QUALITY IMPROVEMENT OF MANUKA HONEY THROUGH THE APPLICATION OF HIGH PRESSURE PROCESSING

NOOR AKHMAZILLAH MOHD FAUZI

A thesis submitted in fulfilment of the requirements for the degree of
Doctor of Philosophy in Chemical and Materials Engineering
University of Auckland

2014

ABSTRACT

The quality of honey is known to be compromised when it goes through thermal processing due to its negative impact on the unstable and thermolabile honey components which originated from the nectar and bees themselves. This present work is undertaken to access the use of an emerging food preservation technique known as "High Pressure Processing" for treating honey, as an alternative to the conventional thermal processing. In this thesis, honey quality has been addressed by measuring the effects of high pressure processing parameters (pressure, time and temperature) on nutritional properties of honey, namely total phenolic content and antioxidant activity. Honey samples, contained in small pouches, were subjected to different pressures (200-600 MPa) at close to ambient temperatures (25-33°C) for different holding times (10 to 30 min). Thermal processing (49-70°C) was also carried out for comparison purpose. Results demonstrated that high pressure processing operated at 600 MPa for 10 min has capability to increase significantly the total phenolic content and antioxidant activity by 47% and 30%, respectively. Besides, the result showed that high pressure processing can maintain the natural colour of honey which relates directly to consumer perception, while retaining its shear-thinning behaviour and viscosity with no significant changes ($p > 0.05$). High pressure processing can also control hydroxymethylfurfural (HMF) concentration in honey during process within the standard limit, 16.93 to 18.76 mg/kg (which is below than the maximum allowed limit of 40 mg/kg). This work also reveals that high pressure processing can enhance antibacterial activity of Manuka honey significantly. It shows an increase in the percentage inhibition of *Staphylococcus epidermidis* from 64.15 ± 5.86% to 84.34 ± 7.62% when honey was subjected to 600 MPa. Storage studies for one year at room temperature (25°C) demonstrated that high pressure-treated samples have a good retention to the physicochemical, nutritional and rheological properties of honey throughout storage, which confirms that the positive effect of high pressure on honey is not a temporary effect. Whereas, an insight study on the safety part showed that the *Saccharomyces cerevisiae* cell varied linearly with ° Brix, indicating that food compressibility has a significant role in the microbial inactivation.

Dedicated to my parents and family for their endless love

"There are no shortcuts to any place worth going."

ACKNOWLEDGEMENTS

Praised be to God the Almighty for infinite blessings

First and foremost, I would like to express my sincerest and greatest gratitude to my principal supervisor, Professor Mohammed Farid for all his unreserved support, untiring effort, patient guidance, enthusiastic encouragement, useful critiques and advice ensured a smooth and ultimate completion of this thesis. Special appreciation also goes to my co-supervisor, Dr Filipa Silva for her valuable advice and encouragement in every step of the way. I am glad to say that it has been a wonderful, challenging and exciting journey. I could not have asked for better supervisors.

My study would have been impossible without the support and financial assistance of my sponsors: Ministry of Education of Malaysia (MOE) and Universiti Tun Huseein Onn Malaysia (UTHM) for making my dream comes true and making my stay here in Auckland, New Zealand a pleasant and rewarding experience. Studying at The University of Auckland (UoA) was a fantastic opportunity and I remain forever indebted to them.

My life at UoA required maximum effort and commitment but was made all the easier by a group of people surrounding me. I am truly blessed to have made acquaintance with helpful and understanding technicians - Mr Allan Celendining, Mr Peter Buchanan, Mr Raymond, Mr Rick Coetzer and Mrs Laura Liang. Special thanks to Professor Lewis, Yimin and Shengpu from Biological School at The University of Auckland for their help in microbiology works. I also gratefully acknowledge Comvita® New Zealand Ltd. for their kind donation of the honey samples used in this research.

To all friends (special to Mr Alifdalino), I would not have survived all the stresses and worries, if not for the beautiful moments you gave me over the past three years. Last but not least, the most special thank goes to my beloved mom, Gayah Sulaiman and dad, Mohd Fauzi Abdul Razak for their unconditional and endless love, and also for being there in times of need. Both of you have showered me with full of love, understanding, support and continuous pray. Also thank you to my beloved brothers and sisters, all my family members and not forgotten, Mr Rasdizarapi Salhudin for their endless support and encouragement throughout my study. I love all of you very much.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

AA	= antioxidant Activity
ANOVA	= analysis of variance
AU	= absorbance Unit
Abs	= absorbance
$Abs_{control}$	= absorbance reading of the control
Abs_{sample}	= absorbance reading of the sample
A_{660nm}	= absorbance at 660 nm
BPF	= brown pigment formation
CFU	= colony forming unit
C*p*	= specific heat capacity
DHA	= dihydroxyacetone
DNA	= deoxyribonucleic acid
DPPH	= 2,2-diphenyl-1-picrylhydrazyl
D-value	= decimal reduction time at specific temperature
FAO	= food and agriculture organization of the United Nations
GAE	= gallic acid equivalent
g	= gram
HHP	= high hydrostatic pressure
HMF	= hydroxymethylfurfural
HPP	= high pressure processing
Hz	= hertz
hrs	= hours
in	= inches
j	= joule
$K_4Fe\ (CN)_6.3H_2O$	= potassium hexacyanoferrate in water

L	= litre
MGO	= methylglyoxal
MIC_{95}	= minimum inhibitory concentration of at least 95% inhibition
MPa	= megapascal
MWCO	= molecular weight cut off
min	= min
mL	= mililitre
mS	= milisiemen
N	= number of microorganism
NaCl	= sodium chloride
Na_2CO_3	= sodium carbonate
N_o	= initial number of microorganisms
nm	= nanometers (wavelength)
OH	= hydroxyl molecules
P	= pressure
pH	= decadic logarithm of acid dissociation
psi	= pounds-force per square inch
R^2	= linear regression coefficient/ coefficient of determination
r	= correlation coefficient
rpm	= revolutions per minute (measure of the frequency of a rotation)
S	= entropy
SD	= standard deviation
S.cerevisiae	= *Saccharomyces cerevisiae*
S. epidermidis	= *Staphylococcus epidermidis*
T	= temperature
TCD	= total colour difference
TPC	= total phenolic content

T_{avg} = average temperature

T_{set} = setting temperature

t = time

UF = ultrafiltration

UHP = ultra high pressure

UV = ultraviolet

V = volume

YPD = yeasts peptone dextrose

$Zn(CH_3COO)_2.2H_2O$ = zinc acetate in water

Z_p- value = Pressure required for one log reduction in the *D*-value.

ºC = degree centigrade

< = less than

≥ = greater than or equal to

± = plus minus

% = percentage

w/v = weight/volume

µ = micro

β = thermal expansion coefficient

W = watt

*L**, *a**, *b** = colour parameters: *L** from 0: black to 100: white; *a** from –80: green to +80: red; *b** from –80: blue to +80: yellow.

Co - Authorship Forms

THE UNIVERSITY OF AUCKLAND
SCHOOL OF GRADUATE STUDIES

Co-Authorship Form

Graduate Centre
ClockTower – East Wing
22 Princes Street, Auckland
Phone: +64 9 373 7599 ext 81321
Fax: +64 9 373 7610
Email: postgraduate@auckland.ac.nz
www.postgrad.auckland.ac.nz

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work. Chapter 3: Akhmazillah, M. F. N., M. M. Farid, Filipa F.V.M (2013). High pressure processing (HPP) of honey for the improvement of nutritional value. Innovative Food Science & Emerging Technologies 20(0): 59-63.

Nature of contribution by PhD candidate	Contribute to the idea, do all experimental works and do writing task.
Extent of contribution by PhD candidate (%)	80%

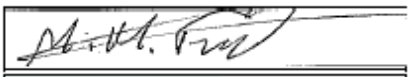
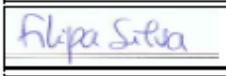
CO-AUTHORS

Name	Nature of Contribution
Prof Mohammed Farid	As a main supervisor, contribute to the main idea, make a review and check the whole paper from beginning untill the paper get accepted.
Dr Filipa Silva	As a co-supervisor, give ideas on experimental works and assist in review and check the whole paper

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
Prof Mohammed Farid		3/12/2014
Dr Filipa Silva		3/12/2014
		Click here
		Click here
		Click here
		Click here

Last updated: 25 March 2013

THE UNIVERSITY OF AUCKLAND
SCHOOL OF GRADUATE STUDIES

Co-Authorship Form

Graduate Centre
ClockTower – East Wing
22 Princes Street, Auckland
Phone: +64 9 373 7599 ext 81321
Fax: +64 9 373 7610
Email: postgraduate@auckland.ac.nz
www.postgrad.auckland.ac.nz

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work. Chapter 4 Fauzi, N., M. Farid, Filipa F.V.M. (2014). High-Pressure Processing of Manuka Honey: Improvement of Antioxidant Activity, Preservation of Colour and Flow Behaviour. Food and Bioprocess Technology 7(8): 2299-2307.

Nature of contribution by PhD candidate	Contribute to the idea, do all experimental works and do writing task.
Extent of contribution by PhD candidate (%)	80%

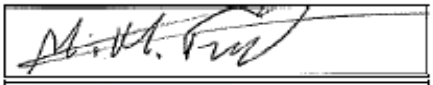
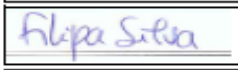
CO-AUTHORS

Name	Nature of Contribution
Prof Mohammed Farid	As a main supervisor, contribute to the main idea, make a review and check the whole paper from beginning untill the paper get accepted.
Dr Filipa Silva	As a co-supervisor, give ideas on experimental works and assist in review and check the whole paper

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
Prof Mohammed Farid		3/12/2014
Dr Filipa Silva		3/12/2014
		Click here
		Click here
		Click here
		Click here

Last updated: 25 March 2013

THE UNIVERSITY OF AUCKLAND
SCHOOL OF GRADUATE STUDIES

Co-Authorship Form

Graduate Centre
ClockTower – East Wing
22 Princes Street, Auckland
Phone: +64 9 373 7599 ext 81321
Fax: +64 9 373 7610
Email: postgraduate@auckland.ac.nz
www.postgrad.auckland.ac.nz

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 5:

N.A. Fauzi & M.M.Farid. High Pressure Processing (HPP) of Manuka honey: Brown pigment formation, improvement of antibacterial activity and hydroxymethylfurfural content

Contents published in International Journal of Food Sciences and Technology.

Nature of contribution by PhD candidate	Contribute to the idea, do all experimental works and do writing task.
Extent of contribution by PhD candidate (%)	80%

CO-AUTHORS

Name	Nature of Contribution
Prof Mohammed Farid	As a main supervisor, contribute to the main idea, make a review and check the whole paper

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
Prof Mohammed Farid		3/12/2014
		Click here
		Click here
		Click here
		Click here

Last updated: 25 March 2013

THE UNIVERSITY OF AUCKLAND
SCHOOL OF GRADUATE STUDIES

Co-Authorship Form

Graduate Centre
ClockTower – East Wing
22 Princes Street, Auckland
Phone: +64 9 373 7599 ext 81321
Fax: +64 9 373 7610
Email: postgraduate@auckland.ac.nz
www.postgrad.auckland.ac.nz

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work. Chapter 6 High Pressure Processed Manuka Honey: Change in Nutritional and Rheological Properties over One Year Storage Contents submitted to journal publication and under review

Nature of contribution by PhD candidate	Contribute to the idea, do all experimental works and do writing task.
Extent of contribution by PhD candidate (%)	80%

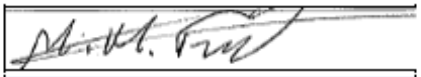
CO-AUTHORS

Name	Nature of Contribution
Prof Mohammed Farid	As a main supervisor, contribute to the main idea, make a review and check the whole paper.

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
Prof Mohammed Farid		Click here
		Click here
		Click here
		Click here
		Click here
		Click here

Last updated: 25 March 2013

THE UNIVERSITY OF AUCKLAND
SCHOOL OF GRADUATE STUDIES

Co-Authorship Form

Graduate Centre
ClockTower – East Wing
22 Princes Street, Auckland
Phone: +64 9 373 7599 ext 81321
Fax: +64 9 373 7610
Email: postgraduate@auckland.ac.nz
www.postgrad.auckland.ac.nz

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in all copies of your thesis submitted for examination and library deposit (including digital deposit), following your thesis Acknowledgements.

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work. Chapter 7 An insight on the relationship between food compressibility and microbial inactivation during high pressure processing Contents submitted to journal publication and under review

Nature of contribution by PhD candidate	Contribute to the idea, do all experimental works and do writing task.
Extent of contribution by PhD candidate (%)	80%

CO-AUTHORS

Name	Nature of Contribution
Prof Mohammed Farid	As a main supervisor, contribute to the main idea, make a review and check the whole paper from beginning untill the paper get accepted.
Dr Filipa Silva	As a co-supervisor, assist in review and check the whole paper

Certification by Co-Authors

The undersigned hereby certify that:

- the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- in cases where the PhD candidate was the lead author of the work that the candidate wrote the text.

Name	Signature	Date
Prof Mohammed Farid		3/12/2014
Dr Filipa Silva		3/12/2014
		Click here
		Click here
		Click here

Last updated: 25 March 2013

CHAPTER 1

Introduction

1.1 Introduction

Honey is an iconic New Zealand product with an excellent export growth potential because of its high nutritional value and unique antibacterial activity. Although increasingly expanding in international markets, ($120 million exports in 2012 and expected future growth potential of $200-300 million) the growth of New Zealand honey industry is at risk due to the unpredictable quality (Farm Monitoring Report, 2013). Honey is known to have wide range of health promoting phytochemicals which can possess antimicrobial, antiviral, antiparasitory, anti-inflammatory, antioxidant, antimutagenic and antitumor effects (Bogdanov et al., 2008). The importance of these bioactive compounds on human health therefore generates a great interest in honey processing research.

In commercial processing plant, honey is usually heated for inhibiting microorganism, facilitating packaging and delaying crystallization. Although thermal processing is a convenient way to protect honey from fermentation (since an increase of water activity during crystallization tends to cause fermentation), high temperature can be detrimental to the quality and biological properties as well as masking its originality. The quality of honey is known to be compromised when it goes through thermal processing due to the unstable and thermolabile components, decomposition of vitamins and also destruction of the integrity of the enzymes particularly when it is heated at 60°C and above (Nagai et al. 2001). Therefore, the possibility of thermal processing to improve the nutritional value look rather limited when honey is exposed to higher temperature.

To maintain honey quality as high as possible, novel processing techniques such as non-thermal processing need to be implemented. High pressure processing (HPP) is a non-thermal food preservation technique that has been cited as one of the best innovations in food processing in 50 years. HPP can be applied without causing significant heating that can damage taste, texture and nutritional value of the food (Farid, 2010).The process involves applying about 400-900 MPa at chilled or mild process temperature, with or without the addition of heat.

The mechanism of HPP is based on the decrease in volume; as pressure increases, free volume decreases so the attractive and repulsive interactions with nearby molecules will change and thus have an affect on the rates of chemical and biochemical reaction. The pressure is uniformLy distributed throughout the food sample. The sample then returns to its original shape when the pressure is released. Besides, samples are not mechanically damaged during pressurization and the initial shape of the product is not affected, which subsequently prevent the food products from being deformed or crushed when treated with pressure. These conditions

allow most foods to be preserved with minimal effects on taste, texture, appearance or nutritional value, hence it preferred as 'minimally preserved'.

1.2 Research objectives

This research project is driven by the recent interest to develop advanced technologies and innovations in honey processing as an effort to improve its quality in comparison with conventional thermal method. The main objectives of this research were to HPP Manuka honey and investigate the effect of HPP on its quality, namely; (i) total phenolic content, (ii) antioxidant activity, (iii) colour, (iv) viscosity, (v) flow behaviour, (vi) antibacterial activity, (vii) brown pigment formation and (viii) hydroxymethylfurfural content.

The specific objectives of this research were to study:

1. The effect of HPP of Manuka honey at ambient temperature for 5 to 30 min on its quality.
2. The combined effect of pressure (200, 400 and 600 MPa) and mild temperatures (50, 60 and 70°C) processing of Manuka honey on its quality.
3. The effect thermal processing of Manuka honey on its quality in comparison to HPP at similar temperatures and time.
4. The effect of storage of HPP treated- Manuka honey for 12 months at 25°C, on its quality.
5. The effect of HPP of Manuka honey on its microbial inactivation, particularly osmophilic yeasts (*Saccharomyces cerevisiae*).
6. The compressibility and sugar concentration effect on the inactivation of *Saccharomyces cerevisiae* in HPP- treated honey.

1.3 Thesis framework

In this thesis, the work was presented following the outline below:

Chapter 2 briefly introduces the background of this study, including (i) an overview of honey (ii) the production, collection and process of honey and (iii) general idea about high pressure processing as a non-thermal treatment in food processing. In this section, the effect of conventional thermal treatment on honey quality, which is determined by its sensorial, chemical, physical and microbiological characteristics, is highlighted. An alternative thermal treatment (microwave heating and infrared heating) and non-thermal treatment (ultrasound, ultraviolet and membrane filter) are also presented.

Chapter 3 introduces a detailed work on the effect of HPP on total phenolic content (TPC) as a main phytochemical in honey. From a nutritional perspective, this is associated with the production of a higher antioxidant honey, known to prevent certain diseases such as cancer. The work in this chapter has been published in *Innovative Food Sciences and Emerging Technologies.*

Chapter 4 then introduces work on the effect of HPP on antioxidant activity and its relation with total phenolic content. While understanding the flow behaviour of honey is of prime importance in all stages of honey production which in turn affects its quality and affects consumers' preference, the change of viscosity as affected by HPP during honey processing is determined. Apart from antioxidant activity and rheological properties, Chapter 4 also reports the change in colour of HPP-treated sample. The work in this chapter has been published in *Food Bioprocess Technology*.

Chapter 5 presents the study on the effect of HPP on brown pigment formation as well as antibacterial activity of Manuka honey against *Staphylococcus epidermidis*. The relationship between brown pigment formation and antibacterial activity in high pressure-treated honey is also established. The study of hydroxymethylfurfural (HMF) which is considered as an important quality parameter in honey is also included in this chapter. The work has been published in *International Journal of Food Science and Technology*.

Chapter 6 is then conducted to provide evidence that the change in physicochemical, nutritional (antioxidant activity, total phenolic content and brown pigment) and rheological properties in honey during HPP is permanent based on one year storage study.

Chapter 7 focuses on an insight study on the relationship between food compressibility (where honey is chosen as a model food) and microbial inactivation (where *Saccharomyces cerevisiae* was selected as the testing microorganism) during high pressure processing.

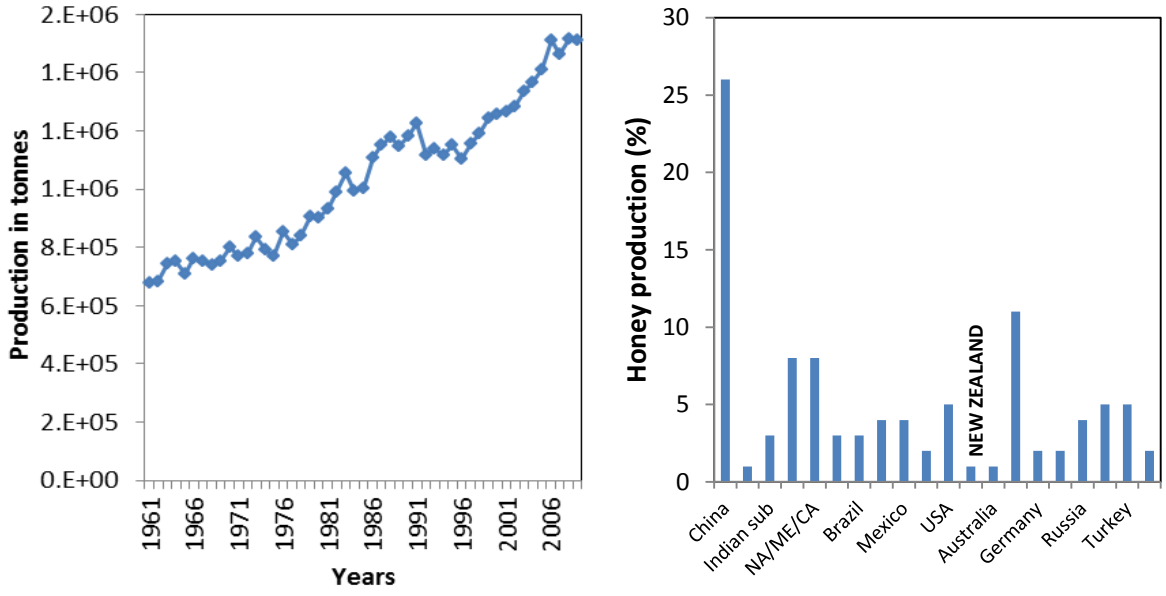
Finally, Chapter 8 summarizes the results in this thesis and their significance in food research and future industrial application. Recommendations for further work are also discussed.

CHAPTER 2

Literature Review

2.1 Honey: A natural sweetener and its valuable properties

Honey is flower nectar which has been collected, regurgitated and dehydrated by honey bees to enhance its nutritional properties making it ready for human consumption. Known as a nature's original sweetener, honey has been used as a food for at least 6000 years. Due to the unique combination of components in honey which makes it a prized addition to the diet, health treatment and medicine, the consumption of honey is on the rise. According to the Food and Agriculture Organization of the United Nations (FAO), total honey production in 1961 was 0.7 million tons and it was steadily increased to about 1.5 million tons in 2009 (Alvarez-Suarez et al., 2010) as presented in Figure 2.1a. It was expected that the demand will increase for the next few years. Honey production is spread throughout the world; China is the largest single producer while Europe is the largest producing region. New Zealand accounts for about 1% of global production (Figure 2.1b).



(a) (b)

Figure 2.1: (a) Production quantity of natural honey in world from 1961 to 2009, (b) Global honey production by select country and region (Source from Food and Agriculture Organization of the United Nations, FAO).

In New Zealand, honey exports are on a roll and growing rapidly. Exports have grown at a 30% compound rate for the past decade, reaching US$70 million in 2010. Exports are strong to Europe (in particular the United Kingdom and Germany) and Asia. New Zealand's

Manuka honey is the most expensive in the world and receives a significant premium over other suppliers. The on-going international success of Manuka honey is driving the growth of the total New Zealand honey industry (Table 2.1)

Table 2.1: Honey export volumes, value and prices of New Zealand market from 2002 to 2013 (Farm Monitoring Report, 2013).

Year ended 30 June	Export volume (tonnes)	Export value ($ million fob)	Average export price ($ per kg fob)
2002	3028	20.6	6.8
2003	3233	25.5	7.87
2004	2394	23.1	9.65
2005	3273	33.5	10.23
2006	3927	38.4	9.77
2007	4411	47.8	10.83
2008	5366	62.6	11.66
2009	7384	81	10.97
2010	7147	97.6	13.66
2011	6721	101.6	15.11
2012	7675	121.1	15.78
2013	8054	144.9	17.99

fob = free on board

Honey composition varies based on its plant origin and weather conditions when the honey was produced (Grane and Kirk Visscher, 2007). A general composition of all honey types can be summarized as in Figure 2.2. About 17% of honey is composed of water (Grane and Kirk Visscher, 2007) but moisture content can vary greatly in the range of 13% to 25% (White, 1961). A moisture content of below 17% in honey is considered the safe level for retarding yeast activity (Subramanian et al., 2007). Honey can be classified as; (i) blossom honey, obtained predominantly from the nectar of flowers; (ii) honeydew honey, produced by bees after they collect pierce plant cells, ingest plant cells, and then secrete it again; (iii) monofloral honey, in which the bees forage predominantly on one type of plant, and which is named according to the plant and (iv) multifloral honey (also known as polyfloral) that has several botanical sources, none of which is predominant, for example: meadow blossom honey and forest honey.

Besides its main components, the carbohydrates (fructose and glucose), honey contains also a great number of other constituents in small and trace amounts, producing numerous nutritional and biological effects. Its high antioxidant content (flavonoids, amino

acids and phenolic acids) has been shown to reduce the risk of heart disease, cancer, cataracts and inflammatory processes (Aljadi and Kamaruddin, 2004; Bogdanov et al., 2008; Socha et al., 2011). These different biological effects of honey with respect to its nutritional, physical, microbiological and medicinal aspects have been summarized and presented in Table 2.2

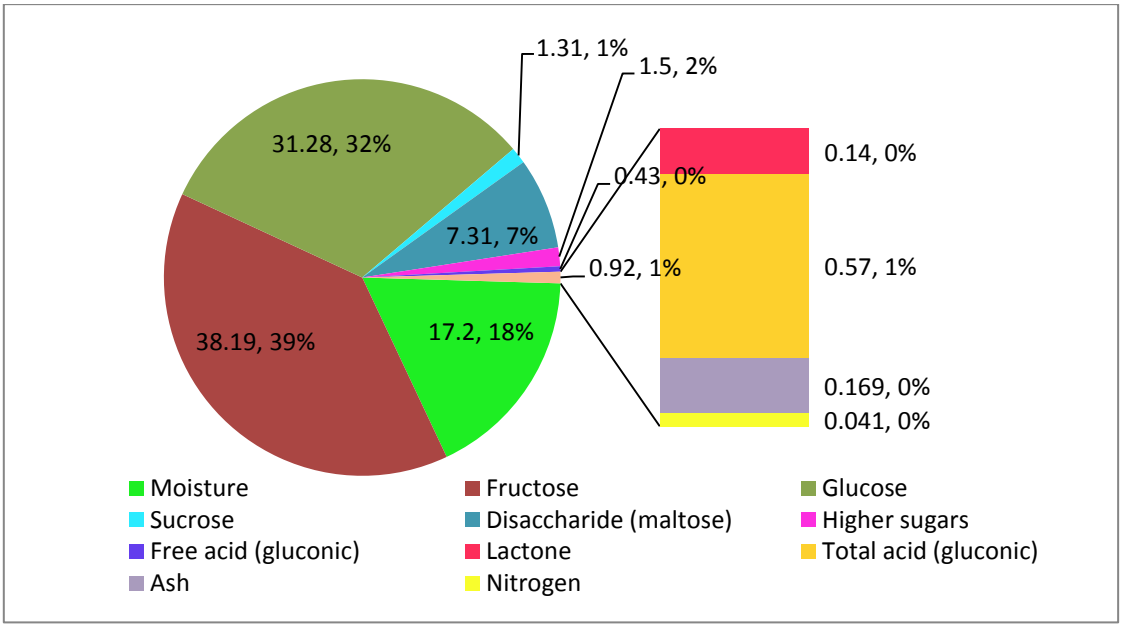


Figure 2.2 Average composition of honey (the data is summarized from Alvarez-Suarez et al., 2010, Jeffrey and Echazaretta, 1996 and Olaitan et al., 2007).

There are several factors on why honey has a great advantage in these biological affect which are:

(i) Osmotic effect of honey. Honey is a saturated or super-saturated solution of a mixture of fructose and glucose sugars (84%), therefore, no fermentation occurs in honey (Molan, 1992).

(ii) The antimicrobial activity of honey is its acidity. The pH being between 3.2 and 4.5 is low enough to be inhibitory to many pathogens (Cooper et al., 2002).

(iii) Presence of antibacterial phytochemical components (Mavric et al., 2008; Yao et al., 2004).

(iv) Antibacterial active fraction of honey derived from the native New Zealand Manuka tree (*Leptospermum scoparium)*, which consists of derivatives of benzoic acids, cinnamic acids and flavonoids, all of which have been identified previously in honeys which do not exhibit non-peroxide residual antibacterial activity.

Table 2.2 The characteristics of honey.

	REMARKS	REFERENCES
Nutritional Aspects		
Carbohydrates/ sugars of honey	About 95% of honey dry weight is composed by sugars which are fructose and glucose, the monosaccharides hexoses produced by hydrolysis of the disaccharide sucrose	White, 1978
	In the process of digestion after honey intake the principal carbohydrates fructose and glucose are quickly transported into the blood and can be utilized for energy requirements by the human body	Bogdanov et al., 2008
Vitamins/Minerals/trace compounds	It is small residue remaining after the honey is burned and it was reported to varies from 0.02 to slightly over 1 %.	Conti, 2000
	Honey contains a number of trace elements such as chrome, manganese, selenium, sulphur, boron, cobalt, fluorine, iodine, molybdenum and silicon which are nutritional importance. Besides, honey also contains choline (0.3 -25 mg/kg) which is essential for cardiovascular and acetylcholine (0.06 -5 mg/kg), which acts as a neurotransmitter. Chromium, manganese and selenium are important, especially for 1 to 15 years old children.	Iskander, 1995; Terrab et al., 2005
Proteins, enzymes and amino acids	Enzymes in honey can conceivably arise from different sources such as bees, pollen, nectar or even yeast and microorganisms.	Bogdanov et al., 2008
	Both diastase and invertase has been reported to play an important role for judging honey quality and have been used as honey freshness indicators	
	Glucose oxidase is an enzyme produced and added by bees. This enzyme converts some glucose into gluconic acid and hydrogen peroxide, which is potent antimicrobial and gives a honey low in pH	Grane and Kirk Visscher, 2007
	Formic, acetic, citric, lactic, maleic, malic, oxalic, pyroglutamic and succinic acid are the acids which	

	have been found in the honey only in small portion but it is important for the honey taste.	
Aroma compounds, taste-building compounds and polyphenols	500 different volatile compounds have been identified in different types of honey. Most aroma building compounds vary in different types of honey depending on its botanical origin. Polyphenols in honey are mainly flavonoids, phenolic acids and phenolic acid derivatives. These are compounds known to have antioxidant properties. The main polyphenols are the flavonoids, their content can vary between 60 and 460 μg/100 g of honey and was higher in samples produced during a dry season with high temperatures.	Kenjeric et al., 2007; Tomas-Barberan et al., 2001;
Physical Aspects		
Sensitivity to heat	The loss of antibacterial activity on exposure of honey to heat was of complete loss of inhibition by 17 % honey after exposure of 50% honey to 100°C for 5 min, 80°C for 10 min or 56°C for 30 min	White et al., 1964
Sensitivity to light	Honey lost its ability to inhibit bacterial growth (tested in a 17 % solution) after exposing a thin film of it to sunlight and it is confirmed that exposure of honey in a layer 1-2 mm thick to sunlight for 15 min was found to result in complete loss of non-osmotic activity	
Storage effect	Enzymatic activity, antimicrobial properties, microbial quality, colour and chemical composition are all influenced by heat and storage	
Microbiological Aspects		
Antimicrobial activity	Honey has both peroxide and non-peroxide antibacterial action, with different non-peroxide antibacterial substances involved: acidic, basic or neutral	Al-Waili, 2004; Al-Waili and Haq, 2004; Bogdanov et al., 1997; Molan, 1997; Mundo et al., 2004
	Antibacterial effects are different due to different substance and compounds with different chemical properties. The high sugar concentration and low in pH is also responsible for the antibacterial activity. Honey has also antiviral activity (Rubella and Herpes virus) and fungicide activity against different dermatophytes	

Medicinal Aspect		
As remedy for diarrhoea	Pure honey has bactericidal activity against many enteropathogenic organisms, including those of the *Samonella* and *Shigella* species, and enteropthogenic *Eschericia coli*	Jeddar et al.,1985
As medicine for gastric ulcer	Gastric ulcers have been successfully treated by the use of honey as a dietary supplement	Ali, 1991
For wound healing	Honey is an effective treatment of wounds because it is non-irritating, non-toxic, self-sterile, bactericidal, nutritive, easily applied and more comfortable than other dressings	Bassam et al., 1997; Bergman et al., 1983;
Rapid healing agent	honey promotes healing of ulcers and burns better than any other local application	Efem, 1988;
	Clinical observations made are that open wounds heal faster and are ready faster for closure by stitching when dressed with honey than when dressed conventionally	Ndayisaba et al., 1993; Tan et al., 2009; Wood et al., 1997)
Stimulation of healing process	Honey has been found to be effective in starting the healing process in non-healing ulcers some of which had been present for a median time of 1 year, or had been treated for up to 2 years, or had shown no healing over more than 5 years despite usual measures including skin grafts. Honey has also been used successfully on chronic foot ulcers in lepers and diabetic foot ulcers.	

Due to good nutritional value as it contains carbohydrates, proteins, vitamins, minerals and various other components, honey has been use as food in different parts of the world and in different ways. Commercial application of honey is summarized in table below:

Table 2.3: Commercial application of honey (Farm Monitoring Report, 2013).

Use of honey	**Remarks**
Sweetener for sport beverages, non-alcoholic fruit, ice tea, yogurt drinks, chocolate milk beverages, fermented beverages, vinegar, vegetable juices; in mead production	Supplies different natural honey flavours and colours; honey sugars are fermentable and give alcoholic drinks unique flavours; prevent browning due to antioxidative properties
Additive to poultry and other meat, to fruit and vegetable processing	Antioxidant and preservative (antibacterial) properties, reduces browning improve sensory properties.
Additive to microwave foods: cakes, muffins, cookies, glazes	Superior microwave sensitivity and water activity managements than synthetic sugars
Additive to flour bagels, cereals, chicken marinades, French fries, bread, pasta, extruded snacks , corn chips, potato chips	Improve sensory properties, adds/retains moisture due to hygroscopic properties; improves browning due to reducing sugars
Additive to frozen ice cream and dough	Better stability and sensory properties
Additive to fruit spreads, peanut butter, nut spread	Better storability and sensory properties
Additive to salsas and sauces	Neutralize sour and burn intensity
Additive to fried or roasted beef, poultry	Reduces the formation of heterocyclic aromatic amines and their mutagenic effects
Dried honey	Convenient as consistent in texture, flavour and colour, allowing blending with other dry ingredients

2.2 Production, Collection and Processing of Honey

Honey is considered as a natural biological product evolved from the nectar. The honey in the nutshell is flower nectar which has been collected, regurgitated and dehydrated by honey bees. The production of honey from nectar is presented in Figure 2.3.

At first, pollen and nectar of the plants and flowers are collected by honey bees. Nectar is sucked out of the flowers using long, tube like tongues called proboscis and the bees store this nectar in their stomach before carried it to the beehive. At this stage, the nectar contains 80% water. While inside the bee's stomach for about half an hour, the nectar mixes with the proteins and enzymes produced by the bees, converting the nectar into honey. Invertase from hypo pharyngeal gland of bees will cleave the disaccharide (sucrose) into fructose and glucose which then doubles the number of molecules contributes to the osmotic potential. Honey was then dropped into the beeswax comb, which are hexagonal cells made of wax produced by the bees. The process is repeated until the combs are full. Bees fan their wings to evaporate and thicken the honey which contains about 14-18% water in order to ensure the long-term storage of honey. When this is done, the bees cap the honeycomb with wax and move on to the next empty comb, starting all over again.

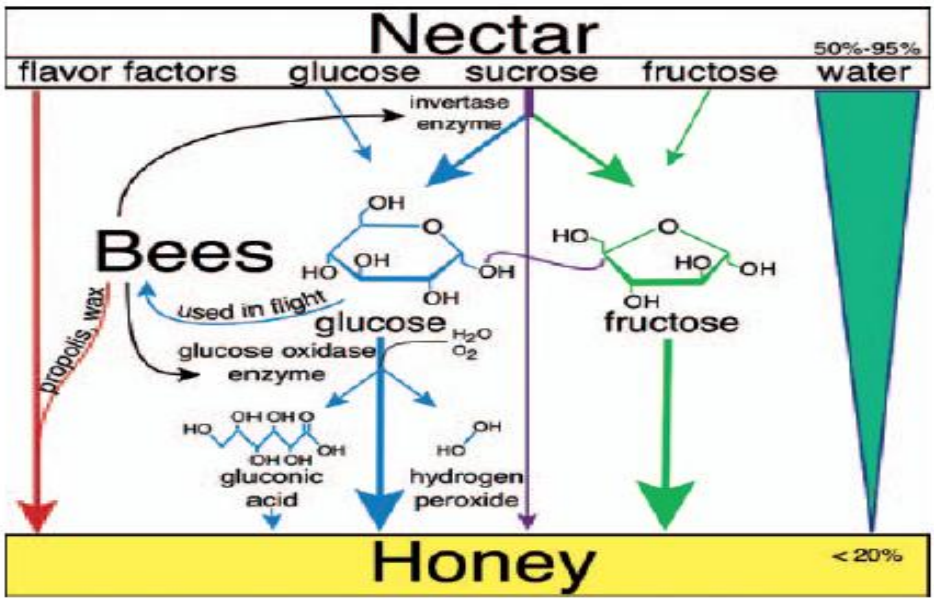


Figure 2.3: The production of honey from nectar (adapted from Grane and Kirk Visscher, 2007).

In conventional honey processing, the use of heat is found to be essential for fast handling, to dissolve large sugar granules and to sustain honey quality. Two stages of heating applied in honey industry are known as liquefaction (to keep honey in liquid form for as long as possible), and pasteurization which is to kill the yeast and other spoilage microorganisms as well as preventing fermentation (Subramanian et al., 2007). Both stages are operated at a temperature of more than 50°C or even up to 77°C.

Apart from conventional treatment, alternative thermal treatment (such as microwave heating and infrared heating) and non-thermal treatment (such as ultrasound, ultraviolet and membrane filter) are proposed, explored and implemented (Barbosa-Cánovas et al., 1998) to ensure the quality and safety aspect of honey (Figure 2.4). These novel food processing technologies could provide safe, fresher-tasting, nutritive foods without the use of heat or chemical preservatives, which totally can satisfy new consumer demand (Barbosa-Canovas and Bermude-Aguirre, 2011).

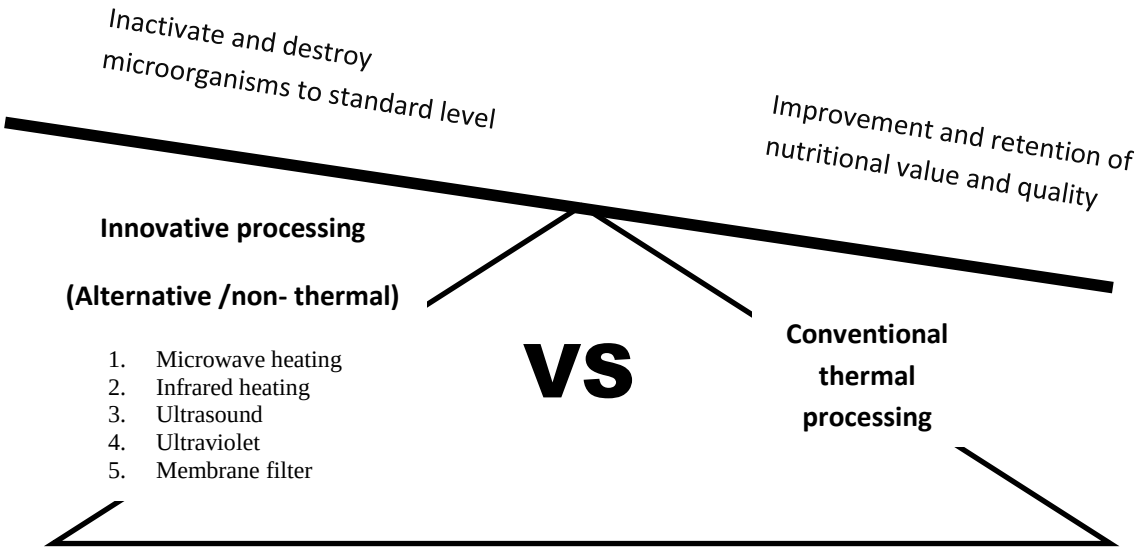


Figure 2.4: Innovative processing which includes alternative thermal processing (1-2) and non-thermal processing (3-5) provide an approach to honey safety and preservation that are designed to retain the natural and as-fresh properties of honey.

2.3 Conventional Thermal Treatment of Honey Processing

Thermal processing is a popular technology for food industry which ensures microbiological safety of the products. The essential principle of these methods is based on the convection and conduction mechanism of heat transfer which is generated either by combustion of fuels or by an electric resistive heateing before it transferred into the product (Pereira and Vicente, 2010).

It is known that the major problem faced by honey producers is its rapid deterioration in quality due to fermentation (Ghazali et al., 1994; Subramanian et al., 2007). Therefore, the primary objective of thermal processing of honey is to keep it stable with an extended shelf life (White, 1964). Liquefaction (operated at approximately 55°C) and pasteurization (operated normally up to 70°C) are two stages of thermal treatment applied to honey to ensure it stay in liquid form and also to destroy the microorganisms which contaminate causing it to ferment (Tosi et al., 2004). The steps of preheating, straining, filtering and indirect heating were performed before it can be stored and produced (Figure 2.5).

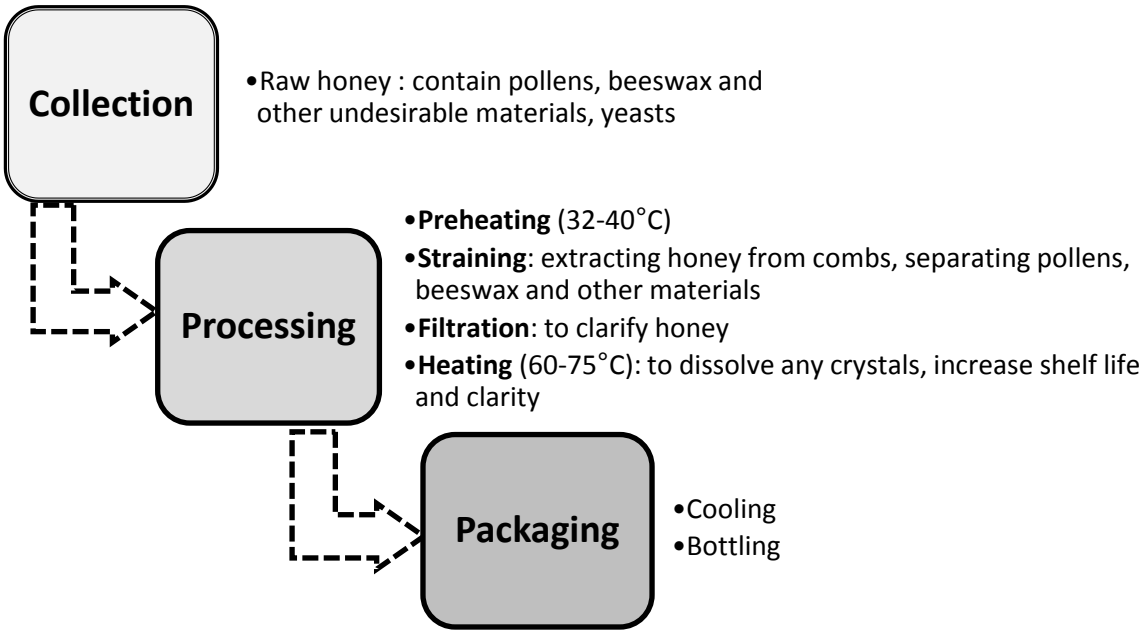


Figure 2.5: The flow of conventional process in honey production.

The quality of honey is mainly determined by its sensorial, chemical, physical and microbiological characteristics. The criteria for ensuring quality honey have been specified by the European Legislation (EC Directive 2001/110) and Codex Almentarius Commission (2001). The effect of heat processing on the quality parameters in honey is well documented and summarized in Table 2.4.

Studies have shown that heating honey at temperature between 60 to 70°C for 10 min as well as indirect heating in conventional process which is in the range of 60 to 65°C for 25-30 min can destroy the yeasts completely. Yeasts can be grown to tens of thousands per gram although honey possesses anti-microbial characteristics, have low water content and high concentration of sugars (Bogdanov et al., 2008). *Saccharomyces cerevisiae* was found as the dominant yeast fermenting honey (Snowdon et al., 1996). It is also known as osmophilic or sugar tolerant yeast because it can withstand the low water content (around 16 - 21%) and high sugar concentration in honey. Food grade honey with a very high yeast count (more than 100 000 CFU/g) is not likely to be marketable (Snowdon et al., 1996). With respect to medical health applications, yeast count must be less than 500 CFU/g.

Tosi et al. (2004) reported the completely inhibition of yeasts and fungi in natural honey when heated at higher temperature, 80°C for 60 s in transient and 30 s in isothermal stage using the technique of high temperature-short time heating. Whereas, Wakhle et al. (1995) showed complete inhibition of yeast using lower temperature ranging from 63 to 65°C but with longer treatment time of 7.5 to 35 min.

Clostridium botulinum is associated with honey and is known to cause a disease called botulism (Helligas and Demirci, 2003; Kuplulu et al., 2006). Honey free of *C. botulinum* spores can potentially be used in products fed to infants (Areekul and Toledo, 2010; Kuplulu et al., 2006; Snowdon, 1996). However, insignificant inactivation of *C. botulinum* spores was found by heat shock (Nakano et al., 1992). In contrast to *C. botulinum* spores, Shimanuki et al. (1984) reported that *Bacillus* spores could be eliminated from honey.

In general, the different combination of time and temperature treatment is necessary to inactivate all types of microbes specially mold and yeasts since they are the only microbes which have been reported to grow in honey.

Table 2.4: The summary on the effects of conventional thermal treatment on honey quality.

