UNIVERSITI PUTRA MALAYSIA

EFFECT OF DIFFERENT PASTEURIZATION AND STORAGE TEMPERATURE ON COLOR, BIOACTIVE COMPOUNDS, ANTIOXIDANT ACTIVITY AND MICROBIOLOGICAL QUALITIES OF RECONSTITUTED POMEGRANATE JUICE (RPJ)

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ABSTRACT

This study emphasized on the effect of pasteurisation of reconstituted pomegranate juice (RPJ) that were conducted at 95°C for 30 seconds (High Thermal Pasteurisation, HTP) and 80°C for 30 seconds (Mild Thermal Pasteurisation, MTP) to investigate their effects on color, bioactive compounds, antioxidant activity and microbiological qualities. Both thermal treatments successfully reduced the bacterial growth to beyond the acceptable limit, with MTP consumed less time to complete the pasteurisation process compared to HTP. In terms of antioxidant properties, MTP was proven to have better antioxidant properties than HTP. Comparing to storage at 25±2°C for 21 days, storage at 4±1°C helped to avoid substantial degradation of RPJ's color, bioactive compound and antioxidant properties. Nevertheless, HTP had a greater impact on RPJ's color and antioxidant properties, with the samples remaining unstable after pasteurisation and undergoing considerable changes over time, most likely due to major changes in bioactive components due to high temperature treatment. Conversely, MTP seemed to have no significant effect on colour and antioxidant properties of RPJ because the samples were more stable after storage. At the completion of storage, both the standard plate count (SPC) and total yeast and mould count (TYMC) had some spoilage except for HTP at 4±1°C. Consequently, MTP and storage at 4±1°C is the best parameter in ensure best quality of RPJ with the least effects on color, bioactive compounds, antioxidant activity and microbiological qualities, according to the findings of this study.

ABSTRAK

Kajian ini menekankan pada kesan pasteurisasi jus delima yang disusun semula (RPJ) yang dilakukan pada suhu 95°C selama 30 saat (High Thermal Pasteurisation, HTP) dan 80°C selama 30 saat (Mild Thermal Pasteurisation, MTP) untuk menyiasat kesannya pada warna, sebatian bio-aktif, aktiviti antioksidan dan kualiti mikrobiologi. Kedua-dua rawatan termal berjaya mengurangkan pertumbuhan bakteria melebihi had yang boleh diterima, dengan MTP memakan lebih sedikit masa untuk menyelesaikan proses pasteurisasi berbanding dengan HTP. Dari segi sifat antioksidan, MTP terbukti mempunyai sifat antioksidan yang lebih banyak daripada HTP. Berbanding dengan penyimpanan pada suhu 25±2°C selama 21 hari, penyimpanan pada suhu 4±1°C membantu mengelakkan penurunan warna, sifat bioaktif dan antioksidan RPJ. Walaupun begitu, HTP memberi kesan yang lebih besar terhadap sifat fizikokimia dan antioksidan RPJ, dengan sampel kurang stabil setelah dipasteurisasi dan mengalami perubahan yang cukup besar, kemungkinan disebabkan oleh perubahan besar komponen bioaktif kerana kesan daripada suhu tinggi. Sebaliknya, MTP tidak mempunyai kesan yang signifikan terhadap warna dan sifat antioksidan RPJ kerana keadaan sampel lebih stabil setelah disimpan. Setelah selesai penyimpanan, kedua-dua bilangan plat aerobik (SPC) dan jumlah ragi dan acuan (TYMC) didapati ada beberapa kerosakan kecuali HTP yang disimpan pada suhu 4±1°C. Oleh itu, kaedah MTP dan suhu penyimpanan pada suhu 4±1°C adalah parameter terbaik dalam memastikan kualiti RPJ terbaik dengan kesan paling sedikit terhadap warna, sebatian bioaktif, aktiviti antioksidan dan kualiti mikrobiologi, menurut penemuan kajian ini.

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EA : ellargic acid 15

EAGs : ellarguc acid glycosides 15

ETs : ellargitannins 15

FRAP : Ferric reducing antioxidant powder 11

HTP :high temperature pasteurisation 8

LDL : Low density lipoprotein 11

MTP : mild-temperature pasteurisation 9

PDA: potato dextrose agar 9

POD : peroxidase 9

PPO : polyphenol oxidase 9

RTD : Ready-to-drink 10

TPC: total phenol content 9

TPTZ : 4,6-tripryridyl-s-triazine 15

TYMC :total yeast & mould count 8

CHAPTER 1

INTRODUCTION

1.1 OVERVIEW

Often referred to as superfruit, pomegranate fruit is extensively and particularly known for its nutritional attributes such as vitamins and antioxidants. Pomegranate juice (PJ) is enriched in polyphenols that are essential for its antioxidant function, such as anthocyanin. The inner structure of a pomegranate fruit is shown in Figure 1.1. The outer part of the fruit contains the peel, while the inner part of the fruit consists of seed and juice, which generally referred to as arils. Most have been fascinated by the high antioxidant content in pomegranate fruit as it is presumed to prevent consumers from oxidative stress and eventually reduce the risk of chronic diseases and also prevent the spread of diseases such as cancer, cardiovascular diseases, autoimmune disorder and neurodegeneration (Zaouay et al., 2012).

Pomegranate fruit is formerly native from Iran and nowadays planted in many countries such as India, Pakistan, Israel, Afghanistan, Egypt, China, Japan, USA, Russia, Australia, South Africa, Saudi Arabia and also in South America's subtropical regions. (Holland et al., 2009). Pomegranate fruit is commonly consumed in non-cultivar countries despite the lack availability in most countries. Consumers are absolutely eager to search for the pomegranate fruit's antioxidant properties. Also, the selling price of pomegranate fruit is higher compared to local fruits in non-cultivar countries. Nevertheless, the consumers are willing to pay for high price of pomegranate fruit because of its high antioxidant quality.

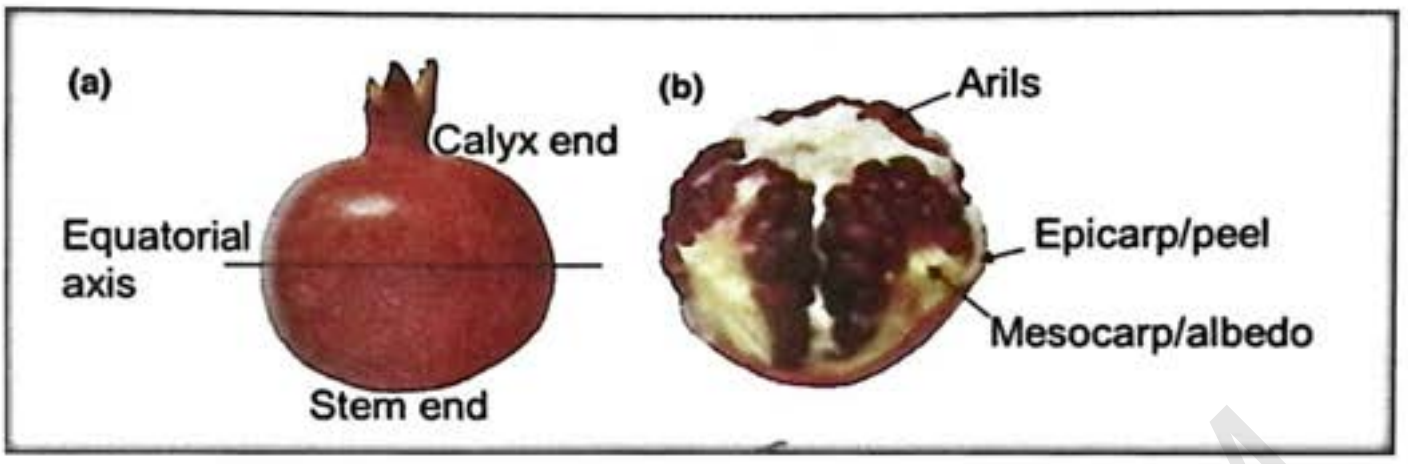


Figure 1.1 : The internal structure of pomegranate fruit (Mukama, M., Ambaw, A., & Opara, U.L., 2018)

Due to the encouraging purchase intention among consumers in non-cultivar countries, many beverage industries have diverted their market towards the production of reconstituted pomegranate juice (RPJ) particularly the small-scale manufacturers. (Bates et al. 2001). This is because the production of reconstituted fruit juice is relatively simple compared to fruit juice. The reconstituted juice is derived from a concentrate of fruit juice and varies slightly from fresh juices in taste, bearing a distinct texture and aroma. The juice is extracted from a juicing machine which by evaporation processes, so then maximum water is removed from it as much possible, reducing it to a concentrate. In terms of economy, flexibility and food safety, juice in the form of concentrate has prolong the shelf-life and makes it simpler for the juice concentrate to be processed, delivered and distributed to secondary producers. The juice is then reconstituted by re-adding the suitable water to the concentrated juice. The juice must then be pasteurized again to prevent recontamination with microorganisms. Unfortunately, reconstituted juice proffer lower nutritional value as important nutrients are mostly knocked down through the heating and reconstitution processes. Thus, it is important for the small-scale

manufacturers to understand the appropriate processing of reconstituted pomegranate juice (RPJ) to avoid further or severe degradation as this is desired by the consumers.

Small-scale factories may overtake the production of reconstituted juice, whereby the technique is not oriented on study but on the sensory recognition, cost-efficiency and short lead time required. Therefore, conditions that degrade the integrity of the health values of pomegranate can be excessively carried out. In particular, compared to freshly pressed pomegranate juice, RPJ has lowered nutritional qualities. Still, it is essential for the industry to acknowledge the fact that, despite its costly price, the consumer's buying intention for the RPJ is because of its antioxidant-rich qualities particularly in non-cultivar countries.

Therefore, from the perspective of consumer health, it is essential to reduce the degradation of antioxidant potential in the RPJ and is a responsibility that producers need to pursue with effective production techniques. For that reason, it is important for local beverage manufacturer to have the awareness on secondary processing of RPJ especially for the thermal pasteurisation and storage condition as to minimise any further degradation on the qualities of RPJ.

One of the processing techniques that is able to achieve certain food quality such as antioxidant retention and microbiological stability is called hurdle preservation. Hurdle technology is a method of removing or controlling pathogens in food products in order to enhance food safety and prolong shelf life. This technology combines various preservation techniques as preservation strategy and the most commonly used are based on controlling temperature, water activity, acidity, redox potential and used of preservatives (Pilizota,

2014). This technique can help to improve product consistency, stability, and safety. The conditions that are considered to maintain food preservation are called as "hurdles" and for RPJ production, high and low temperature are used. RPJ is pasteurized in high temperature and stored in cold storage.

1.2 ANTIOXIDANTS IN THE RPJ AND ITS DEGRADATION

In pomegranate juice (PJ), the common antioxidants come from phenolic compounds, especially flavonoids, namely anthocyanins. (Vegara et al., 2013). Anthocyanins, which are abundant in arils and are responsible for the red color of pomegranate juices, have been found to prevent lipid peroxidation, which can kill cells, as well as to have preventive effects on cardiovascular diseases such as obesity and diabetes (Karimi et al., 2017). Numerous studies have recorded significant changes in consistency over the various stages of RPJ processing, such as astringency, flavor and color, compared to fresh pomegranate juice. The progression of organoleptic quality parameters can be related scientifically to changes in the composition and degradation of antioxidants, which are mostly not preferred by consumers. Inevitably, the antioxidant properties of the RPJ can be significantly reduced more than 20% after pasteurization.

1.3 PROBLEM STATEMENT

Due to increasing purchasing behaviour towards pomegranate juice, many small-scale beverage industries have ventured their business in the manufacturing of RPJ

(Devarci et al., 2019). This includes Company XYZ, a Malaysian SME that is based in Kuala Lumpur and produced pomegranate juice. The demand for their pomegranate juice is rising due to the growing awareness on its nutrients and benefits for the health of the consumers. Nevertheless, the present production techniques used has degraded the health value of the juice in which the juice is added with more than 10% sugar as preservatives. Additionally, the current home-style kitchen production is not sufficient to meet the market demand and has some weakness which lead to prolonged production time. Some of the inefficiencies are too many manual processes, disorganized production facilities and too long waiting time between processes. Thus, resulting to the production of juice fluctuates and lower of the juice quality.

In order to meet the increasing demand, Company XYZ had decided to pursue a high-quality production in a proper factory premise. The concept of the product is reconstituted pomegranate juice (RPJ) without added sugar. Thus, a new process design is required which begins with a lab-scale tests that can minimize the degradation of antioxidants such as phenols and anthocyanin as well as ensure minimum color change and food safety. Since the pomegranate has low pH which act as natural inhibitor for the growth of many types of microorganisms, this study proposes to evaluate the effect of different pasteurization and storage temperature on the color, antioxidants and microbial stability of the RPJ. At present, the industrial practice recommend the pasteurization at 95°C and holding time at 15-30 second (high temperature pasteurization), followed by subsequent aseptic filling and ambient storage. Although this can ensure for food safety, the juice is over-pasteurised and thought to induce excessive antioxidant degradation especially when stored at ambient temperature. A milder heat-treatment is proposed to be

investigated at temperature of 80°C with the same holding time, followed with subsequent cold storage and compared with the effect of industrial recommendation. An insight into possible less degradation of color, antioxidant, microbial stability and energy usage will be investigated for the techniques applied.

1.4 RESEARCH OBJECTIVES

The main aim of the project is to investigate the appropriate pasteurisation and storage that can be combined with aseptic packaging for the production of high-antioxidant RPJ whilst not losing its color and microbiological safety. The RPJ is not added with any additive such as sugar and preservatives in line with the current trend to avoid the addition of sugar and preservative in drinks products. The most suitable preservation technique will be integrated into a complete process design for a small-scale juice production in the future project.

The specific objectives are:

- To evaluate the effect of High temperature pasteurisation (HTP) and Mild-temperature pasteurisation (MTP) on the antioxidant contents, activities, colour and microbiological deactivation of the RPJ
- To evaluate the effect of room and refrigeration temperature on antioxidant contents, activities, colour and microbiological growth during storage after pasteurisation

1.5 SCOPE OF LIMITATION

Regarding on completion of this project work, the pasteurization and storage method for reducing on the degradation of antioxidant and related properties of the RPJ are proposed. The method should be able to reduce alterations in color and degradation of antioxidant capacities and also bioactive compound contents while not compromise the microbiological safety of RPJ.

CHAPTER 2

LITERATURE REVIEW

2.1 POMEGRANATE

2.1.1 Origin of Cultivation

Granatum punica L. (Pomegranate) is a red fruit that is categorised as a berry and has a diameter in the range of 5-12 cm. Sass (1979) states that pomegranate is known as one of the ancient fruits, it is presumably originated in Persia, later expanding the cultivation across the Mediterranean and spread to Arabia, India, and China. Also, it is a drought-tolerant and long-lived crop commonly planted in arid and semiarid zones. They are mainly grown in Iran, India, and Mediterranean countries such as Turkey, Egypt, Tunisia, Spain, and Morocco. Despite being classified as a berry, it stills belongs to its own botanical family, Punicaceae (Zarfeshany et al., 2014).

2.1.2 Economic Importance towards Cultivar Countries

Pomegranate is resistant of high heat, iron chlorosis, salt, and active calcium carbonate, making it adaptable to all types of soil and climate (Kirshenbaum et al., 2015). Pomegranate germination is limited to the tropics and subtropics, and it thrives in arid and semi-arid regions. One of the cultivar countries which classify pomegranate as major fruit is Turkey. Pomegranate production and consumption are minimal in the tropics and subtropics if compared to other fruits, but it is a significant part of the industry because it is used to make fruit juice, citric acid, dye, vinegar, and medication (Ozkan, 2003).

From Table 2.1, Turkey has produced 217,575 tonnes of pomegranate fruit and most of them are consumed in local retailers and over 30% of the whole amount (85,000 tons) is exported to European and Near East countries, mainly Bulgaria, German, Russia and,

Ukraine. The substantial increase in pomegranate exports is influenced by the upward trend in production. According to the Ministry of Economy, this export brought in US$ 70 million in 2011.

Table 2.1 : Pomegranate production balance (Turkish Statistical Institute, 2013)

	Marketing year						
	2005/ 2006	2006/ 2007	2007/ 2008	2008/ 2009	2009/ 2010	2010/ 2011	2011/ 2012
Production (t)	80,000	90,737	106,560	127,760	170,963	208,502	217,572
Usable production (t)	78,640	89,194	104,748	125,588	168,057	204,957	213,873
Imports (t)	142	1,136	1,939	619	311	142	60
Imports - EU 27 (t)	"	"	-	-	-	36	-
Domestic use (t)	67,659	77,343	91,658	96,114	117,565	137,585	148,238
Human consumption (t)	62,246	71,156	84,325	88,425	108,159	126,579	136,379
Losses (t)	5,413	6,187	7,333	7,689	9,405	11,007	11,859
Exports (t)	11,123	12,987	15,029	30,092	50,803	67,514	65,695
Exports - EU 27 (t)	"	"	6,637	11,865	21,298	23,061	20,023
Consumption per capita (kg)	"	"	1.19	1.24	1.49	1.72	1.83
Degree of self sufficiency (%)	116.23	115.32	114.28	130.67	142.95	148.97	144.28

2.1.3 Increasing Popularity of Pomegranate Fruit in Malaysia

Pomegranate production, either it is consumed as fresh fruit or fresh juice, is constantly evolving globally. Despite the high price, pomegranate juice is sought after by many consumers because of its high antioxidant content, and the popularity of the juice has spread to non-cultivar countries. Vegara et al. (2013) state that the pomegranate juice has higher demand in recent years that makes the juice among the first line of functional juice market. In non-cultivar countries, where the pomegranate fruits are hardly obtainable, there

are already ready-to-drink (RTD) juice which can be found in form of reconstituted pomegranate juice, also known as RPJ.

INDIA EXPORT OF AGRO FOOD PRODUCTS

Product Report/Country Wise

Value In US$
Qty In Kg

Product: Pomegranates Fresh(08109010)

	2017-18		2018-19		2019-20	
Country	Qty	US$	Qty	US$	Qty	US$
U Arab Emts	1,97,99,750.00	3,60,02,905.00	1,90,30,400.00	2,97,59,371.00	1,58,97,724.00	2,58,84,094.00
Bangladesh Pr	5,10,740.00	2,60,500.00	1,05,11,535.00	65,18,113.00	3,38,13,124.00	1,95,21,693.00
Netherland	24,34,820.00	90,51,484.00	44,49,506.00	1,30,31,794.00	30,31,864.00	90,00,534.00
Saudi Arab	38,88,139.00	56,42,272.00	59,23,617.00	86,03,750.00	50,83,397.00	78,79,083.00
Nepal	84,81,543.00	36,42,411.00	1,00,18,756.00	53,47,772.00	87,75,983.00	51,54,281.00
Qatar	20,76,565.00	34,13,634.00	31,10,596.00	47,19,043.00	26,33,563.00	42,06,290.00
Oman	19,46,631.00	43,36,642.00	41,99,183.00	54,73,822.00	25,39,062.00	35,61,185.00
Thailand	5,93,676.00	22,16,613.00	13,21,826.00	29,46,461.00	11,44,710.00	26,91,278.00
Sri Lanka Dsr	15,03,861.00	28,31,414.00	12,38,697.00	25,52,207.00	11,89,802.00	25,50,203.00
U K	4,46,654.00	28,37,820.00	5,76,864.00	27,36,008.00	4,64,436.00	21,31,204.00
Kuwait	13,18,226.00	23,38,094.00	13,72,835.00	21,38,367.00	12,62,149.00	20,54,364.00
Malaysia	7,15,101.00	13,42,455.00	7,73,969.00	14,70,534.00	6,58,780.00	13,82,507.00
Belgium	68,512.00	5,08,344.00	2,02,704.00	15,81,455.00	1,95,132.00	12,91,863.00
Switzerland	37,239.00	3,13,897.00	65,128.00	5,59,776.00	1,61,566.00	12,69,182.00

Figure 2.1 : India Export of Agro Food Products (Pomegranate Fresh) to Malaysia from 2017-2020

In Malaysia, there is an advance in total of pomegranate fruit imported from cultivar countries such as India. Based on Figure 2.1, there is an increase in the importing trend of pomegranate fruit. In 2017-2018, about 715 101 kg of pomegranate fruit had been imported and this number is increased to 773969 kg in 2018-2019. Although the number of pomegranate fruit imported decreased for the next two years, the number is still in a huge amount which is 658780 kg. The decreased trend by 2019-2020 is due to Covid-19 pandemic which effects the import of pomegranate fruits. Thus, the increasing trend of pomegranate fruit imported from India describes that Malaysians are eager on the consumption of pomegranate fruit and pomegranate juice.

2.1.4 Chemical composition of Pomegranate Fruit

Pomegranate fruit is a nutrient dense fruit rich with phytochemical compound, low molecular weight compounds widely produced by plant as defense mechanisms (Sreekumar et al., 2014). Based on Table 2.2, about 50% of the total fruit weight correlated from the peel, which is the main source of bioactive compound such as phenolics, flavonoids, ellagitannins, and proanthocyanin compounds, minerals (mostly potassium, nitrogen, calcium, phosphorus, magnesium and sodium) and complex polysaccharides. The arils and seeds of the pomegranate fruit are edible, with the arils containing 85% water, 10% total sugars (primarily fructose and glucose), 1.5% pectin, organic acid (including ascorbic acid, citric acid, malic acid), and bioactive compound (such as phenolics, flavonoids and anthocyanin). The seed cover of the fruit consists of delphinidin-3-glucoside, cyanidin-3-glucoside, pelargonodin-3,5-diglucoside, and pelargonidin-3-glucoside with delphinidin-3,5-diglucoside as the main anthocyanin in pomegranate juice. Phenolic compounds, alongside with flavonoids, anthocyanins and tannins are the major group of antioxidant phytochemical that are essential because of their biological and free radical scavenging activities.

Table 2.2 : The principal constituents of different parts of pomegranate tree and fruit. (BioMed Research International,2014)

TABLE 1: Principal constituents of different parts of pomegranate tree and fruit. The different parts of pomegranate plant like peel, root, bark, flower, leaves, and so forth exhibit different phytochemicals.

Pomegranate peel	Pomegranate juice	Pomegranate root and bark	Pomegranate flower	Pomegranate leaves	Pomegranate seed
(i) Gallic acid (ii) Ellagic acid (iii) Punicalin (iv) Punicalagin (v) Caffeic acid (vi) Ellagitannins (vii) Pelletierine alkaloids (viii) Luteolin (ix) Kaempferol (x) Quercetin	(i) Simple sugars (ii) Aliphatic organic acids (iii) Gallic acid (iv) Ellagic acid (v) Quinic acid (vi) Flavonols (vii) Amino acids (viii) Minerals (ix) EGCG (x) Ascorbic acid	(i) Ellagitannins (ii) Piperidine alkaloids (iii) Pyrrolidine alkaloid (iv) Pelletierine alkaloids	(i) Gallic acids (ii) Ursolic acid (iii) Triterpenoids (iv) Fatty acids	(i) Carbohydrates (ii) Reducing sugars (iii) Sterols (iv) Saponins (v) Flavanoids (vi) Tannins (vii) Piperidine alkaloids (viii) Flavone (ix) Glycoside (x) Ellagitannins	(i) 3,3′-DI-O-methylellagic acid (ii) 3,3′,4′-Tri-O-methylellagic acid (iii) Punicic acid (iv) Oleic acid (v) Palmitic acid (vi) Stearic acid (vii) Linoleic acid (viii) Sterols (ix) Tocopherols (x) Sex steroids

2.1.5 Nutritional value of Pomegranate Fruit on Health Benefit and Cancer

Pomegranate fruit popularity quite significant among the consumers due to the benefits to the health. Certain components in pomegranate such as polyphenols have shown to have potential antioxidant, anti-inflammatory, and anticarcinogenic impact. Pomegranate juice's antioxidant potential is greater than that of red wine or green tea, which is stimulated by ellagitannins and hydrosable tannins (Zarfeshany et al., 2014).

The pomegranates have impressive antioxidant properties, which are correlated to high polyphenol content. Polyphenols' health benefits are mostly due to their ability to scavenge free radicals. Polyphenol-rich diets have been associated with a lower risk of cancer mortality and coronary heart disease (Turfan et al., 2012).

In ancient Egypt, pomegranate fruit is known as a remedy for treating inflammation, diarrhoea, intestinal worms, coughing and infertility (Labib & El-Ahmady, 2015).

