sample.

Total carotenoid content

Carotenoid was extracted according to Lee et al. (2001), while the total carotenoid content (Scott, 2001) using β-carotene as a reference was calculated using the following formula:

$$\mathrm{Total\,carotenoid\,content} = (A \times V_1 \times C^{1\%})/A^{1\%}$$

where A is absorbance reading of the diluted sample, V_1 is dilution factor, $A^{1\%}$ is absorbance of a 1% solution (the extinction coefficient for β-carotene; 2592 AU) and $C^{1\%}$ is concentration of a 1% solution (10 mg/mL).

Ascorbic acid content

Ascorbic acid content in samples was determined based on the DCPIP visual titration method (Ranganna, 1977). Results obtained were expressed as milligrams of ascorbic acid per 100 mL sample.

Antioxidant activity (total polyphenol and flavonoid content, DPPH radical scavenging and reducing power assay)

Antioxidants were extracted according to Santhirasegaram et al. (2013) using 80% methanol.

Total polyphenol content of juice samples was determined using Folin-Ciocalteu assay (Singleton et al., 1965) modified to a microscale (Bae and Suh, 2007). A standard curve of gallic acid ($y = 0.00566x$, $r^2 = 0.9955$) was prepared, and results were reported as milligrams of gallic acid equivalent (GAE) per 100 mL juice extract.

Total flavonoid content of juice samples was determined using a colorimetric method described by Sakanaka et al. (2005). A standard curve of (+)-catechin ($y = 0.0135x$, $r^2 = 0.9943$) was prepared, and results were reported as milligrams of catechin equivalent (CE) per 100 mL juice extract.

DPPH radical scavenging assay was carried out as described by Oyaizu (1986) and Bae and Suh (2007). A standard curve of ascorbic acid ($y = 10.145x$, $r^2 = 0.9907$) was prepared, and results were reported as micrograms of ascorbic acid equivalent (AAE) per millilitre juice extract. The radical scavenging activity was calculated accordingly

$$\%\text{DPPH inhibition} = (A_{control} - A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is absorbance reading of control and A_{sample} is absorbance reading of the sample.

A spectrophotometric method by Oyaizu (1986) was used for measuring the reducing power of juice samples. A standard curve of ascorbic acid ($y = 0.0014x$, $r^2 = 0.9906$) was prepared, and results were reported as micrograms of AAE per milliliter juice extract.

Microbial inactivation analysis

The 3M Petrifilm plate methods are recognised as AOAC International Official Methods of Analysis (3M Food Safety, 2010). Microbial count of juice samples was determined using Petrifilm plates (3M Center, MN, USA) for aerobic bacteria, coliform, yeast and mould according to Santhirasegaram et al. (2013). Results were expressed as log (CFU/mL).

Statistical analysis

Data obtained were subjected to statistical analysis using SPSS 19.0 software (SPSS Inc., IBM). In this study, data were represented as mean values ± standard deviation. The significant differences between mean values of juice samples were determined by analysis of variance (one way-ANOVA) using Tukey's HSD (honestly significant difference) test at a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical analysis (pH, TSS and TA)

The pH, TSS and TA of juice (Table 1) showed no significant changes after thermal and combined treatment. Values for pH (4.58 to 4.63), TSS (14.5 to 14.7 °Brix) and TA (0.19 to 0.22%) of treated juice samples still lie within the range of standards desirable for freshly squeezed Chokanan mango juice. These observations are in agreement with a previous study reporting non-significant deviations of physicochemical parameters of juice processed by either thermal, ultrasound or UV-C, such as in apple and cranberry juice blend as well as orange and carrot juice blend (Caminiti et al., 2011, 2012).

Colour, clarity, NEBI and HMF content

Significant differences in colour of juice samples were observed between control and treatments (combined method and thermal), as shown in Table 2. An increase in lightness (L^*) and decrease in redness ($+a^*$) and yellowness ($+b^*$) were observed after all treatments. The increase in L^* values could be attributed to the brightening effect of juice due to cavitational collapse of bubbles during sonication and UV-C photodegradation of coloured compounds (Bhat et al., 2011; Tiwari et al., 2008). The b^* value is an indicator of the level of natural pigments (mostly carotenoids) responsible for the yellow colour of mango juice. Thus, the decrease in $+b^*$ value may be due to isomerisation of carotenoids, as previously reported by Rattanathanalerk et al. (2005) in thermal-treated pineapple juice. According to Cserhalmi et al. (2006), differences in colour parameters (ΔE) can be categorised as not noticeable ($0 < \Delta E < 0.5$), slightly noticeable ($0.5 < \Delta E < 1.5$), noticeable ($1.5 < \Delta E < 3.0$), well visible ($3.0 < \Delta E < 6.0$) and greatly visible ($6.0 < \Delta E < 12$). In comparison to thermal treatment, combined treated samples S15 + U15, S30 + U15 and S15 + U30 showed lowest variation from the control, therefore falling within the 'slightly noticeable' range. However, an increase in ΔE corresponding to increased ultrasonic treatment time was observed, regardless of the UV-C treatment. Thus, sonication could be responsible for the colour degradation observed in this hurdle system, as previously suggested by Cheng et al. (2007) in guava juice treatment.

Clarity acts as an indicator of the degree of turbidity of fruit juices. The clarity of juice (Table 3) showed significant decrease after thermal treatment. Thermal-treated samples showed the least clarity (7.30), when

Table 1. Effects of thermal and combined treatment on physicochemical analysis (pH, total soluble solids, titratable acidity) of Chokanan mango juice.[a,b]

Treatment	pH	Total soluble solids (°Brix)	Titratable acidity (%)
Control	4.62 ± 0.02 a	14.7 ± 0.06 a	0.20 ± 0.02 a
TT	4.58 ± 0.02 a	14.6 ± 0.05 a	0.19 ± 0.01 a
S15 + U15	4.59 ± 0.01 a	14.6 ± 0.08 a	0.22 ± 0.01 a
S30 + U15	4.60 ± 0.01 a	14.7 ± 0.06 a	0.21 ± 0.02 a
S15 + U30	4.61 ± 0.02 a	14.5 ± 0.05 a	0.21 ± 0.01 a
S30 + U30	4.63 ± 0.01 a	14.5 ± 0.08 a	0.19 ± 0.02 a

[a]Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[b]Control (freshly squeezed or no treatment); TT (thermal treatment); S15 + U15 (combined sonication for 15 min and UV-C treatment for 15 min); S30 + U15 (combined sonication for 30 min and UV-C treatment for 15 min); S15 + U30 (combined sonication for 15 min and UV-C treatment for 30 min); S30 + U30 (combined sonication for 30 min and UV-C treatment for 30 min).

Table 2. Effects of thermal and combined treatment on colour of Chokanan mango juice.[a,b]

	Colour parameters			
Treatment	*L**	*a**	*b**	ΔE
Control	70.28 ± 0.05 a	5.00 ± 0.03 a	54.45 ± 0.04 a	–
TT	72.26 ± 0.11b	3.27 ± 0.11b	51.23 ± 0.15 b	4.16 ± 0.08 a
S15 + U15	70.55 ± 0.15 ac	4.72 ± 0.05 c	53.85 ± 0.03 c	0.72 ± 0.02 b
S30 + U15	70.78 ± 0.05 c	4.89 ± 0.02 ac	53.55 ± 0.08 c	1.04 ± 0.06 c
S15 + U30	70.76 ± 0.12 c	4.64 ± 0.13 c	53.88 ± 0.05 c	0.83 ± 0.12 b c
S30 + U30	71.68 ± 0.07 d	4.46 ± 0.09 d	52.94 ± 0.10 d	2.17 ± 0.10 d

[a]Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[b]Control (freshly squeezed or no treatment); TT (thermal treatment); S15 + U15 (combined sonication for 15 min and UV-C treatment for 15 min); S30 + U15 (combined sonication for 30 min and UV-C treatment for 15 min); S15 + U30 (combined sonication for 15 min and UV-C treatment for 30 min); S30 + U30 (combined sonication for 30 min and UV-C treatment for 30 min).

Table 3. Effects of thermal and combined treatment on clarity, non-enzymatic browning index, HMF, total carotenoid and ascorbic acid content of Chokanan mango juice.[a,b,c]

Treatment	Clarity (transmittance at 660 nm)	NEBI (absorbance at 420 nm)	HMF (mg/L)	Total carotenoid content (μg/100 mL)	Ascorbic acid content (mg/100 mL)
Control	25.50 ± 0.22 a	0.06 ± 0.00 a	0.60 ± 0.03 a	82.03 ± 1.29 a	8.91 ± 0.51 a
TT	7.30 ± 0.14 b	0.13 ± 0.01 b	1.12 ± 0.05 b	48.92 ± 1.32 b	3.10 ± 0.62 b
S15 + U15	27.40 ± 0.22 c	0.07 ± 0.00 a	0.67 ± 0.02 a	90.60 ± 1.52 c d	7.92 ± 0.38 c
S30 + U15	28.55 ± 0.14 d	0.07 ± 0.01 a	0.71 ± 0.05 a	93.65 ± 2.10 d	7.72 ± 0.11 c
S15 + U30	26.02 ± 0.35 e	0.07 ± 0.01 a	0.69 ± 0.02 a	88.29 ± 1.43 c	7.88 ± 0.29 c
S30 + U30	27.64 ± 0.12 c	0.11 ± 0.00 c	0.98 ± 0.07 b	94.70 ± 1.80 d	6.33 ± 0.55 d

[a]Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[b]HMF: 5-hydroxymethyl furfural; NEBI: non-enzymatic browning index.
[c]Control (freshly squeezed or no treatment); TT (thermal treatment); S15 + U15 (combined sonication for 15 min and UV-C treatment for 15 min); S30 + U15 (combined sonication for 30 min and UV-C treatment for 15 min); S15 + U30 (combined sonication for 15 min and UV-C treatment for 30 min); S30 + U30 (combined sonication for 30 min and UV-C treatment for 30 min).

compared to the control (25.50), thus indicating the highest percentage of turbidity. This could be explained by the properties of heat, wherein particle sizes become larger, resulting in protein coagulation and negatively altering the rheology of juice samples (Aguiló-Aguayo et al., 2009). In contrast, significant improvement in clarity was observed in all combined treated samples. The highest increase in clarity (12%) was exhibited by S30 + U15 sample. In addition, there is an increasing trend in clarity corresponding to sonication time. This observation is mainly due to implosion shock waves during cavitation that breakdown macromolecules and colloidal particles, thus reducing the viscosity of juice and improving the clarity (Abid et al., 2013).

The NEBI indicates browning of juice due to Maillard process (Caminiti et al., 2011), while HMF is one of the intermediates produced during this reaction (Rattanathanalerk et al., 2005). A significant increase in NEBI of juice (Table 3) subjected to thermal treatment was observed. Accordingly, HMF content also increased in thermal-treated samples (1.12 mg/L) when compared to the control (0.60 mg/L). This is a clear indication that heat accelerated the formation of browning compounds, consequently darkening the juice thus contributing to the highest increase in NEBI value and HMF. Likewise, enhanced browning index and HMF formation were observed in heat-treated strawberry juice (Aguiló-Aguayo et al., 2009). The results of NEBI and HMF agree with colour analysis for thermal-treated samples, whereby the decrease in $+b^*$ and increase in L^* values denoted the colour change from yellow towards light brown. Conversely, combined treated samples, S15 + U15, S30 + U15 and S15 + U30, except S30 + U30, showed no significant variation in NEBI and HMF content, when compared to the control. This is consistent with the study conducted by Caminiti et al. (2011) on apple and cranberry juice blend subjected to combined non–thermal-processing methods, where no significant browning was promoted. Nevertheless, it is important to highlight that the concentration of HMF in all treated juice samples (thermal and combined method) were minimal and remained below the permitted limit ($\leq$ 5 mg/L) by the Association of the Industry of Juices and Nectars from Fruits and Vegetables (AIJN, 1996).

Total carotenoid content

Juice samples subjected to thermal treatment exhibited significant loss of carotenoids (48.92 µg/100 mL) when compared to the control (82.03 µg/100 mL), as shown in Table 3. This could be attributed to the high temperature-induced degradation of main carotenoid pigments resulting in the rearrangement of structures (*trans*- to *cis*-form) and formation of epoxides (Rodríguez-Amaya, 1997). After combined treatment, juice samples showed significant enhancement (8 to 15%) in carotenoid content. S30 + U30 sample showed the highest increase in extractability of carotenoids (15%) when compared to the control. The modification of carotenoid-binding protein may improve the extraction yield of free carotenoids (Oms-Oliu et al., 2012). These phenomena were due to the sonochemical and UV photochemical reaction as a result of ultrasonic and UV-C treatment, respectively (Demirdoven and Baysal, 2008; Oms-Oliu et al., 2012). Therefore, the increased availability of free carotenoids possessing health promoting properties in combined treated juice samples is beneficial to consumers.

Ascorbic acid content

Ascorbic acid or commonly known as vitamin C possesses antioxidant properties that have been associated with protection against cancer (Block, 1991). There was significant reduction in ascorbic acid content in juice processed by thermal and combined method when compared to control (Table 3). For thermal-treated juice, a maximum reduction (65%) of ascorbic acid was exhibited when compared to the control. Similarly, significant loss of ascorbic acid was observed in thermal-processed orange juice, owing to its thermolabile characteristic (Pala and Toklucu, 2013). The least degradation of ascorbic acid after combined treatment was about 11% in S15 + U15 sample. The depletion of ascorbic acid occurs mainly due to oxidation induced by enzyme activities such as ascorbate oxidase and peroxidase (Oms-Oliu et al., 2012). Formation of hydroxyl radicals during UV photon generation and also by bubble implosion during sonication could be responsible for the decreased content of ascorbic acid (Bhat et al., 2011; Hart and Henglein, 1985). Minimal degradation of ascorbic acid content was observed in ultrasonic or UV-C-treated juices when compared to thermal treatment (Goh et al., 2012; Oms-Oliu et al., 2012). Hence, it is important to emphasise that combined treatment provides better retention of ascorbic acid when compared to thermal treatment due to the absence of heat supply.

