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.
# TABLE OF CONTENTS

ABSTRACT ii
DEDICATION iii
ACKNOWLEDGEMENT iv
TABLE OF CONTENTS v
LIST OF FIGURES x
LIST OF TABLES xiii
NOMENCLATURE xv

CHAPTER 1: Introduction 1
  1.1 Introduction 2
  1.2 Research objectives 3
  1.3 Thesis framework 4

CHAPTER 2: Literature Review 6
  2.1 Honey: A natural sweetener and its valuable properties 7
  2.2 Production, Collection and Process of Honey 14
  2.3 Conventional Thermal Treatment of Honey Processing 16
  2.4 Alternative Thermal Processing Methods for Honey 22
    2.4.1 Microwave heating 22
    2.4.2 Infrared heating 23
  2.5 Non-Thermal Processing Methods for Honey 24
    2.5.1 Ultrasound 24
    2.5.2 Ultraviolet 25
    2.5.3 Membrane filtration 26
  2.6 High Pressure Processing 27
    2.6.1 Evolution of High Pressure Processing 27
    2.6.2 General Principle of High Pressure Processing and
      Adiabatic Heating of Compression 30
    2.6.3 High Pressure Processing Equipment 33
    2.6.4 Impact of high pressure on food quality 36
    2.6.5 Inactivation of microorganisms under pressure 42
  2.7 Advantages and Limitation of HPP 45
    2.7.1 Advantages 45
2.7.2 Limitation

CHAPTER 3: High pressure processing (HPP) of honey for the improvement of nutritional value

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter Abstract</td>
<td>48</td>
</tr>
<tr>
<td>3.1 Introduction</td>
<td>49</td>
</tr>
<tr>
<td>3.2 Material and methods</td>
<td>51</td>
</tr>
<tr>
<td>3.2.1. Honey sample preparation</td>
<td>51</td>
</tr>
<tr>
<td>3.2.2. High pressure processing (HPP) equipment</td>
<td>51</td>
</tr>
<tr>
<td>3.2.2.1. HPP of honey</td>
<td>52</td>
</tr>
<tr>
<td>3.2.2.2 Ambient temperature HPP</td>
<td>52</td>
</tr>
<tr>
<td>3.2.2.3. Combined HPP–thermal processing of honey</td>
<td>52</td>
</tr>
<tr>
<td>3.2.3. Thermal processing of honey</td>
<td>52</td>
</tr>
<tr>
<td>3.2.4. Total phenolic content (TPC) determination</td>
<td>53</td>
</tr>
<tr>
<td>3.2.5. Statistical analysis</td>
<td>53</td>
</tr>
<tr>
<td>3.3 Results and discussion</td>
<td>54</td>
</tr>
<tr>
<td>3.3.1. Total phenolic content (TPC) of unprocessed Manuka honey</td>
<td>54</td>
</tr>
<tr>
<td>3.3.2. High pressure processing (HPP) of Manuka honey</td>
<td>56</td>
</tr>
<tr>
<td>3.3.3. Combined HPP and thermal processing of Manuka honey</td>
<td>58</td>
</tr>
<tr>
<td>3.3.4. Thermal processing of Manuka honey</td>
<td>60</td>
</tr>
<tr>
<td>3.3.5. Comparison of HPP, HPP–thermal and exclusively thermal processing of Manuka honey</td>
<td>61</td>
</tr>
<tr>
<td>3.4 Conclusions</td>
<td>62</td>
</tr>
</tbody>
</table>

CHAPTER 4: High-Pressure Processing of Manuka Honey: Improvement of Antioxidant Activity, Preservation of Colour and Flow Behaviour

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter Abstract</td>
<td>64</td>
</tr>
<tr>
<td>4.1 Introduction</td>
<td>65</td>
</tr>
<tr>
<td>4.2 Material and Methods</td>
<td>67</td>
</tr>
<tr>
<td>4.2.1 Honey Samples Preparation</td>
<td>67</td>
</tr>
<tr>
<td>4.2.2 Processing of Honey</td>
<td>67</td>
</tr>
<tr>
<td>4.2.2.1 High Pressure Processing Equipment</td>
<td>67</td>
</tr>
<tr>
<td>4.2.2.2 HPP of Honey at Ambient Temperature</td>
<td>68</td>
</tr>
<tr>
<td>4.2.2.3 Combined HPP–Thermal Processing of Honey</td>
<td>68</td>
</tr>
<tr>
<td>4.2.3 Thermal Processing of Honey</td>
<td>68</td>
</tr>
<tr>
<td>4.2.4 Quality Determination</td>
<td>69</td>
</tr>
</tbody>
</table>
4.2.4.1 Antioxidant Activity 69
4.2.4.2 Colour 70
4.2.4.3 Rheological Behaviour and Viscosity 70
4.2.5 Statistical Analysis 71
4.3 Results and Discussion 72
4.3.1 Effect of Processes on Honey Antioxidant Activity 72
4.3.2 Effect of Processes on Colour of Honey 75
4.3.3 Effect of Processes on Honey Flow Behaviour 77
4.4 Conclusions 81