Quality properties	Flower/ Country types of honey	Thermal process condition	Remarks	References
Antioxidant activity and phenolic compounds	Clover honey and Buckwheat honey	55°C /12 to 16 hrs	-No significant differences in phenolic profiles of processed and raw buckwheat honeys, except for differences in galangin concentrations, which, unlike clover honey, decreased after processing. -Processing reduced total phenolics of buckwheat honey by 37%. -No significant effect on antioxidant capacity in clover honey but lowered the antioxidant capacity in buckwheat honey (33.4%)	Wang et al., 2004
	Sunflower, cotton and canola honey	50, 60 and 70°C for up to 12 days	Antioxidant activity increased linearly with increasing heating time at 50 and 60°C, logarithmic increase in antioxidant activity at 70°C was observed.	Turkmen et al., 2006
Hydroxymethylfurfural, HMF concentration	Middle Antonia honey	135°C /100s and 150°C / 40 s	HMF concentration is increased. Treatment at 135°C for 100s produces approximately the same HMF concentration at 150°C for 40 s	Turhan et al., 2009
	Sunflower honey	47.5 ± 1°C for 9.5 ± 1 min at pH 5.2 ± 0.15	This optimum condition can keep HMF concentration within the prescribed limit of standard.	Nanda et al., 2006
	Clover honey and Buckwheat honey	55°C for 12 to 16 hrs	HMF value of processed Clover honey was 57% higher than that raw clover honey. However, for processed Buckwheat honey, HMF value was 23% from raw.	Wang et al., 2004

	Unifloral Sicilian honeys (Orange, Eucalyptus, Sulla, Chestnut)	50°C /144 hrs, 70°C / 96 hrs and 100°C / 4 hrs	No measurable amount of HMF in heated-Chesnut honey (50°C for 1 week) while orange honey after 4 days at the same temperature has exceeded the legal limit. During heating at 100°C, all honey showed considerable formation of HMF. The HMF formation increased as heating temperature increased.	Fallico et al., 2004
	Forest honey, multifloral honey	35, 45 or 55°C (672 hrs), 65°C (168 hrs), 75°C (24 hrs) and 80°C (12 hrs)	HMF concentration increases with increased temperature and treatment time	Visquert et al., 2004
	Pine, thymus, cotton, helianthus and orange	35, 45, 55, 65 and 75°C /24 hrs	No significant increase of HMF at 55°C for 24hrs period. At 65°C HMF at pine and orange sample is still low, while at the rest of the samples exceeded the 40 mg/kg. At 75°C the enzyme was almost destroyed and HMF is extremely high except in pine honey which just exceeded 40 mg/kg.	Karabournioti et al., 2001
	Natural honey	100-160°C, 14-60s	HMF increases from 3.9 to 10.1 mg/kg honey by 100°C 60 s heating, and from 3.9 to 32.8 mg HMF/kg honey by 140°C 60 s treating	Tosi et al., 2004
Colour and brown pigment formation	Orange blossom floral honey	50, 60, 70 and 80°C for up to 160 hrs	The increase in browning was rather negligible at 50°C and 60°C, smaller at 70°C but very pronounced at 80°C	Vaikousi et al., 2009
	Sunflower, cotton and canola honey	50, 60 and 70°C for up to 12 days	Brown pigment increase as the temperature increase. The increase was more noticeable in heated samples at 70°C than those at 50 and 60°C. The increase in brown pigment is depends on time and temperature of heating.	Turkmen et al., 2006

	Clover honey and Buckwheat honey	55°C /12 to 16 hrs	Processed Clover honey was darker in colour as compared with raw. Raw Buckwheat honey was significantly darker in colour than processed Buckwheat honey.	Wang et al., 2004
Antibacterial activity	Manuka honey	50 - 70°C / 15 - 120 min	The highest temperature (60 and 70°C), treated for 15, 60 and 120 min exhibited the lowest percentage inhibition of *Staphyloccus aureus*	Al-Habsi and Niranjan, 2012
	Zanthoxylum Fagara honey	40°C/30 days, and 78°C / 5, 10 and 15 min	At 40°C, the antibacterial activity was not affected but it was decreased significantly when honey was exposed to 78°C for 15 min	Rios et al., 2001
	Canola/red stringy bark honey	45°C / 8 hours	Thermal processed honey shows significant reduction in antibacterial activity against *Staphyloccus aureus* as compared with unprocessed	Chen et al., 2012
Physicochemical properties (pH, electrical conductivity, moisture content)	Tahonal honey	55°C / 3, 6, 9 and 12 min/	The acididity of thermal-treated Tahonal honey (55°C for 9 and 15 min) increased during storage	Ramirez et al., 2000
	Forest honey, multifloral honey	35, 45 or 55°C (672 hrs), 65°C (168 hrs), 75°C (24 hrs) , 80°C (12 hrs)	Acidity, electrical conductivity and moisture content were unaffected by heat.	Visquert et al., 2004
	Clover honey and Buckwheat honey	55°C for 12 to 16 hours	No significant difference in moisture content between processed and raw Clover honey. Processed Buckwheat honey showed slightly higher in moisture content as compared with raw	Wang et al., 2004
Crystallization	Natural honey	140-80°C, 60-15s (transient stage) and 30-10s (isothermal stage)	Crystallization is inhibited at 80°C, 6s (transient stage) and 30s (isothermal stage)	Tosi et al., 2004

Enzyme activity	Natural honey	60-100°C /120-1200s	Decrease in the diastase activity related to an increase in temperature. The activity becomes zero at 100°C for both transient and isothermal heating.	Tosi et al., 2008
		47.5 ± 1°C / 9.5 ± 1 min/ pH 5.2 ± 0.15	This optimum condition can keep diastatic activity within the prescribed limit of standard.	Nanda et al., 2006
	Clover I, Clover II, Chinese, Orange, Pepper and Buckwheat	63°C and 85°C/ 5-30 min	Amylase activity was slightly decreased in all heat treated honey Longer exposure for 15 min caused honey to lose more activity in the range 4 to 28 Diastase number	Babacan et al., 2002
	Natural honey	140-80°C, 60-15s (transient stage) and 30-10s (isothermal stage)	An unacceptable diastase number reduction -beyond 140°C during 15 s (transient stage), 30 s (isothermal stage)	Tosi et al., 2004
	Dzidzilche honey	55°C for 3, 6, 9 and 12 min	The diastase activity diminished	Ramirez et al., 2000
	Pine, thymus, cotton, helianthus and orange	35, 45, 55, 65 and 75°C for 24 hrs	The decrease of invertase starts from the temperature of 35°C. The concentration of invertase at 55°C was decreased to less than the half of its initial value at pine, about half of its initial value at orange sample and about seventy percent at cotton, thymus and helianthus. At 75°C the enzyme was almost destroyed.	Karabournioti et al., 2001
Rheological properties and viscosity	Dark and light honey	40, 60, 80 or 90°C /5,10 or 20 min	Both honey showed Newtonian fluids behavior. Viscosity increased with increasing ultimate heating temperature	Abu-Jdayil et al., 2002
	Korean honey	55°C, 60 min	Over the temperature range of 0–30°C, Korean honey varieties exhibited liquid-like rheological behavior	Yoo, 2004

2.4 Alternative Thermal Processing Methods for Honey

2.4.1 Microwave heating

The applications of microwave heating in food industry demonstrate significant advantages over conventional methods in reducing process time and also improving food quality. Microwave heating takes place due to the polarization effect of electromagnetic radiation at frequencies between 300 MHz and 300 GHz (Lorenz and Decareau, 1976).

The effect of microwave on the quality of honey is well documented. Power levels and treatment time are main parameters which influence the quality of honey, namely hydroxymethylfurfural, HMF content and browning (Bath and Singh., 1999; Hebbar et al., 2003). In the formation of HMF and browning pigment, the values increased with increasing both power intensity and heating duration. Hebbar et al (2003) carried out an experiment at different power levels ranging from 10 to 100 (175 - 800 W), and found that heated samples for longer duration of 45- 60s, 60s and 90 s at higher power intensity of 16.0 W/g, 11.9 W/g and 9.1 W/g, respectively resulted in rapidly high HMF (> 5 mg/kg), showing the sensitivity of honey to the power intensity and duration of heating. However, the HMF level obtained was still far below the maximum permissible statutory level of 40 mg/kg of honey. Whereas, Bath and Singh (1999) showed that power level of 280 W with heating time of 270 s give the maximum value on HMF formation with 25.789 mg/kg and 8.548 mg/kg for *Helianthus annuus* and *Eucalyptus lanceolatus* honey, respectively.

Different combinations of heating duration (15 to 90 s) and power intensity (175 -800 W) show the changes in properties. Higher power level and shorter processing time is better than lower power level and longer duration. Power level of 800W for 15s resulted in considerable reduction in yeast count (450 CFU/ mL), which is due to the rapid increase in sample temperature after exposed to the microwave heating which then leads to the rupture of yeast cell walls. They also reported that processed honey with a yeast count of 8.00×10^2 CFU/mL could be stored at 28 ± 2°C for 16 weeks without fermentation, which is due to the reduction of moisture content above 9% at power intensity of 9.1 W/g, 11.9 W/g and 16.0 W/g when the samples heated for 60 seconds.

The study of Hebbar et al. (2003) also showed the reduction of diastase activity to 50% of its original value when heated for long periods (60 to 90 s) and at power intensity of 6.3 W/g, 9.1 W/g, 11.9 W/g. Even though the larger reduction was observed in higher power intensity,

the final moisture content for all samples (19.8-21.2%) were reported in the range of the acceptable level for commercial processed honey (22%).

Ghazali et al. (1994) showed that the microwave energy used to heat samples of starfruit honey at 71°C did not affect the pH, titratable acidity, water activity, moisture, nitrogen, ash, glucose, fructose, maltose and sucrose content of the honey. However when the samples were stored over 16 weeks, major changes in moisture content, titratable acidity, sugar content and diastase activity were observed.

2.4.2 Infrared heating

Infrared heating is gaining popularity and widely applied to various thermal processing operations such as dehydration, frying and pasteurization due to higher thermal efficiency and fast heating rate / response time in comparison to conventional heating. In addition, it provides significant advantages over conventional heating such as reduced quality losses, versatile, simple and compact equipment and significant energy saving. Two conventional types infrared radiators used are electric and gas-fired heaters (Krishnamurthy et al, 2008).

In food processing, the radiant electromagnetic energy may induce changes in the electronic, vibrational and rotational states of atoms and molecules once it impinge upon a food surface. As food is exposed to infrared radiation, it is absorbed, reflected or scattered and the absorption intensities at different wavelength differ by food components. Infrared application heating was reported to be effectively used for enzyme and pathogen inactivation including bacteria, spores, yeasts and molds in both liquid and solid foods (Galindo et al., 2005; Kouzeh-Kanani et al., 1983). The efficacy of this inactivation depends on the parameters which are (i) power level (ii) temperature of food sample (iii) wavelength and bandwidth (iv) sample depth (v) types of microorganism (vi) moisture content (vii) physiological phase of microorganism and (viii) types of food materials.

Hebbar et al. (2003) investigated the effect of infrared heating on the quality of forest bee honey using a near infrared (NIR) batch oven fitted with infrared lamps for 1.0 kW, peak wavelength 1.1-1.2 μm. The result showed that yeasts count was reduced substantially when honey samples were heated continuously for 2, 3, 4, 5 and 8 min. With regards to quality, HMF value was increased 220% with 37% drop in enzyme activity after 5 min heating with product temperature of 85°C. However, when the samples were heated for 8 min which at very high in temperature (110°C) the diastase activity fell drastically, clearly indicating excessive heating of honey. Benefits to this, at this time no viable colony forming units of

yeasts were noticed. They concluded that heating period of 3 to 4 min was adequate to meet all the statutory requirements of honey quality and commercially acceptable product.

2.5 Non-Thermal Processing Methods for Honey

2.5.1 Ultrasound

Ultrasound is generated by the application of a vibration force to the surface of material. Food technologist has discovered that it is possible to employ a more powerful form of ultrasound (>5 W/cm^2) at a lower frequency (generally around 40 kHz), which is usually refer as power ultrasound. When applied to the surface of material, the vibration force is transmitted through the bonds within molecules. In food processing, the application of ultrasound shows considerable promises in various applications such as crystallization of fats and sugars, inhibition of enzyme activity and also enhanced preservation. Sonication in combination with heat and pressure has potential to enhance microbial inactivation (Kaloyereas, 1958; Liebl, 1977).

In honey processing, the application of ultrasound showed it benefits in eliminating the existing crystals with 9 kHz of ultrasound frequency (Kaloyereas, 1958). Liebl (1977) has improved the method for preventing granulation using higher frequency of 18 kHz which can drastically reduce the liquefaction time to less than 30 seconds. Whereas Thrasyvoulou et al. (1994) found that the ultrasound treated samples remained in the liquefied state for 344 ± 36 days, longer as compared with the heat treated sample which is remained in the liquefied state only for 282 ± 86 days. It has been reported that the probe size and cycle have significant effect on liquefaction time (Ipek, 2010).

With regards to honey quality parameters, no significant effect was found on moisture content, electrical conductivity and pH when 23 kHz frequency of ultrasound was applied (Thrasyvoulou et al., 1994). D' Arcy (2007) also reported no significant difference in colour changes between untreated and ultrasound treated sample. Meanwhile, the effect of ultrasound on the HMF concentration in honey is different depending on the probe and cycle used. Ipek (2010) has reported a significant increase of HMF concentration in ultrasound-treated honey (7 mm probe, 100% amplitude, 1 cycle). In contrast, D' Arcy (2007) showed a significant lower range of HMF concentration as compared with heat-treated honey (70°C) when using longer probe (40 mm probe-100% amplitude-cycle 1). This is primarily due to the honey being at the maximum temperature reached of 77.3°C for a much shorter time (434.0

s) than for a heating regime (55°C for 16 h and 72°C for 2 min) which is similar to that presently used by the honey industry. In addition, a lower HMF concentration can be explained by the fact that, ultrasound waves have a suppression effect on Maillard reaction by preventing amino acid and carbohydrate reaction and could break down HMF molecules.

Regarding to microbial content, previous study showed that ultrasound has a destructive effect on microorganism showing a decrease of 50% in total aerobic bacteria (cotton and canola honey) and 3% in total aerobic bacteria for sunflower honey (Ipek, 2010). Most of the yeasts have been destroyed by ultrasound treatment. This result is in an agreement with D' Arcy (2007) who stated that microbial inactivation in ultrasound treated honey was considered to occur due to cavitation, localized heating and free radical formation.

2.5.2 Ultraviolet

Ultraviolet is one of the electromagnetic processes which getting more attention from food industry as an alternative technology especially for inactivation of pathogenic and spoilage microorganism (Demirci and Panico, 2008).

Economically, pulsed mode UV light can provide a cost-effective alternative to inactivate both vegetative cells and spores compared to continuous mode UV light. In pulsed UV light, electrical energy is released as very short period pulses (several nanoseconds) after stored in capacitor over a short period of time (few milliseconds). The electrical energy is transferred through a lamp which is filled with xenon and then the ionization of gases will produces a broad spectrum of light in the wavelength region of UV. When comparing with continuous UV light, Dunn et al. (1995) revealed that pulsed UV light treatment is a more effective and rapid way of inactivating the microorganism.

In honey processing, the use of UV treatment is limited and still in improvement. The purpose of the application of UV is more to inactivate the spores. Helligas and Demirci (2003) applied pulsed light to the *Clostridium sporogenes* inoculated honey using the SterilePulseXL® 3000 Pulsed Light Sterilization System with an equipment setting of 3 pulses/sec. Inoculated honey samples were placed in the pulse UV chamber where they were exposed to pulses of UV-light. The energy released from the UV strobe was 5.6 J/cm^2 per pulse when the input voltage was 3,800 V. The number of pulses, the distance between the food product and the lamp as well as the depth of honey are the parameters which affect the spores inactivation. Increasing the number of pulses showed and increased in the reduction of *C.sporogenes;* from 0% (15 and 135 pulses for 5 and 45 s respectively) to 89.4% (540 pulses,

3 min). However, pulsed UV light was failed to completely inactivate the *C. sporogenes* due to the limited penetration of UV light in honey. In addition, there is no synergistic effect of the heat generated within the pulsed UV light on the spores because *C. sporogenes* were survived. Besides, the percent reduction of the spores increased as the depth of honey was decreased (0.0 and 39.5% were obtained for 8 and 2 mm, respectively). In conclusion, inactivating the spores in UV-treated honey is more effective when the number of pulses was increased.

2.5.3 Membrane filtration

Membrane processing is a technique that permits concentration and separation of macro- and micromolecules based on molecular size and shape. In recent years, membrane process such as microfiltration (MF), ultrafiltration (UF) and reverse osmosis gaining importance for processing liquid foods and natural colours.

Ultrafiltration is an alternate approach to the conventional thermal process and has been used to transform raw honey into a material suitable for food processing. The membranes are made up of polysulphone, polyvinyldene fluoride and cellulose acetate of pore size 1-100 nm. As reported by National Honey Board, the benefits of UF can be pointed as: (i) no cloudiness or sedimentation/granulation, (ii) reduced viscosity, (iii) commercially sterile product and (iv) consistent quality characteristics. In addition to that, ultrafiltration membranes in honey processing could eliminated bacteria (7000, 30 000 and 80 000 molecular weight cut off (MWCO)) and could completely remove yeasts (20 000, 25 000, 50 000 and 100, 000 MCWO) as has been found by Itoh et al (1999) and Barhate et al (2003). It is also reported that microfiltration membrane (MF) with pore size of 200 nm would remove the viable microorganism completely and as such could be used for sterilization (Little et al., 1987).

However, UF have some limitation. Ultrafiltered honey is devoid of desirable enzymes and proteins, and hence, cannot be regarded for applications related to food health. Protein could be eliminated from honey when it was exposed to UF (Itoh et al., 1996). Protein and enzymes are nutritionally important in honey (amylase, invertase and glucose oxidase), which can be used in wounds treatment and gastrointestinal disease.

2.6 High Pressure Processing

Innovative non-thermal processes for preservation of food have attracted the attention of many food manufacturers. In the search for new processing methods, particularly for certain products, the application of high-pressure (HP) processing has shown considerable potential as an alternative technology to heat treatments which aims to satisfy the increasing consumer demands for fresh-like products with minimal degradation of nutritional properties (Palou et al., 1999a; Palou et al., 1999b).

High pressure processing (HPP) which is also identified as high hydrostatic pressure processing (HHP) or ultrahigh pressure processing (UHP) is a non-thermal food preservation technique that has been cited as one of the best innovations in food processing in 50 years (Gould, 2001a). The majority of HPP applications in food industry involves pasteurization of foods at ambient temperature, at about 400-900 MPa and at chilled or mild process temperature, with or without the addition of heat. This condition allowing most foods to be preserved with minimal effects on taste, texture, appearance or nutritional value (Butz, 2010; Matser, Krebbers et al., 2004), hence it preferred as 'minimally preserved'(Gould, 2001b). In addition, it plays a role in inactivating harmful pathogens and vegetative spoilage microorganism as well as enzymes (Hendrickx et al., 1998; Matser et al., 2004). Although HPP is currently more expensive than other conventional method, the use of HPP offers new opportunities and great advantages to food industry in respond to consumers need. Moreover, the cost of HPP is decreasing, mainly due to the new development in equipment and methods of treatment (van den Berg, 2001).

2.6.1 Evolution of High Pressure Processing

Early ideas on the development of high pressure technology were driven by the needs of military to improve the guns or cannons, and not by food technologist (Gould, 2001a). The application of high pressure in food research started by Royer in 1895 was an effort to inactivate bacteria. Four years later, it was followed by Hite, the first person who demonstrates the potential of high pressure technology in milk preservation when it was subjected to 700 MPa for 10 min at ambient temperature (Gould, 2001a).Another study of high pressure on coagulation of egg albumin by Bridgman in 1914 showed that this treatment alters the functional property in different way than heat treatment (Gould, 2001b). An important finding in the inactivation of bacterial spores was then recorded by Larson et al.

(1918). They found that pressure of 607 MPa for 14 hours treatment can destroy vegetative cells. Whereas, about double of this pressure (1200 MPa) is needed to inactivate the spores of *Bacillus subtilis*. In later years, more work was done by different researchers to verify this finding and this was set the scene for later work.

The number of industrial HPP systems for food preservation has steadily increased during the past 20 years. Then there have been significants advances in food preservation applying high pressure when more products were commercially available. A brief history of HPP in food preservation is presented in Figure 2.6.

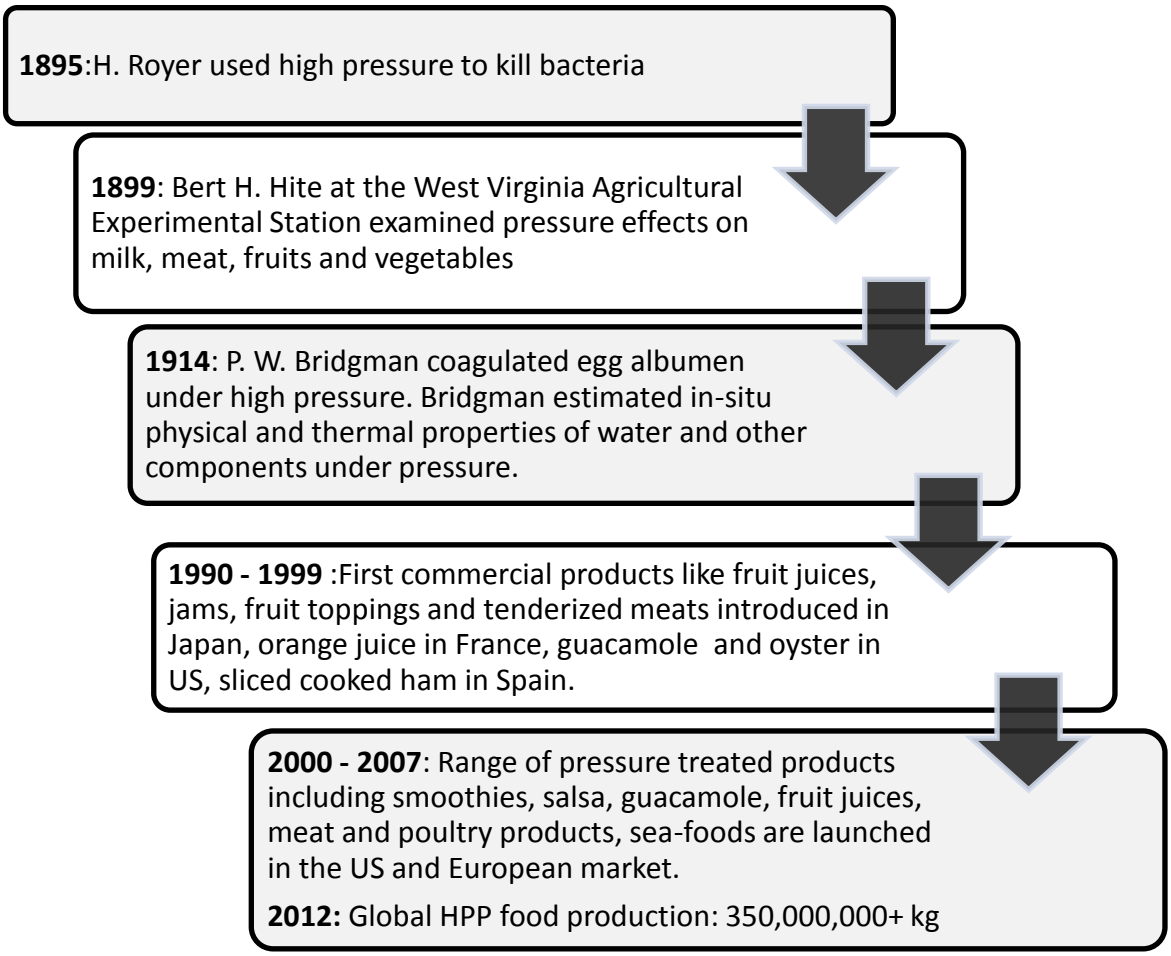
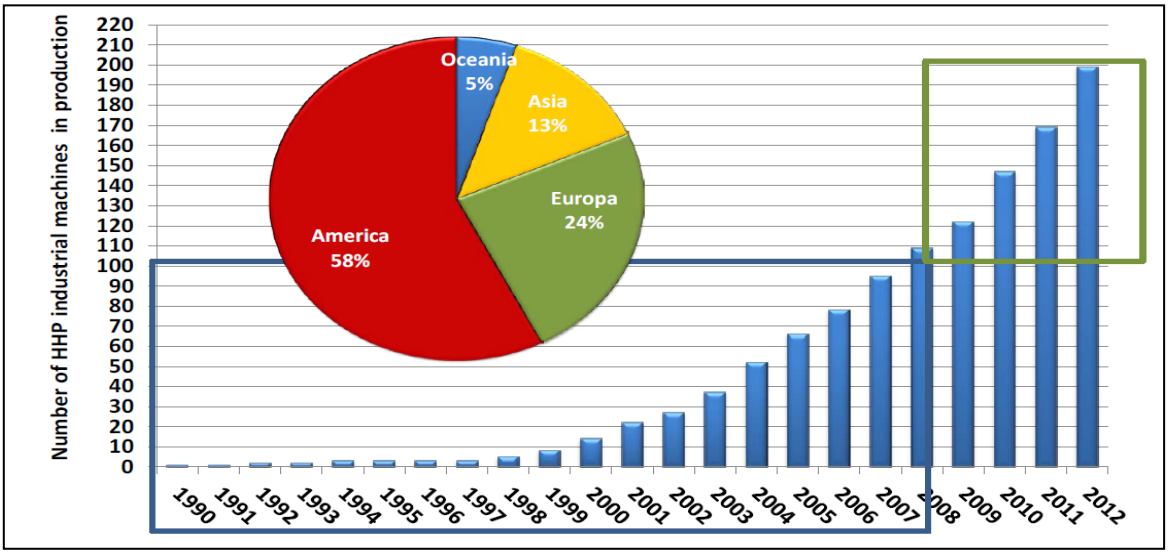


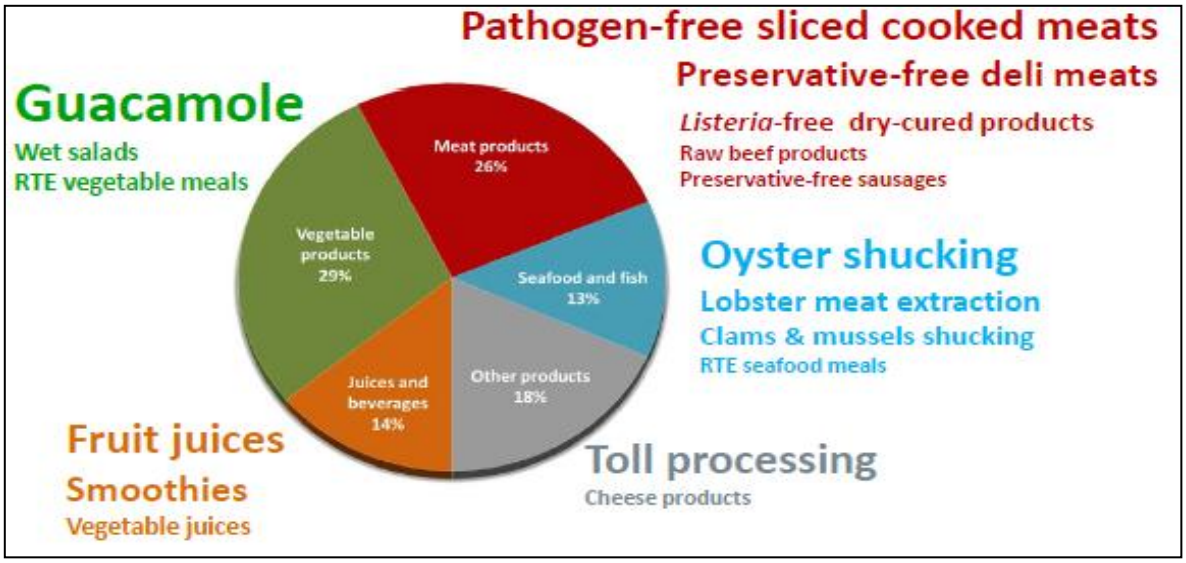
Figure 2.6: A brief history of high pressure food processing.

The HPP units are principally used for the inactivation of vegetative microorganisms to extend the shelf life of food. However, there are numerous other interesting food applications for HPP, such as food structure engineering (Diels and Michiels, 2006; Gould, 2001a; Knorr et al., 2006), enhanced food quality (Ludikhuyze et al., 2002; Oey et al., 2008), stress response utilization (Ananta et al., 2004; Pavlovic et al., 2005), and the control of

bioconversion reactions (Gould, 2001b). In 2012, more than 193 industrial scale installations with a maximum volume of 687 L are used in worldwide and a total annual production of more than 350,000,000+ kg pasteurized products (Purroy, 2013). The production of HPP industrial machines was steadily increases from 1990 to 2008 and the number produce is more pronounce after 2008, with different varieties of food has been produced (Figure 2.7).



(a)



(b)

Figure 2.7: Industrial scale high pressure machines used worldwide and total vessel volume used for different food products; not including 15 dismantled machines installed before 2003. Total number in production in April 2013: 212. Global HPP food production in 2012: 350,000,000+ kg (Purroy, 2013).

2.6.2 General Principle of High Pressure Processing and Adiabatic Heating of Compression

The behaviour of foods under pressure is governed by two fundamentals principles, Le Chatelier's Principle and Isostatic Principle. Le Chatelier's Principle states that any phenomenon of phase transition, change in molecular configuration or chemical reaction accompanied by a decrease in volume is enhanced by pressure, whereas processes involving volume increase are inhibited by pressure (Farkas and Hoover, 2000; Yaldagard, 2008). The mechanism of HPP is based on the decrease in volume; as pressure increase, free volume decreases so the attractive and repulsive interactions with nearby molecules will change and thus have an affect on the rates of chemical and biochemical reaction. It was noted that pressure is an important thermodynamic variable which can affect a wide range of biological structures and processes. As an example, low molecular weight food components which is responsible for nutritional and sensory characteristics remains intact during treatment because the covalent bonds (ionic and hydrophobic bonds) are resistant to pressure. Small molecules such as vitamins and flavours components are mostly unaffected. However, the tertiary structures in high molecular weight components such as enzymes, proteins, lipids and cell membranes are sensitive to pressure (Patterson, 2005; Yaldagard, 2008).

Second is the Isostatic Principle, which states the process time is independent of sample size and shape, assuming uniform thermal distribution within the sample. This is because pressure is uniformly distributed throughout the sample in two different conditions, either in direct contact with the pressurizing medium or insulated from it in a flexible container. This absence of transport limitations gives high pressure processing a unique advantage over all other processing methods. The sample then returns to its original shape when the pressure is released. Besides, samples are not mechanically damaged during pressurization due to the low contraction of liquids and solids (Yaldagard, 2008). Therefore, the initial shape of the product is not affected. These principles prevent the food products from being deformed or crushed when treated with pressure. Thus the chemistry of the food is not changed.

Several authors have reported temperature variations of different foods to compression and expansion during HPP (Kalichevsky et al., 1995; Otero et al., 2000; Rasanayagam et al., 2003) due to the adiabatic heating. Rasanayagam et al. (2003) reported that the temperature increase 3- 4°C for every 100 MPa of pressure rise. However, this temperature peaks can vary significantly, based on the composition of the treated food as presented in Table 2.5. Fluid properties such as viscosity, specific heat and thermal

conductivity, the target pressure, holding time, compressibility, initial temperature and the rate of heat loss due to the surrounding primarily influenced the apparent temperature increase of the pressure transmitting fluid in a vessel during HPP (Balasubramaniam, 2003).

Table 2.5: Overview of temperature increase during compression for some foods (Patazca et al., 2007; Ting et al., 2002).

Substances at 25°C	Temperature increase per 100 MPa (°C)
Water, juice, tomato salsa, 2% fat milk, cream cheese, and other water-like substance	~ 3.0
Tofu	~ 3.1
Egg albumin	~ 3.0
Mashed potato	~ 3.0
Orange juice	~ 3.0
Tomato salsa	~ 3.0
Yogurt	~ 3.0
Salmon	~ 3.2
Honey	~ 3.2
Chicken fat	~ 4.5
Water/glycol	From 4.8 to 3.7
Beef fat	~ 6.3
Olive oil	From 8.7 to < 6.3
Soy oil	From 9.1 to < 6.2

The adiabatic heat of compression which occurs during pressure build up can maximize the benefits of high pressure technology (Toepfl et al., 2006). All materials change temperature during physical compression, depending on their compressibility and specific heat (Ting et al., 2002). Following the first and second law of thermodynamics as well as Maxwell equations, the temperature change (heating during compression and cooling during decompression) can be described as a function of thermo-physical properties of the compressible product.

According to the relevant Maxwell equation:

$$\left(\frac{\partial T}{\partial p}\right) = \left(\frac{\partial V}{\partial S}\right) \quad (2.1)$$

Where *T, p, V* and *S* denote the temperature, pressure, volume and entropy, respectively. The right hand side of equation 2.1 can be rewritten as:

$$\left(\frac{\partial V}{\partial S}\right)_p = \left(\frac{\partial V}{\partial T}\right)_p . \left(\frac{\partial T}{\partial S}\right)_p \quad (2.2)$$

Making use of the definition of the thermal expansion coefficient, β:

$$\beta = \frac{1}{V}\left(\frac{\partial V}{\partial T}\right)_p \quad (2.3)$$

Where, *V* is the specific volume, Equation 2.2 can be modified as:

$$\left(\frac{\partial V}{\partial S}\right)_p = \beta . V . \left(\frac{\partial T}{\partial S}\right)_p = \frac{\beta}{\rho} . \left(\frac{\partial T}{\partial S}\right)_p \quad (2.4)$$

From the second law of thermodynamics, entalphy is given by:

$$\left(\frac{\partial H}{\partial T}\right)_p = T . \left(\frac{\partial S}{\partial T}\right)_p \quad (2.5)$$

Where the left-hand side is defined as the heat capacity at constant pressure:

$$Cp = \left(\frac{\partial H}{\partial T}\right)_p \quad (2.6)$$

By combining equations 2.4, 2.5, 2.6, a general expression for the adiabatic temperature increase upon compression is obtained:

$$\left(\frac{\partial T}{\partial p}\right)_s = \frac{\beta}{p.Cp} . T \quad (2.7)$$

The following plot illustrates the typical trend in a pressure processing cycle in QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Kent, WA, USA). The system is a batch, where the overall cycle time is the sum of the numbers of single steps: filling, closing, pressure build up, pressure holding, pressure releasing, opening and taking out. The temperature increases with pressurization due to adiabatic heating. In the pressurization stage, the pressure is built up so that it reaches the targeted pressure level. At this time, the temperature will increase. During pressure holding time, the pressure and temperature is nearly constant with only slight changes due to heat loss to environment.

Lastly, at depressurization stage, the pressure is released and the temperature rises occurred during pressurization will returns to its initial temperature.

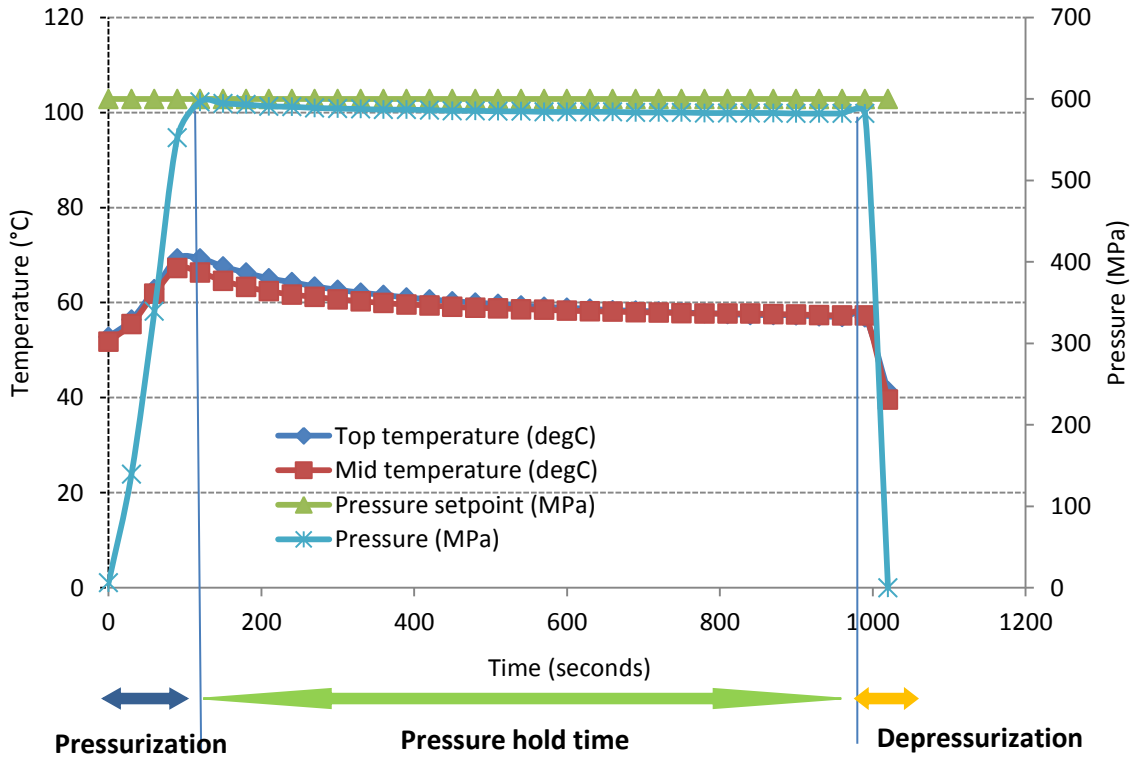


Figure 2.8: Typical plot of pressure, temperature and time during high pressure processing of honey using QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Kent, WA, USA). The setting temperature and pressure is 50°C and 600 MPa, respectively.

2.6.3 High Pressure Processing Equipment

The key components of a high-pressure system consist of pressure vessel and pressure-generating device (Figure 2.9). Pressure vessel, in which products under treatment are subjected to pressure (also known as the heart of the HPP system), is a forged monolithic, cylindrical vessel constructed in low alloy steel of high tensile strength. The primary components HPP consists of (i) pressure vessel (ii) closure (s) for sealing the vessel (iii) a device for holding the closure (s) in place while the vessel is under pressure (iv) high pressure intensifier pumps (v) system for controlling and monitoring the pressure and (optionally) temperature and (vi) product handling system for transferring product to and from the pressure vessel.

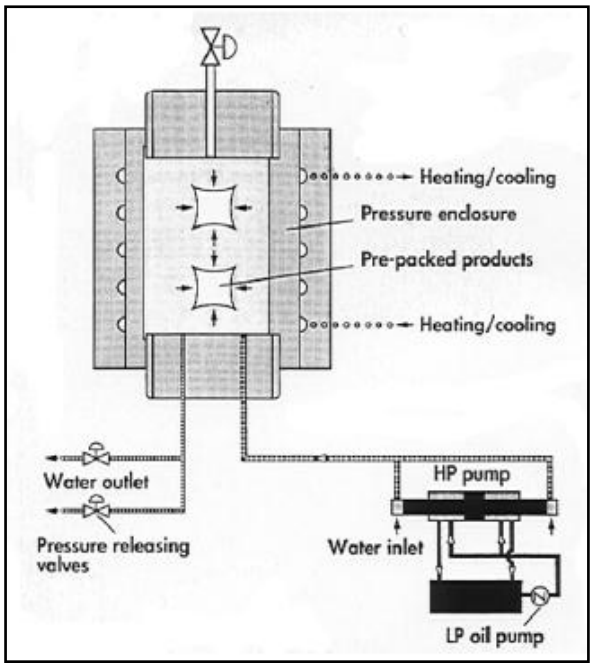


Figure 2.9: A typical high pressure processing system for treating food (Food and Agriculture Organization of the United Nation, (http://www.fao.org/ag/ags/agsi/Npnthermal/nonthermal1.htm).

Two types of pressurization systems defined as indirect and, direct are commonly employed in the industry:

(i) Direct pressurization system- is a piston-type compression where the pressure intensifier is located within the pressure vessel. In this system, both the pressure intensifier and the vessel are fabricated as a single unit, and the total size of the vessel can be quite large. A piston is used to deliver the high pressure to the product. This system requires heavy-duty seals that must withstand repeated opening and closure without leakage. A major limitation of this method is the need for efficient seals between the pressure vessel and the piston.

(ii) Indirect pressurization system- the pressurizing medium (e.g., water) is first pumped through an intensifier, into the pressure vessel. Pressure intensifier force additional water into the container, due to the relative "incompressibility" of water, pressure inside the vessel rises (Heinz et al., 2009). The intensifier is separate from the high-pressure vessel. This system requires high-pressure tubing and appropriate fittings to convey the pressurized medium to the pressure vessel (Mertens, 1995). Pressure increase time (time needed to build up the full pressure inside the vessel) of indirect pressurization is longer compared to direct compression via the piston and depends on pressure intensity, vessel volume and

the number of build-in-intensfiers (Heinz et al., 2009). Most of the industrial cold, warm and hot isostatic pressing systems use the indirect pressurization method

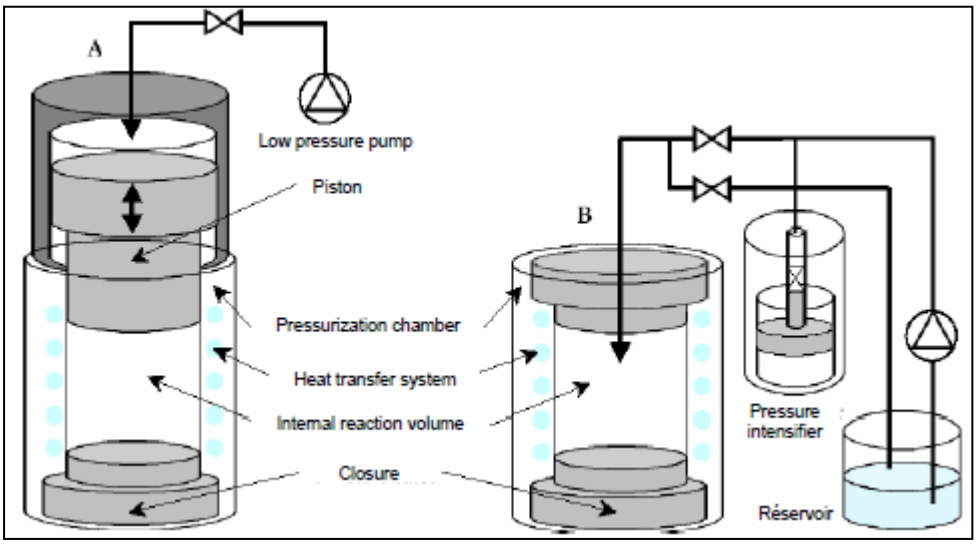


Figure 2.10: (A) Direct system for generation of high isostatic pressure and (B) indirect system. Adopted from Urrutia-Benet (2005).

To operate HPP, the product was first packaged in a flexible container and then loaded into a high pressure chamber which is filled with hydraulic fluid. This fluid in the chamber is pressurized with a pump and consequently this pressure is transmitted through the package into the food. HPP are subjected to ultrahigh hydrostatic pressure which is generally in the range of 100-1000 MPa at adjusted treatment temperature from below 0°C to above 100°C with exposure time ranging from a few seconds to over 20 min (Yaldagard, 2008).

For this PhD project programme, the research equipment available is the QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Kent, WA, USA), which is located in the Food Processing Technology laboratory, Department of Chemical and Materials Engineering, University of Auckland (Figure 2.11). The equipment is a conventional batch system, where the summation of the filling, closing, pressure built up, pressure holding, pressure releasing, opening and taking out is considered as overall cycle. The equipment consists of a 2- litre cylindrical-shaped pressure treatment chamber (inner height = 0.25 m, inner diameter = 0.10 m) with a thermocouple, water circulation, a cooling system, a pumping system and a control system operated through a computer with a software supplied by the manufacturer. Distilled water was used as the medium in the chamber where the packed honey samples were placed. The equipment can operate at maximum pressure and

temperature of 600 MPa and 90°C, respectively. The treatment time was the holding pressure time and did not include the pressure come up and decompression times. The temperature inside the pressure chamber is monitored during treatment using a thermocouple, which was immersed in the pressure medium (distilled water).

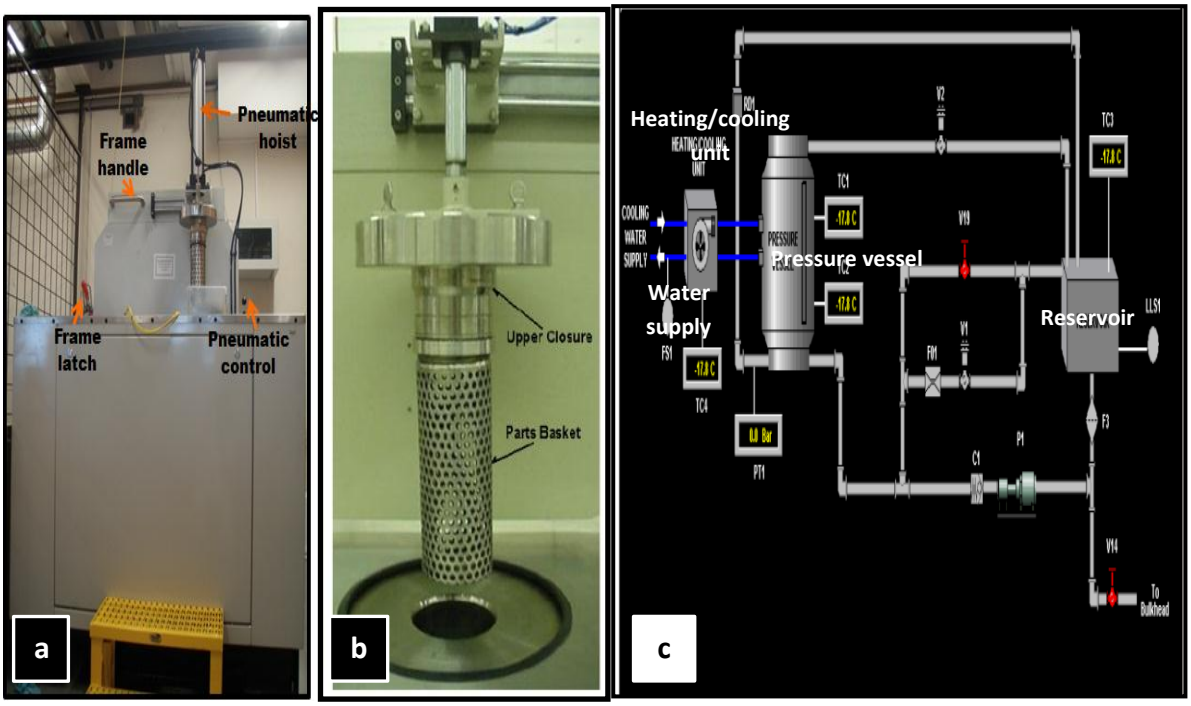


Figure 2.11: (a) QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Kent, WA, USA), (b) 2-litre pressure vessel and (c) schematic diagram of the system.