Besides, pomegranate peel is well-known for its strong astringent, anti-inflammatory properties and being a therapy for traumatic haemorrhage, ulcers, infections, dysentery, dental plaque and as a douche and enema agent. Traditional Chinese treatment also practise pomegranate fruit in assisting haemostasis, killing parasites, overcoming hyperacidity, potent wound healing abilities, therapy of diabetes, cancer and blood pressure control (Khwairakpam et al.,2018).

Pomegranate fruit is also notable for its anti-parasitic properties and has been applied as a blood tonic, as an active agent to cure ulcers and metabolic syndromes. On account of the high antioxidant compounds in pomegranate fruit, this fruit has been a successful remedy in combatting superoxide anion, hydroxyl and proxy radicals and also to preventing low density lipoprotein (LDL) oxidation related to copper sulphate, $CuSO_4$ (Medjakovic & Jungbauer, 2013).

Pomegranate fruit has recently been revealed as a natural remedy for oxidative stress-related diseases like Alzheimer's disease and aging. Pomegranate fruit is also recognized as a "super-fruit" or "youth elixir" for its ability to preserve and improve customer wellbeing. The key benefits of pomegranate fruit to be used as an herbal cure are its high antioxidant content and immunity-boosting properties. Pomegranate fruit also found to be able to oppose with cardiovascular disease and to prevent the development and growth of tumour cells in human body. Pomegranate-mediated antioxidant activity can be viewed as a means of lowering the inflammatory threshold. Pomegranate fruit may have chemotherapeutic and chemo preventive properties due to its antioxidant activity and inflammation suppression (P. Sharma, McClees, & Afaq, 2017).

Bladder cancer is one of the most common and deadly cancers of the urinary tract, with poor diagnoses for patients in advanced stages. Pomegranate is well-known as a

versatile food with various health benefits in humans, and it has been discovered to play an important role in the treatment of bladder cancer (Masci et al., 2016). Furthermore, pomegranate fruit is also used in preventing breast cancer. Pomegranate has been shown to be an effective cure for breast cancer. Experiments have shown that PJ and its components, such as luteolin and punicic acid (PA) can enhance tumour cells adhesion by up-regulating E-cadherin and minimize tumour cell migration while having no effect on normal cells (Rocha, Wang, Penichet, & Martins-Green, 2012).

Pomegranate is renowned for its health benefits, which include anti-cancer qualities. In recent years, several clinical trials on pomegranate's anti-cancer properties in colon and prostate cancer have been performed. Studies has shown that pomegranate juice have protective properties against prostate cancer. The median levels of prostate-specific antigen (PSA) increased by 14.7 percent in patients who took a food supplement containing pomegranate, which is high in polyphenol. For the treatment of prostate cancer patients, pomegranate juice, extracts, and whole fruit powder were widely used (Zhao et al., 2018).

2.2 GENERAL PROCESSING OF RECONSTITUTED POMEGRANATE JUICE (RPJ)

Pomegranate juice is the unfermented but fermented liquid produced from the edible portion of the sound, suitably mature and fresh pomegranate juice. Two distinct methods can be used to acquire pomegranate juice: extraction from separated arils and pressing of the whole and unpeeled fruit. The juice is prepared by adequate processes that still retain

the necessary physical, chemical, organoleptically, and nutritional values of the pomegranate juice.

For the first method, the separated arils are distributed to the juice extraction process while the arils are pressed to extract the juice. Then, the freshly pressed juice is permitted to settle for clarification. The clarification process is essential to remove such macromolecules. Enzyme treatment of raw juice is achieved by using conventional methods with the aid of enzymes such as pectinase and amylase to lower the pectic substance and starch content followed by the addition of fining agents. This enzymatic treatment is used to minimize the cloudiness and viscosity and thus makes the clarification process easier (Bhattacharjee et al., 2017). The clumps of cloud particles produced during juice clarification are due to the breakdown of pectin or polygalacturonic acid and this can be precipitated out. Subsequently, the clarified juice is evaporated to manually remove a sufficient quantity of water inside the concentrate to enhance the Brix level to a value at least 50% greater than the Brix value set for the reconstituted juice from the same fruit. There, pomegranate concentrate is made.

Before packaging and storage, pomegranate concentrates of 65° to 70° Brix are pasteurised. At this stage, the pasteurisation process is referred to as primary industry pasteurisation (Tetra Pak,2014). Pasteurisation of pomegranate concentrate is combined with evaporation as preheating in some cases. Then, the pomegranate concentrate is frozen, packed, and stored in a freezer at a temperature of -18°C or in a chiller temperature of 0°C to 4°C. Hence, the pomegranate concentrate is eligible for the following shipments in frozen or chilled conditions.

Pomegranate concentrate is obtained in either a frozen state or a chilled condition in importing countries. The juice reconstitution is accomplished via diluting the pomegranate concentrate with potable water. The standard formula for juice reconstitution is the ratio of pomegranate concentrate to the water of 1:3 or 1:1.25, consequently forming reconstituted pomegranate juice (RPJ). Also, there are sugar, ascorbic acid, and other flavorings are added into RPJ depends on the manufacture and consumer's undertaking. Based on Codex General Standard for Fruit Juices and Nectar, the minimum Brix level for the RPJ is 12° Brix (Codex Alimentarius Commission, 2005).

Later, RPJ is accustomed to secondary pasteurisation, cooling, and then packaging. RPJ can be hot filled during primary packaging, depending on the packaging material, the material should be able to endure hot temperature. Leading up to secondary packaging and storage, the filled RPJ is cooled first. The finished product of RPJ is kept at ambient temperature or chilled condition by the local manufacturers.

Nevertheless, studies and reports have been conducted in importing countries on the substantial loss of quality in RPJ production. One of the major conventional techniques for the Nevertheless, studies, and reports have been conducted in importing countries on the substantial loss of quality in RPJ production. country (Asadi-gharneh et al., 2017). One of the major conventional techniques for fruit juice concentrate processing is thermal evaporation. To lessen the storage and shipping costs, alongside achieving consistency and a longer storage period, the fruit juices are often concentrated using multiple-stage vacuum evaporation (Provesi, Dias, & Amante, 2011). Still, there are some limitations when applied to fruit juices. Operating temperatures are still high enough to cause substantial deterioration in the product juice such as colour degradation, nutrient loss, and

appearance of a 'cooked' flavour even though under vacuum. For instance, the oxidation of lipids and ascorbic acid, the Maillard browning reaction of amino acids and sugars, and degradation of pigments, mainly anthocyanin, and carotenoids (Maskan, 2006).

Throughout the secondary production of RPJ, the pomegranate concentrate is subjected to further thermal processes, oxidation, and storage condition that further causes the loss of antioxidant content in RPJ. Thermal processing persists as the most widely used method for shelf-life extension and food preservation and concentration (Vegara et al., 2013).

Also, Turfan et al., (2012) explain the pomegranate juice processing using pressing of the whole fruit as shown in Figure 2.2. The pomegranate fruit is harvested from the plantation and is distributed to a processing plant for juice manufacturing. They were only kept at 4°C for roughly 2 days after harvesting before being turned into juice. To avoid microbial contamination, the pomegranates were washed in cold tap water and rinsed before the top and bottom of the rinds were chopped with a sharp knife. To obtain the juice, the pomegranates were chopped into four pieces and squeezed using a rack-and-cloth press. Halves of the juices then were clarified using only gelatin at 5°C while the remaining are direct to the concentration process. For the clarification of the juice, 1% (w/v) gelation solutions were utilized. The juice is concentrated approximately 68° Brix using a rotating low-pressure evaporator at 40°C and 20 mm-Hg pressure during the concentration process.

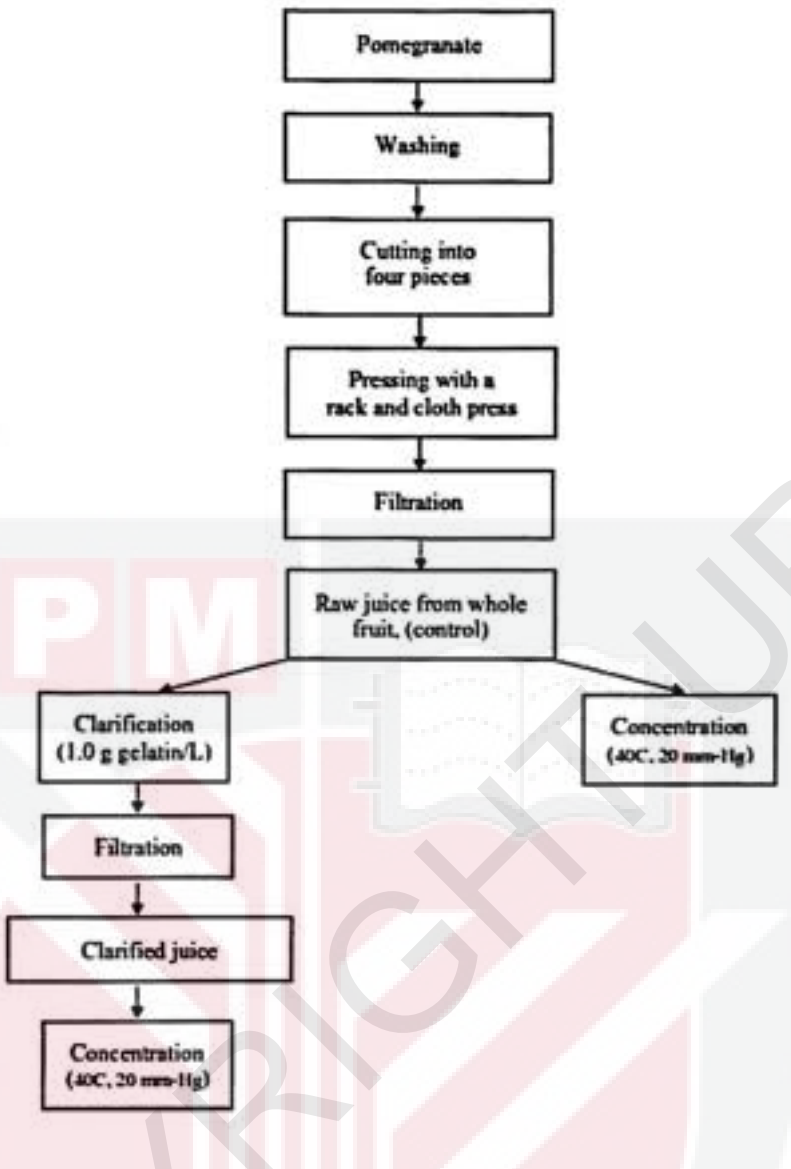


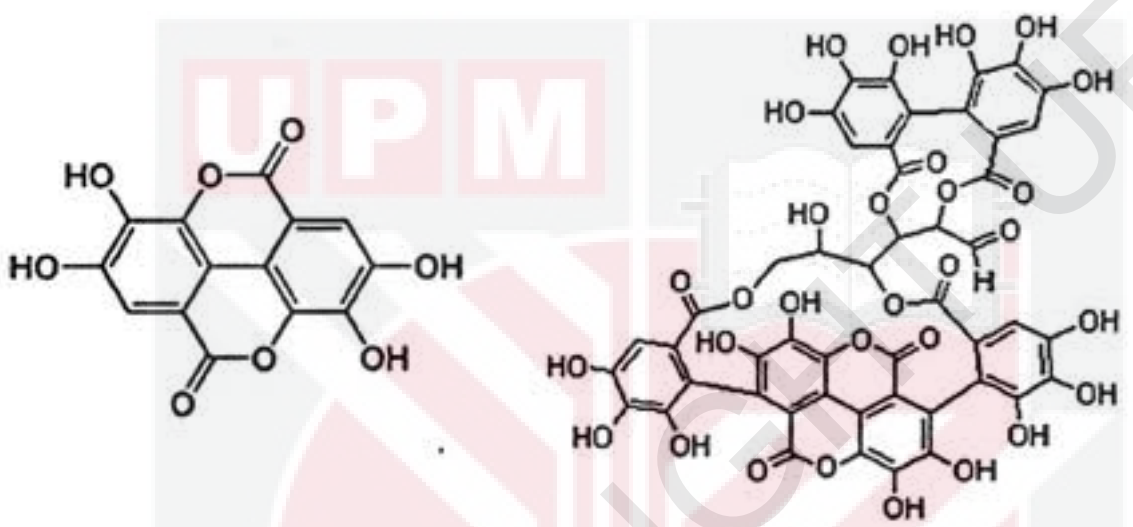
Figure 2.2 : Processing of pomegranate juice concentrate (Turfan et al., 2012)

2.3 BIOACTIVE COMPOUNDS

2.3.1 Polyphenols

Polyphenols are secondary plant metabolites and are typically associated with the prevention of pathogens against ultraviolet radiation or attack. Polyphenols can lead to bitterness, astringency, colour, taste, odour, and oxidative stability in foods. The product of pomegranate juice exhibits strong antioxidant and anti-atherosclerotic properties due to its high content of free and bound polyphenols, including ellagic acids (EA), such as

ellagitannins (ETs) and ellagic acid glycosides (EAGs), gallotannins, and anthocyanins, including cyanidins, glycosides of delphinidin and pelargonidin, as well as other flavonoids such as quercetin, kaempferol, and luteolioli glycosides. The major profuse of these polyphenols is punicalagin, an ET implicated as the bioactive constituent responsible for 50 % of the juice's antioxidant activity (July, Venkata, Prakash, & Prakash, 2011).



Ellagic acid Punicalagin

Figure 2.3 : Structures of Punicalagin, the major polyphenol antioxidant in PJ, and Ellagic Acid (EA) (F.Les et al., 2018)

Pomegranate is rich in polyphenols like flavanoids (anthocyanins, catechins and other complex flavonoids) and hydrolysable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid esters of glucose), which are in charge of the antioxidant activity. Punicalagins A, Punicalagins B and ellagic acid are reported as the key phenolic compounds in pomegranate fruit. Functional and nutritional properties of pomegranate results from the high levels of tannins and other beneficial biochemicals like phenolics, which make pomegranate as essential part of the human diet. About 153

different phytochemicals with the ability to combat diseases have been detected in pomegranate with polyphenols being the most abundant compound (Schaffer et al., 2012).

2.3.2 Anthocyanin

Anthocyanins are the biggest and most important group of flavonoids available in pomegranate arils, which are used to produce the juice. These pigments distribute in producing the red color to the fruit and juice (Afaq et al., 2005). Anthocyanin is odorless, tasteless, and has a strong astringent smell, which is the reason some fruits are astringent. Flowering plants and plants of higher classes are commonly abundant in anthocyanins.

Anthocyanins have physicochemical properties that give them their distinct color and stability. They are highly reactive molecules, rendering them sensitive to degradation. The chemistry of anthocyanins, and thus their stability and color, can be influenced by a number of factors including oxygen, temperature, light, enzymes, and pH (Khoo, Azlan, Tang, & Lim, 2017).

There are a great variety of anthocyanins in the pomegranate such as cyanidin-3-O-glucoside, cyanidin-3,5-di-O-glucoside, delphinidin-3-O- glucoside, delphinidin-3,5-di-O-glucoside, pelargonidin-3-O-glucoside, and pelargonidin-3,5-di-O-glucoside (Lansky & Newman, 2007). The chemical composition is shown in Figure 2.4. The bioactivities of pomegranate are defined by the quantities of the above-mentioned compounds, as well as the pomegranate varieties. The red-purple coloration of pomegranate fruit is attributed to

six anthocyanin pigments. Pomegranate fruit can turn pale due to a lack of or loss of these anthocyanin pigments (Fischer, Dettmann, Carle, & Kammerer, 2011).

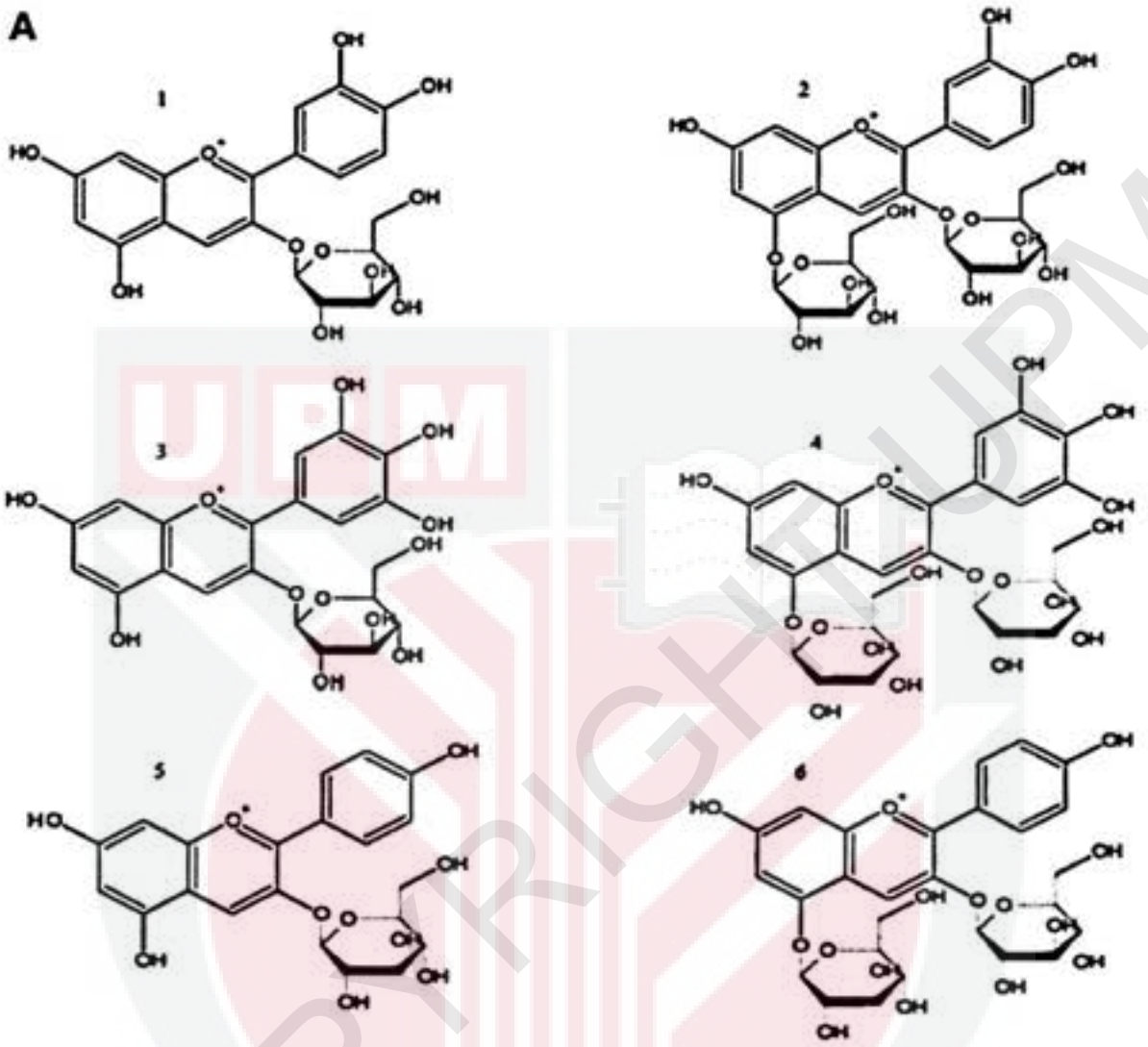


Figure 2.4 : Principal anthocyanins present in pomegranate juice. 1: cyanidin-3-O-glucoside; 2: cyanidin-3,5-di-O-glucoside; 3: delphinidin-3-O- glucoside; 4: delphinidin-3,5-di-O-glucoside; 5: pelargonidin-3-O-glucoside; 6: pelargonidin-3,5-di-O-glucoside pomegranate juice (Fawole & Opara, 2013).

The number and arrangement of aromatic hydroxyl groups, the extent of structural conjugation and the presence of electron-donating and electron-withdrawing substituents

in the ring structure made anthocyanins very effective donors of hydrogen to highly reactive free radicals. This influence preserves cells from oxidative damage, which causes aging and diseases like cancer, neurological and cardiovascular diseases, inflammation, diabetes, and bacterial infections, and others. The antioxidant potential of phenolic compounds is also due to chelate metal ions, which participate in the production of free radicals and thus reduce metal-induced peroxidation (Ceci et al., 2018).

2.4 COLOR CHANGES OF POMEGRANATE JUICE DURING STORAGE

The psychochemical properties of pomegranate fruit and pomegranate juice depend significantly on the cultivar, environment, and the techniques used to extract the juice (Akbarpour, Hemmati & Sharifani, 2009). Pomegranate fruit is usually consumed as fresh fruit, fresh arils, or being processed into products such as juice or concentrate. Hence, progressing maturity which correlates to a variety of integrated physiological, biochemical and structural features that lead to changes in color and flavor during processing, further influencing the taste and sensation during consumption.

Recent studies found that pomegranate juice's physicochemical properties significantly influence by the type of cultivar, growing area, maturity, cultural tradition, storage, and processing conditions. Physicochemical properties including pH, Titratable Acidity (TA), Total Soluble Solids (TTS), Total Sugars, and color are essential aspects to be observed once the processing of pomegranate fruit into pomegranate concentrate.

The physicochemical properties of pomegranate fruits are thus essential parameters in determining the quality of the juice. Besides, pomegranate juice or RPJ color

characteristics have been found to reduce throughout manufacturing processes and storage time. For example, after pasteurisation treatment and higher storage temperature, the red coloration of pomegranate juice decreases. The loss of red pomegranate juice color was because of the loss or degradation of anthocyanin, the primary source of red pigmentation for pomegranate juice (Vegara et al., 2013).

2.5 ANTIOXIDANT PROPERTIES OF POMEGRANATE JUICE

The antioxidant properties of pomegranate juice are higher than any other fruit juices. These antioxidant properties have been associated with a great number of phenolic compounds, which include anthocyanins (3-glucosides and 3,5-diglucosides of delphinidin, cyanidin, and pelargonidin), ellagic acid, punicalin, punicalagin, pedunculagin, and various flavanols (Bazargani-Gilani. B., Tajik. H & Aliakbarlu. J., 2014). Anthocyanin is one of the major antioxidants in the pomegranate juice is which contributes the natural red coloration of the pomegranate fruit and juice. A pomegranate juice that has a higher antioxidant content will have a higher quality compared to other fruit juices that has lower antioxidant content.

However, through thermal processing and storage period, there will be degradation of anthocyanin. According to a study, anthocyanin degrades during the storage period. Anthocyanin degradation is endowed with low anthocyanin stability throughout processing and storage. Besides, the storage temperature of pomegranate juice also significantly influences total anthocyanin pigments. Oxidation and condensation of anthocyanin pigments with ascorbic acid also affect anthocyanin degradation. Hence, loss

of anthocyanin pigments is affected by many factors such as storage temperature, Ph, enzymes, and reaction with by-products of ascorbic acid degradation (Alighourchi & Barzegar, 2009).

Moreover, the increasing storage temperature causes the kinetic parameter of anthocyanin degradation during storage at a specified temperature also to increase. This is because the degradation of anthocyanin is intensified by high temperatures. Degradation of anthocyanin is also usually linked with color loss of pomegranate fruit. As anthocyanin is the natural red pigment presented in the juice, thus loss of anthocyanin will result in the loss of the red color of pomegranate juice. It is suggested that color or chromatic parameters are suitable indicators to evaluate the anthocyanin levels and pomegranate juice quality (Meighani, Ghasemnezhad & Bakhshi, 2017).

2.6 EFFECT OF PASTEURISATION ON POMEGRANATE JUICE

The most common approach to enhancing the shelf life of fruit juices by inhibiting microorganisms with limited 5-log reduction and spoilage enzymes is thermal processing.

According to a study, thermal treatment is a significant contribution to the deactivation of microorganisms in fruit juices. Compared to untreated pomegranate juice, total plate count (TPC) and yeast and mould (Y&M) have decreased in correlation with the thermal treatment time and thermal treatment temperature. The inactivation of microorganisms by heat treatment is primarily due to the changes in the composition of the membrane due to irreversible protein, enzyme denaturation, and intercellular content leakage subjected to high temperature (Ma et al., 2019).

Nonetheless, conventional heat treatments such as pasteurisation often contribute to changes in the physicochemical and antioxidant properties of pomegranate juice. For example, a change or loss of color of pomegranate juice is detected after thermal treatment. This is because of the degradation of sugar products and the rate of deterioration of anthocyanin has increased.