CHAPTER 5: High Pressure Processing (HPP) of Manuka honey: Brown pigment formation, improvement of antibacterial activity and hydroxymethylfurfural content 82

5.1 Introduction 84
5.2 Material and Methods 86
5.2.1 Honey samples preparation 86
5.2.2 Processing honey 86
5.2.2.1 High Pressure Processing (HPP) equipment 86
5.2.2.2 HPP of honey at ambient temperature 87
5.2.2.3 Combined HPP-thermal processing of honey 87
5.2.2.4 Thermal processing of honey 87
5.2.3 Brown pigment 88
5.2.4 Antibacterial activity 88
5.2.5 Hydroxymethylfurfural, HMF content 89
5.2.6 Statistical analysis 90
5.3 Results and discussion 91
5.3.1 Influence of treatments on brown pigment formation 91
5.3.2 Influence of treatments on antibacterial activity 94
5.3.3 Influence of treatments on hydroxymethylfurfural content 96
5.3.4 The relationship between the brown pigment formation, antibacterial activity, antioxidant activity and hydroxymethylfurfural 98
5.4 Conclusions 99
CHAPTER 6: High Pressure Processed Manuka Honey: Change in Nutritional and Rheological Properties over One Year Storage

Chapter Abstract

6.1 Introduction

6.2 Material and Methods

6.2.1 Honey samples preparation

6.2.2 High Pressure Processing (HPP) equipment

6.2.2.1 HPP of honey at ambient temperature

6.2.3 Monitoring changes in honey properties during storage of one year

6.2.4 Physicochemical Analysis

6.2.4.1 Moisture content, total soluble solids and total solids

6.2.4.2 pH measurement

6.2.4.3 Electrical conductivity

6.2.5 Nutritional properties analysis

6.2.5.1 Antioxidant activity

6.2.5.2 Total Phenolic Content

6.2.5.3 Brown pigment

6.2.6 Rheological behaviour and viscosity

6.2.7 Statistical analysis

6.3 Results and Discussion

6.3.1 Changes in physicochemical properties

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

6.3.3 Changes in rheological properties and viscosity

6.4 Conclusions

CHAPTER 7: An insight on the relationship between food compressibility and microbial inactivation during high pressure processing

Chapter Abstract

7.1 Introduction

7.2 Materials and Method

7.2.1 *Saccharomyces cerevisiae* strain and growth medium
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.2.2</td>
<td>Honey inoculation and packaging</td>
<td>123</td>
</tr>
<tr>
<td>7.2.3</td>
<td>Preparation of honey samples with different concentration</td>
<td>124</td>
</tr>
<tr>
<td>7.2.4</td>
<td>High pressure processing equipment and operation</td>
<td>124</td>
</tr>
<tr>
<td>7.2.4.1</td>
<td>HPP of honey samples</td>
<td>124</td>
</tr>
<tr>
<td>7.2.5</td>
<td>Thermal processing of honey</td>
<td>125</td>
</tr>
<tr>
<td>7.2.6</td>
<td><em>Saccharomyces cerevisiae</em> cells enumeration</td>
<td>125</td>
</tr>
<tr>
<td>7.2.7</td>
<td>Compressibility measurement</td>
<td>126</td>
</tr>
<tr>
<td>7.2.8</td>
<td>Statistical analysis</td>
<td>126</td>
</tr>
<tr>
<td>7.3</td>
<td>Results and Discussion</td>
<td>127</td>
</tr>
<tr>
<td>7.3.1</td>
<td>Compressibility and ° Brix during high pressure processing</td>
<td>127</td>
</tr>
<tr>
<td>7.3.2</td>
<td>The combined effect of compressibility and sugar protective nature on the inactivation of <em>Saccharomyces cerevisiae</em></td>
<td>132</td>
</tr>
<tr>
<td>7.4</td>
<td>Conclusions</td>
<td>137</td>
</tr>
</tbody>
</table>