2.6.4 Impact of high pressure on food quality

High pressure offers a technology that can achieve the food safety properties of heat-treated foods while meeting consumer demand for fresher tasting food products. Unlike heating, HPP treatment generally does not change the odour, flavour or other sensory characteristics of foods and could also preserve nutritional value. This is due to its limited effects on covalent bonds of low molecular-mass compounds resulting in minimal modifications in nutritional and sensory quality. During HPP processing which is normally operates at condition of 100-1000 MPa/ - 20°C to 60°C, (i) cell wall and membrane disruption (Michel and Autio, 2001); (ii) enzyme catalysed conversion processes (Ludikhuyze et al., 2000;Verlent et al., 2005); (iii) chemical reactions (Indrawati et al., 2004; Nguyen et al., 2006; Oey et al., 2006); (iv)

modification of enzyme inactivation, protein denaturation and gel formation (Balny et al., 2002; Ludikhuyze et al., 2000) can occur at the same time.

The maintenance of naturally coloured pigments in foods is a major challenge. Colour relates directly to consumer perception of appearance and therefore has to be within an expected range for consumer acceptance. HPP at low or moderate temperatures has limited effects on colours pigment such as chlorophyll, carotenoids and anthocyanins. In fact, the green colours in most vegetables are more intense (decrease in L^*, a^* and b^* values) due to cell disruption during HPP, which then resulting in the leakage of chlorophyll into intercellular space yielding more intense bright green colour on the vegetable surface. Whereas, carotenoids (orange-yellow and red appearance) and anthocyanins (flavonoid pigments responsible for the red to blue colour) are rather pressure stable.

Knowledge of the rheological and/or textural properties of foods is essential for product development, quality control, sensory evaluation and design and evaluation of process equipment (Ahmed et al., 2003). Various types of food resulted in different HPP-effect on their physical structure. For most high-moisture products, their physical structure remains unchanged after exposed to HPP, since no shear force are generated by the pressure. Whereas, for gas-containing products treated under HPP, the texture may be changed due to gas displacement and liquid infiltration. For food not containing air voids, HPP frequently result in minimal or no permanent change in textural characteristics (Ting and Marshall, 2002). Ahmed et al (2005) and Verlent et al (2006) have showed that HPP can affect the rheological properties of mango pulp and tomato homogenate, respectively.

Flavour is the sensory impression of a food that is determined mainly by the chemical sense of taste and smell. Since the structure of small molecular flavour compounds is not directly affected by high pressure, the fresh flavour of fruits and vegetables is assumed not altered by HPP. However, HPP could indirectly alter the content of some flavour compounds and disturb the whole balance of flavour composition since HPP can enhance and retard enzymatic and chemical reactions, as has been observed in a number of studies (Garcia et al., 2001; Lambert et al., 1999; Takahashi et al., 1993; Yen and Lin, 1996). Therefore, HPP could result in undesired changes in flavour.

Past attempts to investigate the effect of HPP on the various quality attributes were carried out and well established. The application of HPP and its effect on quality attributes in various types of foods is summarized and presented in Table 2.6.

Table 2.6: Application of high pressure processing and its effects on the qualities of various types of food

Quality attributes	Product	Pressure (MPa)	Holding time (min)	Temp (°C)	Remarks	References
Colour	Fruit jam	400	5	ambient temp	Colour to be superior to conventionally treated jam in brightness (*L*-value) and redness (*a*-value)	Watanabe et al., 1991
	Guava puree	600	15	25	The original colour is retained	Yen a nd Lin, 1996
	Avocado puree	345, 689	10 to 30	21	HPP-treated puree had a colour equivalent to the freshly prepared puree.	Lopez-Malo et al., 1998
	Banana puree	689	10	21	HPP can preserve the original colour of banana puree	Palou et al., 1999b
	Broccoli juice	200,500,700, 800	5	30-80	Degradation of chlorophyll (green colour) to pheophytin (gray colour)	Van Loey et al., 1998; Weemaes et al., 1999
	Persimmon fruit puree	150, 300, 400	15	25	Increase the extraction yields of carotenes	De Ancos et al., 2000
	Tomato puree	600	60	20	Increase the extraction yields of carotenes	Garcia et al., 2001
	Strawberry jam	400	5	25	Colour was found to be affected by increasing peptin concentration	Dervisi et al., 2001
	Green beans	500	1	ambient	Decrease in *L**, *a** and *b**	Krebbers et al., 2002
	Broccoli juice	600	10-40	25 &75	Significant reduction in chlorophylls	Butz et al., 2002
	Red raspberry and strawberry	800	15	18-22	Anthocyanins are stable during HPP at moderate temperature	Garcia-Palazon et al., 2004
	Green beans and spinach	700	1	90	Significant reduction in chlorophylls	Matser et al., 2004

	Tomato puree	700	60	65	The colour remain unchanged	Rodrigo et al., 2007
Nutritive value	Mandarin orange juice	600	5	20, 47 & 57	The activity of pectin esterase was retained (45,32 and 19 %)	Takahashi et al., 1993
	Potato cubes	400	15	5-50	Retention of ascorbic acid in pressure-treated samples was temperature dependent ranging from = 90% at 5°C to = 35% at 50°C.	Eshtiaghi and Knorr, 1993
	Strawberry jam	400 - 500	10 to 30		About 95% of the vitamin C of fresh strawberry was preserved in pressurized strawberry jam	Kimura et al., 1994
	Orange juice	200 to 500	1	30	Several vitamins, sugars and organic acids found no substantial modification at any of the pressure levels tested	Donsi et al., 1996
	Mushrooms and potatoes	100-800	20,40,60	1-20	The enzyme in potatoes steadily lost activity with increasing applied pressure (10 min at 800 MPa) about 40% of the activity remained. The mushroom extract exhibited a marked increase in activity (400 MPa ,10 min), considerable activity remained (10 min at 800 MPa)	Gomes and Ledward, 1996
	Beet and tomatoes	600, 800	10	50, 35	Antimutagenic activities of beet and tomatoes were affected by extreme pressure only	Butz et al., 1997a
	Egg yolk	400 to 1000	30	20	No variation in Vitamin C content	Sancho et al., 1999
	Milk	600	3	60	HPP-thermal induced 50% loss of active substance, while the non-sweet aspartylphenylalanine and diketopiperazine formed (using aspartame solution of 0.5 g/L)	Butz et al., 1997b

	Pork meat	125 - 600	10 min to 18 hrs	20	Thiamin was not degraded at 125 -600 MPa for 10 min -18 hours at 20°C	Sancho et al., 1999
	Tomato puree	Up to 700	30	Up to 90	HPP caused partial inactivation of polygalacturonase (70%), but activation of pectin methylesterase. HPP improved the colour and viscosity	Krebbers et al., 2003
	Soybean glycinin	0-600	0-35		glycinin had been denatured completely after processed at 400 MPa for 10 min.	Zhang et al., 2005
	Raspberry	200,400,600, 800	15	18-22	The highest stability of the anthocyanins was found when raspberries were pressured under 200 and 800 MPa and stored at 4°C.	Suthanthangjai et al., 2005
Texture	Spinach and cauliflower	400	30	5	Disruption of cell integrity was observed. HPP affects the organization of the parenchyma cells	Pre´stamo and Arroyo, 1998
	Apple, pear, orange, pineapple, carrot, celery, green&red pepper	100-400	5 - 60 min	20	Treatment at low pressure indicated considerable baro-resistance to texture loss. Pressure induced firming of texture with all samples at 100 MPa for a period 30-60 min	Basak and Ramaswamy, 1998
	Tomato juices	500,700, 900	3, 6, 9	Ambient temp	High pressure affected the viscosity of tomato juices and gave rise to jelly like, translucent structure caused by protein-tissue coagulation and compacting	Poretta et al., 1995
	Guava puree	600	15	25	The turbidity, viscosity and cloud content were not significantly affected	Yen and Lin, 1996
	Cherry tomatoes	200-600	20	20	Increased texture damaged (200-400 MPa/ 20min) but pressure of 500 and 600 MPa led to less apparent damage.	Tangwongchai, Ledward and Ames, 2000

	Green beans	1000	80	75	Texture preservation during HPP at elevated temperatures	Krebbers et al., 2002
	Tomato puree	500		60	The viscosity increased	Plaza et al., 2003
	Mango pulp	100, 200,300, 400	15, 30	20	Viscosity increased (100@200 MPa/ 15 @ 30min) while reduction in viscosity was observed at 300 & 400 MPa/ 15 @ 30 min	Ahmed et al., 2005
	Navel orange juice	600	4	40	Pressure treatment results in higher viscosity	Polydera et al., 2005
	Carrot disks	600	90	80	Texture preservation during HPP at elevated temperatures	De Roeck et al., 2008
Flavour	Mandarin juice	400	10	ambient temp	The typical off-flavour of heat-treated mandarin juice was not detected in HPP-treated	Takahashi et al., 1993
	Fresh tomato juices	500,700, 900	3,6,9	ambient temp	HPP-treated sample resulted in the generation of such a strong rancid taste (n-hexanal)	Poretta et al., 1995
	Strawberry puree	800	20	ambient temp	Less pronounced effect on the hexanal content, modified the flavour profile	Lambert et al., 1999
	Strawberry jam	400/ 800	5	22	HPP-treated jam smelled more chemical, rancid and less fruity	Gimenez et al., 2001
	Carrot juice	800	5	ambient temp	The carrot aroma was more intense in HP-treated than in fresh orange-lemon-carrot juice	Garcia et al., 2001
	Orange juice	600	60	18-20	No difference in the concentration of flavour compounds between freshly frozen, heat treated and HPP-treated	Baxter et al., 2005

2.6.5 Inactivation of microorganisms under pressure

The objective of food preservation is not only for quality retention and improvement, but also for the inactivation of spoilage micro-organisms in order to improve shelf-life of the food. Growth of microorganisms in foods can cause spoilage by producing unacceptable changes in taste, odour, appearance and texture.

Process temperature and pressure are not the only process parameters that influences inactivation. Many varieties of microorganisms with different physiological properties may have different pressure-resistant characteristics. The effect of high pressure on microorganisms can be categorized primarily as (Huang et al., 2014):

(i) Change to the cell morphology

Increase of pressure in the cell environment disrupts cells permeability, which is followed by the loss of the membrane integrity and swelling. This eventually leads to cell death. High pressure causes varying levels of damage to microorganism morphology (Furukawa et al., 2003; Huang et al., 2014; Yang et al., 2012).

(ii) Physiological function

When microorganism are affected by high pressure, the cell membrane will damage, the microorganism's absorption of nutrition is affected, elimination of the waste accumulated inside the cell is hindered, and normal metabolic pathway are disrupted (Torres and Velazquez, 2005). Some of the microorganisms' membrane protein had deteriorated (Ritz et al., 2000). Irreversible denaturation of key proteins or the inhibition of protein repairs by high pressure will also causes cellular death (Simpson and Gilmour, 1997).

(iii) Genetic mechanism

High pressure inhibits the activity of DNA replication and enzymes transcription which negatively affect the functionality of genetic materials in microorganisms (Alpas et al., 1999; Kaletunc et al., 2004)

There are the factors which affect the pressure-resistant characteristics of microorganisms:

(i) Microorganism variation

Gram - positive bacteria showed more pressure resistance as compared with gram- negative bacteria. Gram- positive bacteria have a thicker peptidoglycan (about 40 layers) than gram-negative (1 up to 5 layers) and this contributes to the greater structural resistance to mechanical breakage in the former (Madigan et al., 2000). Bacteria of small size and coccid shape are also known to be more pressure resistant to HPP than large rod-shaped one. Whereas, the structure and the thickness of the bacterial spores coat would cause the bacterial spores cannot be inactivated by high pressure alone. It can survive at pressure treatments above 1000MPa unless pressurization is carried out at temperatures close to 100°C (Reddy et al., 2003).

(ii) Growth stage

Microorganism in the stationary stage possesses higher pressure resistance than in exponential phase due to complete cell structures and they are protected by cell membranes (Patterson, 2005). It is also partly due to the synthesis of proteins that protect against a range of adverse condition such as high salt concentrations, elevated temperatures and oxidative stress (Hill et al., 2002). Whereas, microorganism in exponential phase undergo continuous cell division and synthesis, and the stress tolerance of cells in an adverse environment is lower.

(iii) Environmental condition

Bacterial cells are more resistant at neutral pH, with pressure resistance decrease when the pH is either increased or decreased. Whereas, a reduction of bacterial spores in high acid foods can be achieved by moderate heating for a longer time instead of high temperature heating (Vercammen et al., 2012). For ensuring the elimination of spore-forming bacteria, the application of heat combined with high pressure can be more practical (Juhee and Balasubramaniam, 2007; Ramaswamy et al., 2013; Shoa and Ramaswamy, 2011). Besides, the composition of foods such as proteins, sugars and lipids can provide a protective effect, reduce sensitivity of microorganism to high

pressure deactivation, and increase their resistance to pressure (Jordan et al., 2001; Raso et al., 1998). Previous studies also revealed that solid foods provide greater protection for microbial strains than liquid food (Smiddy et al., 2005; Tassou et al., 2007).

(iv) Baroprotective effect

Baroprotective effect of increased solute concentration could be attributed to the lower water activity (Palou et al., 1997), high concentration of sucrose (Glaasker et al., 1998) and membrane lipids and proteins (Crowe et al., 1997; Goh et al., 2005; Iwahashi et al., 1997; Lesli et al., 1995). Pressure resistance of yeasts and moulds increased as sugar concentration (sucrose, fructose, glucose) in media increased (Hashizume et al., 1995; Ogawa et al., 1990; Oxen and Knorr, 1993; Palou et al., 1998).

(v) Different compression method

Increasing pressure or processing pogawaeriods results in increased inhibition results. To inhibit endospores activity, an excess of 600 MPa combined with an appropriate temperature must be used. Combining extremely high pressure and temperature is a method to kill bacterial endospores (Heinz and Buckow, 2010; San martin et al., 2002).

(vi) Compressibility

The solute concentration, ° Brix under pressure is related to the compressibility (Min et al., 2010), where increasing solute concentration significantly decrease its compressibility. They also showed that increasing pressure level significantly decreased compressibility which is due to the loss of secondary and tertiary structure, as well as effect on the hydrogen bonding and carbohydrate composition (Cano and de Ancos, 2005; Li and Akasaka, 2006). As pressure increase, free volume decreases and compressibility becomes governed by the molecule's inherent compressibility and attractive and repulsive interactions with nearby molecules. Min et al. (2010) concluded that variability in compressibility of different materials is probably due difference in concentration, chemical composition and complex interaction between components within a food system.

2.7 Advantages and Limitation of HPP

2.7.1 Advantages

Although high pressure technology is more expensive than traditional processing technology such as thermal processing, the use of high pressure offers new opportunities for food industry to respond consumer's wishes. The merits of HPP as food preservation technology mainly lie in safety, quality and consumer friendliness.

The major advantages of pressure processing food is the absence of severe heating and it does not break covalent bond, hence eliminate undesirable effects that are produced at high temperatures such as texture defects, off-flavours, nutrient destruction and colour changes (Balogh et al., 2004). HPP has merit of reduction in microbial load where the vegetative cells in foods can be fully inactivated since it requires low pressure levels, around 400-600 MPa (Patterson et al., 1995). The most unique property of HPP is its ability to be transfer pressure effect instantly and uniformly throughout food system without any particles escaping the treatment, proving that the application of HPP is independent of size and shape of the foods.

In summary, the advantages of HPP can be pointed as (Buckow, 2013):

(i) *Consumers:* HPP is a consumer acceptable, 'environmental friendly', scientifically recognised method to achieve higher quality in certain foods

(ii) *Processing:* Pressure transmission is instantaneous and uniform (not heat transfer controlled, no 'shadow', depth, or uneven distribution effect) - rapid, short processing times, assured safety in whole pack, suitable for solids and liquids

(iii) *Quality:* Better retains flavour than thermal processing

(iv) *Environmentally:* safe and no process by-products, no emissions

(v) *Opportunities:* HPP can modify food biopolymers (examples: starch and proteins) which allows targeted texture modification without the need of heat.

2.7.2 Limitation

Due to the effectiveness of HPP in retaining and improving foods quality, the use of HPP has the potential to address the requirements of modern consumers to prefer "minimally preserved" foods. However, HPP could also associated with some demerits when deals with structurally fragile foods (high in air content foods). HPP can cause cell-deformation and cell membrane damage which cause undesirable changes, which makes the treated product no longer fresh. Hence, a careful choice on food products is necessary before using HPP as a processing step (Farkas and Hoover, 2000).

With regards to the effect of HPP on the microorganism, some of bacteria spores are very resistant to pressure and require very high pressure for their inactivation. Extreme conditions of pressure (more than 1000 MPa) and temperature and longer treatment time are required to ensure the spores are inactivated (Patterson et al., 1995; Sale et al., 1970). Also, the residual enzyme activity and dissolved oxygen results in enzymatic and oxidative degradation of certain food components. The other limitation is that; most of the pressure-processed foods need low temperature storage and distribution to retain their sensory and nutritional qualities.

Besides, HPP system consist of high pressure vessel, a means to close off, a system for temperature and pressure control and a material handling system, so, machinery is complex and requires extremely high precision in its construction, use and maintenance. Although HPP shows promise in its ultimate usefulness for food processing, limitation with respect to difficulty in data comparison and complexity associated with understanding interactive components of the process currently limit full acceptance of the practice.

Overall, HPP is not likely to replace all traditional processing methods, but it may complement or find niche applications and the novel physicochemical and sensory properties obtained from this technology offer exciting opportunities for industry (Heinz and Buckow, 2010).

CHAPTER 3

High Pressure Processing (HPP) of Honey for the Improvement of Nutritional Value

Akhmazillah, M. F. N., M. M. Farid, Filipa F.V.M (2013). Innovative Food Science & Emerging Technologies 20(0): 59-63.

Chapter Abstract

The present study was undertaken to assess the effect of high pressure processing (HPP) on total phenolic content (TPC) in Manuka honey. Manuka honey is known for its amazing antimicrobial action and antioxidant properties. The effect of HPP (200, 400 and 600 MPa) at ambient and moderate temperatures (53.41 ± 0.30°C, 65.29 ± 1.77°C, 71.92 ± 1.63°C) and their combination for different processing time (5, 10 and 15 min) was investigated. Conventional thermal processing (51.74 ± 0.03°C, 61.90 ± 0.10°C and 71.58 ± 0.04°C) was also carried out as comparison to HPP. Operating HPP at 600 MPa (26.80 ± 0.95°C–30.18 ± 2.14°C) for 10 min was found to be the most effective process with 47.16% increment in TPC as compared with unprocessed honey, whereas no significant increase ($p < 0.05$) was observed in thermal processing as well as in combined HPP–thermal processing. Therefore, HPP at ambient temperatures could be an appropriate method to produce tastier and more nutritive Manuka honey.

3.1 Introduction

Honey is the world's primary sweetener and nature's original sweetener prepared by honey bees. Honey has been used as a food for at least 6000 years. Honey evolved from the nectar, being a very popular natural sweetener consisting of glucose and fructose. The honey is flower nectar which has been collected, regurgitated and dehydrated by honey bees to enhance its nutritional properties making it ready for human consumption. The consumption of honey is on the rise due to the unique combination of components in honey which makes it a prized addition to the diet. According to the Food and Agriculture Organization of the United Nations (FAO), total honey production in 1961 was 0.7 million tons and it was steadily increased to about 1.5 million tons in 2009 (Alvarez-Suarez et al., 2010).

The demand for high quality honey is attracting a lot of attention as it provides health benefits (Alvarez-Suarez et al., 2010) and has been shown to possess antimicrobial, antiviral, antiparasitic, anti-inflammatory, antioxidant, antimutagenic and antitumor effects (Bogdanov et al., 2008). Diseases prevention through consumption of honey is probably due to the presence of more than 181 substances with a wide range of health promoting phytochemicals, some with antioxidant properties. Antioxidants, which are abundant in natural honey, are free-radical scavengers that either reduce or neutralize the formation of free radicals. Phenolics are the components in honey which are responsible for its antioxidative effects. The importance of these honey bioactive compounds and antioxidants on human health therefore generates a great interest in honey processing research.

Honey antioxidant compounds include phenolic acids (Aljadi and Kamaruddin, 2004), flavonoids (Socha et al., 2011) and amino acids (Barbosa-Canovas et al., 1998). Phenolic compounds specifically gallic, p-coumaric, ferulic, syringic, caffeic, synaptic and chlorogenic acids are reported as main antioxidants in honey (Aljadi and Kamaruddin, 2004). Honey with highest amount of phenolic compounds will possess the best radical scavenging activity (Meda et al., 2005; Wang et al., 2004). It is also known that phenolic compounds boost antioxidant activity and there is a strong correlation between antioxidant capacity and total phenolic content (TPC) in honey (Cao et al., 2011; Wang et al., 2004).

High pressure processing (HPP) is a non-thermal treatment which has positive effect on product quality and has capability to inactivate microorganisms in various food matrices (Butz, 2010; Nguyen and Balasubramaniam, 2011). As an alternative to classical thermal processing, HPP has potential to produce high quality foods with ‘fresh-like’ characteristics and improved functionalities (Patras et al., 2009a; Patras et al., 2009b). Although detailed

studies on the effects of HPP on microbial and enzymatic inactivation were widely reported in the literature, the effects of pressure on individual aspects of food quality are much less documented. As there is an increasing demand for better quality and safe foods, the study of innovative high pressure processing is currently one of the most interesting researches in non-thermal food preservation. Due to the almost instantaneous isostatic pressure transmission (Abdul Ghani and Farid, 2007), HPP- treated food has been shown to keep its original freshness with minimal changes in flavor, taste and colour (Butz, 2010).

Past attempts to investigate the effect of HPP on TPC were carried out with orange juice (Concepción et al., 2005), vegetables (McInerney et al., 2007), grape by-products (Corrales et al., 2008), strawberry purees and blackberries purees (Patras et al., 2009a), fruit smoothies (Keenan et al., 2010), apple puree products (Landl et al., 2010), strawberry pulps (Cao et al., 2011) and aloe vera (Vega-Gálvez et al., 2012). Pressures around 400 to 600 MPa and temperatures in between 10°C to 70°C were applied in these studies. Most of the works revealed that the TPC increased for HPP-treated samples. However, there has been no research on the potential application of HPP to obtain higher quality in high sugar content foods particularly honey, dealing with bioactive compounds and antioxidant activity.

Therefore, the aim of this study was to investigate the changes in TPC in HPP-treated Manuka honey as compared to conventional thermal process. The combined effect of pressure and temperature will be also investigated.

3.2. Material and methods

3.2.1. Honey samples preparation

The fresh and unprocessed Manuka honey used in this study (pH of 4.3 ± 0.2, 79 ± 0.3 ° Brix and water content of 16.46 ±1.4%) was kindly donated by Comvita®, Auckland, New Zealand. The jar of honey sample was collected directly from beekeepers and sourced from Manuka tree (*Leptospermum scoparium*), a native of New Zealand.

Honey (5 g) was packed in 5 cm × 5 cm transparent plastic film pouches (Cas-Pak plastic vacuum pouch, Silverdale, New Zealand) and thermosealed under vacuum after manually stirred. A very thin pouch of 3 mm is deliberately used so that honeys temperature approaches the surrounding water temperature in short time. The plastic film is made of cast polypropylene for excellent transparency and heat sealing qualities and can withstand temperatures up to 125°C.

3.2.2. High pressure processing (HPP) equipment

The HPP unit used in this research was QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Kent, WA, USA). The equipment consists of a 2- litre cylindrical-shaped pressure treatment chamber (inner height = 0.25 m, inner diameter = 0.10 m) with a thermocouple, water circulation, a pumping system and a control system operated through a computer with software supplied by the manufacturer. Distilled water was used as the medium in the chamber where the packed honey samples were placed. The equipment can operate at maximum pressure and temperature of 690 MPa and 90°C, respectively. The treatment time was the pressure holding time and did not include the pressure come up and the decompression times. The temperature inside the pressure chamber during treatment was monitored using thermocouples (located at mid and top of the vessel), which were immersed in the pressure medium (distilled water). The compression time, decompression time and average temperature were based on the cycle report which is directly obtained from a control system operated through a computer with software. After processing, the packed honey samples were immediately cooled in ice water before analysed.

3.2.2.1. HPP of honey

Triplicates of 5 g vacuum-packed honey samples were subjected to different conditions of HPP (pressure, time) and HPP–thermal (pressure, temperature, time). The treatment time was the holding pressure time and did not include the pressure come up and the decompression times. The temperature of pressure medium (distilled water) during pressure treatment was measured using a thermocouple. After treatment, the samples were immediately cooled in ice water. All honey samples were taken from the same honey batch and every single treatment was repeated for three times.

3.2.2.2 Ambient temperature HPP

Manuka honey samples were subjected to HPP with pressures of 200 MPa, 400 MPa and 600 MPa, at close to ambient temperature for 5, 10 and 15 min. The adiabatic heating of 200 MPa, 400 MPa and 600 MPa gave an average processing temperature of 26.80 ± 0.95°C, 28.71 ± 0.90°C and 30.18 ± 2.14°C, respectively during the holding pressure phase. Pressure come up times were approximately 1.5 min to reach the desired pressure and the decompression time was < 20 s.

3.2.2.3. Combined HPP–thermal processing of honey

Manuka honey samples were enclosed in the pressure chamber, preset at the desired pressure of 600 MPa for 5, 10 and 15 min. The pressure increase up to the desired pressure took less than 2 min. Initial temperature settings of 50°C, 60°C and 70°C for 600 MPa resulted in the average processing temperature of 53.41 ± 0.30°C, 65.29 ± 1.77°C and 71.92 ± 1.63°C during holding pressure phase.

3.2.3. Thermal processing of honey

The thermal processing in the absence of high pressure was performed at 50°C, 60°C and 70°C for 5, 10, 15 and 30 min using a thermostatic water bath, W28 (Grant Instruments, Cambridge, England). For the treatment, the vacuum-sealed samples were fully submerged into the distilled water bath. A setting temperature of 50°C, 60°C and 70°C resulted in the average temperature of 51.74 ± 0.03°C, 61.90 ± 0.10°C and 71.58 ± 0.04°C with the heat up time of 0.7 min, 1.2 min and 1.5 min respectively. These actual temperatures were obtained by measuring the thermometer readings (which is located in the centre of water bath) during process. It is very reasonable to assume that honey samples temperature is very close to the

measured surrounding water temperature due to the thin pouches used as discussed before. After each treatment, all samples were immediately placed in ice-cooled water before analysis. All honey samples were taken from the same honey batch and every single treatment was repeated for three times.

3.2.4. Total phenolic content (TPC) determination

The total phenolic content in samples was determined using the Folin–Ciocalteu method as adapted by previous work (Singleton et al., 1999). 5 g of each honey samples was diluted to 50 mL with distilled water and filtered through Whatman No. 1 paper. The solution was then mixed with 2.5 mL of 0.2 N Folin– Ciocalteu reagents (Sigma-Aldrich Chemie, Steinheim, Germany) for 5 min and 2 mL of 75 mg/L sodium carbonate (Na_2CO_3) were then added. After incubation at room temperature for 2 hrs, the absorbance of the reaction mixture was measured at 760 nm against a methanol blank. Gallic acid (Sigma-Aldrich Chemie, Steinheim, Germany) was used as standard to produce the calibration curve. A good linearity of gallic acid was obtained ($R^2 = 0.997$) from 5–100 mg/L dissolved in methanol and distilled water (1:1). The honey samples were done in triplicate and the mean of three reading was used. The total phenolic content was expressed in mg of gallic acid equivalents (GAE)/100 g of honey.

3.2.5. Statistical analysis

Results were given as mean ± standard deviation of triplicate measurement. One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered significant at $p < 0.05$. All statistical analyses were performed with Microsoft Excell® 2010 (Microsoft©, Redmond, WA, USA).

3.3. Results and discussion

3.3.1. Total phenolic content (TPC) of unprocessed Manuka honey

Unprocessed Manuka honey TPC was 63.85 ± 0.90 mg GAE/100 g as presented in Table 1. This value was within the range of multifloral honey (Meda et al., 2005), however it was found to be higher com- pared to other types of honey (Ferreira and Aires, 2009; Socha et al., 2011). The differences of TPC observed in different type of floral honeys show that the phytochemical constituents are closely related to the plant or flower sources (Cherchi et al., 1994). The TPC also varies with the geographical location of honey's flower such as Malaysia, Burkina Faso, Turkey and Croatia (Aljadi and Kamaruddin, 2004; Küçük et al., 2007; Meda et al., 2005). This result emphasized the relevance of honey as a source of natural antioxidants and it has proven that New Zealand Manuka honey contains high content of phenolic compounds and antioxidants when compared with other types of honey.

Table 3.1: Total phenolic content from different types of honey obtained from previous works in comparison with this study. Values are mean ± standard deviation (n = 3).

Types of honey	**Total phenolic content (mg GAE/ 100g ± SD)**	**References**
Multifloral	32.59 ± 0.48 – 93.66 ± 0.44	Meda et al., 2005
Leadwood tree (*Combretaceae)*	52.08 ± 0.31 – 59.67 ± 1.35	
Anacardiaceae, *(Lannea)*	42.96 ± 0.63	
Lime	8.31 ± 0.23	Socha et al., 2011
Nectar-honeydew	6.78 ± 0.13	
Rape	4.46 ± 0.16	
Honeydew	6.08 ± 0.14	
Acacia	5.15 ± 0.15	
Buckwheat	15.04 ± 0.63	
Multiflower	6.92 ± 0.61	
Light honey	13.22 ± 0.05	Ferreira et al., 2009
Ambar honey	16.84 ± 1.99	
Dark honey	20.42 ±0.63	
Manuka honey	63.85 ± 0.90	From this study

3.3.2. High pressure processing (HPP) of Manuka honey

The effect of HPP close to ambient temperature (26.80 ± 0.95°C–30.18 ± 2.14°C) on TPC was investigated. The honey samples were subjected to 200, 400 and 600 MPa for different treatment time of 5, 10 and 15 min. The level of TPC in Manuka honey before and after HPP treatments are presented in Figure 3.1. In general, the result showed that HPP-treated honey has higher level of TPC as compared with unprocessed honey. The process condition of 600 MPa for 10 min showed significant increase in TPC (93.96 ± 1.0 mg GAE/100 g honey; $p < 0.05$) as compared with unprocessed honey (63.85 ± 0.9 mg GAE/100 g honey). The highest increment of about 47.16% was noted for this process condition. Similar effects of HPP on TPC have been well documented for aloe vera (Vega-Gálvez et al., 2012), fruit smoothies (Keenan et al., 2010), longan fruit pericarp (Prasad et al., 2009), strawberry and blackberry purees (Patras et al., 2009a) and tomato and carrot purees (Patras et al., 2009b). Meanwhile, Corrales et al. (2008) also have reported the increase in TPC of grape by-product following high pressure processing, ultrasonic as well as pulsed electric field. The increase of TPC in these food matrices is mostly attributed to the disintegration of cell membrane. The cell membranes and organelles are disrupted and enzymes are released from vacuoles which then affect the TPC and antioxidant activity in the samples (Keenan et al., 2010; Prasad et al., 2009). However, since there is no intact cell in honey, the possible reason might be due to the pollen. Proteins, polysaccharides, enzymes or other substances will release or leak out from the pollen (as might be affected by HPP) which subsequently increase the total phenolic content and antioxidant activity in honey. Further work is needed to confirm this.

For all treatment time, pressure treatment at 600 MPa exhibited significantly higher TPC (above 75 mg GAE/100 g honey, $p < 0.05$) as compared with 200 MPa (between 66.58 ± 0.8 to 72.04 ± 0.4 mg GAE/100 g honey) and 400 MPa (between 67.88 ± 0.6 to 70.5 ± 0.9 mg GAE/100 g honey). It is presumed that a higher increase in TPC at 600 MPa is due to the increase in activity of enzyme present in honey, such as amylase, a predominant enzyme in honey. High enzyme activity helps in extracting more antioxidant compounds and also phenolic compounds. Similar finding was observed for apple puree product when TPC was increased significantly ($p < 0.05$) at 600 MPa and remained unchanged at 400 MPa (5 min at 20°C) (Landl et al., 2010). Vega-Gálvez et al. (2012) reported a similar trend for aloe vera gel showing no significant difference in TPC among pressurized samples of 300 MPa–500 MPa (1, 3 and 5 min). The same result was reported for strawberry pulps after treated with HPP at ambient temperature for 25 min (Cao et al., 2011). Prasad et al. (2009) showed the

TPC was greatly influenced by high pressure treatment, particularly at 500 MPa (2.5 to 30 min at 30°C).

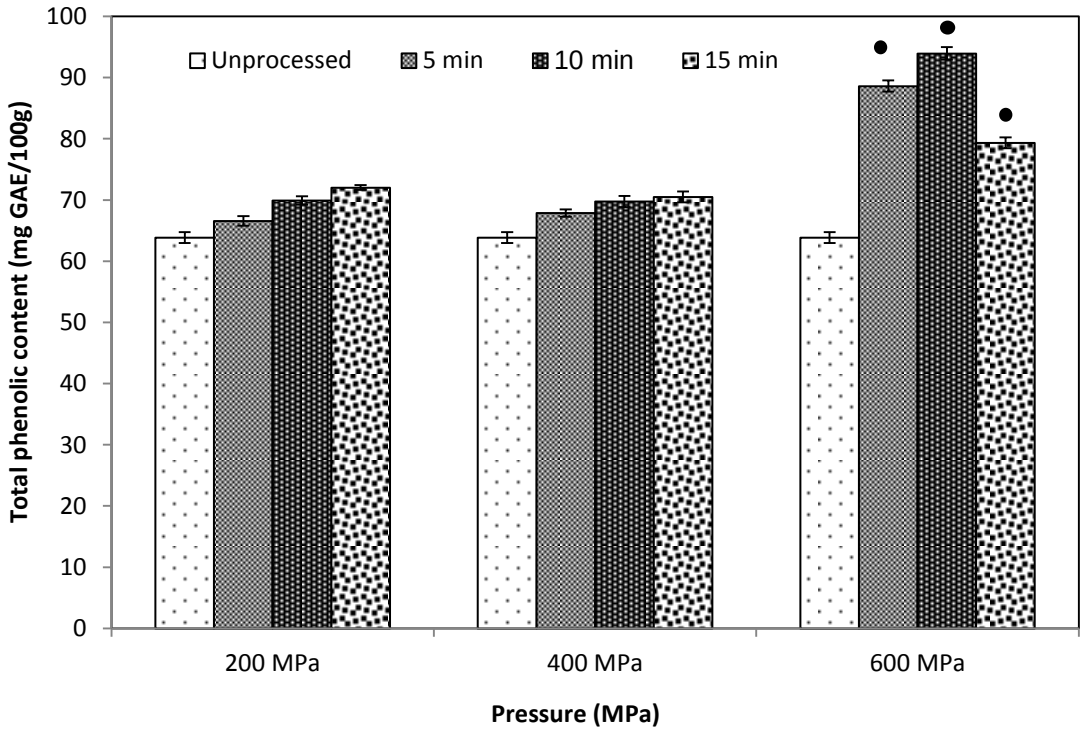


Figure 3.1: Total phenolic content of honey samples after treated with HPP at different pressure and time in comparison to unprocessed. Values are mean ± standard deviation (n = 3). Bullet on processed sample show significant difference ($^{\bullet}p < 0.05$) with respect to unprocessed honey.

The increment of TPC in various food matrices (Cao et al., 2011; Keenan et al., 2010; Landl et al., 2010; Patras et al., 2009; Prasad et al., 2009; Vega-Gálvez et al., 2012) could be attributed to increased extractability of some of antioxidant components, amino acids and protein with phenolic hydroxyl group during HPP (Cao et al., 2011; Keenan et al., 2010). HPP increased TPC and other antioxidant yields due to its ability to deprotonate charged groups and disrupt salt bridges and hydrophobic bonds in cell membrane which then leads to a higher permeability (Barbosa-Canovas et al., 1998).

The increase in apparent TPC is due to HPP possibly denaturing the protein component of conjugated protein-phenols which migh affect the total phenolic content. Phenolic compounds have hydrogen bond which can be affected and changed at very high pressure (Gould, 2001a; Yaldagard et al., 2008). Generally, in most food matrices high pressure can cause conformational changes and denaturation of proteins which results in an

exposure of hydrophic groups. This hydrophobic group may include phenols which then render the phenolic compounds more available (Barbosa-Canovas et al., 1998). It was also found that protein lose their tertiary structure when pressure of more than 300 MPa was applied (Hendrickx et al., 1998). In addition, HPP provides the possibility of inactivating degrading enzymes which may account for higher antioxidant activity (Prasad et al., 2009) and enhance the chemical and biochemical reactions by both desired and undesired modification (Oey et al., 2008).

3.3.3. Combined HPP and thermal processing of Manuka honey

Combined high pressure and mild thermal processing was carried out with the Manuka honey samples. At constant pressure of 600 MPa, the samples were processed for 5, 10 and 15 min at various temperatures as shown in Figure 3.2. The temperatures shown in Figure 3.2 were T_{avg} with standard deviation of the triplicate processes. HPP treatment at ambient temperature was found to have a significant effect ($p < 0.05$) on the increment of TPC in honey (ranging from 79.33 ± 0.9 to 93.96 ± 1.0 mg GAE/100 g). Meanwhile, combined HPP and thermal processing did not increase TPC ($p > 0.05$). The results were found to be in a similar range (between 65.07 ± 8.60 to 71.68 ± 1.06 mg GAE/100 g honey) for all temperatures tested. This phenomenon might be due to the degradation of phenolic compounds as the temperature increased to higher temperatures. The same pattern was reported for phenolic compounds in dried sage heated from 22°C to 40°C (Durling et al., 2007).

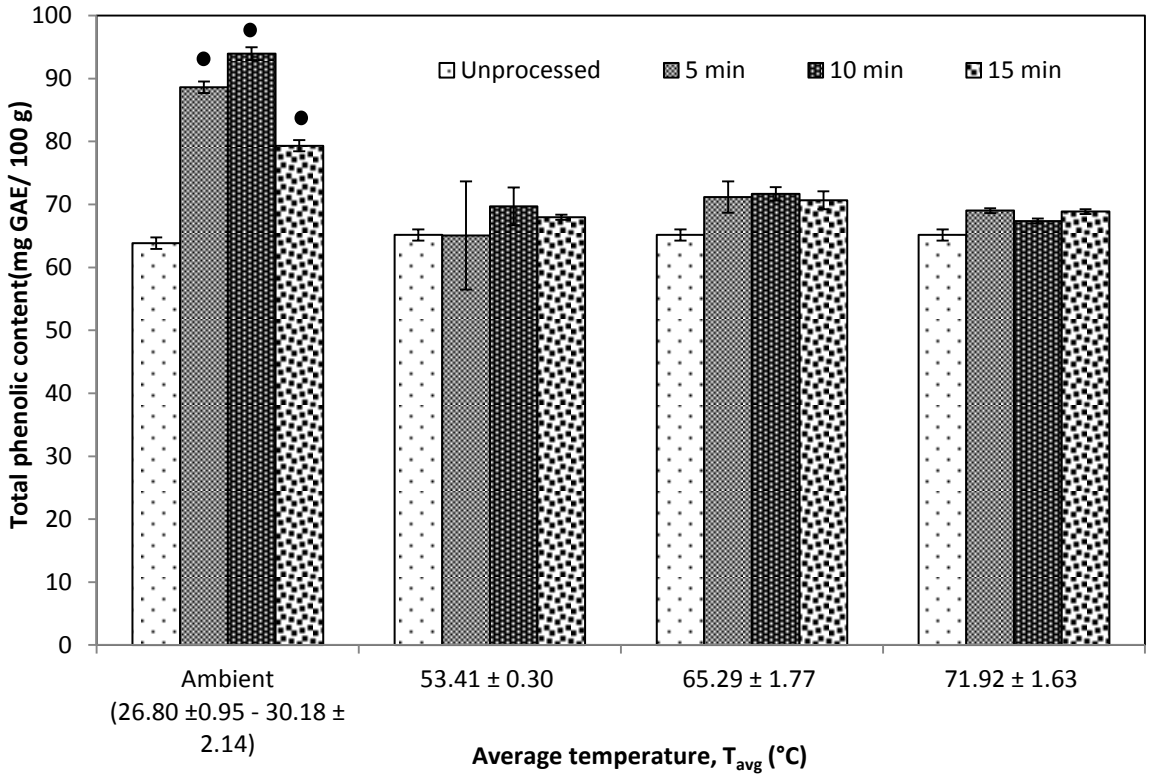


Figure 3.2: Total phenolic content of honey samples after treated with HPP at 600 MPa, for different temperature and time in comparison to unprocessed. Values are mean ± standard deviation (n = 3). Bullet on processed sample show significant difference ($^{\bullet}p < 0.05$) with respect to unprocessed honey.

Quantitative studies with regard to the effect of combined HPP and thermal processing on TPC in honey are lacking. However, previous research has been conducted on other phytochemicals in fruits and vegetables. Loey et al. (1998) and Weemaes et al. (1999) showed that the chlorophyll concentration in broccoli juice was not decreased by the application of high pressure up to 800 MPa combined with temperature of 30°C and 40°C, proving that HPP at ambient temperature can keep the original fresh green colour. When the temperature was increased to 50°C, there was only about 10% greenness loss. This finding can be attributed to the stability of the covalent structure of chlorophylls to high pressure, where the compressibility of covalent bond was negligible. For processing of tomato puree, combined HPP (700 MPa) and thermal processing (80°C and 90°C) showed no degradation effect on the lycopene content (Krebbers et al., 2003). They concluded that this treatment resulted in a quality improvement compared to a conventional sterilization process. Rodrigo et al. (2007) also revealed the same result on tomato puree and strawberry juice. No colour

degradation of tomato puree appeared under combined HPP (300–700 MPa) and thermal processing (65°C). For strawberry samples, maximum increase of 8.8% in *L* a*/b** parameter was found.

In conclusion, results demonstrated that although HPP at ambient temperature leads to an increment in TPC and antioxidant capacity in processed honey, no added benefit could be obtained in honey when combining HPP with thermal processing. This phenomenon could be explained by the ability of the hydroxyl molecules (OH), the functional group of phenolic compounds to react with water molecules. The solubility which is due to hydrogen bonding was enhanced by the application of high pressure whereas the hydration of charged group is loosened at high temperature (Mozhaev et al., 1996).

3.3.4. Thermal processing of Manuka honey

In these experiments, temperatures of 50°C, 60°C and 70°C were chosen considering the standard temperatures to which honey is exposed in industrial liquefaction and pasteurization are 45°C and 80°C, respectively (Escriche et al., 2009). The average temperatures registered throughout thermal treatments (5, 10, 15 and 30 min) were 51.74 ± 0.03°C, 61.90 ± 0.10°C and 71.58 ± 0.04°C (Figure 3.3).The results showed that TPC slightly increased from unprocessed samples without significant difference between the treatment temperatures ($p < 0.05$). Generally, longer treatment time had increased TPC of Manuka honey. For 30 min treatment, the increase was more noticeable in heated samples at 50°C (20.13% increment) and 60°C (19.30% increment) than 70°C (16.35%). The same finding was observed by Turkmen et al. (2006) showing some increase in antioxidant activity during prolonged heating process (50, 60 and 70°C for up to 12 days). The increase of TPC induced by thermal processing was possibly due to the extraction of phenolic compounds during thermal processing (Cao et al., 2011).

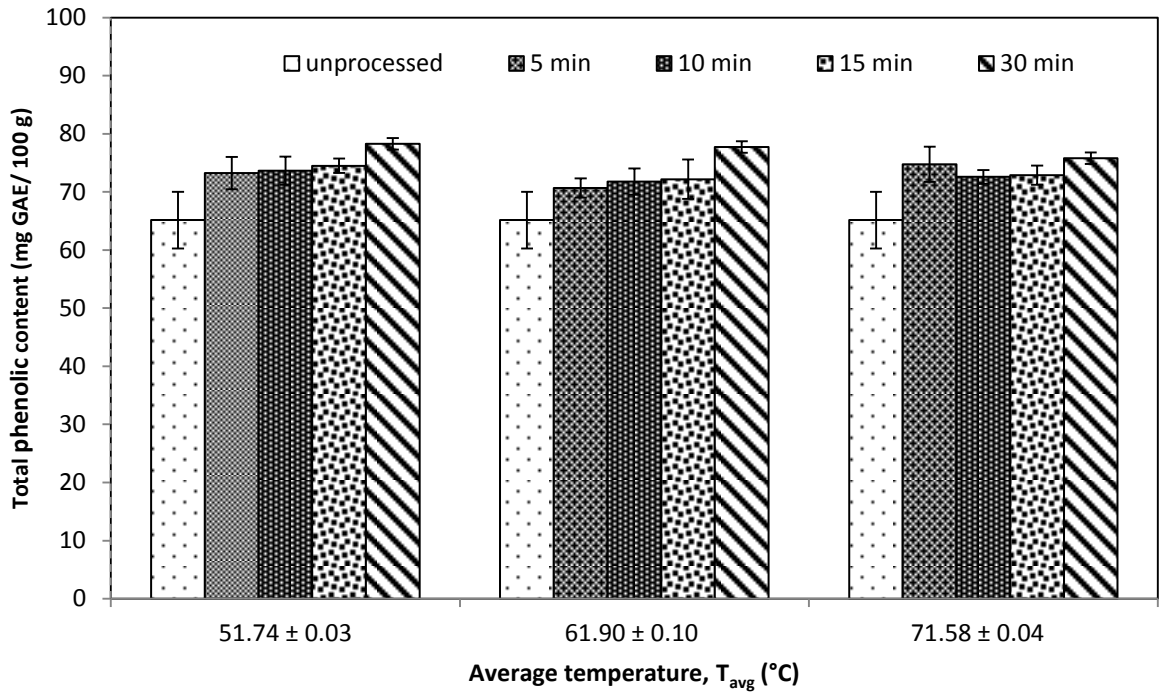


Figure 3.3:.Total phenolic content of honey samples after thermal treatments, for different temperature and time in comparison to unprocessed. Values are mean ± standard deviation (*n* = 3).