Antioxidant activity (total polyphenol and flavonoid content, DPPH radical scavenging and reducing power assay)

The effects of treatments (thermal and combined method) on antioxidant activity in juice extracts are shown in Table 4. After thermal treatment, a significant reduction in total polyphenols and total flavonoids was observed. In contrast, a significant increase in

Table 4. Effects of thermal and combined treatment on antioxidant activity of Chokanan mango juice.[a,b,c]

Treatment	Total polyphenol content (mg GAE/100 mL)	Total flavonoid content (mg CE/100 mL)	DPPH radical scavenging activity (μg AAE/mL)	Reducing power (μg AAE/mL)
Control	97.83 ± 1.76 a	8.74 ± 0.12 a	8.29 ± 0.18 a	360.71 ± 3.20 a
TT	60.87 ± 1.42 b	6.52 ± 0.15 b	8.09 ± 0.15 a	345.10 ± 6.10 a
S15 + U15	120.46 ± 1.08 c	11.24 ± 0.19 c	8.55 ± 0.13 b	442.14 ± 4.12 b
S30 + U15	134.01 ± 1.53d	11.51 ± 0.07 c	9.01 ± 0.22 c	454.29 ± 2.87 c
S15 + U30	129.32 ± 1.38e	11.76 ± 0.23 c	8.63 ± 0.11 b	450.43 ± 5.15 c
S30 + U30	127.95 ± 1.87e	11.79± 0.25 c	8.24 ± 0.20 a	458.66 ± 6.11 c

[a]Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 9$).
[b]AAE: ascorbic acid equivalent; CE: catechin equivalent; GAE: gallic acid equivalent.
[c]Control (freshly squeezed or no treatment); TT (thermal treatment); S15 + U15 (combined sonication for 15 min and UV-C treatment for 15 min); S30 + U15 (combined sonication for 30 min and UV-C treatment for 15 min); S15 + U30 (combined sonication for 15 min and UV-C treatment for 30 min); S30 + U30 (combined sonication for 30 min and UV-C treatment for 30 min).

extraction of polyphenols (23 to 37%) and flavonoids (29 to 35%) was exhibited in all combined treated juice samples when compared to control. The highest increase (37%) was from 97.83 mg GAE/100 mL to 134.01 mg GAE/100 mL in S30 + U15 sample for phenolic compounds. With regards to total flavonoids, the highest increase was for S30 + U30 sample (11.79 mg CE/100 mL), when compared to control (8.74 mg CE/100 mL). These observations are in agreement with a study by Abid et al. (2013), reporting significant increase in extractability of phenolic compounds and flavonoids in sonicated apple juice. The free radical generated by cavitation reacts with the aromatic ring of polyphenolics, thus contributing to their improvement in juice (Ashokkumar et al., 2008). Besides that, UV-C and ultrasound inactivate polyphenol oxidase enzyme, therefore preventing further loss of phenolic compounds (Bhat et al., 2011; Oms-Oliu et al., 2012). This is consistent with the study conducted by Bhat et al. (2011), where higher concentration of polyphenols and flavonoids was observed in starfruit juice after exposure to UV-C.

Antioxidant activity may be evaluated by the ability to donate hydrogen to the stable-free radical DPPH or the reductive capacity by Fe^{3+} to Fe^{2+} transformation (Prabha and Vasantha, 2011). No significant changes were observed in percentage of DPPH inhibition after thermal treatment. Although thermal-treated juice samples showed a decrease in reducing power (345.10 μg AAE/mL), it was non-significant when compared to the control (360.71 μg AAE/mL). Similarly, Caminiti et al. (2012) reported that heat treatment did not induce significant changes in antioxidant capacity of orange and carrot juice blend. However, combined treated samples (S15 + U15, S30 + U15 and S15 + U30) exhibited significant increase in the percentage of DPPH inhibition compared to control. The highest percentage inhibition of DPPH was 91.4% (9.01 μg AAE/mL) for S30 + U15 sample, compared to the control, 84.1% (8.29 μg AAE/mL). Accordingly, all combined treated samples showed increase in reducing capacity. The maximum increase (27%) was from 360.71 μg AAE/mL to 458.66 μg AAE/mL in S30 + U30 sample. Previous studies have reported that non–thermal-processing methods increase extractability of antioxidants, such as observed in sonicated apple juice and UV-C-treated starfruit juice (Abid et al., 2013; Bhat et al., 2011). Therefore, combined treated samples with enhanced antioxidant capacity will be an added advantage to health conscious consumers, as it plays a role in prevention of the degenerative processes (Block et al., 1992).

Microbial inactivation analysis (coliform count, aerobic plate count, total yeast and mould count)

Aerobic bacteria, coliform, yeast and mould counts in freshly squeezed Chokanan mango juice were 2.74 log CFU/mL, 1.00 log CFU/mL and 2.42 log CFU/mL, respectively. After thermal treatment and combined treatment, juice samples showed significant reduction of microbial count ($p < 0.05$), as shown in Table 5. Thermal treatment completely inactivated aerobic bacteria, coliform, yeast and mould in juice samples. This is consistent with the study conducted by Noci et al. (2008) on heat-treated apple juice, where microbial count was reduced to below detection limit (< 1 log CFU/mL). High temperature causes adverse effects on microbes by denaturing essential proteins and altering cell membrane, eventually inactivating microbial growth. Combined treatment reduced coliform count to below detection limits. For aerobic plate count, S30 + U30 sample exhibited 100% reduction of microbial count when compared to other combined treated samples, S15 + U15 (46%), S30 + U15 and S15 + U30

Table 5. Effects of thermal and combined treatment on microbial inactivation analysis of Chokanan mango juice.[a,b,c]

Treatment	Coliform count (log CFU/mL)	Aerobic plate count (log CFU/mL)	Yeast and mould count (log CFU/mL)
Control	1.00 ± 0.02a	2.74 ± 0.01a	2.42 ± 0.02a
TT	NDb	NDb	NDb
S15 + U15	NDb	1.48 ± 0.02c	1.84 ± 0.05c
S30 + U15	NDb	1.00 ± 0.08d	1.50 ± 0.03d
S15 + U30	NDb	1.00 ± 0.02d	1.39 ± 0.03e
S30 + U30	NDb	NDb	1.00 ± 0.05f

[a]Values followed by different letters within the same column are significantly different ($p < 0.05$) ($n = 6$).
[b]CFU, colony-forming unit; ND, not detected.
[c]Control (freshly squeezed or no treatment); TT (thermal treatment); S15 + U15 (combined sonication for 15 min and UV-C treatment for 15 min); S30 + U15 (combined sonication for 30 min and UV-C treatment for 15 min); S15 + U30 (combined sonication for 15 min and UV-C treatment for 30 min); S30 + U30 (combined sonication for 30 min and UV-C treatment for 30 min).

(64%). This could be explained by the properties of cavitation bubbles that induce microstreaming and shear stresses with high-localised pressure and temperature, resulting in the disintegration of the microbial cells (Abid et al., 2013). Additionally, absorption of UV light ray causes formation of cross-links between adjoining pyrimidine bases on the same DNA strand, thus exhibiting bactericidal effects (Pala and Toklucu, 2013; Walkling-Ribeiro et al., 2008).

As a hurdle, ultrasound and UV-C improve the rate of sterilisation of juice samples, which may be due to their synergistic effects. Previous studies have reported promising preservation of fruit juices by combined non–thermal-processing methods (Noci et al., 2008; Walkling-Ribeiro et al., 2008). In this study, the percentage of inactivation of total yeast and mould (24 and 59%) was lower than aerobic bacteria (46 to 100%) for combined treated samples, exhibiting maximum inactivation for S30 + U30 sample (59%). It was clearly exhibited that yeast and mould display greater resistance to sonication and UV-C due to difference in size of microbial cells and thickness of cell wall (Pala and Toklucu, 2013). However, complete inactivation of yeast and mould in juice samples was not observed in this study for all combined treated samples. Therefore, this hurdle sequence (ultrasound and UV-C) could be combined with mild heat or pressure treatment to enhance the decontamination efficiency.

CONCLUSIONS

Chokanan mango juice subjected to combined sonication and UV-C treatment (S15 + U15, S30 + U15 and S15 + U30) exhibited significant improvements in clarity, antioxidant capacity and extractability of carotenoids, phenolic compounds and flavonoids, when compared to freshly squeezed juice. In addition, combined treatment showed complete inactivation of coliforms and aerobic bacteria, along with significant reduction in yeast and mould count. Although thermal treatment was effective in completely inactivating microbial growth in juice, significant quality loss was observed. Combination of ultrasound and UV-C, therefore, is a promising hurdle for better retention of quality and a feasible alternative to thermal treatment. Further research work is needed to develop models to optimise the processing variables of this hurdle sequence, thus producing improved quality juice together with safety standards. Consequently, this study provides more attention for positive implementation of combined treatment on a pilot scale.

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REFERENCES

3M Food Safety. (2010). 3M Petrifilm interpretation guide. Available at: http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/ (accessed 2 July 2013).

Abid M, Jabbar S, Wu T, Hashim MM, Hu B, Lei S, et al.. (2013). Effect of ultrasound on different quality parameters of apple juice. *Ultrasonics Sonochemistry* 20: 1182–1187.

Aguiló-Aguayo I, Oms-Oliu G, Soliva-Fortuny R and Martín-Belloso O. (2009). Changes in quality attributes throughout storage of strawberry juice processed by high-intensity pulsed electric fields or heat treatments. *LWT-Food Science and Technology* 42: 813–818.

Ashokkumar M, Sunartio D, Kentish S, Mawson R, Simons L, Vilkhu K, et al.. (2008). Modification of food ingredients by ultrasound to improve functionality: a preliminary

study on a model system. *Innovative Food Science & Emerging Technologies* 9: 155–160.

Association of the Industry of Juices and Nectars from Fruits and Vegetables (AIJN) (1996). *Association of the Industry of Juices and Nectars of the European Economic Community Code of Practice for Evaluation of Fruit and Vegetable Juices*. Brussels: AIJN.

Bae SH and Suh HJ. (2007). Antioxidant activities of five different mulberry cultivars in Korea. *LWT-Food Science and Technology* 40: 955–962.

Bhat R, Ameran SB, Voon HC, Karim AA and Tze LM. (2011). Quality attributes of starfruit (Averrhoa carambola L.) juice treated with ultraviolet radiation. *Food Chemistry* 127: 641–644.

Block G. (1991). Vitamin C and cancer prevention: the epidemiologic evidence. *The American Journal of Clinical Nutrition* 53: 270–282.

Block G, Patterson B and Subar A. (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutrition and Cancer* 18: 1–29.

Caminiti IM, Noci F, Morgan DJ, Cronin DA and Lyng JG. (2012). The effect of pulsed electric fields, ultraviolet light or high intensity light pulses in combination with manothermosonication on selected physico-chemical and sensory attributes of an orange and carrot juice blend. *Food and Bioproducts Processing* 90: 442–448.

Caminiti IM, Noci F, Munoz A, Whyte P, Morgan DJ, Cronin DA, et al.. (2011). Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. *Food Chemistry* 124: 1387–1392.

Cheng LH, Soh CY, Liew SC and Teh FF. (2007). Effects of sonication and carbonation on guava juice quality. *Food Chemistry* 104: 1396–1401.

Cohen E, Birk Y, Mannheim CH and Saguy IS. (1998). A rapid method to monitor quality of apple juice during thermal processing. *Lebensmittel-Wissenschaft & Technologie* 31: 612–616.

Cserhalmi Z, Sass-Kiss A, Tóth-Markus M and Lechner N. (2006). Study of pulsed electric field treated citrus juices. *Innovative Food Science & Emerging Technologies* 7: 49–54.

Demirdoven A and Baysal T. (2008). The use of ultrasound and combined technologies in food preservation. *Food Reviews International* 25: 1–11.

Food and Agriculture Organisation of the United Nation Statistics Division, FAOSTAT (2013). *Food and Agricultural commodities production*. Available at: http://faostat.fao.org/site (accessed 26 July 2013).

Food and Drug Administration (FDA). (2000). Irradiation in the production, processing and handling of food. *Federal Register–Rules and Regulations* 65: 71056–71058.

Glevitzky M, Pop M, Brusturean GA, Bogdan I, Calisevici M and Perju D. (2008). Efficient use of antioxidants to preserve fruit juice. *Revista de Chimie (Bucure^{o}ti)* 59: 1291–1295.

Gogate PR, Sutkar VS and Pandit AB. (2011). Sonochemical reactors: Important design and scale up considerations with a special emphasis on heterogeneous systems. *Chemical Engineering Journal* 166: 1066–1082.

Goh SG, Noranizan M, Leong CM, Sew CC and Sobhi B. (2012). Effect of thermal and ultraviolet treatments on the stability of antioxidant compounds in single strength pineapple juice throughout refrigerated storage. *International Food Research Journal* 19: 1131–1136.

Guerrero-Beltrán JA and Barbosa-Cánovas GV. (2004). Advantages and limitations on processing foods by UV light. *Food Science and Technology International* 17: 137–147.

Hart EJ and Henglein A. (1985). Free radical and free atom reactions in the sonolysis of aqueous iodide and formate solutions. *The Journal of Physical Chemistry* 89: 4342–4347.

Keyser M, Mű ller IA, Cilliers FP, Nel W and Gouws PA. (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innovative Food Science & Emerging Technologies* 9: 348–354.

Lee HS, Castle WS and Coates GA. (2001). High-performance liquid chromatography for the characterization of carotenoids in the new sweet orange (Earlygold) grown in Florida, USA. *Journal of Chromatography A* 913: 371–377.

Leistner L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology* 55: 181–186.

Noci F, Riener J, Walkling-Ribeiro M, Cronin DA, Morgan DJ and Lyng JG. (2008). Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple juice. *Journal of Food Engineering* 85: 141–146.

O'Donnell CP, Tiwari BK, Bourke P and Cullen PJ. (2010). Effect of ultrasonic processing on food enzymes of industrial importance. *Trends in Food Science & Technology* 21: 358–367.