**CHAPTER 8: Conclusions, Recommendation and Implication of the Project**  
138

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1</td>
<td>Conclusions</td>
<td>139</td>
</tr>
<tr>
<td>8.2</td>
<td>Recommendations for Future Work</td>
<td>141</td>
</tr>
<tr>
<td>8.3</td>
<td>Implications of the Project</td>
<td>142</td>
</tr>
</tbody>
</table>

**Publications and Presentations Based on Thesis Work**  
143

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**References**  
147
LIST OF FIGURES

Figure 2.1: (a) Production quantity of natural honey in world from 1961 to 2009 (b) Global honey production by select country and region (FAO). 7

Figure 2.2: Average composition of honey (the data is summarized from Alvarez-Suarez et al., 2010, Jeffrey and Echaza retta, 1996 and Olaitan et al., 2007). 9

Figure 2.3: The production of honey from nectar (adapted from Grane and Kirk Visscher, 2007). 14

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. 15

Figure 2.5: The flow of conventional process in honey production. 16

Figure 2.6: A brief history of high pressure food processing. 28

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). 29

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, Columbus, OH, USA). 33

Figure 2.9: A typical high pressure processing system for treating food. 34

Figure 2.10: (A) Direct system for generation of high isostatic pressure and (B) Indirect system. 35

Figure 2.11: (a) QFP 2L-700 Laboratory Food Processing System (Avure Technologies, Columbus, OH, USA), (b) 2-litre pressure vessel and (c) schematic diagram of the system. 36

Figure 3.1: Total phenolic content of honey samples after treated with HPP at different pressure and time in comparison to unprocessed. 57

Figure 3.2: Total phenolic content of honey samples after treated with HPP (600 MPa) for different temperature and time in comparison to unprocessed. 59

Figure 3.3: Total phenolic content of honey samples after thermal treatments, for different temperature and time in comparison to unprocessed. 61

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.

Figure 4.1: Antioxidant activity (%) of Manuka honey for different processes. All the processes had the duration of 10 min.

Figure 4.2: Correlation between antioxidant activity of HPP treated Manuka honey (600 MPa at ambient temperature) and its total phenolic content.

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.

Figure 4.4: The viscosity curves as a function of shear rate. (a) Unprocessed and HPP-processed Manuka honey at ambient temperature

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.

Figure 5.2: Percentage inhibition of *S. epidermidis* at different concentration of Manuka honey.

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.

Figure 5.4: Percentage increase of HMF in HPP- treated honey sample (ambient temp) at different pressure and different processing time

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.

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

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.