3.3.5. Comparison of HPP, HPP–thermal and exclusively thermal processing of Manuka honey.

The changes of TPC in Manuka honey due to the three different types of processing (i) HPP (600 MPa) at ambient temperature, (ii) combined HPP (600 MPa) with thermal processing and (iii) thermal processing, are summarized in Figure 3.4. The graph clearly shows that HPP at ambient temperature was found to have a very significant effect ($p < 0.05$) on TPC in Manuka honey as compared to other processing conditions (combined HPP–thermal and thermal alone). Even it was a slightly increased in TPC after being treated, no significant difference ($p > 0.05$) was found between combined HPP–thermal and thermal alone at all temperatures tested.

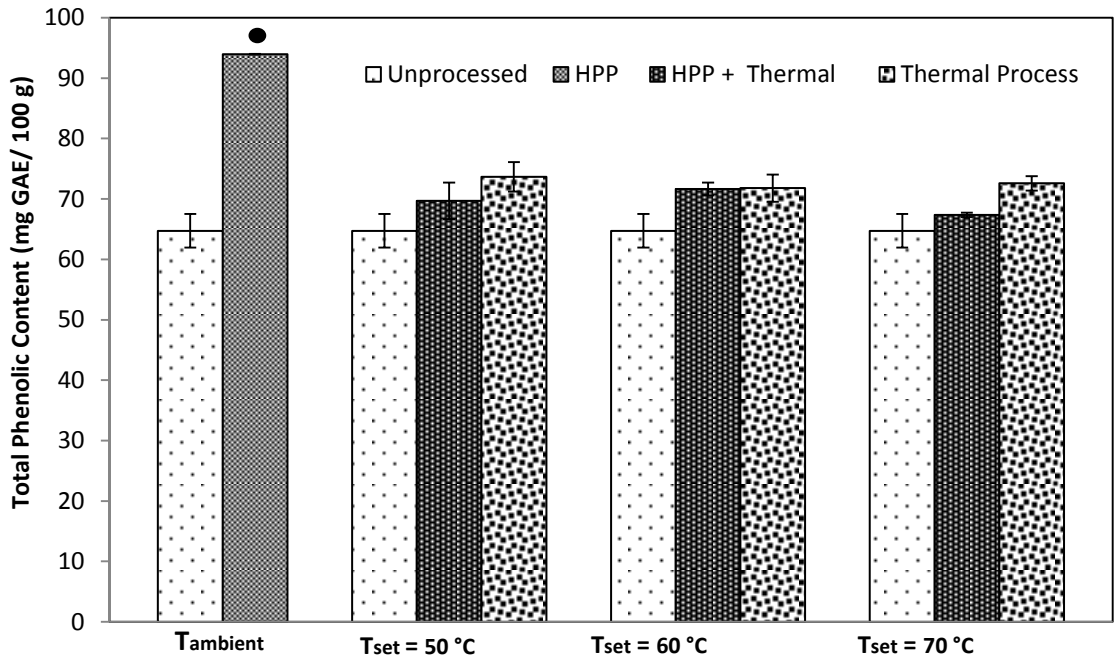


Figure 3.4: The changes in TPC for three different types of treatment at various temperatures for 10 min as compared with unprocessed. The samples were subjected to HPP at 600 MPa. Values are mean ± standard deviation (n = 3). Bullet on processed sample show significant difference ($^{\bullet}p < 0.05$) with respect to unprocessed honey. T_{set} = setting temperature during the processes.

3.4. Conclusions

This work has shown that Manuka honey from New Zealand contains high amount of total phenolics (TPC) compared to other floral sources of honeys. No added benefit was found in TPC when combining HPP with thermal processing, since no significant difference was noted as compared with exclusively thermal treatment. For Manuka honey processing, it is suggested using 600 MPa high pressure processing at ambient temperature (below 40°C), where the increment of TPC was significant. 10 min processing time (at 600 MPa) was found to be the most appropriate treatment time showing a TPC increment of 47.16%.

CHAPTER 4

High-Pressure Processing of Manuka Honey: Improvement of Antioxidant Activity, Preservation of Colour and Flow Behaviour

Fauzi, N., M. Farid, Filipa F.V.M. (2014). Food and Bioprocess Technology 7(8): 2299-2307.

Chapter Abstract

Manuka honey in New Zealand is known for its superior antioxidant properties. However, these valuable properties are known to be compromised when raw honey goes through conventional thermal processing, thus reducing its final quality. As such, this present work is undertaken to assess the effect of high-pressure processing on quality of honey, namely, the antioxidant activity, colour and viscosity. The honey was subjected to different pressures (200 – 600 MPa) at ambient temperatures (25 to 33°C) and combined with moderate temperatures (53 to 74°C) for holding times (10 to 30 min). Thermal processing (49 to 70°C) was also carried out for comparison purpose. In the absence of heat, the antioxidant activity of high pressure-treated samples (600 MPa, 10 min) was found to increase by about 30% with no colour changes detected. The shear-thinning behaviour of the honey was also retained after HPP at ambient temperature, whereas for combined HPP–thermal treatment, no added benefit in antioxidant activity was observed particularly at higher temperature. Colour was significantly degraded when processed for ≥15 min at 70°C and the flow behaviour was brought about from shear thinning to Newtonian. Thus, it can be concluded that the quality of honey can be enhanced by using high-pressure processing at ambient temperature.

4.1 Introduction

In commercial processing plant, honey is usually heated to 60°C or above for inhibiting microorganisms, facilitating packing and delaying crystallization (Bath and Singh 1999; Tosi et al., 2004). However, the quality of honey is known to be compromised when it goes through thermal processing due to the unstable and thermolabile honey components which originated from the nectar and bees themselves (Nagai et al., 2001). Thus, the possibilities of thermal processing to improve the nutritional value look rather limited when honey is exposed to higher temperature. To maintain honey quality as high as possible, there is a need to develop novel processing technique such as non-thermal processing.

Manuka honey in New Zealand is known for its superior health benefits. Antioxidant activity, colour and viscosity are important quality characteristics and major factors affecting consumer acceptance. Honey is regarded as natural nutraceuticals due to its high antioxidant content with added value and has been shown to reduce the risk of heart disease, cancer, cataracts and inflammatory processes (Bogdanov et al., 2008). Flavonoids (Socha et al., 2011), amino acids (Bogdanov et al., 2008) and phenolic acids (Aljadi and Kamaruddin, 2004) are the main antioxidant compounds in honey. However, most of these substances are unstable over time and thermolabile. Heating has been reported to decrease the antioxidative activities of honey due to decomposition of vitamins and also destruction of the integrity of the enzymes, particularly at higher temperature of 100°C (10, 20 and 30 min) (Nagai et al., 2001).

The maintenance of naturally coloured pigments in foods is a major challenge. Colour relates directly to consumer perception of appearance and therefore has to be within an expected range for consumer acceptance. It is known that honey exists in a wide range of colours, varying from pale yellow to dark red. The floral origin and processing/storage temperature and time can affect honey colour (Gonzales et al., 1999). Lynn et al. (1936) reported that the darkening of honey could be due to instability of fructose (caramelization reaction) and oxidation of polyphenols. Ibarz et al. (2000) and Wong and Stanton (1989) revealed that one of the effects of thermal processing is non-enzymatic browning reactions including Maillard reaction. Browning, which occurs by thermal processing, can be unfavourable for some foods (Burdulu and Karadeniz, 2003) but can be preferred in manufacturing of some other foods such as coffee and bread (Shaker et al., 1995).

Understanding the flow behaviour of honey is of prime importance in all stages of honey production, processing, storage and packaging which in turn affects its quality

(Anupama et al., 2003). Some honeys tend to crystallize at low temperature. Crystallized honey has an opaque waxy appearance and less visual impact than liquid honey. These features are not accepted for many consumers who prefer liquid honey. In addition, alteration in the consistency of honey makes it hard to use, handle and process. Although thermal processing is a convenient way to change the consistency of honey and protect it from fermentation (since an increase of water activity during crystallization tends to ferment), high temperature can be detrimental to the quality of honey and its biological properties. Most type of honey show Newtonian behaviour (Abu-Jdayil et al., 2002; Al-Malah et al., 2001; Bhandari et al., 1999; Zaitoun et al., 2001), however, there are some reports in the literature showing a non-Newtonian behaviour of honey (Munroe, 1943; White, 1978).

Losing the antioxidant activity, darkening of colour and changing of rheological properties of honey may occur during processing and these have detrimental effects on its quality as well as masking its originality. Thus, to maintain and improve the quality and nutritional value of honey, high-pressure processing (HPP) will be investigated for the first time as alternative to the conventional thermal processing. Due to the almost instantaneous isostatic pressure transmission (Abdul Ghani and Farid, 2007), HPP-treated food has been shown to keep its original freshness and to improve functionalities (Butz, 2010). To the best of our knowledge, no information is available on the effect of high pressure on the antioxidant activity and quality (namely, colour and rheological properties) of honey.

This paper, therefore determines the effect of HPP and combined HPP–thermal processing on the antioxidant activity of Manuka honey, which has not been investigated so far. The paper also reports on the resulting quality changes occurring in Manuka honey expressed in terms of colour and viscosity.

4.2 Material and Methods

4.2.1 Honey Samples Preparation

The fresh and unprocessed Manuka honey used in this study (pH of 4.3 ± 0.2, 79 ± 0.3 ° Brix and water content of 16.46 ±1.4%) was kindly donated by Comvita®, Auckland, New Zealand. The jar of honey sample was collected directly from beekeepers and sourced from Manuka tree (*Leptospermum scoparium*), a native of New Zealand. Honey (5 g) was packed in 5 cm×5 cm transparent plastic film pouches (Cas-Pak plastic vacuum pouch, Silverdale, New Zealand) and thermosealed under vacuum after manually stirred. A very thin pouch of 3 mm is deliberately used so that honeys temperature approaches the surrounding water temperature in short time. The plastic film is made of cast polypropylene for excellent transparency and heat sealing qualities and can withstand temperatures up to 125°C.

4.2.2 Processing of Honey

4.2.2.1 High-Pressure Processing Equipment

The HPP unit used in this research was QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Kent, WA, USA). The equipment consists of a 2- litre cylindrical-shaped pressure treatment chamber (inner height = 0.25 m, inner diameter = 0.10 m) with a thermocouple, water circulation, a pumping system and a control system operated through a computer with software supplied by the manufacturer. Distilled water was used as the medium in the chamber where the packed honey samples were placed. The equipment can operate at maximum pressure and temperature of 690 MPa and 90°C, respectively. The treatment time was the pressure holding time and did not include the pressure come up and the decompression times. The temperature inside the pressure chamber during treatment was monitored using thermocouples (located at mid and top of the vessel), which were immersed in the pressure medium (distilled water). The compression time, decompression time and average temperature were based on the cycle report which is directly obtained from a control system operated through a computer with software. After processing, the packed honey samples were immediately cooled in ice water before analysed. All honey samples were taken from the same honey batch and every single treatment was done twice.

4.2.2.2 HPP of Honey at Ambient Temperature

Five grams of vacuum-packed Manuka honey samples were subjected to HPP with pressures of 200, 400 and 600 MPa, at close to ambient temperature (25 to 35°C) for 10 min. Pressure come-up times were approximately 1.5 min and the decompression time was < 20 s. The adiabatic heating of 200, 400 and 600 MPa gave an average processing temperature of 26.80 ± 0.95°C, 28.71 ± 0.90°C and 30.18 ± 2.14°C, respectively, during the holding pressure phase. The samples were then taken for the analysis of antioxidant activity, colour, rheological behaviour and viscosity.

4.2.2.3 Combined HPP–Thermal Processing of Honey

5 grams of vacuum-packed Manuka honey samples were submitted to high pressure of 600 MPa and processing time of 10–30 min after initial heating. The samples were enclosed in the pressure chamber for each condition tested. Pressure come-up times were approximately 1.5 min to reach 600 MPa, and adiabatic heating was observed during pressurization phase. The pressure increase up to the desired took less than 2 min. Initial temperature settings of 50, 60 and 70°C for 600 MPa resulted in the average processing temperature of 53.62 ± 0.30°C, 62.65 ± 0.47°C and 72.99 ± 0.38°C during holding pressure phase. The samples were then taken for the analysis of antioxidant activity, colour, rheological behaviour and viscosity.

4.2.3 Thermal Processing of Honey

The thermal processing in the absence of high pressure was performed at 50, 60 and 70°C for 10 min using a thermostatic water bath, W28 (Grant Instruments, Cambridge, UK). For the treatment, the vacuum-sealed samples were fully submerged into the water bath. Setting temperatures of 50, 60 and 70°C resulted in average temperatures of 51.74 ± 0.03°C, 61.90 ± 0.10°C and 71.58 ± 0.04°C, respectively. The come-up time of the centre of the packed honey was less than 1.5 min. These actual temperatures were obtained by measuring the thermometer readings (which is located in the centre of water bath) during process. It is very reasonable to assume that honey samples temperature is very close to the measured surrounding water temperature due to the thin pouches used as discussed before. After each treatment, all samples were immediately placed in ice cooled water before analysis. All honey samples were taken from the same honey batch and every single treatment was repeated for two times. After processing, the packed honey samples were cooled in ice water

before analysed. The samples were then taken for the analysis of antioxidant activity, colour, rheological behaviour and viscosity.

4.2.4 Quality Determination

4.2.4.1 Antioxidant Activity

Antioxidant activity in the sample was determined using the 2, 2, diphenyl-2-picryl-hydrazyl (DPPH) method (Brand-Williams et al., 1995; Rauter et al., 2012; Turkmen et al., 2006).One gram of each honey sample was dissolved in 5 mL of distilled water. The solution was then centrifuged for 10 min at 10 000xg and filtered through Whatman No. 1 before precisely diluted to 4 ° Brix with distilled water using an Atago RX-5000a digital refractometer (Atago®, London, UK). A 0.5 mL of honey extract was mixed with an aliquot of 1.5 mL of 0.1 mM DPPH radical (Sigma-Aldrich Chemie, Steinheim, Germany) in methanol. The reaction mixture was vortex-mixed and left to stand at 25°C in the dark for 60 min. Absorbance at 517 nm was measured using a spectrophotometer (Shimadzu, Santa Clara, California, North America) using methanol as blank, whereas distilled water was used as a control. The experiment was carried out two times, each time with duplicate samples. Antioxidant activity (DPPH scavenging activity) was expressed as percentage inhibition of the DPPH radical and was determined by the following equation (Yen and Duh, 1994):

$$AA\ (\%) = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} x100 \qquad (4.1)$$

where AA (%) is the antioxidant activity in percentage, $Abs_{control}$ is the absorbance reading of the control and Abs_{sample} is the absorbance reading of the sample.

4.2.4.2 Colour

Colour characteristics were assessed by the CIE *L*a*b** method where lightness, *L**, and two colour coordinates, *a* and b**, were measured by means of the Minolta CR-400 chromameter. The calibration was done using calibration plate with white background and 2° angle observer by taking Y, x, y as standard values (Y= 85.9, x = 0.3188, y = 0.33578). The honey samples were heated to 50°C for 30 min in a water bath to dissolve sugar crystals and decrease their viscosity (Gonzales, 1999). The sample was placed in a transparent plastic container (2.5 cm diameter, 1.0 cm thickness) and covered with a transparent plastic plate. The sample thickness was 0.4 cm. *L**, *a** and *b** values were measured against a white background and were directly obtained from the equipment. The measurement of the *L**, *a** and *b** values for each duplicate samples were read five times at five different locations. The experiment was carried out two times. Total colour difference (TCD) is a parameter that quantifies the overall colour difference of a processed sample (*L**, *a**, *b**) when compared to unprocessed sample (*L_0**, *a_0**, *b_0*)* and calculated as:

$$TCD = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

(4.2)

4.2.4.3 Rheological Behaviour and Viscosity

Viscosity of unprocessed and processed honey samples were measured using a rheometer (AR-G2, TA Instrument, Texas, USA) which was connected to a computer with software (TA Instrument Advantage™ software, Texas, USA). The measuring system consists of Smart Swap geometry with 40 mm, 2° steel cone. The method was adapted from Yanniotis et al. (2006) with some modification on shear rate range. Honey samples of 2 g were poured onto the sample plate. The rotational speed was increased to provide a shear rate in the range of 0.1 to 100 s^{-1}. Shear stress and viscosity were measured and recorded at different shear rates. The experiment was conducted at room temperature (25°C) and carried out two times, each time with duplicate samples.

4.2.5 Statistical Analysis

Results were presented as mean ± standard deviation of duplicate measurements. One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered significant at $p < 0.05$. The separation of treatment means was carried out with Tukey's honestly significant difference (HSD) test. All statistical analyses were performed with Statistica, version 11 (Statsoft® Texas, Austin, USA) and Microsoft Excell® 2010 (Microsoft©, Redmond, WA, USA).

4.3 Results and Discussion

4.3.1 Effect of Processes on Honey Antioxidant Activity

The antioxidant activity of unprocessed Manuka honey obtained from this study was 52.36 ± 0.03%, which exhibited the highest antioxidant activity in comparison with commercial Polish honey, 18.21–46.40% (Socha et al., 2011), Malaysian floral honey, 22.4–41.3% (Aljadi and Kamaruddin, 2004; Hussein et al., 2011; Mahaneem et al., 2010) and Romanian honey, 35.8–49.19% (Al et al., 2009). The results from this study emphasized the relevance of Manuka honey as a healthy food supplement and a source of natural antioxidants due to its high antioxidant activity and total phenolic content. The total phenolic content in unprocessed Manuka honey was
recorded as 63.85 ± 0.90 mg GAE/100 g (Akhmazillah et al., 2013) which was higher than other types of honey, 4.46 – 20.42 mg GAE/100 g (Ferreira and Aires, 2009; Socha et al., 2011).

The antioxidant activity of Manuka honey as affected by HPP, combined HPP–thermal and thermal processes are presented in Figure 4.1. From the graph, all processed samples showed a significant increase in antioxidant activity as compared with unprocessed ($p < 0.05$), except for combined HPP–thermally processed (600 MPa, 70°C) and thermally processed samples (60°C). Generally, the antioxidant activity for HPP-treated samples (at ambient temperature) showed the
highest increment as compared with other treatments. This result is in agreement with measurements of the total phenolic content where HPP treatment (600 MPa/ ambient temperature/ 10 min) gave a significant increase of 47.16% as compared with untreated (Akhmazillah et al., 2013). A good correlation between the antioxidant activity and total phenolic content ($r = 0.889$) was found in this study (Figure 4.2). The graph was constructed based on the values of antioxidant activity and total phenolic content obtained from HPP-treated honey (600 MPa/ ambient temperature) at seven different treatment times (ranging from 2-30 min). The same findings were also reported for Malaysian floral honeys (Aljadi and Kamaruddin, 2004; Mahaneem et al., 2010). Since phenolic compounds play a major role in increasing antioxidant activity in honey, high pressure is expected to create significant increase in antioxidant activity.

Results demonstrated that although HPP-treated samples (at ambient temperature) lead to a significant increase to about 30% in antioxidant activity, no added benefit was

observed when combining HPP with thermal processing. This phenomenon could be explained by the ability of the hydroxyl molecules (OH), the functional group of phenolic compounds, to react with water molecules. The solubility which is due to hydrogen bonding was enhanced by the application of high pressure, whereas the hydration of charged group is loosened at high temperature (Mozhaev et al., 1996).

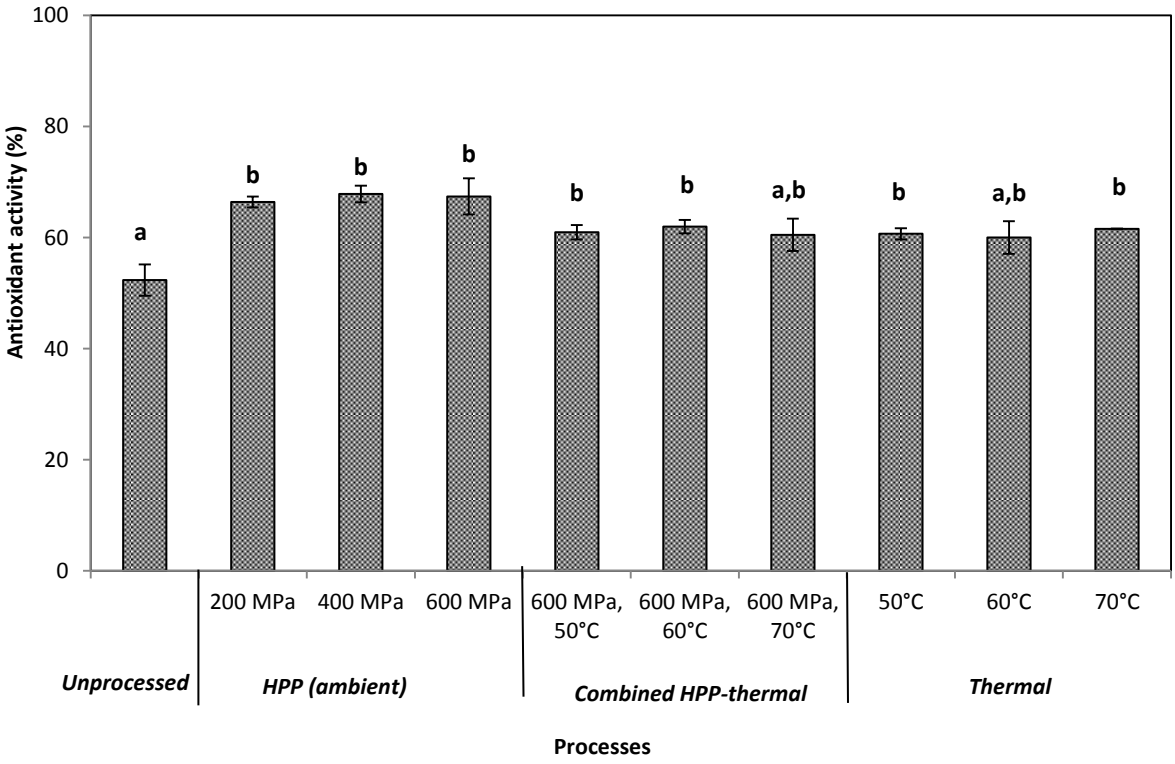


Figure 4.1: Antioxidant activity (%) of Manuka honey for different processes. All the processes had the duration of 10 min. [a,b] values are means ± standard deviation, different letters are significantly difference according to Tukey HSD test (Statistica version 11, Statsoft®) with n = 2.

Previous studies revealed that HPP and combined HPP–thermal processing showed a different effect on antioxidant activity for different food products such as persimmon (de Begoa et al., 2000), apple juice (Fernández García et al., 2000), orange juice and tomato puree (Fernández García et al., 2000; Garcia et al., 2001), carrot juice (Van Loey Indrawati and Hendrickx, 2004), legume seeds (Doblado et al., 2007), vegetables (McInerney et al., 2007) and aloe vera gel (Vega-Gálvez et al., 2012). The increase of antioxidant activity and phenolic content in these food matrices is mostly attributed to the disintegration of cell

membrane. The cell membranes and organelles are disrupted and enzymes are released from vacuoles which then affect the phenolic content and antioxidant activity in the samples (Keenan et al. 2010; Prasad et al. 2009).

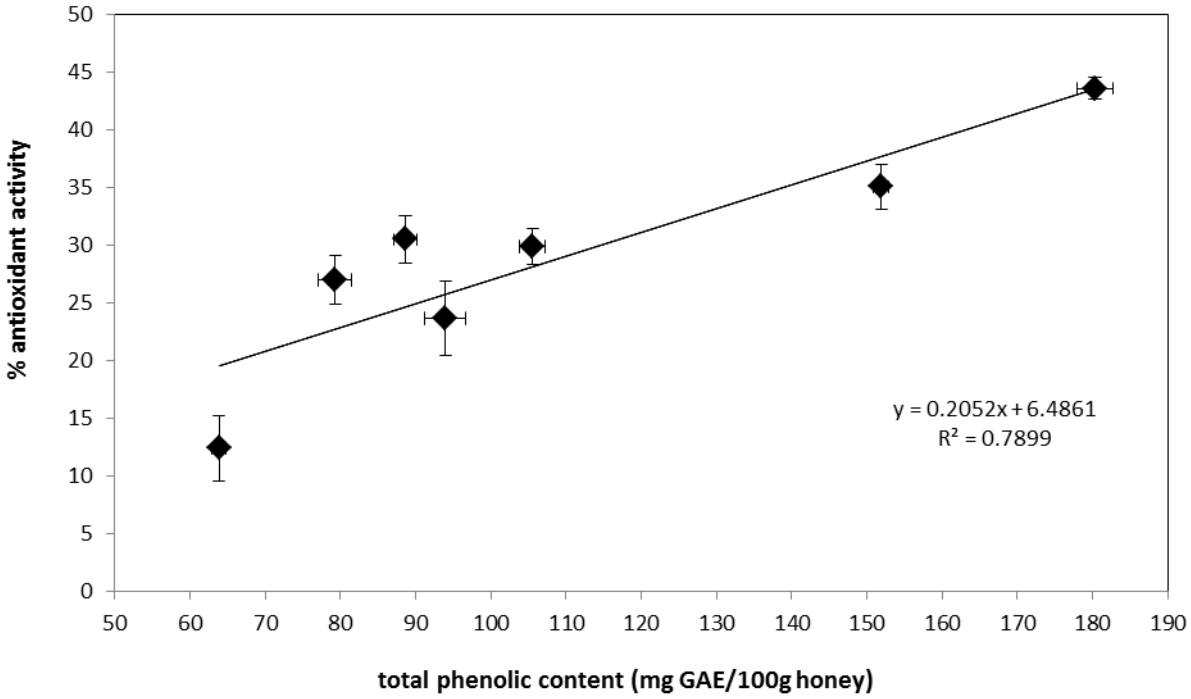


Figure 4.2: Correlation between antioxidant activity of HPP treated Manuka honey (600 MPa at ambient temperature) and its total phenolic content. The error bars are means ± standard deviation with n = 2 for both antioxidant activity and total phenolic content.

However, since there is no intact cell in honey, the possible reason might be due to the presence of pollen. As enzymes and protein in honey conceivably arise from pollen, this can contribute to the phenolic content increment when HPP is applied.

The present study found that HPP treatment (600 MPa/ ambient temperature/10 min) is beneficial not only in retaining antioxidant activities in honey but also in improving them by increasing significantly ($p < 0.05$) the value to about 30% from the unprocessed sample.

4.3.2 Effect of Processes on Colour of Honey

The colour parameters (*L**, *a** and *b**) and TCD of Manuka honey as affected by conventional thermal processing, HPP and combined HPP–thermal are presented in Table 4.1. The result showed that there is no significant difference ($p < 0.05$) in *L**, *a** and *b** values for all treatments as compared with unprocessed sample.

With respect to TCD, the result from this study shows that HPP-treated samples at ambient temperature had the lowest effect on TCD (range between 0.41 and 0.83) compared to others. While the colour change of combined HPP–thermal (600 MPa/70°C) showed significant difference ($p < 0.05$) where the TCD value was more intense (> 6) as the processing time was longer (15 and 30 min). The difference in colour could be due to the presence of heat sensitive compounds particularly at higher temperature of 70°C. These compounds involve the degradation of thermolabile pigments which in turn result in the formation of dark compounds that reduces luminosity (Barreiro et al., 1997). Besides, it also might be due to caramelization because of high sugar content present in honey. Monosaccharides particularly fructose will go initial enolization and progress to subsequent complex reactions, like dehydration, dicarboxylic cleaving and aldol condensation (Kroh, 1994).

The results from this study suggest that HPP at ambient temperature preserves the original colour of honey. However, for combined HPP–thermal process and in order to minimize colour change, it is recommended to treat at temperatures below 70°C with shorter time of less than 15 min.

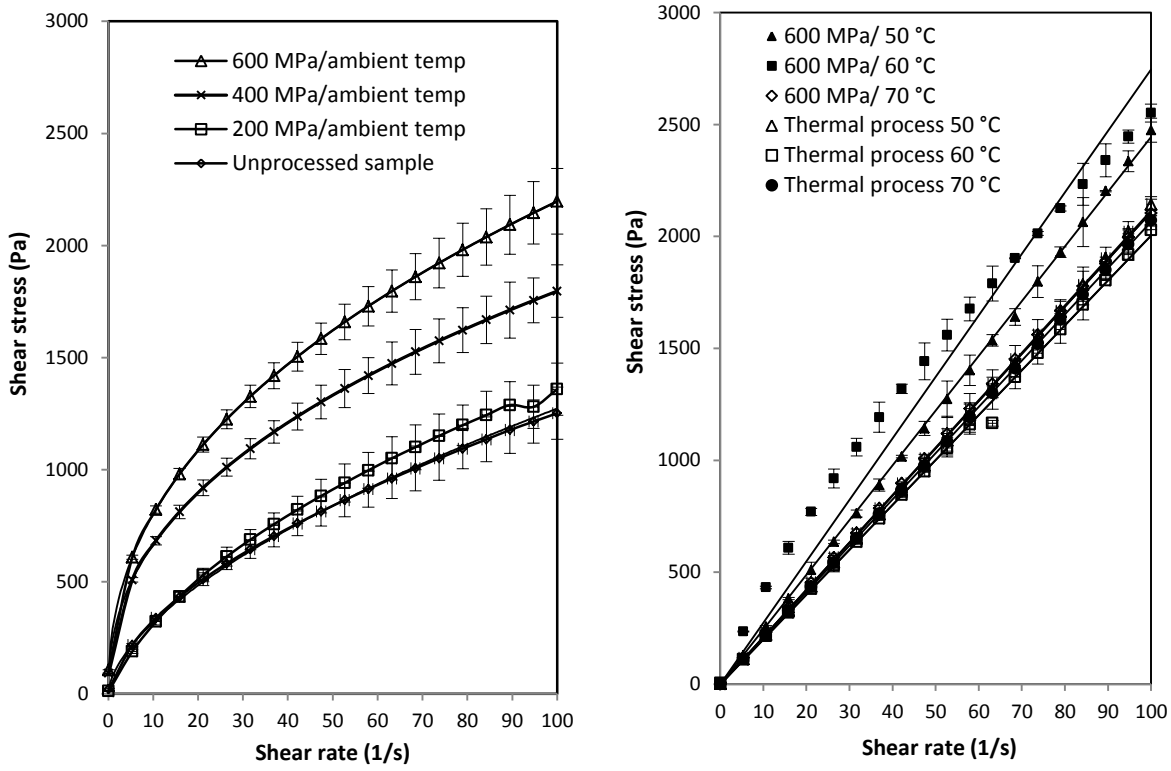
Table 4.1: The values of *L**, *a**, *b** and total colour difference (TCD) for HPP, combined HPP-thermal and thermal treated Manuka honey.

TREATMENT	***L****	***a****	***b****	**Total Colour Difference, TCD**
Unprocessed	58.41 ± 0.36[a]	3.61 ± 0.1[a]	35.14 ± 0.16[a]	0[a]
HPP at ambient temperature				
200 MPa, 10 min	57.97 ± 2.74[a]	3.47 ± 0.37[a]	34.89 ± 1.83[a]	0.53 ± 0.12[a,b]
400 MPa, 10 min	58.00 ± 4.83[a]	3.64 ± 0.19[a]	35.00 ± 3.50[a]	0.43 ± 0.30[a,b]
600 MPa, 10 min	57.88 ± 0.12[a]	3.55 ± 0.04[a]	34.65 ± 0.09[a]	0.72 ± 0.11[a,b]
Combined HPP –thermal				
600 MPa, 50°C,10 min	55.13 ± 2.31[a]	4.25 ± 0.24[a]	33.30 ± 1.58[a]	3.81 ± 0.68[a,b]
600 MPa, 60°C, 10 min	56.06 ± 0.53[a]	3.76 ± 0.24[a]	33.59 ± 0.32[a]	2.82 ± 0.58[a,b]
600 MPa, 70°C, 10 min	60.18 ± 3.70[a]	3.29 ± 0.54[a]	36.26 ± 2.45[a]	2.12 ± 0.12[a,b]
600 MPa, 70°C, 15 min	63.45 ± 0.27[a]	3.35 ± 0.09[a]	38.85 ± 0.06[a]	6.26 ± 0.02[b]
600 MPa, 70°C, 30 min	63.70 ± 0.29[a]	3.28 ± 0.34[a]	38.68 ± 0.46[a]	6.38 ± 0.01[b]
Thermal process at ambient pressure (0.1 MPa)				
50°C, 10 min	59.60 ± 2.66[a]	3.48 ± 0.29[a]	36.18 ± 1.83[a]	1.59 ± 0.20[a,b]
60°C, 10 min	59.90 ± 4.42[a]	3.18 ± 0.41[a]	35.84 ± 3.20[a]	1.70 ± 0.12[a,b]
70°C, 10 min	62.35 ± 0.27[a]	3.28 ± 0.05[a]	38.11 ± 0.04[a]	4.95 ± 0.21[a,b]

[a,b]Mean values (means ± standard deviation) within the same column with different letters are significantly different according to Tukey's HSD test (Statistica version 11, Statsoft®) with n = 2.

4.3.3 Effect of Processes on Honey Flow Behaviour

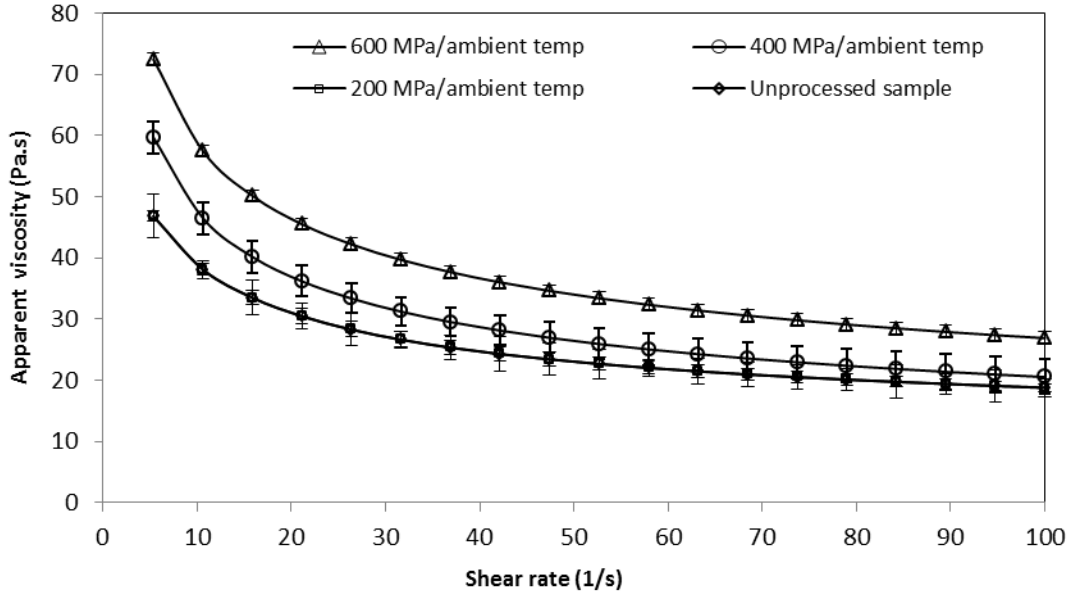
The unprocessed Manuka honey investigated in this study displayed a non-Newtonian behaviour. Figure 4.3a shows the graph of shear stress (Pa) against shear rate (s^{-1}) for unprocessed honey, indicating a shear-thinning or pseudoplastic behaviour. This was confirmed by the value of flow behaviour index "n" which is less than 1, showing that the viscosity of unprocessed honey decreases with increasing shear rate. The non-Newtonian behaviour has been attributed to the presence of colloids or high molecular weight compounds such as proteins or polysaccharides (Juszczak and Fortuna, 2006; Mossel et al., 2000). Although this result differs from some published studies where honey is reported as Newtonian fluid (Abu-Jdayil et al., 2002; Bhandari et al., 1999; Juszczak and Fortuna, 2006; Lazaridou et al., 2004; Mossel et al., 2000; Pan and Ji 1998; Yanniotis et al., 2006; Zaitoun et al., 2001), there are some reports in the literature showing similar behaviour to the unprocessed Manuka honey investigated in this study such as heather honey (*Calluna vulgaris*), buckwheat honey (*Fagopyrum esculentum*), white clover honey (*Trifolium repens*) and Indian Karvi honey (*Carvia callosa*). Nigerian honey (*Opuntia engelmannii*) and several eucalyptus honeys (e.g. *Eucalyptus ficifolia*) were also reported to have non-Newtonian behaviour but with dilatancy type. The HPP-treated samples (200, 400 and 600 MPa) at ambient temperature for 10 min displayed shear thinning or pseudoplastic behaviour, similar to unprocessed honey (Figure 4.3a). On the contrary, HPP–thermally and thermally - treated samples displayed a Newtonian behaviour (n=1), where the shear stress directly proportional to the shear rate as shown in Figure 4.3b.



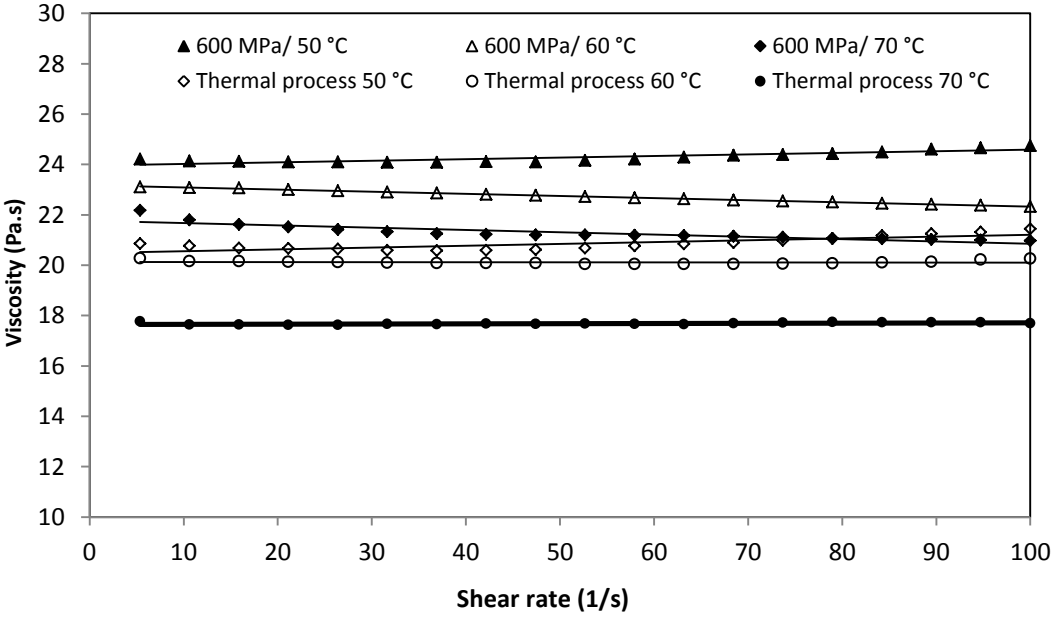
(a) (b)

Figure 4.3: Flow curves of (a) unprocessed sample and HPP- treated samples at ambient temperature and (b) combined HPP-thermal and thermal treated Manuka honey. The error bars are means ± standard deviation with n = 2.

The viscosity curve of the pressure-treated samples (200, 400 and 600 MPa at ambient temperature) as a function of shear rate is shown in Figure 4.4a. The viscosity decreased with an increase of the shear rate. The difference in viscosity of the samples was largest at a lower shear rate and becomes flatter at a higher shear rate. Meanwhile, Figure 4.4b shows the viscosity of heated honey with and without HPP as a function of shear rate. The apparent viscosity for non-Newtonian honey samples and viscosity for Newtonian honey samples were presented in Tables 4.2 and 4.3, respectively. The important finding is that at low shear rate (usually when the honey is consumed), the viscosity is not affected by pressure treatment particularly at pressure < 400 MPa. However, it was reduced by more than a factor of 2 through the thermal treatment.



(a)



(b)

Figure 4.4: The viscosity curves as a function of shear rate. (a) Unprocessed and HPP-processed Manuka honey at ambient temperature. The error bars are means ± standard deviation with n =4. (b) Combined HPP-thermal and thermal processing. The range of standard deviation (n = 2) for the treatments were found as follows: closed triangle= 2.58–2.65; open triangle =0.12–93; closed diamond =2.86–3.34; open diamond =1.88–2.01; open circle =0.83–1.07; closed circle =1.02–1.18.

Table 4.2: Apparent viscosity of unprocessed and HPP treated Manuka honey (ambient temperature, 10 min treatment) at shear rate 5.39 s^{-1}.

	Apparent Viscosity (Pa.s)
Unprocessed	46.86 ± 1.42[a]
HPP (ambient temperature)	
HPP, 200 MPa	46.73 ± 2.23[a]
HPP, 400 MPa	59.71 ± 2.56[a,b]
HPP, 600 MPa	72.30 ± 3.20[b]

Mean values (means ± standard deviation) within the same column with different letters are significantly different according to Tukey's HSD test (Statistica version 11, Statsoft®) with n =2

Table 4.3: Viscosity of combined HPP - thermally treated and thermally- treated Manuka honey (10 min treatment) at shear rate 5.39 s^{-1}.

	Viscosity (Pa.s)
Combined HPP- Thermal	
HPP (600 MPa), 50°C	24.29 ± 0.21[a]
HPP (600 MPa), 60°C	22.82 ± 0.26[a]
HPP (600 MPa), 70°C	21.31 ± 0.32[a,b]
Thermal (ambient pressure, 0.1 MPa)	
Thermal, 50°C	20.68 ± 0.26[a,b]
Thermal, 60°C	20.16 ± 0.07[b,c]
Thermal, 70°C	17.64 ± 0.06[c]

[a,b,c]Mean values (means ± standard deviation) within the same column with different letters are significantly different according to Tukey's HSD test (Statistica version 11, Statsoft®) with n = 2.

Previous studies have reported that viscosity was correlated with high pressure for many fruits and vegetables products such as tomato juices (Porretta et al., 1995), guava puree (Yen and Lin, 1996), chopped tomatoes (Rovere et al., 1997), spinach and cauliflower (Prestamo and Arroyo, 1998), mango pulp (Ahmed et al., 2005), navel orange juice (Polydera et al., 2005), fruit yogurt (Walker et al., 2006), tomato puree (Krebbers et al., 2003; Sánchez-Moreno et al., 2006) and apple puree (Landl et al., 2010). Sánchez-Moreno et al. (2006) and Oey et al. (2008) revealed that the increase in viscosity was attributed to an increase in linearity of the cell walls and volumes of particles due to the permeabilization of the cell walls. However, in the case of honey, the increase of viscosity as affected by HPP (400 and 600 MPa/ambient temperature/10 min) might be due to the high molecular weight sugars (oligosaccharides) and also residual bees wax present in honey (Assil et al., 1991). HPP may affect oligosaccharides and may also induce a phase shift of the wax under pressure and hence can cause the change in viscosity. Further work is needed to verify this hypothesis.

This study has shown that in the absence of heat, high pressure can retain the shear-thinning behaviour of Manuka honey, whereas combined HPP–thermal and thermal process brought about the Manuka honey from shear-thinning behaviour to a Newtonian behaviour.

4.4 Conclusions

The determination of antioxidant activity, colour and rheological properties of Manuka honey as affected by HPP is reported here for the first time. HPP at ambient temperature shows a great potential in increasing antioxidant activity which, in turn, improves the natural nutritional value of honey. The original colour of honey was also preserved at this condition, whereas the combined HPP–thermal process at higher temperature and longer time alters the original colour of the honey. Rheological measurement indicated that combining HPP with thermal brought about Manuka honey from shear thinning behaviour to a Newtonian behaviour, unlike HPP treatment alone in which the viscosity at lower shear rate was not affected by HPP treatment, while thermal treatment (70°C/ 10 min) has reduced it to less than half. Based on the work presented in this paper, showing significant improvement in the nutritional value of honey, we expect that the HPP treatment could lead to a higher-value product.

CHAPTER 5

High Pressure Processing (HPP) of Manuka honey: Brown Pigment Formation, Improvement of Antibacterial Activity and Hydroxymethylfurfural Content

Fauzi, N. A. and M. M. Farid (2014). International Journal of Food Science & Technology. DOI: 10.1111/ijfs.12630

Chapter Abstract

Present work was undertaken to assess the effects of high pressure processing on brown pigment formation, antibacterial activity and hydroxymethylfurfural content in Manuka honey. The honey was subjected to different pressures (200–600 MPa) at ambient temperatures (25-33°C) and with moderate temperatures (53-74°C) for holding times (10-30 min). The brown pigment formation in high pressure treated sample showed a significant increase (0.16-0.17, $p < 0.05$), whereas maximum percentage inhibition of *Staphylococcus epidermidis* (84.34 ± 7.62%) was achieved when 5% (w/v) of honey dilution was subjected to 600 MPa (at ambient temperature for 10 min) as compared with control (64.15 ± 5.86%). The percentage inhibition in high pressure treated samples correlated linearly ($r = 0.941$) with brown pigment. No significant increase in hydroxymethylfurfural as affected by high pressure was found ($p > 0.05$). Thus, high pressure processing has no adverse effect on the honey quality since it can control HMF concentration during process. Increasing brown pigment formation is associated with the enhancement of antibacterial activity.

5.1 Introduction

In honey commercial applications, heating is an important operation and is known to have a potential for eliminating spoilage microorganisms, facilitating packaging and delaying crystallization (Subramanian et al., 2007). However, heating honey to higher temperatures of more than 70°C is not suitable because it causes alteration of flavour, colour and granulation of honey; also degrade bioactive compounds and antioxidants which could result in product quality deterioration (Subramanian et al., 2007; Visquert et al., 2004). The deterioration of honey quality and its nutritional properties is due to the unstable and thermolabile honey components (Nagai et al., 2001).

Formation of brown pigment, reduction of antibacterial activity and accumulation of hydroxymethylfurfural, HMF are main concerns during honey processing. Darkening of honey is generally associated with a loss in quality which directly affects the consumer's acceptance of the product. The colour of honey may result from non-enzymatic browning or the Maillard reaction, which involves amino groups of free amino acids/ proteins and carbonyl groups of reducing sugars, resulted in thousands of reaction intermediates and products (Turkmen et al., 2006). In practical application, brown pigment formation is an internal parameter used as a control so as to limit thermal treatment and also during honey storage (Bult and Kilic, 2009).