Through thermal treatment, the antioxidant properties of pomegranate juice are greatly reduced. Research has shown that the decrease in total phenol content (TPC) in pomegranate juice by thermal treatment is linked to the degradation of phenolic compounds by non-enzymatic oxidation and thermal instability of these compounds. The progression of chemical reactions, which effectively promoted the oxidative degradation of the total phenols is stimulated by the high temperature. Degradation products from organic acids formed by thermal treatment are condensed with anthocyanin, thus reducing the content of anthocyanin in pomegranate juice. These condensation reactions include the covalent interaction of anthocyanins with other organic acids present in juices, resulting in the formation of a new pyran ring by cycloaddition, which is necessary for the color changes of pomegranate juice (Marszalek & Wo, 2017).

2.7 EFFECT OF STORAGE TEMPERATURE ON POMEGRANATE JUICE

The temperature of storage has an important influence on the physicochemical and antioxidant properties of the juice. Research proves that there is no major difference in the physicochemical properties of lower-temperature stored fruit juice. For chromatic parameters, the red color of pomegranate juice deteriorated after storage with higher

storage temperature. The loss of the red color of pomegranate juice at high storage temperature can lead to an increase in browning reactions alongside the degradation of anthocyanin, the red pigments. The higher storage temperature causes the browning reaction, which then reduces the redness of pomegranate juice. This is illustrated in the decreasing L* values and the shifts in a* and b* values. The formation of acidophilic microorganisms such as lactic acid bacteria and yeasts and moulds enhances the spoilage of pomegranate juice during higher storage temperature, then resulting in changes in the pomegranate juice quality (Ampofo-asiama & Quayq, 2019).

The stability of anthocyanin in pomegranate juice is also affected by the increasing storage temperature (Mena, Marti & Garcia-Viguera, 2014). The higher rate constants of the degradation process depend on the temperature increase. According to the study, it is proven that there is a decrease in the total phenol content of fruit juice during storage at a higher temperature. The reduction of the polyphenolic content of fruit juice at high temperatures is because of the increased oxidation of these bioactive components. The rates of these oxidation reactions increase as temperature increases. Hence, the degradation rate of polyphenols is more rapid in higher storage temperatures compared to lower storage temperatures. Furthermore, anthocyanin has a higher degradation rate compared to polyphenols at higher storage temperatures. This shows that polyphenols have higher stability compared to anthocyanin (Popa, 2016).

2.8 ASEPTIC FILLING

The major factors of food deterioration and spoilage are improper handling, chemical reactions and growth of microorganisms (Driscoll & Peterson, 1999). It is important to provide optimum protective measures during filing process to maintain good condition of juice throughout its shelf life. If sealing and filling are done in accessible areas, it can lead to recontamination of the product when the product cold. The fundamental concept in aseptic filling is shown in Figure 2.5, the pumpable food is continuously heated to pasteurized temperature , and after holding, continuously being cooled. Also, the bottle and the lid as the package are sterilized with steam or a mixture of superheated steam and air. The pasteurized food and sterile bottle are put in an aseptic enclosure for filling process. Certain measures, such as disinfectants or a steady stream of superheated steam, are utilized to maintain aseptic conditions while kept at a modest over-pressure to avoid air penetration from outside (Food Process Engineering and Technology, 2013).

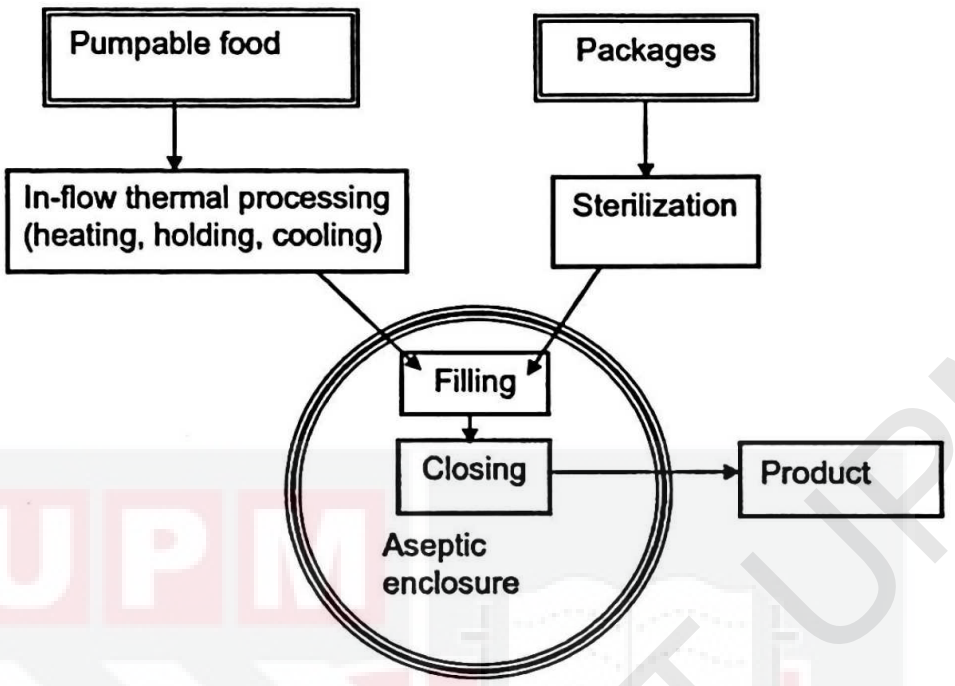


Figure 2.5 : The schematic flow of aseptic filling

In aseptic processing systems, the product is transported to a sterile container and cooled relatively close to ambient temperature as soon as sterilisation process. Thus, commercial sterility of the product need to be preserve starting from the heat processing until packaging operations. The product is a commercially sterile product in a hermetically sealed container that can be stored at ambient temperatures for longer period, which up to several months.

CHAPTER 3

METHODOLOGY

3.1 DESIGN OF RESEARCH

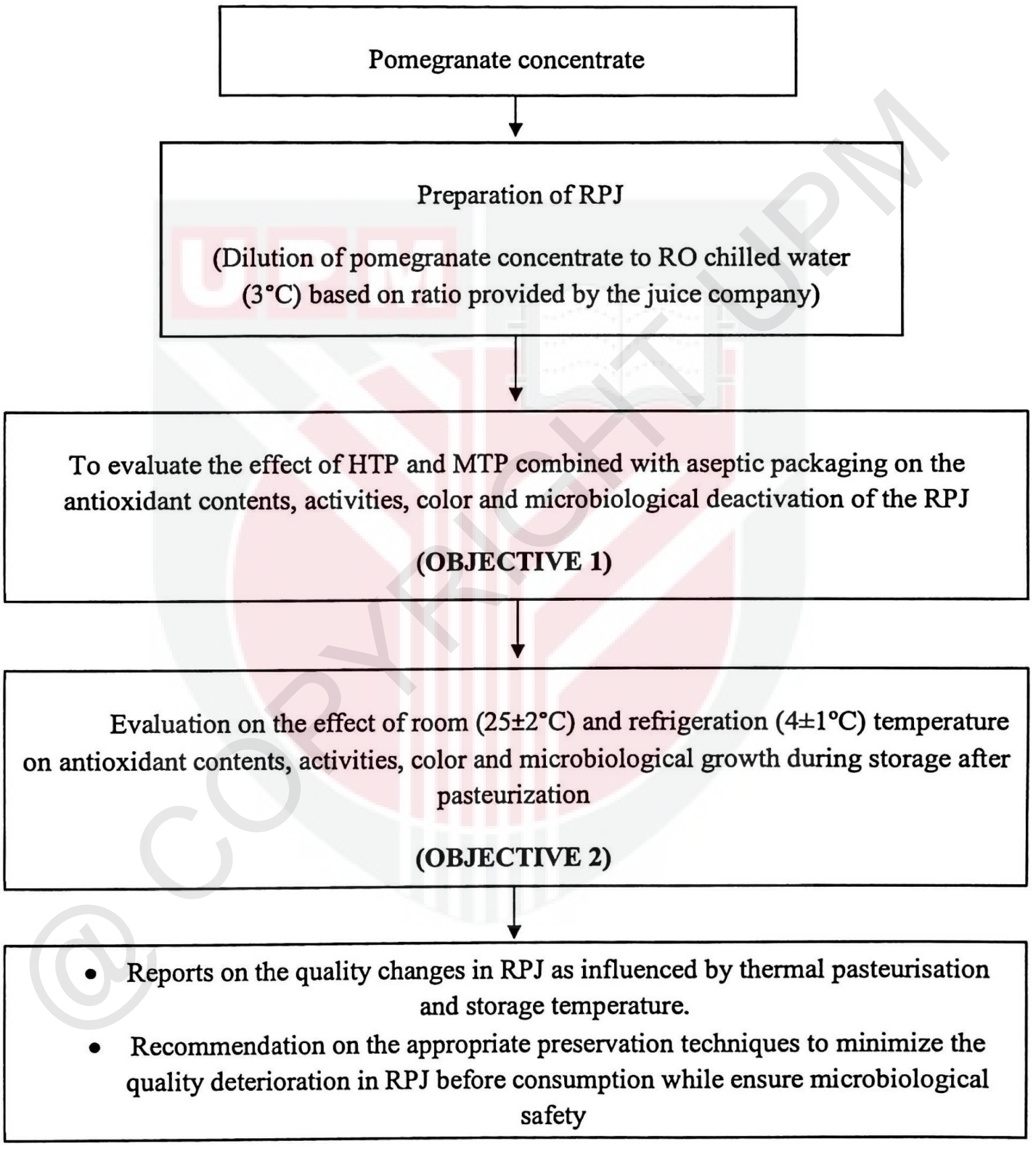


Figure 3.1 : The process flow of design research RPJ

3.2 POMEGRANATE CONCENTRATE PROCUREMENT

Pomegranate concentrates were purchased from the regular supplying company, whereby they were imported from origin country, Turkey.

3.3 PREPARATION OF RPJ

Reconstituted pomegranate juice was prepared by diluting the pomegranate concentrate with chilled water (3°C), treated using reverse osmosis process based on the ratio provided by the juice manufacturer and through thorough stirring.

3.4 PASTEURISATION OF RPJ

Pasteurisation of RPJ was done at different pasteurisation conditions. For industrial pasteurisation, the pasteurisation temperature used was 95°C with a holding time of 30 seconds (Memet, 2011). This is also known as high-temperature pasteurisation (HTP) according to Mena et al. (2013), who studied different categories of pasteurisation.

Before proceeding with pasteurization process, the PH of RPJ is measured using pH meter. This is to ensure the RPJ has pH below of 4.2. Smith & Stratton (2007) states that it is significant in juice preservation for the juice to have pH of 4.6 or lower as this condition will not assist the growth of Clostridium botulinum and prefer a less extreme heat treatment to produce shelf-stable and safe RPJ. The values obtained for control, HTP and MTP were 3.10, 3.11 3.02 respectively.

The RPJ was poured into a glass bottle that had been pre-sterilised in an autoclave. A water bath is heated until constant temperature of 95°C. Then, the bottle was immersed in the water bath until RPJ reached constant temperature of 95°C. After 30 seconds, the bottle is taken out and cooled rapidly to room temperature. For reduced pasteurisation, the pasteurisation temperature used was 80°C with a holding time of 30 seconds. This is also known as mild-temperature pasteurisation (MTP) according to Mena et al. (2013). The RPJ was poured into a bottle that had been pre-sterilised. A water bath is heated until the temperature reached constant temperature of 80°C. The bottle was immersed in the water bath until RPJ also reached temperature 80°C. After 30 seconds, the bottle was taken out and cooled rapidly to temperature below 60°C. The RPJ was poured into pre-sterilised glass bottle using aseptic filling.

For aseptic filling, this process is conducted fully under laminar hood. The laminar hood's surface is sprayed with alcohol before turned on the Bunsen burner. The bottle's mouth and lid are flame sterilized to ensure no microorganisms enter the mouth of the bottle. The aim of flaming is to warm the bottle's opening and allow an air convection currents rise and away from the opening. The heated, rising air kept out any dust particles and other contaminants away (Sanders,2012). The RPJ is poured until about full to minimize headspace which can lead to contamination. Sultan et al. (2019) suggested to perform filling with minimum head space in the bottle and make sure the bottle is tightly closed to allow anaerobic condition to preserve the RPJ.

Figure 3.2 : RPJ in glass bottles after pasteurisation

Both pasteurised RPJ (HTP- and MTP-samples) were evaluated for the following qualities and compared with untreated juice (control):

- Color
- Total phenol contents
- Total anthocyanin contents
- Antioxidant activity
- Microbiological deactivation (total plate count, yeast and mould)
-

3.5 STORAGE STABILTY DUE TO DIFFERENT TEMPERATURE

Both HTP and MTP-samples were stored at room temperature (25±2°C) and at refrigeration (4±1°C) for 21 days. At an interval of 7 days, the samples were analysed to investigate the color changes, antioxidant activity (using FRAP method), total anthocyanin and total polyphenol content in order to investigate their degradation in different storage conditions. The samples were also analysed for microbiological growth namely total plate count, yeast and mold.

3.6 ANALYSIS ON COLOR CHANGES OF RPJ

A portable calorimeter (FRU WR18 Universal Portable Colorimeter, Shenzhen) used for color measurements. The instrument is ensured clean from any dust or particle that may affect the reading. A petri dish containing the samples was placed below the light source. L^* (lightness), a^* (redness) and b^* (yellowness) values of each sample were determined. Chroma, C^*, hue angle, h° and colour difference, ΔE were calculated as follows:

$$\text{Chroma, } C^* = (a^{*2} + b^{*2})^{1/2}$$

$$\text{Hue angle, } h^\circ = \arctan b^*/a^*$$

$$\text{Colour difference, } \Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

3.7 ANALYSIS ON ANTIOXIDANT PROPERTIES OF RPJ

The analysis is done using spectrophotometer (Figure 3.3) to measure the absorbance. The sample is diluted to 1:10 ratio with distilled water to achieve absorbance value which lies between the standard range.

Figure 3.3 : Spectrophotometer used to measure absorbance

Figure 3.4 : Each sample were triplicate for analysis

3.7.1 Total Polyphenol Content

Procedure for the determination of total polyphenol was adapted and modified from Johari & Khong (2019).

Preparation of Folin Ciocalteu Reagent

20 ml of Folin Ciocalteu's reagent was placed into a beaker and was diluted 10-fold with distilled water.

Preparation of Standard Curve of Gallic Acid

Procedure for the preparation of standard curve of gallic acid was adapted and modified from Kamtekar, Keer, & Patil (2014). 10mg of gallic acid powder was dissolved into 10ml of distilled water to produce a gallic acid stock solution. Next, several dilutions of gallic acid in distilled water were carried out to give off solutions with concentration between 0 mg/ml and 1 mg/ml. The standard gallic acid solutions were positioned into small reagent bottles. 5ml of Folin Ciocalteu's reagent was mixed into the small reagent bottles and were allowed to incubate in the dark for 5 minutes. Later, 4ml of 7.5% sodium carbonate solution was added into the small reagent bottles and were left to incubate in the dark for 2 hours. After incubation, absorbance was measured at 765nm using UV spectrophotometer (Varian, Cary 50, Australia). The standard calibration curve of gallic acid was plotted.

Preparation of Sample

Procedure for the preparation of sample was adapted and modified from Alhakmani, Kumar, & Khan (2013), Baba & Malik (2015) and Johari & Khong (2019). The RPJ samples were first diluted by liquefying 1ml of the RPJ samples in 5ml of distilled water. Next, 1ml of diluted RPJ samples with 5ml of Folin Ciocalteu's reagent were mixed into the small reagent bottles and were allowed to incubate in the dark for 5

minutes. Later, 4ml of 7.5% sodium carbonate solution was added into the small reagent bottles and were left to incubate in the dark for 2 hours. After incubation, absorbance was measured at 765nm using UV spectrophotometer (Varian, Cary 50, Australia). The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as gallic acid equivalent (GAE) and was later multiplied with the dilution factor.

$$\text{Total concentration (mgGAE/ml)} = [(A - c)/m] \times DF$$

Where A = absorbance, c = y-intercept on standard curve of gallic acid, m = gradient of standard curve of gallic acid and DF = dilution factor

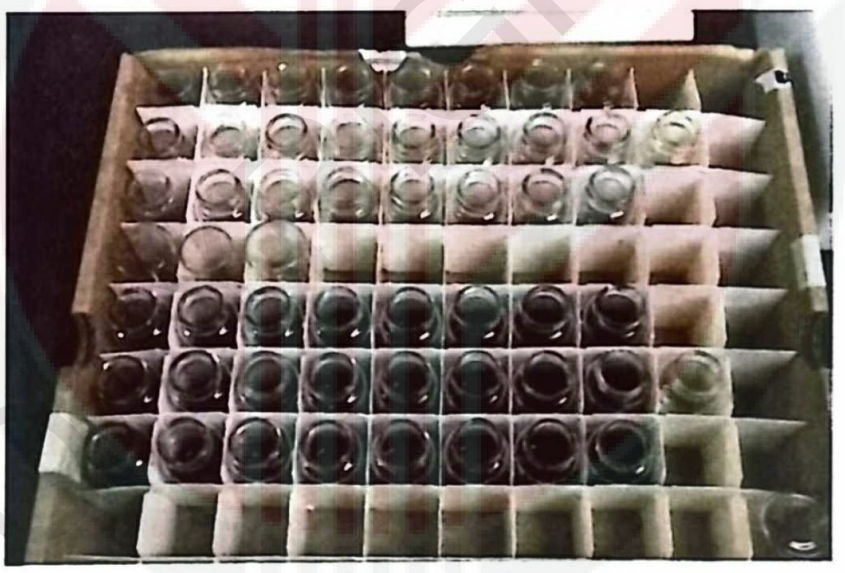


Figure 3.5 : Measurement on Total Phenol Content of RPJ Using Folin Ciocalteu's Reagent

3.7.2 Total Anthocyanin

Procedure for determination of anthocyanin content was adapted and modified from Mayuoni Kirshenbaum et al. (2016). Anthocyanin content was evaluated by means of UV visible spectroscopy. 1ml RPJ sample were added to 20ml of 0.025 mol/l (0.025M) potassium chloride at pH 1.0 and 0.4 mol/l (0.4M) sodium acetate at pH 4.5. The sample was incubated for 15 min at room temperature and absorbance at 510 and 700 nm was measured with an UV spectrophotometer (Varian, Cary 50, Australia). The absorbance was calculated as:

$$A = (A_{510}\,\text{nm} - A_{700}\,\text{nm})_{\text{pH 1.0}} - (A_{510}\,\text{nm} - A_{700}\,\text{nm})_{\text{pH 4.5}}$$

The monomeric anthocyanin pigment concentration was calculated as cyanidin-3-glucoside equivalent according to the following equation:

$$\text{Total concentration (mg/l)} = A \times \text{MW} \times \text{DF} \times 1000 / (\varepsilon \times l)$$

Where A = absorbance, MW = molecular weight (449.2g/mol), DF = dilution factor and ε = molecular absorptivity (26,900L/cm-mol) and l = path length (cm)

Figure 3. 6: Measurement on Total Anthocyanin of RPJ

3.7.3 Antioxidant Activity using Ferric Reducing Antioxidant Powder (FRAP) method

The procedure for determining antioxidant activity was adapted and modified from Martins et al. (2013) and Wootton-Beard, Moran, & Ryan (2011). The FRAP method works based on the principle of reduction by antioxidant on the complex ferric ion – TPTZ. The binding of Fe^{2+} to a ligand or an antioxidant creates a very intense navy-blue colour. The absorbance can be measured to test the amount of iron reduced and can be correlated with the number of antioxidants. The absorbance of the navy-blue coloration depends linearly on the antioxidant concentration. The higher the antioxidant concentration, the darker the navy-blue colour (Pisoschi & Negulescu, 2012).

Preparation of FRAP Working Solution

0.0625g of 4,6-tripryridyl-s-triazine (TPTZ) powder was dissolved in 20ml of 40mM hydrochloric acid solution to produce a TPTZ solution. Next, 150ml of acetate buffer at pH 3.6 and 15ml of iron (III) chloride solution were mixed into the TPTZ solution. This FRAP working solution was later shaken in a water bath at 37°C.

Preparation of Standard Curve of Ascorbic Acid

10mg of ascorbic acid powder was dissolved into 50ml of distilled water to produce an ascorbic acid stock solution. Next, several dilutions of ascorbic acid in distilled water was carried out to give off solutions with concentration between 0 mg/ml and 1 mg/ml. The standard ascorbic acid solutions were positioned into small reagent bottles. 2.850ml of FRAP working solution was mixed into the small reagent bottles and were allowed to

incubate in the dark for 30 minutes. After incubation, absorbance was measured at 515nm using UV spectrophotometer (Varian, Cary 50, Australia). The standard calibration curve of ascorbic acid was plotted.

Preparation of Sample

The RPJ samples were first diluted by liquefying 1ml of the RPJ samples in 5ml of distilled water. Next, 1ml of diluted RPJ samples with 2.850ml of FRAP working solution were placed in small reagent bottles. The samples were allowed to incubate in the dark for 30 minutes. After incubation, absorbance was measured at 515nm using UV spectrophotometer (Varian, Cary 50, Australia). The antioxidant activity were determined from the linear equation of a standard curve prepared with ascorbic acid. The antioxidant activity was expressed as ascorbic acid equivalent (AAE) and was later multiplied with the dilution factor.

$$\text{Total concentration (mgAAE/ml)} = [(A - c)/m] \times DF$$

Where A = absorbance, c = y-intercept on standard curve of ascorbic acid, m = gradient of standard curve of ascorbic acid and DF = dilution factor

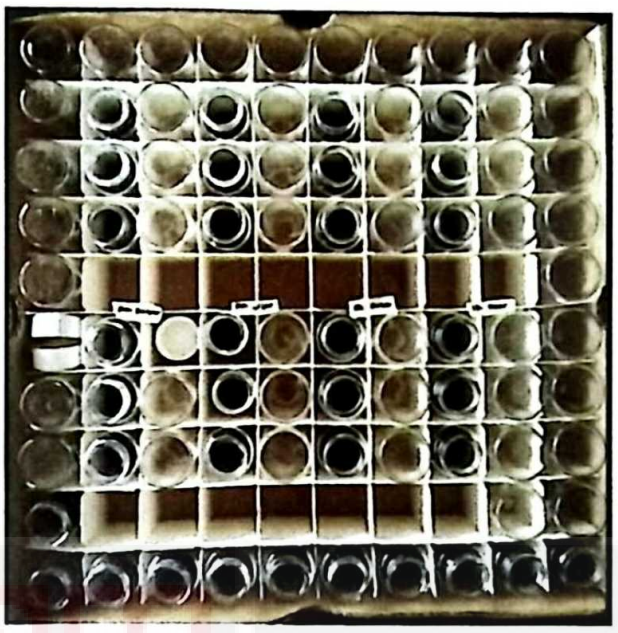


Figure 3.7: Measurement on Antioxidant Activity of RPJ Using FRAP method

3.8 ANALYSIS ON MICROBIOLOGICAL QUALITIES

Colony forming units (CFU) were determined by standard spread-plate methodologies. Decimal dilutions from unpasteurised, industrial pasteurised and reduced pasteurised pomegranate juice were made in buffered peptone water and then 0.1 ml of appropriate dilutions were plated in duplicate for standard plate counts (SPC) on standard plate count agar. For total yeast and mould counts (TYMC), 0.1 ml of appropriate dilutions were plated on Potato Dextrose agar (PDA) (Vet Food Agro Diagnostics Sdn Bhd, Malaysia).

3.9 STATISTICAL ANALYSIS

An analysis of variance (ANOVA) and correlation coefficient were obtained by the MINITAB (Version of Release, 13) statistical computer programme. Tukey's multiple range taste was used to obtain comparisons among sample means. Evaluation were based on the $p<0.05$ significance level.

CHAPTER 4

RESULT AND DISCUSSION

4.1 EFFECT OF MILD-TEMPERATURE AND HIGH-TEMPERATURE PASTEURISATION ON RPJ'S QUALITIES

This section reports and discusses on the evaluation of the effect of High temperature pasteurisation (HTP) and Mild-temperature pasteurisation (MTP) on the antioxidant contents, activities, color and microbiological deactivation of the RPJ. Also, the effect of different pasteurisation and storage temperature on color, bioactive compounds, antioxidant activity and microbiological qualities of RPJ are discussed.