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.
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>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.</td>
<td>118</td>
</tr>
<tr>
<td>7.1</td>
<td>Compressibility of honey solution at different pressure levels as a function of ° Brix after subjected to high pressure processing.</td>
<td>127</td>
</tr>
<tr>
<td>7.2</td>
<td><em>Saccharomyces cerevisiae</em> cell survivor curves in honey (80° Brix) subjected to high pressure treatment (ambient temperature) at (i) ◊ = 200; □ = 400 and Δ = 600 MPa.</td>
<td>129</td>
</tr>
<tr>
<td>7.3</td>
<td>Comparison of log reduction of <em>Saccharomyces cerevisiae</em> after pressure treatment at 600 MPa for 2 and 30 min and thermal treatment at 55°C for 10 min at different ° Brix.</td>
<td>132</td>
</tr>
<tr>
<td>7.4</td>
<td>Effect of compressibility and ° Brix on inactivation of <em>Saccharomyces cerevisiae</em> subjected to pressure treatment at 600 MPa for 2 and 30 min.</td>
<td>135</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 2.1: Honey export volumes, value and prices of New Zealand market from 2002 to 2013 (Farm Monitoring Report, 2013). 8
Table 2.2: The characteristics of honey. 10
Table 2.3: Commercial application of honey (Farm Monitoring Report, 2013). 13
Table 2.4: The summary of effects of conventional thermal treatment on honey quality. 18
Table 2.5: Overview of temperature increase during compression for some foods. 31
Table 2.6: Application of high pressure processing and its effects on the qualities of various types of food. 38
Table 3.1: Total phenolic content from different types of honey obtained from previous works in comparison with this study. 55
Table 4.1: The values of $L^*$, $a^*$, $b^*$ and total colour difference (TCD) for HPP, combined HPP-thermal and thermal treated Manuka honey. 76
Table 4.2: Apparent viscosity of unprocessed and HPP treated Manuka honey (ambient temperature, 10 min treatment) at shear rate 5.39 s$^{-1}$. 80
Table 4.3: Viscosity of combined HPP - thermally treated and thermally- treated Manuka honey (10 min treatment) at shear rate 5.39 s$^{-1}$. 80
Table 5.1: Brown pigment formation and HMF content of untreated and treated honey samples after 10 min treatment. 92
Table 5.2: Correlation matrices for four variables (antibacterial activity, brown pigment, HMF and antioxidant activity) of high pressure-treated honey using Pearson correlation. 99
Table 6.1: The physicochemical properties of untreated and HPP-treated Manuka honey (600 MPa/ambient temperature/10 min) after 12 months storage at room temperature (approximately 24°C). Values are presented as mean ± standard deviation of two measurements. 109
Table 6.2: Apparent viscosity of untreated honey and HPP-treated honey at shear rate 0.1 s$^{-1}$ during 12 months storage. 117
Table 7.1: Correlation between compressibility (MPa$^{-1}$) and ° Brix at different pressure levels after subjected to high pressure processing. The data were reassembled from Min et al. (2010). 128
Table 7.2: ° Brix effect of solute concentration on Saccharomyces cerevisiae cells in...
Table 7.3: The changes in log reduction (Δ log reduction) of *Saccharomyces cerevisiae* subjected to high pressure and thermal treatment with respect to different °Brix.

Table 7.4: Correlation matrices for °Brix, compressibility and *Saccharomyces cerevisiae* cells reduction subjected to high pressure treatment (600 MPa, ambient temperature), obtained using Pearson correlation from Statistica version 11, Statsoft®.
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
Cp = 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
Nomenclature

L = litre
MGO = methylglyoxal
MIC\textsubscript{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\textsubscript{2}CO\textsubscript{3} = sodium carbonate
N\textsubscript{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\textsuperscript{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.

°C = degree centigrade

< = less than

$\geq$ = 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
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:**


<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Contribution to the idea, do all experimental works and do writing task.</td>
<td></td>
</tr>
</tbody>
</table>

| Extent of contribution by PhD candidate (%) | 80% |

### CO-AUTHORS

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td>As a main supervisor, contribute to the main idea, make a review and check the whole paper from beginning until the paper get accepted.</td>
</tr>
<tr>
<td>Dr Filipa Silva</td>
<td>As a co-supervisor, give ideas on experimental works and assist in review and check the whole paper</td>
</tr>
</tbody>
</table>

### 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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td>[Signature]</td>
<td>3/12/2014</td>
</tr>
<tr>
<td>Dr Filipa Silva</td>
<td>[Signature]</td>
<td>3/12/2014</td>
</tr>
</tbody>
</table>

Last updated: 23 March 2013
Co-Authorship Form

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

| 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

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td>As a main supervisor, contribute to the main idea, make a review and check the whole paper from beginning until the paper get accepted.</td>
</tr>
<tr>
<td>Dr Filipa Silva</td>
<td>As a co-supervisor, give ideas on experimental works and assist in review and check the whole paper</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td>[Signature]</td>
<td>3/12/2014</td>
</tr>
<tr>
<td>Dr Filipa Silva</td>
<td>[Signature]</td>
<td>3/12/2014</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Last updated: 25 March 2013
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.

<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th>Contribute to the idea, do all experimental works and do writing task.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of contribution by PhD candidate (%)</td>
<td>80%</td>
</tr>
</tbody>
</table>

**CO-AUTHORS**

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td>As a main supervisor, contribute to the main idea, make a review and check the whole paper</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td></td>
<td>3/12/2014</td>
</tr>
<tr>
<td>Click here</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Click here</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Click here</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Click here</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Last updated: 25 March 2013
Co-Authorship Form

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td>As a main supervisor, contribute to the main idea, make a review and check the whole paper.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td>[Signature]</td>
<td>Click here</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Click here</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Click here</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Click here</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Click here</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Click here</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Click here</td>
</tr>
</tbody>
</table>

Last updated: 25 March 2013
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