As an alternative to therapeutic agents, the antibacterial activity in Manuka honey is a major interest to honey industry and is gaining a high consumer's demand (Mavric et al., 2008). Dissimilar with other types of honey, Manuka honey has special characteristics that its antibacterial activity is exhibited from a non-peroxide activity, reportedly attributed to methylglyoxal (MGO) which formed from condensation of dihydroxyacetone (DHA) (Adams et al., 2009). Prolong the storage time and subject honey to mild heat is a traditional approach to allow enough time for the condensation of DHA, and facilitate the build- up of MGO (Adams et al., 2009). However, the quality of honey might be deteriorates due to the prolonged storage and the application of heat.

Hydroxymethylfurfural (HMF) is undesirably produced as a result of Maillard reaction and/or hexose dehydration. Low level in HMF shows honey is in fresh condition. Controlling the formation of HMF is a major challenge since its amount can increase during honey processing and storage (Singh and Bath, 1998). High levels of HMF may be the results of inadequate storage, adulteration with sugar additives or severe heat treatments (Khalil et al., 2010; Tosi et al., 2004). Although HMF is not harmful substance, food standards in many

countries regulate the levels of HMF, which has become a practise during recent years in the international European Union Directive (110/2001) regulatory standards fixed HMF limit in honey to 40 mg/kg, with the exceptions of 80 mg/kg for honey coming from tropical countries and 15mg/kg for honey with low enzymatic level.

Thus, to maintain and improve the quality and nutritional value of honey, a non-thermal processing, high pressure processing (HPP) will be investigated. HPP- treated food has been shown to keep its original freshness without damaging taste, texture and appearance of the food (Butz, 2010). Although detailed studies on the effect of HPP on quality and safety of varieties food have already been made by very large numbers of investigators, the effect of pressure on the high sugar content food such as honey are much less documented. Akhmazillah et al (2013), Al-Habsi and Niranjan (2012) and Fauzi et al. (2014) have reported the effect of HPP on quality attributes in honey, however, there has no previous study reporting the effect of HPP on brown pigment formation. In addition, the relationship between brown pigment and antibacterial activity in honey as affected by HPP is not yet investigated although a good correlation between these properties has been found in untreated honey (Brudzynski and Miotto, 2011).

This paper, therefore determines the effect of HPP on the brown pigment formation of Manuka honey in comparison with conventional thermal treatment. The paper also reports on the relationship between brown pigment formation and its antibacterial activity as affected by high pressure, which has not been studied so far. The effect of HPP on HMF will also be presented.

5.2 Material and Methods

5.2.1 Honey samples preparation

The fresh and unprocessed Manuka honey used in this study (pH of 4.3 ± 0.2, 79 ± 0.3 ° Brix and water content of 16.46 ±1.4%) was kindly donated by Comvita®, Auckland, New Zealand. The jar of honey sample was collected directly from beekeepers and sourced from Manuka tree (*Leptospermum scoparium*), a native of New Zealand.

Honey (5 g) was packed in 5 cm×5 cm transparent plastic film pouches (Cas-Pak plastic vacuum pouch, Silverdale, New Zealand) and thermosealed under vacuum after manually stirred. A very thin pouch of 3 mm is deliberately used so that honeys temperature approaches the surrounding water temperature in short time. The plastic film is made of cast polypropylene for excellent transparency and heat sealing qualities and can withstand temperatures up to 125°C.

5.2.2 Processing honey

5.2.2.1 High Pressure Processing (HPP) equipment

The HPP unit used in this research was QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Kent, WA, USA). The equipment consists of a 2- litre cylindrical-shaped pressure treatment chamber (inner height = 0.25 m, inner diameter = 0.10 m) with a thermocouple, water circulation, a pumping system and a control system operated through a computer with software supplied by the manufacturer. Distilled water was used as the medium in the chamber where the packed honey samples were placed. The equipment can operate at maximum pressure and temperature of 690 MPa and 90°C, respectively. The treatment time was the pressure holding time and did not include the pressure come up and the decompression times. The temperature inside the pressure chamber during treatment was monitored using thermocouples (located at mid and top of the vessel), which were immersed in the pressure medium (distilled water). The compression time, decompression time and average temperature were based on the cycle report which is directly obtained from a control system operated through a computer with software. After processing, the packed honey

samples were immediately cooled in ice water before analysed. All honey samples were taken from the same honey batch and every single treatment was repeated twice.

5.2.2.2 HPP of honey at ambient temperature

Five grams of vacuum-packed Manuka honey samples were subjected to HPP with pressures of 200, 400 and 600 MPa, at close to ambient temperature (25 to 35°C) for 5-30 min. It may be noted that the pressure range employed in this work is common for HPP applications in food industry (100 – 800 MPa). Pressure come-up time was approximately 1.5 min while the decompression time was < 20 s. The adiabatic heating 200, 400 and 600 MPa gave an average processing temperatures of 26.80 ± 0.95°C, 28.71 ± 0.90°C and 30.18 ± 0.98°C, respectively, during the holding pressure period. The samples were then taken for the analysis of brown pigment formation, antibacterial activity and hydroxymethylfurfural content.

5.2.2.3 Combined HPP- thermal processing of honey

Five grams of vacuum-packed Manuka honey samples were submitted to high pressure of 600 MPa and processing time of 10 min. Initial temperature settings of 50, 60 and 70°C for 600 MPa resulted in average processing temperature of 53.62 ± 0.30°C, 62.65 ± 0.47°C and 72.99 ± 0.38 °C during holding pressure phase. Pressure come-up time was approximately 1.5 min to reach 600 MPa. The samples were then taken for the analysis of brown pigment formation and hydroxymethylfurfural content.

5.2.2.4 Thermal processing of honey

The thermal processing in the absence of high pressure was performed at 50, 60 and 70 °C for 10 min using a water bath, W28 (Grant Instruments, Cambridge, UK). The temperatures were chosen considering the standard temperatures to which honey is exposed in industrial (45 – 80°C). For the treatment, the vacuum-sealed samples were fully submerged into the water bath. Setting temperatures of 50, 60 and 70°C resulted in average temperatures of 51.74 ± 0.30°C, 61.90 ± 0.10°C and 71.58 ± 0.40°C, respectively. The come up time of the centre of the packed honey was less than 1.5 min. These actual temperatures were obtained by measuring the thermometer readings (which is located in the centre of water bath) during process. It is very reasonable to assume that honey samples temperature is very close to the measured surrounding water temperature due to the thin pouches used as discussed before. After each treatment, all samples were immediately placed in ice-cooled water before

analysis for brown pigment, antibacterial activity and hydroxymethylfurfural content. The samples were taken from the same honey batch and every single treatment was repeated twice.

5.2.3 Brown pigment

The method for determination of brown pigment formation was adapted from Turkmen et al. (2006). One gram of honey samples was dissolved in 5 mL of distilled water using a vortex-mixer. The solution was then centrifuged for 10 min at 10, 000xg and filtered through Whatman No. 1 before precisely diluted to 4 ° Brix with distilled water using a RX-5000a digital refractometer (Atago®, London, UK). Brown pigment formation of honey sample was then determined by measuring the absorbance of diluted honey at 420 nm using a spectrophotometer (Shimadzu, Santa Clara, California, North America). The experiment was carried out two times, each time with duplicate samples.

5.2.4 Antibacterial activity

The antibacterial activity determination method was adapted from Brudzynski and Miotto (2011) with some modification. Instead of using optical density, the plate counting method was used to measure bacterial growth. The honey was diluted to 50% (w/v) using Nutrient broth (BD Difco, North Ryde, Australia). Further dilutions were done to achieve honey concentrations of 20%, 10%, 6.25%, 5%, 1.0% and 0.5%.

Staphylococcus epidermidis (ATCC® CRM-12228™, Manassas, VA, USA) was obtained from Microbiology Lab, University of Auckland was used as a strain since it has been used most frequently to test honey antibacterial action. One mL stock was spread on a Nutrient Agar (BD Difco, North Ryde, Australia) plate and incubated overnight at 37°C. Then, a loop of bacterial colony on the plate was transferred into 100mL fresh Nutrient Broth (BD Difco, North Ryde, Australia) and incubated at 37°C for 24 hrs in shaking room (120 rev min^{-1}). The bacteria suspension with concentration of 1 x 10^8 CFU/mL was used as inoculum.

A fixed volume of a 0.90 mL of a given honey concentration is inoculated with 0.1 mL inoculum and incubated at 37°C for 18 hours. One mL of bacteria suspension was then serial diluted with 9 mL of 0.1% NaCl (w/v) in test tubes from dilutions of 10^0 to 10^{-8}. Then, 1.0mL of each dilution was spread plated twice in Nutrient Agar (BD Difco, North Ryde,

Australia) plates, and incubated at 37°C for 18 hours. After incubation, the colonies formed were counted and the numbers were then used to construct growth inhibition profiles. The minimal inhibitory concentration which represents the lowest concentration of honey that inhibits the bacterial growth by 95% was determined from the growth inhibition profile curves. The growth inhibition was determined using the following formula:

$$\%\ growth\ \ inhibition = \frac{Number\ of\ \ colonies\,(control) - Number\ of\ \ colonies\,(experimental)}{Number\ of\ \ colonies\,(control)}\ x100 \qquad (5.1)$$

5.2.5 Hydroxymethylfurfural, HMF content

The HMF value was determined by using the proposed formula reported by International Honey Commission (Bogdanov, 2002). Five gram of each honey samples was approximately weighed (± 0.05) and dissolved in 25 mL of water before transferred into 50mL volumetric flask. Then, 0.5mL of Carrez solution I (consisting of 15g of potassium hexacyanoferrate (II), $K_4Fe(CN)_6.3H_2O$ in 100mL of water) was added and mixed. It was followed by the addition of 0.5mL Carrez solution II (consisting of 30 g zinc acetate, $Zn(CH_3COO)_2.2H_2O$ in 100 mL of water), mixed and made up to the mark with water. The solution was filtered using Whatman No. 1 filter paper after rejecting the first 10 mL of the filtrate. Then, aliquots of 5 mL were pipetted into two test tubes each. To the first test tube, 5 mL of water was added and mixed well (sample solution), while 5.0mL of 0.2% sodium bisulphite solution (reference solution) was added to the second test tube and mixed well (reference solution). The absorbance of the samples solution at 284nm and 336nm was determined using UV-vis spectrophotometer (Shimadzu, Santa Clara, California, North America) in 10mm quartz cells. The readings were expressed as mg/kg honey. The experiment was carried out two times, each time with duplicate samples.

5.2.6 Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered significant at $p < 0.05$. The separation of treatments means was carried out with Tukey honestly significant difference (HSD) test. All statistical analyses were performed with Statistica, version 11 (Statsoft® Texas, Austin, USA) and Microsoft Excell ® 2010 (Microsoft©, Redmond, WA, USA).

5.3 Results and discussion

5.3.1 Influence of treatments on brown pigment formation

The brown pigment in untreated Manuka honey was registered as 0.108 ± 0.010 which is higher than the values reported by previous work (Turkmen et al., 2006), as indication to its high total phenolic content (Akhmazillah et al., 2013) and antioxidant potential (Fauzi et al., 2014).

The results on the effects of HPP, combined HPP- thermal and thermal treatment on brown pigment formation obtained from this study are tabulated in Table 5.1. In general, all processes show significant difference ($p < 0.05$) in brown pigment formation as compared with untreated sample. The result also shows an increase in brown pigment is more intense (> 0.18) when honey was exposed to combined HP-thermal (0.179 – 0.191) and thermal treatment (0.188 – 0.202). This observation showed that development of brown colour is strongly dependent on temperature (Vaikousi et al., 2009), where higher temperature will increase the reaction between sugar and amino acids (Manzocco et al., 2001). It was also reported that an increase in brown pigment is related to the increasing of the antibacterial activity (Aljadi and Yusof, 2003). However, some studies reported the contradictory effect where at higher temperature, the antibacterial activity was decreased (Al-Habsi and Niranjan, 2012). With regards to high pressure, HPP-treated samples at ambient temperature have lower impact on brown formation (0.158 to 0.170) and showed a significant difference ($p < 0.05$) as compared with thermal treatment. Theoretically, this might be due to the effect of pressures on the carboxylic acid group of the amino acid present in honey. Since honey has lower pH (3.5 – 4.0), the system will buffered mainly by carboxylic acid and high pressure will favours the ionic form. In general, pressure increases the acidity of the solution in the food system, for example; the pH will drop to about ≤ 0.3 unit for buffer solution and 0.6 unit for distilled water when pressure of 784.6 MPa HPP was applied (Samaranayake and Sastry, 2010). This condition will decreases the pressure-induce pH (Hill et al., 1996), which expected to slow the brown formation (Lee et al., 1984).

Table 5.1: Brown pigment formation and HMF content of untreated and treated honey samples after 10 min treatment.

	Brown pigment (AU_{420nm})	HMF (mg/kg)
Untreated	0.108 ± 0.010^{a}	17.16 ± 0.60^{a}
HPP at ambient temp		
200 MPa, 26.80 ± 0.95°C	$0.164 \pm 0.008^{b,c}$	$17.72 \pm 0.65^{a,b}$
400 MPa, 28.71 ± 0.90°C	0.162 ± 0.004^{b}	$17.83 \pm 0.93^{a,b}$
600 MPa, 30.18 ± 0.98°C	$0.167 \pm 0.003^{b,c,d}$	$17.74 \pm 0.81^{a,b}$
Combined HPP-thermal		
600 MPa, 53.62 ± 0.30°C	$0.184 \pm 0.005^{d,e}$	$18.59 \pm 0.16^{a,b}$
600 MPa, 62.65 ± 0.47°C	$0.182 \pm 0.003^{c,d,e}$	$18.68 \pm 0.12^{a,b}$
600 MPa, 72.99 ± 0.38°C	0.188 ± 0.003^{e}	19.36 ± 1.45^{b}
Thermal treated at ambient pressure (0.1 MPa)		
51.74 ± 0.30°C	0.189 ± 0.001^{e}	$17.71 \pm 0.69^{a,b}$
61.90 ± 0.10°C	0.191 ± 0.002^{e}	$18.93 \pm 0.94^{a,b}$
71.58 ± 0.40°C	0.199 ± 0.003^{e}	26.89 ± 0.88^{c}

[a, b, c, d, e]Mean values (means ± standard deviation, n =2) within the same column with different letters are significantly different ($P < 0.05$) according to Tukey's HSD test.

With respect to percentage increment in brown pigment, thermally treated samples at 70°C showed the highest absorbance increase (84.69 ± 0.002%) as compared with 50°C (74.94 ± 0.002%) and 60°C (77.26 ± 0.001%) (Figure 5.1). This finding is in agreement with Vaikousi et al. (2009) where the increase in absorbance was pronounced at higher temperature of 80°C, due to an increase of the reactivity between the sugar and the amino group in the food system (Manzocco et al., 2001).

The browning reaction in honey was found to be less sensitive to heat, as compared with usual non enzymatic browning in other food matrices such as peach puree (thermally treated at 80, 85, 90, 95 and 98°C for 480 min) (Garza et al., 1999). This is due to the different contents and types of amino acids and reducing sugars present in honey. It also might be due to the variations in thermal sensitivity of the foods and processing conditions (Manzocco et al., 2001).

The brown pigment formation can be unfavourable for intermediate moisture, dehydrated or concentrated foods (Burdulu and Karadeniz, 2003). On the contrary, browning can be preferred in the manufacturing of coffee and baking of bread for improvement of colour and flavour (Shaker et al., 1995). In the case of honey, the brown pigment formation can be an advantage because previous works have reported that Maillard reaction products which include brown pigment are associated with antibacterial activity (Aljadi and Yusof, 2003).

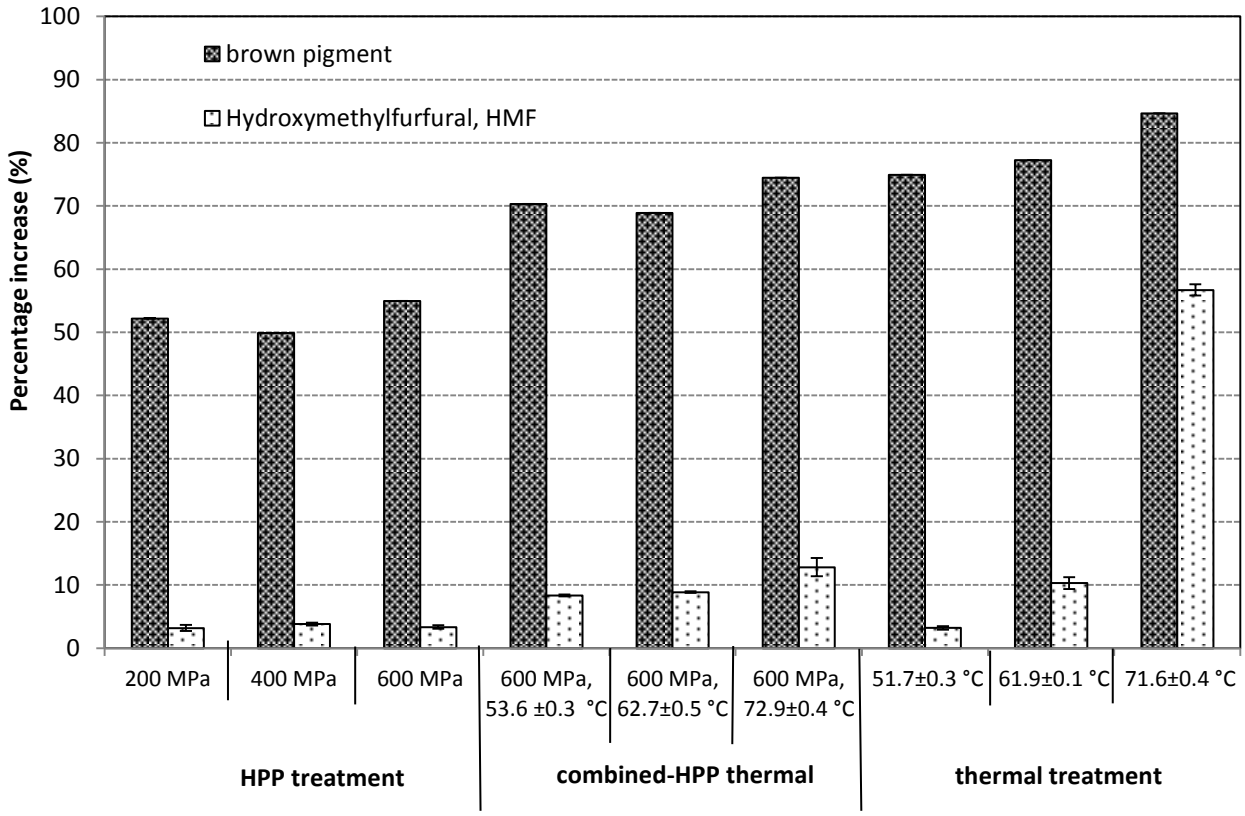


Figure 5.1: Percentage increase of brown pigment and hydroxymethylfurfural content in HPP- treated honey sample (ambient temperature) at different pressure in comparison to combined HPP-thermal and thermal treatment for 10 min treatment time. The values are means ± standard deviation with n = 2. The error bars were not clearly visualized in the graph due to the small variation (0.001 - 0.008). Untreated sample is a reference sample.

5.3.2 Influence of treatments on antibacterial activity

Figure 5.2 shows the percentage inhibition of *S. epidermidis* at different concentrations of Manuka honey investigated, ranging from 0.1 to 50% (w/v). From the result obtained, the honey concentration between 10% and 20% (w/v) gave the minimum inhibitory concentration of at least 95% inhibition (MIC_{95}) with 95.96 ± 0.41% and 95.50 ± 2.12% inhibition respectively. Since it is difficult to notice any change in antibacterial activity at concentrations higher than 10% w/v (because these concentration resulted in nearly 100% and 100% inhibition), 5% (w/v) of honey concentration, which gave 65% inhibition is chosen to investigate the effect of HPP and thermal treatment on the antibacterial activity.

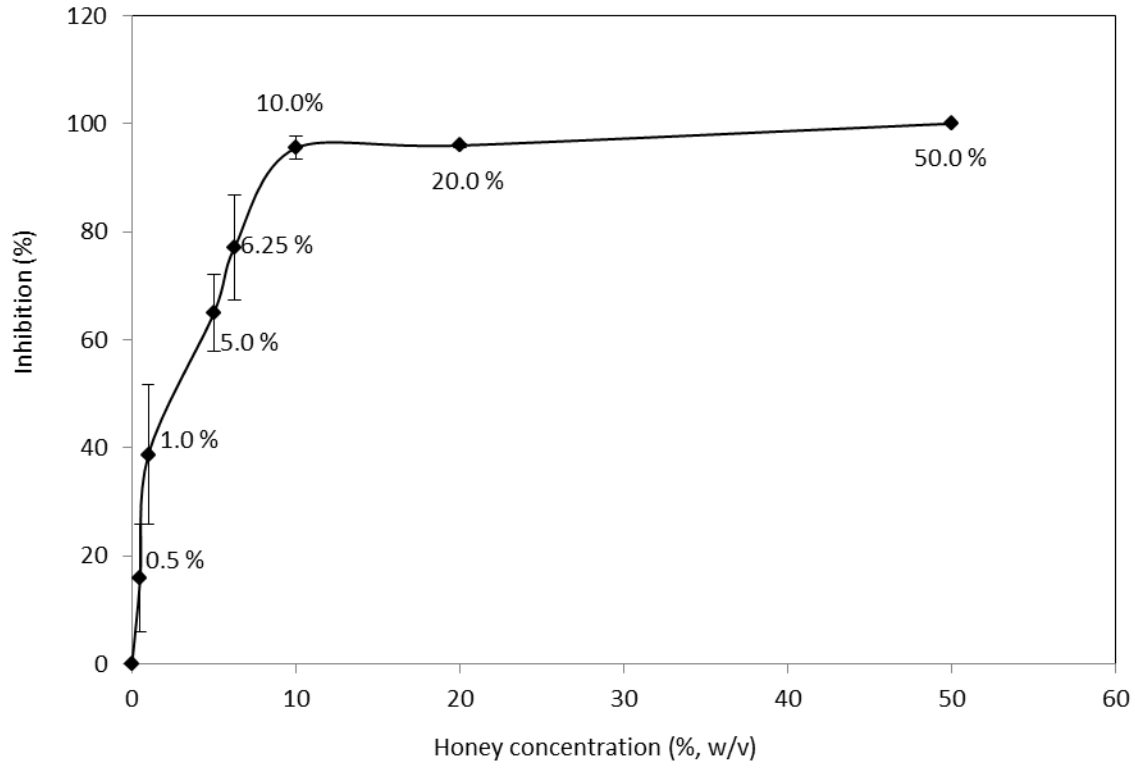


Figure 5.2: Percentage inhibition of *S. epidermidis* at different concentration of Manuka honey. The values are means ± standard deviation with n = 2

The percentage inhibition of *S. epidermidis* in HPP treated Manuka honey at different pressures level of 200, 400 and 600 MPa (with 83.71 ± 4.68%, 84.43 ± 6.01% and 84.34 ± 7.62%, respectively) for 10 min treatment showed no significant difference ($p > 0.05$) amongst them. However, it shows a significant increase in inhibition ($p < 0.05$) as compared with untreated honey, 65.0 ± 7.07% (Figure 5.3). The increase in antibacterial activity in HPP-treated samples is might be due to the molecular structure change of some compounds after subjected to high pressure. The conjugated systems of double bonds present in flavonoids and long chain in polyphenols compounds might be affected since HPP can enhance chemical and biochemical reactions through both desired and undesired modifications (Oey et al., 2008). In addition, Aljadi and Yusof (2003) noted that the antibacterial activity may results from the interactions of polyphenols, flavonoids and Maillard reaction products. Tannin which is high molecular weight phenol is known as an antimicrobial component in honey that can be affected by HPP. Since the total phenolic content and flavonoids are predictive markers of the antioxidant activity in honey (Estevinho et al., 2008), the change in these compounds will then affect honey antibacterial activity.

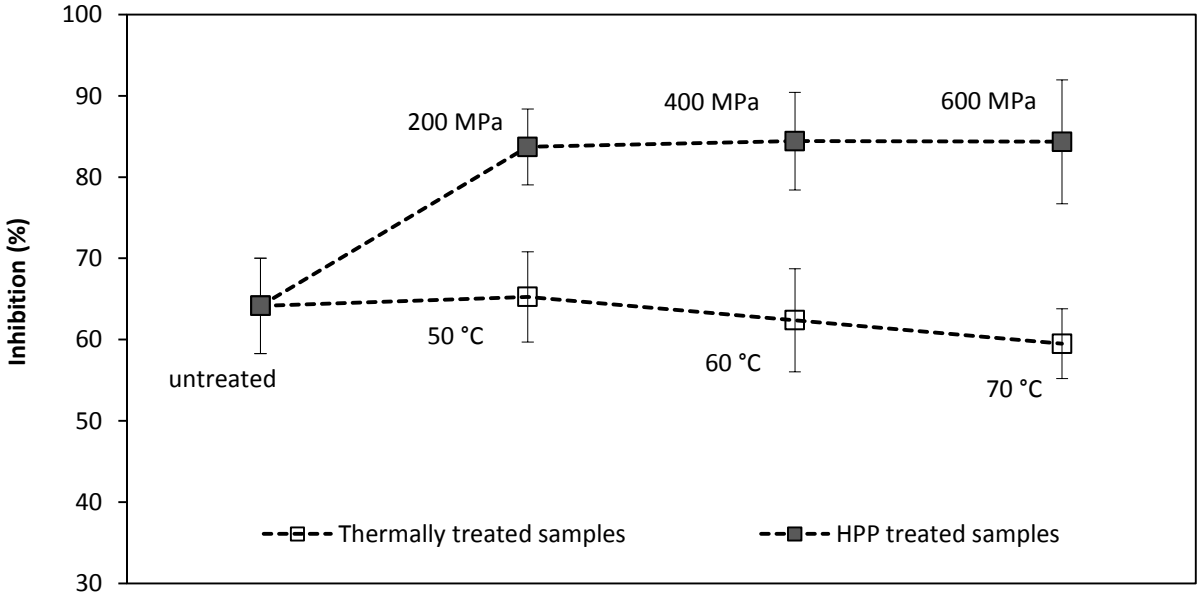


Figure 5.3: Percentage inhibition of *S. epidermidis* in HPP- treated (200, 400 and 600 MPa) and thermally treated Manuka honey (50, 60 and 70°C) for 10 min. The values are in means ± standard deviation with n = 2.

Although Al- Habsi and Niranjan (2012) suggested the condensation reaction of DHA might have occurred during HPP (800 MPa/ 25°C/15 min) which then leads to the increase of methylglyoxal, MGO (compound responsible for antibacterial activity), Grainger et al. (2014) have shown that HPP does not accelerate the conversion of DHA to MGO. It can thus be suggested that, the possible increase in antibacterial activity as affected by HPP might be due to release of MGO reversibly bound to amino acids or protein. Generally, HPP can cause conformational changes and denaturation of proteins which the tertiary structure can be affected when pressure was applied (Hendrickx et al., 1998). This might be related to the more availability in MGO. Further work is needed to verify this hypothesis.

On the contrary, the percentage inhibition of *S. epidermidis* is slightly decreased when Manuka honey was subjected to thermal treatments of 50°C (65.26 ± 5.56%), 60°C (62.38 ± 6.34%) and 70°C (59.49 ± 4.29%). However, no significant difference ($p > 0.05$) was observed as compared with untreated. The highest temperature of 70°C exhibited the lowest percentage inhibition. This is in agreement with Al-Habsi and Niranjan (2012) who found that honey losses its antibacterial activity when exposed to higher temperature. Likewise, Rios et al. (2001) has observed that, at lower temperature of 40°C, the antibacterial activity of honey was not affected.

5.3.3 Influence of treatments on hydroxymethylfurfural content

HMF value for untreated Manuka honey was registered as 17.16 ± 0.60 mg/kg, which is lower than the maximum allowed limit of 40 mg/kg as recommended by European Legislation (EC Directive 2001/110).

The HMF content of Manuka honey as affected by different treatments is presented in Table 5.1. All treated samples showed no significant increase ($p > 0.05$) in HMF concentration as compared with untreated, except for combined HPP-thermal and thermally treated sample (70 °C). HMF concentration in HPP-treated samples at ambient temperature showed the lowest HMF content (16.93 to 18.76 mg/kg) as compared with other treatments. This is in agreement with earlier study (Al- Habsi and Niranjan, 2012) where HPP operating at 25°C for different pressure levels did not influence HMF concentration. A possible explanation for this is that high pressure has insignificant effect on acid-catalysed dehydration of hexose, the reaction in which HMF is formed. This hypothesis is related to the effect of HPP on sugars/polysaccharides in honey, which need further work to verify.

Regarding thermal treatment, the result shows a significant difference ($p < 0.05$) in HMF concentration when sample was thermally treated at higher temperature of 70°C (Table 5.1). The percentage increase of HMF at this temperature is registered as 56.71 ± 0.88% (Figure 5.1), which showed the highest as compared with other treatments. This is consistent with earlier study (Fallico et al., 2004), showing that at higher temperature, the reaction will be accelerated more.

Overall, the result showed that there is no correlation ($r = 0.342$, $p > 0.05$) between HMF concentrations and pressure levels tested (200, 400 and 600 MPa). However, HMF formation during HPP treatment was significantly influenced ($p < 0.05$) by treatment time, where 30 min give the highest increase in HMF formation at all pressure levels tested (Figure 5.4).

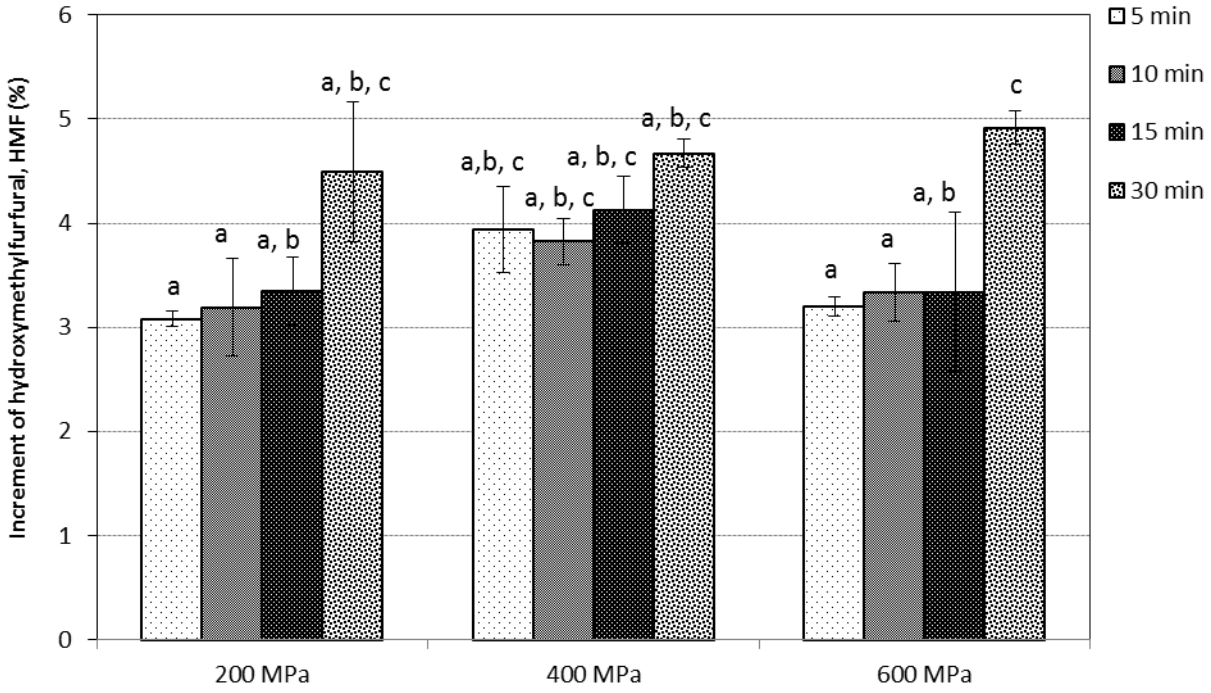


Figure 5.4: Percentage increase of HMF in HPP- treated honey sample (ambient temperature) at different pressure and different processing time; a, b and c values are means ± standard deviation. Different letters are significantly different according to Tukey's HSD test with n = 2.Untreated sample is a reference sample.

Besides processing temperature and time, the increase in HMF is related to chemical properties of honey, its botanical origin and also due to acidic decomposition of monosaccharide, specifically fructose (Raminez Cervantes et al., 2000; Singh and Bath, 1998). Different honey sources vary in their fructose to glucose ratio, where it has been known that HMF formation is faster from fructose than glucose because fructose enolizes faster than glucose (Lee and Nagy, 1990). Another reason for increase in HMF is might be due to the condensation of carbohydrates that have free amine groups, according with the well-known Maillard reaction.

5.3.4 The relationship between the brown pigment formation, antibacterial activity, antioxidant activity and hydroxymethylfurfural

A significant relationship between antioxidant activity, Maillard reaction products, phenolic content and colour in raw honey has been reported by previous investigators (Brudzynski and Miotto, 2010). However, the correlation between these compounds in HPP- treated honey is yet unknown. Theoretically, the functional properties of honey including antibacterial activity may results from the interactions of biologically active groups of compounds such as polyphenols, flavonoids and Maillard reaction products (Brudzynski and Miotto, 2011). Whereas, Shaker et al. (1995) reported that Maillard reaction products have antioxidant activity.

We have previously shown that the total phenolic content and antioxidant activity were significantly increased when honey sample was subjected to HPP (Akhmazillah et al., 2013; Fauzi et al., 2014). Thus, the increase of antibacterial activity in this study could implicate brown pigment and antioxidant as conferring growth inhibitor activity against *S. epidermidis*. The novel observation made in this study was a strong and significant ($p < 0.05$) relationship between antibacterial activity, antioxidant activity and brown pigment formation of HPP-treated samples as revealed by correlation matrix (Table 5.2). However, no correlation was observed between these three variables and HMF content.

Table 5.2 Correlation matrices for four variables (antibacterial activity, brown pigment, HMF and antioxidant activity) of high pressure-treated honey using Pearson correlation.

	Antibacterial activity	Brown pigment	HMF	Antioxidant activity
Antibacterial activity	1.0000	*0.9404	0.4486	*0.8635
Brown pigment	*0.9404	1.0000	0.1934	*0.8826
HMF	0.4486	0.1943	1.0000	0.5032
Antioxidant activity	*0.8635	*0.8826	0.5032	1.0000

*Marked correlations are significant at $p < 0.05$

As tabulated in Table 5.2, a strong correlation between antibacterial activity and brown pigment was found. Whereas, the antioxidant activity (measured as DPPH scavenging activity from previous work, Fauzi et al. (2014)) correlated reasonably well with antibacterial activity and brown pigment formation. This finding suggests that brown pigment formation leads to higher antibacterial activity of *S. epidermidis* and antioxidant activity in HPP-treated Manuka honey.

5.4 Conclusions

The application of HPP (600 MPa/ambient temperature/ 10 min) significantly increased the brown pigment formation and antibacterial activity against of *S. epidermidis*. New information is presented on the strong and significant correlation between brown pigment and antibacterial activity of HPP-treated Manuka honey. HPP treatment at ambient temperature is beneficial in controlling HMF concentration during treatment, particularly at shorter time of less than 30 min. HPP technology has no adverse effect on the quality of Manuka honey, particularly HMF content.

CHAPTER 6

High Pressure Processed Manuka Honey: Change in Nutritional and Rheological Properties over One Year Storage

Contents submitted to publication.

Chapter Abstract

To elucidate the permanent effect of high pressure processing (HPP) on the quality of Manuka honey, the quality properties such as physicochemical (moisture content, pH, total soluble solids and total solids), nutritional (total phenolic content, antioxidant activity and brown pigment formation) and flow behaviour as well as viscosity of HPP-treated Manuka honey were tested after storage. The process condition of HPP (600 MPa at ambient temperature for 10 min) was selected and treated samples were stored at 25°C in a dark, dry place for one year. Periodically, a portion of honey was taken out and analysed. The result shows that the physicochemical properties (moisture content, pH and total solid) of HPP-treated honey were changed but statistically insignificant ($p > 0.05$) after one year. However, significant increases ($p < 0.05$) in total soluble solids were found in untreated and HPP-treated samples with 7.27% and 8.17%, respectively. An increase in antioxidant activity, total phenolic content and brown pigment during storage were found in HPP-treated samples with a maximum value of 90.24 ± 2.4%, 111.19 ± 4.25 mg GAE/kg and 0.26 ± 0.02 AU, respectively. HPP was able to able to maintain flow behaviour with insignificant increase ($p > 0.05$) in viscosity. In conclusion, HPP-treated sample stored at room temperature retained an acceptable overall quality for one year storage which confirmed that the positive effect of high pressure on honey is not a temporary effect.

6.1 Introduction

Consumer demand has increasingly requiring minimally processed foods with high nutritional quality and extended storage. In honey processing, conventional thermal treatment is known to have a detrimental effect on the quality of honey (specifically antioxidant activity) due to its effect on the unstable and thermolabile honey components (Nagai et al., 2001). As such, high pressure processing (HPP), a novel non-thermal treatment has recently been applied to honey. It is known that HPP has been widely used in food processing since it has shown to keep foods original freshness and improve its functionalities (Butz, 2010) due to the almost instantaneous isostatic pressure transmission in the system (Ghani and Farid 2010). Previous studies demonstrated the HPP capability to increase nutritional properties of Manuka honey, namely total phenolic content and antioxidant activity, while maintaining its rheological properties and colour (Akhmazillah et al., 2013; Fauzi et al., 2014). HPP was also found to control the marker of honey age/ heating used to downgrade honey in the commercial marketplace known as hydroxymethylfurfural, HMF by slowing down its formation under HPP conditions (Al-Habsi and Niranjan 2012).

The quality of honey might change when it is stored for long time. The effect of storage of unprocessed and thermally processed honey on its quality is well documented (Bath and Singh, 1999; Cavia et al., 2002; Cherchi et al., 1997; Gheldof and Engeseth 2002; Jiménez et al., 1994; Lynn et al., 1936; Ramirez Cervantes et al., 2000; Wang et al., 2004; White et al., 1961), however, there has been some contradictory findings related to impact of storage on the quality of honey. Some have reported a reduction in antioxidant activity of thermally processed clover and buckwheat honey after 6 months of storage (Wang et al., 2004). While, no significant alteration in antioxidant activity of clover honey over two years storage has been reported (Gheldof and Engeseth, 2002). Brudzynski and Kim (2011) have reported the chemical changes in colour, Maillard reaction products and antibacterial activity of unprocessed honey for a period of 1-3 years. These findings may be due to the use of various type of honey having different sugar contents, physicochemical characteristics, phytochemical compositions and storage conditions (Cavia et al., 2002; Jiménez et al., 1994; Rabahah et al., 2014; White et al., 1961).

Antioxidant activity, total phenolic content and brown pigment are the components in honey which has direct impact on nutritional value (Aljadi and Yusoff 2003; Aljadi and Kamaruddin, 2004; Cao et al., 2011; Wang et al., 2004). Although there is sufficient evidence that HPP could improve the quality of honey (Akhmazillah et al., 2013; Al-Habsi and

Niranjan, 2012; Fauzi et al., 2014), however, up to date there is no information on how these properties change during storage. Therefore, the aim of this work was to find if the significant improvement in antioxidant activity, total phenolic content and brown pigment formation of HPP-treated honey is permanent or not, following one-year of storage at room temperature (where honey is normally stored). The effect of storage on physicochemical and rheological properties of high pressure processed Manuka honey will also be studied.

6.2 Material and Methods

6.2.1 Honey samples preparation

Untreated Manuka honey used in this study was kindly donated by Comvita®, Auckland, New Zealand. The jar of honey sample was collected directly from beekeepers and sourced from Manuka tree (*Leptospermum scoparium*), a native tree of New Zealand.

Honey (5 g) was packed in 5 cm x 5 cm transparent plastic film pouches (Cas-pak plastic vacuum pouch, Silverdale, New Zealand) and thermo sealed under vacuum. This formed very thin pouch of 3 mm thick to ensure no temperature distribution within the honey sample. The plastic film is made of cast polypropylene for excellent transparency and heat sealing qualities. These pouches can withstand temperatures up to 125°C, being suitable for high pressure processing and with low oxygen transmission rate (1.3×10^{-3} g/ m^2/day).

6.2.2 High Pressure Processing (HPP) equipment

The HPP unit used in this research was QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Kent, WA, USA). The equipment consists of a 2- litre cylindrical-shaped pressure treatment chamber (inner height = 0.25 m, inner diameter = 0.10 m) with a thermocouple, water circulation, a pumping system and a control system operated through a computer with software supplied by the manufacturer. Distilled water was used as the medium in the chamber where the packed honey samples were placed. The equipment can operate at maximum pressure and temperature of 690 MPa and 90°C, respectively. The treatment time was the pressure holding time and did not include the pressure come up and the decompression times. The temperature inside the pressure chamber during treatment was monitored using thermocouples (located at mid and top of the vessel), which were immersed

in the pressure medium (distilled water). After processing, the packed honey samples were immediately cooled in ice water before analysed. All honey samples were taken from the same honey batch and every single treatment was repeated twice.

6.2.2.1 HPP of honey at ambient temperature

Five grams of vacuum-packed Manuka honey samples were subjected to HPP with pressures of 600 MPa, at close to ambient temperature for 10 min. It may be noted that the process condition was chosen considering the optimum condition obtained from previous works (Akhmazillah et al., 2013; Fauzi et al., 2014). Pressure come-up time was approximately 1.5 min while the decompression time was < 20 s. The adiabatic heating of 600 MPa gave an average processing temperature of 30.18 ± 1.98°C during the holding pressure period. After processing, the packed honey samples were immediately cooled in ice water before analyzed. All honey samples were taken from the same honey batch and every treatment was repeated twice.

6.2.3 Monitoring changes in honey properties during storage of one year

Upon arrival to the laboratory, honey samples were vacuum sealed packaged (as mentioned in honey preparation sample section), processed using HPP and stored at room temperature (approximately at 25°C), in the dark for 1 year. For unprocessed sample, vacuum-sealed honey was directly stored in the same place. At certain time intervals, a portion of honey was analysed for its nutritional and rheological properties. The changes of physicochemical properties were determined between two time points: at the arrival to the laboratory and only after 12 months storage. Unlike physicochemical, the nutritional and rheological properties were analysed periodically (at 5, 11 and 12 months) for monitoring the trend during storage, which is a main concern of this study.

6.2.4 Physicochemical Analysis

The following physicochemical properties (moisture content, total soluble solids, total solids, pH, and electrical conductivity) of honey were measured in duplicate. The results were reported as mean ± standard deviation.

6.2.4.1 Moisture content, total soluble solids and total solids

The moisture content and total soluble solids were measured using a RX-5000a digital refractometer (Atago®, London, UK) at 20°C. The moisture content was indirectly assessed via measuring the refractive index, *nD* of the sample. The water content corresponding to the refractive index was calculated using Wedmore's table from International Honey Commission (Bogdanov, 2002). The total soluble solids of the samples were expressed in °Brix whereas the percentage total solid was determined from measured moisture content (Amin et al., 1999)

6.2.4.2 pH measurement

pH of honey samples were measured using a pH meter (Mettler Toledo, Leicester,UK) for a 10% (w/v) solution of honey prepared in milliQ water (Brandstead Easy Pure II, Thermoscientific, Ohio, USA) as proposed in Harmonised Methods of the International Honey Commision (Bogdanov, 2002).

6.2.4.3 Electrical conductivity

The measurement of electrical conductivity is defined as that of 20% weight in volume solution in water at 20°C, where 20% refers to honey dry matter (Bogdanov 2002). The electrical conductivity of honey sample was measured at 20°C in a 20% (w/v) honey solution in milliQ water (Brandstead Easy Pure II, Thermoscientific, Ohio, USA) using conductivity meter (OAKTON, CON 10 series, USA) as described by Bogdanov et al. (1997). The electrical conductivity of milliQ water is 5.5 x 10^{-5}mS/cm. The readings were expressed in mS/cm.

6.2.5 Nutritional properties analysis

The following nutritional properties of honey (antioxidant activities, total phenolic content and brown pigment) were measured in duplicate. The results were reported as mean ± standard deviation.

6.2.5.1 Antioxidant activity

The antioxidant activity of the sample was determined according to procedure previously described in Section 4.2.4.1 in Chapter 4. The experiment was repeated twice. Antioxidant activity (DPPH scavenging activity) was expressed as percentage inhibition of the DPPH radical and was determined by the following equation (Yen and Duh, 1994):

$$AA\ (\%) = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} x100 \qquad (6.1)$$

where AA (%) is the antioxidant activity in percentage, $Abs_{control}$ is the absorbance reading of the control and Abs_{sample} is the absorbance reading of the sample

6.2.5.2 Total Phenolic Content

Total phenolic content in samples was measured according to procedure previously described in Section 3.2.4 in Chapter 3. The honey samples were measured in duplicate and were reported as mean ± standard deviation. The total phenolic content was expressed in mg of gallic acid equivalents (GAE)/100 g of honey. The experiment was repeated twice

6.2.5.3 Brown pigment

The method for determination of brown pigment formation was measured according to procedure previously described in Section 5.2.3 in Chapter 5. The experiment was repeated twice.

6.2.6 Rheological behaviour and viscosity

Apparent viscosities of samples were measured using AR-G2 rheometer (TA Instrument, USA) which was connected to a computer with software (TA Instrument Advantage™ software, USA). The measuring system consists of Smart Swap Geometry with 40 mm, 2° steel cone. The method was adapted from Yanniotis et al. (2006) with some modification. Honey samples of 2 g were poured onto the sample plate. The rotational speed was increased to provide a shear rate in the range of 0.1 to 35.0 s^{-1}, with a controlled temperature of 25°C. Shear stress and viscosity were measured and recorded at different shear rates. The experiment was repeated twice.