Oms-Oliu G, Odriozola-Serrano I and Martín-Belloso O. (2012). The effects of non-thermal technologies on phytochemicals. In: Rao V (ed.) *Phytochemicals: A Global Perspective of Their Role in Nutrition and Health*. Croatia: InTech, pp. 108–120.

Oyaizu M. (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition* 103: 413–419.

Pala CU and Toklucu AK. (2013). Microbial, physicochemical and sensory properties 606 of UV-C processed orange juice and its microbial stability during refrigerated storage. 607. *LWT-Food Science and Technology* 50: 426–431.

Prabha MR and Vasantha K. (2011). Antioxidant, cytotoxicity and polyphenolic content of Calotropis procera (Ait.) R. Br. Flowers. *Journal of Applied Pharmaceutical Science* 1: 136–140.

Ranganna S. (1977). Chapter 5: ascorbic acid. In: Ranganna S (ed.) *Manual of Analysis of Fruit and Vegetable Products*. New Dehli: Tata McGrawHill Publishing Company, pp. 94–96.

Rattanathanalerk M, Chiewchan N and Srichumpoung W. (2005). Effect of thermal processing on the quality loss of pineapple juice. *Journal of Food Engineering* 66: 259–265.

Rawson A, Patras A, Tiwari BK, Noci F, Koutchma T and Brunton N. (2011). Effect of thermal and non-thermal processing technologies on the bioactive content of exotic fruits and their products: review of recent advances. *Food Research International* 44: 1875–1887.

Rivera AP and Cabornida IMB. (2008). Development of ready-to-drink green mango juice. *University of Southern Mindanao R&D Journal* 16: 71–77.

Rodríguez-Amaya DB. (1997). *Carotenoids and Food Preparation: The Retention of Provitamin A Carotenoids in Prepared, Processed and Stored Foods*. Washington, DC: OMNI.

Sadler GD and Murphy PA. (2010). pH and titratable acidity. In: Nielsen SS (ed.) *Food Analysis*. New York: Springer, pp. 231–233.

Sakanaka S, Tachibana Y and Okada Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chemistry* 89: 569–575.

Salleh-Mack SZ and Roberts JS. (2007). Ultrasound pasteurization: the effects of temperature, soluble solids, organic acids and pH on the inactivation of Escherichia coli ATCC 25922. *Ultrasonics Sonochemistry* 14: 323–329.

Santhirasegaram V, Razali Z and Somasundram C. (2013). Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. *Ultrasonics Sonochemistry* 20: 1276–1282.

Scott KJ. (2001). Detection and measurement of carotenoids by UV/VIS spectrophotometry. In: Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DA, Schwartz SJ, et al. (eds) *Current Protocols in Food Analytical Chemistry*. New York: John Wiley and Sons Inc, pp. F2.2.1–F2.2.10.

Singleton VL, Joseph A and Rossi JRJA. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* 16: 144–158.

Spreer W, Ongprasert S, Hegele M, Wunsche JN and Muller J. (2009). Yield and fruit development in mango (Mangifera indica L. cv. Chok Anan) under different irrigation regimes. *Agricultural Waste Management* 96: 574–584.

Sutkar VS and Gogate PR. (2009). Design aspects of sonochemical reactors: techniques for understanding cavitational activity distribution and effect of operating parameters. *Chemical Engineering Journal* 55: 26–36.

Tiwari BK, Muthukumarappan K, O'Donnell CP and Cullen PJ. (2008). Color degradation and quality parameters of sonicated orange juice using response surface methodology. *LWT-Food Science and Technology* 41: 1876–1883.

Walkling-Ribeiro M, Noci F, Cronin DA, Riener J, Lyng JG and Morgan DJ. (2008). Reduction of Staphylococcus aureus and quality changes in apple juice processed by ultraviolet irradiation, pre-heating and pulsed electric fields. *Journal of Food Engineering* 89: 267–273.

Effects of Ultraviolet Light (UV-C) and Heat Treatment on the Quality of Fresh-Cut Chokanan Mango and Josephine Pineapple

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Abstract: The effects of ultraviolet (UV-C) and medium heat (70 °C) treatments on the quality of fresh-cut Chokanan mango and Josephine pineapple were investigated. Quality attributes included physicochemical properties (pH, titratable acidity, and total soluble solids), ascorbic acid content (vitamin C), antioxidant activity, as well as microbial inactivation. Consumers' acceptance was also investigated through sensory evaluation of the attributes (appearance, texture, aroma and taste). Furthermore, shelf-life study of samples stored at 4 ± 1 °C was conducted for 15 d. The fresh-cut fruits were exposed to UV-C for 0, 15, 30, and 60 min while heat treatments were carried out at 70 °C for 0, 5, 10 and 20 min. Both UV-C and medium heat treatments resulted in no significant changes to the physicochemical attributes of both fruits. The ascorbic acid content of UV-C treated fruits was unaffected; however, medium heat treatment resulted in deterioration of ascorbic acids in both fruits. The antioxidants were enhanced with UV-C treatment which could prove invaluable to consumers. Heat treatments on the other hand resulted in decreased antioxidant activities. Microbial count in both fruits was significantly reduced by both treatments. The shelf life of the fresh-cut fruits were also successfully extended to a maximum of 15 d following treatments. As for consumers' acceptance, UV-C treated fruits were the most accepted as compared to their heat-treated counterparts. The results obtained through this study support the use of UV-C treatment for better retention of quality, effective microbial inactivation and enhancement of health promoting compounds for the benefit of consumers.

Keywords: antioxidant activity, consumer acceptance, heat treatment, UV-C treatment

Practical Application: The results of this study suggest that UV-C and medium heat treatment are feasible treatments for shelf-life extension of fresh-cut fruits. UV-C treatment provides better retention of quality and has the capability to enhance antioxidant activities. Both treatments were successful in extension of shelf life to a maximum of 15 d. The ability of UV-C treatment to retain quality and enhance antioxidant activity in fruits could be well exploited for the benefit of consumers. Combination of UV-C and medium heat treatment with various packaging methods could be further exploited for the benefit of the fresh-cut industry.

Introduction

The increasing health consciousness as well as the growing interest in the role of food in maintaining and improving human well-being and health has led to the increase in fruit consumption. According to epidemiologic studies, there is an inverse relationship between the intake of fruits and vegetables and the risk of developing diseases such as cancers, hypertension as well as cardiovascular diseases (Liu and others 2000; Hung and others 2004; He and others 2007; Bhupathiraju and others 2013). Furthermore, current consumers' profile of increased consumption of readily available food as compared to cooked food has led to the demand for fresh cut as well as processed fruit products. However, a major problem that is faced by the industry is the limited shelf life which is largely a result of rapid deterioration caused by microbial growth as well as physiological disorders (Das and Kim 2010).

Current preservation techniques employed by the fresh-cut industry such as antioxidants, chlorine and modified atmosphere packaging which is known for its inadequacy has led to the development of alternative methods for preservation. Based on previous reports, it is evident that heat treatment is an effective means for preservation due to its efficacy in reducing microbial growth in fresh-cut fruits and vegetables (Li and others 2001; Klaiber and others 2005; Aguayo and others 2008). Drying at elevated temperatures and immersion of horticultural crops in hot water baths has been observed to reduce prevalence of storage diseases (Loaiza-Velarde and others 1997; Delaquis and others 1999; Loaiza-Velarde and others 2003). Unfortunately, these processes result in adverse effects on the organoleptic, textural and nutritional qualities and hence, the need for technologies which are less degradative. An ideal alternative should be effective in the inactivation of enzymes and vegetative micro-organisms while having minimal deleterious effects on food quality attributes (for example color, flavor, and nutritional value). Minimal heat treatment or nonthermal treatment could prove to be better alternatives for preservation of fresh-cut fruits whilst minimizing degradation of its quality.

Shortwave ultraviolet light, 200 to 280 nm (UV-C) radiation has been reported to be successful in reducing the number of surface microorganisms of fresh and cut fruits and vegetables (Yaun

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2004; Fonseca and Rushing, 2006; Shama 2006; Rodoni and others 2012). Although the effects of UV-C treatments on fruit juices have been widely studied, studies on its effect on fresh-cut fruits remain scarce. The UV technology can be used as an alternative to thermal treatment or the application of antimicrobial compounds whereby a better retention of quality of fruits can be achieved (Gomez and others 2010). The major advantages of using UV-C treatment are that it does not require extensive safety equipments and does not leave residues (Yaun and others 2004). The potential for commercial use of UV-C light in minimal processing of fruits is dependent on its ability to contribute to the food safety while minimizing undesirable changes in quality. Accordingly, the use of UV-C treatment and heat treatment, alone or in combination, could prove invaluable in minimizing quality losses while extending shelf life during storage of fresh-cut produce (Allende and Artes 2003).

The purpose of this study was to investigate the effect of UV-C treatment and medium heat treatment (70 °C) on the quality of fresh-cut "Chokanan" mangoes and "Josephine" pineapples. The quality attributes include physicochemical analysis, ascorbic acid content, antioxidant activity, microbial inactivation as well as shelf-life studies. Taking into consideration that the fresh-cut industry is a consumer-based industry; the consumers' acceptance test was carried out to ensure that besides successful retention of quality and extension of shelf life, the differently treated fresh-cut fruits were also acceptable to its consumers.

Materials and Methods

Sample preparation

Mature mango (*Mangifera indica* L. cv. Chokanan) and pineapple (*Ananas Comosus* L. Merr. Josephine) fruits free from external defects were harvested from a mango plantation in Kangar, Perlis, and a pineapple plantation in Batu Pahat, Johor. The fruits were rinsed under running water, dipped in a 5% benomyl solution for 1 min, rinsed again with water, air dried, and left to ripen at room temperature (25 ± 2 °C). Fruits were peeled, cleaned, and thinly sliced (5 mm) with the aid of a dial thickness gauge (Mitutoyo MI 7305, Kawasaki, Japan). The fruits were then cut into uniform discs with a borer (diameter of 2 cm). The uniform sample discs were used for subsequent food processing.

UV-C treatment

Twelve fruit disc samples were placed on sterilized Petri dishes (15 × 100 mm; 10 mm depth) and exposed to a UV light (germicidal fluorescent lamp with a peak emission of 254 nm, Biological Safety Cabinet Class II, 240 V, 50 Hz, 10A) with a distance of 15 cm from the lamp to the surface of the samples. The samples were exposed to UV-C for 0, 15, 30, and 60 min. Ten grams of treated fruit discs were mashed into a paste using a mortar and pestle, for quality analysis.

Heat treatment

Twelve fruit disc samples were placed on sterilized Petri dishes (15 × 100 mm; 10 mm depth) and heat treated at 70 °C in an oven for 5, 10 and 20 min. Ten grams of treated fruit discs were mashed into a paste using a mortar and pestle, for quality analysis.

Physicochemical analysis (pH, total soluble solids [TSS] and titratable acidity [TA])

Following treatments, physiochemical analysis were carried out. The pH of samples was determined using a pH meter (Hanna Microprocessor pH 211, Italy) at 25 ± 1 °C. TSS were determined using a digital refractometer (Atago PR-1 digital refractometer, Tokyo, Japan) at 25 ± 1 °C according to the manufacturer's protocol and results were expressed in standard °Brix unit. As for the determination of TA, sample paste was diluted with ddH_2O and titrated with standardized 0.1 N sodium hydroxide to a definite faint pink end point (color should persist for ≥15 s) using phenolphthalein as an indicator. The volume of sodium hydroxide used for titration was converted to grams of citric acid per 100 g of fruit and the %TA was calculated according to Sadler and Murphy (2010).

Ascorbic acid content

The ascorbic acid content in samples was determined based on the 2,6-dichlorophenol-indophenol (DCPIP) visual titration method (Ranganna 1977). Ascorbic acid was extracted from 5 g of sample paste using 3% metaphosphoric acid. The extract was then filtered with Whatman No. 1 filter paper and the resulting filtrate was titrated with a standardized dye solution (2,6-dichloroindophenol-indophenol and sodium bicarbonate) to a pink end point (color should persist for at least 15 s). The results obtained were expressed as milligrams of ascorbic acid per 100 g sample.

Total polyphenol content

Total polyphenol content of the samples was determined using Folin–Ciocalteu assay modified to a microscale (Bae and Suh 2007). Crude extracts were prepared according to the method developed by Xu and others (2008) using 90% acetone with a sample to acetone ratio of 1:1. The resulting extract was then centrifuged at 5000 rpm (7462 × *g*) for 20 min at 5 °C using a Beckman J2-MI refrigerated centrifuge. The supernatant was saved as crude extract. Following extraction, 0.79 mL of ddH_2O, 0.01 mL sample or standard solution of gallic acid and 0.05 mL Follin-Ciocalteu reagent was added into a 1.5 mL Eppendorf tube and mixed. After exactly 1 min, 0.15 mL of sodium carbonate was added to the solution, mixed well and allowed to stand at room temperature (25 °C) for 2 h. The absorbance of the samples and gallic acid standards were measured at a wavelength of 750 nm using a Shimadzu MRC UV-200-RS spectrophotometer. A standard curve of gallic acid ($y = 0.0056x$, $R^2 = 0.9955$) was prepared and results were reported as milligrams of gallic acid equivalent per 100 g of fruit extract.

Total antioxidant capacity

Total Antioxidant Capacity was evaluated by the phosphomolybdenum method according to Prieto and others (1999). At different concentration, methanol extracts were prepared in water and combined in an Eppendorf tube with 1 mL of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, 4 mM ammonium molybdate mixture). The tubes were incubated for 90 min at 95 °C. The mixture was cooled to room temperature and the absorbance was read at 695 nm against blank. A standard curve of ascorbic acid ($y = 0.0018x$, $R^2 = 0.9923$) was prepared and results were reported as micrograms of ascorbic acid equivalent per g fruit sample.