<table>
<thead>
<tr>
<th>Nature of contribution by PhD candidate</th>
<th>Contribute to the idea, do all experimental works and do writing task.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of contribution by PhD candidate (%)</td>
<td>80%</td>
</tr>
</tbody>
</table>

## CO-AUTHORS

<table>
<thead>
<tr>
<th>Name</th>
<th>Nature of Contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td>As a main supervisor, contribute to the main idea, make a review and check the whole paper from beginning until the paper get accepted.</td>
</tr>
<tr>
<td>Dr Filipa Silva</td>
<td>As a co-supervisor, assist in review and check the whole paper</td>
</tr>
</tbody>
</table>

### 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.

<table>
<thead>
<tr>
<th>Name</th>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof Mohammed Farid</td>
<td>[Signature]</td>
<td>3/12/2014</td>
</tr>
<tr>
<td>Dr Filipa Silva</td>
<td>[Signature]</td>
<td>3/12/2014</td>
</tr>
</tbody>
</table>

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.
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).

![Figure 2.1](image.png)

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).

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

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 enzymes) is of interest for human health.
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.

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.

<table>
<thead>
<tr>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nutritional Aspects</strong></td>
<td></td>
</tr>
<tr>
<td>Carbohydrates/ sugars of honey</td>
<td>White, 1978</td>
</tr>
<tr>
<td>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</td>
<td></td>
</tr>
<tr>
<td>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</td>
<td>Bogdanov et al., 2008</td>
</tr>
<tr>
<td>Vitamins/Minerals/trace compounds</td>
<td>Conti, 2000</td>
</tr>
<tr>
<td>It is small residue remaining after the honey is burned and it was reported to varies from 0.02 to slightly over 1%</td>
<td></td>
</tr>
<tr>
<td>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</td>
<td>Iskander, 1995; Terrab et al., 2005</td>
</tr>
<tr>
<td>Proteins, enzymes and amino acids</td>
<td>Bogdanov et al., 2008</td>
</tr>
<tr>
<td>Enzymes in honey can conceivably arise from different sources such as bees, pollen, nectar or even yeast and microorganisms.</td>
<td></td>
</tr>
<tr>
<td>Both diastase and invertase has been reported to play an important role for judging honey quality and have been used as honey freshness indicators</td>
<td>Grane and Kirk Visscher, 2007</td>
</tr>
<tr>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Formic, acetic, citric, lactic, maleic, malic, oxalic, pyroglutamic and succinic acid are the acids which</td>
<td></td>
</tr>
</tbody>
</table>
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 |
| 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 |
| White et al., 1964 |

### Microbiological Aspects

<p>| Antimicrobial activity | Honey has both peroxide and non-peroxide antibacterial action, with different non-peroxide antibacterial substances involved: acidic, basic or neutral |
| Antimicrobial 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 |
| Al-Waili, 2004; Al-Waili and Haq, 2004; Bogdanov et al., 1997; Molan, 1997; Mundo et al., 2004 |</p>
<table>
<thead>
<tr>
<th>Medicinal Aspect</th>
<th>Pure honey has bactericidal activity against many enteropathogenic organisms, including those of the <em>Samonella</em> and <em>Shigella</em> species, and enteropathogenic <em>Eschericia coli</em></th>
<th>Jeddar et al., 1985</th>
</tr>
</thead>
<tbody>
<tr>
<td>As remedy for diarrhoea</td>
<td>Gastric ulcers have been successfully treated by the use of honey as a dietary supplement</td>
<td>Ali, 1991</td>
</tr>
<tr>
<td>As medicine for gastric ulcer</td>
<td>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</td>
<td>Bassam et al., 1997; Bergman et al., 1983; Efem, 1988; Ndayisaba et al., 1993; Tan et al., 2009; Wood et al., 1997)</td>
</tr>
<tr>
<td>For wound healing</td>
<td>Honey promotes healing of ulcers and burns better than any other local application</td>
<td></td>
</tr>
<tr>
<td>Rapid healing agent</td>
<td>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</td>
<td></td>
</tr>
<tr>
<td>Stimulation of healing process</td>
<td>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.</td>
<td></td>
</tr>
</tbody>
</table>
Due to good nutritional value as it contains carbohydrates, proteins, vitamins, minerals and various other components, honey has been used as food in different parts of the world and in different ways. Commercial application of honey is summarized in the table below:

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

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

<table>
<thead>
<tr>
<th>Quality properties</th>
<th>Flower/ Country types of honey</th>
<th>Thermal process condition</th>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antioxidant activity and phenolic compounds</td>
<td>Clover honey and Buckwheat honey</td>
<td>55°C /12 to 16 hrs</td>
<td>-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%)</td>
<td>Wang et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Sunflower, cotton and canola honey</td>
<td>50, 60 and 70°C for up to 12 days</td>
<td>Antioxidant activity increased linearly with increasing heating time at 50 and 60°C, logarithmic increase in antioxidant activity at 70°C was observed.</td>
<td>Turkmen et al., 2006</td>
</tr>
<tr>
<td>Hydroxymethylfurfural, HMF concentration</td>
<td>Middle Antonia honey</td>
<td>135°C /100s and 150°C / 40 s</td>
<td>HMF concentration is increased. Treatment at 135°C for 100s produces approximately the same HMF concentration at 150°C for 40 s</td>
<td>Turhan et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Sunflower honey</td>
<td>47.5 ± 1°C for 9.5 ± 1 min at pH 5.2 ± 0.15</td>
<td>This optimum condition can keep HMF concentration within the prescribed limit of standard.</td>
<td>Nanda et al., 2006</td>
</tr>
<tr>
<td></td>
<td>Clover honey and Buckwheat honey</td>
<td>55°C for 12 to 16 hrs</td>
<td>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.</td>
<td>Wang et al., 2004</td>
</tr>
</tbody>
</table>
### Chapter 2: Literature Review