4.1.1 Effect of Different Pasteurisation Temperature on RPJ's Qualities

The effect of both mild-temperature pasteurisation (MTP, at 80°C, 30s) and high-temperature pasteurization (HTP, at 95°C, 30s) on RPJ's qualities is shown in Table 4.1. As mentioned earlier in Section 3.3, the classification of pasteurization into mild-temperature and high-temperature is consistent with Mena et al. (2013) and Vegara et al. (2013) that studied different level of pasteurization and not reaching the temperature of sterilization. This section discusses the changes in color, bioactive compound, antioxidant activity and microbiological qualities that occur after pasteurization. The qualities are also compared with the unpasteurized sample.

Changes in the characteristics between the unpasteurised juice and the pasteurised juice (MTP and HTP) are important since they determine the quality of juice as affected by the different heat load.

Table 4. 1: Effect of HTP and MTP on qualities of reconstituted pomegranate juice

Properties	**Unpasteurised**	**HTP**	**MTP**
		(95°C, 30s)	**(80°C, 30s)**
TPC (mgGAE/ml)	908.52±18.31[a]	875.34±33.76[a]	879.29±41.87[a]
Total anthocyanin (mg/L)	0.0150±0.002[a]	0.0033±0.002[b]	0.0067±0.001[b]
Antioxidant activity based on FRAP-method (mgAAE/ml)	912.01±67.24[a]	856.21±12.09[a]	940.34±42.36[a]
Color CIE *L*	30.21±4.72[a]	28.37±0.80[a]	30.19±1.04[a]
Color CIE *a*	0.59±0.22[a]	0.21±0.12[a]	0.47±0.09[a]
Color CIE *b*	-0.41±0.39[a]	-0.77±0.08[a]	-0.94±0.09[a]
ΔE	-	1.91	0.54

*Values followed by the same letter within the same row are not significantly different from each other ($p>0.05$). All values are reported as mean ± standard deviation.

** GAE, gallic acid equivalent; AAE, ascorbic acid equivalent

Effect on Color

In term of color changes, the CIE *L* (lightness) value for MTP-treated RPJ did not alter substantially compared to HTP-treated RPJ which decrease to the lowest value of 28.37±0.80 . Also, the CIE *a* (redness) value of MTP-treated juice was significantly

greater than the HTP sample, with a CIE *a* value of 0.47±0.09. Noted that, CIE *a* value is a measurement of a substance's redness.

The decreasing CIE *a* and CIE *b* (yellowness) values in both pasteurised samples, as compared to unpasteurised samples, implying a decrease in red color as anthocyanin content decreases. Anthocyanin is responsible for the red color in pomegranate juice (Mena et al., 2013). However, there are no significant changes ($p>0.05$) between HTP- and MTP-treated juice with unpasteurized juice on CIE *a* and CIE *b* were determined, these indicates that the characteristics changes in heat-treated RPJ are insignificant. Cserhalmi et al (2006) reported that thermal treatment leads to unnoticeable effect to the color changes.

Also, this result is in agreement with findings by Turfan et al. (2011), who observed decreasing values of CIE *a* as the result of the thermal treatment at 95°C. Also, the changes in CIE *a* is predicted to be to be associated with changes of CIE *L* values. The insignificant color changes after pasteurization is a desired condition as the redness tones is considered to attract the consumers (Mayuoni Kirshenbaum et al., 2016). In terms of chroma and hue angle, it is observed to have insignificant changes between unpasteurized and pasteurized samples. To summarize, mild-temperature pasteurization is more efficient in maintaining the color tones of the juice alongside consume less energy for the process.

Effect on Total Phenol Content (TPC)

Based on Table 4.1, the total phenol content (TPC) of unpasteurized and both HTP- and MTP-pasteurized RPJ samples were 908.52±18.31 mgGAE/mL, 875.34±33.76 mgGAE/mL and 879.29±41.87 mgGAE/mL respectively. These values have statistically

insignificant differences ($p>0.05$). This indicates that although the TPC values had been decreased after pasteurization process, the degradation of phenolic compound is quite low. This result is in agreement with the study conducted by Vegara et al (2013), who found that the thermal treatment has insignificant impact to the total phenol content of fruit juice. Yet the TPC content shows decreasing value in this study as the temperature increases. The decrease in TPC might be attributed to a balance between increased extractability and phenolic compound non-enzymatic oxidation degradation (Nayak et al., 2015). In addition, thermal instability of these compounds could be causing the reduction in total phenols in the juices. The rapid progress of chemical processes was assisted by the high temperature, which significantly enhanced the oxidative breakdown of total phenols (Zhang et al, 2016;Ma et al., 2019).

Effect on Total Anthocyanin

Based on Table 4.1, it is reported that the total anthocyanin of unpasteurized and both HTP- and MTP-pasteurized RPJ samples are 0.0150 mg/L, 0.0033 mg/L and 0.0067 mg/L respectively. These values have statistically significant differences ($p=0.000$).

The values seem to decrease after the RPJ is being pasteurized, with the lowest value was observed for the HTP-treated RPJ, followed by MTP-treated RPJ and the highest amount of these pigments was in unpasteurized RPJ. Lopez et al. (1996) reported that the decrease value of anthocyanin may be due to incomplete inactivation of enzymes during anthocyanin degradation from the thermal treatment.

Also, CIE *a* value correlated to with anthocyanin contents, which explained the same pattern between anthocyanin and CIE *a*. According to Belitz, Grosch & Schieberle

(2009), when heated under acidic conditions, the effect of heat may be caused to the conversion of colorless leucoanthocyanins into red anthocyanins. As HTP- treatment consumed more heat, the leucoanthocyanin may degrade to which caused higher CIE *a* difference. In short, the conversion is more likely to occur which contribute to lower CIE *a* value.

The increase value of CIE *a* is linked with the CIE *b* value reduction which contribute to significant changes between unpasteurized and pasteurized juices. Thus, mild-treatment pasteurization is more appropriate method as it leads to lower anthocyanin degradation and redness tones, as the red coloration of juice will be the captivating factor to attract the consumer.

Effect on Antioxidant Activity

As for high-temperature pasteurization, the FRAP value shows a decrement (856.21±12.09 mgAAE/ml) after pasteurization. According to Michalczyk and Macura (2010), the total polyphenols content or anthocyanin was well correlated with antioxidant activity. For HTP, the anthocyanin decrease drastically which leading to decrease in antioxidant activity. The decrease in antioxidant activity may be caused by the destruction of heat sensitive phenolic compounds in the juice (Cortes et al., 2008). Also, Farahmand at al. (2017) state that the antioxidant activity changes is because of the changes in total phenol content which consider all flavonoid, anthocyanin, and non-flavonoid compounds.

In contrast, there was an increment of FRAP value for mild-temperature sample which indicates the increase in antioxidant activity. Rechkemmer (2007) explained that any increment of antioxidant activity may be due to reactions of biochemical occurred

during heat treatment which then resulting to the release of bound phenolics and formation of new compound by structural rearrangement.

From this analysis, the FRAP values for all the samples had no significant changes ($p=0.158$) which indicates that the temperature difference of pasteurization lead to low degradation of antioxidant activity. Noted that, FRAP assay is used to measure the presence of compounds using reducing power in the juice. Thus, it is more efficient to use mild-temperature treatment as it led to greater antioxidant activity and less energy consuming.

4.1.2 Effect of Different Pasteurisation Temperature on Microbial Deactivation

The effect of both MTP (80°C, 30s) and HTP (95°C, 30s) on microbial deactivation is shown in Table 4.2

Table 4.2 : Effects of Pasteurisation on Microbiological Properties of RPJ

Properties	**Unpasteurised**	**HTP (95°C, 30s)**	**MTP (80°C, 30s)**
Standard Plate Count (CFU/ml)	6	Not detected (<1)	1
Yeast and Mold (CFU/ml)	Not detected (<10)	Not detected (<10)	Not detected (<10)

*Values followed by the same letter within the same row are not significantly different from each other ($p>0.05$). All values are reported as mean ± standard deviation.

Due to its nature as reconstituted juice derived from rapidly pasteurized concentrate, the unpasteurised RPJ was observed to have a standard plate count of only 6 CFU/ml.

As the reconstituted juice had been pasteurized, it was discovered that there was zero microbial count for mesophilic aerobic bacteria as well as yeast and mould in HTP-treated RPJ, indicating that they had been effectively inactivated to below detection level. This also prove that HTP treatment were successful in achieving the microbiological load criterion for food safety. Ma et al., (2019) states that thermal inactivation of microorganisms is mostly caused by changes in the membrane structures of the microbes as a result of irreversible protein, enzyme denaturation, and intracellular content leakage when exposed to high temperatures. However, there is estimated 1 CFU/ml of standard plate count in MTP-treated RPJ. This result similar to that reported by Mena et al. (2011) which observed microbial counts after treatment were always $\leq$1 CFU and this emphasize the efficiency of the heat treatment, with the holding time and temperature and were set for pasteurization.

As for HTP, it consumes about 50$\pm$2 min while MTP took less time in about 42$\pm$1.5 min to complete pasteurization process for 200 mL sample. This meant higher energy usage, which may be unnecessary for the juice reconstitution companies, which are frequently used by small-scale businesses.

However, it is crucial to acknowledge that some bacteria and yeast spores may have survived pasteurization and are waiting for a favorable environment to grow, so this

study proceed with more experimental work in Section 4.2 on quality changes and shelf-life analysis for both pasteurized samples during 21-day storage.

4.1.3 Summary

In this study, three different treatments of RPJ were conducted which were control, high-temperature pasteurization (HTP) and mild-temperature pasteurisation (MTP) to compare the effect on color, bioactive compounds, antioxidant activity and microbiological qualities. Firstly, both thermal treatments had succeeded inactivate microbial growth in the RPJ with MTP consume less time and therefore, energy in pasteurize 200 mL juice compared to HTP.

In color changes, it was observed that the thermal treatment had insignificant changes with MTP resulting less changes in color. The redness tone is linked to the total anthocyanin in RPJ. MTP-treated juice had higher total anthocyanin compared to HTP-treated juice and there was significantly difference of total anthocyanin between pasteurized and unpasteurized juice. For total phenol content (TPC), there was insignificant difference with MTP had higher value than HTP. The low TPC value was due to thermal instability of the compounds that could be causing the reduction in total phenols in the juices. On the other hand, in terms of antioxidant activity, MTP-treated juice caused increased in FRAP value, in contrast with HTP-treated juice. This increment was totally desirable in RPJ as the antioxidant are important and one of the attraction factors to the consumer. Thus, the results from this analysis conclude that MTP is more efficient as it successfully inactivate microbial growth, maintain the color, antioxidant and

bioactive compounds as well as consume less heating time and more energy saving compared to HTP.

4.2 CHANGES IN RPJ'S QUALITIES DURING STORAGE AT DIFFERENT TEMPERATURE

This section discusses the results on the qualities changes and microbial growth of the pasteurised RPJ during storage. The effects of storage at different temperatures for HTP- and MTP-treated juice were reported and discussed for the following changes of quality parameters:

- Color
- Total phenol content (TPC)
- Total anthocyanin content
- Antioxidant activity
- Microbial count (growth)

The storage temperature investigated were 25±2°C (ambient) and 4±1°C (chilled). All samples were stored in transparent glass bottles.

4.2.1 Changes in Color Properties for Pasteurised Juice during Storage at Different Temperatures

Effect on Color

The color changes were investigated for CIE *L* (lightness), CIE *a* (redness), CIE *b* (yellowness), and color changes throughout the storage period in ambient (25±2°C) and chilled temperature (4±1°C).

Effect on CIE *L* (Lightness)

The changes of CIE *L* for both HTP- and MTP-samples were observed for 21 days storage at ambient temperature (25±2°C) and chilled temperature (4±1°C). Overall, the CIE L values decrease for all samples at the end of storage (day 21), yet the values differ insignificantly (p=0.347). According to Ramli (2012), the reduction in CIE *L* was due to non-enzymatic browning upon storage which may be resulting from oxidation or polymerization of polyphenol that leads to darker juice.

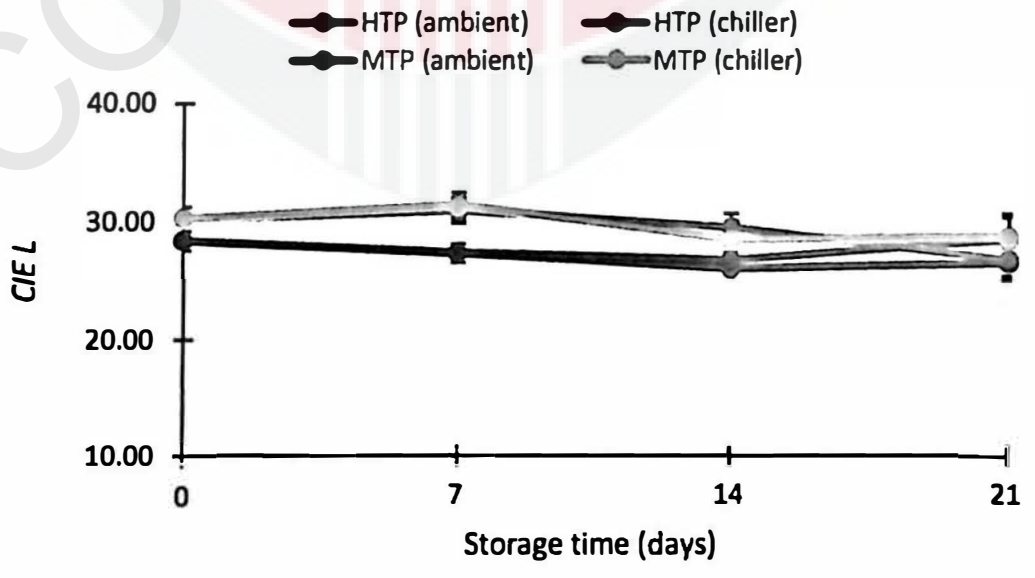


Figure 4.1: CIE (*L*) changes in RPJ at storage of 25±2°C and 4±1°C

Effect on CIE *a* (Redness)

High CIE *a* value corresponds with more reddish tonalities in pomegranate juice and is desired. At the end of storage, MTP-sample stored in the chiller has the highest CIE a value followed by MTP-sample stored in ambient storage, HTP-sample in room and lastly HTP-sample in the chiller. Statistically, the CIE *a* values have moderate strong correlation with anthocyanin content ($r=0.643$, $p= 0.024$) that is responsible for the red color in pomegranate juice. As for HTP-samples, the CIE a values fluctuated during storage perhaps due to instable anthocyanin after the high temperature pasteurisation. The little redness difference in MTP-sample stored in chilled temperature may be due to less reaction of biochemical occurred in chiller compared to ambient temperature, which resulting to more stable anthocyanin in chiller. Also, this is reciprocated with the low anthocyanin degradation in sample stored at chilled temperature.

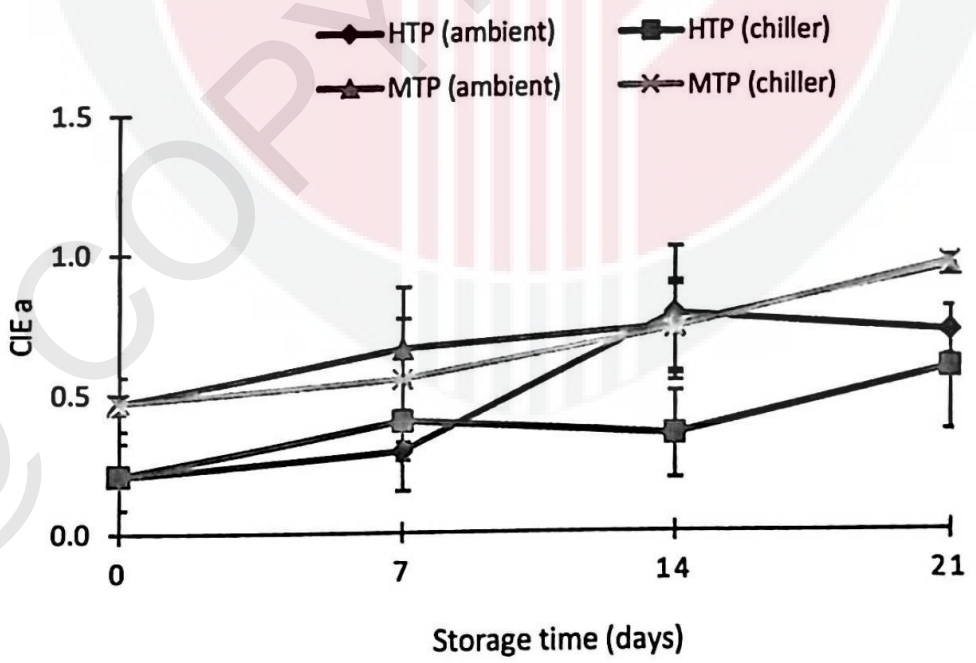


Figure 4.2: CIE *a* changes in RPJ at storage of 25±2°C and 4±1°C

Effect on CIE *b* (Yellowness)

The changes of CIE *b* for both HTP- and MTP-treated RPJ during storage at ambient temperature (25±2°C) and chilled temperature (4±1°C) for 21 days shows no significant changes ($p>0.05$) for all samples.

On the last day, HTP-treated juice stored at 25±2°C showed greater value in CIE *b* which indicates that it has more increase in yellowness compared to HTP sample at 4±1°C. The instance of pigmentation and precipitation during storage at warmer temperature aid in increasing the yellow color of RPJ, resulting in darker and more intense color. Also, the oxidation of polyphenol oxidase (PPO) could lead a reaction of o-diphenol to *o*-quinone. The quinone formation undergoes polymerization and gave rise yellow and brown coloring (Minatel et al., 2017). This finding was similar to Ans, Dioni & Julia (2019), who found that there will be an increase in CIE *b* values after fruit juice storage. In short, it can conclude that lower temperature is more appropriate to store RPJ in order to preserve the color of fruit juice .

Still, the insignificant changes in CIE *b* values indicates that either MTP-treated or HTP-treated juice had no major effect in yellowness which also means there are less degradation and compound reaction in the juice.

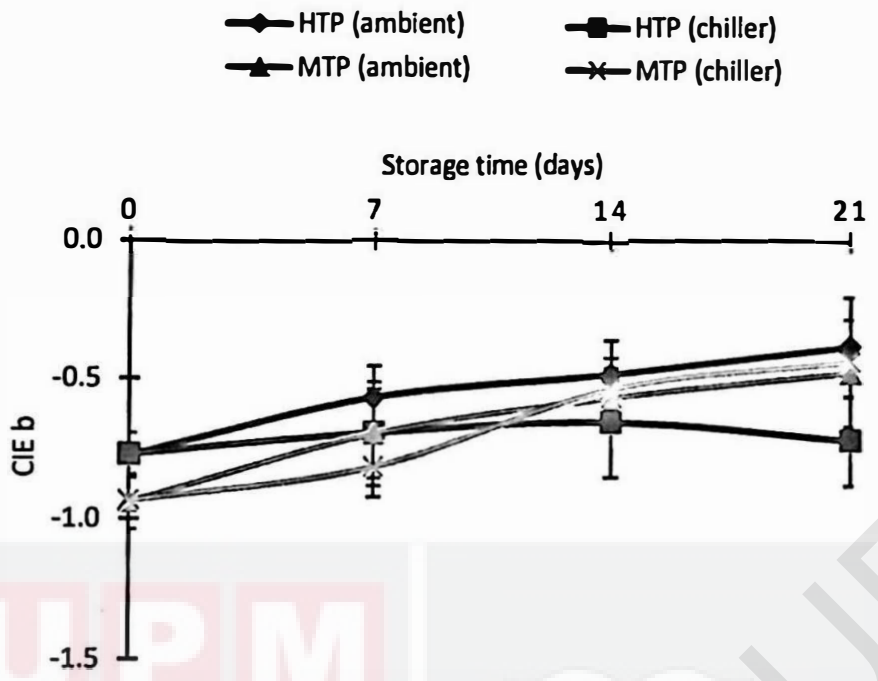


Figure 4.3: CIE *b* changes in RPJ at storage of 25±2°C and 4±1°C

Effect on Color Difference

The color changes for both HTP- and MTP-treated RPJ during storage at ambient temperature (25±2°C) and chilled temperature (4±1°C) for 21 days showed no significant changes ($p>0.05$). After 21 days, the MTP-sample stored in the ambient storage showed the highest color difference value from the initial color on day 0 ($\Delta E= 1.71$). This is followed by MTP-sample in chiller ($\Delta E= 1.04$), HTP-sample in chiller ($\Delta E= 0.95$) and lastly, HTP-sample in ambient storage ($\Delta E= 0.40$).

There was an increasing of color difference especially for MTP-treated juice, as both samples had the higher changes on day 21 . Throughout the entire storage, MTP-treated juice stored at 25±1°C had the higher color changes (3.694) while HTP-treated juice stored at 4±1°C has the lowest total color changes. The great changes is observed

due to the great changes in CIE *a* and CIE *b* of MTP samples in ambient temperature. Allghourchi & Barzegar (2009) emphasized that higher temperature storage gave greater influence on color change of pomegranate juice.

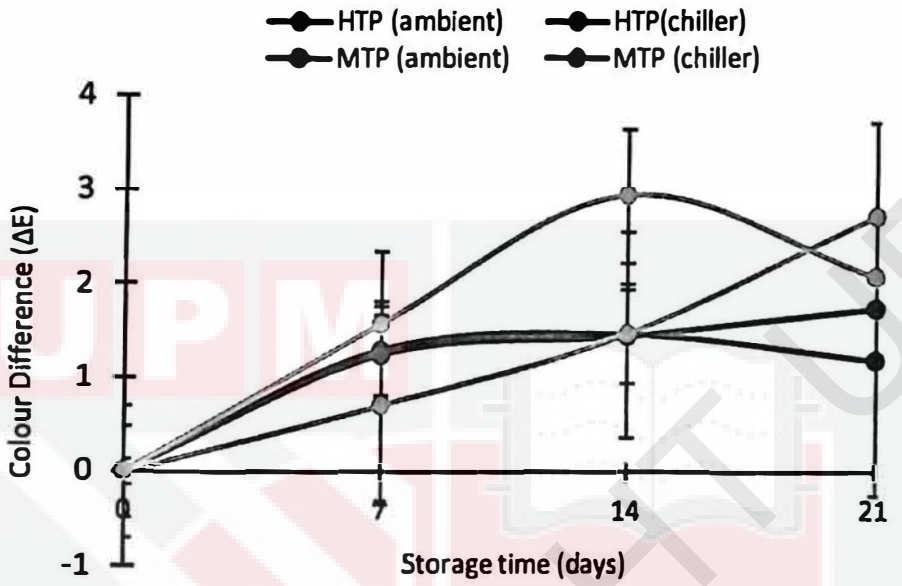


Figure 4.4: Color changes (ΔE) in RPJ at storage of 25±2℃ and 4±1℃

4.2.2 Changes in Bioactive Compounds and Antioxidant Activity of Pasteurised Juice During Storage at Different Temperature

Effect on Total Phenol Content (TPC)

The changes of total phenolic content (TPC) for both HTP- and MTP-samples were observed during storage at ambient temperature (25±2°C) and chilled temperature (4±1°C) for 21 days are shown in Figure 4.5. All samples showed decreasing TPC values, before increasing again only slightly after day 14. Throughout the storage period, the MTP-sample stored in the chiller always had the highest TPC value (837.5±28.59 mg GAE/ml), followed by MTP-sample in the ambient storage (825.25±19.47 mg GAE/ml),

HTP-sample in the chiller (4±1°C) and lastly HTP-sample in the ambient storage (25±2°C). Yet, statistically, the TPC value in MTP-sample in cold storage only showed significant difference when compared with HTP-sample in ambient storage (day 21).

The reduction in TPC may because of degradation and browning of polyphenols which took place during storage, as the result of oxidation and polymerization reaction (Ameena Ali et al., 2018). According to Minatel et al. (2017), the oxidation of phenolic compound led to the production of dark compounds, which also decrease the antioxidant capacity in the juice. The oxidation would produce polyphenol oxidase (PPO) and peroxidase (POD). The POD enzyme could lead to polyphenol degradation and known for its high thermal resistance and high concentration. Thus, the successful inactivation of POD could also mean the indirectly inhibit PPO enzymatic activity.

HTP-samples showed the lowest stability and therefore degraded the most, probably due to high-temperature heating that took place previously that caused the reaction involving phenols to be more actively. Additionally, the high value of TPC in chiller shows that the TPC was less degraded as compared to storage in warmer temperature. (Castro-Lopez et al., 2016). There was significant difference in TPC values between all samples on day 7 ($p = 0.006$) and on day 21.($p = 0.008$). Meanwhile, no significant difference in TPC values for all samples on day 14.