DPPH radical scavenging assay

The DPPH assay is based on the method described by Bae and Suh (2007). A 0.1 mM DPPH solution (Sigma, U.S.A.) was prepared in 80% methanol, and then 1ml of this solution was added

Table 1–Effects of UV-C and medium heat (70 °C) on physicochemical analysis (pH, total soluble solids, titratable acidity) of Chokanan mango and Josephine pineapple. Values followed by different letters within the same column are significantly different for each fruit ($P < 0.05$; $n = 9$).

Treatments	Fruit	Samples	pH	Total soluble solids (°Brix)	Titratable acidity (%)
UV-C	Mango	Control	4.68 ± 0.04[a]	13.44 ± 0.73[a]	0.30 ± 0.01[a]
		15 min	4.67 ± 0.05[a]	13.72 ± 0.8[a]	0.28 ± 0.01[a]
		30 min	4.59 ± 0.04[a]	12.64 ± 0.83[a]	0.29 ± 0.02[a]
		60 min	4.64 ± 0.05[a]	12.52 ± 0.78[a]	0.30 ± 0.01[a]
	Pineapple	Control	3.98 ± 0.05[a]	12.72 ± 0.63[a]	0.84 ± 0.02[a]
		15 min	4.02 ± 0.06[a]	12.26 ± 0.73[a]	0.82 ± 0.02[a]
		30 min	4.00 ± 0.05[a]	12.46 ± 0.68[a]	0.83 ± 0.02[a]
		60 min	4.03 ± 0.06[a]	12.59 ± 0.66[a]	0.83 ± 0.01[a]
Medium heat (70 °C)	Mango	Control	4.80 ± 0.04[a]	16.7 ± 0.24[a]	0.21 ± 0.01[a]
		5 min	4.83 ± 0.03[a]	16.9 ± 0.28[a]	0.20 ± 0.01[a]
		10 min	4.88 ± 0.02[a]	17.2 ± 0.31[a]	0.19 ± 0.01[a]
		20 min	4.92 ± 0.02[a]	17.6 ± 0.33[a]	0.19 ± 0.01[a]
	Pineapple	Control	3.77 ± 0.04[a]	13.6 ± 0.50[a]	0.86 ± 0.02[a]
		5 min	3.77 ± 0.03[a]	14.6 ± 0.53[a]	0.85 ± 0.02[a]
		10 min	3.74 ± 0.02[a]	15.3 ± 0.42[ab]	0.84 ± 0.02[a]
		20 min	3.74 ± 0.03[a]	15.7 ± 0.36[b]	0.82 ± 0.02[a]

to 500 μL of samples. Results were reported as% inhibition. The radical scavenging activity was calculated accordingly:

$$\% \text{ DPPH inhibition} = (A_{control} - A_{sample}/A_{control}) \times 100$$

where $A_{control}$ is absorbance reading of control and A_{sample} is absorbance reading of the sample.

Microbial inactivation analysis (total plate counts of aerobic bacteria, yeast, and mold count)

The 3M Petrifilm plate methods are recognized as AOAC Intl. Official Methods of Analysis (3M Food Safety 2010). Microbial count of samples were determined using Petrifilm plates (3M Center, Minn., U.S.A.) for aerobic bacteria, yeast, and mold according to Santhirasegaram and others (2013) and were calculated as colony forming units. The results were expressed as log (CFU/mL).

Sensory analysis

A> panel of 90 untrained panelists was used to evaluate consumer's acceptance of the UV-C and heat-treated fruit samples. Fruit samples were cut into bite-size pieces and placed in glass custard cups and covered with 125 mm watch glasses. Watch glasses allow the headspace to trap volatiles for sniffing. Panelists were asked to evaluate 4 sets of samples with a 10 min break between sets to minimize carryover tastes. Each set composed of 5 samples. The samples were evaluated individually in partitioned booths under fluorescent light at room temperature. Acceptance test were performed using a 1 to 9 hedonic scale (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely) to evaluate acceptability of the treated fruits' attributes (appearance, texture, aroma, and taste/flavor). The panelists were required to cleanse their palate with lime juice between samples (Bayarri and others 2006).

Statistical analysis

The data obtained were subjected to statistical analysis using SPSS 19.0 software (SPSS Inc., IBM). In this study, data were represented as mean values ± standard error (SE) for 9 batches ($n = 9$). The significant differences between mean values of samples were determined by analysis of variance (one-way ANOVA) using Tukey's Honestly Significant Difference test at a significance level of $P < 0.05$.

Results and Discussion

Physicochemical (pH, TSS, and TA)

The changes in pH of mangoes and pineapples post-UV-C and medium heat (70 °C) are shown in Table 1. There were no significant changes in pH observed in any of the fruit samples following UV-C treatment and mild heat treatment as compared to untreated samples ($P < 0.05$). The pH of fruits

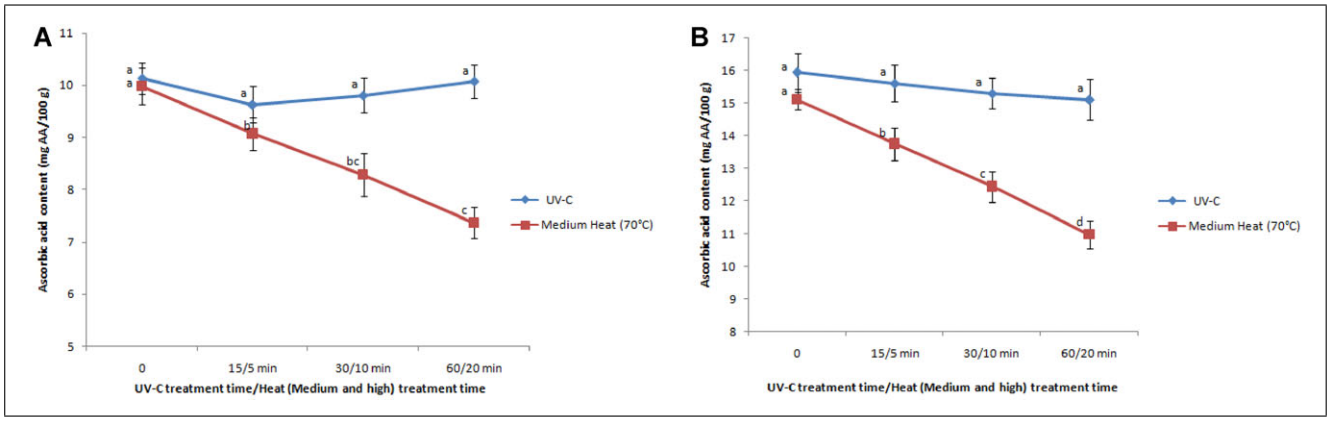

Figure 1–Effects of UV-C and medium heat (70 °C) on ascorbic acid content of (A) Chokanan mango and (B) Josephine pineapple. Values followed by different letters within the same line are significantly different ($P < 0.05$; $n = 9$).

Table 2–Effects of UV-C and medium heat (70 °C) on antioxidant activity of Chokanan mango and Josephine pineapple. Values followed by different letters within the same column are significantly different for each fruit ($P < 0.05$; $n = 9$).

Treatments	Fruit	Samples	Total polyphenol content (mg GAE/100 mL)	Total antioxidant capacity (mg AAE/g)	DPPH radical scavenging activity (% inhibition)
UV-C	Mango	Control	77.89 ± 2.04^{a}	951.67 ± 3.49^{a}	94.63 ± 0.35^{a}
		15 min	79.42 ± 2.03^{ab}	975 ± 3.18^{b}	95.95 ± 0.30^{b}
		30 min	79.46 ± 2.09^{ab}	983.33 ± 2.99^{c}	96.87 ± 0.25^{c}
		60 min	80.91 ± 2.05^{b}	1082.67 ± 3.98^{c}	96.66 ± 0.20^{c}
	Pineapple	Control	39.87 ± 2.02^{a}	1005 ± 3.09^{a}	90.98 ± 0.25^{a}
		15 min	40.37 ± 2.09^{a}	1111.11 ± 2.98^{b}	92.60 ± 0.35^{b}
		30 min	41.16 ± 2.01^{ab}	1136.67 ± 3.17^{c}	94.10 ± 0.30^{c}
		60 min	42.67 ± 2.08^{b}	1174.34 ± 3.23^{c}	94.23 ± 0.40^{c}
Medium heat (70 °C)	Mango	Control	80.97 ± 2.67^{a}	951.67 ± 3.45^{a}	95.30 ± 0.30^{a}
		5 min	77.03 ± 1.94^{a}	950.56 ± 3.33^{a}	94.90 ± 0.35^{a}
		10 min	75.68 ± 1.99^{ab}	833.34 ± 1.23^{b}	93.78 ±0.20^{b}
		20 min	71.97 ± 2.49^{b}	821.12 ± 2.87^{c}	90.74 ± 0.45^{c}
	Pineapple	Control	40.39 ± 1.93^{a}	1196.67 ± 1.98^{a}	90.17 ± 0.30^{a}
		5 min	37.94 ± 1.90^{a}	1136.12 ± 2.34^{b}	88.95 ± 0.30^{b}
		10 min	35.07 ± 1.29^{ab}	1111.12 ± 3.17^{c}	87.53 ± 0.35^{c}
		20 min	32.27 ± 1.18^{b}	1021.12 ± 3.15^{d}	85.50 ± 0.45^{d}

Table 3–Effects of UV-C and medium heat (70 °C) on microbial inactivation of Chokanan mango and Josephine pineapple. Values followed by different letters within the same column are significantly different ($P < 0.05$; $n = 9$).

Treatments	Fruit	Samples	Aerobic plate count (log CFU/mL)	Yeast and mold count (log CFU/mL)
UV-C	Mango	Control	3.92 ± 0.02^{a}	3.82 ± 0.01^{a}
		15 min	3.33 ± 0.01^{b}	3.43 ± 0.01^{b}
		30 min	3.11 ± 0.02^{c}	3.05 ± 0.02^{c}
		60 min	2.85 ± 0.03^{d}	2.69 ± 0.01^{d}
	Pineapple	Control	3.51 ± 0.01^{a}	3.62 ± 0.01^{a}
		15 min	3.02 ± 0.03^{b}	3.31 ± 0.02^{b}
		30 min	2.75 ± 0.02^{c}	2.95 ± 0.01^{c}
		60 min	2.21 ± 0.04^{d}	2.22 ± 0.01^{d}
Medium heat (70 °C)	Mango	Control	3.91 ± 0.01^{a}	3.82 ± 0.02^{a}
		5 min	3.71 ± 0.02^{b}	3.23 ± 0.02^{b}
		10 min	3.56 ± 0.03^{c}	2.98 ± 0.02^{c}
		20 min	3.19 ± 0.02^{d}	2.55 ± 0.01^{d}
	Pineapple	Control	3.55 ± 0.01^{a}	3.62 ± 0.02^{a}
		5 min	3.22 ± 0.03^{b}	3.20 ± 0.01^{b}
		10 min	2.99 ± 0.04^{c}	3.00 ± 0.02^{c}
		20 min	2.56 ± 0.03^{d}	2.40 ± 0.01^{d}

can be used as reliable indicators to evaluate the overall quality of the fruits. Maintaining the pH of fruits has been proven to be invaluable in prolonging shelf life (Anthon and others 2011). The findings of this study pertaining UV-C treatment on pH were similar to those of previous research on kiwifruits (Bal and Kok 2009).

As shown in Table 1, no significant changes in the TSS of mangoes and pineapples were observed after UV-C treatment. As for medium heat treatment, increases of up to 15% in pineapples were observed while no change was observed in mangoes. TSS is used to indicate the percentage of soluble solid and is one of the important factors for grading the quality of fruits (McAllister 1980). According to Davies and Hobson (1981), an estimated half of the water soluble portion of the fruit dry matter is in the form of the reducing sugars fructose (25%) and glucose (22%). Furthermore, previous research on kiwi fruit suggests that TSS are often associated with the eating quality of ripe fruits (Mitchell and others 1991). In addition, it has also been established that fruits with higher TSS contents are generally more preferred by consumers (Rossiter and others 2000; Burdon and others 2004). In the present research, increases were seen in heat-treated samples. An increase in TSS of fruits post heat treatment may be attributed to hydrolysis of polysaccharides (Bal 2012). In addition, heat increases the solubility of certain solids which in turn may have led to the increases of TSS observed in heat-treated samples.

There were no significant changes observed in the TA of both the UV-C and medium heat-treated fruits. In most fruits, TA is responsible for the distinct taste and flavors (Yamaki 1989) and hence, is also a reliable indicator to evaluate the overall quality of fruits (Anthon and others 2011). Results observed in the present study for UV-C treated fruits were similar to those of previous researches on blueberry and persimmon (Perkins-Veazie and others 2008; Khademi and Zamani 2013).

Ascorbic acid content

Consumers' awareness of the importance of vitamin C for human nutrition is on the rise and hence, vitamin C content is considered to be a quality index for fruits. Ascorbic acid (vitamin C) is one of the most abundant antioxidants in plants and is a cofactor of many plant dioxygenases. Loss of Vitamin C can be interpreted as a loss in the fruits' quality (Mahieddine and others 2011). In this study, UV-C irradiation resulted in no significant

changes in ascorbic acid content of both the fruits (mango and pineapple; Figure 1). As for heat treatments of 70 °C, significant changes in the ascorbic acid content were observed. Ascorbic acid content loss of 26% in mangoes and 27% in pineapples was observed in medium heat-treated (70 °C) samples. Decreases in the ascorbic acid content of heat-treated samples are largely due to the fact that ascorbic acid is a heat-sensitive bioactive compound. It is known as the least stable vitamin which is easily destroyed through processing and storage (Ercan and Soysal 2011). Ascorbic acid is also known to degrade by oxidative processes by enzymes which include ascorbate oxidase and peroxidase (Davey and others 2000). Results obtained in this study of mangoes and pineapples are similar to results recorded in heat-treated strawberries and tomatoes (Musto and Satriano 2010; Ercan and Soysal 2011).