6.2.7 Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the means. Differences were considered significant at $p < 0.05$. The separation of treatments means was carried out with Tukey honestly significant difference (HSD) test. All statistical analyses were perform with Statistica, version 11 (Statsoft® Texas, Austin, USA) and Microsoft Excell ® (2010 Microsoft©, Redmond, WA, USA).

6.3 Results and Discussion

6.3.1 Changes in physicochemical properties

As tabulated in Table 1, the results show that moisture content, pH and electrical conductivity remained within the international standard limit of European Legislation (EC Directive 2001/110) and Codex Almentarius Commission after 12 months storage, which states that the moisture content limit is ≤ 20%, pH (3.2-4.5) and electrical conductivity (< 0.8 mS/cm).

The moisture content, pH and total solids in untreated and HPP-treated samples were changed but statistically insignificant after storage ($p > 0.05$). Over one year, moisture content was decreased from 16.41 ± 1.30% to 15.68 ± 1.02% for untreated sample. Similar reduction was observed for HPP-treated sample from 16.46 ± 0.12% to 15.92 ± 0.20% was found at the end of storage. This is in agreement with Assil et al. (1999) findings which showed a decrease in moisture content in processed liquid honey, however, the percentage change is higher (about 9%) after one year. Whereas, a relatively smaller change in moisture content of coconut honey have been reported by Ghazali and Kin (1986) after stored at 28 ± 2°C and 50 ± 2°C for 18 weeks. The insignificant changes in moisture content could be attributed to the moisture permeability of the packaging material (Assil et al. 1999). Dissimilar with moisture content, the pH was slightly increased about 0.16 unit pH (untreated sample) and 0.33 unit pH for HPP-treated sample. Whereas, the percentage increase in total solids were registered as 3.75% and 5.08% for untreated and HPP-treated samples respectively. No significant change ($p > 0.05$) was observed in the electrical conductivity after one year storage.

Apart from the above properties, total soluble solids in both samples were significantly affected by storage. Untreated and HPP-treated samples showed a significant increase ($p < 0.05$) in total soluble solids of 7.27% and 8.17%, respectively after storage.

Table 6.1: The physicochemical properties of untreated and HPP-treated Manuka honey (600 MPa at ambient temperature for 10 min) after 12 months storage at room temperature (approximately 25°C). Values are presented as mean ± standard deviation of two measurements.

	Untreated sample		HPP-treated sample	
	Initial	After 12 months	Initial	After12 months
Moisture content (%)	16.41 ± 1.30[a]	15.68 ± 1.02[a]	16.46 ± 0.12[a]	15.92 ± 0.20[a]
pH	3.88 ± 0.20[a]	4.04 ± 0.12[a]	3.80 ± 0.08[a]	4.13 ± 0.04[a]
Electrical conductivity (mS/cm)	0.47 ± 0.03[a]	0.50 ± 0.01[a]	0.49 ± 0.02[a]	0.51 ± 0.02[a]
Total soluble solid (°Brix)	79.67 ± 0.50[a]	85.00 ± 0.46[b]	79.07 ± 0.30[a]	85.53 ± 0.21[b]
Total solid (%)	83.54 ± 1.98[a]	86.64 ± 0.67[a]	82.89 ± 2.69[a]	87.02 ± 0.15[a]

[a.b] Mean values (means ± standard deviation) within the same row with different letters are significantly different according to Tukey's HSD test (Statistica version 11, Statsoft®)

6.3.2 Changes in nutritional properties: antioxidant activity, total phenolic content and brown pigment formation

The antioxidant activity and total phenolic content of untreated and HPP-treated samples increased during storage, and it seems the same trend with untreated sample throughout the year (Figure 6.1a and 6.1b). The significant increase in antioxidant activity and total phenolic content after HPP treatment, which is before storage (Akhmazillah et al., 2013; Fauzi et al., 2014) did not show any significant difference ($p < 0.05$) as compared with after 12 months storage.

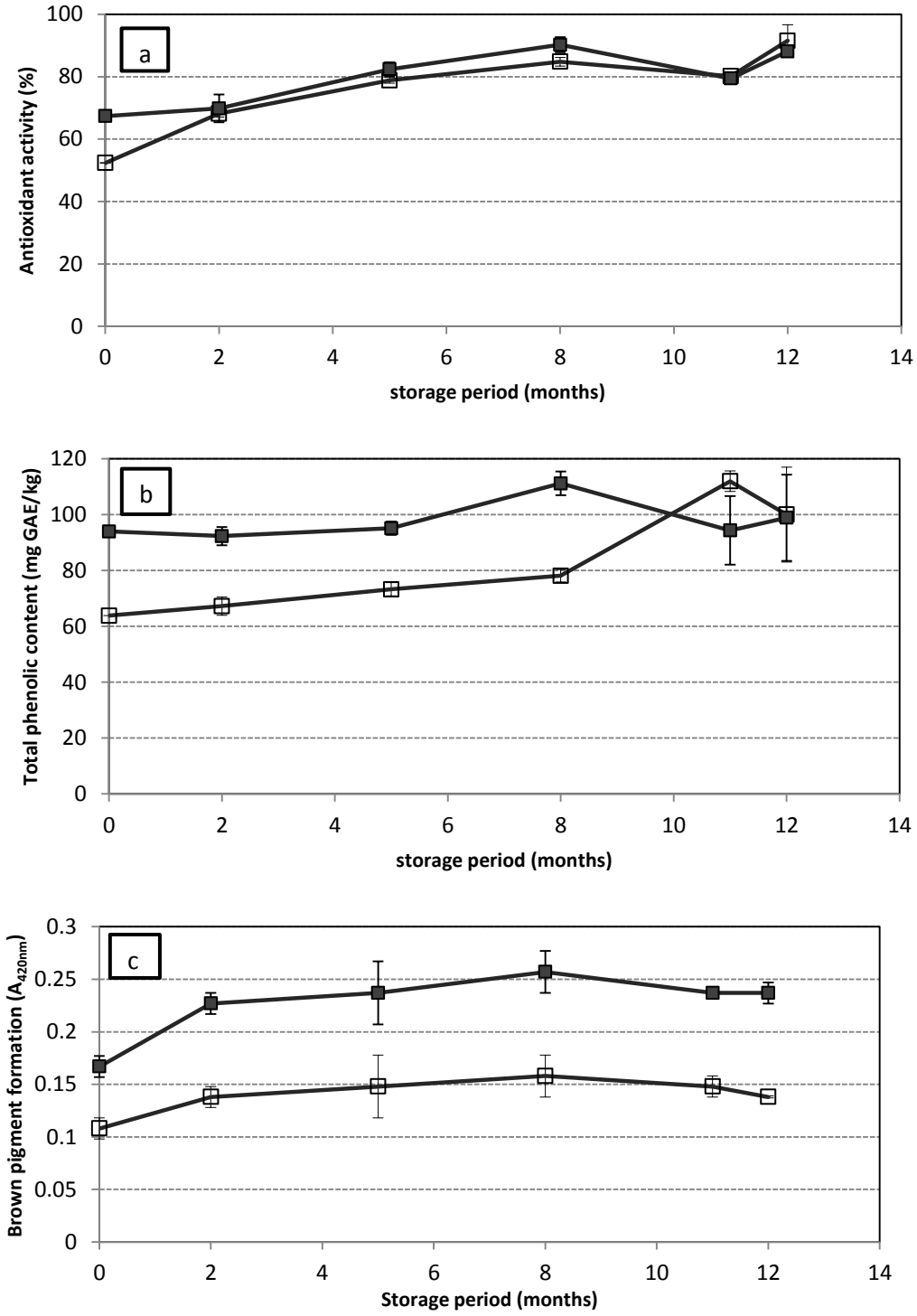


Figure 6.1: Changes in (a) total phenolic content, (b) antioxidant activity and (c) brown pigment formation in (□) untreated and (■) HPP-treated honey during 12 months storage. Values are presented as mean ± standard deviation of two measurements. Error bars represents standard deviation.

The increase in antioxidant activity and total phenolic content of both samples supports the previous finding of Polydera et al. (2004) who described the total antioxidant activity as a function of storage time. The increase in antioxidant activity and total phenolic content during storage may be explained by the fact that prolonged storage time can promote or enhance the progressive enzymatic or chemical oxidation of phenolic compounds (Nicoli et al., 1997). With respect to effect of processing of other food products, it is known that HPP can cause conformational changes and denaturation of proteins which then render the phenolic compounds more available (Gould, 2001b; Yaldagard et al., 2008). Another possible explanation for this is the formation of Maillard reaction products (MRP's) as a consequence of long storage. This MRP's generally exhibited strong antioxidant properties (Nicoli et al., 1997) which can be attributed to the high molecular weight brown compounds formed in the advanced stage of the reaction (Anese et al., 1999). Further experiments on proteins and Maillard reaction need to be done in order to verify this hypothesis.

The percentage increase in antioxidant activity and total phenolic content in both samples during one year storage is shown in Figure 6.2a and Figure 6.2b. Although HPP-treated honey exhibited a higher antioxidant activity (90.24 ± 2.4%) and total phenolic content (111.19 ± 4.25 mg GAE/kg) during storage, the percentage increase recorded is lower (35.8 % for antioxidant acivity and 5.22% for total phenolic content) than untreated honey (74.81 % for antioxidant acivity and 56.66% for total phenolic content). In contrast to earlier findings, Wang et al. (2004) reported that antioxidant capacity of heat-processed clover and buckwheat honey reduced by approximately 26% and 24%, respectively after 6 months of storage (at room temperature). Whereas, Gheldof and Engeseth (2002) indicated no significant alteration of antioxidant capacity in clover honey over long term (> 2 years) storage. These different findings show that the impact of processing and storage on honey antioxidant activity could vary depending on the type of honey. This is due to the complicated chemical composition that varies between honeys from different floral sources (Wang et al., 2004).

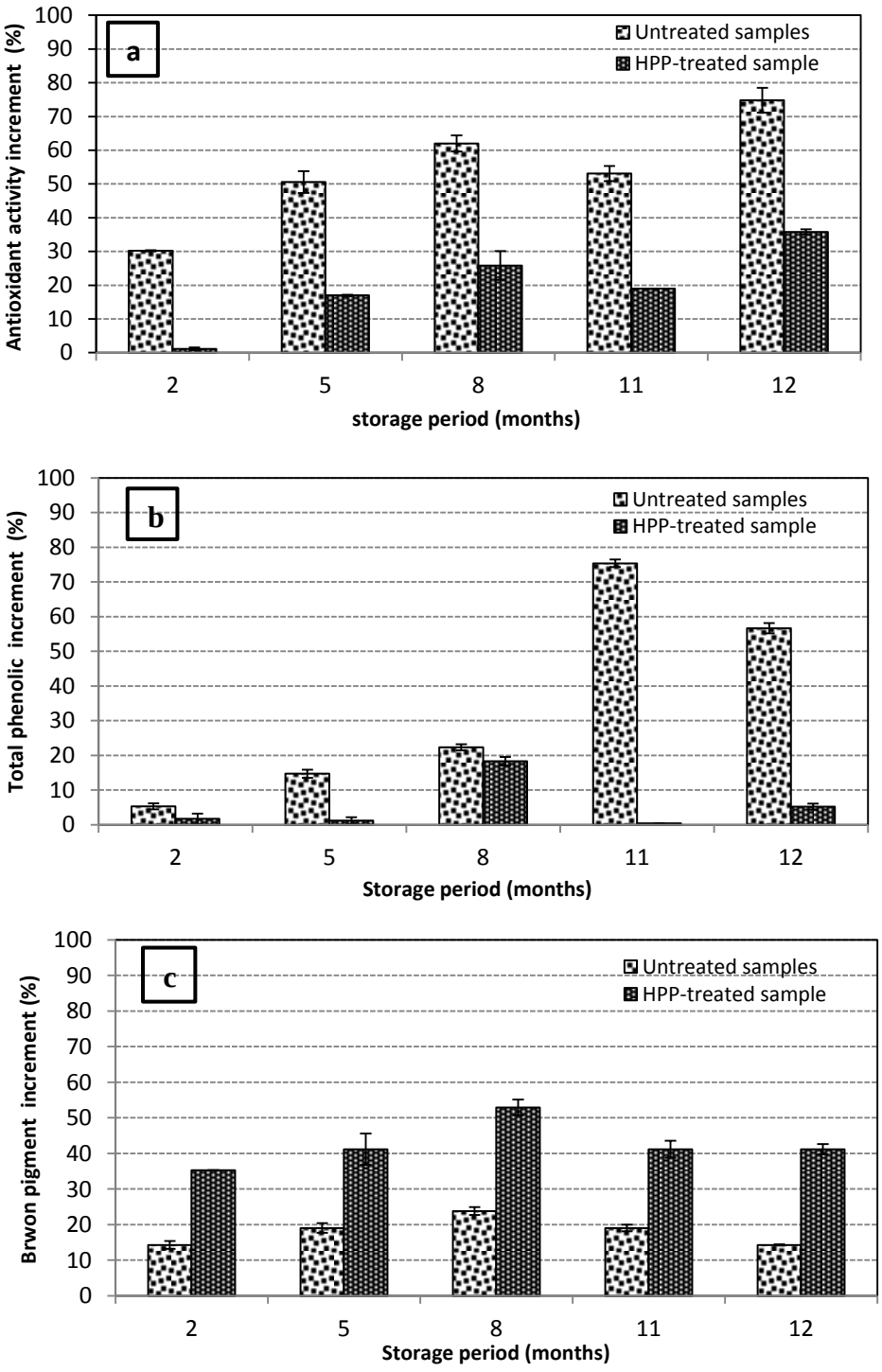


Figure 6.2: Percentage increase in a) antioxidant activity, b) total phenolic content and c) brown pigment formation of untreated and HPP-treated honey during 12 months storage. Values are presented as mean ± standard deviation of two measurements. Error bars represents standard deviation.

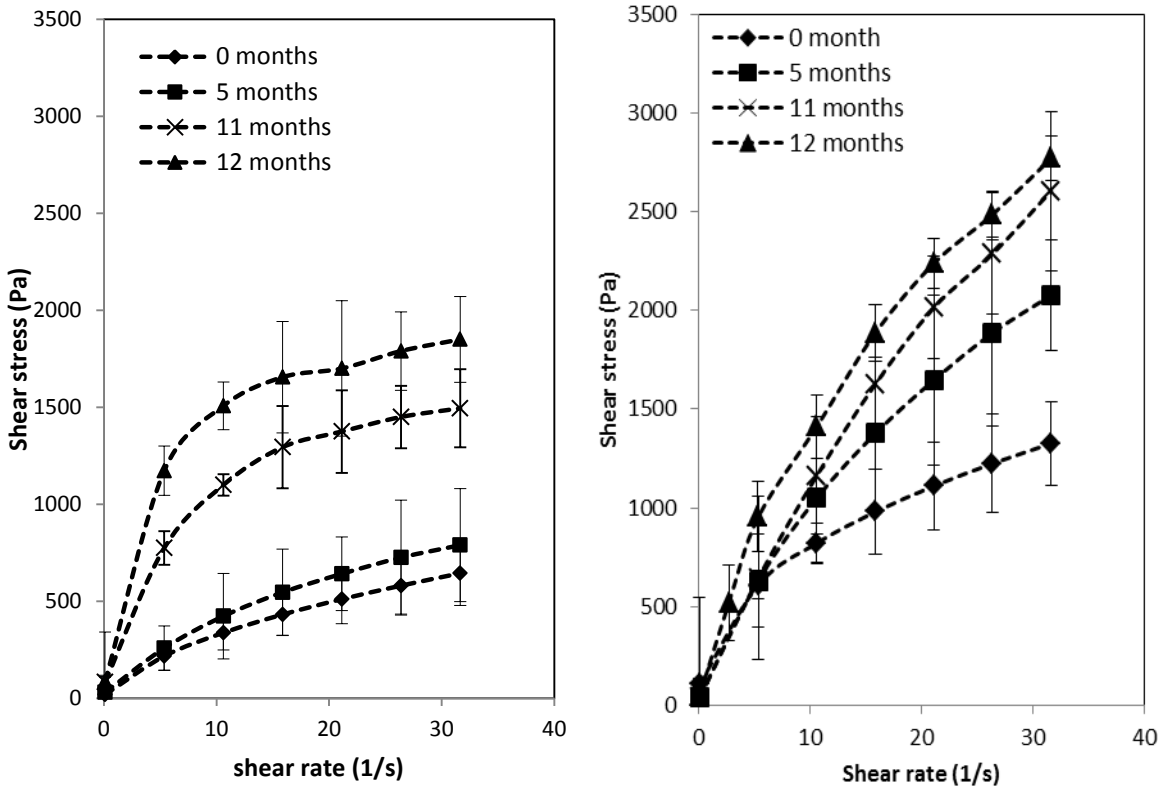
The brown pigment formation can be unfavourable for intermediate moisture, dehydrated or concentrated foods (Burdulu and Karadeniz 2003). However, the brown pigment formation in honey, caused by Maillard reaction is associated with antibacterial activity (Aljadi and Yusoff, 2003). HPP-treated samples (600 MPa/ambient temperature/10 min) showed a higher but constant level in brown pigment formation (within the range of 0.16-0.26 AU) as compared with untreated samples (0.10-0.16 AU) throughout storage period (Figure 6.1c). A possible explanation for this might be the possible pressure enhancement to Maillard reaction. It has been reported that Maillard reactions are enhanced at pressures as high as 600 MPa, under certain pH condition (Hill et al., 1996). Further study is needed to verify this.

Theoretically, Maillard reaction caused the formation of brown pigments with antioxidant properties which then minimised the loss of natural antioxidants and even enhanced the overall antioxidant properties in honey, which has been proved by previous work (Fauzi et al., 2013). Besides, it also might be due to the effect of HPP on the release of brown pigment from protein complexes (Dede et al., 2007). Although formal conclusion can not be drawn from this study, these results suggest further works which may provide insights to the effect of HPP on protein structure in pressure-treated honey. Dissimilar to total phenolic content and antioxidant activity, the percentage increase in brown pigment in HPP-treated honey is considerably higher than untreated honey throughout storage with 41.18% of increment after 12 months. (Figure 6. 2c).

HPP- treated honey not only have high phenolic content, antioxidant activity and brown pigment formation as compared with untreated honey, but also these properties are considerably stable over long storage period. These increases in total phenolic content, antioxidant activity and brown pigment formation are in agreement with previous work reported by Brudzynski and Miotto (2011).

6.3.3 Changes in rheological properties and viscosity

The flow properties of honey are influenced by several factors such as its composition, processing and storage temperature and also the amount and size of crystals present in honey. Our previous study has shown that the untreated and HPP-treated Manuka honey displayed a non-Newtonian behaviour which is shear thinning behaviour (Fauzi et al., 2014), where the value of flow behaviour index "n" is less than 1. The present study showed that there was no change in shear thinning behaviour of untreated and HPP-treated Manuka honey samples after 12 months storage at room temperature as shown in Figure 6.3.

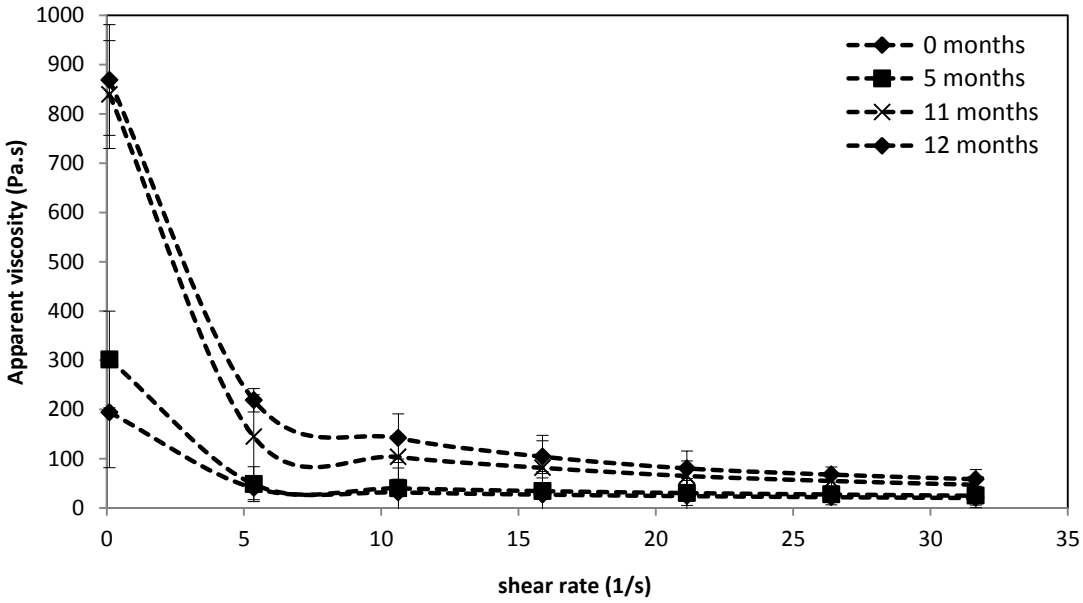


(a) (b)

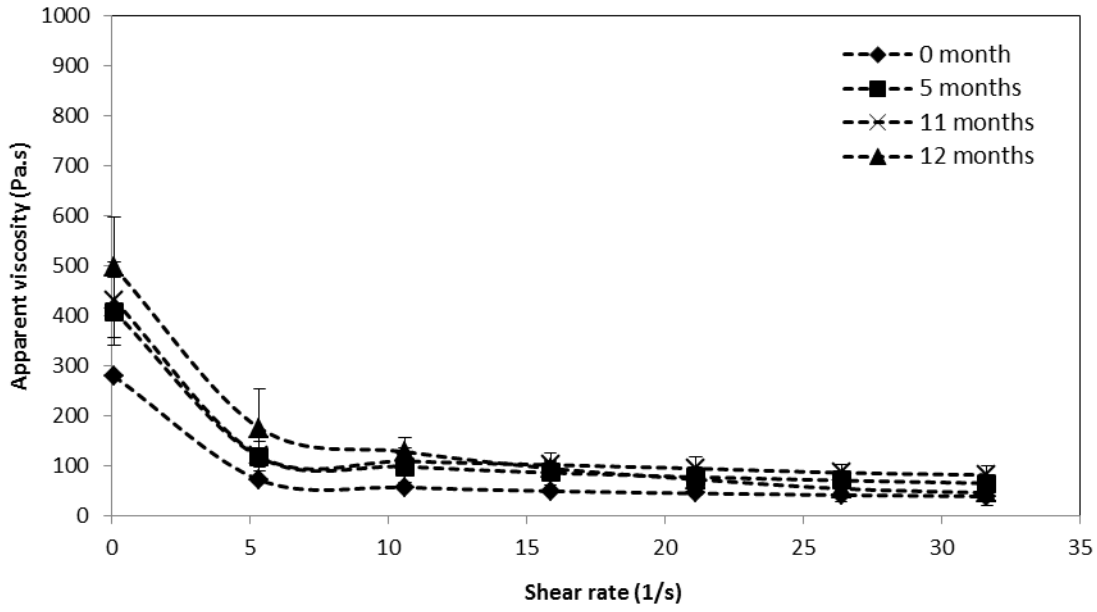
Figure 6.3: Flow curves of (a) untreated honey and (b) HPP-treated honey at different storage time. The storage time is indicated as ♦ = 0 month; ■= 5 months; χ = 11 months and ▲= 12 months. Values are presented as mean ± standard deviation of two measurements. Error bars represents standard deviation.

The apparent viscosity change of untreated and HPP-treated honey as a function of shear rate (1/s) during 12 months storage is illustrated in Figure 6.4. The apparent viscosity of the two samples was largest at lower shear rate but it then decreased with an increase in shear rate. Comparing both samples, untreated honey showed clearer change in apparent viscosity throughout the storage period, whereas, no remarkable change was found in HPP-treated samples. Referring at the lowest shear rate (0.1 s^{-1}), untreated and HPP-treated honey showed a maximum apparent viscosity at 12 months storage with value of 868.27 ± 72.4 Pa.s and 496.78 ± 100 Pa.s, respectively (Figure 6.4).

Table 6.2 compares the apparent viscosity of untreated and HPP-treated samples at lower shear rate of $0.1s^{-1}$ (when usually honey is consumed) during storage. The apparent viscosity for untreated samples increased considerably with storage time (from 195.46 ± 89.39 to 868.27 ± 72.4 Pa.s), where a significant increase ($p < 0.05$) was found during 11 months storage. The apparent viscosity increment in untreated honey samples might be due to the composition of individual sugars namely fructose and glucose which are converted into more complex disaccharides during storage, as has been reported by previous researchers (Chirife and Buera, 1997; White et al., 1961). White et al. (1961) found that when untreated honey is stored for two years at temperatures between 23-28°C, an average of 18.5% of the free monosaccharide content (13% of free glucose and 5.5% of free fructose) is lost**.** It was also reported that, reducing disaccharides sugar has increased to about 69% of the amount initially present. This is due to high sugar concentration and considerable acidity over a period of time, which would promote the combination of monosaccharide. The active enzymes in honey such as α- glucosidase and transglucosylase play an important role in the formation of reducing disaccharide (maltose) and accumulation of oligosaccharides material**.** A higher amount of disaccharides sugars as compared with monosaccharide in stored honey will contributes to a higher viscosity due to the increased complexity of sugars (White et al., 1961). Theoretically, honey is sugar syrup and its viscosity characteristics can be governed by the molecular chain length of sugar present in it.



(a)



(b)

Figure 6.4: Effect of storage time on apparent viscosity of (a) untreated (b) HPP-treated Manuka honey during storage, starting at shear rate of 0.1 s^{-1}. The storage time is indicated as ♦ = 0 month; ■ = 5 months; χ = 11 months and ▲= 12 months. Values are presented as mean ± standard deviation of two measurements. Error bars represents standard deviation.

On contrary, there was an insignificant change ($p > 0.05$) in apparent viscosity of HPP-treated honey at different storage period. A possible explanation for this might be that the rate conversion of monosaccharide to disaccharides in high pressure treated honey is less pronounced during storage. Theoretically, disaccharides are formed when two monosaccharides are joined together by glycosidic bond (is a type of covalent bond) which is not affected by HPP treatment (Bermude and Barbosa, 2012). Pressure usually enhances hydrogen bonds and weakens hydrophobic interactions and salt bridges (Masson, 1992). The observed insignificant increase of viscosity in HPP-treated samples could be also attributed to the insignificant changes in moisture content during storage. Higher moisture level results in reduction of viscosity (Bhandari et al., 1999).

Table 6.2: Apparent viscosity of untreated honey and HPP-treated honey at shear rate 0.1 s^{-1} during 12 months storage. Values are presented as mean ± standard deviation of two measurements.

Storage time	Honey samples	
	untreated honey	HPP-treated honey
0 month	195.46 ± 89.39[a]	279.50 ± 0.05[a]
5 months	311.35 ± 18.60[a]	408.95 ± 69.07[a]
11 months	839.35 ± 32.45[b]	431.80 ± 75.24[a]
12 months	868.27 ± 72.4[b]	496.78 ± 100.[a]

[a,b] Mean values (means ± standard deviation) within the same column with different letters are significantly different according to Tukey's HSD test (Statistica version 11, Statsoft®).

In conclusion, both untreated and HPP-treated honey maintained their flow behaviour after 12 months of storage. However, the increase in apparent viscosity is more intense and significant ($p < 0.05$) in untreated samples as compared with HPP-treated samples (Figure 6.5). This support the fact that no significant alteration in the consistency of HPP- treated honey occurs during storage, which is an important finding since viscosity affects the consumer acceptance.

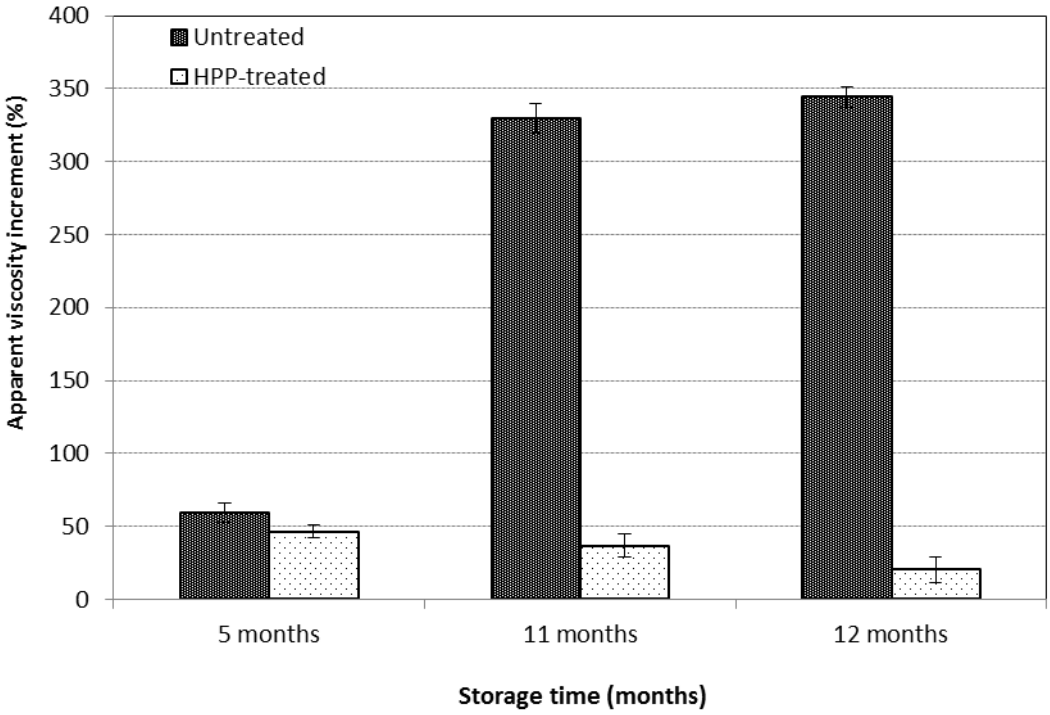


Figure 6.5: Percentage increase of viscosity of untreated and HPP-treated honey (600 MPa, 10 min, ambient temperature) at shear rate $0.1s^{-1}$ during 12 months storage. Values are presented as mean ± standard deviation of two measurements. Control samples are the samples before storage, for untreated and HPP-treated, respectively.

6.4 Conclusions

Overall, the results demonstrated that one year storage can increase the nutritional properties in both untreated and HPP-treated Manuka honey. However, HPP-treated honey exhibited a higher antioxidant activity (90.24 ±2.4%) and higher total phenolic content (111.19 ± 4.25 mg GAE/kg) as compared with untreated sample. Whereas, brown pigment showed an increment of 41.18% after 12 months of storage. No significant changes in flow behaviour and viscosity were observed in HPP-treated honey over one year storage. The important finding form this study is that the effect of HPP treatment (600 MPa/ 10 min/ ambient temperature) on nutritional properties of honey was not temporary.

CHAPTER 7

An Insight on the Relationship between Food Compressibility and Microbial Inactivation during High Pressure Processing

Contents submitted to publication.

Chapter Abstract

This paper investigates the effect of liquid food compressibility on the high pressure inactivation of a microorganism. Honey with various adjusted sugar contents, ° Brix and hence different compressibility was selected as a model food while *Saccharomyces cerevisiae* was selected as the testing microorganism. Higher ° Brix products experience baroprotection against pressure treatment. *S.cerevisiae* cells, in different honey concentrations from 0 to 80° Brix, subjected to 600 MPa pressure (at ambient temperature), showed an increasing resistance to inactivation with ° Brix. Whereas, the *D*-values of *Saccharomyces cerevisiae* cells at different pressure of 200, 400 and 600 MPa for 2 to 30 min were registered as 137.0, 29.2 and 23.5 min, respectively. A significant correlation ($p < 0.05$) between cell reduction, ° Brix and compressibility was found. Cell reduction in high pressure-treated sample varied linearly with ° Brix, suggesting that the effect is not solely due to sugar content, but also due to its compressibility.

7.1 Introduction

High pressure processing (HPP) has been widely used in food industry, since it has shown to keep food original freshness, enhance its functionalities and improve its shelf-life (Butz, 2010; Denys et al., 2000; Ghani and Farid, 2007). Ramaswamy and Balasubramaniam (2007) stated that HPP food pasteurization could result in a change in thermodynamic and rheological properties due to compression heating that impact safety and quality.

The efficacy of high pressure treatment is known to depend on the composition of the food; mainly sugars, fats and salts (Molina-Höppner et al., 2004; Senhajit & Loncins, 1977). High solute concentration food has a baroprotective effect on microorganism cells undergoing pressure treatment (Basak et al., 2002; Goh et al., 2007; Isaacs et al., 1995). Baroprotective effect of increased solute concentration could be attributed to the lower water activity (Palou et al., 1997). Oxen and Knorr (1993), Satomi et al. (1995), Palou et al. (1997) and Mousse et al (2006) showed the pressure sensitivity of microorganism such as *E. Coli*, *Rhotodorula rubra* and *Zygosaccharomyces baiili* were highly dependent on the water activity of the system. Also, high concentration of sucrose and sodium chloride (Glaasker et al., 1998; Molina-Höppner et al., 2004) and nature of membrane lipids and proteins (Crowe et al., 1997; Goh et al., 2007; Iwahashi et al., 1997; Lesli et al., 1995) are contributed to the baroprotective effect on microorganism cells.

The use of high pressure treatment for the inactivation of pathogenic microorganisms is well documented (Alpas et al., 1999; Shoa & Ramaswamy, 2011), however only limited discussion has been made on the baroprotective effects of sodium chloride and sugar in microorganisms (Oxen and Knorr, 1993; Hashizume et al., 1995; Palou et al., 1997). Also, there has been little discussion about the inactivation mechanism of spoilage microorganisms such as osmophilic yeasts in a very high sugar content food such as honey. However, it has been reported that pressure resistance of yeasts and moulds increased as sugar concentration (sucrose, fructose, and glucose) increased in the media containing them (Ogawa et al., 1990; Oxen and Knorr, 1993; Palou et al., 1997). Meanwhile, Hashizume et al. (1995) has reported that sucrose does not protect against pressure inactivation of yeasts when pressurized at sub-zero temperatures, while it protects from the inactivation when pressurized at ambient temperature.

Moussa et al (2006) described the parallel change with pressure and temperature of protein behaviour, microbial inactivation and water structure. They also described the involvement of water compression during pressure treatment on the cell's inactivation. The

limited free volume between molecules contributes to the high compressibility of liquids at low pressure (Bridgman, 1970; Isaac, 1981).

The increase in solute concentration (° Brix, referring to sugar content) of liquid product decrease its compressibility (Min et al., 2010). Min et al. (2010) also showed that increasing pressure significantly decrease liquid compressibility, which is due to the loss of its secondary and tertiary structure, as well as pressure effect on the hydrogen bonding and carbohydrate composition (Cano and de Ancos, 2005; Li and Akasaka, 2006; Shimada et al., 1993). As pressure increases, free volume decreases and compressibility becomes governed by the molecule's inherent compressibility and attractive and repulsive interactions with nearby molecules. Min et al. (2010) concluded that variability in compressibility of different materials is probably due to differences in concentration, chemical composition and complex interaction between components within a food system.

Although there are a number of studies concerning the baroprotective effect of increased solute concentration on osmophilic microorganism (yeasts cells) inactivation during high pressure processing (Basak et al., 2002; Campos and Cristianini, 2007; Goh et al., 2007; Hashizume et al., 1995; Parish, 1998), it is not clear yet if the cells protection is completely due to the baroprotective effect of solute concentration or due to the decrease in its compressibility or both. The study on compressibility of foods under pressure by Min et al. (2010) leads to the hypothesis that, compressibility might contribute to the microbial inactivation in high viscous pressure-treated food.

This paper, therefore, aims to investigate if compressibility of honey (as a model food) has a major influence on the inactivation of *S.cerevisiae* (as the testing microorganism) after subjecting it to high pressure processing, which has not been investigated before. The effect of different sugar contents (° Brix) on cells destruction in high pressure-treated honey will be presented. The study will also establish the effect of compressibility and sugar concentration (° Brix) on the inactivation of *S. cerevisiae*. The thermal treatment at 55°C for 10 min (which is conventionally applied in honey pasteurisation in industry) was carried out for comparison purpose. This research could have significant implications on the success of high pressure treatment of food containing high sugars content.

7.2 Materials and Method

7.2.1 *Saccharomyces cerevisiae* strain and growth medium

Saccharomyces cerevisiae was chosen as testing microorganism due to its osmophilic characteristic, which makes it withstand low water content and high sugar concentration. *S.cerevisiae* ATCC 2601 (American Type Culture Collection, Rockville, MD, USA) was obtained freeze dried from Fort Richard Laboratories, Otahuhu, New Zealand. *S. cerevisiae* cells were prepared by pre culturing them on yeast extract peptone dextrose (YPD) agar plates (BD Difco, North Ryde, Australia) at 30°C for 2 days. Following this process, a yeast colony was transferred into 20 mL YPD broth (BD Difco, North Ryde, Australia) contained in 50 mL Erlenmeyer flask using a sterilized inoculation loop. The flask was then continuously agitated (150 rev min^{-1}) at 30°C in a temperature-controlled shaker (Excella E24 Incubator Shaker Series, USA) for overnight. This constituted the stock cell culture. Inocula was prepared from the overnight culture by resuspending 5 mL of stock solution into 100 mL fresh YPD broth and incubated with shaking (150 rev min^{-1} at 30°C) until it reached stationary phase (24 h). The cell growth was also checked by measuring the absorbance (A_{660nm} = 1.0) in order to ensure a similar inoculum level in all experiments (~10^8 CFU/ mL).The cells were washed three times by centrifugation at 10°C for 5 min at 4000 x g, and sedimented cells were aseptically resuspend in a sterilized distilled water before stored at 4 °C until use (less than 24 hours).

7.2.2 Honey inoculation and packaging

Unprocessed Manuka honey used in this study (with pH of 4.3 and 80° Brix) was kindly donated by Comvita® New Zealand. Honey and transparent plastic film pouches used to pack the honey were sterilized at 121°C for 15 min to ensure the samples were free from any microorganisms before being inoculated. Then, 0.2 mL of *S. cerevisiae* cell suspension with initial count of 10^8 CFU/mL was added aseptically to 2 g of the sterilized honey packed in 5 cm x 5 cm sterilized transparent plastic film pouches. This formed a very thin pouch of 3 mm thick to ensure an even distribution of the cells when samples were manually compressed using fingers and also to minimise temperature distribution within the samples. These pouches can withstand temperatures up to 125°C, being suitable for high pressure processing

and present low oxygen transmission rate (1.3×10^{-3} g/ m^2/day). The sample pouches were then thermosealed under vacuum (Vacuum sealer C200, MULTIVAC, Germany).

7.2.3 Preparation of honey samples with different concentration

Honey samples with different sugar concentrations (20, 30, 40, 50, 60 and 70° Brix) were prepared aseptically using sterilized distilled water. The degree of soluble solids (in ° Brix) for each dilution was measured using a RX-5000a digital refractometer (Atago®, London, UK) at a constant temperature of 20°C. No dilution of honey was made for the 80° Brix sample. While, for 0°Brix (control), sterilized distilled water was used.

7.2.4 High pressure processing equipment and operation

The HPP unit used in this research was QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Kent, WA, USA). The equipment consists of a 2- litre cylindrical-shaped pressure treatment chamber (inner height = 0.25 m, inner diameter = 0.10 m) with a thermocouple, water circulation, a pumping system and a control system operated through a computer with software supplied by the manufacturer. Distilled water was used as the medium in the chamber where the packed honey samples were placed. The equipment can operate at maximum pressure and temperature of 690 MPa and 90°C, respectively. The treatment time was the pressure holding time and did not include the pressure come up and the decompression times. The temperature inside the pressure chamber during treatment was monitored using thermocouples (located at mid and top of the vessel), which were immersed in the pressure medium (distilled water). The compression time, decompression time and average temperature were based on the cycle report which is directly obtained from a control system operated through a computer with software. It is very reasonable to assume that honey samples temperature is very close to the measured surrounding water temperature due to the thin pouches used as discussed before.

7.2.4.1 HPP of honey samples

Duplicate of 2 g vacuum-packed honey samples were subjected to different conditions of HPP (200-600 MPa of pressure, 2-30 min of treatment time). The treatment time was the holding pressure time and did not include the pressure come up and the decompression times, which were of limited effect in most of the experiments. Pressure come up times were of the order of 1.0 min and the decompression time was less than 30 s. The adiabatic heating at 200, 400 and 600 MPa gave average processing temperatures of 26.80 ± 2.1°C, 30.18 ± 1.14 °C and 32.6 ± 2.1°C, respectively during the holding pressure phase. The maximum temperature attained did not exceed 35 °C, which is well within the growth range for the *S. cerevisiae* and hence would not contribute to inactivation. After the treatment, the samples were immediately cooled in ice water. All honey samples were taken from the same honey batch and every single treatment was repeated for three times.

7.2.5 Thermal processing of honey

The thermal processing in the absence of high pressure was performed at 55°C for 10 min using a thermostatic water bath, W28 (Grant Instruments, Cambridge, England). It may be noted that the process condition was chosen considering the condition which is normally applied industrially and also the condition obtained from preliminary works (55°C for 10 min treatment time gave an acceptable range of cell inactivation to compared with high pressure treatment). The vacuum-sealed samples were fully submerged into the distilled water bath. A setting temperature of 55°C resulted in an average temperature of 55 ± 0.10 °C. After each treatment, all samples were immediately placed in ice-cooled water before analysis. As in HPP treatment, the high surface area of the pouches bags compared to the small volume of the honey packed, enhanced the quick heat transfer and minimized temperature distribution within the sample (come up time was less than 50 sec). All honey samples were taken from the same honey batch and every single treatment was repeated for three times.

7.2.6 *Saccharomyces cerevisiae* cells enumeration

S. cerevisiae cell numbers in honey samples were counted before and after treatment. The number of surviving cells was determined after a proper dilution of the untreated/treated samples by the plate count method. 2 g of each sample was mixed and homogenized with 18

mL of sterilized 0.1% peptone water (0.1 g/L) to prepare the initial dilution, which is used as the mother dilution. Serial dilutions from 10^{-1} to $10^{-7}$of sample were done. Then, 1 mL of each dilution was aseptically inoculated onto YPD (Yeasts peptone Dextrose) agar plates by spread-platting technique. The plating was done in duplicate for each series of dilution. The inoculated plates were then incubated in an incubator (Heraeus, Osterode, Germany) at 30 °C for 48 hours. Then, microbial growth was examined and colonies were counted using a colony counter (Suntex Colony Counter 570, Suntex Instrument Co., New Taipei City, Taiwan). Only plates having 30-300 colonies were used for analysis. The colonies were counted as CFU/mL:

$$\frac{(Count\ plate1 + Count\ plate\ 2)}{2} \times dilution\ factor \tag{7.1}$$

7.2.7 Compressibility measurement

Compressibility- pressure data for various sugar concentrations was obtained from the literature. Compressibility of honey solution at different ° Brix and pressure levels was determined by re-plotting the graphs obtained by Min et al (2010). The graphs of compressibility versus pressure for different sucrose concentration (0, 2.5%, 10%, 50% and 80%) were re-plotted in the form of compressibility - ° Brix diagram.

7.2.8 Statistical analysis

One-way analysis of variance (ANOVA) was used to compare the microbial survivor's means. Differences were considered significant at $p < 0.05$. The separation of treatments means was carried out with the Tukey honestly significant difference (HSD) test. The Pearson correlation was performed using Statistica version 11, Statsoft® software (Statsoft® Texas, Austin, USA). Other analyses were also performed using Microsoft Excell ® 2010 (Microsoft©, Redmond, WA, USA).

7.3 Results and Discussion

7.3.1 Compressibility and ° Brix during high pressure processing

Previous study has shown that an increase in concentration (° Brix) significantly ($p < 0.05$) decreased sucrose solution's compressibility over the entire pressure range (Min et al., 2010). ° Brix refers to the sugar content of an aqueous solution, where 1 ° Brix is 1 gram of sucrose in 100 grams of solution. In this study, we assumed that the compressibility is approximately the same for glucose and fructose. To understand the relation between compressibility and ° Brix and their effect on the destruction of microorganisms through the application of high pressure treatment, previous measurements from Min et al (2010) were re-plotted in the form of compressibility - ° Brix diagram as shown in Figure 7.1. A very good correlation between compressibility and ° Brix at the tested pressures were found with a correlation coefficient, r better than 0.98 (Table 7.1).

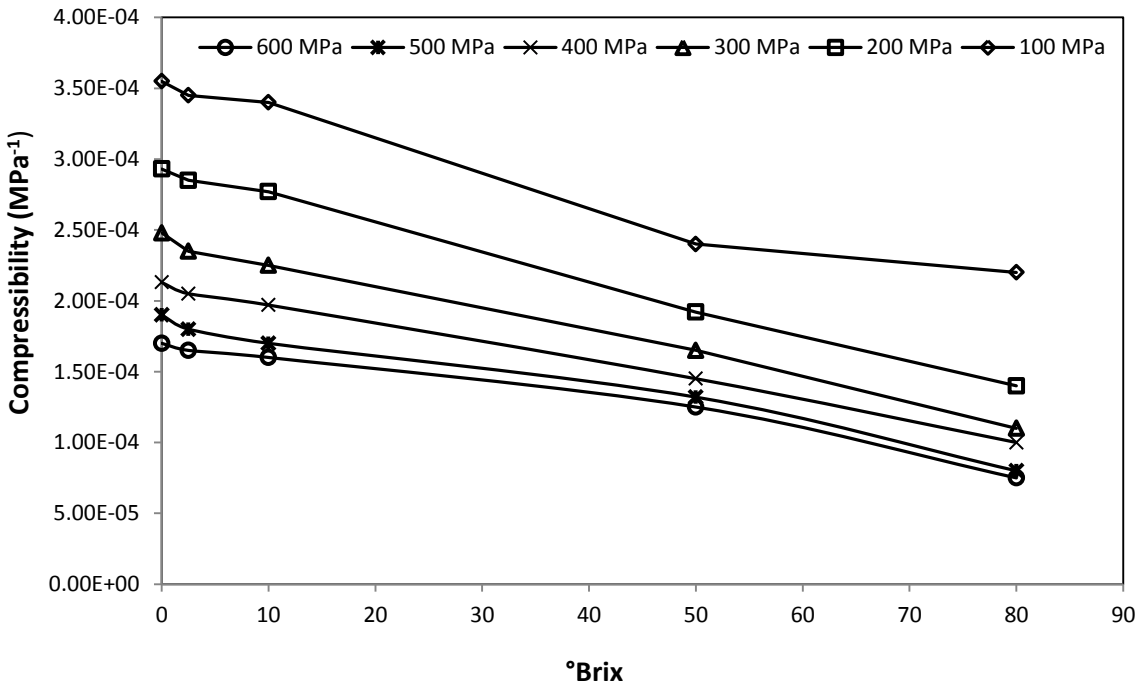


Figure 7.1 Compressibility of honey solution at different pressure levels as a function of °Brix. Data were taken from Min et al. (2010)

Figure 7.1 shows that increasing pressure significantly ($p < 0.05$) decreased compressibility for each ° Brix tested, which is expected. Bridgman (1970) and Isaacs (1981) stated that the relatively high compressibility of liquids at low pressure results from considerable free volume between molecules. Theoretically, as pressure increases, free volume decreases and compressibility becomes governed by the molecule's inherent compressibility as well as attractive and repulsive interactions with nearby molecules. Meanwhile, increasing sucrose concentration (° Brix) decreased solution compressibility over the entire pressure range. This is because, compressibility at lower sugar concentration was likely dominated by water compressibility with little solute effect. Ogawa et al. (1990) and Oxen and Knorr (1993) reported a higher sensitivity of microorganisms to pressure inactivation in the presence of lower concentration of solutes.