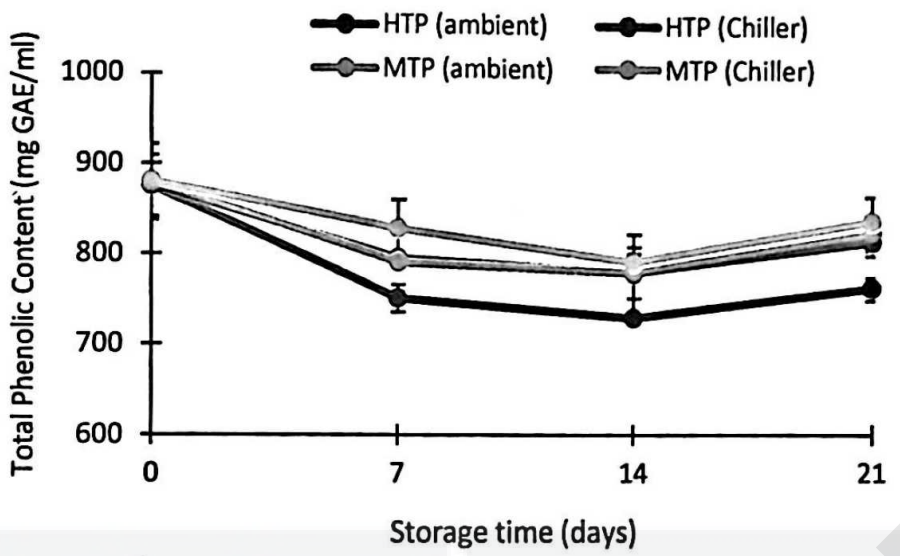


Figure 4.5: The changes of total Phenolic content (TPC) of pomegranate juice at storage 25±2°C or 4±1°C.

Effect on Total Anthocyanin

The changes of total anthocyanin of HTP- and MTP-samples for 21-day-storagewere shown in Figure 4.6. At the end of storage period (day 21), the MTP-sample stored in the chiller had the highest total anthocyanin value (0.0718 mg/L), followed by MTP-sample in the ambient storage (0.0468 mg/L), HTP-sample in the chiller (0.0284 mg/L) and lastly HTP-sample in the ambient storage (0.005 mg/L). Interestingly, the anthocyanin content differs significantly ($p<0.05$) throughout the storage period from day 0 to 21 between all samples. This indicates that the storage temperature effect the anthocyanin content significantly and the cold storage helps to minimize the anthocyanin degradation.

The total anthocyanins seems to increase in the beginning before gradually decrease throughout the storage period. The initial increase is perhaps due to the effect

of heat which aid in the conversion of colorless leucoanthocyanins into red anthocyanins, when heated under acidic condition (Belitz, Grosch & Schieberle, 2009). De Rosso and Mercadante (2007) state that copigmentation could also increase the stability of anthocyanin in presence of certain compounds such as phenolics and metal ions.

The anthocyanin is responsible for the red color of pomegranate juice and may degrade during the storage due to the action of ß-glucosidase (ß-GLC), polyphenol oxidase (PPO) and peroxidase (POD). Anthocyanin would be first hydrolysed by the ß-glucosidase, forming anthocyanidins which then could be oxidized by PPO or POD. At presence of oxygen, the anthocyanin degradation could be faster, which PPO plays the vital role in the anthocyanin degradation (Marszalek et al., 2016).

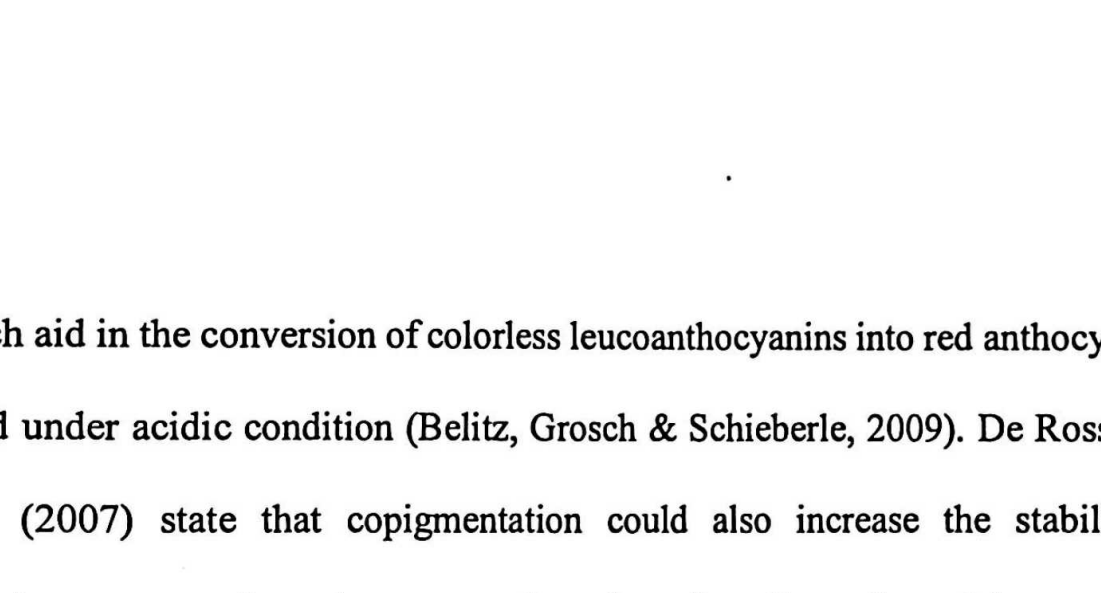


Figure 4. 6:The changes of total anthocyanin of pomegranate juice at storage 25±2°C or 4±1°C.

Effect on Antioxidant Activity Based on FRAP-Assay

Figure 4.4 shows the changes in antioxidant activity based on FRAP-assay in HTP- and MTP-samples during storage at ambient temperature (25±2°C) and chilled temperature (4±1°C) for 21 days. At the end of the storage period, the MTP-sample stored in the chiller always had the highest antioxidant activity value (776.63±50.29 mgAAE/ml), followed by MTP-sample in the ambient storage (720.96±15.51 mgAAE/ml), HTP-sample in the chiller (712.42±8.5 mgAAE/ml) and lastly HTP-sample in the ambient storage (639.13±30.90 mgAAE/ml). Yet, statistically, the value in MTP-sample in chiller only showed significant difference when compared with HTP-sample in ambient storage (day 21).

All the readings shows increase until day 14 and then gradually decrease until day 21.). The significant tendency of polyphenols to undergo polymerization processes, where the resulting oligomers have greater regions available for charge delocalization, could explain the increase in antioxidant activity observed by the FRAP assay in the beginning. The FRAP method is based on the concept of antioxidant reduction of the complex ferric ion TPTZ. As a result, polymerization of polyphenol increases the area available for charger delocalization, which improves the TPTZ reduction reaction. This finding is in agreement with Castro-Lopez et al (2016), who reported the decreasing trend in antiradical capacity of fruit juice after storage. The following reduction in antioxidant activates correlate strongly with the reduction in TPC for all samples ($r = 0.922$, $p=0.000$) while moderately with the reduction in total anthocyanin ($r=0.607$, $p=0.037$). Additionally, MTP-treated juice stored in 4±1°C has the highest FRAP values at the end may be due to less severe degradation of bioactive compound during pasteurization process (80°C)

because of low heat consumption compared to high pasteurization (95°C) as well as low temperature storage.

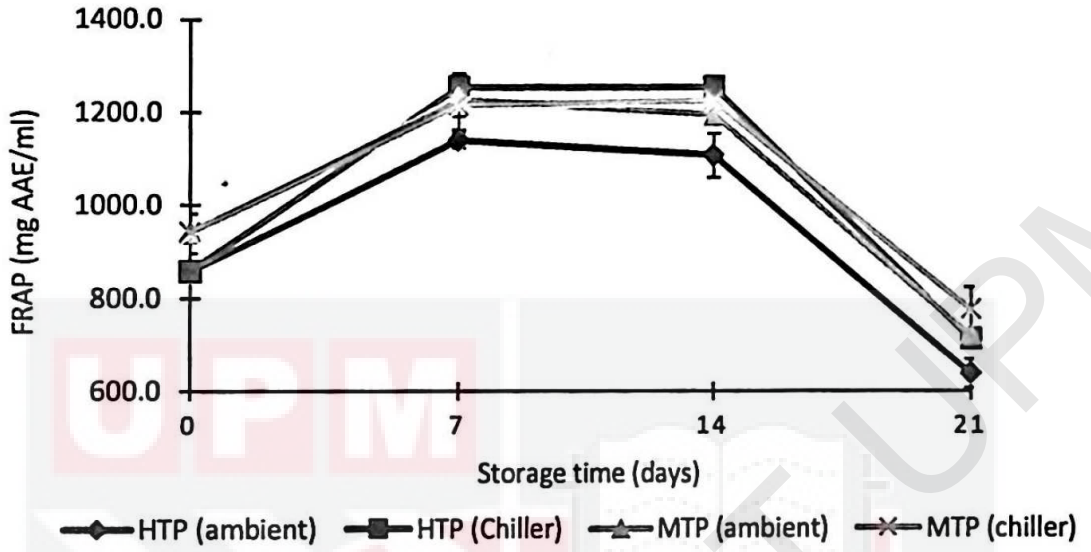


Figure 4.7: The changes of FRAP of pomegranate juice at storage 25±2°C or 4±1°C.

4.2.3 Changes in Microbial Population Pasteurised Juice during Storage at Different Temperatures

On day 21, HTP-sample had nil total plate count except for the sample stored in ambient temperature, which had been observed to have about 1 CFU/ml. Meanwhile, in the MTP-sample has shown 1 CFU/ml MTP-treated RPJ and the growth of microbial count keep increasing and more noticeable. Also, the samples stored at ambient temperature has estimation of 1 and 2 CFU /ml at the end of storage. The increasing standard plate count after storage at (25±2) °C was caused to by the warm environment that encourages microbial growth. At the completion of storage, all the standard plate count values were over the allowed level, spoiling all of the samples (200 ml) except for

HTP-sample stored at 4±1°C. This shows that sample treated with high-temperature and stored in cold storage would be able to hurdle the microbial growth safely for a long period. These results are consistent with prior research by Ampofo-asiama & Quaye (2019), which found no significant changes in standard plate count while stored at 4°C but significant alterations when stored at 25°C. Despite being stored at varying temperatures, TYMC is observed none in the sample. If there are any presence of yeast and mould, this may be due to the low pH of RPJ, which favors the growth of acidophilic microbes, may be the cause of yeast and mould growth.

Table 4.3 : The changes of standard plate count of pomegranate juice at storage 25±2°C or 4±1°C

Treatment	Storage days			
	0	7	14	21
HTP (room)	Not detected (<1)	Not detected (<1)	Not detected (<1)	1 est.
HTP (chiller)	Not detected (<1)	Not detected (<1)	Not detected (<1)	Not detected (<1)
MTP (room)	1 est.	2 est.	1 est.	2 est.
MTP (chiller)	1est	3 est.	1 est.	1 est.

Table 4.4 : The changes of total yeast & mould of pomegranate juice at storage 25±2°C or 4±1°C

Treatment	Storage days			
	0	7	14	21
HTP (room)	Not detected (<10)	Not detected (<10)	Not detected (<10)	Not detected (<10)
HTP (chiller)	Not detected (<10)	Not detected (<10)	Not detected (<10)	Not detected (<10)
MTP (room)	Not detected (<10)	Not detected (<10)	Not detected (<10)	Not detected (<10)
MTP (chiller)	Not detected (<10)	Not detected (<10)	Not detected (<10)	Not detected (<10)

4.2.4 Summary

In this study, both thermal treatment (HTP and MTP) samples were stored at different temperature storage, ambient temperature (25±2) ℃ and chilled temperature (4±1) ℃ to compare the effect on color, bioactive compounds, antioxidant activity and microbiological qualities.

Firstly, both thermal treatments had succeeded inactivate microbial growth in the RPJ but throughout 21 days storage, HTP- samples that stored at (4±1) ℃ had nil microbial count until the end of storage. The low microbial count may be due to aseptic filling which was a vital step after pasteurisation to ensure zero microbial in the juice and produce high-quality fresh juice as well as the use of high temperature that manages to reduce the microorganism effectively to a safe level. In color changes, it was observed that the storage temperature had insignificant changes with HTP-treated juice stored at 4±1℃ has the lowest total color changes. The lower temperature storage gave minimum effects on color changes of pomegranate juice. Although only CIE *a* (redness) had significant changes upon different storage temperature, the color changes resulting to no significant changes. This indicates that storage temperature did not greatly influenced color changes of the juice. For total anthocyanin, the readings were fluctuated which has increase at earlier storage but then decrease until the end of storage. As the total anthocyanin had significant changes, this indicates that storage temperature gave great impact on anthocyanin and anthocyanin is a sensitive compound which crucial to be preserve through the storage.

For total phenol content (TPC), there was significant difference with MTP-treated RPJ that are stored at cold storage showed higher values at the end of the storage. The changes in TPC were caused by the reaction between oxidized polyphenols over storage period affect the increment of polyphenolic compounds in the RPJ. On the other hand, in terms of antioxidant activity, MTP-treated juice stored at 4±1℃ had highest value of FRAP. This was resulted by polymerization process by polyphenols, which also enhance

the antioxidant reduction reaction. The high antioxidant activity is desirable in the pomegranate juice due to its benefit to health.

For this analysis, it was concluded that chilled temperature (4±1)°C was a better storage temperature compared to ambient temperature (25±2)°C as it was more efficient in successfully inactivated microbial growth until the end of 21 days storage, maintained the color by minimize color changes, preserved antioxidant and bioactive compound

CHAPTER 5

CONCLUSION AND RECOMMENDATION

This research highlighted the necessity to study on the effect of improper pasteurisation and storage during the manufacturing of reconstituted pomegranate juice (RPJ), from concentrate, which results in nutritional and quality losses. Reconstitution production normally occurs in small-scale manufacturing which then cause degradation of the quality of RPJ product. In response to this problem statement, the current industrial-pasteurisation procedure (95°C,30s), which is known as high-temperature pasteurisation (HTP) was analysed and compared with a moderate pasteurisation treatment (80°C, 30s), which is known as mild-temperature pasteurisation. In comparison to reduced-pasteurisation approaches, the evaluated effect highlighted the main attributes of RPJ, namely color, antioxidant activities, bioactive compound contents, and microbiological deactivation. Furthermore, the changes of those mentioned quality attributes also were being evaluated during storage, as the temperature was varied. Upon the completion of the study, the following are given as the summary, contribution, project limitation, and future work recommendation.

5.1 SUMMARY

The work is divided into two parts, specifically

5.1.1 Evaluation and comparison on the effect of high-temperature pasterusiation (HTP) and MTP on RPJ's qualities

5.1.2 Evaluation and comparison on the effect of ambient and cold storage temperatures on RPJ's qualities

5.1.1 Evaluation and Comparison on the Effect of HTP- and MTP-Thermal Treatment on RPJ's qualities

The results showed that heat treatment at 95°C for 30 minutes and 80°C for 30 minutes was adequate to successfully inactivate microbial count to beneath detection levels in both HTP and MTP, with MTP taking less time (-16 percent) to complete pasteurization for the 200mL sample than HTP. The findings shows that MTP can successfully inactivate microbial populations and has a pasteurization effect equal to HTP. As a result, MTP is recommended for usage since it requires less energy and takes less time to heat, resulting in less avoidable energy waste in juice reconstitution companies, which is common in small businesses. Furthermore, there was no significant difference in color changes between the HTP- and MTP- thermal treatments. In terms of antioxidant activity and bioactive chemical extractability, both treatments also had insignificant changes in TPC and FRAP values. Still, MTP produced better results in total anthocyanin in the juice as the degradation is lower, which may be due to less heat treatment.

Nevertheless, it's important to keep in mind that some bacteria and yeast spores may have survived pasteurization and are waiting for the right conditions to flourish during storage. Hence, the second part of this project performs the analysis of the quality and microbial growth of the pasteurized samples during storage

5.1.2 Evaluation and Comparison on the Effect of Ambient and Cold Storage Temperatures on RPJ's qualities

In general, the HTP-thermal treatment has a greater degradation impact on RPJ's physicochemical and antioxidant properties than the MTP-thermal treatment. In terms of bioactive compounds, HTP-samples stored in ambient storage had the lowest total anthocyanins and antioxidant capacity. Despite the degradation of the bioactive compounds, only HTP- samples that stored at (4±1) ℃ had nil microbial count at the end of storage. The low microbial count may be due the use of high temperature that manages to reduce the microorganism effectively to a safe level while the cold temperature has impeded the growth of microorganism during storage.

In color changes, it was observed that the storage temperature had insignificant changes with HTP-treated juice stored at 4±1℃ has the lowest total color changes. The lower temperature storage gave minimum effects on color changes of pomegranate juice. This indicates that storage temperature did not greatly influenced color changes of the RPJ. For total anthocyanin, the readings were fluctuated which has increased at earlier storage but then decreased until the end of storage. As the total anthocyanin had significant changes, this indicates that storage temperature gave substantial impact on anthocyanin.

For total phenol content (TPC) and antioxidant activity, the MTP-treated RPJ that are stored at cold storage showed highest values at the end of the storage. The changes in TPC were caused by the reaction between oxidized polyphenols over storage period and also, due to polymerization process by polyphenols, which also enhance the antioxidant reduction reaction.

For this analysis, it was concluded that chilled temperature (4±1)°C was a better storage temperature compared to ambient temperature (25±2)°C as it was more efficient in successfully inactivated microbial growth until the end of 21 days storage, maintained the color by minimize color changes, preserved antioxidant and bioactive compounds.

5.2 RESEARCH CONTRIBUTION

The results of this research has provided useful knowledge for the industry about the effect of pasteurisation and storage temperature on the important qualities of pomegranate juice. Comparing both thermal treatments, MTP-samples had resulted better nutritional value than HTP-samples during the storage. Yet, the sample treated with MTP was contaminated with microbial growth during the storage, while the HTP-sample stored in cold storage showed satisfactory results in term of microbiological safety.

5.3 RESEARCH LIMITATION

There were some limitations in completing this study. Firstly, in the terms of time-interval analysis. The analysis previously was proposed to be done at 14-day-interval analysis for two-month-period. Unfortunately, due to Covid-19 outbreak, there were some limitations to enter the laboratory and there was also time limitation to perform the analysis. Also, there was some delay for the analysis as the laboratory was forbidden during the peak of Covid-19 outbreak. The analysis was continued after the movement control order (MCO) was lifted. As the solution, the color, bioactive compound and

antioxidant capacity analysis of RPJ samples were done in 7-day-interval with 4 readings obtained.

5.4 FUTURE WORK RECOMMENDATION

Future research is required to determine the exact shelf life for RPJ treated at 80°C for 30 seconds with an emphasis on the total plate count and total yeast and mold count during the storage period. This is essential for estimating the RPJ expiry date because no preservatives are used.

Moreover, as society has become more concerned about daily healthy food intake, it is also encouraging to learn more about the effect of heat treatment and storage on the qualitative features of Vitamin C, reducing sugars, and minerals. As a result, more study is necessary to support up the results and persuade society.

Also, a study related to comparison of HTP, and ultra-high pasteurization (UHT) can be done as temperature was proven as crucial parameter to ensure nil microbial count. However, high temperature may also lead to degradation of bioactive compound and antioxidant activities. Thus, an analysis can be done to investigate the effect of UHT to color, bioactive compound and antioxidant activities in reconstituted pomegranate juice (RPJ) and the findings could be compared to the result of HTP samples. The finding also can determine the suitability of UHT thermal treatment on RPJ as zero microbial count can be achieved in terms of food safety without compromise the nutritional value in reconstituted pomegranate juice.

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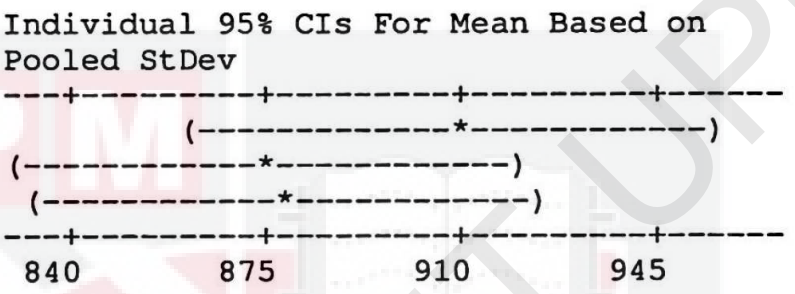
APPENDIX

DATA A-1 : TPC DAY 0

```
Source      DF     SS     MS      F      P
treatment    2   1971    985   0.92  0.450
Error        6   6456   1076
Total        8   8427

S = 32.80    R-Sq = 23.39%    R-Sq(adj) = 0.00%
```




```
Pooled StDev = 32.80

Grouping Information Using Tukey Method

treatment  N    Mean  Grouping
control    3  908.52  A
MTP        3  879.29  A
HTP        3  875.34  A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of treatment

Individual confidence level = 97.80%

treatment = control subtracted from:

treatment    Lower  Center  Upper
HTP       -115.37  -33.18  49.02
MTP       -111.42  -29.23  52.97
                                  -60         0         60        120

treatment = HTP subtracted from:

treatment   Lower  Center  Upper
MTP       -78.24    3.95  86.14
                                  -60         0         60        120
```

DATA A-2 : FRAP DAY 0

Source	DF	SS	MS	F	P
treatment	2	10994	5497	2.55	0.158
Error	6	12924	2154		
Total	8	23917			

S = 46.41 R-Sq = 45.97% R-Sq(adj) = 27.95%

Level	N	Mean	StDev
control	3	912.0	67.2
HTP	3	856.2	12.1
MTP	3	940.3	42.4

Individual 95% CIs For Mean Based on Pooled StDev

```
--------+---------+---------+---------+-
          (----------*----------)
(----------*----------)
                    (----------*----------)
--------+---------+---------+---------+-
         840       900       960      1020
```

Pooled StDev = 46.4

Grouping Information Using Tukey Method

treatment	N	Mean	Grouping
MTP	3	940.34	A
control	3	912.01	A
HTP	3	856.21	A

Means that do not share a letter are significantly different.