Antioxidant activity (total polyphenol content, DPPH radical scavenging activity, and total antioxidant capacity)

Antioxidant activities in fruits are contributed by phenolic compounds. These phenolic compounds due to their biological properties are able to exhibit antioxidant, anti-inflammatory, antiviral,

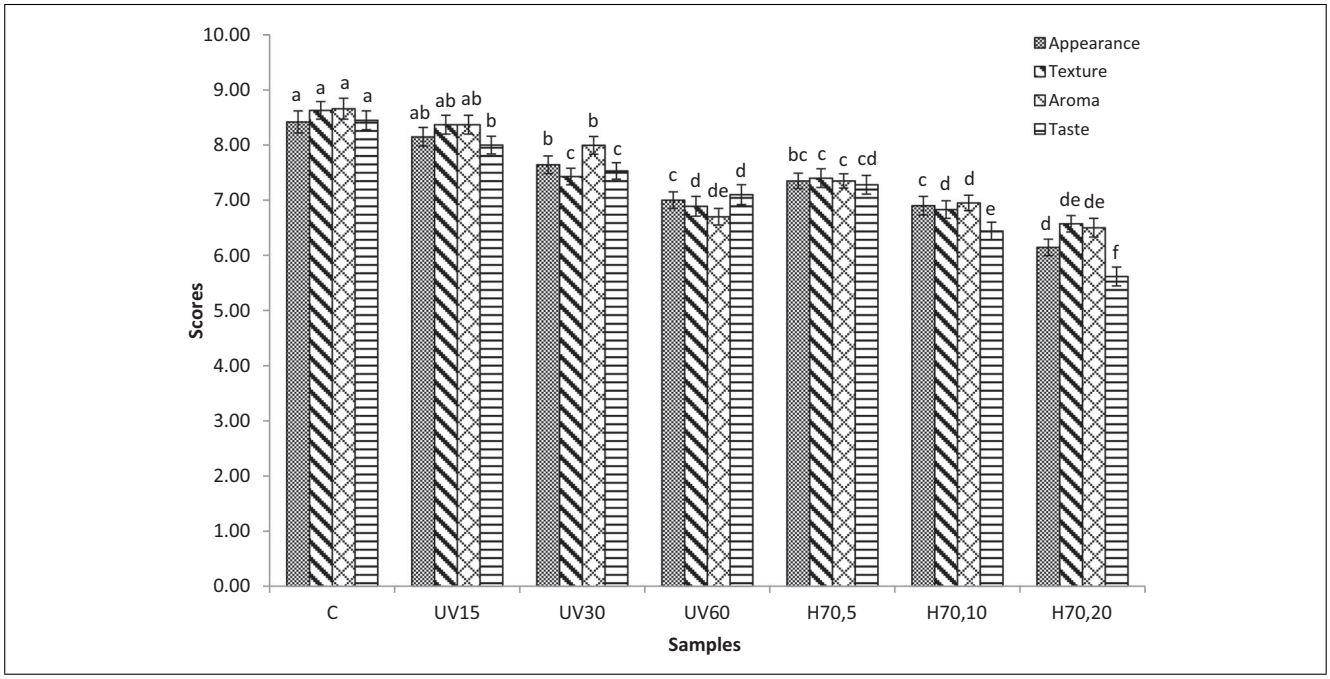

Figure 2–Effects of UV-C and medium heat (70 °C) on sensory evaluation of Chokanan mango. Values followed by different letters for vertical bars of the same pattern are significantly different ($P < 0.05$; $n = 90$). *1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely.

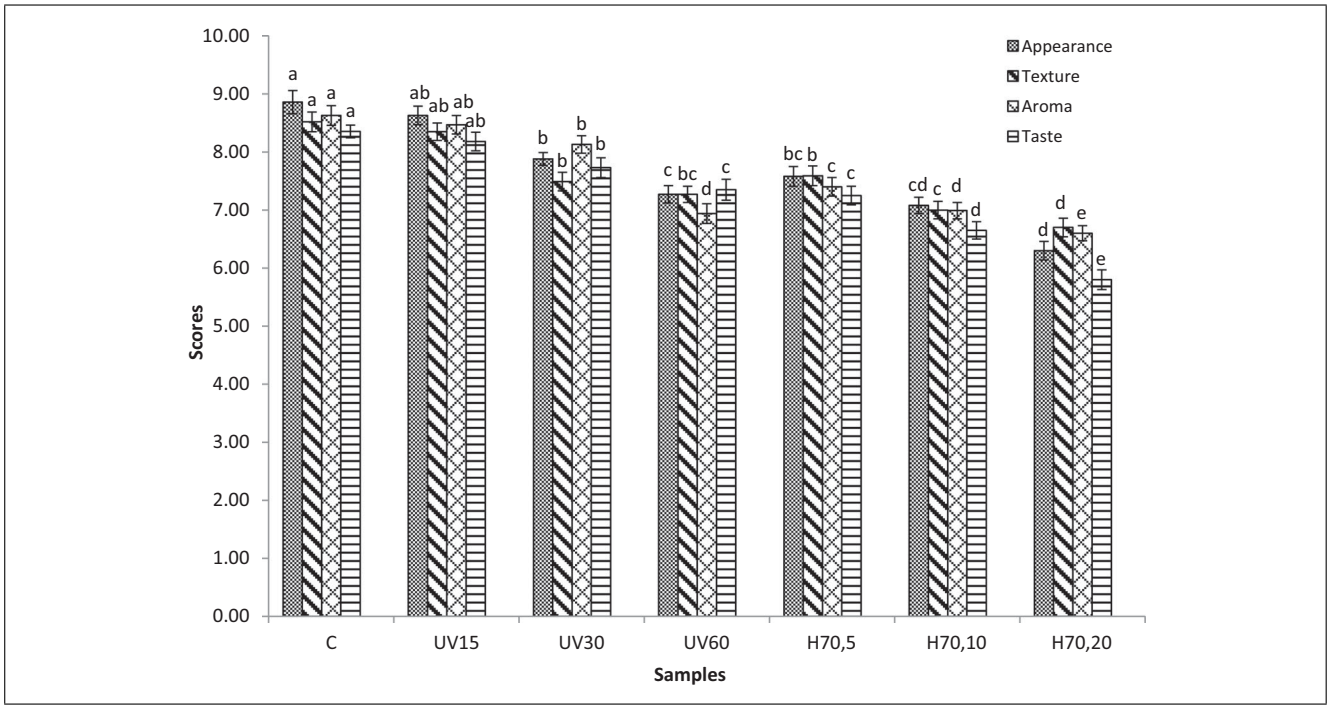

Figure 3–Effects of UV-C and medium heat (70 °C) on sensory evaluation of Josephine pineapple. Values followed by different letters for vertical bars of the same pattern are significantly different ($P < 0.05$; $n = 90$). *1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely.

and anticancer actions (Alothman and others 2009a). The effects of UV-C treatment and medium heat (70 °C) treatment on the polyphenol content are shown in Table 2. The Folin–Ciocalteu method was preferred as it encompasses the detection of phenolic compounds by the reduction of the reagent, which contains tungsten and molybdenum oxides (Waterhouse 2003). According to results, total polyphenols increased as UV-C treatment was carried out for 60 min. An increase of up to 4% in mangoes and up to 7% in pineapples was observed following 60 min of UV-C treatment. Observation for polyphenols following UV-C treatment is similar to those observed in blueberry and pepper (Vicente and others 2005; Perkins-Veazie and others 2008). Heat treatment of 70 °C resulted in decreases of total polyphenol content. Decrease of up to 10% in mangoes and 16% in pineapples were observed in medium heat-treated (70 °C) samples. Thermal treatments have been shown to significantly decrease the concentration of polyphenols in apples (Aguilar-Rosas and others 2007). This reduction in polyphenols of heat-treated fruits is undesirable as polyphenols give added value to fruits.

Total antioxidant capacities of treated samples are shown in Table 2. UV-C treated samples resulted in increases of total antioxidant capacities in both fruits. An increase of up to 14% was observed in mangoes while an increase of up to 17% was observed in pineapples following UV-C irradiation. Medium heat treatments on the other hand resulted in adverse effects to the total antioxidant capacities of both fruits. A decrease of up to 14% was observed in mangoes while a decrease of up to 15% was observed in pineapples following medium heat treatment.

The DPPH radical scavenging assay results are shown in Table 2. The assay is used to measure the hydrogen donating capacity of the antioxidant to the stable free radical DPPH, which results in the formation of diphenylpicrylhydrazine (Shon and others 2003). In this study, UV-C treatments resulted in an increase in DPPH inhibition capacity as compared to controls. An increase of up to 2% was observed in mangoes while an increase of up to 4% was observed in UV-C irradiated pineapples. Conversely, medium heat samples resulted in decreases of DPPH inhibition capacity. A total reduction in DPPH inhibition capacity of up to 5% was observed in both medium heat-treated mangoes and pineapples. The antioxidant activity of UV-C treated samples increased significantly as compared to respective controls as suggested by result of this study. This increase could be due to the formation of free radicals which led to the accumulation of phenolic compounds as a stress response of these fruits against UV-C irradiation. In addition, exposure to UV-C has been linked to increased activity of the enzyme phenylalanine ammonia lyase which contributes to the activation of phenolic biosynthesis pathway, which leads to enhancement of phenolic compounds (Alothman and others 2009b). Furthermore, UV-C irradiation may inactivate polyphenol oxidase activity which in turn prevents further loss of polyphenols (Oms-Oliu and others 2012). It has been reported in tomatoes that UV-C treatment induces the production and

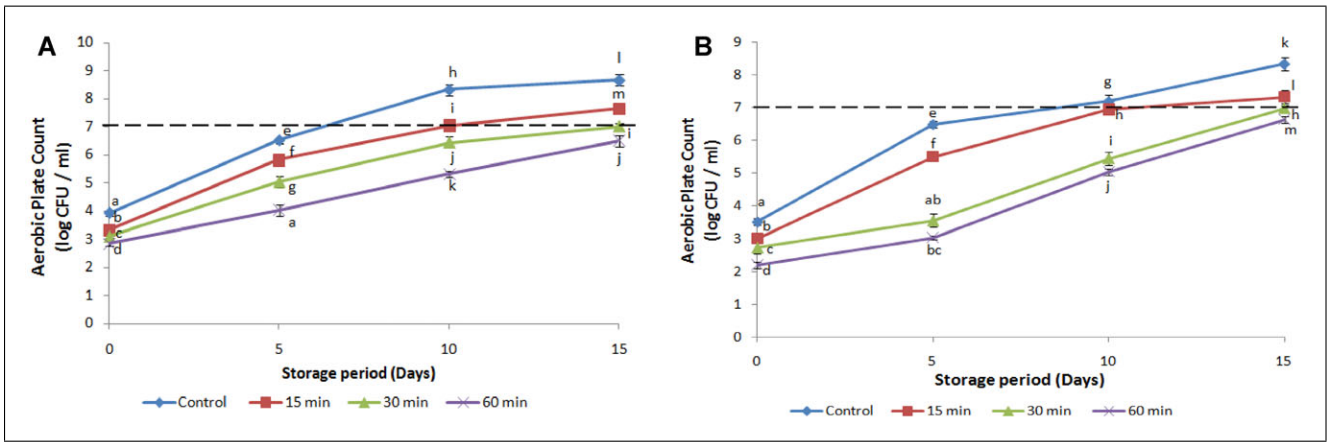

Figure 4–Changes in aerobic plate count of UV-C treated (A) Chokanan mango and (B) Josephine pineapple during storage at 4 °C. *Values followed by different letters are significantly different ($P < 0.05$; n = 9). The dashed line (- – -) indicates the limit of microbial shelf life; CFU, colony forming unit.

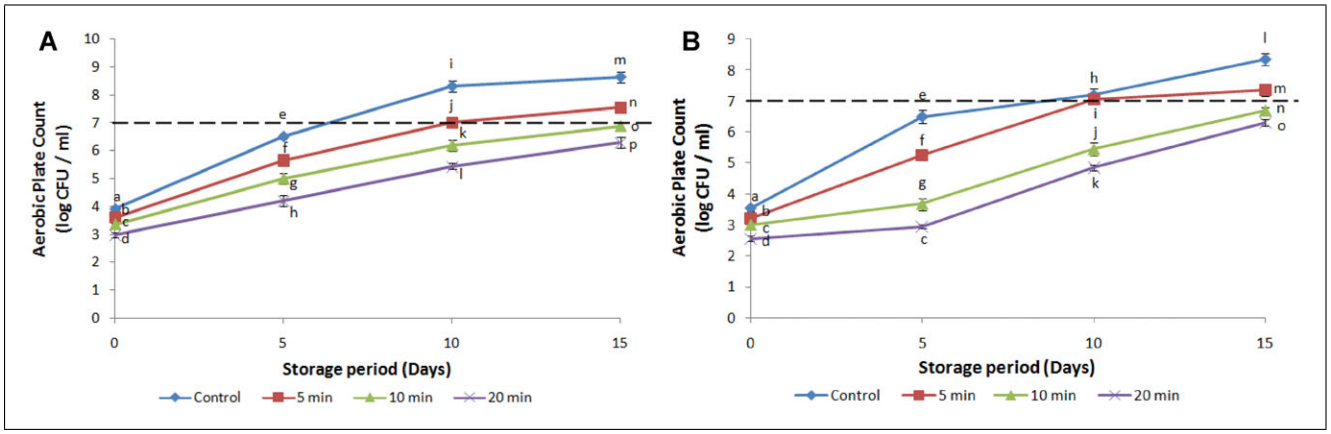

Figure 5–Changes in aerobic plate count of medium heat-treated (A) Chokanan mango and (B) Josephine pineapple during storage at 4 °C. *Values followed by different letters are significantly different ($P < 0.05$; $n = 9$). The dashed line (- – -) indicates the limit of microbial shelf life; CFU, colony forming unit.

accumulation of phenolic compounds in epicarp and mesocarp cells as well as biochemical reinforcement of the cell wall (Narayanasamy 2013).