<table>
<thead>
<tr>
<th>Type of Honey</th>
<th>Heating Conditions</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unifloral Sicilian hones (Orange, Eucalyptus, Sulla, Chestnut)</td>
<td>50°C / 144 hrs, 70°C / 96 hrs and 100°C / 4 hrs</td>
<td>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.</td>
<td>Fallico et al., 2004</td>
</tr>
<tr>
<td>Forest honey, multifloral honey</td>
<td>35, 45 or 55°C (672 hrs), 65°C (168 hrs), 75°C (24 hrs) and 80°C (12 hrs)</td>
<td>HMF concentration increases with increased temperature and treatment time</td>
<td>Visquert et al., 2004</td>
</tr>
<tr>
<td>Pine, thymus, cotton, helianthus and orange</td>
<td>35, 45, 55, 65 and 75°C / 24 hrs</td>
<td>No significant increase of HMF at 55°C for 24 hrs 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.</td>
<td>Karabournioti et al., 2001</td>
</tr>
<tr>
<td>Natural honey</td>
<td>100-160°C, 14-60s</td>
<td>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</td>
<td>Tosi et al., 2004</td>
</tr>
<tr>
<td>Colour and brown pigment formation</td>
<td>Orange blossom floral honey</td>
<td>50, 60, 70 and 80°C for up to 160 hrs</td>
<td>The increase in browning was rather negligible at 50°C and 60°C, smaller at 70°C but very pronounced at 80°C</td>
</tr>
<tr>
<td></td>
<td>Sunflower, cotton and canola honey</td>
<td>50, 60 and 70°C for up to 12 days</td>
<td>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.</td>
</tr>
<tr>
<td>Chapter 2: Literature Review</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Clover honey and Buckwheat honey</strong></td>
<td>55°C / 12 to 16 hrs</td>
<td>Processed Clover honey was darker in colour as compared with raw. Raw Buckwheat honey was significantly darker in colour than processed Buckwheat honey.</td>
<td>Wang et al., 2004</td>
</tr>
<tr>
<td><strong>Antibacterial activity</strong></td>
<td>Manuka honey</td>
<td>50 - 70°C / 15 - 120 min</td>
<td>The highest temperature (60 and 70°C), treated for 15, 60 and 120 min exhibited the lowest percentage inhibition of Staphylococcus aureus</td>
</tr>
<tr>
<td></td>
<td>Zanthoxylum Fagara honey</td>
<td>40°C/30 days, and 78°C / 5, 10 and 15 min</td>
<td>At 40°C, the antibacterial activity was not affected but it was decreased significantly when honey was exposed to 78°C for 15 min</td>
</tr>
<tr>
<td></td>
<td>Canola/red stringy bark honey</td>
<td>45°C / 8 hours</td>
<td>Thermal processed honey shows significant reduction in antibacterial activity against Staphylococcus aureus as compared with unprocessed</td>
</tr>
<tr>
<td><strong>Physicochemical properties (pH, electrical conductivity, moisture content)</strong></td>
<td>Tahonal honey</td>
<td>55°C / 3, 6, 9 and 12 min</td>
<td>The acidity of thermal-treated Tahonal honey (55°C for 9 and 15 min) increased during storage</td>
</tr>
<tr>
<td></td>
<td>Forest honey, multifloral honey</td>
<td>35, 45 or 55°C (672 hrs), 65°C (168 hrs), 75°C (24 hrs), 80°C (12 hrs)</td>
<td>Acidity, electrical conductivity and moisture content were unaffected by heat.</td>
</tr>
<tr>
<td></td>
<td>Clover honey and Buckwheat honey</td>
<td>55°C for 12 to 16 hours</td>
<td>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</td>
</tr>
<tr>
<td><strong>Crystallization</strong></td>
<td>Natural honey</td>
<td>140-80°C, 60-15s (transient stage) and 30-10s (isothermal stage)</td>
<td>Crystallization is inhibited at 80°C, 6s (transient stage) and 30s (isothermal stage)</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td>Source</td>
<td>Conditions</td>
<td>Observations</td>
</tr>
<tr>
<td>-----------------</td>
<td>--------</td>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Natural honey</td>
<td>Tosi et al., 2008</td>
<td>60-100°C /120-1200s</td>
<td>Decrease in the diastase activity related to an increase in temperature. The activity becomes zero at 100°C for both transient and isothermal heating.</td>
</tr>
<tr>
<td>Clover I, Clover II, Chinese, Orange, Pepper and Buckwheat</td>
<td>Nanda et al., 2006</td>
<td>47.5 ± 1°C / 9.5 ± 1 min/ pH 5.2 ± 0.15</td>
<td>This optimum condition can keep diastatic activity within the prescribed limit of standard.</td>
</tr>
<tr>
<td>Natural honey</td>
<td>Babacan et al., 2002</td>
<td>63°C and 85°C/ 5-30 min</td>
<td>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.</td>
</tr>
<tr>
<td>Natural honey</td>
<td>Babacan et al., 2002</td>
<td>140-80°C, 60-15s (transient stage) and 30-10s (isothermal stage)</td>
<td>An unacceptable diastase number reduction -beyond 140°C during 15 s (transient stage), 30 s (isothermal stage)</td>
</tr>
<tr>
<td>Dzidzilche honey</td>
<td>Ramirez et al., 2000</td>
<td>55°C for 3, 6, 9 and 12 min</td>
<td>The diastase activity diminished</td>
</tr>
<tr>
<td>Pine, thymus, cotton, helianthus and orange</td>
<td>Karabournioti et al., 2001</td>
<td>35, 45, 55, 65 and 75°C for 24 hrs</td>
<td>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.</td>
</tr>
<tr>
<td>Rheological properties and viscosity</td>
<td>Abu-Jdayil et al., 2002</td>
<td>40, 60, 80 or 90°C /5,10 or 20 min</td>
<td>Both honey showed Newtonian fluids behavior. Viscosity increased with increasing ultimate heating temperature</td>
</tr>
<tr>
<td>Korean honey</td>
<td>Yoo, 2004</td>
<td>55°C, 60 min</td>
<td>Over the temperature range of 0–30°C, Korean honey varieties exhibited liquid-like rheological behavior</td>
</tr>
</tbody>
</table>
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 x 10² 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,
Chapter 2: Literature Review

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
Chapter 2: Literature Review

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/cm²) 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
References


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


References

Food, and Biological Engineering (pp. 819–822). New York: CRC Press.
Campos, F.P. and Cristianini, M. (2007). Inactivation of Saccharomyces cerevisiae and
Lactobacillus plantarum 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.
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-Muño, M.A., Gómez-Alonso E., Montes- Pérez M.J., Huidobro,
J.F. and Sancho, M.T. (2002). Evolution of fructose and glucose in honey over one
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.
high-performance liquid chromatographic determination of organic acids in honey.
Journal of Chromatography A, 669(1–2), 59–64.
Codex Alimentarius Commission. Revise codex standard for honey. Codex Standards 12–
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.


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.


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.


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.


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


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.


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.


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.


References


stability of L-ascorbic acid and/or 5-methyltetrahydrofolic acid: a kinetic study. European and Food Research and Technology, 223, 71-77.


References


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), 165

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


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.


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.


References


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