Individual confidence level = 97.80%

treatment = control subtracted from:

treatment	Lower	Center	Upper
HTP	-172.09	-55.80	60.50
MTP	-87.96	28.33	144.62

```
-------+---------+---------+---------+---
     (--------*---------)
              (--------*---------)
-------+---------+---------+---------+---
      -120         0        120       240
```

treatment = HTP subtracted from:

treatment	Lower	Center	Upper
MTP	-32.16	84.13	200.42

```
-------+---------+---------+---------+---
                (---------*---------)
-------+---------+---------+---------+---
      -120         0        120       240
```

DATA A-3 : CIE L DAY 0

Source	DF	SS	MS	F	P
treatment	2	6.69	3.34	0.42	0.677
Error	6	48.07	8.01		
Total	8	54.76			

S = 2.831 R-Sq = 12.21% R-Sq(adj) = 0.00%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
control	3	30.207	4.722	(---------*---------)
HTP	3	28.370	0.803	(---------*---------)
MTP	3	30.190	1.045	(---------*---------)
				25.0 27.5 30.0 32.5

Pooled StDev = 2.831

Grouping Information Using Tukey Method

treatment	N	Mean	Grouping
control	3	30.207	A
MTP	3	30.190	A
HTP	3	28.370	A

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of treatment

Individual confidence level = 97.80%

treatment = control subtracted from:

treatment	Lower	Center	Upper	----------+---------+---------+---------+-
HTP	-8.929	-1.837	5.256	(---------*---------)
MTP	-7.109	-0.017	7.076	(---------*---------)
				-5.0 0.0 5.0 10.0

treatment = HTP subtracted from:

treatment	Lower	Center	Upper	----------+---------+---------+---------+-
MTP	-5.273	1.820	8.913	(---------*---------)
				-5.0 0.0 5.0 10.0

DATA A-4 : CIE A DAY 0

Source	DF	SS	MS	F	P
treatment	2	0.2332	0.1166	4.69	0.059
Error	6	0.1490	0.0248		
Total	8	0.3822			

S = 0.1576 R-Sq = 61.01% R-Sq(adj) = 48.01%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
control	3	0.5933	0.2250	(-------*-------)
HTP	3	0.2067	0.1201	(-------*-------)
MTP	3	0.4667	0.0971	(-------*-------)
				0.00 0.25 0.50 0.75

Pooled StDev = 0.1576

Grouping Information Using Tukey Method

treatment	N	Mean	Grouping
control	3	0.5933	A
MTP	3	0.4667	A
HTP	3	0.2067	A

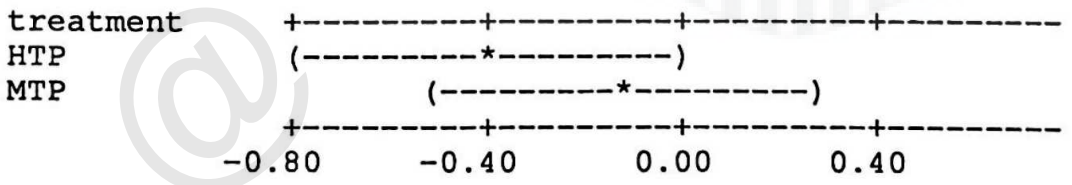
Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of treatment

Individual confidence level = 97.80%

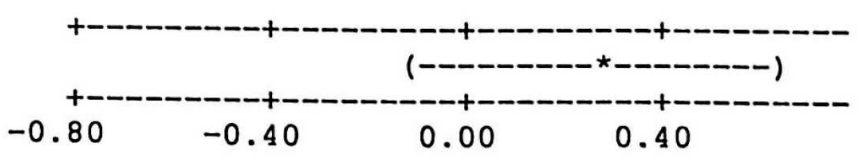
treatment = control subtracted from:

treatment	Lower	Center	Upper
HTP	-0.7815	-0.3867	0.0082
MTP	-0.5215	-0.1267	0.2682



treatment = HTP subtracted from:

treatment	Lower	Center	Upper
MTP	-0.1349	0.2600	0.6549



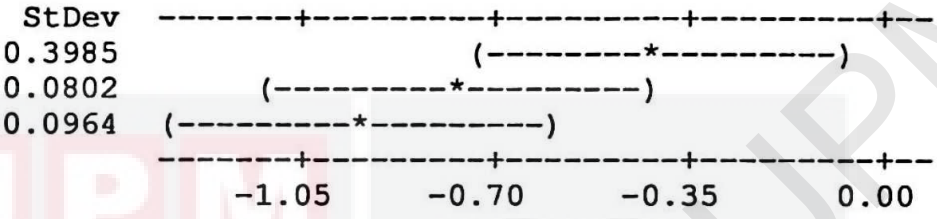
DATA A-5 : CIE b DAY 0

Source	DF	SS	MS	F	P
treatment	2	0.4407	0.2203	3.79	0.086
Error	6	0.3491	0.0582		
Total	8	0.7898			

S = 0.2412 R-Sq = 55.80% R-Sq(adj) = 41.07%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
control	3	-0.4100	0.3985
HTP	3	-0.7733	0.0802
MTP	3	-0.9400	0.0964



Pooled StDev = 0.2412

Grouping Information Using Tukey Method

treatment	N	Mean	Grouping
control	3	-0.4100	A
HTP	3	-0.7733	A
MTP	3	-0.9400	A

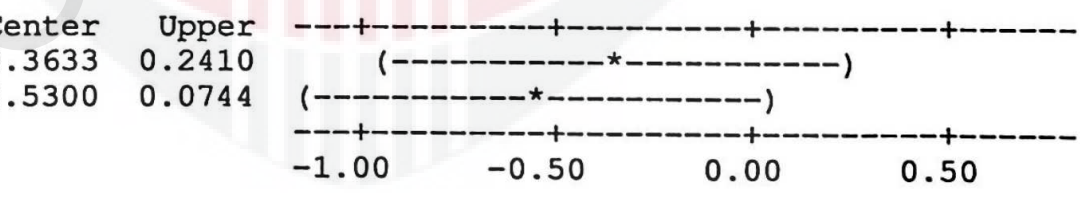
Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of treatment

Individual confidence level = 97.80%

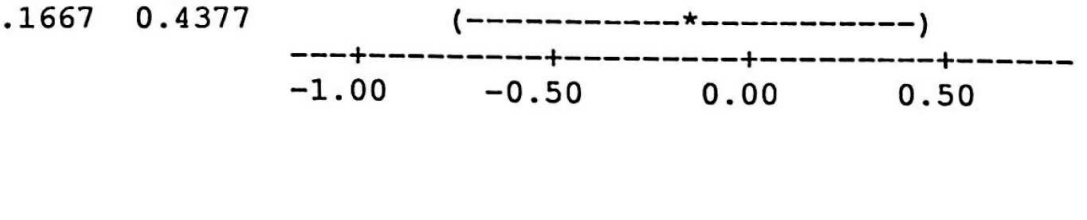
treatment = control subtracted from:

treatment	Lower	Center	Upper
HTP	-0.9677	-0.3633	0.2410
MTP	-1.1344	-0.5300	0.0744



treatment = HTP subtracted from:

treatment	Lower	Center	Upper
MTP	-0.7710	-0.1667	0.4377



DATA A-6 : Anthocyanin DAY 0

Source	DF	SS	MS	F	P
treatment	2	0.0002805	0.0001402	35.26	0.000
Error	6	0.0000239	0.0000040		
Total	8	0.0003043			

S = 0.001994 R-Sq = 92.16% R-Sq(adj) = 89.55%

Level	N	Mean	StDev
control	3	0.017633	0.002281
HTP	3	0.004333	0.002146
MTP	3	0.008233	0.001457

Individual 95% CIs For Mean Based on Pooled StDev

```
-------+---------+---------+---------+--
                                (----*-----)
(-----*----)
        (----*-----)
-------+---------+---------+---------+--
     0.0050    0.0100    0.0150    0.0200
```

Pooled StDev = 0.001994

Grouping Information Using Tukey Method

treatment	N	Mean	Grouping
control	3	0.017633	A
MTP	3	0.008233	B
HTP	3	0.004333	B

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of treatment

Individual confidence level = 97.80%

treatment = control subtracted from:

treatment	Lower	Center	Upper
HTP	-0.018297	-0.013300	-0.008303
MTP	-0.014397	-0.009400	-0.004403

```
treatment ------+---------+---------+---------+---
HTP       (------*------)
MTP         (-------*------)
          ------+---------+---------+---------+---
              -0.0140   -0.0070   -0.0000    0.0070
```

treatment = HTP subtracted from:

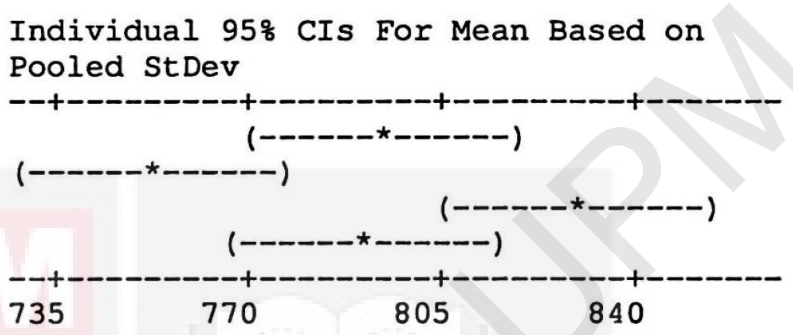
treatment	Lower	Center	Upper
MTP	-0.001097	0.003900	0.008897

```
treatment ------+---------+---------+---------+---
MTP                               (-------*------)
          ------+---------+---------+---------+---
             -0.0140   -0.0070   -0.0000    0.0070
```

DATA B-1 : TPC DAY 7

Source	DF	SS	MS	F	P
treatment	3	8947	2982	8.82	0.006
Error	8	2706	338		
Total	11	11653			

S = 18.39 R-Sq = 76.78% R-Sq(adj) = 68.07%



Pooled StDev = 18.39

Grouping Information Using Tukey Method

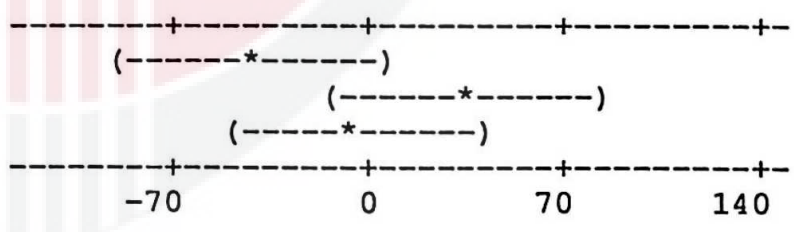
treatment	N	Mean	Grouping
MTP (chiller)	3	828.06	A
HTP (chiller)	3	795.00	A B
MTP (room)	3	791.20	A B
HTP (room)	3	751.08	B

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

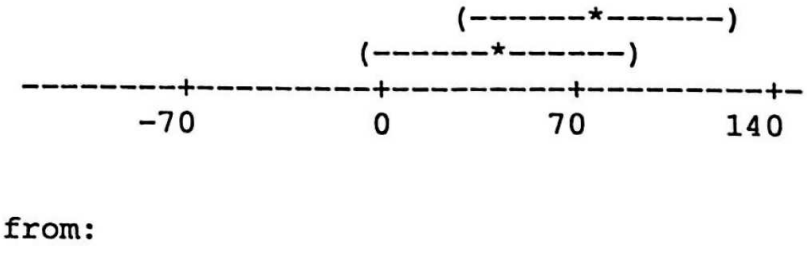
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-92.02	-43.92	4.18
MTP (chiller)	-15.04	33.06	81.15
MTP (room)	-51.89	-3.80	44.30



treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	28.88	76.98	125.07
MTP (room)	-7.97	40.12	88.22



treatment = MTP (chiller) subtracted from:

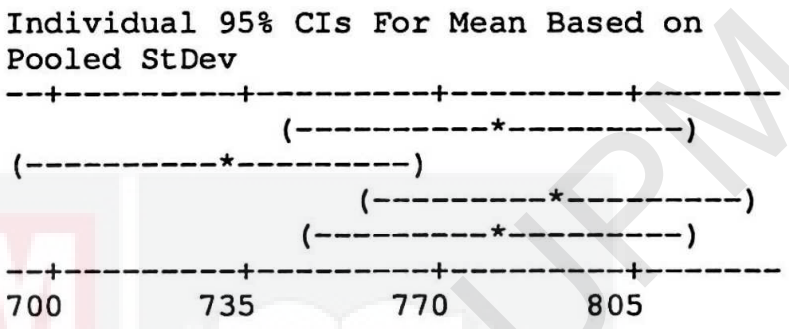
treatment	Lower	Center	Upper
MTP (room)	-84.95	-36.85	11.25

DATA B-2 : TPC DAY 14

Source	DF	SS	MS	F	P
treatment	3	6807	2269	3.17	0.085
Error	8	5732	716		
Total	11	12538			

S = 26.77 R-Sq = 54.29% R-Sq(adj) = 37.15%

Level	N	Mean	StDev
HTP (chiller)	3	779.38	43.67
HTP (room)	3	730.06	5.47
MTP (chiller)	3	791.57	10.84
MTP (room)	3	781.02	28.48



Pooled StDev = 26.77

Grouping Information Using Tukey Method

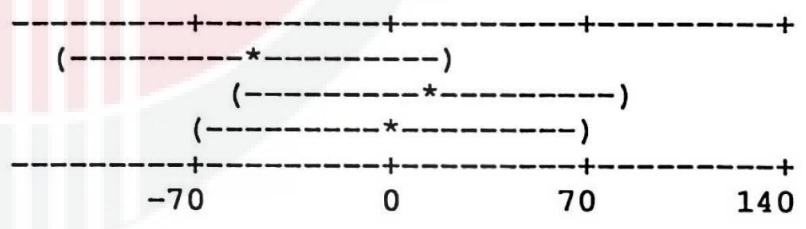
treatment	N	Mean	Grouping
MTP (chiller)	3	791.57	A
MTP (room)	3	781.02	A
HTP (chiller)	3	779.38	A
HTP (room)	3	730.06	A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

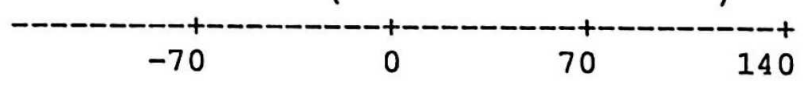
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-119.33	-49.32	20.68
MTP (chiller)	-57.81	12.19	82.20
MTP (room)	-68.37	1.64	71.64



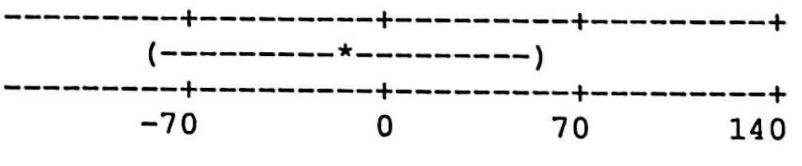
treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	-8.49	61.51	131.52
MTP (room)	-19.05	50.96	120.96



treatment = MTP (chiller) subtracted from:

treatment	Lower	Center	Upper
MTP (room)	-80.56	-10.56	59.45

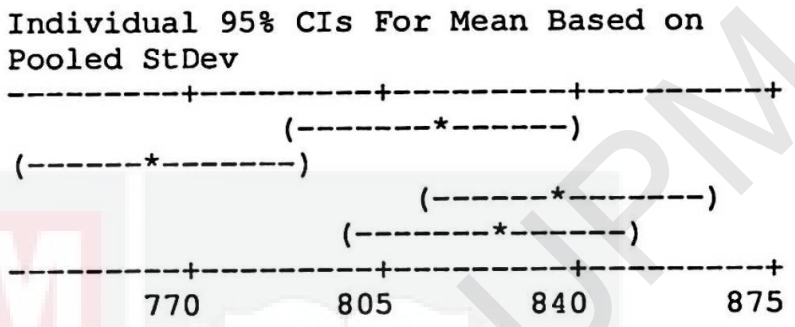


DATA B-3 : TPC DAY 21

Source	DF	SS	MS	F	P
treatment	3	9485	3162	8.08	0.008
Error	8	3130	391		
Total	11	12615			

S = 19.78 R-Sq = 75.19% R-Sq(adj) = 65.89%

Level	N	Mean	StDev
HTP (chiller)	3	814.29	14.22
HTP (room)	3	763.58	12.90
MTP (chiller)	3	837.50	28.59
MTP (room)	3	825.25	19.47



Pooled StDev = 19.78

Grouping Information Using Tukey Method

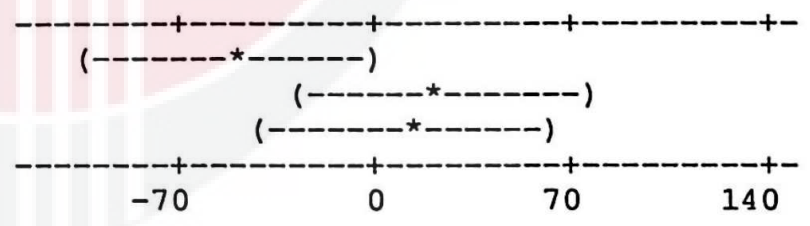
treatment	N	Mean	Grouping
MTP (chiller)	3	837.50	A
MTP (room)	3	825.25	A
HTP (chiller)	3	814.29	A B
HTP (room)	3	763.58	B

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

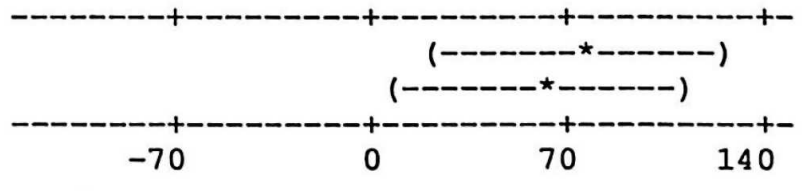
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-102.44	-50.71	1.02
MTP (chiller)	-28.52	23.21	74.94
MTP (room)	-40.77	10.96	62.69



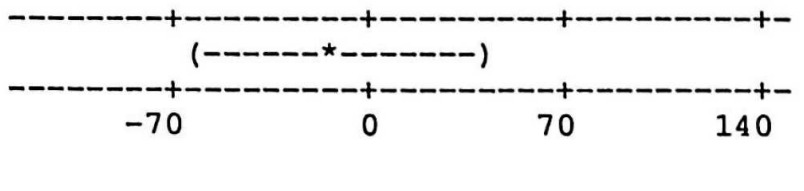
treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	22.19	73.92	125.65
MTP (room)	9.94	61.67	113.40



treatment = MTP (chiller) subtracted from:

treatment	Lower	Center	Upper
MTP (room)	-63.98	-12.25	39.48

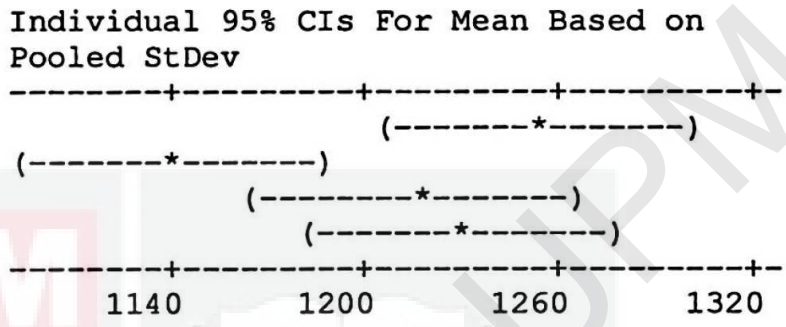


DATA B-4 : FRAP DAY 7

Source	DF	SS	MS	F	P
treatment	3	21029	7010	5.25	0.027
Error	8	10678	1335		
Total	11	31707			

S = 36.53 R-Sq = 66.32% R-Sq(adj) = 53.69%

Level	N	Mean	StDev
HTP (chiller)	3	1253.9	31.9
HTP (room)	3	1141.8	15.4
MTP (chiller)	3	1215.3	51.9
MTP (room)	3	1230.2	37.3



Pooled StDev = 36.5

Grouping Information Using Tukey Method

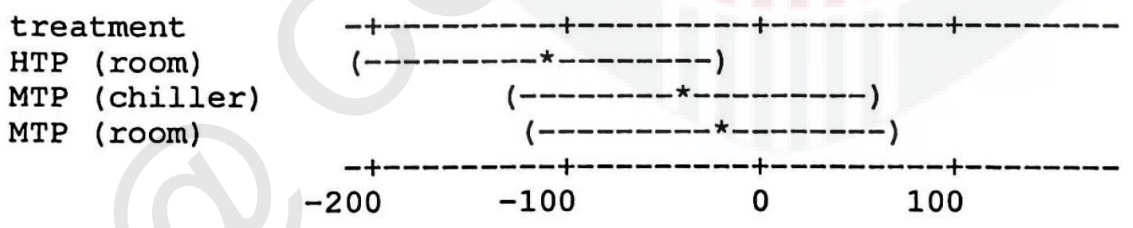
treatment	N	Mean	Grouping
HTP (chiller)	3	1253.90	A
MTP (room)	3	1230.15	A B
MTP (chiller)	3	1215.27	A B
HTP (room)	3	1141.82	B

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

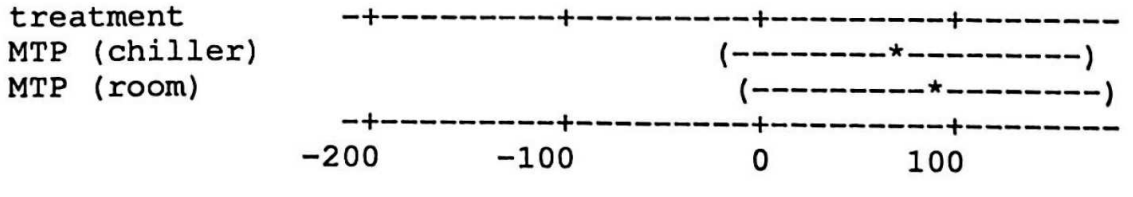
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-207.64	-112.08	-16.53
MTP (chiller)	-134.19	-38.64	56.92
MTP (room)	-119.30	-23.75	71.80



treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	-22.11	73.45	169.00
MTP (room)	-7.22	88.33	183.89



```
treatment = MTP (chiller) subtracted from:

treatment     Lower  Center   Upper    -+---------+---------+---------+--------
MTP (room)   -80.67   14.89  110.44                (--------*---------)
                                       -+---------+---------+---------+--------
                                     -200       -100          0        100
```

DATA B-5 : FRAP DAY 14

```
Source      DF      SS      MS       F      P
treatment    3   36044   12015   18.55  0.001
Error        8    5182     648
Total       11   41226

S = 25.45    R-Sq = 87.43%    R-Sq(adj) = 82.72%


Level            N    Mean  StDev
HTP (chiller)    3  1257.4    8.1
HTP (room)       3  1111.0   48.3
MTP (chiller)    3  1228.3   10.7
MTP (room)       3  1199.9    8.9

                 Individual 95% CIs For Mean Based on Pooled StDev
Level               +---------+---------+---------+---------
HTP (chiller)                                  (-----*-----)
HTP (room)             (-----*-----)
MTP (chiller)                             (-----*-----)
MTP (room)                          (-----*-----)
                    +---------+---------+---------+---------
                  1080      1140      1200      1260

Pooled StDev = 25.5


Grouping Information Using Tukey Method

treatment       N    Mean  Grouping
HTP (chiller)   3  1257.39  A
MTP (chiller)   3  1228.30  A
MTP (room)      3  1199.89  A
HTP (room)      3  1110.98    B

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

treatment = HTP (chiller) subtracted from:

treatment         Lower   Center   Upper
HTP (room)     -212.97  -146.40  -79.84
MTP (chiller)   -95.66   -29.09   37.47
MTP (room)     -124.07   -57.50    9.07

treatment         -+---------+---------+---------+--------
HTP (room)        (-----*------)
```

```
MTP (chiller)                (------*------)
MTP (room)                 (-----*------)
                  -+---------+---------+---------+--------
                -200       -100          0        100
treatment = HTP (room) subtracted from:

treatment       Lower  Center   Upper
MTP (chiller)  50.74  117.31  183.88
MTP (room)     22.34   88.90  155.47

treatment         -+---------+---------+---------+--------
MTP (chiller)                              (------*-----)
MTP (room)                               (------*------)
                  -+---------+---------+---------+--------
                -200       -100          0        100


treatment = MTP (chiller) subtracted from:

treatment     Lower  Center  Upper    -+---------+---------+---------+--------
MTP (room)  -94.97  -28.41  38.16                  (-----*------)
                                     -+---------+---------+---------+--------
                                   -200       -100          0        100
```

DATA B-6 : FRAP DAY 21

```
Source      DF     SS     MS      F      P
treatment    3  28701   9567  10.08  0.004
Error        8   7594    949
Total       11  36295

S = 30.81    R-Sq = 79.08%    R-Sq(adj) = 71.23%


Level            N    Mean  StDev
HTP (chiller)    3  712.42   8.50
HTP (room)       3  639.13  30.90
MTP (chiller)    3  776.63  50.29
MTP (room)       3  720.95  15.51

 Individual 95% CIs For Mean Based on Pooled StDev
Level             +---------+---------+---------+---------
HTP (chiller)                   (------*------)
HTP (room)         (------*-----)
MTP (chiller)                                (-----*------)
MTP (room)                       (------*------)
                  +---------+---------+---------+---------
                600       660       720       780


Pooled StDev = 30.81
```

Grouping Information Using Tukey Method

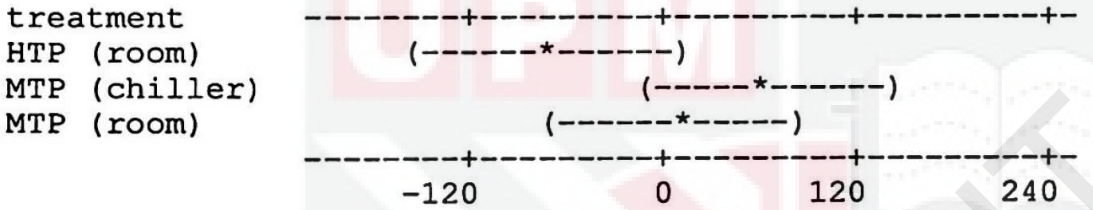
treatment	N	Mean	Grouping
MTP (chiller)	3	776.63	A
MTP (room)	3	720.95	A
HTP (chiller)	3	712.42	A B
HTP (room)	3	639.13	B

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

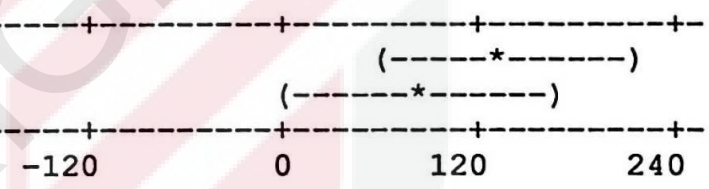
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-153.87	-73.30	7.28
MTP (chiller)	-16.37	64.20	144.78
MTP (room)	-72.06	8.52	89.10