Microbial inactivation analysis (aerobic plate count [APC])

In this study, a reduced microbial count in both fruits following both treatments (UV-C and medium heat) was observed (Table 3). As for UV-C treatment, a total reduction in microbial count of up to 1.07 log CFU/g in mango and up to 1.3 log CFU/g in pineapple were recorded. Following UV-C treatment, yeast and mold count (YMC) was successfully reduced by 1.13 log CFU/g in mango and 1.40 log CFU/g in pineapples. Medium heat treatment (70 °C) was successful in reducing microbial count of up to 0.72 log CFU/g in mango and up to 0.99 log CFU/g in pineapple. YMC was reduced by 1.27 log CFU/g in mango and 1.22 log CFU/g in pineapple following 20 min of medium heat treatment. Successful microbial inactivation in heat-treated fruits may be due to the fact that heat disrupts the integrity of the cellular membrane and damages nucleic acids which eventually lead to cytolytic effects (Santhirasegaram and others 2013). UV-C treatment on the other hand achieves microbial inactivation in a more complex manner which ultimately leads to cell damage. Absorption of UV-C light by nucleic acids causes pyrimidine bases of cytosine and thymine to form crosslinks which are forms of mutation that are fatal to microbial cells (Bintsis and others 2000; Shama 2006).

Sensory analysis

The sensory analysis of mango and pineapple following the different treatments are shown in Figure 2 and 3, respectively. In this study, controls resulted in the most acceptable score, followed by UV-C treatment in all attributes (appearance, texture, aroma, and taste). UV-C treatments of 15 and 30 min were more acceptable as compared to medium heat treatment. Scores for Heat treatment were below acceptable levels. A decline in acceptance of appearance may be due to changes in color which might have been caused by enzymatic browning or Maillard reactions. According to a report, drying at high temperature may degrade the color of fruits (Nowak and Lewicki 2005). Darker undesirable color in fruits may also be due to significant Maillard reaction which involves hydrolysis of hexose and sucrose (Rhim and others 1989). A reduced acceptability of texture was also observed in heat-treated samples as compared to UV-C treated samples. UV-C treated samples received higher scores for acceptability of texture among the 3 different treatments. The texture can be considered as one of the most important quality characteristics of edible fruits and vegetables (Waldron and others 2003). Heat treatment has been reported to affect the degradation of pectin which is a major component related to texture (Roeck and others 2009). The texture of fruits is perceived based on several attributes out of which some of the most important attributes include crispness, hardness, and juiciness or moisture release (Meilgaard and others 1999). Treatments

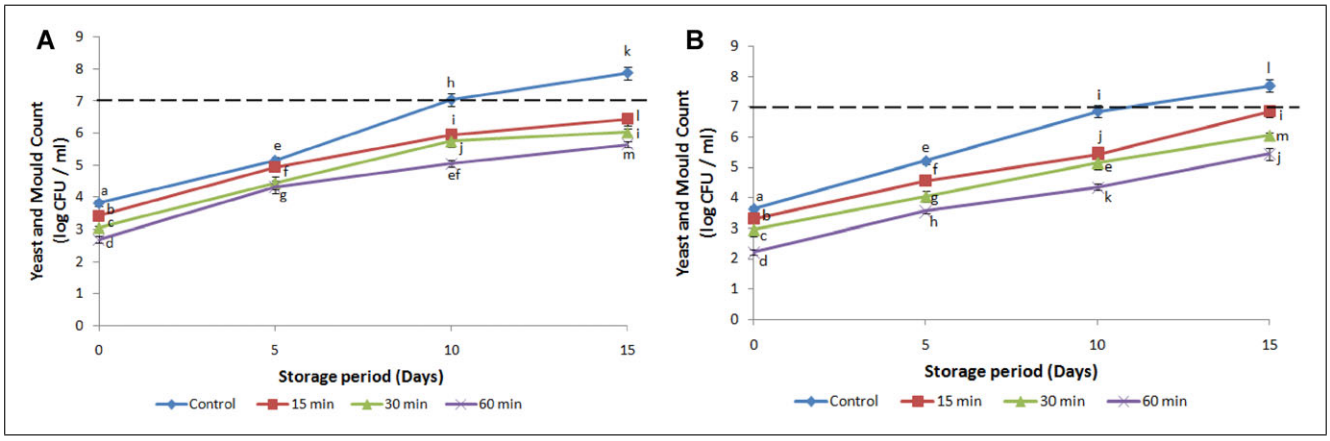

Figure 6–Changes in yeast and mold count of UV-C treated (A) Chokanan mango and (B) Josephine pineapple during storage at 4 °C. *Values followed by different letters are significantly different ($P < 0.05$; $n = 9$). The dashed line (- – -) indicates the limit of microbial shelf life; CFU, colony forming unit.

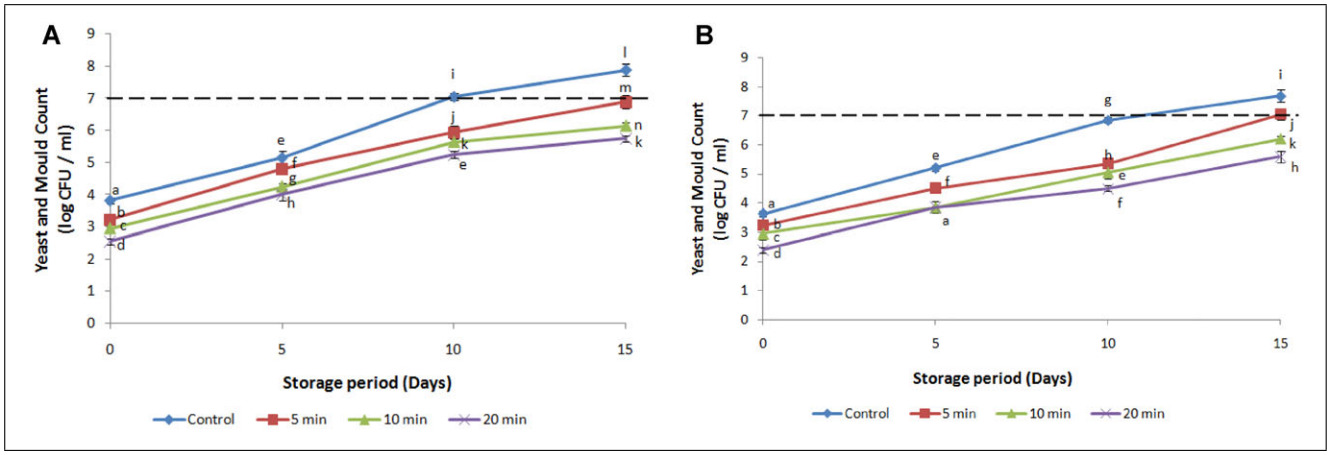

Figure 7–Changes in yeast and mold count of medium heat-treated (A) Chokanan mango and (B) Josephine pineapple during storage at 4 °C. *Values followed by different letters are significantly different ($P < 0.05$; $n = 9$). The dashed line (- – -) indicates the limit of microbial shelf life; CFU, colony forming unit.

at elevated temperatures may result in nonenzymatic conversion of pectins which may affect the hardness and crispness of the fruits which are both attributes of fruit texture (Sila and others 2006). Elevated temperatures also results in dehydration of fruits which affects the juiciness and moisture release attributes of the fruit's texture. As for the aroma and taste of the differently treated fruits, UV-C treated samples resulted in scores that were closest to controls suggesting better retention of aroma and taste posttreatment. Both 10 and 20 min of medium heat (70 °C) resulted in low scores, suggesting that the effects of these treatments on the fruits' aroma and taste were unacceptable by consumers. As both aroma and taste are interrelated, the changes or deterioration of these attributes may be due to the changes in the TSS and TA as reported in earlier sections. Heat generated water loss may also be a key factor in the deterioration of aroma and taste. As a whole, the overall acceptability of the treated fruits, taking into consideration the 4 attributes (appearance, texture, aroma, and taste) suggest that UV-C treated samples are the most acceptable as compared to their heat-treated counterpart.

Shelf-life study

Changes in microbial counts (aerobic bacteria, yeast, and mold) in Chokanan mango and Josephine pineapple stored at refrigeration temperature (4 ± 1 °C) for 15 d are shown in Figure 4–7. Shelf life of the samples was evaluated according to the Public Health Laboratory Service, PHLS (2000) where the acceptable maximum microbial load including aerobic bacteria, and total yeast and mold in fresh-cut fruits are 7 log CFU/mL. The APC in control (untreated mangoes) increased from 3.92 to 8.66 log CFU/mL after 15 d of storage. While, YMC increased from 3.82 to 7.89 log CFU/mL after 15 d of storage. The shelf life of UV-C treated mangoes, 15 min (10 d), 30 min (15 d), 60 min (15 d) were longer than control (6 d), in terms of microbial load (APC and YMC) limit. The shelf life of medium heat-treated (70 °C) mangoes on the other hand was 9 d (5 min of treatment), 15 d (10 min of treatment) and 15 d (20 min of treatment). As for pineapples, APC, YMC in control (untreated pineapples) increased from 3.51 to 8.35 log CFU/mL and from 3.62 to 7.69 log CFU/mL, respectively. The shelf life of UV-C treated pineapples, 15 min (10 d), 30 and 60 min (15 d) were longer than control (8 d), in terms of microbial load (APC and YMC) limit. The shelf life of medium heat-treated (70 °C) mangoes on the other hand was 9 d (5 min of treatment), 15 d (10 min of treatment) and 15 d (20 min of treatment). Hence, the shelf life of UV-C treated and medium heat-treated Chokanan mango and Josephine pineapples stored at 4 ± 1 °C was extended to a maximum of 15 d. Results obtained are in agreement with previous studies that reported prolonged shelf life in UV-C treated carrots and fresh-cut melon (Manzocco and others 2011; Alegria and others 2012).

Conclusions

UV-C treatment exhibited better retention in most quality parameters as compared to medium heat (70 °C) treatment. UV-C irradiated samples showed significant improvement of antioxidant activities (total polyphenols, DPPH inhibition and total antioxidant capacity) as compared to controls while medium heat treatment posed adverse effects to antioxidant activities and vitamin C content. Medium heat-treated samples also received lower scores as compared to UV-C treated samples. Furthermore, a significant reduction in microbial load was also achieved by both UV-C and medium heat treatment. Both treatments were successful in extending the shelf life of fruits stored at 4 °C to a maximum of 15 d which may prove invaluable to the fresh-cut industry. Both UV-C and medium heat treatment with appropriate optimization of processing variables are feasible treatments for shelf-life extension of fresh-cut fruits. Further research work is needed for combining UV-C and medium heat treatment as well as various packaging methods to guarantee improved quality of fresh-cut fruits together with safety standards. Consequently, this study provides more attention for positive implementation of UV-C and medium heat treatment on a pilot scale.

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References

3M Food Safety, 3M Petrifilm interpretation guide. 2010. Available from: http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/. Accessed 2013 May 2.

Aguayo E, Escalona VH, Artés F. 2008. Effect of hot water treatment and various calcium salts on quality of fresh-cut 'Amarillo' melon. Postharvest Biol Technol 47:397–406.

Aguilar-Rosas SF, Ballinas-Casarrubias ML, Nevarez-Moorillon GV, Martin-Belloso O, Ortega-Rivas E. 2007. Thermal and pulsed electric fields pasteurization of apple juice: effects on physicochemical properties and flavor compounds. J Food Eng 83:41–6.

Alegria C, Pinheiro J, Duthoit M, Gonçalves EM, Moldão-Martins M, Abreu M. 2012. Fresh-cut carrot (cv. Nantes) quality as affected by abiotic stress (heat shock and UV-C irradiation) pre-treatments. LWT-Food Sci Technol 48:197–203.

Allende A, Artes F. 2003. UV-C radiation as a novel technique for keeping quality of fresh processed "Lollo Rosso" lettuce. Food Res Intl 36:739–46.

Alothman M, Bhat R, Karim AA. 2009a. UV radiation-induced changes of antioxidant capacity of fresh-cut tropical fruits. Innov Food Sci Emerg Technol 10:512–6.

Alothman M, Bhat R, Karim AA. 2009b. Effects of radiation processing on phytochemicals and antioxidants in plant produce. Trends Food Sci Technol 20:201–12.

Anthon GE, LeStrange M, Barrett DM. 2011. Changes in pH, acids, sugars and other quality parameters during extended vine holding of ripe processing tomatoes. J Sci Food Agric 91(7):1175–81.

Bae SH, Suh HJ. 2007. Antioxidant activities of five different mulberry cultivars in Korea. LWT–Food Sci Technol 40:955–62.

Bal E. 2012. Effect of postharvest UV-C treatments on quality attributes of fresh fig. Bulgarian J Agric Sci 18:191–6.

Bal E, Kok D. 2009. Effects of UV-C treatment on kiwifruit quality during the storage period. J Central Eur Agric 10(4):375–82.

Bayarri S, Taylor AJ, Hort J. 2006. The role of fat in flavor perception: effect of partition and viscosity in model emulsions. J Agric Food Chem 54:8862–8.

Bhupathiraju SN, Wedick NM, Pan A, Manson JE, Rexrode KM, Willett WC, Rimm EB, Hu FB. 2013. Quantity and variety in fruit and vegetable intake and risk of coronary heart disease. Am J Clin Nutr 98:1514–23.

Bintsis T, Litopoulou-Tzanetaki E, Robinson R. 2000. Existing and potential applications of ultraviolet light in the food industry. A critical review. J Sci Food Agric 80:1–9.

Burdon J, McLeod D, Lallu N, Gamble J, Petley M, Gunson A. 2004. Consumer evaluation of 'Hayward' kiwifruit of different at-harvest dry matter contents. Postharvest Biol Technol 34:245–55.

Das BK, Kim JG. 2010. Microbial quality and safety of fresh-cut broccoli with different sanitizers and contact times. J Microbiol Biotechnol 20(2):363–9.

Davey MW, Van Montagu M, Inzé D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IFF, Strain JJ, Favell D, Fletcher J. 2000. Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. J Sci Food Agric 80:825–60.

Davies JN, Hobson GE. 1981. The constituents of tomato fruit-the influence of environment, nutrition and genotype. Crit Rev Food Sci Nutr 15:205–80.