Table 7.1: Correlation between compressibility (MPa^{-1}) and ° Brix at different pressure levels after subjected to high pressure processing (at 25°C). The data were reassembled from Min et al (2010).

Pressure (MPa)	**Equation**	**R^2**	**Correlation, r**
100	y = -2E-06x + 0.0004	0.959	-0.979
200	y = -2E-06x + 0.0003	0.998	-0.999
300	y = -2E-06x + 0.0002	0.995	-0.997
400	y = -1E-06x + 0.0002	0.998	-0.999
500	y = -1E-06x + 0.0002	0.982	-0.991
600	y = -1E-06x + 0.0002	0.976	-0.988

y = Compressibility (MPa^{-1}); x= ° Brix

Our preliminary work demonstrated that model food having 80° Brix (honey) had baroprotection against pressure treatment at 200, 400 and 600 MPa (at ambient temperature), where it showed less than 1-$\log_{10}$ reduction in *S.cerevisiae* even at 600 MPa and processing time of 20 min (Figure 7.2). This is in agreement with previous studies conducted on the fruit juices and sucrose syrup (Basak et al., 2002; Campos and Cristianni, 2007; Goh et al., 2007; Parish, 1998), where at higher ° Brix, microbial inactivation showed significant resistance to pressure.

This observation may be explained by the fact that; higher ° Brix could be attributed to the lower water activity in honey. The survival of cells in lower water activity is due to the cell shrinkage which causes thickening of the cell membrane, reducing the membrane permeability and protecting the cells from destruction by high pressure (Palou et al., 1998) and temperature. Another reason might be due to disaccharide (sucrose) which may impose only a transitory osmotic stress because the cells are able to equilibrate the extra- and intracellular concentration of sucrose and lactose (Gibson, 1973; Glaasker et al., 1998).

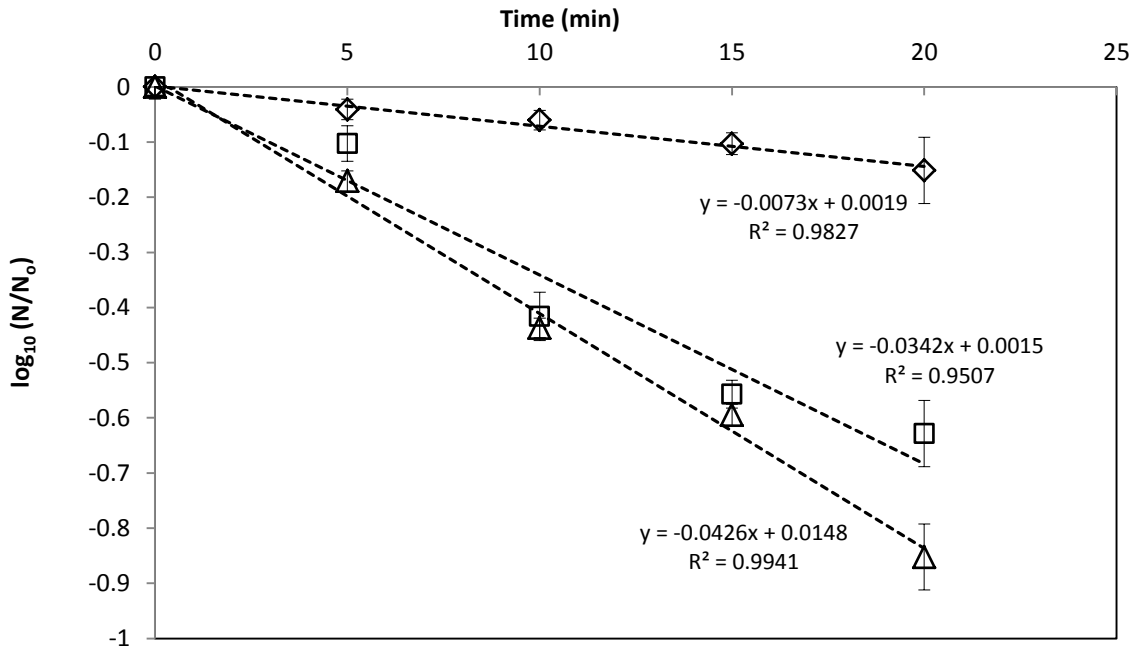


Figure 7.2 *Saccharomyces cerevisiae* cell survivor curves in honey (80 °Brix) subjected to high pressure treatment (ambient temperature) at (i) ◊ = 200; □ = 400 and Δ = 600 MPa. The errors bars are mean ± standard deviation with n = 3.

From the thermodynamics point of view, the mechanical energy transferred to the cell during the pressure treatment can be characterized by the change in volume of the system. The amount of mechanical energy transferred to the cell system is strongly related to the compressibility which depends on the water quantity in the cytoplasm. Water compression is involved in the antimicrobial effect of high pressure (Moussa et al., 2006). An increase in water content in cytoplasm (higher a_w) results in an increase in compressibility and thus in the mechanical energy transferred to the cell system. So, more microorganisms will inactivate. On the other hand, lower water content in cytoplasm will reduce the compressibility and thus in the mechanical energy transferred to the cell system. So, less inactivation of microorganism was observed.

Iwahashi et al. (1997), Goh et al. (2007) and Lesli et al. (1995), stated that sugars protect against changes in the physical state of membrane lipids and in the structure of sensitive proteins during physical stresses. Likewise, sucrose lowers the transmission temperature of the membrane by replacing the water between the lipid head groups, which then prevent the phase transition and inhibit the fusion between the liposome due to glass formation. Therefore, the protein is stabilized in its native state and preserving the integrity of the membranes during pressure treatment (Crowe et al., 1997).

Table 7.2 summarizes the effect of high pressure treatment on the inactivation of *S.cerevisiae* in juices and also buffer, as compared with honey (investigated in this study). *D*-value is the time required at a certain temperature to kill 90% of *S.cerevisiae*; whereas Z_p-value refers to the temperature required for one-log reduction in the *D-value*. In comparison with others, honey samples showed the largest *D*- and Z_p-values, indicating higher ° Brix caused cells to have higher resistance to pressure during treatment. This was claimed to be due to the protective nature of the sugar (Goh et al., 2007). However, it is not certain if the low inactivation in *S.cerevisiae* is solely due to the protective nature of sugar or if the decrease in juice compressibility has some contribution. This cannot be confirmed unless sugar solutions with different ° Brix are treated using high pressure, which has been done and results are shown as follows.

Table 7.2 ° Brix effect of solute concentration on *Saccharomyces cerevisiae* cells in various types of medium during HPP. The errors bars are mean ± standard deviation with n = 3.

° Brix	Media/ Food matrices	Process condition			log reduction ($-\log_{10}N/N_o$)	*D*-value (min)	Z_p-value (MPa)	References
		Pressure (MPa)	Temperature (°C)	Time (min)				
10.5	Orange juice	100 200 300	84 (the maximum temperature reached)		0.60 ± 0.20 4.90 ± 1.00 > 5.60 ± 0.03			Campos and Cristianini, 2007
10.7	Orange juice	350 400 450 500		0.02-5		0.63 ± 0.27 0.12 ± 0.03 0.07 ± 0.02 0.02 ± 0.002	106 ± 6	Parish, 1998
11.4	Single strength juice	100 150 200 250	20	30-120 20-80 10 - 40 5 -20		82.2 38.2 26 5.4	135	Basak et al., 2002
42	Concentrated orange juice	200 300 350 400	20	15-60		119 96.8 45.8 23.5	287	
50 55 60	Sucrose syrup in citrate-phosphate buffer	600	ambient temperature (18-20)	0.25- 2	1(15 s) 3 (120 s) < 1 (120 s)			Goh et al., 2007
80	Manuka honey	200 400 600	ambient temperature (25-35)	5,10,15,20	0.15 (20 min) 0.63 (20 min) 0.85 (20 min)	136.99 ± 7.97 29.24 ± 6.44 23.47± 0.86	526 ± 39	Current work

*D-value = reciprocal of the slope log survivors versus time regression line; Z_p-values = reciprocal of the slope of log D versus pressure line

7.3.2 The combined effect of compressibility and sugar protective nature on the inactivation of *Saccharomyces cerevisiae*.

To investigate the osmoprotection phenomenon of sugar concentration on the destruction of *S.cerevisiae* cells in high pressure- treated honey, different concentrations of honey solution up to 80° Brix were studied by subjecting the cells suspensions to 600 MPa (ambient temperature) for 2 and 30 min. Short and long treatment times of 2 and 30 min, respectively, were selected as usually done in HPP treatment. As expected, inactivation was more rapid at lower ° Brix (0, 10, 20 and 30° Brix) where bacteria concentration was below detection limit (not shown). A higher number of surviving cells was observed when cells were pressure-treated in increasing sugar concentration of honey from 40 to 80° Brix (Figure 7.3).

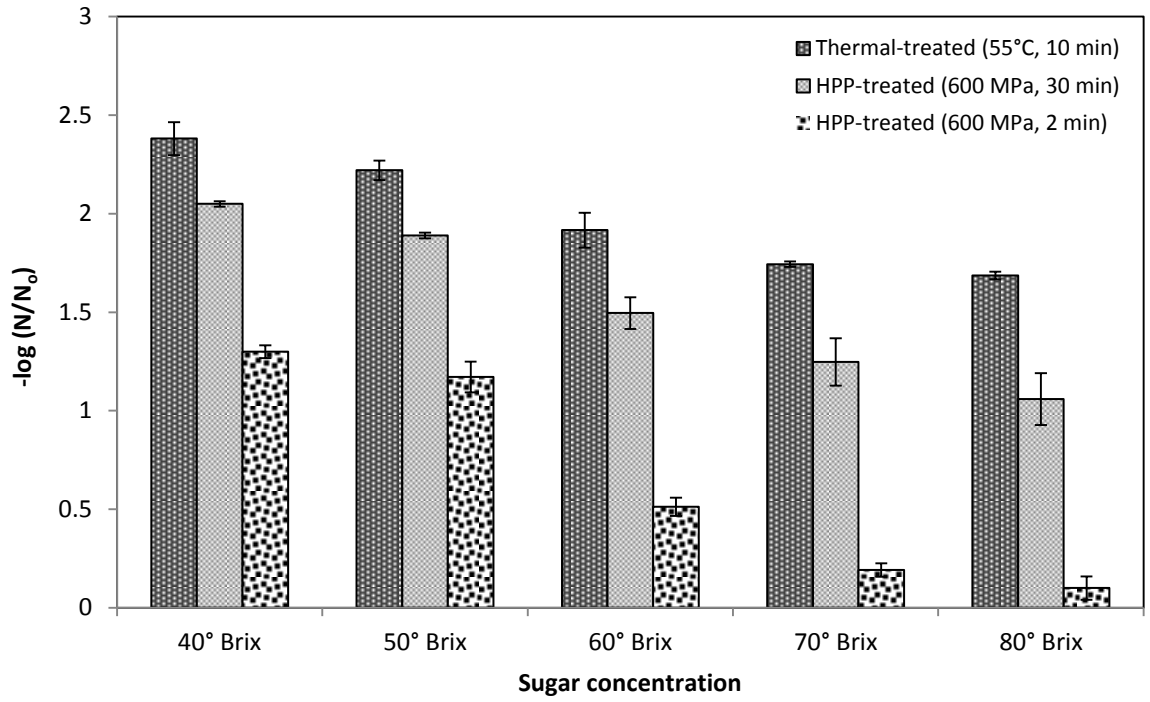


Figure 7.3 Comparison of log reduction of *Saccharomyces cerevisae* after pressure treatment at 600 MPa for 2 and 30 min and thermal treatment at 55°C for 10 min at different ° Brix. Bars represents mean ± standard deviation with n = 3.

The *S.cerevisiae* cells showed a gradual increase in the resistance to pressure treatment with increasing sugar concentration. With an increase in sugar concentration to 60° Brix and above, less than 1.0-$\log_{10}$ reduction (for 2 min treatment) and less than 1.5- $\log_{10}$ reduction (for 30 min treatment) were observed. As expected, longer treatment time of 30 min in high pressure- treated sample showed more cells reduction as compared with 2 min treatment at all honey concentrations. Whereas, the log reduction of *S.cerevisiae* cells in thermal-treated honey (for 10 min treatment) is slightly higher than high pressure-treated honey although longer treatment time (30 min) was applied, reflecting the baroprotective effect of honey concentration during high pressure treatment. Hashizume et al. (1995) reported that sugars, particularly sucrose do protect against pressure inactivation of yeasts when pressurized at ambient temperature, however it is still not clear if the effect is solely due to sugar concentration or due to the decrease in the compressibility as sugar concentration increase.

Delta (Δ) log reduction provides a measurement of the cells sensitivity to the change in the ° Brix, as affected by processes. It is calculated as a difference between log reduction at 40° Brix and log reduction at particular ° Brix (log reduction $_{40\,^\circ\,\text{Brix}}$ - log reduction $_{50/60/70/80\,^\circ\,\text{Brix}}$). A larger value in Δ log reduction indicates a higher microbial sensitivity to sugar changes. Table 2 shows a change in log reduction (Δ log reduction) of *S.cerevisiae* cells with respect to different sugar concentrations for both high pressure and thermal treatment. In general, *S.cerevisiae* cells in high pressure-treated samples (600 MPa/ ambient temp/ 2 and 30 min) are more sensitive to sugar changes than thermally-treated samples (55°C, 10 min), presenting a higher Δ log reduction values (within the range of 0.128 ± 0.01 to 1.151 ± 0.05). The change in cells reduction due to ° Brix change was significantly different ($p < 0.05$) between all treatments, particularly at higher honey concentration of 70 and 80 ° Brix. The highest change in *S.cerevisiae* cells (at 40 → 80 ° Brix) is more pronounced for HPP-treated sample (2 min) of $\Delta = 1.151$ log reduction. A shorter pressure holding time (2 min in this work) coupled with rapid decompression will lead to a fast adiabatic expansion of water that could result in rupturing the cell wall more effectively (Balasubramanian and Balasubramaniam, 2003; Hayakawa et al., 1998). Whereas, Δ log reduction for thermally-treated sample showed the lowest value of 0.695. These results indicate that the inactivation of *S.cerevisiae* is not solely depend on the baroprotective effect of honey, but also the compressibility effect when honey samples were subjected to high pressure processing.

Table 7.3: The changes in log reduction (Δ log reduction) of *Saccharomyces serevisiae* subjected to high pressure and thermal treatment with respect to different ° Brix.

° Brix	Processes		
	600 MPa, 2 min	600 MPa, 30 min	55°C, 10 min
40 → 50	0.128 ± 0.01^{a}	0.161 ± 0.01^{a}	0.161 ± 0.01^{a}
40 → 60	0.787 ± 0.03^{a}	0.554 ± 0.04^{b}	0.465 ± 0.01^{b}
40 → 70	1.108 ± 0.02^{a}	0.803 ± 0.03^{b}	0.637 ± 0.01^{c}
40 → 80	1.151 ± 0.05^{a}	0.991 ± 0.01^{b}	0.695 ± 0.01^{c}

Mean values (mean ± standard deviation) within the same row with different letters are significantly different according to Turkey's HSD Test (Statistica version 11, Satsoft®) with n = 3

The effect of compressibility on *S. cerevisiae* cells reduction at different °Brix subjected to high pressure processing (at 600 MPa for 2 and 30 min) is shown in Figure 7.4. The five points resulted from pressure treatments performed on honey having different ° Brix and hence different compressibility (data were extrapolated from Figure 7.1). The graph show a gradual increase in the inactivation of the cells with increasing compressibility, showing a higher reduction cells when they were in lower sugar concentration (lower ° Brix).

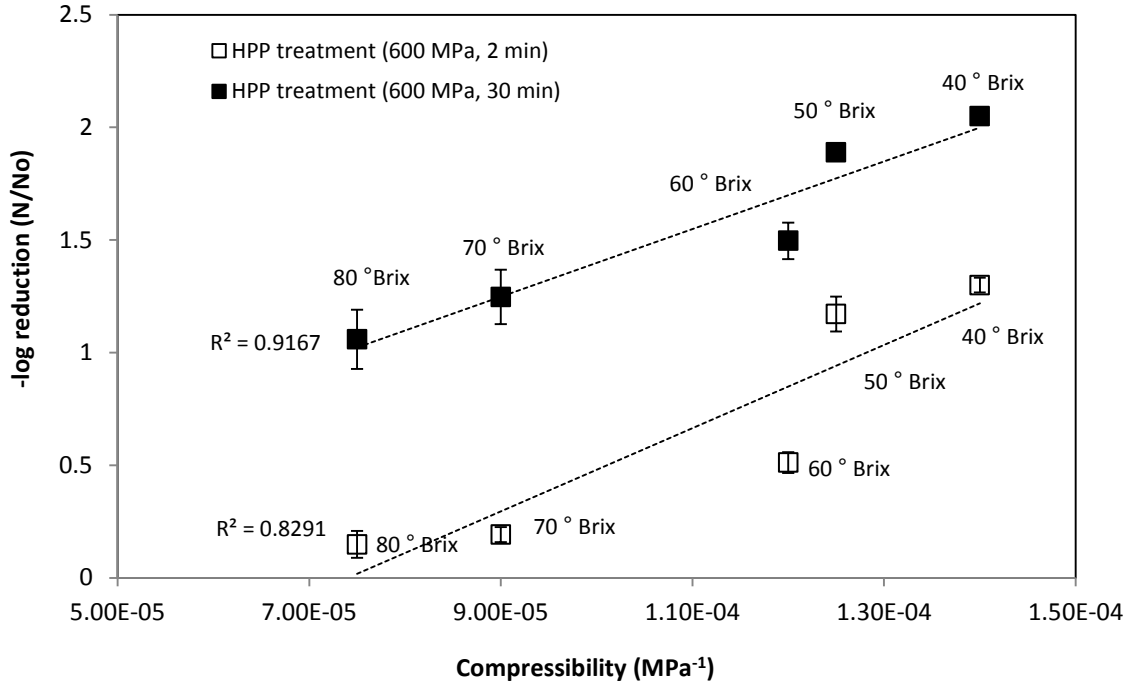


Figure 7.4 Effect of compressibility and ° Brix on inactivation of *Saccharomyces cerevisiae* subjected to pressure treatment at 600 MPa for 2 and 30 min.

A correlation coefficient, r determines the extent to which values of two variables are proportional, linearly related to each other. It measures how good is the relationship between those variables (higher r value indicates how well the relationship between those variables). Table 7.4 shows the relationship between sugar concentration in honey and compressibility on the reduction of *S. cerevisiae* cells as affected by high pressure treatment. As tabulated, a very strong correlation ($r > 0.9$) was found between sugar concentration, compressibility and cell reduction. The r value between cell reduction and sugar concentration for 2 min and 30 min were registered to about 0.95 ($p < 0.05$), whereas, r value between cell reduction and compressibility were found between 0.91 to 0.96 ($p < 0.05$). Cell reduction varied linearly with compressibility and sugar concentration, indicating that these two factors play a significant role in high pressure-treated honey. A strong and significant correlation, $p < 0.05$ between all these parameters (compressibility, sugar concentration in honey and cell reduction) provides more evidence that microbial inactivation during high pressure processing was not solely influenced by ° Brix but also due to compressibility.

Table 7.4: Correlation matrices for ° Brix, compressibility and *Saccharomyces cerevisiae* cells reduction subjected to high pressure treatment (600 MPa, ambient temperature).

	Sugar concentration (° Brix)	Compressibility (MPa^{-1})	Cell reduction ($-\log_{10} N/N_o$)
Honey concentration (° Brix)			
2 min	1.000000	*0.956315	*0.957717
30 min	1.000000	*0.977376	*0.957438
Compressibility (MPa^{-1})			
2 min	*0.956315	1.000000	*0.910550
30 min	*0.977376	1.000000	*0.957453
Cell reduction ($-\log N/N_o$)			
2 min	*0.957717	*0.910550	1.00000
30 min	*0.957438	*0.957453	1.00000

*Correlation is significant at the 0.05 level, obtained using Pearson correlation from Statistica version 11, Statsoft®.

7.4 Conclusions

The effects of pressure and ° Brix on the inactivation of osmophilic microorganism (*S. cerevisiae*) in model food (honey) were studied. The study has confirmed that, at a higher ° Brix of food system (80 ° Brix), the resistance of *S. cerevisiae* to high pressure treatment (600 MPa at temperature less than 35°C) was increased. This study is indispensable in proving that; the cells reduction is not only due to the baroprotective nature of sugar but also due to compressibility effect when samples were subjected to high pressure. The study also demonstrated that high sugar concentration in honey significantly limits the effect of high pressure processing on microbial inactivation, particularly osmophilic yeasts. The results reported from this study have practical implications in establishing efficient process design for commercial manufacturing of food preparation containing high concentration of sugars.

CHAPTER 8

Conclusions, Recommendations for Future Works and Implications of the Project

8.1 Conclusions

High pressure processing of New Zealand honey could provide an effective alternative to thermal processing for improving the nutritional quality while maintaining a better shelf life. Results demonstrated the HPP capability to increase total phenolic content, antioxidant activity, brown pigment and viscosity while maintaining colour. HPP was also found to limit a formation of a marker of honey age/ heating used to downgrade honey in the commercial marketplace such as hydroxymethylfurfural, HMF. The other important finding was that HPP can enhance the antibacterial activity of Manuka honey in an effective way: reduce storage time and cost. Since storage is time consuming and costs money (especially if temperature is controlled), the beekeepers would benefit from HPP, which can increase the antibacterial activity in only few min of processing so that the best price and quality could be obtained.

The final conclusions from the research work are as follows:

1. The increment of total phenolic content (TPC) and antioxidant activity as affected by HPP (600 MPa at ambient temperature for 10 min) has definite advantage in terms of preservation and improvement in nutritional value of honey.
2. HPP (below 600 MPa at ambient temperature for 10 min) maintain the natural colour of honey which relates directly to consumer perception of appearance.
3. HPP (200, 400 and 600 MPa at ambient temperature for 10 min) can retain the shear-thinning behaviour of honey and the viscosity is not affected by high pressure (at lower shear rate when honey is usually consumed).
4. HPP (200, 400 and 600 MPa at ambient temperature for up to 15 min) can inhibit the increment of hydroxymethylfurfural (HMF) so that its concentration in honey is within the limit of standard.
5. The high pessure effect on brown pigment formation in honey can be an advantage since it enhances its antibacterial activity.
6. HPP (200, 400 and 600 MPa at ambient temperature for 10 min) can enhance the antibacterial activity in honey which can extend its use as a therapeutic product.
7. A good correlation has been developed between brown pigment and antioxidant activity (0.8826) and antibacterial activity (0.9404) against of *S.epidermidis* in honey as affected by HPP (600 MPa at ambient temperature for 10 min).

8. Storage study shows the merit of high pressure in extending the stability and better nutrient retention in honey.
9. HPP-treated sample (600 MPa at ambient temperature for 10 min) stored at ambient temperature (approximately 24°C) retained an acceptable overall quality for one year storage; which confirmed that the effect of high pressure on honey is permanent.
10. Insignificant alteration in physicochemical properties showed that HPP treatment (600 MPa at ambient temperature for 10 min) can be an alternative method for preserving honey as freshly processed for up to one year or more.
11. The effect of HPP on microbial inactivation is significantly affected by compressibility and sugar concentration on change compressibility.

8.2 Recommendations for Future Work

High pressure processing is currently an emerging technology and new processing technology often leads to new products. Hence, new product development ideas could be explored with the knowledge gained:

1. Further analysis on high molecular weight compounds such as enzymes, oligosaccharide and protein as affected by HPP could be used to understand the main reasons on how HPP can retain and improve the nutritional quality (total phenolic content, antioxidant activity and brown pigment formation) in honey.
2. The structural analysis on pollen as affected by HPP should also be carried out as enzymes and proteins in honey conceivably arise from pollen. This will provide an understanding of the impacts on the total phenolic content, antioxidant activity and brown pigment formation.
3. Analysis on bees wax could also be used to understand the effect of HPP on the rheological properties and viscosity of honey. HPP may induce a phase shift of the wax under pressure and hence can cause the change in viscosity.
4. Further analysis on methylglyoxal, MGO (the inhibin responsible for the antibacterial activity of Manuka honey) should be conducted in order to get a clearer understanding on how HPP can increase the antibacterial activity. Since HPP does not accelerate the conversion of DHA to MGO, another possible reason might be due to release of MGO reversibly bound to amino acids or protein. The structure or mechanism of MGO compound as affected by HPP should be investigated.
5. Comprehensive sensory analysis is also needed to determine the potential benefit of high pressure processing of honey.

8.3 Implications of the Project

In order to assure provenance and protect quality of New Zealand honey, this project has been highly successful as the results produced have provided good understanding towards the future of high pressure processed New Zealand honey. The major significance for commercialization of high pressure processed honey is not preservation but rather improvement of its nutritional value and also extending its stability for long term storage. This project has shown the capability of HPP as a tool for postharvest optimization of bioactive and nutritive compounds in Manuka honey. Hence, it has implications for further scientific research building on medical credibility of Manuka honey. And also the extension use of honey as other products such as unique flavour for food and beverages, ingredients in cosmetics and nutraceutical/ natural health products with high bioactivity and nutritional value. Besides, HPP generates a new approach in honey processing which will have a strong potential to its continued growth. The non- thermal technology of HPP can also protect New Zealand Manuka honey in a global market from substitution and fraud with other non Manuka products as well as would be able to compete with other countries launching ‘active’ honeys. Apart from that, the project also have practical implications in establishing efficient process design for commercial manufacturing of food preparation containing high concentration of sugars.

Publications and Presentations Based on Thesis Work

Publications:

Articles published:

M.F. Noor Akhmazilah, M.M. Farid and F.V.M Silva (2012). High Pressure Processing of Honey: Preliminary Study of Total Microorganism Inactivation and Identification of Bacteria. *Journal of Science and Technology, 2*(4): 2229-8460.

Akhmazillah, M. F. N., M. M. Farid, F.V.M. Silva (2013). High pressure processing (HPP) of honey for the improvement of nutritional value. *Innovative Food Science & Emerging Technologies, 20*(0): 59-63.

Fauzi, N., M. Farid, F.V.M. Silva (2014). High-Pressure Processing of Manuka Honey: Improvement of Antioxidant Activity, Preservation of Colour and Flow Behaviour. *Food and Bioprocess Technology, 7*(8): 2299-2307.

Grainger, M. N. C., M. Manley-Harris, Noor A.M. Fauzi, Mohammed M. Farid (2014). Effect of high pressure processing on the conversion of dihydroxyacetone to methylglyoxal in New Zealand Mānuka (*Leptospermum scoparium*) honey and models thereof. *Food Chemistry, 153*(0): 134-139.

Fauzi, N. A. and M. M. Farid (2014). High-pressure processing of Manuka honey: brown pigment formation, improvement of antibacterial activity and hydroxymethylfurfural content. *International Journal of Food Science & Technology:* DOI: 10.1111/ijfs.12630

Noor Akhmazillah, MF, Farid, MM and Silva, FVM. High pressure processing of honey: Preliminary study of saccharomyces cerevisiae inactivation and total phenolic content. In: Chemeca 2012: Quality of life through chemical engineering: 23-26 September 2012, Wellington, New Zealand. Barton, A.C.T.: Engineers Australia, 2012. : [1117]-[1126] EISBN: 9781922107596.

Noor Akhmazillah, MF; Farid, MM and Silva, FVM. High pressure processing of Manuka honey: improvement of nutritional value. NZ conference of Chemical and Materials Engineering 2013 (NZCCME 2013), Auckland, New Zealand. ISBN 978-0-473-25614-2.

Articles submitted:

Noor Akhmazillah, MF, Farid, MM and Silva, FVM (2014). High Pressure Processed Manuka Honey: Change in Nutritional and Rheological Properties over One Year Storage. Submitted International Food Research.

Noor Akhmazillah, MF, Farid, MM and Silva, FVM (2014). An Insight on the Relationship between Food Compressibility and Microbial Inactivation during High Pressure Processing. Submitted to Food and Bioprocess Technology.

Presentations (oral):

Noor, M.F, Farid, M.M., Silva, F.V.M. Microbiology and Quality of Honey in Thermal and High Pressure processing. 2011 Beijing International Conference on Non-Thermal processing Technologies & High Hydrostatic Pressure Forum", 21-23 September 2011, Beijing, China.

Noor Akhmazillah, MF; Farid, MM and Silva, FVM. High pressure processing of honey: Preliminary study of *Saccharomyces cerevisiae* inactivation and total phenolic content. In: Chemeca 2012: Quality of life through chemical engineering: 23-26 September 2012, Wellington, New Zealand.

M.F. Noor Akhmazilah, M.M. Farid and F.V.M Silva High Pressure Processing of Honey: Preliminary Study of Total Microorganism Inactivation and Identification of Bacteria. Chemical and Materials Engineering Conference 2012, University of Auckland, New Zealand

Noor Akhmazillah, MF; Farid, MM and Silva, FVM. High pressure processing of honey: improvement of antioxidant activity and preservation of colour. Innovative Food Conference (IFood), 8-10 October 2013, Hannover, Germany.

Noor Akhmazillah, MF; Farid, MM and Silva, FVM. High pressure processing of Manuka honey: improvement of nutritional value.NZ conference of Chemical and Materials Engineering 2013 (NZCCME 2013), Auckland, New Zealand.

References

References

Abdul Ghani, A. G. and Farid, M. M. (2007). Numerical simulation of solid–liquid food mixture in a high pressure processing unit using computational fluid dynamics. *Journal of Food Engineering*, *80*(4), 1031–1042.

Abu-Jdayil, B., Al-Majeed Ghazawi, A., Al-Malah, K. I. M. and Zaitoun, S. (2002). Heat effect on rheology of light- and dark-coloured honey. *Journal of Food Engineering*, *51*(1), 33–38.

Adams, C. J., Manley-Harris, M. and Molan, P. C. (2009). The origin of methylglyoxal in New Zealand Manuka (*Leptospermum scoparium*) honey. *Carbohydrate Research*, *344* (8), 1050–1053.

Ahmed, J., Ramaswamy, H. S., Alli, I. and Ngadi, M. (2003). Effect of high pressure on rheological characteristics of liquid egg. *LWT - Food Science and Technology*, *36*(5), 517-524.

Ahmed, J., Ramaswamy, H. S. and Hiremath, N. (2005). The effect of high pressure Treatment on rheological characteristics and colour of mango pulp. *International Journal of Food Science & Technology, 40*(8), 885–895.

Akhmazillah, M. F. N., M. M. Farid and Filipa, S.V.M. (2013). High pressure processing (HPP) of honey for the improvement of nutritional value. *Innovative Food Science & Emerging Technologies, 20*(0), 59-63.

Al, M. L., Daniel, D., Moise, A., Bobis, O., Laslo, L. and Bogdanov, S. (2009). Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chemistry*, *112*(4), 863–867.

Aljadi, A.M. and Yusoff, K. (2003). Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. *Turkish Journal of Medical Sciences*, *33*, 229–236.

Aljadi, A. M. and Kamaruddin, M. Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry*, *85*(4), 513–518.

Al-Habsi, N. A. and Niranjan, K. (2012). Effect of high hydrostatic pressure on antimicrobial activity and quality of Manuka honey. *Food Chemistry*, *135*, 1448-1454.

Al-Malah, K. I.M., Abu-Jdayil, B., Zaitoun, S. and Al-Majeed Ghazwi, A. (2001).

Application of WLF and Arrhenius kinetics to rheology of selected dark-coloured honey. *Journal of Food Process Engineering*, *24*, 341–357.

Alpas, H., Kalchayanand, N., Bozoglu, F., Sikes, A., Dunne, C. P. and Ray, B. (1999). Variation in resistance to hydrostatic pressure among strains of food-borne pathogens. *Applied and Environmental Microbiology*, *65*, 4248-4251.

Alvarez-Suarez, J., Tulipani, S., Romandini, S., Bertoli, E. and Battino, M. (2010). Contribution of honey in nutrition and human health: a review. *Mediterranean Journal of Nutrition and Metabolism, 3*(1), 15–23.

Al-Waili, N.S. (2004). Investigating the Antimicrobial Activity of Natural Honey and Its Effects on the Pathogenic Bacterial Infections of Surgical Wounds and Conjunctiva. *Journal of Medicinal Food, 7*(2), 210-222

Al-Waili, N.S. and Haq, A. (2004). Effect of Honey on Antibody Production Against Thymus-Dependent and Thymus-Independent Antigens in Primary and Secondary Immune Responses. *Journal of Medicinal Food, 7*(4), 491-494.

Amin, W.A., Safwat, M. and Ei-Iraki, S.M. (1999). Quality criteria of treacle (black honey). *Food Chemistry, 67*, 17–20.

Ananta, E., V. Heinz and D. Knorr (2004). Assessment of high pressure induced damage on Lactobacillus rhamnosus GG by flow cytometry. *Food Microbiology*, *21*(5), 567-577.

Anese M, Manzocco L, Nicoli M.C. and Lerici C.R. (1999). Antioxidant Properties of Tomato Juice as Affected by Heating. *J. Sci. Food Agric*, *79*(5), 750-754.

Anupama, D., Bhat, K. K. and Sapna, V. K. (2003). Sensory and physicochemical properties of commercial samples of honey. *Food Research International*, *36*(2), 183–191.

Areekul, V. and Toledo R.T (2010). Sporicidal effect of a continuous –flow high pressure throttling process on honey, Phd thesis. Department of Food Science and Technology, University of Georgia.

Assil, H. I., Sterling, R. and Sporns, P. (1991). Crystal control in processed liquid honey. *Journal of Food Science*, *56*(4), 1034–1037.

Babacan, S., L. F. Pivarnik, and A.G. Rand (2002). Honey Amylase Activity and Food Starch Degradation. *Journal of Food Science, 67*(5), 1625-1630.

Balasubramanian, S. and V. M. Balasubramaniam (2003). Compression heating influence of pressure transmitting fluids on bacteria inactivation during high pressure processing. *Food Research International, 36*(7), 661-668.

Balny, C., Masson, P. and Heremans, K. (2002). High pressure effects on biological

macromolecules: from structural changes to alteration of cellular processes. Biochimica Biophysica. *Acta Protein Structure and Molecular Enzymology*, *1595*(1-2), 3-10.

Balogh, T., Smout, C., Nguyen, B. L., Van Loey, A. M., and Hendrickx, M. E. (2004). Thermal and high-pressure inactivation kinetics of carrot pectinmethylesterase: from model system to real foods. *Innovative Food Science & Emerging Technologies, 5,* 429-436.

Barbosa-Canovas, G. V., Pothakamury, U. R., Palou, E. and Swanson, B. G. (1998). In *Nonthermal preservation of foods*. New York: Marcel Decker.

Barbosa-Canovas, G. V and Bermude-Aguirre, D. (2011). Introduction Non-thermal processing technologies for food, IFT Press,UK: Wiley-BlackWell.

Barhate, R.S., Subramanian, R., Nandini, K.E. and Umesh Hebbar, H. (2003). Processing of Honey using Polymeric Microfiltration and Ultrafiltration Membranes. *Journal of Food Engineering, 60,* 49–54.

Barreiro, J. A., Milano, M. and Sandoval, A. J. (1997). Kinetics of colour change of double concentrated tomato paste during thermal treatment. *Journal of Food Engineering, 33,* 359–371.

Basak, S., Ramaswamy, H.S. and Piette, J.P.G (2002). High pressure destruction kinetics of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* in single strength and concentrated orange juice. *Innovative Food Science & Emerging Technologies, 3,* 223-231.

Bassam, Z., Zohra, B.I and Saada, A. (1997). The Effects of Honey on Leishmania Parasites: An In Vitro Study. *Trop Doct, 27*(1), 36-38.

Bath, P. K. and Singh, N. (1999). A comparison between *Helianthus annuus* and *Eucalyptus lanceolatus* honey. *Food Chemistry, 67,* 389–397.

Baxter, I. A., Easton, K., Schneebeli, K. and Whitfield, F. B. (2005). High pressure processing of Australian navel orange juices: sensory analysis and volatile flavour profiling. *Innovative Food Science and Emerging Technologies, 6*, 372-387.

Bergman, A., Yanai, J., Weiss, J., Bell, D. and David, M. P. (1983). Acceleration of wound healing by topical application of honey. An animal model. *American Journal of Surgery, 145,* 374-6.

Bermude- Aguirre, D., and Barbosa- Canovas. (2012). In Nurhan Turgut Dunford (Ed.) *Recent advances in non-thermal Technologies, Food and Industrial Bioproducts and Bioprocessing,* (pp 314-325). USA: Wiley-Blackwell.

Bhandari, B., D'Arcy, B. and Chow, S. (1999). Rheology of selected Australian honeys. *Journal of Food Engineering, 41*(1), 65–68.

Bogdanov S, Martin , and LüLlmann C. (1997). Harmonised methods of the European Honey Commission. *Apidologie* (extra issue), 1–59.

Bogdanov, S. (2002). Harmonised Methods of the International Honey Commision. International Honey Commision. *Apidologie*, (extra issue), 9-11.

Bogdanov, S., Jurendic, T., Sieber, R. and Gallmann, P. (2008). Honey for nutrition and health: a review. *Journal of the American College of Nutrition*. *27*(6), 667–689.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie*, *28*, 25–30.

Bridgman, P.W., (1970). The Physics of High Pressure. New York: Dover Publications.

Brudzynski, K. and Kim, L. (2011). Storage-induced chemical changes in active Components of honey de-regulate its antibacterial activity. *Food Chemistry*, *126*(3), 1155-1163.

Brudzynski, K. and Miotto, D. (2011). The relationship between the content of Maillard reaction-like products and bioactivity of Canadian honeys. *Food Chemistry 124*(3), 869-874.

Buckow, R. (2013). HPP Application to Fruit and Vegetables. The FoodBowl - High Pressure Processing Seminar/Workshop, 16-17 April 2013.

Bult, L and Kilic, M. (2009). Kinetics of hydroxymethylfurfural accumulation and colour changes in honey during storage in relation to moisture content. *Journal of Food Processing and Preservation*, *33*, 22-32.

Burdurlu, S.H. and Karadeniz, F. (2003). Effect of storage on nonenzymatic browning of apple juice concentrates. *Food Chemistry*, *80* (1), 91–97.

Butz, P., Edenharder, R., Fister, H. and Tauscher, B. (1997a). The influence of high pressure processing on antimutagenic activities of fruit and vegetable juices. *Food Research International*, *30*(3–4), 287-291.

Butz, P., Edenharder, R., Fister, H. and Tauscher, B. (1997b). Influence of High Hydrostatic Pressure on Aspartame: Instability at Neutral pH. *J. Agric. Food Chem*., *45* (2), 302–303.

Butz, P., Edenharder, R., Ferna´ndez Garcia, A., Fister, H., Merkel, C., et al. (2002). Changes in functional properties of vegetables induced by high pressure treatment. *Food Research International*, *35*, 295-300.

Butz, P. (2010). High pressure: Minimal processing. In: Encyclopedia of Agricultural,

Food, and Biological Engineering (pp. 819–822). New York: CRC Press.

Campos, F.P. and Cristianini, M. (2007). Inactivation of Saccharomyces cerevisiae and Lactobacillus plantarumin orange juice using ultra high-pressure homogenisation. *Innovative Food Science and Emerging Technologies*, *8*, 226–229.

Cano, M.P. and de Ancos, B. (2005). Advances in use of high pressure to processing and preservation of plant foods. In: Barbosa-Cánovas, G.V., Tapia, M., Pilar Cano, M. (Eds.), *Novel Food Processing Technologies*. New York: CRC Press.

Cao, X., Zhang, Y., Zhang, F., Wang, Y., Yi, J. and Liao, X. (2011). Effects of high Hydrostatic pressure on enzymes, phenolic compounds, anthocyanins, polymeric colour and colour of strawberry pulps. *Journal of the Science of Food and Agriculture*, *91*(5), 877–885.

Cavia, M.M., Fernández-Muiño, M.A., Gömez-Alonso E., Montes- Pérez M.J., Huidobro, J.F. and Sancho, M.T. (2002). Evolution of fructose and glucose in honey over one year: influence of induced granulation. *Food Chemistry*, *78*(2), 157-161.

Chen Cuilan, Campbell Liona T., Blair, Shona and A.Carter Dee (2012). The effect of standard heat and filtration processing procedures on antimicrobial activity and hydrogen peroxide levels in honey. *Front Microbiol*., *3*, 265.

Cherchi, A., Spanedda, L., Tuberoso, C. and Cabras, P. (1994). Solid-phase extraction and high-performance liquid chromatographic determination of organic acids in honey. *Journal of Chromatography A*, *669*(1–2), 59–64.

Chirife, J., and Buera, M.P. (1997). A simple model for predicting the viscosity of sugar and oligosaccharide solutions. *Journal of Food Engineering, 33*, 221 – 226.

Codex Alimentarius Commission. Revise codex standard for honey. Codex Standards 12–1982, Rev.1 (1987), Rev.2 (2001)1, COMESA/FDHS 002:2004.

Concepción, Sánchez-Moreno, Plaza, Lucía, Elez-Martínez, Pedro, De Ancos, Begoña, Martín-Belloso, Olga, and Pilar Cano, M. (2005). Impact of high pressure and pulsed electric fields on bioactive compounds and antioxidant activity of orange juice in comparison with traditional thermal processing. *Journal of Agricultural and Food Chemistry*, *53*(11), 4403–4409.

Conti, M. E. (2000). Lazio region (central Italy) honeys: a survey of mineral content and typical quality parameters. *Food Control*, *11*(6), 459-463.

Cooper, R. A., Halas, E. and Molan, P. C. (2002). The Efficacy of Honey in Inhibiting Strains of Pseudomonas Aeruginosa from Infected Burns. *Journal of Burn Care & Rehabilitation, 23* (6), 366-370.

Corrales, M., Toepfl, S., Butz, P., Knorr, D. and Tauscher, B. (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: a comparison. *Innovative Food Science & Emerging Technologies*, *9*(1), 85–91.

Council Directive of the European Union. Council directive 2001/110/ec of 20 December 2001 relating to honey. Official Journal of European Communities 2002, 10, 47–52.

Crowe, J. H., A. E. Oliver, F.A. Hoekstra and L.M. Crowe (1997). Stabilization of Dry Membranes by Mixtures of Hydroxyethyl Starch and Glucose: The Role of Vitrification. *Cryobiology, 35*(1), 20-30.

D'Arcy, B.R (2007). High-power Ultrasound to Control of Honey Crystallisation. Rural Industries Research and Development Corporation. ISSN 1440-6845.

De Ancos, B., Gonzalez, E. and Pilar Cano, M. (2000). Effect of highpressure treatment on the carotenoid composition and the radicalscavenging activity of persimmon fruit purees. *Journal of Food Chemistry, 48*, 3542-3548.

de Begoa, A., Gonzalez, E. and Pilar Cano, M. (2000). Effect of high pressure treatment on the carotenoid composition and the radical scavenging activity of persimmon fruit purees. *Journal of Agricultural and Food Chemistry, 48*(8), 3542–3548.

Dede S., Alpas H. and Bayindirli A., (2007). High hydrostatic pressure treatment and storage of carrot and tomato juices: Antioxidant activity and microbial safety. *Journal of the Science of Food and Agriculture*, *87*(5), 773-782.

Demirci, A. and Panico, L (2008). Pulsed Ultraviolet Light. *Food Science and Technology International*, *14* (5), 443-446.

Denys, S., Ludhikhuyze, L. Van Loey, A. and Hendrickx, M. (2000). Modeling conductive heat transfer and process uniformity during batch high pressure processing of foods. *Biotechnology Progress, 16*, 92–101.

Dervisi, P., Lamb, J. and Zabetakis, I. (2001). High pressure processing in jam manufacture: effects on textural and colour properties. *Food Chemistry*, *73*(1), 85-91.

De Roeck, A., D. N. Sila, T. Duvetter, A. Van Loey and Hendrickx, M. (2008). Effect of high pressure/high temperature processing on cell wall pectic substances in relation to firmness of carrot tissue. *Food Chemistry, 107*(3), 1225-1235.

Diels, A.M.J and Michiels, C.W. (2006). High-Pressure Homogenization as a Non-Thermal Technique for the Inactivation of Microorganisms. *Critical Reviews in Microbiology, 32* (4), 201-216.

Doblado, R., Frías, J. and Vidal-Valverde, C. (2007). Changes in vitamin C content and

antioxidant capacity of raw and germinated cowpea (Vigna sinensis var. carilla) seeds induced by high pressure treatment. *Food Chemistry*, *101*(3), 918–923.

Donsi, G., Ferrari, Matteo, D. and M. (1996). High pressure stabilization of orange juice: Evaluation of the effects of process conditions (Vol. 8). Pinerolo, ITALIE: Chiriotti.

Dunn J., Ott, T., and Clark, W. (1995). Pulsed light treatment of food and packaging. *Food Technology*, *49*, 95- 98.

Durling, N. E., Catchpole, O. J., Grey, J. B., Webby, R. F., Mitchell, K. A., Foo, L. Y. and Nigel B. Perryb (2007). Extraction of phenolics and essential oil from dried sage (Salvia officinalis) using ethanol–water mixtures. *Food Chemistry*, *101*(4), 1417–1424.

Efem, S. E. E. (1988). Clinical observations on the wound healing properties of honey. *British Journal of Surgery*, *75*, 679-81.

Eshtiaghi, M. N. and Knorr, D. (1993). Potato Cubes Response to Water Blanching and High Hydrostatic Pressure. *Journal of Food Science*, *58*(6), 1371-1374.

Escriche, I., Visquert, M., Juan-Borrás, M. and Fito, P. (2009). Influence of simulated Industrial thermal treatments on the volatile fractions of different varieties of honey. *Food Chemistry*, *112*(2), 329–338.

Estevinho, L., Pereira, A. P., Moreira, L., Dias, L. G. and Pereira, E. (2008). Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. *Food and Chemical Toxicology*, *46*(12), 3774-3779.

Fallico, B., M. Zappalà, E. Arena, and A. Verzera (2004). Effects of conditioning on HMF content in unifloral honeys.*Food Chemistry*, *85*(2), 305-313.

Farid, M. M. (2010). Mathematical Modeling of Food Processing. New York: CRC Press.

Farkas, D. F. and Hoover, D. G (2000). High Pressure Processing. *Journal of Food Safety*, *65*, 47-64.

Farm Monitoring Report 2013 (2013): Apiculture Monitoring (Apiculture), ISBN Print: 978-0-478- 42319-8.

Fauzi, N., M. Farid and Silva, F.V.M. (2014). High-Pressure Processing of Manuka Honey: Improvement of Antioxidant Activity, Preservation of Colour and Flow Behaviour. *Food and Bioprocess Technology*, *7*(8), 2299-2307.

Fernández García, A., Butz, P. and Tauscher, B. (2000). Does the antioxidant potential of high pressure treated apple juice change during storage?. *High Pressure Research*, *19*(1–6), 153–160.

Ferna´ndez Garcı´a, A., Butz, P., Bogna` r, A. and Tauscher, B. (2001). Antioxidative

capacity, nutrient content and sensory quality of orange juice and an orange lemon carrot juice product after high pressure treatment and storage in different packaging. *European of Food Research and Technology*, *213*, 290-296.

Ferreira, I. C. F. R. and Aires, E. (2009). Antioxidant activity of Portuguese honey samples: different contributions of the entire honey and phenoic extract. *Food Chemistry*, *114*(4), 1438–1443.

Food and Agriculture Organization of the United Nations, FAO http://www.fao.org/ag/ags/agsi/Npnthermal/nonthermal1.htm. Retrieved on 25 July 2012.

Furukawa, S., Shimoda, M. and Hayakawa, I. (2003). Mechanism of the inactivation of bacterial spores by reciprocal pressurization treatment. *Journal of Applied Microbiology*, *94*, 836-841.

Galindo, F. G., R. T. Toledo, and I. Sjöholm (2005). Tissue damage in heated carrot slices. Comparing mild hot water blanching and infrared heating. *Journal of Food Engineering*, *67*(4), 381-385.

Garcia, A. F., Butz, P. and Tauscher, B. (2001). Effects of high-pressure processing on carotenoid extractability, antioxidant activity, glucose diffusion, and water binding of tomato puree (Lycopersicon esculentum). *Journal of Food Science*, *66*(7), 1033–1038.

Garcia-Palazon, A., Suthanthangjai, W., Kajda, P. and Zabetakis, I. (2004). The effects of high hydrostatic pressure on b-glucosidase,peroxidase and polyphenoloxidase in red raspberry (Rubusidaeus)and strawberry (Fragaria x ananassa). *Food Chemistry*, *88*, 7-10.

Garza, S., Ibarz, A., Pagan, J. and Giner, J. (1999). Non-enzymatic browning in peach puree during heating. *Food Research International*, *32* (5), 335–343.

Ghani, A. G. and Farid, M. M. (2007). Numerical simulation of solid–liquid food mixture in a high pressure processing unit using computational fluid dynamics". *Journal of Food Engineering*, *80*(4), 1031-1042.

Ghazali, H.M. and Sin M.K. (1986). Coconut honey: the effect of storage temperature on some of its physico-chemical properties. *Journal of Apicultural Research*, *25* (2), 109-112.

Ghazali, H.M., Ming, T.C. and Hashim, D.M. (1994). Effect of Microwave Heating on the Storage and Properties of Starfruit Honey. *ASEAN Food J*., *9*, 30–35.

Gheldof, N. and Engeseth N.J.(2002). Antioxidant capacity of honeys from various floral

sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitrolipoprotein oxidation in human serum samples. *Journal of Agricultural and Food Chemistry*, *50*, 3050–3055.

Gibson, B. (1973). The effect of high sugar concentrations on the heat resistance of vegetative Microorganisms. *Journal of Applied Bacteriology*, *36*, 365-375.

Gimenez, J., Kajda, P., Margomenou, L., Piggott, J. R. and Zabetakis, I. (2001). A study on the colour and sensory attributes of highhydrostatic- pressure jams as compared with traditional jams. *Journal of Science and Food Agriculture*, *81*, 1228-1234.

Glaasker, E., Tjan, F. S. B., Ter Steeg, P. F., Konings, W. N. and Poolman, B. (1998). Physiological response of Lactobacillus plantarum to salt and nonelectrolyte stress. *Journal of Bacteriology*. *180*, 4718−4723.

Goh, E. L. C., A. D. Hocking, C.M. Stewart, K.A. Buckle and G.H., Fleet (2007). Baroprotective effect of increased solute concentrations on yeast and moulds during high pressure processing. *Innovative Food Science & Emerging Technologies*, *8*(4), 535-542.

Gomes, M. R. A. and Ledward, D. A. (1996). Effect of high-pressure treatment on the activity of some polyphenoloxidases. *Food Chemistry*, *56*(1), 1-5.

Gonzales, A. P. (1999). Colour changes during storage of honeys in relation to their composition and initial colour. *Food Research International*, *32*(3), 185–191.

Gould, G.W. (2001a). The Evolution of High Pressure Processing of Foods. In Marc E.G Hendrickx and Dietrich Knorr (Ed.) *Ultra High Pressure Treatments of Foods*. New York: Kluwer Acadeim/Plenum Publishers.

Gould, G.W. (2001b). New processing technologies: an overview. *P. Nutr. Soc*., *60*(4), 463–474.

Grainger, M. N. C., Manley-Harris, M., Fauzi, N. A. M. and Farid, M. M. (2014). Effect of high pressure processing on the conversion of dihydroxyacetone to methylglyoxal in New Zealand mānuka (Leptospermum scoparium) honey and models thereof. *Food Chemistry*, *153*, 134-139.

Grane, E. and Kirk Visscher, P. (2007). Honey. what-when-how.com/insects/homoptera-insects: 459-461. Retrived on 30 May 2011.

Hashizume, C, Kimura K. and Hayashi R. (1995). Kinetic analysis of yeast inactivation by high pressure treatment at low temperatures. *Biosci Biotechnol Biochem*., *59*(8), 1455-8.

Hayakawa, I., Furukawa, S., and Midzunaga, A. Horiuchi H., Nakashima, T., Ysuaku Fujio, Y., Yano, Y., Ishikura, T. and Sasaki, K. (1998). Mechanisms of heat tolerant of spores of Bacillus stearothermophilus IFO 12250 by rapid decompression. *Journal of Food Scienc*e, *63*, 371-374.

Hebbar, U.H., Nandini, K.E., Lakshmi, M.C. and Subramanian, R. (2003). Microwave and Infrared Heat Processing of Honey and its Quality. *Food Sci. Technol. Res., 9*, 49–53.

Hillegas, S. L. and Demirci, A. (2003). Inactivation of Clostridium sporogenes in clover honey by pulsed UV-light treatment. *Agricultural Engineering International*, V. Manuscript FP 03 009.

Heinz, V., Knoch, A. and Lickert, T. (2009). Product innovation by high pressure Processing. *New Food*, *2*, 42-47.

Heinz, V and Buckow, R. (2010). Food Preservation by High Pressure. *Journal of Consumer Protection and Food Safety*, *5*, 73–81.

Hendrickx, M., Ludikhuyze, L., Van den Broeck, I. and Weemaes, C. (1998). ffects of high pressure on enzymes related to food quality. *Trends in Food Science & Technology*, *9*(5), 197–203.

Hill, V.M, Ledward, D.A. and Ames, J.M (1996). Influence of High Hydrostatic Pressure and pH on the Rate of Maillard Browning in a Glucose−Lysine System. *Journal of Agricultural and Food Chemistry*, *44* (2), 594–598.

Hill, C., Cotter, P. D., Sleator, R. D and Gahan, C. G. M. (2002). Bacterial stress response in Listeria monocytogenes: jumping the hurdles imposed by minimal processing. *International Dairy Journal*, *12*, 273-283.

Huang, H.-W., Lung, H.M., Yang, B. B. and Wang, C.Y. (2014). Responses of microorganisms to high hydrostatic pressure processing. *Food Control*, *40*(0), 250-259.

Hussein, S. Z., Yusoff, K. M., Makpol, S. and Yusof, Y. A. M. (2011). Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of Malaysian honey. *Molecules, 16*, 6378–6395.

Ibarz, A., Paga´n, J. and Garza, S. (2000). Kinetic models of non- enzymatic browning in apple puree. *Journal of the Science of Food and Agriculture, 80*, 1162–1168.

Indrawati, Arroqui, C., Messagie, I., Nguyen, M. T., Van Loey, A. and Hendrickx, M. (2004). Comparative study on pressure and temperature stability of 5-methyltetrahydrofolic acid in model systems and in food products. *Journal of Agriculture and Food Chemistry, 52*, 485-492.

Ipek, B. (2010). Effect of Ultrasound and High Hydrostatic Pressure (HHP) on Liquefaction and Quality Parameters of Selected Honey Varieties (pp. 1-107). Master of Science Theses. The Graduate School of Natural and Applied Sciences of Middle East Technical University.

Isaacs, N. (1981), Liquid Phase High Pressure Chemistry (pp. 63–105). UK: John Wiley and Sons.

Isaacs, N. S., P. Chilton, and B. Mackey. (1995). Studies on the inactivation by high pressure of micro-organisms. In D. A. Ledward, D. E. Johnston, R. G. Earnshaw, and A. P. M. Hasting (Ed.), *High pressure processing of foods* (p. 65-79). Nottingham: Nottingham University Press.

Iskander, F. Y. (1995). Trace and minor elements in four commercial honey brands. *Journal of Radioanalytical and Nuclear Chemistry, 201*(5), 401-408.

Itoh, S., Watanabe, A., Terakawa, M. and Yoshioka, K. (1996). In a Honey Processing Technology with UF Membrane, *Proceedings of the International Congress on Membranes and Membrane Processes* (ICOM-96) (p.867). Yokohama, Japan: The Membrane Society of Japan.

Itoh, S., Yoshioka, K.,Terakawa, M., Sekiguchi, Y., Kokubo, K.I. and Watanabe, A. (1999). The Use of Ultrafiltration Membrane Treated Honey in Food Processing. *J. Jap. Soc. Food Sci. Technol*. (Nippon Shokuhin Kagaku Kogaku Kaishi), *46*, 293–302.

Iwahashi, H., Obuchi, K., Fujii, S. and Komatsu, Y (1997). Barotolerance is dependent on both trehalose and heat shock protein 104 but is essentially different from thermotolerance in *Saccharomyces cerevisiae*. *Letters in Applied Microbiology*, *5*, 43–47.

Jeddar, A. Kharsany A. Ramsaroop U G. Bhamjee A. Haffejee and I. E. Moosa A. (1985). The antibacterial action of honey. An in vitro study. *South African Medical Journal*, *67*, 257-258.

Jeffrey, A.E. and Echazaretta M. (1996). Medical uses of honey. *Rev Biomed, 7*, 43-49.

Jiménez, M., Mateo J.J., Huerta T. and Mateo R. (1994). Influence of the storage conditions on some physicochemical and mycological parameters of honey. *Journal of the Science of Food and Agriculture, 64*(1), 67-74.

Jordan, S. L., Pascual, C., Bracey, E. and Mackey, B. M. (2001). Inactivation and injury of pressure-resistant strains of E. coli 0157 and Listeria monocytogens in fruit juices. *Journal of Applied Microbiology*, *91*, 463-469

Juhee, A. and Balasubramaniam, V. M. (2007). Physiological responses of Bacillus amyloliquefaciens spores to high pressure. *Journal of Microbiology and Biotechnology*, *17*, 524-529.

Juszczak, L. and Fortuna, T. (2006). Rheology of selected Polish honeys. *Journal of Food Engineering*, *75*(1), 43–49.

Kaletun, G., Lee, J., Alpas, H. and Bozoglu, F. (2004). Evaluation of structural changes induced by high hydrostatic pressure in Leuconostoc mesenteroides. *Applied and Environmental Microbiology*, *70*, 1116-1122.

Kalichevsky, M. T., Knorr, D. and Lillford, P. J. (1995). Potential food applications of high-pressure effects on ice–water transitions. *Trends Food Science and Technology*, 6, 253–259.

Kaloyereas, S.A. and Oertel, E. (1958).Crystallization of Honey as Affected by Ultrawaves, Freezing and Inhibitors. *Am. Bee J*., *38*, 442–443.

Karabournioti S. and Zervalaki, P. (2001). The Effect of Heating on Honey HMF and Invertase. *Apiacta*, *4*, 1-3.

Keenan, D. F., Brunton, N. P., GormLey, T. R., Butler, F., Tiwari, B. K. and Patras, A. (2010). Effect of thermal and high hydrostatic pressure processing on antioxidant activity and colour of fruit smoothies. *Innovative Food Science & Emerging Technologies*, *11*(4), 551–556.

Kenjerić, D., M. L. Mandić, L Primorac, L., Bubalo, D. and Perl, A. (2007). Flavonoid profile of Robinia honeys produced in Croatia. *Food Chemistry*, *102*(3), 683-690.

Khalil, M. I., S. A. Sulaiman and S.H. Gan (2010). High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year. *Food and Chemical Toxicology*, *48* (8-9), 2388-2392.

Kimura, K., Ida, M., Yosida, Y., Ohki, K., Fukumoto, T. and Sakui, N. (1994). Comparison of Keeping Quality between Pressure-processed Jam and Heat-processed Jam: Changes in Flavour Components, Hue, and Nutrients during Storage. *Bioscience, biotechnology, and biochemistry, 58*(8), 1386-1391.

Knorr, D., V. Heinz and Buckow, R. (2006). High pressure application for food Biopolymers. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics 1764*(3), 619-631.

Kouzeh-Kanani, M., D. J. van Zuilichem, J. P. Roozen,W. Pilnik and J. R. van Delden, W. Stolp (1983). Infrared processing of soybeans. *Plant Foods for Human Nutrition 33*(2-3), 139-143.

Krebbers, B., A. M. Matser, M Koets, R.W. Van den Berg (2002). Quality and storage-stability of high-pressure preserved green beans. *Journal of Food Engineering*, *54*(1), 27-33.

Krebbers, B., Matser, A. M., Hoogerwerf, S. W., Moezelaar, R., Tomassen, M. M. M. and Van Den Berg, R. W. (2003). Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Innovative Food Science & Emerging Technologies*, *4*, 377–385.

Krishnamurthy, K., H. K. Khurana, J. Soojin, J. Irudayaraj and A. Demirci. (2008). Infrared Heating in Food Processing: An Overview. *Comprehensive Reviews in Food Science and Food Safety, 7*(1), 2-13.

Kroh, L. W. (1994). Caramelization in food and beverages. *Food Chemistry*, *51*, 373.

Küçük, M., Kolaylı, S., Karaoğlu, Ş., Ulusoy, E., Baltacı, C. and Candan, F. (2007). Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry, 100*(2), 526–534.

Kuplulu, O, Go¨ncu¨og˘lu, M., O¨ zdemir, H. and Ahmet Koluman (2006). Incidence of Clostridium botulinum spores in honey in Turkey. *Food Control, 17*, 222–224.

Landl, A., Abadias, M., Sárraga, C., Viñas, I. and Picouet, P. A. (2010). Effect of high Pressure processing on the quality of acidified Granny Smith apple purée product. *Innovative Food Science & Emerging Technologies*, *11*(4), 557–564.

Lambert, Y., Demazeau, G., Largeteau, A. and Bouvier, J.-M. (1999). Changes in aromatic volatile composition of strawberry after high pressure treatment. Food Chemistry, *67*, 7-16.

Larson, W.P., Hartzell, T.B. and Diel, H.S (1918). The effect of high pressure on bacteria. *J. Infect.Dis*., *22*, 271-279.

Lazaridou, A., Biliaderis, C. G., Bacandritsos, N. and Sabatini, A. G. (2004).Composition, thermal and rheological behaviour of selected Greek honeys. *Journal of Food Engineering*, *64*(1), 9–21.

Lee, C.M, Sherr, B. and Koh, Y.N. (1984). Evaluation of kinetic parameters for a glucose lysine Maillard reaction. *Journal of Agricultural and Food Chemistry, 32*, 379-382.

Lee, H.S and Nagy, S. (1990). Relative reactivities of sugars in the formation of 5-Hydroxymethylfurfural. *Journal of Food Processing and Preservation, 14*, 171-178.

Leslie, S.B., Israeli,E., Lighthart,B., Crowe, J.H. and Crowe, L.M (1995). Trehalose and sucrose protect both membranes and proteins in intact bacteria during drying. *Appl. Environ. Microbiol., 61* (10), 3592-3597.

Li, H. and Akasaka, K., (2006). Conformational fluctuations of proteins revealed by variable pressure NMR. *Biochimica et Biophysica Acta*, 331–345.

Liebl, D.E. Method of Preserving Honey (1977). US Patent 4050952. Filed 19 November 1976; issued 27 September 1977.

Little, B., Gerchakov, L., and Udey, L. A (1987). Method for Sterilization of Natural Seawater. *J. Microbiol. Methods, 7*, 193–200.

Loey, A. V., Weemas, C., Broeck, V. D., Ludikhuyze, L., Indrawati, Deny, S., and M. Hendrickx. (1998). Thermal and pressure-temperature degradation of chlorophyll in Broccoli (Brassica oleracea L.italica) juice: a kinetic study. *Journal of Agricultural and Food Chemistry, 46*, 5289–5294.

López-Malo, A., Palou, E., Barbosa-Cánovas, G. V., Welti-Chanes, J. and Swanson, B. G. (1998). Polyphenoloxidase activity and colour changes during storage of high hydrostatic pressure treated avocado puree. *Food Research International, 31*(8), 549-556.

Lorenz, K. and Decareau, R.V (1976). Microwave heating of foods - changes in nutrient and chemical composition. *C R C Critical Reviews in Food Science and Nutrition, 7* (4), 339-370.

Ludikhuyze, L., Rodrigo, L., and Hendrickx, M. (2000). The activity ofmyrosinase from broccoli (Brassica oleracea L. cv. Italica): influence of intrinsic and extrinsic factors. *Journal of Food Protection, 63* (3), 400-403.

Lynn, E. G., Englis, D. T. and Milum, V. G. (1936). Effect of processing and storage on composition and colour of honey. *Food Research, 1*, 2–27.

Madigan, M.T., Martinko, J.M., and Parker, J. (2000). Brock biology of microorganisms. Upper Saddle River, NJ: Prentice-Hall Inc.

Mahaneem, M., Sirajudeen, K. N. S., Swamy, M., Yaacob, N. S. and Sulaiman, S. A. (2010). Studies on the antioxidant properties of Tualang honey of Malaysia. Journal of Traditional. *Complementary and Alternative Medicines, 7*(1), 59–63.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C. and Lerici, C. R. (2001). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science and Technology, 11*, 340–346.

Masson, P. (1992) Pressure denaturation of proteins, in Balny C, Hayashi R, Heremans K, Masson P. (Ed.), *High Pressure and Biotechnology by Colloques INSERM* (pp 89-99). France: John Libbey Eurotext Ltd.

Matser, A. M., B. Krebbers, Robert W van den Berg, Paul V Bartels (2004). Advantages of high pressure sterilisation on quality of food products. *Trends in Food Science & Technology*, *15*(2), 79-85.

Mavric, E., Wittmann, S., Barth, G. and Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand. *Mol Nutr Food Res*., *52*(4), 483-9.

McInerney, J. K., Seccafien, C. A., Stewart, C. M. and Bird, A. R. (2007). Effects of high Pressure processing on antioxidant activity, and total carotenoid content and availability, in vegetables. *Innovative Food Science & Emerging Technologies*, *8*(4), 543–548.

Meda, A., Lamien, C. E., Romito, M., Millogo, J. and Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, *91*(3), 571–577.

Mertens, B (1995). Hydrostatic pressure treatment of food: equipment and processing. In: Gould GW (Ed.), *New Methods of Food Preservation* (pp. 135-158). Glasgow: Blackie, Academic and Professional.

Michel, M. and Autio, K. (2001). Effects of high pressure on protein- and polysaccharide-based structures. In M. Hendrickx. and D. Knorr (Eds.), *Ultra high pressure treatments of foods* (pp. 189-214). New York, United States: Kluwer Academic/Plenum Publishers.

Min, S., S. K. Sastry, V.M. Balasubramaniam (2010). Compressibility and density of select liquid and solid foods under pressures up to 700 MPa. *Journal of Food Engineering*, *96*(4), 568-574.

Molan, P.C. (1992). The antibacterial activity of honey. 1. The nature of the antibacterial Activity. *Bee World*, *73*(1), 5-28.

Molan, P. C. (1997). Honey as an Antimicrobial Agent. In A. Mizrahi and Y. Lensky (Eds.), *Bee Products* (pp 27-37). US: Springer.

Molina-Ho¨ppner, A., Doster, W., Vogel, R.F. and Michael G. Ganzle. (2004). Protective Effect of Sucrose and Sodium Chloride for *Lactococcus lactis* during Sublethal and Lethal High-Pressure Treatments. *Applied and Environmental Microbiology*, *70* (4), 2013–2020.

Mossel, B., Bhandari, B., D'Arcy, B. and Caffin, N. (2000). Use of an Arrhenius model to predict rheological behaviour in some Australian honeys. *LWT - Food Science and Technology*, *33*(8), 545–552.

Moussa, M., Perrier-Cornet, JM and Gervais, P. (2006). Synergistic and antagonistic effects of combined subzero temperature and high pressure on inactivation of *Escherichia Coli*. *Applied and Environmental Microbiology*, *7*(2), 150-156.

Mozhaev, V. V., Lange, R., Kudryashova, E. V. and Balny, C. (1996). Application of high hydrostatic pressure for increasing activity and stability of enzymes. *Biotechnology and Bioengineering*, *52*(2), 320–331.

Mundo, M. A., O. I. Padilla-Zakour, and R.W. Worobo (2004). Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *International Journal of Food Microbiology*, *97*(1), 1-8.

Munroe, J. A. (1943). The viscosity and thixotropy of honey. *Journal of Entomology*, *36*, 769–777.

Nagai, T., Sakai, M., Inoue, R., Inoue, H. and Suzuki, N. (2001). Antioxidative activities of some commercially honeys, royal jelly, and propolis. *Food Chemistry*, *75*, 237–240.

Nakano, H., Y. Yoshikuni, et al. (1992). Detection of Clostridium botulinum in natural Sweetening. *International Journal of Food Microbiology*, *16*(2), 117-121.

Nanda, V., M. B. Bera and Bakhshi, A.K. (2006). Optimization of the process parameters to establish the quality attributes of hydroxymethylfurfural content and diastatic activity of sunflower (Helianthus annus) honey using response surface methodology. *European Food Research and Technology*, *222*(1-2), 64-70.

National Honey Board (1991). Ultrafiltered Honey; N. H. B.: Longmont, CO.

Ndayisaba, G. Bazira, L. Habonimana, E. and Muteganya, D. (1993). Clinical and bacteriological results in wounds treated with honey. *Journal of Orthopaedic Surgery*, *7*(2), 202-4.

Nguyen, M. T., Oey, I., Hendrickx, M. and Van Loey, A. (2006). Kineticsfor isobaric-isothermal degradation of (6R,S) 5-formyltetrahydrofolicacid in a model system. *European and Food Research and Technology*, *223*(3), 325- 332.

Nguyen, L. C. and Balasubramaniam, V.M. (2011). Chapter 1: fundamentals of food Processing using high pressure. Non-thermal processing technologies for food (pp. 3–19). UK: IFT Press,Wiley-BlackWell.

Nicoli, M.C., Anese, M., Parpinel, M.T., Franceschi S. and Lerici C.R. (1997). Study on Loss and/or Formation of Antioxidants during Processing and Storage. *Cancer Letters*, *114*, 71-74.

Oey, I., Verlinde, P., Hendrickx, M. and Van Loey, A. (2006). Temperature and pressure

stability of L-ascorbic acid and/or [6s] 5-methyltetrahydrofolicacid: a kinetic study. *European and Food Researchand Technology*, *223*, 71-77.

Oey, I., Lille, M., Van Loey, A. and Hendrickx, M. (2008). Effect of high-pressure Processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. *Trends in Food Science & Technology*, *19*(6), 320–328.

Ogawa, H., Fukuhisa, K., Kubo, Y. and Fukumoto, H. (1990). Pressure inactivation of yeasts, molds, and pectinesterase in Satsuma mandarin juice: Effects of juice concentration, pH, and organic acids, and comparison with heat sanitation. *Agricultural and Biological Chemistry*, *54*(5), 1219−1225.

Olaitan, P.B, Adeleke, O.E. and Iyabo, O.O (2007). Honey: a reservoir for microorganisms and an inhibitory agent for microbes. *African Health Sciences*, *7*(3), 159-165.

Otero, L., Molina-Garcı´a, A. D. and Sanz, P. D. (2000). Thermal effect in foods during quasi-adiabatic pressure treatments. *Innovative Food Science and Emerging Technologies*, 1, 119–126.

Oxen, P., and Knorr, D. (1993). Baroprotective effects of high solute concentrations against inactivation of Rhodotorula rubra. *Lebensmittel-Wissenchaft und Technology, 26*, 220-223.

Palou, E., López-Malo, A., Bárbosa-Canovas, G. V., Welti-Chanes, J. and Swanson, B. G. (1997). Effect of water activity on high hydrostatic pressure inhibition of Zygosaccharomyces bailii. *Letters in Applied Microbiology*, *24*, 417−420.

Palou, E., López-Malo, A., Barbosa-Cánovas, G. V., Welti-Chanes, J., Davidson, P. M. and Swanson, B. G. (1998). High Hydrostatic Pressure Come-Up Time and Yeast Viability. *Journal of Food Protection*, *12*, 1597-1697.

Palou, E., Lopez-Malo, A., Barbosa-Canovas, G. V., Welti-Chanes, J., and Swanson, B. G. (1999a). High-pressure treatment in food preservation. In M. S. Rahman (*Ed*.) *Handbook of Food Preservation* (pp. 533-575). New York: Marcel Dekker.

Palou, E., López-Malo, A., Barbosa-Cánovas, G. V., Welti-Chanes, J. and Swanson, B. G. (1999b). Polyphenoloxidase Activity and Colour of Blanched and High Hydrostatic Pressure Treated Banana Puree. *Journal of Food Science*, *64*(1), 42-45.

Pan, J. and Ji, C. (1998). General rheological model for natural honeys in China. *Journal of Food Engineering*, *36*(2), 165–168.

Parish, M. E. (1998). High pressure inactivation of *Saccharomyces cerevisiae*, endogenous microflora and pectinmethylesterase in orange juice. *Journal of Food Safety, 18*, 57−65.

Patazca, E., T. Koutchma, Balasubramaniam, V.M. (2007). Quasi-adiabatic temperature increase during high pressure processing of selected foods. *Journal of Food Engineering*, *80*(1): 199-205.

Patras, A., Brunton, N. P., Da Pieve, S. and Butler, F. (2009a). Impact of high pressure Processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies*, *10*(3), 308–313.

Patras, A., Brunton, N., Da Pieve, S., Butler, F. and Downey, G. (2009b). Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées. *Innovative Food Science & Emerging Technologies*, *10*(1), 16–22.

Patterson, M. F., M. Quinn, R. Simpson, and A. Gilmour (1995). Sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate-buffered saline and foods. *J. Food Prot*., *58,* 524-529.

Patterson, M. F. (2005). Microbiology of pressure-treated foods. *Journal of Applied Microbiology*, *98*, 1400-1409.

Pavlovic, M., S. Hörmann, Rudi F. Vogel and Matthias A. Ehrmann (2005). Transcriptional response reveals translation machinery as target for high pressure in Lactobacillus sanfranciscensis. *Archives of Microbiology*, *184*(1), 11-17.

Pereira, R.N. and Vicente, A.A. (2010). Environmental impact of novel thermal and non-thermal technologies in food processing. *Food Research International, 43*, 1936–1943.

Plaza, L., Mun˜oz, M., de Ancos, B. and Pilar Cano, M. (2003). Effect of combined treatments of high-pressure, citric acid and sodium chloride on quality parameters of tomato puree. *European and Food Research and Technology*, *216*, 514-519.

Polydera, A. C., Stoforos, N. G. and Taoukis, P. S. (2005). Quality degradation kinetics of pasteurised and high pressure processed fresh navel orange juice: nutritional parameters and shelf life. *Innovative Food Science & Emerging Technologies*, *6*(1), 1–9.

Porretta, S., Birzi, A., Ghizzoni, C. and Vicini, E. (1995). Effects of ultrahigh hydrostatic pressure treatments on the quality of tomato juice. *Food Chemistry*, *52*(1), 35–41.

Prasad, K. N., Yang, E., Yi, C., Zhao, M. and Jiang, Y. (2009). Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp. *Innovative Food Science & Emerging Technologies*, *10*(2),

155–159.

Prestamo, G. and Arroyo, G. (1998). High hydrostatic pressure effects on vegetable structure. *Journal of Food Science, 63*(5), 878–881.

Purroy, F. High Pressure Processing: Evolution From Novel To… Mainstream?. The FoodBowl - High Pressure Processing Seminar/Workshop, 16-17 April 2013.

Ramaswamy, R and Balasubramaniam V.M. (2007). Effect of polarity and molecular structure of selected liquids on their heat of compression during high pressure processing. *High Pressure Research*, *27* (2), 1–9.

Ramaswamy, H. S., Shao, Y., Bussey, J. and Austin, J. (2013). Screening of twelve Clostridium botulinum (group I) spores for high-pressure resistance at elevated temperatures. *Food and Bioproducts Processing*, *91*, 403-412.

Ramirez Cervantes, M.A., S.A. Gonzales Novelo and E.Saur Duch (2000). Effect of temporary thermic treatment of honey on variation of quality of the same during storage. *Apiacta, 35*, 162-170.

Rasanayagam, V., V.M. Balasubramaniam, E. Ting, C.E. Sizer, C. Anderson and C. Bush (2003). Compression heating of selected fatty food substances during high pressure processing. *Journal of Food Science*, *68* (1), 254 -259.

Raso, J., Góngora-Nieto, M. M., Barbosa-Cánovas, G. V. and Swanson, B. G. (1998). Influence of several environmental factors on the inhibition of germination and inactivation of *Bacillus cereus* by high hydrostatic pressure. *International Journal of Food Microbiology*, *44*, 125-132.

Rauter, A.P., Dias, C., Martins, A., Branco, I., Neng, N.R. and Nogueira, J.M. (2012). Non-toxic Salvia Sclareoides Brot. extracts as a source of functional food ingredients: phenolic profile, antioxidant activity and prion binding properties. *Food Chemistry*, *132* (4), 1930–1935.

Reddy, N.R., Solomon H.M., Tezloff R.C. and Rhodehamel E.J. (2003). Inactivation of *Clostridium botulinum* type A spores by high pressure processing at elevated temperatures. *J Food Prot*, *66*, 1402–7.

Rios, A. M., Novoa, M. L. and Vit, P. (2001). Effects of extraction, storage conditions and heating treatment on antibacterial activity of Zanthoxylumfagara honey from Cojedes, Venezuela. *Revista Cientifica-Facultad De CienciasVeterinarias*, *11*(5), 397–402.

Ritz, M., Freulet, M., Orange, N. and Federighi, M. (2000). Effects of high hydrostatic pressure on membrane proteins of Salmonella typhimurium. *International Journal of Food Microbiology*, *55*,115-119.

Rodrigo, D., Van Loey, A. and Hendrickx, M. (2007). Combined thermal and high pressure colour degradation of tomato puree and strawberry juice. *Journal of Food Engineering*, *79*, 553–560.

Rovere, P., Sandei, L., Colombi, A., Munari, M., Ghiretti G. and Carpi, G. (1997). Effects of high-pressure treatment on chopped tomatoes. *Industrial Conserve*, *72*(1), 3–12.

Sale, A.J.H, Gould G.W. and Hamilton, W.A (1970). Inactivation of bacterial spores by hydrostatic pressure. *J. Gen. Microb*. *60*, 323-334.

Samaranayake, C.P and Sastry, S.K. (2010). In Situ Measurement of pH Under High Pressure. *J. Phys. Chem. B, 114*, 13326–13332.

San Martín, M. F., Barbosa-Cánovas, G. V. and Swanson, B. G. (2002). Food processing by high hydrostatic pressure. *Critical Reviews in Food Science and Nutrition*, *42*, 627-645.

Sánchez-Moreno, C., Plaza, L., De Ancos, B. and Cano, M. P. (2006). Impact of high-pressure and traditional thermal processing of tomato puree on carotenoids, vitamin C and antioxidant activity. *Journal of the Science of Food and Agriculture*, *86*(2), 171–179.

Sancho F, Lambert Y, Demazeau G, Largeteau A, Bouvier J.M and Narbonne J.F. (1999). Effect of ultra-high hydrostatic pressure on hydrosoluble vitamins. *Journal of Food Engineering*, *39*(3), 247-253.

Satomi, M., T. Yamaguchi, M. Okuzumi, and T. Fujii. (1995). Effect of conditions on the barotolerance of *Escherichia coli*. *J. Food Hyg. Soc. Jpn*. *36*, 29–34.

Senhajit, A.F. and Loncins, M (1977). The protective effect-of fat on the heat resistance of bacteria (I). *Journal of Food Technol*, *12*, 203-216.

Shaker, E.S., Ghazy, M.A. and Shibanoto, T. (1995). Antioxidants activity volatile browning reaction products and related compounds in a hexanol/hexanoic acid system. *Journal of Agricultural and Food Chemistry*, *43*, 1017–1022.

Shao, Y. and Ramaswamy, H. S. (2011). Clostridium sporogenes-ATCC 7955 spore destruction kinetics in milk under high pressure and elevated temperature treatment conditions. *Food and Bioprocess Technology*, *4*, 458-468.

Shimada, S., Andou, M., Naito, N., Yamada, N., Osumi, M. and Ž.Hayashi, R. (1993). Effects of hydrostatic pressure on the ultra-structure and leakage of internal substances in the yeast *Saccharomyces cerevisiae*. *Applied Microbiology and Biotechnology*, *40*, 121-131.

Shimanuki, H., Herbert, E.W., Jr., and Knox, D.A. (1984). High velocity electron beams for bee disease control. *Apicultural Res*., *124*(12), 865-867.

Simpson, R. K. and Gilmour, A. (1997). The effect of high hydrostatic pressure on the activity of intracellular enzymes of Listeria monocytogenes. *Letters in Applied Microbiology*, *25*, 48-53.

Singh, N. and Bath, P. K. (1998). Relationship between heating & hydroxymethylfurfural formation in different honey types. *Journal of Food Science and Technology*, *35*, 154–156.

Singleton,V. L., Orthofer, R. and Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. In P.Lester (Ed.), *Methods in Enzymology* (pp 152–178). USA: Academic Press.

Smiddy, M., O'Gorman, L., Sleator, R. D., Kerry, J. P., Patterson, M. F., Kellya, A. L. and Colin Hill, (2005). Greater high-pressure resistance of bacteria in oysters than in buffer. *Innovative Food Science and Emerging Technologies, 6*, 83-90.

Snowdon, J. A. and Cliver, D. O. (1996). Microorganisms in honey. *International Journal of Food Microbiology*, *31*, 1-26.

Socha, R., Juszczak, L., Pietrzyk, S., Gałkowska, D., Fortuna, T. and Witczak, T. (2011). Phenolic profile and antioxidant properties of Polish honeys. *International Journal of Food Science and Technology*, *46*(3), 528–534.

Subramanian, R., Umeshhebbar, H. and Rastogi, N.K. (2007). Processing of Honey: A Review. *International Journal of Food Properties*, *10*, 127-143.

Suthanthangjai, W., Kajda, P. and Zabetakis, I. (2005). The effect of high hydrostatic pressure on the anthocyanins of raspberry (Rubusidaeus). *Food Chemistry, 90*(1–2), 193-197.

Takahashi, Y., Ohta, H., Yonei, H. and Ifuku, Y. (1993). Microbicidal effect of hydrostatic pressure on satsuma mandarin juice. *International Journal of Food Science & Technology*, *28*(1), 95-102.

Tan, H.T, Abdul Rahman, R, Gan, S.H., Halim, A.S., Hassan, S.A., Sulaiman, S.A. and Kaur, K. (2009). The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to Manuka honey. *BMC Complementary and Alternative Medicine*, *9*, 34.

Tangwongchai, R., Ledward, D. A. and Ames, J. M. (2000). Effect of high-pressure Treatment on the texture of cherry tomato. *Journal of Agriculture and Food Chemistry*, *48*, 1434-1441.

Tassou, C. C., Galiatsatou, P., Samaras, F. J. and Mallidis, C. G. (2007). Inactivation kinetics of a piezotolerant *Staphylococcus aureus* isolated from high-pressuretreated sliced ham by high pressure in buffer and in a ham model system: evaluation in selective and non-selective medium. *Innovative Food Science & Emerging Techno*logies, *8*, 478-484.

Terrab, A., A. F. Recamales, M. L. González-Miretc, and F.J. Herediac (2005). Contribution to the study of avocado honeys by their mineral contents using inductively coupled plasma optical emission spectrometry. *Food Chemistry*, *92*(2), 305-309.

Thrasyvoulou, A.,Manikis, J., and Tselios, D. (1994). Liquefying Crystallized Honey with Ultrasonic Waves. *Apidologie*, 25: 297–302.

Ting, E.Y. and Marshall, R.G. (2002) Production issues related to UHP food. In Welti-Chanes J, Barbosa-Canova, G.V., Aguilera, J.M (Ed.) *Engineering and Food for the 21 st century. Food Preservation Technology Series* (pp. 727 -738). Boca Raton: CRC Press.

Ting, E., Balasubramaniam and V. M., Raghubeer, E. (2002). Determining thermal effects in high pressure processing. *Journal of Food Technology*, *56*(2), 31-35.

Toepfl, S., Mathys, A., Heinz, V. and D. Knorr (2006). Review: Potential of emerging technologies for energy efficient and environmentally friendly food processing. *Food Reviews International*, *22*(4), 405- 423.

Tomás-Barberán, F. A., I. Martos, F. Ferreres, B.S. Radovic and E. Anklam (2001). HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. *Journal of the Science of Food and Agriculture*, *81*(5), 485-496.

Torres, J. A. and Velazquez, G. (2005). Commercial opportunities and research challenges in the high pressure processing of foods. *Journal of Food Engineering*, *67*,95-112.

Tosi,E., Ciappini, M.C and Ré, H. Lucero. (2001). Honey thermal treatment effects on hydroxymethylfurfural content. *Food Chemistry*, *77*, 71–74.

Tosi, E. A., Re, E. and Bulacio, L. (2004). Effect of honey high temperature short-time heating on parameters related to quality, crystallisation phenomena and fungal inhibition. *LWT- Food Science and Technology*, *37*(6), 669–678.

Tosi, E., Martinet, R., Ortega, M., Lucero, H. and Ré, E. (2008). Honey diastase activity modified by heating. *Food Chemistry*, *106*(3), 883-887.

Turhan, K. (2009). Effects of Thermal Treatment and Storage on Hydroxymethylfurfural

(HMF) Content and Diastase Activity of Honeys Collected from Middle Anatolia in Turkey. In B. Şener (*Ed*). *Innovations in Chemical Biology* (pp. 233-239). Netherlands: Springer.

Turkmen, N., Sari, F., Poyrazoglu, E. S. and Velioglu, Y. S. (2006). Effects of prolonged heating on antioxidant activity and colour of honey. *Food Chemistry*, *95*, 653–657.

Urrutia-Benet, G. (2005). High-pressure-low-temperature processing of foods: Impact of metastable phases of process and quality parameters. PhD thesis, Berlin University of Technology.

Vaikousi, H., Koutsoumanis, K. and Biliaderis, C. G. (2009). Kinetic modelling of non-enzymatic browning in honey and diluted honey systems subjected to isothermal and dynamic heating protocols. *Journal of Food Engineering*, *95*(4), 541-550.

Van den Berg, R., and H. Hoogland (2001). High Pressure Equipment Designs for Food Processing Applications. In M. G. Hendrickx, D. Knorr, L. Ludikhuyze, A. Van Loey and V. Heinz *(Ed.)Ultra High Pressure Treatments of Foods* (pp. 297-313). New York: Springer-Verlag.

Van Loey Indrawati, A. and Hendrickx, M. (2004). Pressure and temperature stability of water-soluble antioxidants in orange and carrot juice: a kinetic study. *European Food Research and Technology*, *219*(2),161–166.

Vega-Gálvez, A., Giovagnoli, C., Pérez-Won, M., Reyes, J. E., Vergara, J., Miranda, M., E. Uribea and K. D. Scalad (2012). Application of high hydrostatic pressure to aloe vera (Aloe barbadensis Miller) gel: microbial inactivation and evaluation of quality parameters. *Innovative Food Science & Emerging Technologies*, *13*, 57–63.

Vercammen, A., Vivijs, B., Lurquin, I. and Michiels, C. W. (2012). Germination and inactivation of Bacillus coagulans and Alicyclobacillus acidoterrestris spores by high hydrostatic pressure treatment in buffer and tomato sauce. *International Journal of Food Microbiology*, *152*: 162-167.

Verlent, I., Hendrickx, M., Rovere, P., Moldenaers, P. and Van Loey, A.(2006). Rheological properties of tomato-based products after thermal and high-pressure treatment. *Journal of Food Science*, *71*(3), 243-248.

Visquert, M., Escriche, I., Andres, A. and Fito, P. (2004). Changes in the Quality Parameters of Honey Caused by Thermal Processes. *Alimentacion-Equipos-y-Tecnologia*., *23*, 87–92.

Wakhle, D.M. and Phadke, R.P. (1995). Design for Honey Processing Unit Part I. Indian *Bee J. 57*, 144–146.

Walker, M. K., Farkas, D. F., Loveridge, V and Meunier-Goddik, L. (2006). Fruit yogurt processed with high pressure. *International Journal of Food Science & Technology*, *41*(4), 464–467.

Wang, X. H., Gheldof, N. and Engeseth, N. J. (2004). Effect of processing and storage on antioxidant capacity of honey. *Journal of Food Science*, *69*(2), 96–101.

Watanabe, M., Arai, E., Kumeno, K. and Honma, K. (1991). A New Method for Producing a Non-heated Jam Sample: The Use of Freeze Concentration and High-pressure Sterilization (Food & Nutrition). *Agricultural and biological chemistry*, *55*(8), 2175-2176.

Weemaes, C., Ooms, V., Indrawati, Ludikhuyze, L., Broeck, V. D., Loey, A. V. and M. Hendrickx (1999). Pressure–temperature degradation of green colour in broccoli juice. *Journal of Food Science*, *64*(3), 504–508.

White J.W., Riethof M.L and Kushnir I., (1961). Composition of Honey. VI. The Effect of Storage on Carbohydrates, Acidity and Diastase Content. *Journal of Food Science 26*(1), 63-71.

White, J.W., Jr., Kushnir, I. and Subers, H.M. (1964). Effect of Storage and Processing Temperature on Honey Quality. *Food Technol*., *18*, 555–55.

White, J. W. (1978). Honey. *Advances in Food Research*, *24*, 288–354.

Wong, M. and Stanton, D. W. (1989). Nonenzymic browning in kiwifruit juice concentrate systems during storage. *Journal of Food Science*, *54*, 669–673.

Wood B., Rademaker M and Molan P C (1997). Manuka honey, a low cost leg ulcer Dressing. *New Zealand Medical Journal*, 110, 107.

Yaldagard, M., Mortazavi, S.A. and Tabatabaie, F. (2008).The principles of ultra high pressure technology and itsapplication in food processing/preservation: A review of microbiological and quality aspects. *African Journal of Biotechnology*, *7* (16), 2739-2767.

Yang, B., Shi, Y., Xia, X., Xi, M., Wang, X., Ji, B., and J. Meng (2012). Inactivation of foodborne pathogens in raw milk using high hydrostatic pressure. *Food Control*, *28*, 273-278.

Yanniotis, S., Skaltsi, S. and Karaburnioti, S. (2006). Effect of moisture content on the viscosity of honey at different temperatures. *Journal of Food Engineering*, *72*(4), 372–377.

Yen, G. C. and Duh, P. D. (1994). Scavenging effect of methanolic extracts of peanut hulls On free-radical and active oxygen species. *Journal of Agricultural and Food Chemistry*, *42*, 629–632.

Yen, G.C. and Lin, H.T. (1996). Comparison of high pressure treatment and thermal pasteurization effects on the quality and shelf life of guava puree. *International Journal of Food Science & Technology*, *31*(2), 205-213.

Zaitoun, S.,Ghzawi, A.A.,Al-Malah, K.I.M., and Abu-Jdayil, B (2001). Rheological Properties of Selected Light Colored Jordanian Honey. *Int. J. Food Prop*., *4*, 139–148.

Zhang, H., L. Li, Tatsumic, E., Isobea, S. (2005). High-pressure treatment effects on proteins in soy milk. *LWT - Food Science and Technology*, *38*(1), 7-14.