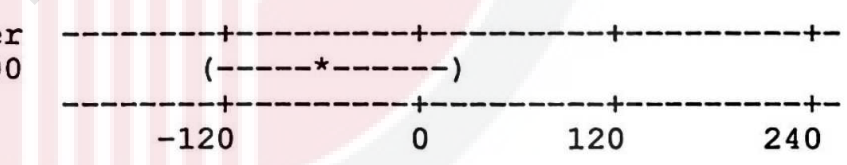
treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	56.92	137.50	218.08
MTP (room)	1.24	81.82	162.40



treatment = MTP (chiller) subtracted from:

treatment	Lower	Center	Upper
MTP (room)	-136.26	-55.68	24.90



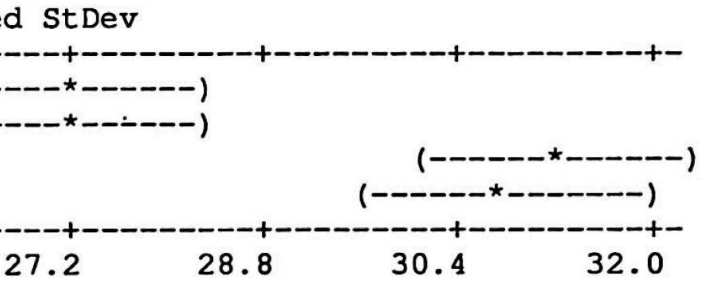
DATA B-7 : CIE L DAY 7

Source	DF	SS	MS	F	P
treatment	3	44.445	14.815	19.44	0.000
Error	8	6.096	0.762		
Total	11	50.541			

S = 0.8730 R-Sq = 87.94% R-Sq(adj) = 83.41%

Individual 95% CIs For Mean Based on Pooled StDev

Level	N	Mean	StDev
HTP (chiller)	3	27.163	0.762
HTP (room)	3	27.123	0.264
MTP (chiller)	3	31.183	1.111
MTP (room)	3	30.780	1.079



Pooled StDev = 0.873

Grouping Information Using Tukey Method

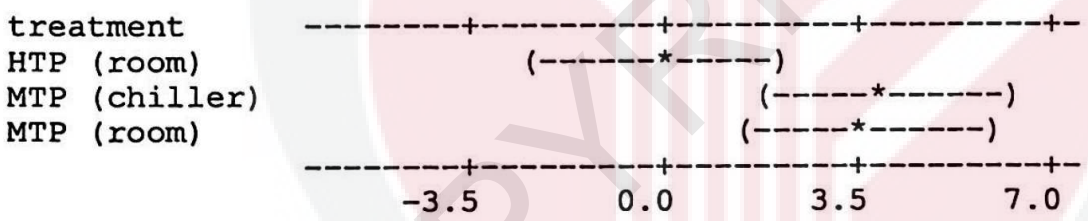
treatment	N	Mean	Grouping
MTP (chiller)	3	31.1833	A
MTP (room)	3	30.7800	A
HTP (chiller)	3	27.1633	B
HTP (room)	3	27.1233	B

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

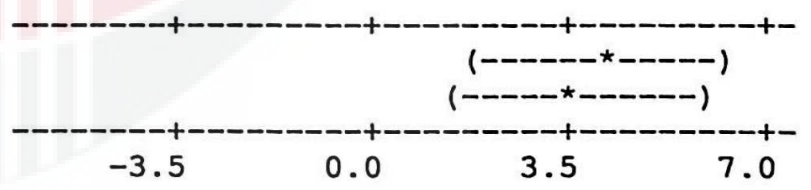
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-2.3231	-0.0400	2.2431
MTP (chiller)	1.7369	4.0200	6.3031
MTP (room)	1.3335	3.6167	5.8998



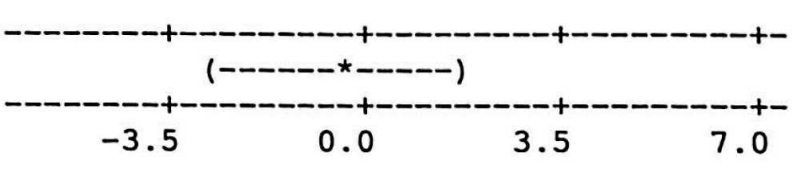
treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	1.7769	4.0600	6.3431
MTP (room)	1.3735	3.6567	5.9398



treatment = MTP (chiller) subtracted from:

treatment	Lower	Center	Upper
MTP (room)	-2.6865	-0.4033	1.8798

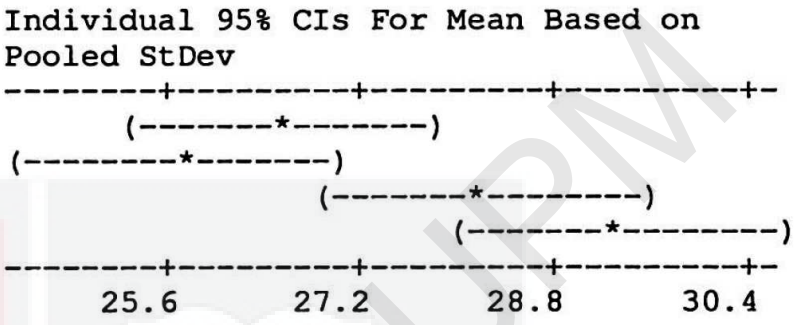


DATA B-8 : CIE L DAY 14

Source	DF	SS	MS	F	P
treatment	3	23.19	7.73	7.54	0.010
Error	8	8.20	1.03		
Total	11	31.39			

S = 1.012 R-Sq = 73.87% R-Sq(adj) = 64.08%

Level	N	Mean	StDev
HTP (chiller)	3	26.557	1.207
HTP (room)	3	25.747	0.439
MTP (chiller)	3	28.230	1.024
MTP (room)	3	29.300	1.185



Pooled StDev = 1.012

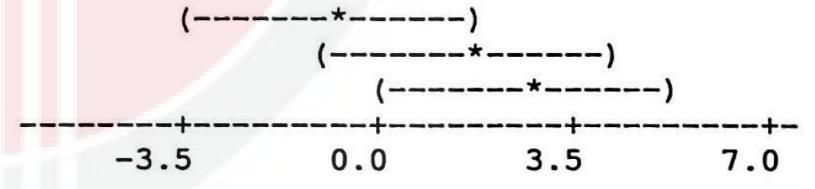
Grouping Information Using Tukey Method

treatment	N	Mean	Grouping
MTP (room)	3	29.300	A
MTP (chiller)	3	28.230	A B
HTP (chiller)	3	26.557	B
HTP (room)	3	25.747	B

Means that do not share a letter are significantly different.

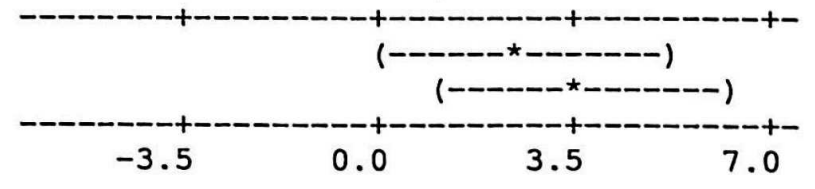
Individual confidence level = 98.74%
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-3.458	-0.810	1.838
MTP (chiller)	-0.975	1.673	4.321
MTP (room)	0.095	2.743	5.391



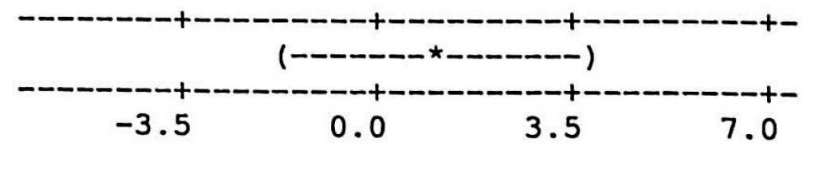
treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	-0.165	2.483	5.131
MTP (room)	0.905	3.553	6.201



treatment = MTP (chiller) subtracted from:

treatment	Lower	Center	Upper
MTP (room)	-1.578	1.070	3.718

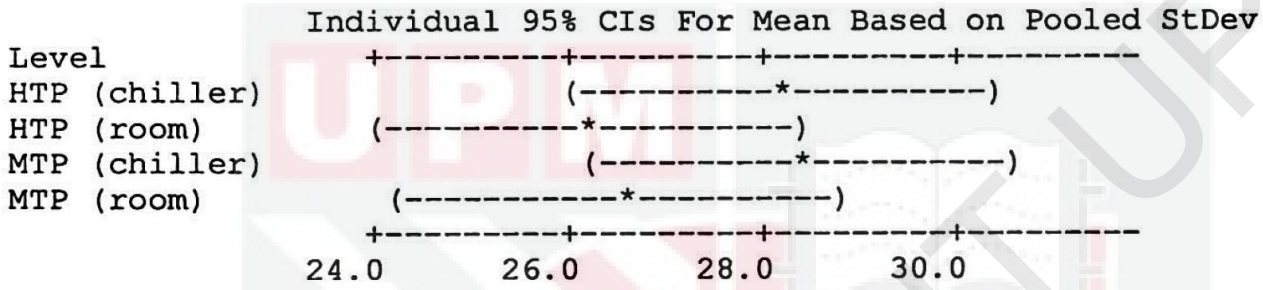


DATA B-9 : CIE L DAY 21

Source	DF	SS	MS	F	P
treatment	3	11.11	3.70	1.27	0.347
Error	8	23.24	2.91		
Total	11	34.35			

S = 1.705 R-Sq = 32.33% R-Sq(adj) = 6.96%

Level	N	Mean	StDev
HTP (chiller)	3	28.177	1.980
HTP (room)	3	26.193	1.337
MTP (chiller)	3	28.377	1.989
MTP (room)	3	26.557	1.400



Pooled StDev = 1.705
Grouping Information Using Tukey Method

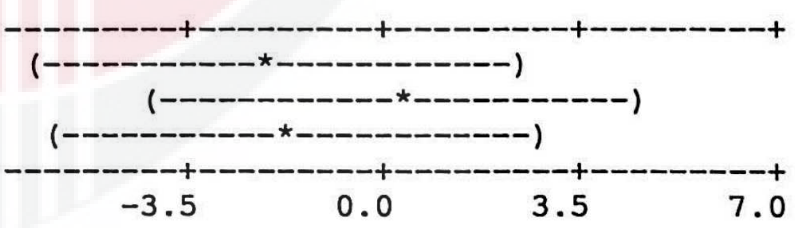
treatment	N	Mean	Grouping
MTP (chiller)	3	28.377	A
HTP (chiller)	3	28.177	A
MTP (room)	3	26.557	A
HTP (room)	3	26.193	A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

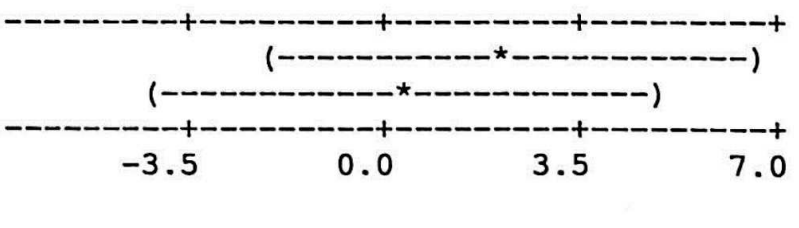
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-6.441	-1.983	2.475
MTP (chiller)	-4.258	0.200	4.658
MTP (room)	-6.078	-1.620	2.838



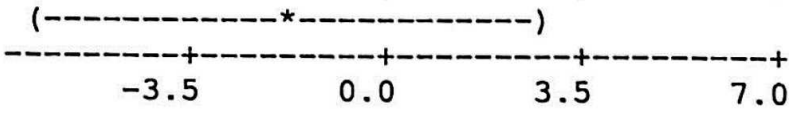
treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	-2.275	2.183	6.641
MTP (room)	-4.095	0.363	4.821



treatment = MTP (chiller) subtracted from:

treatment	Lower	Center	Upper
MTP (room)	-6.278	-1.820	2.638

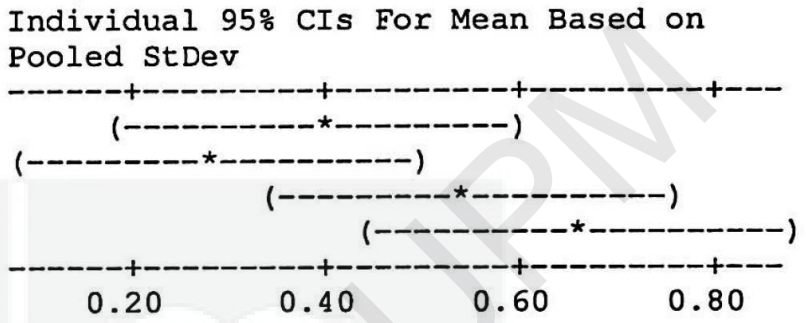


DATA B-10 : CIE a DAY 7

Source	DF	SS	MS	F	P
treatment	3	0.2391	0.0797	3.12	0.088
Error	8	0.2041	0.0255		
Total	11	0.4433			

S = 0.1597 R-Sq = 53.95% R-Sq(adj) = 36.68%

Level	N	Mean	StDev
HTP (chiller)	3	0.3967	0.1365
HTP (room)	3	0.2900	0.1400
MTP (chiller)	3	0.5467	0.1222
MTP (room)	3	0.6600	0.2211



Pooled StDev = 0.1597

Grouping Information Using Tukey Method

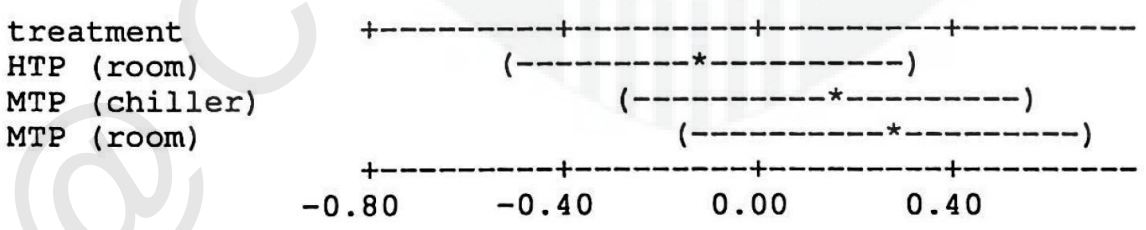
treatment	N	Mean	Grouping
MTP (room)	3	0.6600	A
MTP (chiller)	3	0.5467	A
HTP (chiller)	3	0.3967	A
HTP (room)	3	0.2900	A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

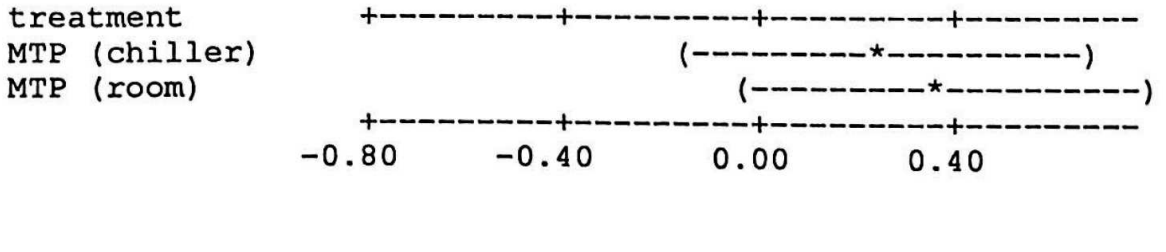
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-0.5244	-0.1067	0.3111
MTP (chiller)	-0.2678	0.1500	0.5678
MTP (room)	-0.1544	0.2633	0.6811



treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	-0.1611	0.2567	0.6744
MTP (room)	-0.0478	0.3700	0.7878



```
treatment = MTP (chiller) subtracted from:

treatment      Lower  Center   Upper
MTP (room)  -0.3044  0.1133  0.5311

treatment      +---------+---------+---------+---------
MTP (room)                    (----------*---------)
               +---------+---------+---------+---------
            -0.80     -0.40      0.00      0.40
```

DATA B-11 : CIE a DAY 14

```
Source      DF      SS      MS     F      P
treatment    3  0.3653  0.1218  4.13  0.048
Error        8  0.2360  0.0295
Total       11  0.6013

S = 0.1718   R-Sq = 60.75%   R-Sq(adj) = 46.03%

                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level           N    Mean   StDev  -----+---------+---------+---------+----
HTP (chiller)   3  0.3467  0.1550  (--------*--------)
HTP (room)      3  0.7800  0.2390            (--------*--------)
MTP (chiller)   3  0.7233  0.1050          (--------*--------)
MTP (room)      3  0.7367  0.1607          (--------*---------)
                                  -----+---------+---------+---------+----
                                     0.25      0.50      0.75      1.00

Pooled StDev = 0.1718

Grouping Information Using Tukey Method

treatment      N    Mean  Grouping
HTP (room)     3  0.7800  A
MTP (room)     3  0.7367  A
MTP (chiller)  3  0.7233  A
HTP (chiller)  3  0.3467  A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

treatment = HTP (chiller) subtracted from:

treatment        Lower  Center   Upper
HTP (room)     -0.0159  0.4333  0.8825
MTP (chiller)  -0.0725  0.3767  0.8259
MTP (room)     -0.0592  0.3900  0.8392

treatment      ----+---------+---------+---------+-----
HTP (room)                     (----------*----------)
MTP (chiller)             (----------*----------)
MTP (room)                (----------*----------)
               ----+---------+---------+---------+-----
                -0.35      0.00      0.35      0.70
```

```
treatment = HTP (room) subtracted from:

treatment          Lower   Center   Upper
MTP (chiller)   -0.5059  -0.0567  0.3925
MTP (room)      -0.4925  -0.0433  0.4059

treatment       ----+---------+---------+---------+-----
MTP (chiller)   (-----------*-----------)
MTP (room)      (-----------*-----------)
                ----+---------+---------+---------+-----
                 -0.35      0.00      0.35      0.70


treatment = MTP (chiller) subtracted from:

treatment      Lower  Center   Upper  ----+---------+---------+---------+-----
MTP (room)  -0.4359  0.0133  0.4625     (-----------*-----------)
                                       ----+---------+---------+---------+-----
                                        -0.35      0.00      0.35      0.70
```

DATA B-12 : CIE a DAY 21

```
Source      DF       SS       MS      F      P
treatment    3   0.3059   0.1020   6.37  0.016
Error        8   0.1280   0.0160
Total       11   0.4339

S = 0.1265   R-Sq = 70.50%   R-Sq(adj) = 59.44%

                                        Individual 95% CIs For Mean Based on
                                        Pooled StDev
Level           N    Mean   StDev  ---------+---------+---------+---------+
HTP (chiller)   3  0.5800  0.2207  (-------*-------)
HTP (room)      3  0.7133  0.0862        (--------*-------)
MTP (chiller)   3  0.9567  0.0850                 (--------*-------)
MTP (room)      3  0.9467  0.0252                 (-------*--------)
                                    ---------+---------+---------+---------+
                                           0.60      0.80      1.00      1.20

Pooled StDev = 0.1265


Grouping Information Using Tukey Method

treatment       N    Mean  Grouping
MTP (chiller)   3  0.9567  A
MTP (room)      3  0.9467  A
HTP (room)      3  0.7133  A B
HTP (chiller)   3  0.5800    B

Means that do not share a letter are significantly different.


Individual confidence level = 98.74%


treatment = HTP (chiller) subtracted from:
```

```
treatment         Lower  Center   Upper
HTP (room)      -0.1975  0.1333  0.4642
MTP (chiller)    0.0458  0.3767  0.7075
MTP (room)       0.0358  0.3667  0.6975

treatment       ------+---------+---------+---------+---
HTP (room)              (---------*--------)
MTP (chiller)                   (---------*--------)
MTP (room)                      (--------*---------)
                ------+---------+---------+---------+---
                   -0.35      0.00      0.35      0.70
```

```
treatment = HTP (room) subtracted from:

treatment         Lower  Center   Upper
MTP (chiller)   -0.0875  0.2433  0.5742
MTP (room)      -0.0975  0.2333  0.5642

treatment       ------+---------+---------+---------+---
MTP (chiller)                 (--------*--------)
MTP (room)                  (---------*--------)
                ------+---------+---------+---------+---
                   -0.35      0.00      0.35      0.70
```

```
treatment = MTP (chiller) subtracted from:

treatment      Lower   Center   Upper  ------+---------+---------+---------+---
MTP (room)  -0.3408  -0.0100  0.3208         (---------*--------)
                                      ------+---------+---------+---------+---
                                         -0.35      0.00      0.35      0.70
```

DATA B-13 : CIE b DAY 7

```
Source      DF       SS       MS      F      P
treatment    3   0.0938   0.0313   1.50  0.288
Error        8   0.1671   0.0209
Total       11   0.2608

S = 0.1445    R-Sq = 35.95%    R-Sq(adj) = 11.93%
```

```
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level             N     Mean    StDev  ---+---------+---------+---------+------
HTP (chiller)     3  -0.6933   0.1582          (-----------*-----------)
HTP (room)        3  -0.5667   0.1159                    (-----------*-----------)
MTP (chiller)     3  -0.8167   0.1041  (-----------*-----------)
MTP (room)        3  -0.6933   0.1850          (-----------*-----------)
                                  ---+---------+---------+---------+------
                                  -0.96     -0.80     -0.64     -0.48

Pooled StDev = 0.1445
```

```
Grouping Information Using Tukey Method
```

```
treatment       N     Mean  Grouping
HTP (room)      3  -0.5667  A
MTP (room)      3  -0.6933  A
HTP (chiller)   3  -0.6933  A
MTP (chiller)   3  -0.8167  A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

treatment = HTP (chiller) subtracted from:

treatment        Lower   Center   Upper
HTP (room)     -0.2513   0.1267  0.5046
MTP (chiller)  -0.5013  -0.1233  0.2546
MTP (room)     -0.3780   0.0000  0.3780

treatment      -------+--------+--------+--------+-
HTP (room)              (----------*---------)
MTP (chiller)     (---------*----------)
MTP (room)           (----------*----------)
               -------+--------+--------+--------+-
                   -0.35     0.00     0.35     0.70


treatment = HTP (room) subtracted from:

treatment        Lower   Center   Upper
MTP (chiller)  -0.6280  -0.2500  0.1280
MTP (room)     -0.5046  -0.1267  0.2513

treatment      -------+--------+--------+--------+-
MTP (chiller)       (----------*----------)
MTP (room)             (---------*----------)
               -------+--------+--------+--------+-
                   -0.35     0.00     0.35     0.70


treatment = MTP (chiller) subtracted from:

treatment      Lower  Center   Upper  -------+--------+--------+--------+-
MTP (room)  -0.2546  0.1233  0.5013                (----------*---------)
                                      -------+--------+--------+--------+-
                                          -0.35     0.00     0.35     0.70
```

DATA B-14 : CIE b DAY 14

```
Source      DF      SS      MS     F      P
treatment    3  0.0470  0.0157  0.83  0.511
Error        8  0.1500  0.0188
Total       11  0.1970


S = 0.1369   R-Sq = 23.84%   R-Sq(adj) = 0.00%
                             Individual 95% CIs For Mean Based on
                             Pooled StDev
```