Delaquis PJ, Stewart S, Toivonen PMA, Moyls AL. 1999. Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. Food Res Intl 32:7–17.

Ercan SS, Soysal C. 2011. Effect of ultrasound and temperature on tomato peroxidase. Ultrason Sonochem 18:689–95.

Fonseca JM, Rushing JW. 2006. Effect of ultraviolet-C light on quality and microbial population of fresh-cut watermelon. Postharvest Biol Technol 40:256–61.

Gómez PL, Alzamora SM, Castro MA, Salvatori DM. 2010. Effect of ultraviolet-C light dose on quality of cut-apple: microorganism, color and compression behavior. J Food Eng 98:60–70.

He FJ, Nowson CA, Lucas M, MacGregor GA. 2007. Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. J Hum Hypertens 21:717–28.

Hung HC, Joshipura KJ, Jiang R, Hu FB, Hunter D, Smith-Warner SA, Colditz GA, Rosner B, Spiegelman D, Willett WC. 2004. Fruit and vegetable intake and risk of major chronic disease. J Natl Cancer Inst 96(21):1577–84.

Khademi O, Zamani Z. 2013. Poor Ahmadi, E. and Kalantari, S. Effect of UV-C radiation on postharvest physiology of persimmon fruit (Diospyros kaki Thunb.) cv. 'Karaj' during storage at cold temperature. Intl Food Res J 20(1):247–53.

Klaiber RG, Baur S, Wolf G, Hammes WP, Carle R. 2005. Quality of minimally processed carrots as affected by warm water washing and chlorination. Innov Food Sci Emerg Technol 6:351–62.

Li Y, Brackett RE, Shewfelt RL, Beuchat LR. 2001. Changes in appearance and natural microflora on iceberg lettuce treated in warm, chlorinated water and then stored at refrigeration temperature. Food Microbiol 18:299–308.

Liu S, Manson JE, Lee IM, Cole SR, Hennekens CH, Willett WC, Buring JE. 2000. Fruit and vegetable intake and risk of cardiovascular disease: the Women's Health Study. Am J Clin Nutr 72(4):922–8.

Loaiza-Velarde JG, Mangrich ME, Campos-Vargas R, Saltveit ME. 2003. Heat shock reduces browning of fresh-cut celery petioles. Postharvest Biol Technol 27:305–11.
Loaiza-Velarde JG, Tomás-Barberán FA, Saltveit ME. 1997. Effect of intensity and duration of heat-shock treatments on wound-induced phenolic metabolism in Iceberg lettuce. J Am Soc Hortic. Sci. 122:873–7.
Mahieddine B, Faouzi SM, Hedjer S, Moussa H, Aïssa B, Mahmoud S. 2011. Heat treatment effect on the technological quality of processed tomato paste. Can J Chem Eng Technol 2(3):27–40.
Manzocco L, Pieve SD, Maifreni M. 2011. Impact of UV-C light on safety and quality of fresh-cut melon. Innov Food Sci Emerg Technol 12:13–17.
McAllister JW. 1980. Methods of determining the quality of citrus juice. In: Nagy S, Attaway JA, editors. Citrus nutrition and quality. Washington, DC: American Chemical Society. p.291–317.
Meilgaard M, Civille GV, Carr BT. 1999. Sensory evaluation techniques. 3rd ed. Boca Raton, Fla.: CRC Press. p. 195–208.
Mitchell FG, Mayer G, Biasi W. 1991. Effect of harvest maturity on storage performance of 'Hayward' kiwifruit. Acta Hortic 297:617–25.
Musto M, Satriano ML. 2010. Fruit responses to postharvest heat treatment time: characterisation of heat-treated strawberry (Fragaria x ananassa) cv. 'Candonga' fruits. Agron Res 8(1):815–26.
Narayanasamy P 2013. Biological management of diseases of crops: Volume 2: integration of biological control strategies with crop disease management system. Netherlands: Springer Science & Business Media.
Nowak D, Lewicki PP. 2005. Quality of infrared dried apple slices. Drying Technol 23:831–46.
Oms-Oliu G, Odriozola-Serrano I, Martín-Belloso O. 2012. The effects of non-thermal technologies on phytochemicals. In: Rao V, editor. Phytochemicals: a global perspective of their role in nutrition and health. Croatia: InTech.
Perkins-Veazie P, Collins JK, Howard L. 2008. Blueberry fruit response to postharvest application of ultraviolet radiation. Postharvest Biol Technol 47:280–5.
PHLS (Public Health Laboratory Service). 2000. Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. Commun Dis Public Health(CDPH) 3:163–7.
Prieto P, Pineda M, Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 269:337–41.
Ranganna, S. 1977. Chapter 5: ascorbic acid. In: Ranganna S, editor., Manual of analysis of fruit and vegetable products. New Dehli: Tata McGrawHill Publishing Comp., p. 94–6.
Rhim JW, Nunes RV, Jones VA, Swartzel KR. 1989. Kinetics of colour change of grape juice generated using linearly increasing temperature. J Food Sci 54(3):776–7.
Rodoni LM, Concellón A, Chaves AR, Vicente AR. 2012. Use of UV-C treatments to maintain quality and extend the shelf life of green fresh-cut bell pepper (Capsicum annuum L.). J Food Sci 77(6):632–9.
Roeck AD, Duvetter T, Fraeye I, Plancken IV, Sila DN, Loey AV, Hendrickx M. 2009. Effect of high-pressure/high-temperature processing on chemical pectin conversions in relation to fruit and vegetable texture. Food Chem 115:207–13.
Rossiter KL, Young H, Walker SB, Miller M. 2000. The effects of sugars and acids on consumer acceptability of kiwifruit. J Sens Stud 15:241–50.
Sadler GD, Murphy PA. 2010. pH and titratable acidity. In: Nielsen SS, editor. Food analysis. 4th ed. New York: Springer. p. 231–3.
Santhirasegaram V, Razali Z, Somasundram C. 2013. Effects of thermal treatment and sonication on quality attributes of Chokanan mango (Mangifera indica L.) juice. Ultrason Sonochem 20:1276–82.
Shama G. 2006. Ultraviolet light. In: Hui YH, editor, Handbook of food science, technology and engineering, Vol. 3. Boca Raton, Fla.: CRC/Taylor and Francis. p. 122–1–122–14.
Shon MY, Kim TH, Sung NJ. 2003. Antioxidants and free radical scavenging activity of Phellinus baumii (Phellinus of Hymenochaetaceae) extracts. Food Chem 82:593–7.
Sila DN, Smout C, Elliot F, Van Loey A, Hendrickx M. 2006. Non-enzymatic depolymerization of carrot pectin: toward a better understanding of carrot texture during thermal processing. J Food Sci 71(1):E1–E9.
Vicente AR, Pineda C, Lemoine L, Civello PM, Martinez GA, Chaves AR. 2005. UV-C treatments reduce decay, retain quality and alleviate chilling injury in pepper. Postharvest Biol Technol 35:69–78.
Waldron KW, Parker ML, Smith AC. 2003. Plant cell walls and food quality. Compr Rev Food Sci Food Safety 2:101–19.
Waterhouse AL. 2003. Determination of total phenolics. In: Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid DA, Schwartz SJ, Shoemaker CF, Smith D, Sporns P, editors. Current protocols in food analytical chemistry. New York: John Wiley and Sons Inc. p. 1111–1118.
Xu G, Liu D, Chen J, Ye X, Maa Y, Shi J. 2008. Juice components and antioxidant capacity of citrus varieties cultivated in China. Food Chem 106:545–51.
Yamaki YT. 1989. Organic acids in the juice of citrus fruits. J Jap Hortic Sci 58:587–94.
Yaun BR, Summer SS, Eifert JD, Marcy JE. 2004. Inhibition of pathogens on fresh produce by ultraviolet energy. Intl J Food Microbiol 90:1–8.

APPENDICES

Appendix 1 Sample of questionnaire used for sensory analysis

Appendix 2 HMF standard curve

Appendix 3 Gallic acid standard curve for TPC

Appendix 4 Catechin standard curve for TFC

Appendix 5 Ascorbic acid standard curve for DPPH assay

Appendix 6 Ascorbic acid standard curve for ABTS assay

Appendix 7 Ascorbic acid standard curve for RPA

Appendix 8 Ascorbic acid standard curve for TAC

SENSORY ANALYSIS OF CHOKANAN MANGO JUICE SAMPLES

Instruction: Please circle one answer ONLY.

PERSONAL DETAILS

Name: .....

Age: ……..

Gender: Male / Female

SENSORY EVALUATION

You are being presented with 13 Chokanan mango juice samples that are labelled with three-digit numbers). The juice samples are untreated (freshly squeezed) and treated. Please assess the sensory attributes and rate them using a 1-9 hedonic scale provided below.

1	2	3	4	5	6	7	8	9
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

A) COLOUR

Sample code	Rating								
Sample 001	1	2	3	4	5	6	7	8	9
Sample 002	1	2	3	4	5	6	7	8	9
Sample 003	1	2	3	4	5	6	7	8	9
Sample 004	1	2	3	4	5	6	7	8	9
Sample 005	1	2	3	4	5	6	7	8	9
Sample 006	1	2	3	4	5	6	7	8	9
Sample 007	1	2	3	4	5	6	7	8	9
Sample 008	1	2	3	4	5	6	7	8	9
Sample 009	1	2	3	4	5	6	7	8	9
Sample 010	1	2	3	4	5	6	7	8	9
Sample 011	1	2	3	4	5	6	7	8	9
Sample 012	1	2	3	4	5	6	7	8	9

Sample 013	1	2	3	4	5	6	7	8	9

B) ODOUR

Sample code	Rating								
Sample 001	1	2	3	4	5	6	7	8	9
Sample 002	1	2	3	4	5	6	7	8	9
Sample 003	1	2	3	4	5	6	7	8	9
Sample 004	1	2	3	4	5	6	7	8	9
Sample 005	1	2	3	4	5	6	7	8	9
Sample 006	1	2	3	4	5	6	7	8	9
Sample 007	1	2	3	4	5	6	7	8	9
Sample 008	1	2	3	4	5	6	7	8	9
Sample 009	1	2	3	4	5	6	7	8	9
Sample 010	1	2	3	4	5	6	7	8	9
Sample 011	1	2	3	4	5	6	7	8	9
Sample 012	1	2	3	4	5	6	7	8	9
Sample 013	1	2	3	4	5	6	7	8	9

C) TASTE

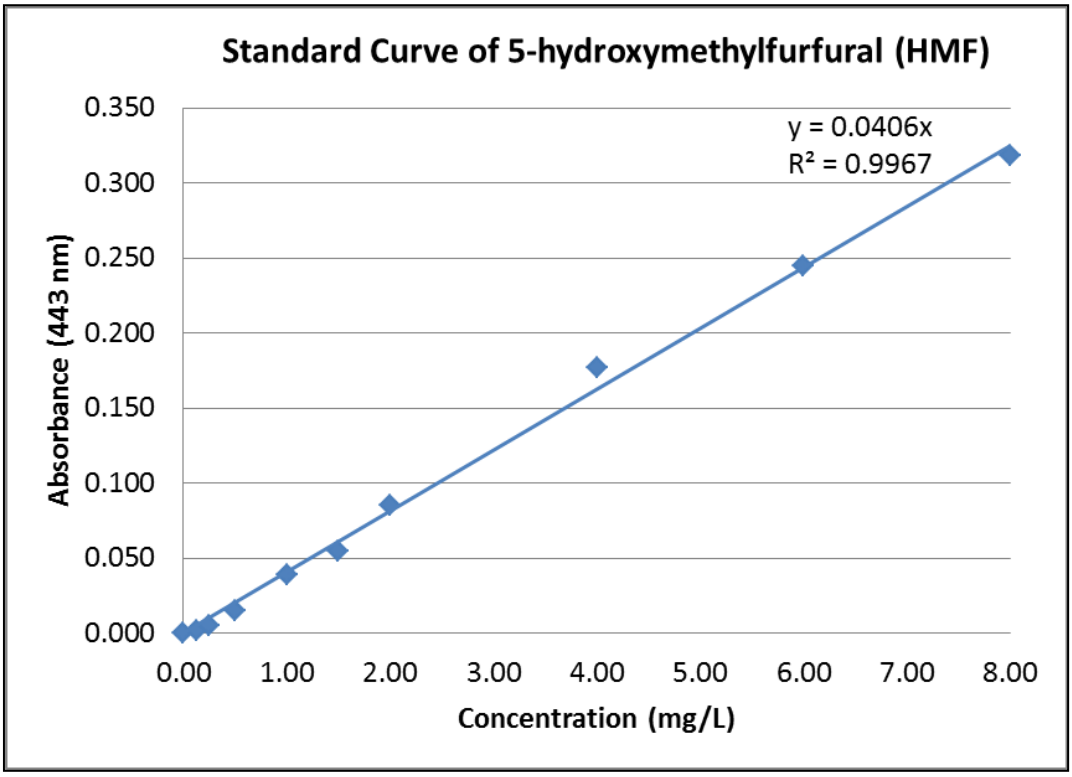
Sample code	Rating								
Sample 001	1	2	3	4	5	6	7	8	9
Sample 002	1	2	3	4	5	6	7	8	9
Sample 003	1	2	3	4	5	6	7	8	9
Sample 004	1	2	3	4	5	6	7	8	9
Sample 005	1	2	3	4	5	6	7	8	9
Sample 006	1	2	3	4	5	6	7	8	9
Sample 007	1	2	3	4	5	6	7	8	9
Sample 008	1	2	3	4	5	6	7	8	9
Sample 009	1	2	3	4	5	6	7	8	9
Sample 010	1	2	3	4	5	6	7	8	9

Sample 011	1	2	3	4	5	6	7	8	9
Sample 012	1	2	3	4	5	6	7	8	9
Sample 013	1	2	3	4	5	6	7	8	9

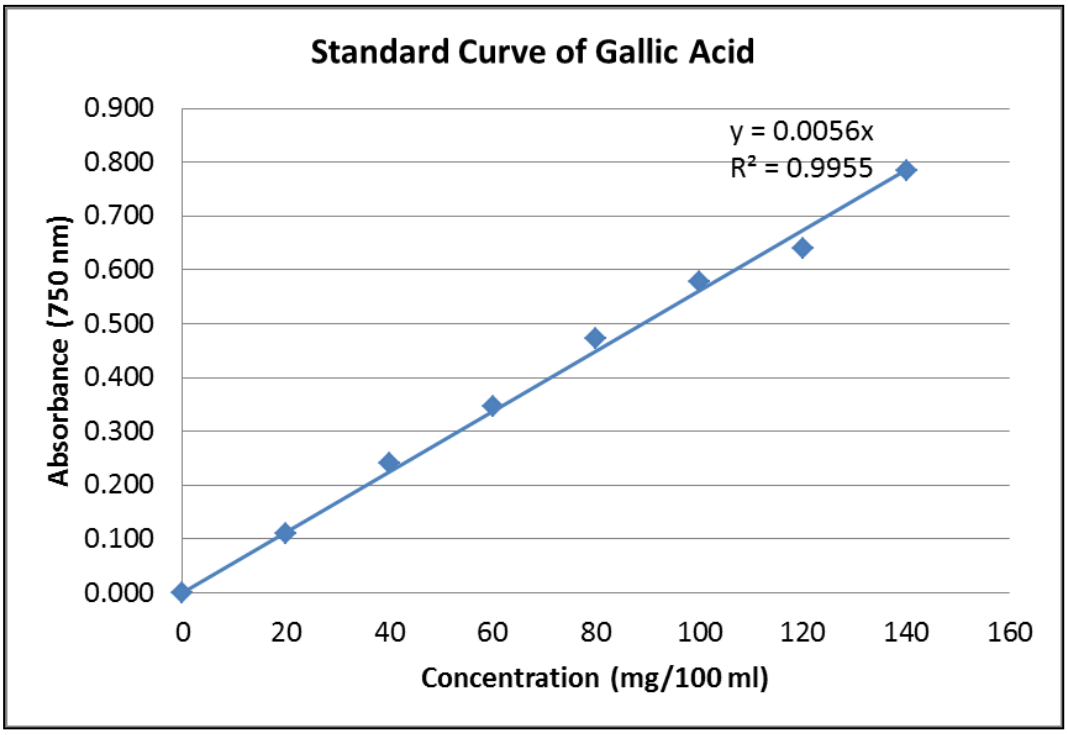
D) OVERALL ACCEPTABILITY

Sample code	**Rating**								
Sample 001	1	2	3	4	5	6	7	8	9
Sample 002	1	2	3	4	5	6	7	8	9
Sample 003	1	2	3	4	5	6	7	8	9
Sample 004	1	2	3	4	5	6	7	8	9
Sample 005	1	2	3	4	5	6	7	8	9
Sample 006	1	2	3	4	5	6	7	8	9
Sample 007	1	2	3	4	5	6	7	8	9
Sample 008	1	2	3	4	5	6	7	8	9
Sample 009	1	2	3	4	5	6	7	8	9
Sample 010	1	2	3	4	5	6	7	8	9
Sample 011	1	2	3	4	5	6	7	8	9
Sample 012	1	2	3	4	5	6	7	8	9
Sample 013	1	2	3	4	5	6	7	8	9

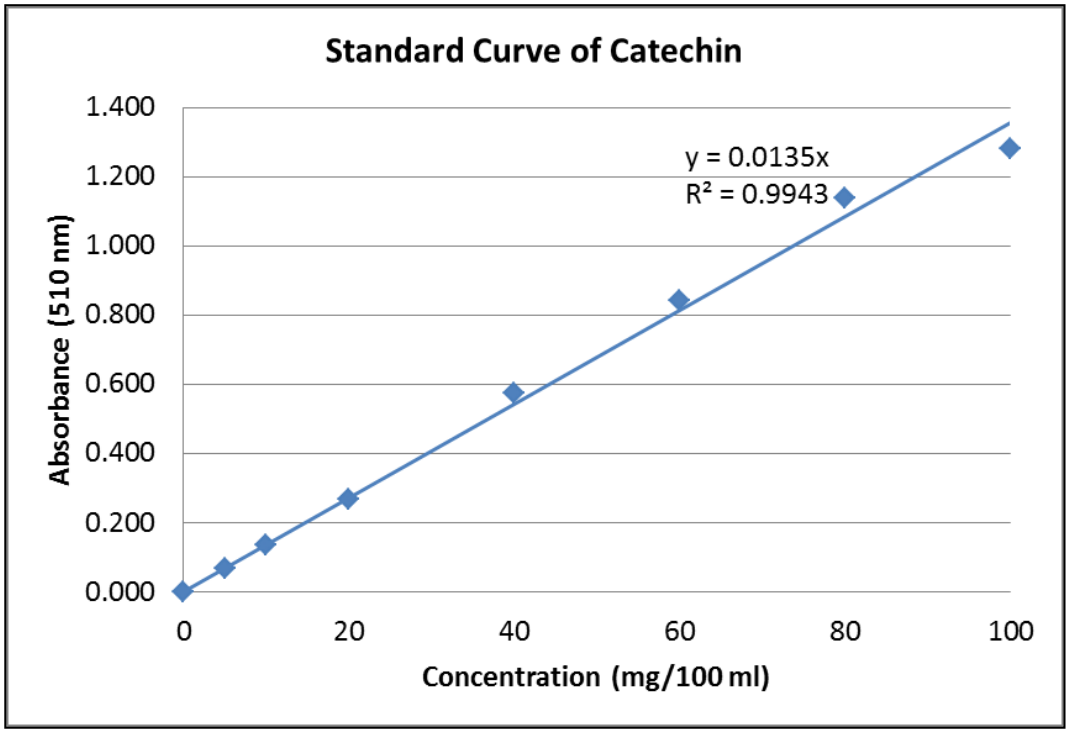
APPENDIX 2: HMF standard curve



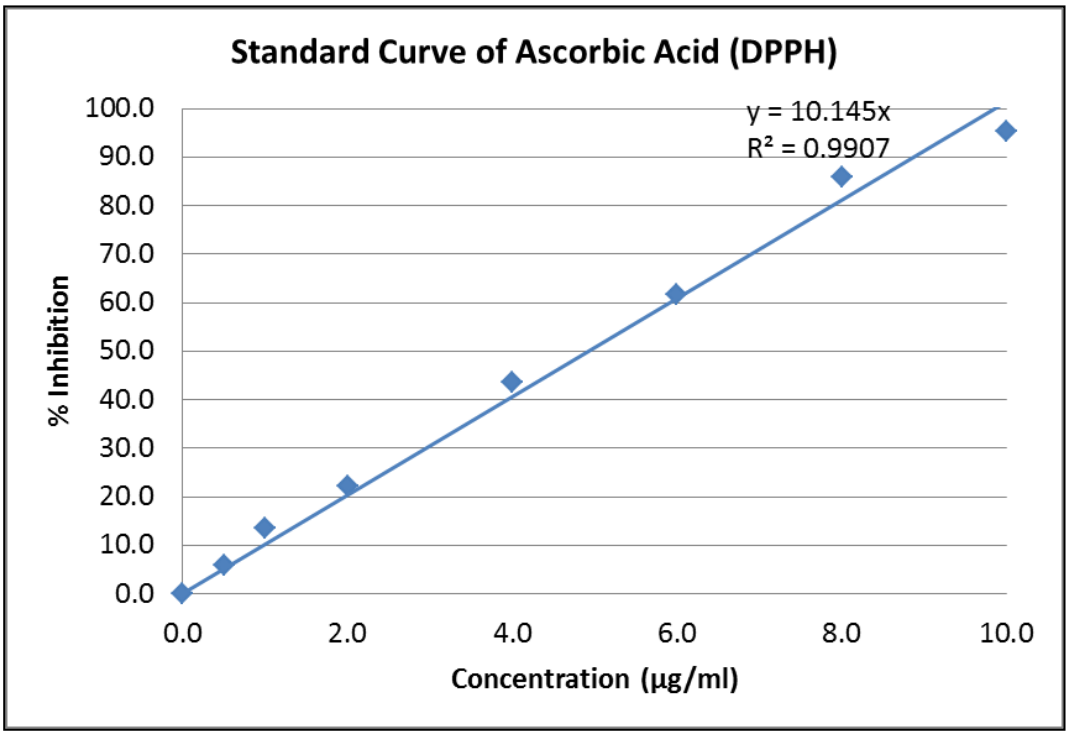
APPENDIX 3: Gallic acid standard curve for TPC



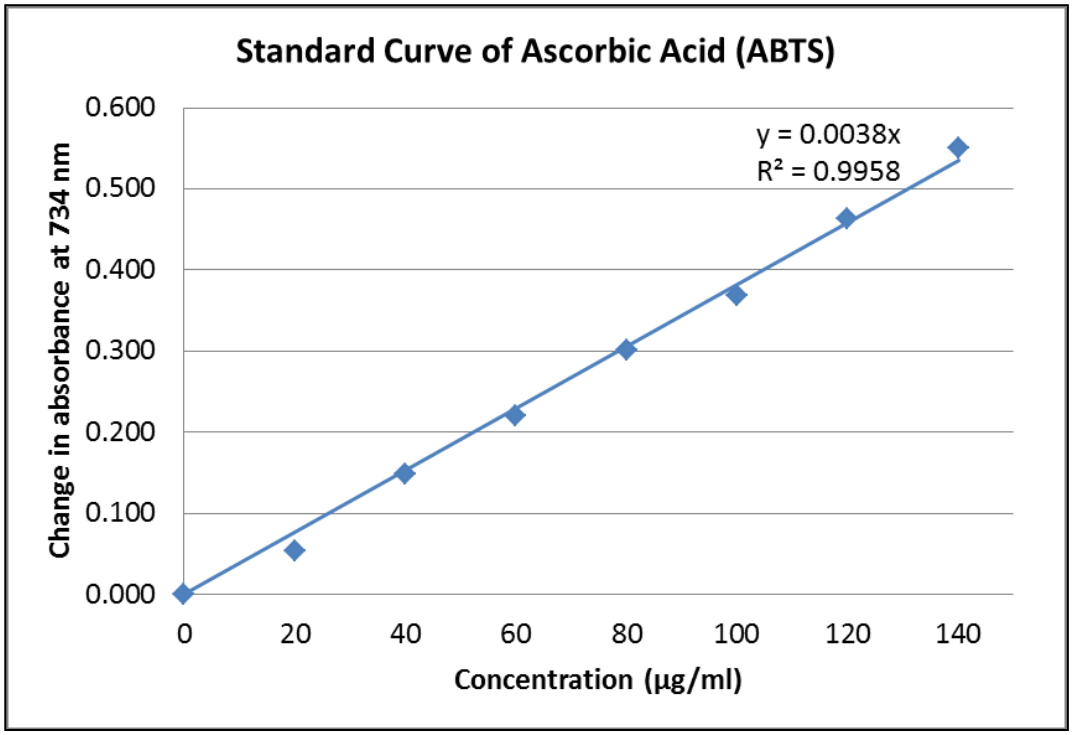
APPENDIX 4: Catechin standard curve for TFC



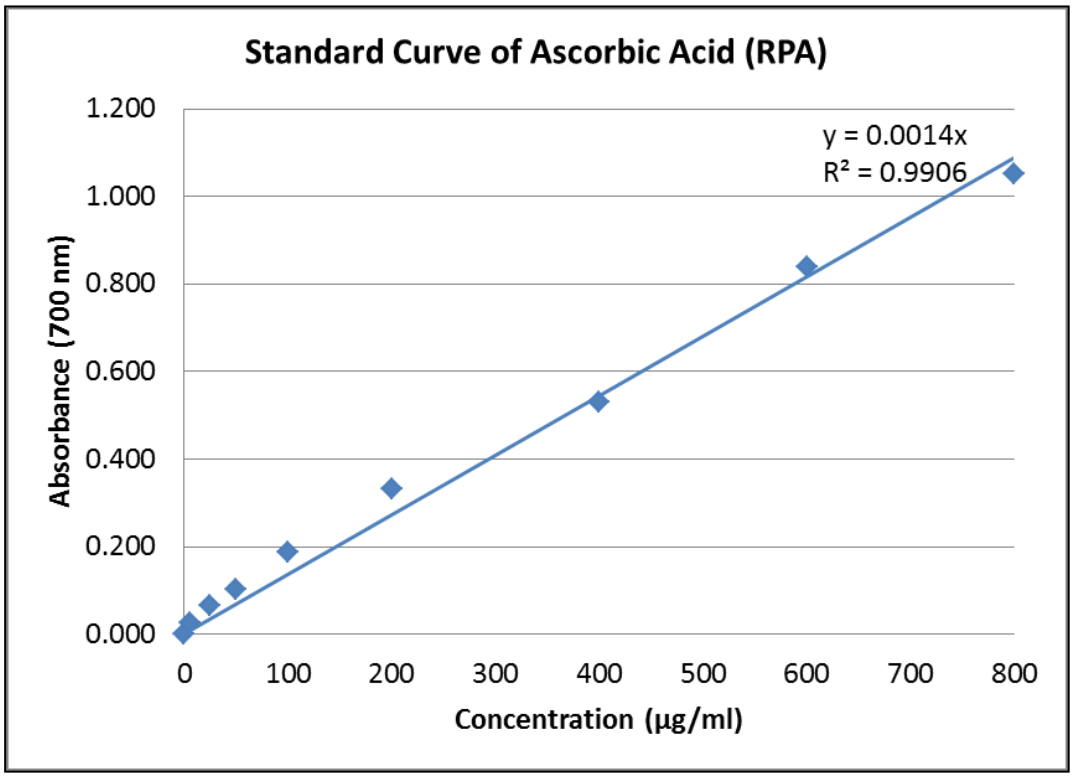
APPENDIX 5: Ascorbic acid standard curve for DPPH assay



APPENDIX 6: Ascorbic acid standard curve for ABTS assay



APPENDIX 7: Ascorbic acid standard curve for RPA



APPENDIX 8: Ascorbic acid standard curve for TAC