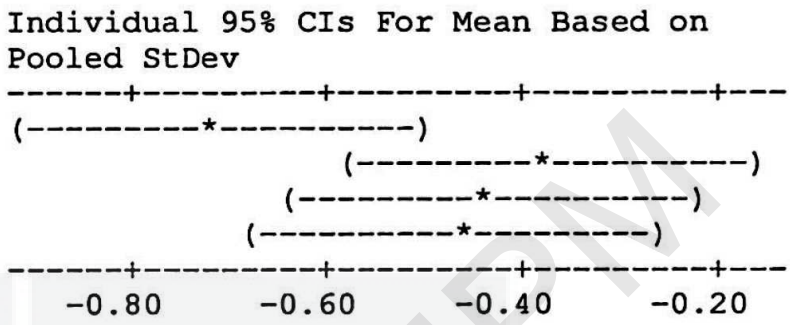
```
Level           N     Mean   StDev  ------+---------+---------+---------+---
HTP (chiller)   3  -0.6533  0.1950  (-----------*-----------)
HTP (room)      3  -0.4800  0.1217              (-----------*-----------)
MTP (chiller)   3  -0.5367  0.1150         (-----------*-----------)
MTP (room)      3  -0.5633  0.0945       (-----------*-----------)
                                       ------+---------+---------+---------+---
                                          -0.75     -0.60     -0.45     -0.30

Pooled StDev = 0.1369


Grouping Information Using Tukey Method
treatment       N     Mean  Grouping
HTP (room)      3  -0.4800  A
MTP (chiller)   3  -0.5367  A
MTP (room)      3  -0.5633  A
HTP (chiller)   3  -0.6533  A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

treatment = HTP (chiller) subtracted from:

treatment         Lower  Center   Upper
HTP (room)      -0.1848  0.1733  0.5315
MTP (chiller)   -0.2415  0.1167  0.4748
MTP (room)      -0.2681  0.0900  0.4481

treatment       --------+---------+---------+---------+-
HTP (room)                (-------------*-------------)
MTP (chiller)          (--------------*-------------)
MTP (room)           (--------------*-------------)
                --------+---------+---------+---------+-
                     -0.25      0.00      0.25      0.50


treatment = HTP (room) subtracted from:

treatment         Lower   Center   Upper
MTP (chiller)   -0.4148  -0.0567  0.3015
MTP (room)      -0.4415  -0.0833  0.2748

treatment       --------+---------+---------+---------+-
MTP (chiller)     (--------------*-------------)
MTP (room)      (--------------*-------------)
                --------+---------+---------+---------+-
                     -0.25      0.00      0.25      0.50


treatment = MTP (chiller) subtracted from:

treatment      Lower   Center   Upper  --------+---------+---------+---------+-
MTP (room)  -0.3848  -0.0267  0.3315      (-------------*-------------)
                                        --------+---------+---------+---------+-
                                             -0.25      0.00      0.25      0.50
```

DATA B-15 : CIE b DAY 21

Source	DF	SS	MS	F	P
treatment	3	0.2025	0.0675	2.80	0.109
Error	8	0.1928	0.0241		
Total	11	0.3953			

S = 0.1552 R-Sq = 51.23% R-Sq(adj) = 32.94%

Level	N	Mean	StDev
HTP (chiller)	3	-0.7167	0.1595
HTP (room)	3	-0.3767	0.1779
MTP (chiller)	3	-0.4333	0.0451
MTP (room)	3	-0.4700	0.1931



Pooled StDev = 0.1552

Grouping Information Using Tukey Method

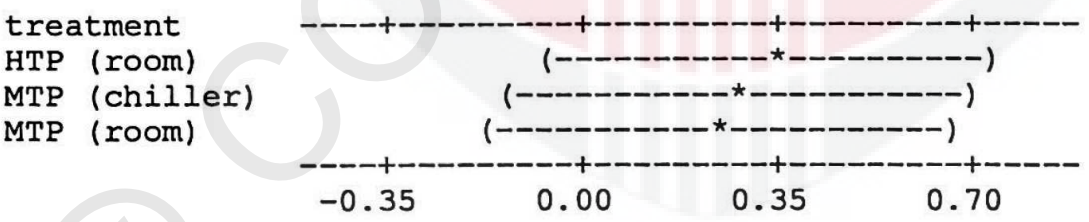
treatment	N	Mean	Grouping
HTP (room)	3	-0.3767	A
MTP (chiller)	3	-0.4333	A
MTP (room)	3	-0.4700	A
HTP (chiller)	3	-0.7167	A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

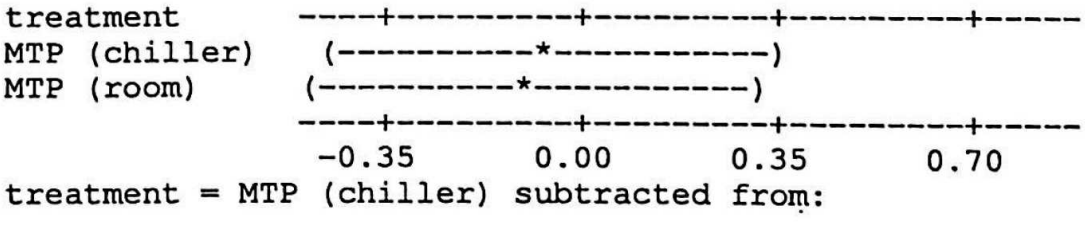
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-0.0660	0.3400	0.7460
MTP (chiller)	-0.1227	0.2833	0.6894
MTP (room)	-0.1594	0.2467	0.6527



treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	-0.4627	-0.0567	0.3494
MTP (room)	-0.4994	-0.0933	0.3127



treatment = MTP (chiller) subtracted from:

```
treatment      Lower   Center   Upper  ----+---------+---------+---------+-----
MTP (room)  -0.4427  -0.0367  0.3694    (-----------*-----------)
                                       ----+---------+---------+---------+-----
                                        -0.35      0.00      0.35      0.70
```

DATA B-16 : Anthocyanin DAY 7

```
Source      DF         SS          MS         F      P
treatment    3  0.0736273   0.0245424  1573.90  0.000
Error        8  0.0001247   0.0000156
Total       11  0.0737520

S = 0.003949   R-Sq = 99.83%   R-Sq(adj) = 99.77%

                                        Individual 95% CIs For Mean Based on
                                        Pooled StDev
Level           N     Mean     StDev    --------+---------+---------+---------+-
HTP (chiller)   3  0.14187  0.00186                        (*)
HTP (room)      3  0.03473  0.00350       (*)
MTP (chiller)   3  0.01577  0.00536    (*)
MTP (room)      3  0.20693  0.00424                                    *)
                                        --------+---------+---------+---------+-
                                              0.060     0.120     0.180     0.240

Pooled StDev = 0.00395

Grouping Information Using Tukey Method

treatment       N     Mean  Grouping
MTP (room)      3  0.20693  A
HTP (chiller)   3  0.14187     B
HTP (room)      3  0.03473        C
MTP (chiller)   3  0.01577           D

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

treatment = HTP (chiller) subtracted from:

treatment          Lower    Center     Upper
HTP (room)     -0.11746  -0.10713  -0.09681
MTP (chiller)  -0.13643  -0.12610  -0.11577
MTP (room)      0.05474   0.06507   0.07539

treatment      -------+---------+---------+---------+--
HTP (room)             (*)
MTP (chiller)        *)
```

```
MTP (room)                              *)
                  -------+---------+---------+---------+--
                      -0.12      0.00      0.12      0.24
```

```
treatment = HTP (room) subtracted from:

treatment          Lower    Center     Upper
MTP (chiller)  -0.02929  -0.01897  -0.00864
MTP (room)      0.16187   0.17220   0.18253

treatment         -------+---------+---------+---------+--
MTP (chiller)                  *)
MTP (room)                                    (*)
                  -------+---------+---------+---------+--
                      -0.12      0.00      0.12      0.24
```

```
treatment = MTP (chiller) subtracted from:

treatment     Lower   Center    Upper  -------+---------+---------+---------+--
MTP (room)  0.18084  0.19117  0.20149                                      (*)
                                       -------+---------+---------+---------+--
                                         -0.12      0.00      0.12      0.24
```

DATA B-17 : Anthocyanin DAY 14

```
Source      DF         SS         MS       F      P
treatment    3  0.0198081  0.0066027  527.41  0.000
Error        8  0.0001002  0.0000125
Total       11  0.0199082

S = 0.003538   R-Sq = 99.50%   R-Sq(adj) = 99.31%
```

```
                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level           N     Mean    StDev ------+---------+---------+---------+---
HTP (chiller)   3  0.06577  0.00455                (-*)
HTP (room)      3  0.01560  0.00354  (*-)
MTP (chiller)   3  0.03310  0.00089      (-*-)
MTP (room)      3  0.12227  0.00401                                    (-*)
                                  ------+---------+---------+---------+---
                                      0.030     0.060     0.090     0.120

Pooled StDev = 0.00354

Grouping Information Using Tukey Method

treatment       N     Mean  Grouping
MTP (room)      3  0.12227  A
HTP (chiller)   3  0.06577    B
MTP (chiller)   3  0.03310      C
HTP (room)      3  0.01560        D
Means that do not share a letter are significantly different.

Individual confidence level = 98.74%
```

```
treatment = HTP (chiller) subtracted from:

treatment          Lower    Center     Upper
HTP (room)     -0.05942  -0.05017  -0.04091
MTP (chiller)  -0.04192  -0.03267  -0.02341
MTP (room)      0.04725   0.05650   0.06575

treatment      ---------+---------+---------+---------+
HTP (room)               (-*)
MTP (chiller)               (-*)
MTP (room)                                (*-)
               ---------+---------+---------+---------+
                     -0.060     0.000     0.060     0.120


treatment = HTP (room) subtracted from:

treatment          Lower    Center     Upper
MTP (chiller)  0.00825   0.01750   0.02675
MTP (room)     0.09741   0.10667   0.11592

treatment      ---------+---------+---------+---------+
MTP (chiller)                        (-*)
MTP (room)                                     (-*)
               ---------+---------+---------+---------+
                     -0.060     0.000     0.060     0.120


treatment = MTP (chiller) subtracted from:

treatment      Lower    Center     Upper
MTP (room)   0.07991   0.08917   0.09842

treatment    ---------+---------+---------+---------+
MTP (room)                                  (-*)
             ---------+---------+---------+---------+
                   -0.060     0.000     0.060     0.120
```

DATA B-18 : Anthocyanin DAY 21

```
Source      DF          SS          MS        F      P
treatment    3   0.0077896   0.0025965   114.15  0.000
Error        8   0.0001820   0.0000227
Total       11   0.0079716

S = 0.004769   R-Sq = 97.72%   R-Sq(adj) = 96.86%


Level            N      Mean     StDev
HTP (chiller)    3  0.026933  0.001680
HTP (room)       3  0.006433  0.001563
MTP (chiller)    3  0.045800  0.004093
MTP (room)       3  0.075667  0.008305

                 Individual 95% CIs For Mean Based on Pooled StDev
Level              +---------+---------+---------+---------
HTP (chiller)              (--*-)
```

```
HTP (room)       (--*-)
MTP (chiller)                (-*--)
MTP (room)                             (-*--)
                +---------+---------+---------+---------
              0.000     0.025     0.050     0.075

Pooled StDev = 0.004769
```

Grouping Information Using Tukey Method

treatment	N	Mean	Grouping
MTP (room)	3	0.075667	A
MTP (chiller)	3	0.045800	B
HTP (chiller)	3	0.026933	C
HTP (room)	3	0.006433	D

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-0.032974	-0.020500	-0.008026
MTP (chiller)	0.006393	0.018867	0.031341
MTP (room)	0.036259	0.048733	0.061207

```
treatment       ------+---------+---------+---------+---
HTP (room)             (--*-)
MTP (chiller)                  (--*-)
MTP (room)                            (--*-)
                ------+---------+---------+---------+---
                  -0.050     0.000     0.050     0.100
```

treatment = HTP (room) subtracted from:

treatment	Lower	Center	Upper
MTP (chiller)	0.026893	0.039367	0.051841
MTP (room)	0.056759	0.069233	0.081707

```
treatment       ------+---------+---------+---------+---
MTP (chiller)                  (--*-)
MTP (room)                            (--*-)
                ------+---------+---------+---------+---
                  -0.050     0.000     0.050     0.100
```

treatment = MTP (chiller) subtracted from:

treatment	Lower	Center	Upper
MTP (room)	0.017393	0.029867	0.042341

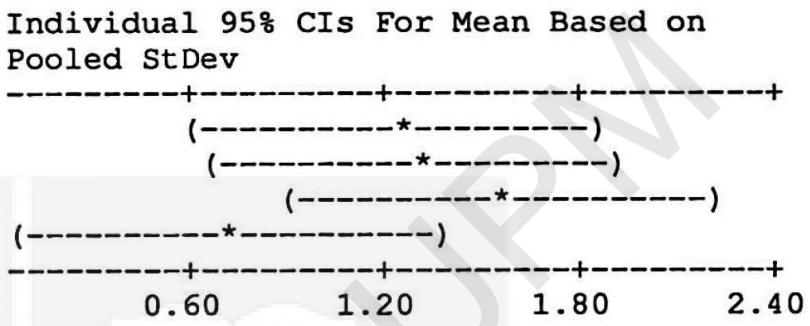
```
treatment       ------+---------+---------+---------+---
MTP (room)                     (--*-)
                ------+---------+---------+---------+---
                  -0.050     0.000     0.050     0.100
```

DATA B-19 : ΔE DAY 7

Source	DF	SS	MS	F	P
treatment	3	1.167	0.389	1.68	0.248
Error	8	1.857	0.232		
Total	11	3.024			

S = 0.4818 R-Sq = 38.60% R-Sq(adj) = 15.57%

Level	N	Mean	StDev
HTP (chiller)	3	1.2391	0.4334
HTP (room)	3	1.2993	0.4879
MTP (chiller)	3	1.5693	0.6961
MTP (room)	3	0.7087	0.1350



Pooled StDev = 0.4818

Grouping Information Using Tukey Method

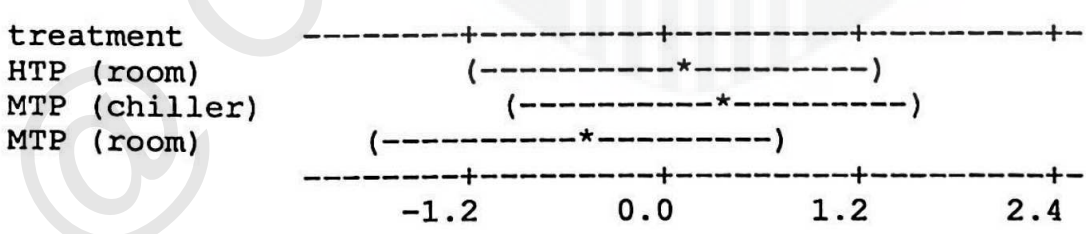
treatment	N	Mean	Grouping
MTP (chiller)	3	1.5693	A
HTP (room)	3	1.2993	A
HTP (chiller)	3	1.2391	A
MTP (room)	3	0.7087	A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

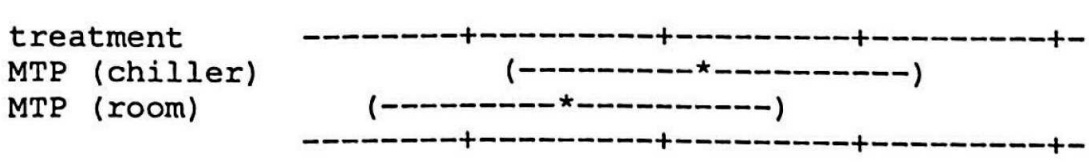
treatment = HTP (chiller) subtracted from:

treatment	Lower	Center	Upper
HTP (room)	-1.2000	0.0602	1.3203
MTP (chiller)	-0.9299	0.3302	1.5903
MTP (room)	-1.7905	-0.5304	0.7297



treatment = HTP (room) subtracted from:

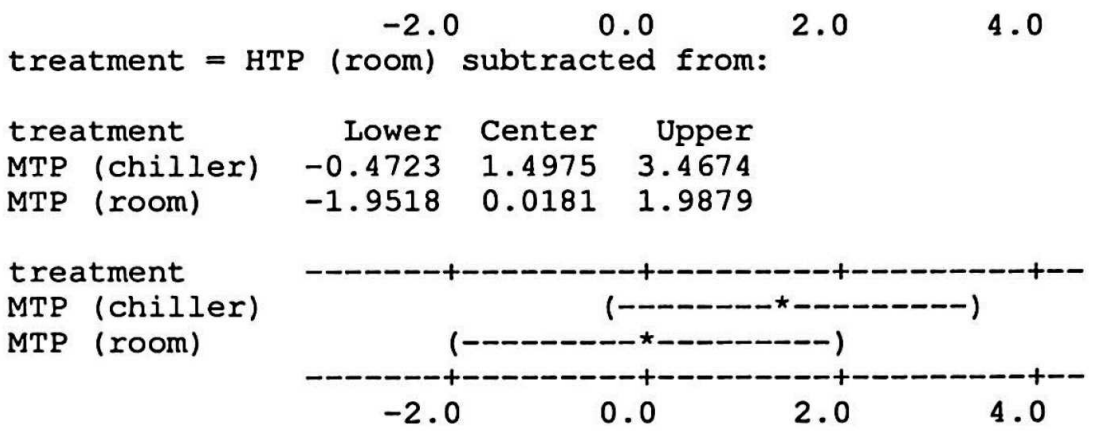
treatment	Lower	Center	Upper
MTP (chiller)	-0.9901	0.2700	1.5302
MTP (room)	-1.8507	-0.5906	0.6695

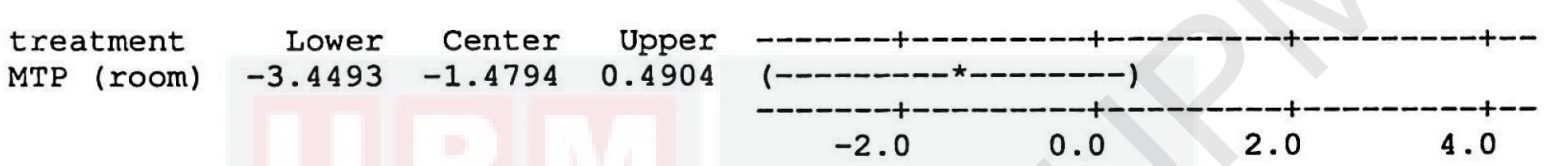


```
                    -1.2        0.0        1.2        2.4

treatment = MTP (chiller) subtracted from:

treatment      Lower   Center   Upper  --------+---------+---------+---------+-
MTP (room)   -2.1207  -0.8606  0.3995  (----------*---------)
                                        --------+---------+---------+---------+-
                                             -1.2        0.0        1.2        2.4
```

DATA B-20 : ΔE DAY 14

```
Source      DF      SS      MS      F      P
treatment    3   5.034   1.678   2.96  0.098
Error        8   4.538   0.567
Total       11   9.572

S = 0.7532   R-Sq = 52.59%   R-Sq(adj) = 34.81%

                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level             N    Mean   StDev  -----+---------+---------+---------+----
HTP (chiller)     3  1.4636  0.5043  (---------*---------)
HTP (room)        3  1.4758  0.5230  (---------*---------)
MTP (chiller)     3  2.9733  0.7827            (---------*---------)
MTP (room)        3  1.4938  1.0624  (---------*---------)
                                     -----+---------+---------+---------+----
                                        1.0       2.0       3.0       4.0

Pooled StDev = 0.7532


Grouping Information Using Tukey Method

treatment       N    Mean  Grouping
MTP (chiller)   3  2.9733  A
MTP (room)      3  1.4938  A
HTP (room)      3  1.4758  A
HTP (chiller)   3  1.4636  A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%


treatment = HTP (chiller) subtracted from:

treatment         Lower  Center   Upper
HTP (room)     -1.9577  0.0122  1.9821
MTP (chiller)  -0.4601  1.5097  3.4796
MTP (room)     -1.9396  0.0303  2.0001

treatment      -------+---------+---------+---------+--
HTP (room)             (---------*---------)
MTP (chiller)                   (---------*--------)
MTP (room)             (---------*---------)
               -------+---------+---------+---------+--
```

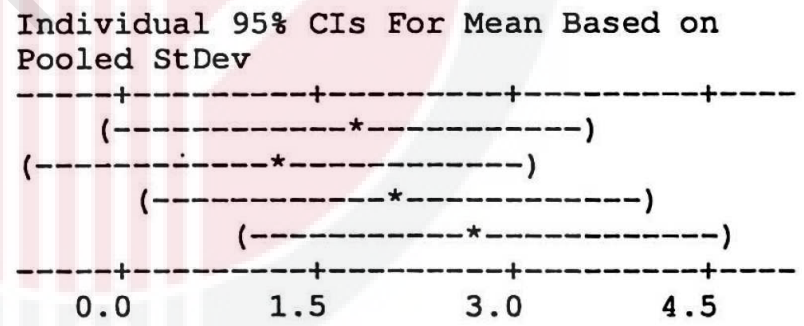




DATA B-21 : ΔE DAY 21

Source	DF	SS	MS	F	P
treatment	3	3.79	1.26	0.63	0.617
Error	8	16.08	2.01		
Total	11	19.87			

S = 1.418 R-Sq = 19.08% R-Sq(adj) = 0.00%



Pooled StDev = 1.418

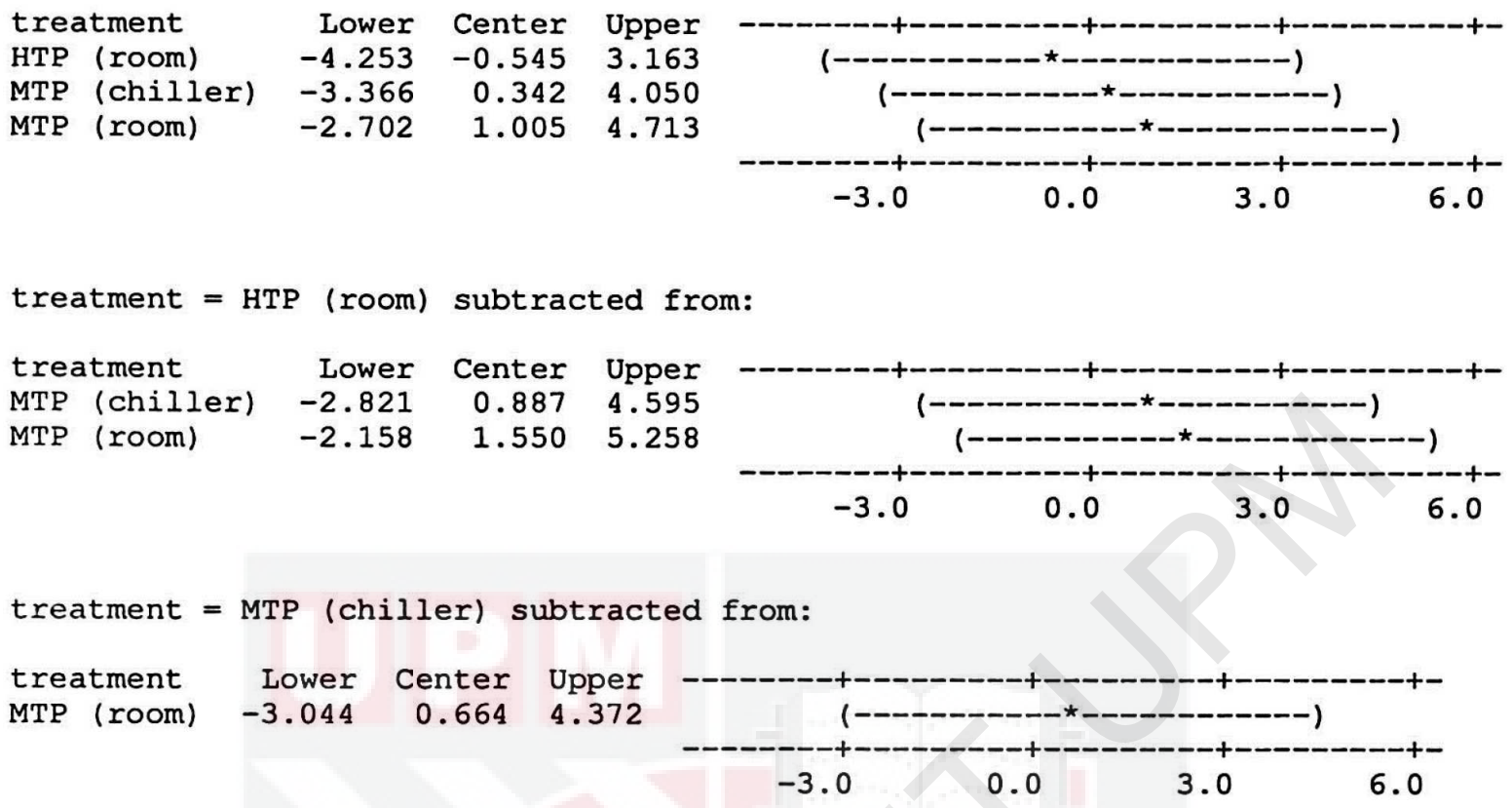
Grouping Information Using Tukey Method

treatment	N	Mean	Grouping
MTP (room)	3	2.763	A
MTP (chiller)	3	2.099	A
HTP (chiller)	3	1.757	A
HTP (room)	3	1.212	A

Means that do not share a letter are significantly different.

Individual confidence level = 98.74%

treatment = HTP (chiller) subtracted from:



treatment = HTP (room) subtracted from:

treatment = MTP (chiller) subtracted from: