A Mechanistic Insight into Supercritical Fluid – Substrate Interaction in Supercritical CO\textsubscript{2} Extraction of Oils from \textit{Moringa oleifera} and \textit{Eucalyptus}

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgement has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

Signature: ........................

Date ...............................
Publications


[6] Zhao, S. and Zhang, D.K., *Supercritical fluid extraction and its Applications*, *Chemeca 2009* September 27\textsuperscript{th}-30\textsuperscript{th}, Perth, Western Australia.
Abstract

Supercritical CO$_2$ extraction of oils from *Moringa oleifera* and *Eucalyptus* was studied using a Supercritical Fluid Technologies Inc. customized Supercritical Water - Supercritical Fluid Extraction (SCW-SFE) system. This new experimental technique was validated by comparing against the results from the conventional methods of Soxhlet extraction and hydro-distillation. Firstly, the yields and compositions of the oils from *Moringa oleifera* seeds and leaves were extracted using supercritical CO$_2$ and compared with those obtained by Soxhlet extraction. Experimental results revealed that the oil yield from the supercritical fluid extraction was lower than that from the Soxhlet extraction. The chemical compositions of all oils were analysed and the results indicated that the fatty acid compositions of the seed oil extracted by these two methods were very similar. However, there was a significant difference in the *Moringa oleifera* leaf oil, in which the number of compounds identified in the Soxhlet extraction was significantly greater than that of the supercritical fluid extraction. Secondly, the *Eucalyptus* leaves were subjected to extraction using all of the three methods. The experimental results showed that Soxhlet extraction achieved the highest yield, while hydro-distillation produced the lowest yield among the three methods. With respect to the oil compositions, the hydro-distillation method extracted only volatile compounds, while the supercritical fluid extraction and Soxhlet extraction methods extracted not only volatile compounds, but also heavy molecular weight compounds.

In order to gain a mechanistic insight into the interaction between supercritical CO$_2$ and the substances, the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide was investigated at temperatures of 333 K, 353 K, 373 K and pressures of 20, 30, 40, 50 MPa. The oil solubility was found to be between 0.64 g oil/kg CO$_2$ and 12.68 g oil/kg CO$_2$. The results showed that the solubility of *Moringa oleifera* seed oil increased with increasing pressure under isothermal conditions. However, the effect of
temperature showed different trends depending on the pressure. At 20 and 30 MPa, increasing temperature led to a decrease in the solubility while at 40 and 50 MPa, an increase in temperature increased the solubility. To generalize the experimental results, four literature models were employed to correlate the measured solubility data, including the Peng-Robinson equation of state and three density based models, namely, the Chrastil, del Valle and Aguilera, Adachi and Lu models. While all four models correlated the experimental data very well, the del Valle and Aguilera model showed the best fit. The Peng-Robinson equation of state, with two adjustable parameters of $k_{ij}$ and $l_{ij}$ estimated from the measured solubility data, enabled a thermodynamic explanation of the observed experimental trends of the variations in the *Moringa oleifera* seed oil solubility in supercritical CO$_2$.

The effects of pressure, temperature, CO$_2$ flow rate, and extraction time on the supercritical fluid extraction of oils from plants of *Moringa oleifera* and *Eucalyptus* were investigated. The experimental results indicated that increasing pressure, extraction time, and CO$_2$ flow rate significantly increased the oil yield. However, the effect of temperature on the oil yield was dependent on the pressure, with the oil yield increasing with increasing temperature at high pressures but decreasing at low pressures. In addition, the effect of particle size on the *Moringa oleifera* seed oil yield was also investigated and it was found that the oil yield increased with decreasing particle size, suggesting that the intra-particle diffusion played an important role. The fatty acid compositions of the *Moringa oleifera* seed oil were analysed using a gas chromatograph (GC), and the chemical compositions of the oils extracted from *Moringa oleifera* leaves and *Eucalyptus* leaves were analysed using a gas chromatograph – mass spectrometer (GC-MS). The results revealed that all the oil compositions varied under different extraction conditions. The oleic acid and the total unsaturated fatty acids of the *Moringa oleifera* seed oil decreased with increasing pressure and temperature, while for
Eucalyptus leaf oil, more volatile compounds compared to higher molecular-weight compounds were extracted at lower CO\textsubscript{2} densities.

The effect of co-solvents or modifiers on supercritical fluid extraction was also investigated. Ethanol and water were chosen as the modifiers. The experimental results revealed that the addition of ethanol increased the extraction efficiency of supercritical fluid extraction of Moringa oleifera seed oil and Eucalyptus leaf oil, the oil yields of both of them increased with increasing ethanol concentrations. Unfortunately, the introduction of ethanol did not improve the selectivity of fatty acid in Moringa oleifera seed oil and the selectivity of 1,8-cineole in Eucalyptus leaf oil. The effect of water was only evaluated in the Moringa oleifera seed oil extraction process and it was found that the yield of the oil decreased with increasing water concentration.

This thesis work as a whole indicated that the supercritical fluid extraction technique was a feasible processing method to extract oil from plants-based substrates as modelled with Moringa oleifera and Eucalyptus. The supercritical fluid extraction was shown to be superior to the conventional hydro-distillation method in terms of the essential oil yield and to be much cleaner than the traditional Soxhlet extraction method in which organic solvents are used as the extraction fluids.
Table of Contents

Declaration ...................................................................................................................................... I

Publications ..................................................................................................................................... II

Abstract .......................................................................................................................................... III

Table of Contents ............................................................................................................................ VI

List of Figures .................................................................................................................................. X

List of Tables ................................................................................................................................... XIV

Acknowledgement ............................................................................................................................ XVII

Chapter 1 Introduction .................................................................................................................... 1

Chapter 2 Literature Review .......................................................................................................... 6

2.1 Basics of supercritical fluids .................................................................................................. 6
  2.1.1 Supercritical fluids and their properties ........................................................................... 6
  2.1.2 Supercritical fluid extraction and its advantages ............................................................. 8
  2.1.3 Choice of supercritical fluid for extraction ..................................................................... 11

2.2 System and Equipment .......................................................................................................... 16
  2.2.1 Principles of supercritical fluid extraction systems ......................................................... 16
  2.2.2 Effect of temperature and pressure .................................................................................. 19
  2.2.3 Effect of flow rate .............................................................................................................. 22
  2.2.4 Effect of sample particle size ............................................................................................ 23
  2.2.5 Effect of plant materials’ structure ................................................................................... 24
  2.2.6 Effect of modifiers on supercritical fluid extraction ......................................................... 25

2.3 Applications of supercritical fluid extraction ........................................................................ 27

2.4 *Moringa oleifera* ................................................................................................................... 29

2.5 *Eucalyptus* leaves .................................................................................................................. 32

2.6 Research and development needs .......................................................................................... 35

2.7 Specific objectives of the present research .......................................................................... 37

Chapter 3 Methodology, Approach and Techniques ..................................................................... 39

3.1 Materials and reagents .......................................................................................................... 39
  3.1.1 *Moringa oleifera* seed samples ....................................................................................... 39
3.1.2 *Moringa oleifera* leaves samples ......................................................... 40
3.1.3 *Eucalyptus* leaves samples ................................................................. 40
3.1.4 Chemicals and compounds .................................................................. 40

3.2 Analytical instruments ............................................................................ 42
3.2.1 Gas chromatography (GC) ................................................................. 43
3.2.2 Gas chromatography and mass spectrometry (GC-MS) ....................... 44
3.2.3 Nuclear magnetic resonance (NMR) ..................................................... 45

3.3 Extraction apparatus .............................................................................. 46
3.3.1 Supercritical fluid extraction system .................................................. 46
3.3.2 Soxhlet apparatus .............................................................................. 48
3.3.3 Hydro-distillation apparatus ............................................................... 50

3.4 Experimental procedures ...................................................................... 51
3.4.1 Extraction of *Moringa oleifera* seed oil ........................................... 51
3.4.2 Extraction of *Moringa oleifera* leaf oil ............................................ 57
3.4.3 Extraction of *Eucalyptus* leaf oil ...................................................... 60

3.5 Data analysis ........................................................................................... 64

Chapter 4 Supercritical CO\textsubscript{2} Extraction of *Moringa oleifera* and *Eucalyptus* Oil and Comparison with Soxhlet Extraction and Hydro-distillation Methods .................................................................................. 65

4.1 Yield and composition differences of the *Moringa oleifera* seed oil obtained by supercritical fluid extraction and Soxhlet extraction with different solvents 66
4.1.1 Yield and composition differences of the *Moringa oleifera* seed oil obtained by supercritical fluid extraction and Soxhlet extraction ........................................ 66
4.1.2 The effects of different solvents on Soxhlet extraction of *Moringa oleifera* seed oil ....................................................................................................................... 71

4.2 Yield and composition differences of the *Moringa oleifera* leaf oil obtained using supercritical fluid extraction and Soxhlet extraction ........................................ 75

4.3 Yield and composition differences of the *Eucalyptus* leaf oil obtained by supercritical fluid extraction, Soxhlet extraction with different solvents and hydro-distillation ................................................................................................................................. 79
4.3.1 Comparison of SFE, Soxhlet extraction and hydro-distillation............ 79
4.3.2 The effect of operating parameters on Soxhlet extraction ...................... 86
4.3.3 Hydro-distillation ................................................................................. 88
4.4 Conclusions ............................................................................................. 88

Chapter 5 Solubility of Moringa oleifera Seed Oil in Supercritical Carbon Dioxide ................................................................................................................................. 90
  5.1 Solubility calculations .............................................................................. 92
  5.2 Effect of flow rate on solubility .............................................................. 92
  5.3 Comparison of Moringa oleifera seed oil with other vegetable oil .......... 94
  5.4 Fatty acid composition ......................................................................... 96
  5.5 Effect of pressure and temperature on solubility ................................... 98
  5.6 Thermodynamic analysis of experimental data ....................................... 99
  5.7 Correlation of the experimental solubility data with density based model . 106
      5.7.1 Chrastil Equation ............................................................................ 106
      5.7.2 del Valle and Aguilera Equation .................................................... 107
      5.7.3 Solubility correlation with the Adachi and Lu Equation ................... 108
      5.7.4 Correlation results with the three density based models............... 109
  5.8 Conclusions ............................................................................................ 113

Chapter 6 The Effects of Different Parameters on Supercritical Carbon Dioxide Extraction of Oils from Moringa oleifera and Eucalyptus ................................................................................................................. 114
  6.1 Parametric study of Moringa oleifera seed oil extraction ....................... 114
      6.1.1. Response surface analysis ............................................................ 114
      6.1.2 Chemical composition of Moringa oleifera seed oil ..................... 125
  6.2 Parametric study of Moringa oleifera leaf oil extraction ......................... 130
      6.2.1 Yields of Moringa oleifera leaf oil .................................................. 130
      6.2.2 Effect of SFE conditions on extraction yield .................................... 132
  6.3 Parametric study of Eucalyptus leaf oil extraction .................................. 136
      6.3.1. Response surface analysis ............................................................ 136
      6.3.2 Chemical compositions of Eucalyptus leaf oil and selectivity of 1,8-cineole ..................................................................................................................... 142
Chapter 7 The Effect of Modifiers on Supercritical Fluid Extraction as Applied to *Moringa oleifera* Seeds and *Eucalyptus* Leaves

7.1 Effect of modifiers on the *Moringa oleifera* seed oil extraction

7.1.1 Effect of modifiers on the yield of *Moringa oleifera* seed oil

7.1.2 Effect of modifiers on seed oil composition

7.2 The effects of modifier on the *Eucalyptus* leaf oil extraction

7.2.1 Effect of modifier on extraction yield

7.2.2 Effect of modifiers on composition and selectivity of *Eucalyptus* leaf oil

7.3 Conclusions

Chapter 8 Evaluation and Implication

8.1 Experimental results evaluation

8.2 Contributions to science and technology

8.3 Implications and research gap identifications

8.4 General discussion of the supercritical fluid extraction performance

Chapter 9 Conclusions and Recommendations

9.1 Summary of conclusions

9.1.1 Supercritical CO₂ extraction of *Moringa oleifera* and *Eucalyptus* leaf oil and comparison with Soxhlet extraction and hydro-distillation methods

9.1.2 Solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide

9.1.3 The effects of different parameters on supercritical carbon dioxide extraction of *Moringa oleifera* and *Eucalyptus*

9.1.4 The effects of modifiers on supercritical fluid extraction as applied to *Moringa oleifera* and *Eucalyptus*

9.2 Future works

References

Appendix
List of Figures

Figure 1-1  Thesis map ........................................................................................................... 5
Figure 2-1  Typical T-P phase diagram for a pure substance. ............................................. 7
Figure 2-2  Schematic of the instrumentation required for SFE ......................................... 16
Figure 2-3  The effect of extraction pressure on lycopene yield at constant temperature
of 343 K and flow rate of 2.5 ml/min (Topal et al., 2006) .................................................... 19
Figure 2-4  The effect of extraction temperatures on vitamin E yield at different
pressures .................................................................................................................................. 21
Figure 2-5  Scanning electron micrographs of surface structure, disrupted epidermal
cell and papillae of fresh, oven dried and freeze dried of ground pandan leaves (Yahya
et al., 2010) 25
Figure 3-1  Picture of Gas Chromatography ...................................................................... 43
Figure 3-2  Picture of Gas Chromatography-Mass Spectrometry ..................................... 44
Figure 3-3  Schematic diagram and the picture of the supercritical fluid extraction
apparatus ............................................................................................................................... 47
Figure 3-4  Schematic diagram of the Soxhlet extraction apparatus ................................. 49
Figure 3-5  A schematic and the picture of the hydro-distillation apparatus used in this
study ......................................................................................................................................... 50
Figure 4-1  $^1$H-NMR spectra of oils extracted by supercritical fluid extraction and
Soxhlet extraction .................................................................................................................... 68
Figure 4-2  2D COSY spectra of the oil extracted by supercritical fluid extraction ... 69
Figure 4-3  Extraction yields of Eucalyptus leaf oil obtained by different methods ... 80
Figure 4-4  Total ion compound (TIC) chromatograms of the Eucalyptus leaf oil
obtained by Soxhlet extraction with ethanol and hexane, supercritical fluid extraction
and hydro-distillation ............................................................................................................ 81
Figure 4-5  Composition profiles of the *Eucalyptus* leaf oil extracted by SFE (under conditions of 40 MPa, 343 K and time of 60 minutes), hydro-distillation, and Soxhlet extraction ................................................................................................................... 82
Figure 4-6  Schematic diagrams of the hydro-distillation process, Soxhlet extraction process and supercritical fluid extraction process ................................................................................................................... 84
Figure 4-7  Effect of solvent type and extraction time on the yield of Soxhlet extraction of *Eucalyptus* leaf oil ................................................................................................................... 87
Figure 4-8  Effect of extraction time on the yield of hydro-distillation of *Eucalyptus* leaf oil ................................................................................................................... 88
Figure 5-1  Overall extraction yield curves obtained at different flow rates at pressure 40 MPa and temperature 353 K ................................................................................................................. 93
Figure 5-2  Effects of pressure and temperature on the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide ................................................................................................................. 98
Figure 5-3  Solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide. Lines are predicted solubility using PR-EoS with $k_{ij}$ and $l_{ij}$ of triolein ......................... 103
Figure 5-4  Solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide. Lines are predicted solubility using PR-EoS with $k_{ij}$ and $l_{ij}$ of the *Moringa oleifera* seed oil ................................................................................................................. 105
Figure 5-5  Comparison of the experimental and correlated solubility data with the three density based models. ................................................................................................................. 112
Figure 6-1  Effect of particle size on extraction yield of the *Moringa oleifera* seed oil (40 MPa, 353 K and 3.68 g/min) ................................................................................................................. 118
Figure 6-2  The three-dimensional plot of the response surface for the oil yield as related to pressure and temperature at the flow rate of 5.52 g/min and extraction time of 90 minutes ................................................................................................................. 120
Figure 6-3  The three-dimensional plot of the response surface for the oil yield as related to flow rate and pressure at the fixed temperature of 353 K and extraction time of 150 minutes ............................................................................................................... 123

Figure 6-4  The three-dimensional plot of the response surface for the oil yield as related to flow rate and temperature at 40 MPa and 150 minutes ................................................................. 124

Figure 6-5  A schematic diagram of the supercritical fluid extraction process ....... 124

Figure 6-6  The three-dimensional plot of the response surface for the oil yield as related to time and pressure at temperature of 353 K and flow rate of 5.52 g/min ...... 125

Figure 6-7  Contour plot showing the effects of operating parameter on fatty acid composition .................................................................................................................................. 129

Figure 6-8  Effect of pressure (a), temperature (b) and extraction time (c) on the yield of the extracted oil from Moringa oleifera leaves .............................................................. 134

Figure 6-9  Response surface for the oil yield as related to temperature and pressure at fixed 90 minutes extraction ......................................................................................... 139

Figure 6-10  Response surface for the oil yield as related to pressure and time and temperature of 333K ............................................................................................................. 141

Figure 6-11  Response surface for the selectivity of 1,8-cineole as related to pressure and temperature at time of 90 minutes ................................................................................... 150

Figure 7-1  Effect of ethanol’s concentrations on the yield of Moringa oleifera seed oil at 40 MPa, 353 K, 3.68 g/min of CO$_2$ and with particle size under 1000µm ............. 154

Figure 7-2  Effect of water’s concentrations on the yield of Moringa oleifera seed oil at 40 MPa, 353 K, 3.68 g/min and with particle size under 1000µm ................................. 157

Figure 7-3  Fatty acid compositions of the Moringa oleifera seed oil extracted using supercritical fluid extraction with different co-solvents ................................................. 160

Figure 7-4  Effect of ethanol’s concentration on the yield of Eucalyptus leaf oil at 333 K ...................................................................................................................................... 162
Figure 7-5  Effect of ethanol concentrations on the content of 1,8-cineole..............163
Figure 7-6  Effect of ethanol concentrations on the selectivity of 1,8-cineole .........164
Figure 8-1  Mechanism comparisons of hydro-distillation, Soxhlet extraction and supercritical fluid extraction .................................................................174
Figure 8-2  The interactions of the fluid properties, process conditions and sample matrix characteristics and their effect on the performance of a SFE process ..........179
List of Tables

Table 2-1  Typical properties of gases, supercritical fluids and liquids .................. 7
Table 2-2  Critical properties of several solvents used in SFE ................................. 12
Table 2-3  Dipole moment and solubility parameters of modifiers and mixture of modifiers with carbon dioxide (Huang et al., 2013) .................................................... 26
Table 2-4  Summaries of applications of SFE in different areas ............................... 29
Table 3-1  Description of the chemicals used in this study ..................................... 41
Table 3-2  Central composite design for the supercritical fluid extraction of *Moringa oleifera* seed oil ........................................................................................................ 54
Table 3-3  Description of solvents used for Soxhlet extraction ............................... 55
Table 3-4  Three levels orthogonal design for extraction of *Moringa oleifera* leaf oil with supercritical CO$_2$ extractions ................................................................. 58
Table 3-5  Central composite design for the supercritical fluid extraction of *Eucalyptus* leaf oil ............................................................................................................. 62
Table 4-1  Yield and fatty acid compositions of *Moringa oleifera* seed oil obtained by supercritical fluid extraction and Soxhlet extraction ............................ 67
Table 4-2  Assignment of signals and percentage of total hydrogen of $^1$H NMR spectra from the *Moringa oleifera* seed oil extracted by supercritical fluid extraction and Soxhlet extraction ................................................................. 70
Table 4-3  Solvent effect on the yield of *Moringa oleifera* seed oil extracted by the Soxhlet method ........................................................................................................... 72
Table 4-4  Fatty acid contents of *Moringa oleifera* seed oil obtained by Soxhlet extraction with different solvents ................................................................. 74
Table 4-5  Chemical compositions of the *Moringa oleifera* leaf oil obtained by Soxhlet extraction and supercritical fluid extraction ........................................... 77
Table 5-1  Solubility of *Moringa oleifera* seed oil in supercritical CO$_2$..................94
Table 5-2  Solubility values of *Moringa oleifera* seed oil and other plant seed oil in supercritical carbon dioxide .................................................................................95
Table 5-3  Fatty acid compositions of *Moringa oleifera* seed oil and other plant oils...
........................................................................................................................................97
Table 5-4  Estimated critical parameters and acentric factors used in the equation of state for calculations.................................................................................................................102
Table 5-5  The optimal fitted binary parameters of triolein .......................................102
Table 5-6  The optimal fitted binary parameters of *Moringa oleifera* seed oil.......103
Table 5-7  The obtained fitting constants for three density based correlations......111
Table 5-8  Experimental results for the supercritical fluid extraction of *Moringa oleifera* seed oil using central composite design.........................................................116
Table 5-9  Analysis of variance of the regression parameters.................................117
Table 5-10 Fatty acid composition (percent fatty acid) of *Moringa oleifera* seed oil extracted by supercritical fluid extraction (SFE) under different conditions............127
Table 5-11 Experimental results for supercritical fluid extraction of *Moringa oleifera* leaf oil by three level orthogonal design.................................................................131
Table 5-12 The compositions (wt%) of compounds from *Moringa oleifera* leaf oil obtained by supercritical CO$_2$ extractions from three level orthogonal design ........135
Table 5-13 Experimental results for supercritical fluid extraction of *Eucalyptus* leaf oil by central composite design.......................................................................................137
Table 5-14 Analyses of variance of the regression parameters...............................138
Table 5-15 The compositions (wt%) of compounds from *Eucalyptus* leaf oil obtained by supercritical CO$_2$ extraction by centre composite design ..........................143
Table 5-16 The selectivity of 1,8-cineole under every conditions .........................148
Table 6-10  Regression coefficients and corresponding t and p-values for the selectivity of 1,8-cineole ................................................................. 149

Table 7-1  The solubility parameter of CO$_2$ modified with ethanol at 40 MPa and 353K ................................................................................................................. 155

Table 7-2  Fatty acid contents of *Moringa oleifera* seed oil obtained by supercritical fluid extraction (SFE) with different concentration of modifiers ......................... 159

Table 7-3  The solubility parameter of the pure and modifier CO$_2$...................... 162

Table 8-1  Comparison between the present work and the literature results on the *Moringa oleifera* seed oil extraction ................................................................. 167

Table 8-2  Comparison between the present work and the literature results on the Eucalyptus leaf oil extraction ................................................................................. 168

Table 8-3  Comparison of supercritical fluid extraction with Soxhlet extraction and hydro-distillation methods ................................................................................. 171
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Chapter 1 Introduction

In the past few decades, supercritical fluid extraction (SFE) has received increasing attention for the recovery of various compounds from distinct natural and food resources. Supercritical fluid extraction is a solvent extraction process which uses a supercritical fluid as the solvent. A supercritical fluid is any compound at a temperature and pressure above its critical point. Under supercritical conditions, the fluid possesses both gas and liquid like properties (Dean, 1993). Due to the environmental and food processing regulations, supercritical fluid extraction is a very attractive alternative to the conventional extraction methods, such as Soxhlet extraction that uses organic solvents and steam distillation (Reverchon and De Marco, 2006). The use of organic solvents in the Soxhlet extraction process can lead to product contamination with the solvent residue, while the steam distillation is usually carried out at high temperatures, which can cause destruction of valuable substances (Francisco and Sivik, 2002, Ammon et al., 1985).

The commonly used fluid in SFE is CO₂, which has several unique characteristics and physico-chemical properties (Dean, 1993). For example, CO₂ is non-toxic, non-flammable, inexpensive, odour-less and it has low critical pressure (7.38 MPa) and temperature (304.3 K). Therefore, supercritical CO₂ extraction leaves no solvent residue in the products, thus providing extracted products with superior quality (Dean, 1993). In addition, supercritical fluid extraction has other advantages over traditional extraction techniques such as operation at low temperatures thus preserving the thermally labile components in the extracts. Moreover, the selectivity of carbon dioxide in relation to some components can be easily adjusted by changing the temperature and pressure (Smith, 2003). For these reasons, supercritical fluid extraction has been considered to be an ideal technique to extract various products from natural sources.
Supercritical fluid extraction has been applied to extract oils and essential oils from a variety of plants. *Moringa oleifera* is a fast-growing perennial plant that bears seeds containing edible oil (Abdulkarim *et al.*, 2005). The extraction of *Moringa oleifera* seed oil using traditional Soxhlet extraction has been extensively studied (Anwar and Bhanger, 2003, Mani *et al.*, 2007). However, very little information was found on the supercritical fluid extraction of this seed oil. Only one published paper has investigated the extraction of oil from *Moringa oleifera* kernels using ethanol modified supercritical carbon dioxide (Nguyen *et al.*, 2011). Therefore one part of this research is to investigate the possibility of using supercritical fluid extraction to extract oil from *Moringa oleifera* seeds and compare with the traditional Soxhlet extraction method.

*Eucalyptus Loxophleba ssp. Lissophloia* (*Eucalyptus Loxliss*) is a species of *Eucalyptus* that is native to Australia. It is known to contain high amounts of 1,8-cineole, one of the most valuable compounds present in *Eucalyptus* oil (Babu and Singh, 2009). This oil can be extracted using a variety of methods. Currently, the most popular extraction method is steam extraction or hydro-distillation; however, this technique has several disadvantages, such as incomplete extraction of essential oil from plant materials, high operating temperatures with the consequent breakdown of thermally labile components and requirement for a post-extraction process to remove water (Francisco and Sivik, 2002). Therefore supercritical fluid extraction could represent a green and valuable alternative to extract oils from *Eucalyptus* leaves.

There are many variables to be considered in a supercritical fluid extraction process. One important area that must be assessed is the solubility of the extracted solute in a supercritical fluid. A great deal of research has been conducted to measure the solubility of different compounds in supercritical carbon dioxide and several correlations have been developed in order to correlate and extrapolate the solubility data at various conditions (Soares *et al.*, 2007, Machmudah *et al.*, 2007, Gaspar *et al.*, 2002, Chrastil,
Some of these correlations are highly empirical while some others have strong fundamental basis. In addition to the solubility behaviour, it is also essential to understand the effects of some other variables such as operating pressure, temperature, particle size, sample moisture content, extraction time and flow rate on the performance of supercritical fluid extraction. Therefore, the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide, the effects of different operation parameters on the extraction of *Moringa oleifera* seed oil, *Moringa oleifera* leaf oil and *Eucalyptus* leaf oil were discussed in the present thesis.

Furthermore, carbon dioxide is a non-polar fluid and it has been used primarily to extract non-polar compounds. In order to overcome this limitation, a modifier or co-solvent which consists a small amount of organic solvents such as methanol or ethanol, may be added directly to the fluid or the sample matrix. Modifiers generally serve three functions (Casas *et al*., 2007): 1) increase the solvating power of the supercritical fluids, 2) increase the intermolecular interactions between the co-solvent and the solute, and 3) swell the cellular structure of the matrix thus distort the matrix-solute diffusion process and favour the penetration of the supercritical fluid inside the matrix. The ideal modifier and modifier’s concentration utilized for each sample should be determined experimentally. Water and ethanol are regarded as attractive solvents over other organic-based solvents due to their relatively safe nature and the advantage of cleanness. Therefore these two solvents were chosen as the co-solvent in the supercritical carbon dioxide extraction process in this study.

There are a total of nine chapters in this thesis, as outlined below. The structure of the thesis is graphically shown in Figure 1-1.

Chapter 1 introduces the properties of the supercritical fluid extraction and why it is chosen for this study. The overall aims and the thesis structure are provided as well.
Chapter 2 reviews the current knowledge status of the supercritical fluid science and supercritical fluid extraction technology. The extraction methods to extract oil and essential oil from *Moringa oleifera* and *Eucalyptus* are reviewed as well, which lead to the identification of research gaps and establish specific objectives for the present work.

Chapter 3 describes the supercritical fluid extraction system, Soxhlet extraction and hydro-distillation apparatus as well as the analytical instruments used for this thesis. The detailed experimental procedures are presented. The data analysis methods including response surface methodology and orthogonal design are explained.

Chapter 4 examines the effects of different extraction methods, including supercritical fluid extraction, Soxhlet extraction and hydro-distillation on the yields and compositions of the oils extracted from *Moringa oleifera* and *Eucalyptus*.

Chapter 5 investigates the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide under different conditions.

Chapter 6 studies the effects of extraction conditions such as pressure, temperature, flow rate, extraction time and particle size on the supercritical fluid extraction of oil and essential oil from *Moringa oleifera* and *Eucalyptus* using the response surface methodology and orthogonal design.

Chapter 7 investigates the effects of modifiers, including ethanol and water, on the supercritical fluid extraction of oil and essential oil from plants of *Moringa oleifera* and *Eucalyptus*.

Chapter 8 evaluates the findings from the present research and discusses their practical implications for the supercritical fluid extraction applied in the plant extraction industry. New research gaps are identified and recommended for the future research work.

Chapter 9 draws conclusions from the present study and outlines the recommendations for further works.
Chapter 1 Introduction
- Define the scope of the overall project on SFE.
- Provide a background of SFE, Moringa oleifera and Eucalyptus.
- Define the overall aims.
- Thesis map

Chapter 2 Literature Review
- Review of supercritical fluids
- Review of SFE technologies
- Review of research on Moringa oleifera and Eucalyptus leaf oil extraction
- Identify the gaps.
- Define the specific objectives of the thesis.

Chapter 3 Approaches/Experimental Techniques
- Overall research strategy
- Experimental systems and instrumentations
- Data analysis and error analysis methods

Chapter 4 Comparison of different extraction methods on yield and compositions of Moringa oleifera and Eucalyptus leaves oil
- Soxhlet extraction
- Hydro-distillation
- Supercritical fluid extraction

Chapter 5 Solubility of Moringa oleifera seed oil in supercritical CO₂
- Experimental works
- Modelling

Chapter 6 Optimization of operating parameters for supercritical fluid extraction of Moringa oleifera seed oil and Eucalyptus leaf oil by using response surface methodology
- Pressure
- Temperature
- Flow rate
- Extraction time

Chapter 7 Effects of modifiers on the yield and compositions of Moringa oleifera seed oil and Eucalyptus leaf oil
- Ethanol
- Water

Chapter 8 Evaluations
- Integrate the results/findings from our experiments
- Evaluate our own work against the objectives and the literature
- Identify the significant findings and contributions to the science
- Identify new gaps

Chapter 9 Conclusions & Future works
Chapter 2 Literature Review

Supercritical fluids with both gas and liquid like properties have been adopted as an extraction media to extract many useful substances from various types of complex matrices (Reverchon and De Marco, 2006). Compared to the conventional extraction methods such as hydro-distillation or Soxhlet extraction, supercritical fluid extraction (SFE) is preferred because it is simple, fast, inexpensive, effective and selective (Pourmortazavi and Hajimirsadeghi, 2007). Although the most commonly used supercritical fluid is pure or modified carbon dioxide, a number of alternative solvents, such as nitrous oxide, ethane and water, possess critical properties that could permit convenient applications in SFE (Herrero et al., 2006, Dean, 1993). This review chapter firstly describes the basic principles of supercritical fluids, including their thermodynamic properties and the choice of supercritical fluids that can be used for extraction. The effects of different operation conditions such as pressure, temperature and flow rate et al. on the SFE process are also described. The applications of SFE in various areas such as environmental remediation, food processing, pharmaceutical products, bioenergy, and many other areas are discussed in this chapter. Furthermore, recently progresses in the research and development of SFE in Moringa oleifera and Eucalyptus extraction are also reviewed.

2.1 Basics of supercritical fluids

2.1.1 Supercritical fluids and their properties

A supercritical fluid (SCF) is a term which can be applied to any compound at a temperature and pressure above its critical point (Dean, 1993). The definition can be better understood through the use of a phase diagram as shown in Figure 2-1. The phase diagram for a pure substance shows the temperature and pressure regions where the substance occurs as a single phase (solid, liquid and gas) and the phase transition lines
where two of the phases can exist in equilibrium. These two phase regions between the solid-gas, solid-liquid and liquid-gas interfaces involve the phase transition of sublimation, melting and vaporisation respectively. The three curves intersect at the triple point, where the solid, liquid and gaseous phases coexist in equilibrium. The gas-liquid equilibrium curve ends at the critical point. At the critical point the density of both phases are equal and the two phases are indistinguishable. At this point, the substance is termed as supercritical fluid and consists of a single phase. If the temperature and pressure are above the critical parameters, the substance will always be single phase, and no further liquefaction or vaporisation will occur (Dean, 1993).

![T-P phase diagram](image)

Figure 2-1 Typical T-P phase diagram for a pure substance. The critical temperature (Tc) and pressure (Pc) are represented by the dotted lines; the top right-hand corner represents the supercritical region of the substance

| Table 2-1 Typical properties of gases, supercritical fluids and liquids |
|-----------------------------|-----------------|-----------------|
|                            | Density (kg/m³) | Viscosity (cP)  | Diffusivity (mm²/s) |
| Gases                      | 1               | 0.01            | 1-10               |
| Supercritical Fluids       | 100-1000        | 0.05-0.1        | 0.01-0.1           |
| Liquids                    | 1000            | 0.5-1.0         | 0.001              |
Under supercritical conditions, the fluid possesses both gas and liquid-like properties. It is gas-like in that it is a compressible fluid while it is liquid-like in that it has comparable density and solvating power (Dean, 1993). Characteristic values for the gases, liquids and supercritical fluids are listed in Table 2-1. From this table, it can be seen that the density of supercritical fluids is much greater than that of typical gases and comparable to that of liquids which enable them to act as solvents. However, note that supercritical fluids exhibit significantly lower viscosity but higher diffusivity than liquids. The combination of viscosity and diffusivity properties result in supercritical fluids being more penetrating, whilst retaining desirable transport properties (Smith, 2003). In addition, the solvent power of a supercritical fluid can be easily controlled by changing pressure and temperature. Therefore, it can achieve a remarkably high selectivity. This tunable extraction power of the supercritical fluid is very useful for the extraction of complex samples such as plant materials.

2.1.2 Supercritical fluid extraction and its advantages

Supercritical fluid extraction is the process of separating one component (the extractant) from another (the matrix) using a supercritical fluid as the extraction solvent. Because SFE has several distinct properties, it is regarded as a promising alternative technique to replace conventional solvent extraction methods. Some of its major advantages are summarised as follows:

(1) SFE is fast (Hawthorne, 1990). Supercritical fluids have relatively low diffusivity and viscosity as listed in Table 2-1. The low diffusivity is a significant property since the extraction rate is ultimately determined by the internal diffusion of the extracted substances from the sample matrix into the bulk fluid (Dean, 1993). On the other hand, supercritical fluids exhibit significantly lower viscosities than liquids, thus they can penetrate into a porous solid material more effectively than conventional solvents. Therefore, they can result in faster extractions. For instance, with comparable or better
recoveries, the extraction time could be reduced from hours or days with the conventional solvent extraction to as little as 30 minutes with SFE (Scalia et al., 1999, Özcan and Özcan, 2004, Kotnik et al., 2007, Khosravi-Darani and Vasheghani-Farahani, 2005).

(2) The solvent power of a supercritical fluid can be easily controlled. The solvent strength of a liquid is essentially constant regardless of extraction conditions, but the solvent strength of a supercritical fluid is a function of its density, which, in turn, is a function of pressure and temperature. Therefore, it may achieve a remarkably high selectivity by changing pressure and temperature. This tuneable solvation power of supercritical fluids is very useful for the extraction of complex samples such as plant materials. For example, a vindoline component can be extracted from among more than 100 alkaloid compounds from the leaves of catharanthus roseus by supercritical fluid extraction (Song et al., 1992). Increasing pressure at a constant temperature will increase the density of the supercritical fluid. However, increasing temperature at a constant pressure will result in a reduction in density and hence solvent strength (Hawthorne, 1990, Dean, 1993). Therefore, this allows an extraction to be optimized by changing the pressure or temperature; this also enables multi-step operation by simply varying pressure or temperature in each process step. For example, it is possible to perform a first extraction operation at low CO₂ density (e.g. 290 kg/m³, 9 MPa, 323 K) followed by a second extraction step at high density (e.g. 870 kg/m³, 30 MPa, 323 K) just by changing the pressure (Reverchon and De Marco, 2006). As a result the most soluble solute can be extracted in the first step while the less soluble ones extracted in the second one.

(3) Ease of removal from solutes. Since a large amount of solvent has to be used in Soxhlet extractions (≈50-200ml) (Luque de Castro and Jiménez-Carmona, 2000), the extracts obtained need to be concentrated prior to analysis in order to obtain adequate
solute concentrations. This step is usually time-consuming and often results in the loss of volatile components. In contrast, many supercritical fluids such as carbon dioxide (CO\textsubscript{2}) and nitrous oxide (N\textsubscript{2}O) are gaseous under ambient conditions, hence they can be easily separated from the solutes by depressurization. Therefore, the concentration steps after SFE can be greatly simplified.

(4) SFE can bring environmental benefits. SFE uses no or significantly less environmentally hostile organic solvents, most substances used for supercritical fluid extraction are relatively inert, available in high purity, nontoxic and inexpensive. In addition, in situations where an organic solvent is employed as a modifier, the SFE method may only need a few millilitres (≈20–40ml) while a conventional extraction technique such as Soxhlet extraction would require tens to hundreds of millilitres (≈50–200ml) (Luque de Castro and Jiménez-Carmona, 2000, Özcan and Özcan, 2004). Therefore the organic solvent usage and disposal cost can be minimized. In many large scale SFE processes, the fluid, usually CO\textsubscript{2}, can be recycled or reused thus minimising waste generation.

(5) SFE can preserve the bioactive properties of the solute and may lead to the discovery of new natural compounds. Supercritical fluids such as CO\textsubscript{2} and N\textsubscript{2}O have relatively low critical temperatures (304 K and 309 K, respectively), thus SFE may be a good technique to extract thermally sensitive materials. In addition, the extraction system provides an oxygen-free environment; thereby it can minimise degradation and preserve the bioactive properties of the extracts. For example, when SFE was used to extract ginger, many undesirable reactions such as hydrolysis, oxidation, degradation and rearrangement could be effectively prevented. By doing this, the common difficulties of quality assessment in classical hydro-distillation could be avoided (Lang and Wai, 2001, John P Bartley, 1994). SFE can prevent the oxidation of lipids. Bernardo-Gil et al. (2002) found that the contents of free fatty acids, sterols,
triaclylglycerols and tocopherols were comparable with those obtained with n-hexane solvent extraction; however, the oil obtained by supercritical fluid extraction was slightly more protected against oxidation than the n-hexane extracted oil.

(6) Other considerations. In SFE, a fresh fluid can be continuously forced to flow through the sample; therefore, it can provide quantitative or complete extraction (Stashenko et al., 1996). In addition, SFE can be directly coupled with on-line chromatographic techniques which makes SFE highly suitable for fast, routine analyses (Zougagh et al., 2004). It also provides quantitative transfer of extracted solutes to the analytical instruments and reduces the potential for contamination.

2.1.3 Choice of supercritical fluid for extraction

There are a wide range of substances that can be used for supercritical fluid extraction. Table 2-2 lists the critical properties of several solvents commonly used in SFE (Herrero et al., 2006, Dean, 1993, Hawthorne, 1990).

Whether a substance can be used as a supercritical solvent or not is influenced by the polarity of the target solute. Practical considerations, such as temperature and pressure, are also very important in order to choose a suitable supercritical fluid, since they are the basic requirements to push a substance into its critical region. Supercritical CO\(_2\) has been chosen for most SFE studies, primarily because of its relative low critical temperature and pressure, non-toxic and non-flammable properties. Besides, it is easily available in high purity with low cost, and suitable for extracting heat labile natural compounds. On an industrial scale, carbon dioxide can be recovered and reused. In general, carbon dioxide is an excellent extraction medium for nonpolar species such as alkanes and terpenes (Phelps et al., 1996). It is reasonably good for moderately polar species, including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), aldehydes, esters, alcohols and organochlorine pesticides (Lee and Markides,
However, it is still a challenge for polar solutes (such as antibiotics) because of their lower solubility in supercritical carbon dioxide (King and Hopper, 1992).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Critical temperature (K)</th>
<th>Critical pressure (MPa)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>carbon dioxide</td>
<td>304.2</td>
<td>7.29</td>
<td>Non-toxic, non-flammable, inert to most materials</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low critical temperature and pressure, easy separation, low cost, easily available at high purity</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Less effective in extraction of polar compounds</td>
</tr>
<tr>
<td>nitrous oxide</td>
<td>309.7</td>
<td>7.17</td>
<td>Low critical temperature and pressure, A better solvent than carbon dioxide for many solutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>May cause explosion</td>
</tr>
<tr>
<td>water</td>
<td>647.3</td>
<td>21.76</td>
<td>Non-toxic, environmental-friendly, low costs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High critical temperature and pressure, corrosive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not suitable for thermally sensitive compounds</td>
</tr>
<tr>
<td>ethane</td>
<td>305.3</td>
<td>4.81</td>
<td>Low critical temperature and pressure, suitable for food products</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Good extracting solvent for lipids and complex hydrocarbons mixtures</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>High cost, highly flammable</td>
</tr>
<tr>
<td>methane</td>
<td>513.25</td>
<td>8.20</td>
<td>High solvent strength , High critical temperature</td>
</tr>
<tr>
<td>chlorodifluoromethane</td>
<td>369.7</td>
<td>4.11</td>
<td>Suitable for more polar solutes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Toxic, ozone depletion effect</td>
</tr>
<tr>
<td>NH₃</td>
<td>405.6</td>
<td>11.25</td>
<td>Favourable solvent strength and moderate critical temperature and pressure</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Corrosive and toxic</td>
</tr>
</tbody>
</table>

N₂O, which has low toxicity and low critical parameters, is considered better suitable for polar compounds because of its permanent dipole moment (Hawthorne, 1990). Since
N₂O is not acidic like CO₂, it could have a real benefit for the extraction or elution of organic bases, some of which tend to react with CO₂ and form insoluble carbonates. Therefore there was a particular interest in its application for the separation of amine. Amine has been proven to have a high solubility in supercritical N₂O and both aliphatic and aromatic amines can be easily extracted by this solvent (Ashraf-Khorassani et al., 1990). For some solutes, such as, tetrachlorodibenzo-p-dioxins TCDDs and PAHs (Hawthorne and Miller, 1987), extraction efficiencies can be increased by using supercritical N₂O than that would have been expected with CO₂. Alexandrous and Pawliszyn’s (1989) studied the extraction of TCDDs from incinerator fly ash, they found that pure carbon dioxide did not extract dioxins from untreated fly ash, but N₂O extracted nearly 100% of TCDDs from untreated fly ash, whereas CO₂ yielded good recoveries after the fly ash surface was modified by acid treatment. They explained that the increased extraction efficiency of N₂O was caused by the displacement of the solute from the desorption site on the fly ash. Unfortunately, the oxidizing strength of this fluid can cause a violent explosion when used on samples that have high organic content. Raynie (1993) reported an explosive experiment when nitrous oxide was used in the supercritical fluid extraction of ground coffee. Therefore, it is only to be used when absolutely necessary.

Another alternative to supercritical CO₂ is supercritical water. In recent years, several researchers have studied the effect of supercritical water on coal or oil shale conversions. They found that carbon conversation and oil yield using SFE are much higher than those using conventional methods. Water in a supercritical state acts not only as a solvent but also as a reactant, participating actively in the decomposition of organic macromolecules into dissolution of fragments, it can also reacts with the double and triple bonds of the decomposition products resulting in alcohols and aldehydes (Yanik et al., 1995). Water is a potentially useful solvent for the extraction of several
classes of environmental pollutants, Hawthorne et al. (1994) revealed that subcritical water at 523 K and 5 MPa can allow quantitative extraction of PAHs in 15 minutes from soil and urban air particulates. The polarity of water can be decreased by sequentially raising the extraction temperature from 323 to 523 K (subcritical water) and finally to 673 K (supercritical water if P>22.1 MPa). This can allow class-selective extraction of polar organics (e.g., chlorinated phenols), low polarity organics (e.g., PAHs), and nonpolar organics (alkanes) to be performed. Water is an attractive medium for industrial chemical reactions because it is an environmentally benign. A number of papers have reported the aqueous conversion of biomass to hydrogen through supercritical water extraction (Antal et al., 2000, Demirbas, 2005, Demirbas, 2004). Demirbas (2004, 2005) investigated hydrogen production from nut shells, olive husk and tea waste through supercritical water extraction. He found that compared with other biomass thermochemical processes such as pyrolysis, gasification, air gasification or steam gasification, supercritical water extraction can directly deal with wet biomass without drying and results in a higher gasification efficiency at lower temperatures. However, superheated water is not suitable for thermally labile compounds. If oxygen is not carefully purged, water at high pressure can be corrosive and may cause damage to extraction vessels (Basile et al., 1998).

Ethane, also an acceptable solvent for supercritical fluid extraction, has approximately the same critical temperature as CO₂. Despite its higher cost, it has a lower critical pressure than CO₂ and could, therefore, be used as a supercritical solvent at lower operating pressures and consequently lower energy cost. Ethane is also more efficient in the extraction of lipids when compared to CO₂ (Saldaña et al., 2002). Some researchers (Mohamed et al., 2002, Saldaña et al., 2002) have used supercritical ethane and CO₂ to extract cocoa butter from cocoa beans. They found that the extraction yield of cocoa butter obtained with ethane was much higher than that obtained with CO₂ because of the
higher solubility of this fat in ethane. Supercritical ethane was also reported to be a better solvent in the extraction of fat from fermented seeds and in the reduction of cholesterol in milk (Mohamed et al., 2000, de Azevedo et al., 2003). The benefit of employing supercritical ethane to extract hydrocarbons has also been reported by several papers (Ávila-Chávez et al., 2007, Rose et al., 2001). Ávila-Chávez et al. (2007) studied the extraction of hydrocarbons from crude oil tank bottom sludge using supercritical ethane. They found that extraction yields were up to 58.5%, the quality of the extracts obtained should enable supercritical ethane extraction to be a promising technique to treat the large amount of crude oil tank bottom sludge that was annually produced by the oil industry.

A number of exotic and frequently toxic solvents have also been used in the supercritical fluid extraction. Supercritical ammonia would be very attractive from a solvent strength standpoint. Supercritical fluid extraction of C-labelled metabolites from biological tissues using ammonia was reported (Jacobson et al., 1997) to be a rapid and efficient extraction method. However, as ammonia is such a strong solvent, it will dissolve silica-based materials in most cases, therefore it is difficult to be pumped (it tends to dissolve the pump seal) and careful consideration must be taken in choosing the instrumentation. It is also toxic and as a consequence it is likely to be considered too dangerous for routine use (Hawthorne, 1990). Freon, like CHCLF₂ (Freon—22) and CHF₃, can increase the extraction efficiency of several compounds when compared to the extraction with CO₂. Combs et al. (1996) compared the extraction of sulfonamides from various food matrices using supercritical CHF₃ and CO₂. They found that extraction efficiencies of sulfamethazine and sulfadimethoxine using pure CHF₃ were more than 45% higher from spiked sand samples and over 200% higher from fortified beef liver samples than pure CO₂. Hawthorne et al. (1992) compared the extraction recoveries of pollutants (including PCBs and PAHs) from environmental matrices using
supercritical CHClF₂ (Freon-22), N₂O and CO₂. In this study, he found that the extraction rate and recoveries of PCBs from river sediment using CHClF₂ were higher than those using pure CO₂ or methanol modified CO₂. For PAHs especially for the higher molecular weight PAHs from the petroleum waste sludge and the railroad bed soil, improved recoveries and faster extractions were obtained with Freon when compared to CO₂ and N₂O. However, Freon is no longer available commercially now because of its ozone depletion effect in the upper atmosphere.

2.2 System and Equipment

2.2.1 Principles of supercritical fluid extraction systems

![Schematic of the instrumentation required for SFE](image)

Figure 2-2 Schematic of the instrumentation required for SFE

Supercritical fluid extraction system can be divided into analytical system and preparative system (pilot or industrial scale). The analytical system is utilized in sample preparations process, for example, prior to a chromatographic analysis, to obtain milligrams or grams samples. This system usually can be easily assembled in the laboratory. The preparative system can extract grams or kilograms of compounds...
depending on the scale of the system (Herrero et al., 2006). However, the basic components of these systems are similar and a schematic diagram of such a system is shown in Figure 2-2.

The main components of the system include (a) supply of CO$_2$ or some other potential solvents, (b) gas compressor or pump for solvent transportation, (c) heated zone or oven, (d) extraction vessel or thimble, (e) outlet restrictor, and (f) a collection device. Individual units may have different configurations, valves, bypasses, gas purge systems and safety features are not shown here. Pure CO$_2$ is commonly used for the majority of extractions. A high-purity gas supply (free of water, halocarbons and hydrocarbons) of carbon dioxide is required and now is available from commercial outlets worldwide. The fluid must be pressurized to above the critical pressure by using a pump or a gas compressor. Reciprocation pumps or syringe pumps are often used for small scale extractions (up to a few grams). Gas compressors are often useful for larger scale extractions (e.g., samples>50g) (Hawthorne, 1990). The extraction vessel can range from simple tubing to a more sophisticated purpose built vessel designed to withstand high pressure (34.4-68.8 MPa). It is often constructed with stainless steel or similar inert material. The vessel must be equipped with a means of heating to control the temperature. This can be achieved by placing it inside an oven or in a single tube heater for small vessels, or an oil or electrically heated jacket for large vessels.

There are two main types of supercritical fluid extraction: countercurrent extraction from liquid samples and extraction from solid particles. In the countercurrent extraction, the supercritical solvent is introduced from the bottom of the extractor, the liquid sample is introduced from the top of the extractor to create a countercurrent flow between the liquid sample and the supercritical solvent. When the sample is in the form of solid, the supercritical fluid solvent is continuously passed through the extractor either in downward or in upward direction. The ways of loading samples in the extractor
will have a great effect on the extraction process. Rubio-Rodríguez et al. (2008) studied the influence of different contact devices used in the extractor on the oil yield extracted from hake (Merluccius capensis - Merluccius paradoxus). Two different devices were used to place the hake by product inside the extractor. One was a basket where the raw material was placed as a placed bed which provided a continuous contact, and the other one was a tray contacting device which provided intermittent contact. Their experiments results indicated that the extraction rate was higher in the tray contacting device than that in a basket. They thought the contact between the sample and supercritical CO₂ was better in the tray than that in the basket. Nguyen et al. (2011) evaluated the effect of different packing methods on the oil extraction from Moringa oleifera seeds. Two methods were applied when they loaded the ground kernels in the extractor. One method was random packing where the ground kernels were dumped into the extractor forming a layer. The other one was loading the kernels arranged in a multi-layers, the number of layers and the thickness of each layer were varied as well. They found that significant increase in oil yield was observed when multiple-layered packing method was employed; also decreasing the thickness of the sample layer and increasing the number of layers enhanced the extraction yield. This was because the multi-layer packed bed prevented the formation of preferential channels thus allowing supercritical CO₂ to be distributed uniformly in the extractor. The multi-layer packing arrangement allowed supercritical CO₂ to penetrate wider surface areas of the ground kernels thus promoting better extraction of the oil.

Once the supercritical fluid and its solubilised solute have passed out of the extraction cell, a means of separating the solute from the supercritical fluid is required; this is usually achieved by reducing the pressure using a restrictor, as illustrated in Figure 2-2. Both fixed and variable restrictors are currently employed (Dean, 1993). Physically, a piece of stainless steel or fused silica capillary tube can be used as a fixed restrictor and
the inner diameter of the fixed restrictor could regulate the flow rate and back pressure, the smaller of the inner diameter, the greater the back pressure in line and the greater the mass flow rate. The main advantages of using a fixed restrictor are they are cheap and generally easy to replace. However, a fixed restrictor is more likely to be plugged at its end where the depressurization of the supercritical fluid causes the solutes to drop out of the solution. Another problem for a fixed restrictor is sample contamination, resulting from carryover into the transfer lines due to the inaccessibility to clean fixed restrictors. As for the variable restrictor, its main advantage over the fixed restrictor is that the size of the orifice in the variable restrictor can change and therefore the mass flow rate is independent of the system back pressure, however, the adiabatic expansion of the depressurized fluid may still result in ice formation and therefore cause blockage, as a result both fixed and variable restrictors in commercial systems are usually heated to minimize plugging.

2.2.2 Effect of temperature and pressure

![Figure 2-3 The effect of extraction pressure on lycopene yield at constant temperature of 343 K and flow rate of 2.5 ml/min (Topal et al., 2006)]
Pressure and temperature are the most important physical parameters for SFE and they have both theoretical and practical implications for the extraction process. They together determine the density of the supercritical fluid. The majority of the literature concludes that an increase in the extraction pressure leads to an increase in the extraction yield. This is because an increase of pressure at a given temperature will result in an increase in the fluid density, which means an enhanced solubility of solutes, thus higher pressure is responsible for quantitative recoveries and stronger interactions between the fluid and the matrix. However, some studies have found that there is an optimum pressure point, beyond which a further increase in pressure could lead to a decrease in the yield. Topal et al. (2006) studied the effect of pressure on supercritical carbon dioxide extraction of lycopene from tomato skins. They observed that a steady increase in operating pressure from 20 to 40 MPa resulted in a steady increase in lycopene yield. However, further increasing the pressure from 40 MPa to 50 MPa did not improve the recovery of lycopene as shown in Figure 2-3. Therefore, high pressure is not always recommended for supercritical fluid extraction process.

The influence of temperature on supercritical fluid extraction process is more difficult to predict than that of pressure because of its two counter effects. First of all, increasing temperature reduces the solvent density and thus its solvation power at constant pressure. On the other hand, increasing temperature increases the vapour pressure of the solutes, therefore increasing their solubility in the supercritical solvent (Couto et al., 2009). Furthermore, high temperatures can increase the mass transfer. Therefore, the increase in temperature can have either a positive or a negative effect, which is dependent on whether the solvent density or the solute vapour pressure is predominant (Thana et al., 2008). Ge et al. (2002) studied the effects of temperature and pressure on the extraction of vitamin E (VE) from wheat germ. They found that there was a transformation pressure at about 26.2 MPa during the extraction process as shown in
Figure 2-4. At pressures below the “transformation pressure”, VE yield decreased with increasing temperature, in contrast, at pressures above the “transformation pressure”, VE yield increased with increasing temperature. The main reason for this observation was that the solubility was determined by the vapour pressure of the extracted vitamin E and the density of supercritical CO₂. At pressures above the “transformation pressure”, the density of supercritical CO₂ was high, although the solvent power of supercritical CO₂ decreased when the temperature increased, the vapour pressure, the diffusion coefficient and the molecular transfer of the extracted VE greatly increased at the same time. As a result, solvent-solute affinity increased and the solubility of the solvent eventually increased as well. In contrast, at pressures below the “transformation pressure”, the compressibility of supercritical CO₂ was high, increased temperature resulted in a rapid reduction in the density of supercritical CO₂ and the enhanced volatility and diffusion coefficient of the extracted VE did not fully counteract with the reduced density of supercritical fluid and therefore resulted in a decreased solvation power of the fluid.

![Figure 2-4](image)

Figure 2-4 The effect of extraction temperatures on vitamin E yield at different pressures (SFE conditions: flow rate = 2.0 mL/min; restrictor temperature = 328 K; sample amount = 5g; extraction time = 90 minutes) (Ge et al., 2002)
2.2.3 Effect of flow rate

The CO$_2$ flow rate has a significant effect on the supercritical fluid extraction process. In general, increasing the CO$_2$ flow rate will increase the extraction yield. However, further increasing the flow rate beyond an optimal point will not increase the extraction yield or even lower the yield. Yang et al. (2013) investigated the effect of the CO$_2$ flow rate on the extraction of protocatechuic acid from Scutellaria Barbata D. Don. Their results indicated that an increase in the CO$_2$ flow rate within the range of 0.8-2.2 ml/min increased the amount of protocatechuic acid extracted. However, increasing the flow rate from 2.2 ml/min to 2.7 ml/min did not significantly increase the yield of protocatechuic acid extracted. Topal et al. (2006) tested CO$_2$ flow rate ranging from 1.5 to 4.5 ml/min when they extracted lycopene from tomato skin. They found that when the flow rate was increased from 2.5 to 4.5 ml/min, it resulted in a decrease in the amount of lycopene extracted. This is because the extraction process is divided into four general stages: (1) the internal diffusion of CO$_2$ into the sample matrix, (2) the solubility of the extracted components into supercritical CO$_2$, (3) the diffusion and transportation of the extracted substance to the outside of the sample surface and (4) transportation of the extracted substances from the external surface of the sample to the bulk solution (external mass transfer). How the flow rate affects the SFE process is dependent on which stage to be the limiting step for the whole mass transfer process. Increasing the CO$_2$ flow rate will increase the number of CO$_2$ molecules that come into contact with the extracted substances and enhance the solubility of the substances. However, the higher the flow rate, the shorter is the residence time. The shorter resident time does not allow for CO$_2$ to remain in contact with the matrix and diffuse through the pores of the matrix, thus resulting in a lower extraction yield.
2.2.4 Effect of sample particle size

Sample particle size is another critical factor for a satisfactory SFE process. In general, decreasing particle size will increase the surface area per unit mass between the particle and the solvent. Because more solute will be located on the surface and exposed to the solvent, thus the total yield is higher when small particle is used. Also there is an intra-particle diffusion process as discussed in Section 2.2.3, the intra-particle diffusion resistance is smaller for smaller particle due to the shorter diffusion path of the solute across to the matrix (Cheah et al., 2006). Therefore, grinding a sample into fine powder can speed up the process and improve the extraction efficiency. Zuo et al. (2008) studied the effect of particle size on the supercritical CO\(_2\) extraction of soybean isoflavones from soybean meal. Four particle sizes were studied which included 10-20 mesh, 20-30 mesh, 30-40 mesh and 40-60 mesh, respectively. The results showed that the isoflavone recovery increased significantly when the particle size decreased from 1.19mm (mean diameter, 10-20 mesh) to 0.68mm (20-30 mesh). However, when particles became too small, the isoflavone recovery decreased sharply. This was because small particles aggregated together during extraction which caused the fluid to channel or short circuit. The experiments from Sabio et al. (2003) also showed that smaller particle size reduced the yield of lycopene extracted from the mixture of tomato skin and seeds. They verified that particle sizes that were too small could result in inhomogeneous extraction because of channelling effects in the fixed bed. Furthermore, very small particles might have poor flow properties and could retard mass transfer by forming flat, dense layers, leaving no pathways for liquid flow. However, this can be resolved by packing the sample with glass beads or other rigid inert materials (Cheah et al., 2006). As a rule, particles with mean diameters between 0.25 and 2.0mm are often used in supercritical fluid extraction experiments (Reverchon and De Marco, 2006).
2.2.5 Effect of plant materials’ structure

The cell structure is another factor that affects the supercritical fluid extraction process. For example, Gutiérrez et al. (2008) investigated the effects of different drying methods on the oil yield extracted from Quebec sea buckthorn seeds and pulps, they found that the oil yields of the air dried and freeze dried pulps were significantly different. They explained that the difference between the oil yields was due to the drying effects on the pulp cellular structure. They observed that air drying caused breakage and destruction of cell walls, and consequently large cavities and intercellular spaces were formed. On the other hand, no broken cell walls were observed in freeze-dried pulp. Therefore, the mass transfer resistance in the air dried samples were smaller than that in the freeze dried samples which could contribute to the yield differences between the two drying methods. The cell structure effect was also taken into account in the extraction of essential oil from plant leaves. According to Palavra et al (2011), the solubility of essential oil in supercritical CO₂ is much higher than that of waxes. However, waxes are located on the surface of the leaves, essential oil compounds are found in intracellular spaces which represents less favourable positions for extraction. Therefore, grinding or other pre-treatment methods are necessary for essential oil extraction. Yahya et al. (2010) studied the effect of pre-treatment on the structure of Pandan leaf. Figure 2-5 shows scanning electron micrographs of fresh, oven dried and freeze dried of ground pandan leaves. They found that grinding pre-treatment destroyed Pandan leaves epidemis cells which therefore made the essential oil more easily to be extracted. Moreover, the size of oven dried papillae was smaller than fresh and freeze-dried ones. This could due to water been removed from the papillae during the oven drying operations. However, the papillae of freeze dried leaves did not differ much from that of the fresh leaves.
Figure 2-5 Scanning electron micrographs of surface structure, disrupted epidermal cell and papillae of fresh, oven dried and freeze dried of ground pandan leaves (Yahya et al., 2010)

2.2.6 Effect of modifiers on supercritical fluid extraction

Supercritical carbon dioxide is suitable for non-polar compounds extraction, but many polar natural products such as natural phenolic antioxidants have low solubility in this solvent and variations in pressure and temperature have limited effects on the extraction process. The introduction of a suitable modifier may increase the solvation power of supercritical carbon dioxide and improve the extraction yield. Huang et al. (2013) discussed the change in the solubility parameter and the dipole moment of the supercritical carbon dioxide with the introduction of a modifier. The dipole moment of commonly used modifiers, the solubility parameters of pure modifiers and mixtures of different modifiers with carbon dioxide are listed in Table 2-3.
It can be seen from Table 2-3 that carbon dioxide is a non-polar solvent with dipole moment of 0. The introduction of a modifier with dipole moment bigger than zero can significantly improve the polarity of the modified supercritical carbon dioxide fluid. The introduction of a small amount of a polar modifier can significantly increase the solubility parameter of supercritical carbon dioxide. Not only enhancing the solvent strength of supercritical carbon dioxide, the modifier may also interact with the analyte/matrix by lowering the activation energy of analyte desorption (Roy et al., 1996), promote the accessibility of remote sites to the extraction fluid and facilitate the transportation of the analyte to the bulk fluid (Lalas and Tsaknis, 2002). For these reasons, the introduction of a polar modifier makes supercritical CO\textsubscript{2} extraction more attractive in various potential applications.

The most frequently encountered modifier in the literature is methanol because it is an effective polar modifier and is up to 20% miscible with CO\textsubscript{2}. Ethanol, although not as polar as methanol, may be a better choice for food applications because it has low toxicity and is generally recognized as safe. For example, resveratrol can be selectively

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Dipole moment of pure modifier</th>
<th>Solubility parameter of pure solvent (MPa)</th>
<th>Solubility parameter of modifier/SCCO\textsubscript{2} at 338 K and 15 MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO\textsubscript{2}</td>
<td>0</td>
<td>10.45</td>
<td>10.45</td>
</tr>
<tr>
<td>water</td>
<td>1.85</td>
<td>47.86</td>
<td>14.19</td>
</tr>
<tr>
<td>methanol</td>
<td>1.70</td>
<td>29.66</td>
<td>12.37</td>
</tr>
<tr>
<td>ethanol</td>
<td>1.69</td>
<td>26.39</td>
<td>12.05</td>
</tr>
<tr>
<td>2-propanol</td>
<td>1.58</td>
<td>24.34</td>
<td>11.84</td>
</tr>
<tr>
<td>acetone</td>
<td>2.88</td>
<td>20.05</td>
<td>11.41</td>
</tr>
<tr>
<td>dichloromethane</td>
<td>1.60</td>
<td>19.84</td>
<td>11.39</td>
</tr>
</tbody>
</table>
extracted from the pomaces of palomino fino grapes with the introduction of ethanol, which is not possible with pure supercritical carbon dioxide at low pressures (Casas et al., 2010). In addition, numerous other modifiers with different chemical characteristics have been used to enhance recoveries in SFE, such as water, hexane, ethyl acetate and dichloromethane. The utilization of two or three modifiers has been proposed for certain situations. Ethanol and isopropyl alcohol have been proposed for turmeric extracts (Leal et al., 2003). Mixture of methanol-water-acetonitrile has been reported to be an effective modifier to extract amino acids from birch leaves (Klejdus et al., 2008).

By manipulating the types and ratios of modifiers, one may obtain different extraction results. For instance, Shi et al. (2009) tested the effects of several modifiers such as ethanol, water and olive oil on the yield of lycopene extracted from tomato paste waste. They found that the extraction yield of lycopene increased when the concentration of the three modifiers increased from 5% (w/w) to 15% (w/w). Under the same conditions and the same concentration level, the degree in which the modifier improved the extraction efficiency of lycopene was given in the following order: olive oil > ethanol > water. The use of increasing amounts of modifier can also allow extraction of low polar compounds first and then more polar compounds (Diaz-Reinoso et al., 2006). It has been reported that mixtures of supercritical carbon dioxide and a low percentage of modifier could extract some of the low molecular weight phenols while a progressive increase in pressure and percentage of modifier would allow extraction of increasing molecular weight phenols (Murga et al., 2000).

### 2.3 Applications of supercritical fluid extraction

The applications of supercritical fluid extraction in different areas are summarized in Table 2-4. The most extended use of supercritical fluid extraction is in the food and pharmaceutical area. This is because supercritical fluids are typically less damaging to delicate flavour, fragrances and biologically active compounds than conventional steam
distillation and solvent extraction, and SFE does not leave undesirable or toxic solvent residues in the extract. As can be seen from Table 2-4, supercritical fluid extraction has been applied to extract oils, essential oils, flavours, colours and medicinal components from different natural plants. The analytical use of SFE in the food industry has also involved the detection of undesired residues in food. Many of the analyses traditionally used in the food industry rely on large quantities of organic solvents and tedious, labour-intensive sample handling techniques. The introduction of the supercritical fluid extraction in the food residue analysis can significantly reduce the solvent consumption and analysis time (Sahena et al., 2009).

Supercritical fluid extraction has also been successfully utilized for environmental remediation in laboratory and pilot-scale plants (Sunarso and Ismadji, 2009). For example, supercritical fluid has been adopted as an extraction media to remove hydrocarbons, PAHs, PCBs, herbicides, pesticides and metals from soils, sediments, sludge and similar samples. Reviews of the SFE approach on environmental analysis have been presented by several authors (Sunarso and Ismadji, 2009, Garcia-Rodriguez et al., 2008, Anitescu and Tavlarides, 2006). The superiority of supercritical fluid extraction over conventional extraction methods lie on its flexibility to adjust the solvent power of supercritical fluid and the inherent nullification of organic solvent demand. The addition of cosolvent and surfactant to supercritical CO₂ can boost its extraction efficiency, especially for several hazardous organic compounds (Sunarso and Ismadji, 2009). Supercritical fluids modified by the addition of complex agents can extract metal ions from various solid and liquid matrices. In addition, there are other applications of SFE in biofuel productions and polymer productions, e.g., production of hydrogen by supercritical water extraction, separation of ethanol from fermentation broth, the oligomeric material extraction and removal of additives from polymer. These areas are still being studied.
### Table 2-4  Summaries of applications of SFE in different areas

<table>
<thead>
<tr>
<th>Application areas</th>
<th>Examples of the application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food processing</td>
<td>Vegetable oil and essential oil extraction (Sahena <em>et al</em>., 2009) natural colour extraction, such as carotenoids from carrot (Sun and Temelli, 2006), lycopene from tomato skin (Topal <em>et al</em>., 2006) chemical residues such as pesticides (Chuang <em>et al</em>., 2001) and herbicides (Pensabene <em>et al</em>., 2000)</td>
</tr>
<tr>
<td>Pharmaceutical</td>
<td>Medicinal components extraction from natural plants (Chen and Ling, 2000) Drugs and metabolites from tissues, fluids and other biological matrices (Brewer <em>et al</em>., 2001) Pollutants extraction such as hydrocarbons, PAHs, and PCBs (Barnabas <em>et al</em>., 1995) herbicides and pesticides removal (Izquierdo <em>et al</em>., 1996) heavy metals removal, such as extraction of Zn, Cu, Pb, Cd and Cr from fly ash (Kersch <em>et al</em>., 2000)</td>
</tr>
<tr>
<td>Environmental</td>
<td>Hydrogen production by supercritical water extraction (Demirbas, 2005) separation of ethanol from the fermentation broth (Güvenç <em>et al</em>., 1998)</td>
</tr>
<tr>
<td>Biofuel</td>
<td>Oligomeric material extraction and additives removal (Taylor, 1996)</td>
</tr>
<tr>
<td>Polymer</td>
<td></td>
</tr>
</tbody>
</table>

#### 2.4 *Moringa oleifera*

*Moringa oleifera*, commonly referred to as “Moringa”, is one of the most widely cultivated tree species in the family Moringaceae. This species originally comes from northern India but is now distributed worldwide in the tropics and subtropics (Lalas and Tsaknis, 2002). The height of the trees range from 5 to 10m and sometimes can be even 15m. The trees can grow in hot dry lands or destitute soils and are reasonably drought resistant. *Moringa oleifera* is a traditional important source of food as its leaves, flowers, pods, seeds and roots are locally used as vegetables. *Moringa oleifera* also has multiple medicinal qualities (Anwar *et al*., 2007). For example, nitrile, mustard oil glycosides and thiocarbamate glycosides which are extracted from *Moringa oleifera* leaves have been found to be beneficial for high blood pressure treatment. The root
extracts possess antimicrobial activity which has been attributed to the presence of 4-α-L-rhamnosyloxy benzyl isothiocyanate. The extracts from *Moringa oleifera* flowers are also found to have a significant hepatoprotective effect which may be due to the presence of quercetin (Anwar et al., 2007).

*Moringa oleifera* leaves have been taken to combat malnutrition, especially among infants and nursing mothers, because they contain more calcium than milk, more iron than spinach, more Vitamin C than oranges and more potassium than bananas (Fahey, 2005). Recent research has demonstrated that the extracts from *Moringa oleifera* leaves possess several useful biological properties, such as antihypertensive (Faizi et al., 1994), anti-fungal (Chuang et al., 2007), antiulcer (Pal et al., 1995), antitumor and anticancer (Makonnen et al., 1997) properties. Aqueous leaf extracts can regulate thyroid hormone and can be used to treat hyperthyroidism (Tahiliani and Kar, 2000). A recent report also shows that *Moringa oleifera* leaves may be used as a prophylactic or therapeutic anti-HSV (Herpes simplex virus type 1, or HSV) medicine and may be effective against the acyclovir-resistant variant (Lipipun et al., 2003). These properties make *Moringa oleifera* a promising candidate in the pharmaceutical industry.

The seeds of *Moringa oleifera* are one of the best natural coagulants. The aqueous extract of *Moringa oleifera* seeds has a flocculating protein that can be effectively utilized for treatment and purification of highly turbid water (Manzoor et al., 2007). *Moringa oleifera* seeds contain between 33% and 41% oil which is commercially known as “Ben oil” (Mani et al., 2007). This oil has been used in the manufacturing of perfume and hair care products, as lubricant in watch making and precision equipment, and as coagulation for water purification and as medicine for improvement of cardiac function (Mani et al., 2007). Additionally, the oil can also be used for edible purpose and it is considered to be equivalent to olive oil in terms of its fatty acid compositions (Abdulkarim et al., 2005). Oleic acid which is a monounsaturated omega-9 fatty acid is
the predominant fatty acid in *Moringa oleifera* seed oil, accounting for more than 70% of the total fatty acids (Anwar and Bhanger, 2003). The high oleic acid vegetable oil has been reported to be very stable even in highly demanding applications like frying.

The extraction of oil from *Moringa oleifera* seeds using organic solvents in a Soxhlet extractor is a well-studied area, and n-hexane is a commonly used solvent. da Silva *et al.* (2010) extracted dried seeds from Brazil using n-hexane at 353 K for twenty-four hours, resulting in an oil yield of 39wt%. The main component of the oil was oleic acid (78%) followed by palmitic acid (7.0%) and behenic acid (4.0%). Rashid *et al.* (2008) obtained a slightly lower yield of 35wt% from seeds sourced from Pakistan and the oil consisted of 72.2% oleic acid, 6.5% palmitic acid and 7.1% behenic acid. The lower yield could be due to the use of a shorter extraction time of only six hours. Such variation in oil yield could also be attributed to the possible variations in seasonal temperatures, soil conditions, nutrients and water availability as well as the maturity of the trees (Anwar *et al.*, 2006). However, the incomplete elimination of the toxic solvents could cause safety concerns in the use of the extracted oil. Although the extraction of oil from *Moringa oleifera* seeds by cold pressing and aqueous enzymatic methods has been studied (Abdulkarim *et al.*, 2005, Tsaknis *et al.*, 1999), these two methods were reported to produce very low oil yield compared to the Soxhlet extraction.

Using supercritical carbon dioxide extraction can overcome the disadvantage of the above extraction methods. However, very limited research has been conducted on the supercritical fluid extraction of *Moringa oleifera* seed oil. Until now only one paper has been found regarding the extraction of oil from *Moringa oleifera* seeds using ethanol modified supercritical carbon dioxide (Nguyen *et al.*, 2011). The authors studied the influence of different sample loading methods on the yield of the oil. The samples were loaded in a multi-layered packing arrangement or randomly charged into the vessel.
They found that loading the seeds in multiple-staged trays increased the yield by 26.89% compared to a randomly packed configuration. Additionally, the extraction yield and the fatty acid components of the *Moringa oleifera* seed oil extracted using supercritical carbon dioxide, ethanol modified supercritical carbon dioxide and n-hexane were compared. It was found that adding 10wt% ethanol increased 10% in oil yield. However, there was no significant difference in the yield between SFE using supercritical carbon dioxide-ethanol and Soxhlet extraction using n-hexane. The fatty acid compositions of the oils extracted by supercritical carbon dioxide, supercritical carbon dioxide-ethanol and n-hexane were similar.

### 2.5 Eucalyptus leaves

*Eucalyptus* is a large genus of the Myrtaceae family that includes over 700 species. Although *Eucalyptus* is widely grown in many countries all over the world, most of the species are native to Australia. *Eucalyptus* is a prospective new tree crop for the Western Australia wheatbelt. In this region, agriculture has been traditionally based on annual crops and pastures which have generated adverse water balances and thus caused dryland salinity. Deep rooted, long lived perennials could help reduce this problem (Wildy *et al*., 2000). Beginning in the 1990s, many wheatbelt farmers began planting *Eucalyptus* as part of landcare efforts to tackle salinity and land degradation problems. This is because this plant is a long lived perennial which can be harvested over many years on a short rotation basis. Currently, over 20,000 hectares of *Eucalyptus* have been established in Western Australia. Planting of these trees also provides shelter belts that reduce wind erosion.

The *Eucalyptus* plant is a genus of tall, evergreen and magnificent tree cultivated all over the world for its oil, gum, pulp, timber, medicine and aesthetic value (Batish *et al*., 2008). *Eucalyptus* trees are among the most important hard wood forestry crops worldwide and provide a major source of pulp wood for high quality paper production.
Eucalyptus timber is used for construction and fuel, and Eucalyptus gum is used for diarrhea and as an astringent in dentistry (Marzoug et al., 2011). Amongst all of the wood and non-wood products, Eucalyptus oil found in its foliage is the most important one. Eucalyptus leaf oil has been shown to contain a relatively high concentration of 1,8-cineole, which has chemical and physical properties that make it suitable for a range of applications. The content of 1,8-cineole in the oil from different Eucalyptus species varies from 25% to 90% (Giamakis et al., 2001). Apart from 1,8-cineole, the Eucalyptus leaf oil contains, in relatively high amounts of several monoterpenic hydrocarbons (α-pinene, limonene, p-cymene, β-pinene, α-phellandrene, camphene, γ-terpinene, etc., with the first three in major amounts) and in lower percentages of several sesquiterpenic hydrocarbons (aromadendrene, allo-aromadendrene, globulol, etc.), oxygenated monoterpenes (e.g. myrtenal, carvone and pinocarvone) and others aromatic phenols, oxides, ethers, alcohols, esters, aldehydes and ketones. The quantity and strength of the oil varies across Eucalyptus species (Batish et al., 2008). The most-known compound, terpenoid, gives Eucalyptus foliage its characteristic smell. Eucalyptus leaf oil is used mostly as an ingredient in general pharmacy products (e.g. liniments, inhalants, expectorants) throughout the world due to its broad biological properties including anti-inflammatory, anti-allergenic, anti-asthmatic, anticonvulsant, antiseptic, aquaculture antiviral, anti-bacterial and anti-malarial. It has also been used as a flavour and aroma enhancer in food and cleaning products and in cosmetic formulations (Gilles et al., 2010). The chemical composition and biological effects of Eucalyptus leaf oil, including their antimicrobial properties and potential applications in food products, have been reviewed by Bakkali et al. (2008) and Burt (2004).

The extraction of Eucalyptus leaf oil from leaves is conventionally performed by steam distillation. The extraction is a long process using water and steam as distillation media. However, this technique has disadvantages because of artefacts produced during the
process, especially when thermo labile components are involved (da Cruz Francisco et al., 2001). Recently, supercritical fluid extraction has become a valid alternative to the steam distillation. This is because the commonly used fluid in SFE is CO₂, which has several unique characteristics and physic-chemical properties. For example, CO₂ is non-toxic, non-flammable, inexpensive, and odourless and has low critical pressure (7.38 MPa) and temperature (304 K) and leaves no solvent residue in the products, thus providing an oil of superior quality. SFE also has advantages over the traditional extraction techniques including operation at low temperatures thus enabling the preservation of the thermally labile components in the extracts. Moreover, the selectivity of carbon dioxide can be easily adjusted by changing the temperature and pressure. Several authors have studied the supercritical fluid extraction of oil from *Eucalyptus* leaves (Della Porta et al., 1999, da Cruz Francisco et al., 2001). The *E. camaldulensis* Dehn. leaf oil obtained by hydro-distillation and by supercritical fluid extraction were compared by da Cruz Francisco et al. (2001). Their results revealed that the oil obtained by hydro-distillation contained higher concentration of 1,8-cineole (43%), α-pinene (5.5%), β-pinene (3.4%), p-cymene (5.2%), terpinen-4-ol (3.1%), and globulol (4.1%). The extracted oil obtained by supercritical carbon dioxide extraction had lower amounts of 1,8-cineole, α-pinene, β-pinene, and terpinen-4-ol, but higher amounts of allo-aromadendrene and globulol. Della Porta et al. (1999) also isolated *Eucalyptus* leaf oil by supercritical CO₂ extraction with a fractional separation technique. They found that a good extraction performance was obtained by operating at 9 MPa and 323 K for 510 minutes. The optimum fractionation was achieved by operating at 9 MPa and 263 K in the first separator and at 1.5 MPa and 288 K in the second one.
2.6 Research and development needs

An important drawback of supercritical carbon dioxide extraction is that carbon dioxide is a non-polar extraction fluid which makes the extraction of polar compounds very difficult. Therefore, how to decrease the solute polarity to make them more soluble in non-polar supercritical fluids has become a research trend to widen the application of SFE technique. In this sense, chemical in situ derivatization has been applied to improve the selectivity of a specific group of compounds, as it promotes the conversion of the solute polar groups (hydroxyl and carboxyl) into other less polar function (ether, ester and silyl derivatives) which makes the derivative more readily soluble in supercritical CO₂ (Luque de Castro and Tena, 1996). The use of a supercritical carbon dioxide reaction medium to determine the (R)-and (S)-enantiomers of mandelic acid (MA) was proposed by Zougagh et al. (2006). The process involved a previous derivatization step under supercritical conditions by which the carboxyl group was esterified with methanol, and then followed by acylation of the hydroxyl group into methyl MA with pentafluoropropionic anhydride in the absence of a catalyst. The proposed method was used to analyse urine samples from 10 volunteers and the results convinced that this process could be reliably applied to real samples. Nevertheless, a more common practice in SFE is to change the polarity of the supercritical fluid and increase their solvating power towards the solute of interest. This can be achieved by changing the physical parameters of the supercritical fluid or by employing polar modifiers. For example, the addition of relatively small percentages (1-10%) of methanol to carbon dioxide expands its extraction range to include more polar solutes (Mendiola et al., 2007).

On the other hand, experimental method development is more difficult for SFE since many parameters have to be optimized and the optimal extraction conditions tend to be unique for each particular sample type. The use of optimum extraction conditions for
the SFE extractions could significantly increase the extraction yield of a target compound. Varying different extraction parameters such as pressure, temperature, modifier volume and extraction time will bring about quantitative and qualitative differences in the extracts (Cheah et al., 2006). A common practice to set up robust extraction methods is to use experimental design. For instance, a mixture design was employed to optimize the temperature, pressure, dynamic time and modifier volume in order to obtain the highest yield of the Valerian essential oil (Safaralie et al., 2010). Based on the graphical and statistical analysis of the results, temperature and pressure were to be the influential variables on the extraction process and the optimal conditions were found at temperature (310 K), pressure (24.3-25.0 MPa), dynamic time (19-24 min) and modifier volume (100-200µl). Response surface methodology (RSM) is another popular possibility. The RSM is a collection of mathematical and statistical technique useful for the modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Yim et al., 2012). This procedure involves fitting a function to the experimental data and then using optimization techniques to obtain the optimum parameters (Ramalhosa et al., 2012). It is much faster and more economical to gather research results than classic one variable-at-a time or full-factors experimentation (Li et al., 2012). This methodology has been employed to optimize the extraction of various samples, such as Cyperus rotundus Linn. to obtain essential oil (Wang et al., 2012) and Yellow Horn seeds to obtain oil (Zhang et al., 2010). Orthogonal array design can also be effectively used to study the influence of five different factors simultaneously. This design has been used to study the effect of temperature, pressure, modifier volume and extraction time (dynamic and static) on the supercritical fluid extraction of S. mirzayanill oil (Yamini et al., 2008). Five levels of each variable were considered and their optimal values were determined through the analysis of variable (ANOVA) analysis. These designs can
provide a good tool to determine the main affective parameters during the extraction process by using only a few experimental runs.

2.7 Specific objectives of the present research

Following from the preceding review of the literature, four main specific objectives were identified and pursued in this thesis:

(1) To investigate the possibility of using supercritical fluid extraction to extract valuable oils from the plants of *Moringa oleifera* and *Eucalyptus* and to compare supercritical fluid extraction with the other two main conventional extraction methods, namely Soxhlet extraction and hydro-distillation. Through the comparison studies, the advantages and mechanisms of supercritical fluid extraction could be better understood.

(2) To investigate the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide. The experimental solubility data was analysed using the Peng-Robinson equation of state with the conventional van der Waals mixing rules. Three density based models, proposed by Chrastil (1982), del Valle and Aguilera (1988), and Adachi and Lu (1983) were also used to correlate the solubility data in this study.

(3) To optimise the conditions for the supercritical fluid extraction of *Moringa oleifera* seed oil, *Moringa oleifera* leaf oil and *Eucalyptus* leaf oil. As the efficiency of supercritical carbon dioxide extraction depends on several variables, including extraction temperature, pressure, flow rate, particle size and time et al. To investigate the effects of these parameters on the yield of *Moringa oleifera* seed oil, *Moringa oleifera* leaf oil and *Eucalyptus* leaf oil and find out the optimum conditions, the response surface methodology using a central composition design and the orthogonal array design were employed in this thesis.
(4) To study the effect of different modifiers on the supercritical fluid extraction process. Ethanol and water were chosen as the modifiers in this study as they are safe and of low costs.
Chapter 3 Methodology, Approach and Techniques

Two species of plants were studied in this thesis, namely *Moringa oleifera* and *Eucalyptus*. *Moringa oleifera* has been planted as an important commercial tree in many parts of Australia including Queensland, Western Australia and northern parts of the northern territory. *Eucalyptus* is native to Australia and it is an essential part of Australian agriculture, forest and industry. Due to their industrial and commercial values, these two plants were studied in this thesis. However, it should be emphasised that the two plants employed in this thesis were only as examples for the application of supercritical fluid extraction, Soxhlet extraction and hydro-distillation. These extraction methods can be applied to other plant species as well. Supercritical fluid extraction was employed to extract oil and essential oil from *Moringa oleifera* seeds and leaves and *Eucalyptus* leaves. The yield and compositions of the extracted oil using SFE was compared with those obtained using the traditional Soxhlet extraction and hydro-distillation methods. The facilities and operating procedures for these three methods were described in details in this chapter. Moreover, all the extracted oils were characterised using various analytical techniques including gas chromatography (GC), gas chromatography and mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). The analysis procedures and conditions were summarized in this chapter. Furthermore, the data analysis method was explained at the end of this chapter.

3.1 Materials and reagents

3.1.1 Moringa oleifera seed samples

The *Moringa oleifera* seeds used in this work were provided by Department of Agriculture and Food of Western Australia (DAFWA). Prior to processing, the samples were dried at 323 K for 8 hours and the moisture of the dried samples was determined to be 4.6%. The dried samples were ground in a Waring blender (model W-800S, Waring,
Torrington, CT) and the ground samples were then sieved using a sieved shaker (model EFL2000/2, Endecotts Ltd., London, England) into four fractions, namely, <500µm, 500-710µm, 710-1000µm, and 1000-2000µm. These fractions were used to study the effect of particle size on the extraction yield. The unsieved fraction <1000µm was selected for the response surface methodology experiments. The samples were kept in darkness in a refrigerator before experimentation.

3.1.2 *Moringa oleifera* leaves samples

The *Moringa oleifera* leaves used in this work were also provided by DAFWA. Prior to experimentation, the samples were air-dried at 313 K for 8 hours. The final water content of the dried leaves were determined to be 15.2% by drying a sample to 378 K for 5 hours. The air-dried samples were then ground with a knife grinder (Model 3383-L30, Thomas Scientific, USA) and the fraction of particles under 250µm was selected for all extraction experiments. The final samples were kept in a sealed plastic container and placed in a refrigerator before experimentation.

3.1.3 *Eucalyptus* leaves samples

*Eucalyptus loxophleba* subspecies *lissophloia* leaves were collected from trees in the south west region of Western Australia. The leaves were air dried for two days and the final moisture content of the leaves was determined to be 12.3%. The samples were then ground with a knife grinder (Model 3383-L30, Thomas Scientific, USA) and the fraction of particle sizes under 400µm was selected for all subsequent extraction experiments. The final samples were kept in a sealed container in the refrigeration at 277 K before experimentations.

3.1.4 Chemicals and compounds

The details and descriptions of the chemicals used in this work are listed in Table 3-1.
<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n-alkenes (C8-C20)</strong></td>
<td>Standard solution from Sigma Aldrich, used to calculate the retention indices of the components in <em>Moringa oleifera</em> leaf oil and <em>Eucalyptus</em> leaf oil through GC-MS analysis</td>
</tr>
<tr>
<td><strong>n-alkene (C21-C40)</strong></td>
<td>Standard solution from Sigma Aldrich, used to calculate the retention indices of the components in <em>Moringa oleifera</em> leaf oil and <em>Eucalyptus</em> leaf oil through GC-MS analysis</td>
</tr>
<tr>
<td><strong>myristic acid methyl ester (C14:0)</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
</tr>
<tr>
<td><strong>palmitic acid methyl ester (C16:0)</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
</tr>
<tr>
<td><strong>palmitoleic acid methyl ester (C16:1, Δ9 cis)</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
</tr>
<tr>
<td><strong>stearic acid methyl ester (C18:0)</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
</tr>
<tr>
<td><strong>oleic acid methyl ester (C18:1, Δ9 cis)</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
</tr>
<tr>
<td><strong>linoleic acid methyl ester (C18:2, Δ9 cis, Δ12 cis)</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
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<td><strong>linolenic acid methyl ester (C18:3, Δ9 cis, Δ12 cis, Δ15 cis)</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
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<tr>
<td><strong>arachidic acid methyl ester (C20:0)</strong></td>
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<td><strong>behenic acid methyl ester (C22:0)</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
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<td><strong>cis-11-eicosenoic acid methyl ester (C20:1)</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
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<td><strong>lignoceric acid methyl ester C24:0</strong></td>
<td>Analytical standard from Sigma Aldrich, used to analyse the fatty acid compositions of <em>Moringa oleifera</em> seed oil</td>
</tr>
<tr>
<td><strong>boron trifluoride- methanol solution(14% in methanol)</strong></td>
<td>Sigma Aldrich, used in the esterification process of fatty acid</td>
</tr>
<tr>
<td><strong>dichloromethane</strong></td>
<td>HPLC grade from Sigma Aldrich, dilution solvent for GC-MS analysis of <em>Moringa oleifera</em> leaf oil</td>
</tr>
<tr>
<td><strong>distilled water</strong></td>
<td>Ultrapure water was generated by the Ibis Alpha Ultrapure system (Mt. Hawthorn, WA, Australia), used in the esterification process of <em>Moringa oleifera</em> leaf oil</td>
</tr>
<tr>
<td>Chemical</td>
<td>Description</td>
</tr>
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<td>-----------------------------------------------------------------------------</td>
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<tr>
<td>ethyl acetate</td>
<td>Analytical grade from Chem-Supply Pty Ltd, extraction solvent for Soxhlet extraction</td>
</tr>
<tr>
<td>ethanol</td>
<td>Analytical grade from Chem-Supply Pty Ltd, extraction solvent for Soxhlet extraction and modifiers for supercritical fluid extraction</td>
</tr>
<tr>
<td>heptane</td>
<td>HPLC grade from Sigma Aldrich, used in the esterification process of fatty acid</td>
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<tr>
<td>hexane</td>
<td>Extraction grade from Shell Company, extraction solvent for Soxhlet extraction</td>
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<tr>
<td>methanol</td>
<td>HPLC grade from Sigma Aldrich, used in the esterification process of fatty acid</td>
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<td>sodium hydroxide</td>
<td>Analytical reagent from Chem-Supply Pty Ltd, used in the esterification process of fatty acid</td>
</tr>
<tr>
<td>sodium chloride</td>
<td>Analytical reagent from Chem-Supply Pty Ltd, used in the esterification process of fatty acid</td>
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<td>sodium sulphite anhydrous</td>
<td>Analytical reagent from Ajax Finechem, used in the esterification process of fatty acid</td>
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<td>liquid carbon dioxide</td>
<td>High purity grade from BOC (99.99%), extraction solvent for supercritical fluid extraction</td>
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<tr>
<td>helium</td>
<td>Ultra high purity grade from BOC (99.999-99.9999%), carrier gas for GC-MS and GC</td>
</tr>
<tr>
<td>nitrogen</td>
<td>Ultra high purity grade from BOC (99.999-99.9999%), used in the GC-FID detector</td>
</tr>
<tr>
<td>hydrogen</td>
<td>Analytical grade made by hydrogen generator, used in the GC-FID detector</td>
</tr>
</tbody>
</table>

### 3.2 Analytical instruments

Three analytical techniques were employed in this thesis. Gas Chromatograph (GC) analysis was employed to measure the fatty acid compositions of the extracted *Moringa oleifera* seed oil. Gas Chromatography (GC)–Mass Spectrometry (MS) was used to analyse the compositions of *Moringa oleifera* leaf oil and *Eucalyptus* leaf oil. Nuclear magnetic resonance (NMR) was introduced to study the bonding structure of the *Moringa oleifera* seed oil.
3.2.1 Gas chromatography (GC)

![Figure 3-1 Picture of Gas Chromatography](image)

GC is the use of a carrier gas to convey the sample into a vapour state through a narrow column. The column is usually made from fused silica tubes (0.1-0.3mm ID) that have refined stationary phase films (0.1-5µm) bound to the surface and cross linked to increase thermal stability. The column is installed in an oven that has temperature control. The column can be heated up slowly from ambient temperature to 623-723 K to provide separation of a wide range of compounds. The carrier gas is usually hydrogen or helium under pressure, and the eluting compounds can be detected by several ways, including by flames (flame ionization detector), by changing properties of the carrier (thermal conductivity detector), or by mass spectrometry. The universal detectors FID (flame ionization detector) was available in our laboratory which made GC the appropriate tool to analyse and identify the fatty acid methyl esters.

In this study, the fatty acid methyl ester was analysed by an Agilent 5890 GC unit which was equipped with an auto sampler (HP7653), a split/splitless inlet, a flame ionization detector (FID) and a J&W (Folsom, CA, USA) DB-Wax capillary column (30m×0.25mm ID, fused-silica column coated with 0.25µm polyethylene glycol film).
The carrier gas used was ultrahigh purity helium and the detector gases were ultrahigh purity nitrogen, ultrahigh purity hydrogen and industry grade air. A picture of the Gas Chromatography used in this study was shown in Figure 3-1.

### 3.2.2 Gas chromatography and mass spectrometry (GC-MS)

![Picture of Gas Chromatography-Mass Spectrometry](image)

In GC/MS, a mixture of compounds to be analysed is initially injected into the GC where the mixture is vaporised in a heated chamber (injector). The gas mixture travels through a GC column carried by a carrier gas, where the compounds become separated as they interact with the stationary phase of the column. The separated compounds then immediately enter the mass spectrometer which generates the mass spectrum of the individual compound. Mass spectrometer is an analytical technique that involves generating charged particles (ions) from molecules of the analytes. The generated ions are analysed to provide information about the molecular weight of the compound and its chemical structure. GC-MS is routinely employed to analyse compositions of various
essential oils. In this study, all the oils extracted from *Moringa oleifera* and *Eucalyptus* leaves were analysed using an Agilent 7890N series gas chromatograph as shown in Figure 3-2. This GC-MS was equipped with an Agilent 5975 mass selective detector and a HP-5MS column (30×0.25mm (5%-Phenyl) - methylpolysiloxane column, film thickness×0.25μm).

### 3.2.3 Nuclear magnetic resonance (NMR)

Two types of nuclear magnetic resonance instruments were used for this study. One dimensional spectra was performed on a Bruker Advance 500 nuclear magnetic resonance to study the structure of the *Moringa oleifera* seed oil, and the two dimensional COSY was operated on a Bruker Advance 600 nuclear magnetic resonance to study the nuclear spin-spin coupling interactions between nuclei from the seed oil samples.

The Bruker Advance 500 nuclear magnetic resonance was equipped with three RF channels and a Z gradient accessory. This spectrometer had ultra-stable temperature control and could be used for variable temperature operations to study molecular dynamics, reaction kinetics or diffusion studies. In addition, this instrument could perform experiments with 5mm $^1$H ($^{13}$C, $^{15}$N) indirect detection probe with Z gradient, 5mm $^1$H ($^{31}$P-$^{109}$Ag) indirect detection probe, 5mm $^{13}$C($^1$H) dual probe and 10mm BB ($^{31}$P-$^{183}$W) observe $^1$H decouple.

The Bruker Advance 600 nuclear magnetic resonance was equipped with three RF channels plus 2H decoupling capabilities and triple axis (XYZ) gradients accessory. It could perform 5mm $^1$H ($^{13}$C, $^{15}$N) indirect detection probe with XYZ gradients and 5mm BB ($^{31}$P-$^{109}$Ag) observe $^1$H/$^{31}$P decouple with Z gradient. As the same with Bruker Advance 500 nuclear magnetic resonance, this instrument also could be used for variable temperature operations to study molecular dynamics, reaction kinetics or diffusion studies.
3.3 Extraction apparatus

3.3.1 Supercritical fluid extraction system

Supercritical fluid extraction experiments were carried out by using an SFT Custom SCW-SFE system (Newark, DE, USA) as shown in Figure 3-3. This system was designed to perform both supercritical carbon dioxide extraction and supercritical water oxidation experiments. Liquid CO\textsubscript{2} was supplied from CO\textsubscript{2} cylinder by a siphon tube and then introduced into the system through a dual piston pump. The pump was designed and operated at pressure up to 68 MPa with flow rate ranging from 0.1 ml/min to 24 ml/min. The CO\textsubscript{2} extraction can be performed in a temperature range of 273-473 K.

This system accommodated four parallel extraction vessels which were two vessels for supercritical CO\textsubscript{2} extraction and two vessels for supercritical water oxidation. The CO\textsubscript{2} fluid was heated to its supercritical status through a preheater and a heating jacket around the vessel. A robust variable restrictor valve was used to control the flow rate. In order to prevent sample plugging, the restrictor was warmed electrically. A flow meter was provided to indicate the flow rate of CO\textsubscript{2} being passed through the system. The supercritical CO\textsubscript{2} with dissolved compounds passed through a heated restrictor and were subsequently expanded to ambient pressure. The extracts were precipitated in a collection vial at ambient pressure and temperature. A volume totalizer (Model 33400-58 Cole Parmer Instrument Company, Vernon Hills, IL, USA) was used to measure the total volume of CO\textsubscript{2} used in the extraction. A second pump was used for adding a modifier into the system.
Figure 3-3  Schematic diagram and the picture of the supercritical fluid extraction apparatus: (1) CO₂ cylinder; (2) CO₂ and H₂O pump; (3) co-solvent reservoir; (4) co-solvent pump; (5) preheater; (6) 100 ml jacketed H₂O vessel; (7) 50ml H₂O vessel; (8) 100ml CO₂ vessel; (9) 50ml CO₂ vessel; (10) pressure gauge; (11) condenser; (12) restrictor; (13) collection bottle; (14) flow meter
3.3.2 Soxhlet apparatus

Soxhlet extraction is one of the most common laboratory procedures for solid-liquid extraction, it has been a standard technique for over a century. And at present, it is still the main reference to which the performance of other extraction methods is compared (Luque de Castro and Priego-Capote, 2010). Soxhlet extraction has some attractive advantages. Firstly, the sample is repeatedly brought into contact with fresh solvent, which facilitates displacement of the transfer equilibrium. Secondly, no filtration is required after the extraction step and the sample throughput can be increased by simultaneous extraction in parallel since the basic equipment is inexpensive (Luque de Castro and Priego-Capote, 2010). However, compared with other extraction techniques, long time is required for the extraction and a large amount of wasted solvents are generated, which are not only expensive to dispose of but also cause additional environmental problems. Moreover, in many cases, Soxhlet extraction is not selective because interfering compounds (which could hinder good separation of interested analytes during extraction) are also extracted. Although the hardware required for a Soxhlet extraction is relatively simple, extreme care must be taken to prevent contaminants in the extract and to minimize losses during sample transfer and solvent exchange.
Figure 3-4 shows a sketch of the Soxhlet extraction apparatus used in this study. Normally a solid material containing some of the desired compounds was placed inside a cellulose or ceramic thimble, which was loaded into the main chamber of the Soxhlet extractor. The extractor was connected to a flask containing the solvent, and a condenser was placed on top of the extractor. The solvent was heated to reflux, the vapour passed through a bypass arm to reach the condenser, which it condensed and dripped down into the thimble housing the solid material. The extractor containing the solid material slowly filled with warm solvent and some of the desired compounds would then dissolve into the warm solvent. Once the solvent level in the extractor reached the top of the siphon arm, the solvent and the extract were siphoned back into the lower flask. The completed extraction produced a high-volume; dilute solution which usually needed to be concentrated by a rotary evaporator. The non-soluble portion of the extracted solid remained in the thimble, and was usually discarded.
3.3.3 Hydro-distillation apparatus

Hydro-distillation is the main method used to extract essential oil from different plant matrixes. The process is usually carried out in a Clevenger-type apparatus. This simple Clevenger-type apparatus used in this study is shown in Figure 3-5. This apparatus contained a 500ml round-bottomed flask, a condenser, a measuring tube with stopcock and a return tube (which connected the bottle of the measuring tube and the vertical tube) for the aqueous part of the distillate. The samples to be extracted would immerse in water, which was then boiled. During hydro-distillation, the essential oil components form an azeotropic mixture with water. The vapours of the volatile components were carried away by the steam to the condenser. Upon condensation, the droplets were continuously accumulated in the measuring tube. The extraction period influenced not only the yield but also the extraction composition. The sample was exposed to
temperatures close to 373 K, which could lead to changes in ‘thermolabile’ components. Prolonged heating in contact with water could lead to hydrolysis of esters, polymerization of aldehydes, or decomposition (e.g. dehydration) of other components.

3.4 Experimental procedures

3.4.1 Extraction of *Moringa oleifera* seed oil

3.4.1.1 Supercritical fluid extraction

In a typical experiment, about 10 grams of *Moringa oleifera* seed samples were weighed and loaded into the 50ml supercritical CO₂ vessel. About 1.5 grams glass wool were packed at both ends of the extractor and sandwiched the seed samples to prevent transfer of the solid samples to the tubing and clogging of the system. Supercritical fluid extraction started as soon as the desired extraction pressure and temperature had been reached, and the oil was collected in an amber glass bottle. The outlet restrictor valve was set at 348 K. The oil yield, on a dry basis, was defined as percentage of the mass of the extracted oil divided by the mass of the *Moringa oleifera* seed loaded in the extraction column. All the extracted oils were stored in a fridge before further analysis.

The SFE assays were divided into three groups, (i) the solubility study of the *Moringa oleifera* seed oil in supercritical carbon dioxide, (ii) the supercritical fluid extraction with pure carbon dioxide and (iii) the supercritical fluid extraction with modified carbon dioxide. In group (i), the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide were obtained experimentally by a dynamic method. In the dynamic method, supercritical carbon dioxide flows continuously through the extraction vessel which contains the solid material of large surface, the operation conditions are chosen so that the outlet stream can be assumed to be at equilibrium. The extracted oil is usually precipitated out and weighed, and the gaseous flow out of the restrictor is measured using a wet or dry flow meter to determine the total amount of fluid used to dissolve the
oil (Graham et al., 1998, del Valle and de la Fuente, 2006). The dynamic method is based on the assumption that the solute-solvent system reaches equilibrium as the solvent flows over the solute. This is usually achieved at a relatively low flow rate of the solvent. In this thesis, the influence of the solvent flow rate on the solubility of Moringa oleifera seed oil in supercritical CO$_2$ was firstly investigated at conditions of pressure 40 MPa, temperature 353 K and four different flow rates ranging from 1.84 g/min to 7.36 g/min. The experimental results revealed that the flow rate at 3.68 g/min was low enough to enable the solute-solvent system reach equilibrium. Then the solubility measurements were carried out at conditions of temperatures at 333, 353, 373 K and pressures at 20, 30, 40, 50 MPa. The flow rate of carbon dioxide was kept at 3.68 g/min. The extracted oil was collected into a previously weighted glass vial at every 5-30 minutes for 4 hours, and weighted immediately after the collection.

The pure supercritical carbon dioxide extractions were conducted in group (ii). Initially, the extractions were conducted to study the effect of particles size on Moringa oleifera seed oil yield. The experiments were performed at various particle sizes (1000-2000µm, 710-1000µm, 500-710µm and <500µm) at 40 MPa, 353 K and a constant CO$_2$ flow rate of 3.68 g/min. Then a central composite design of the response surface methodology was applied to investigate the effects of pressure, temperature, CO$_2$ flow rate and extraction time on the oil yield. The central composite design has been demonstrated to be an effective and powerful statistical method for studying the relationships between measured responses and independent factors (Xu et al., 2008). The design could also reduce the number of experimental trials and investigate the critical processes and correlations between factors that can be used for process optimizations. This design consists of three distinct sets of experimental runs which are a factorial design, a set of centre points runs and a set of star point runs (Wang et al., 2012). The centre replicates are used to estimate the pure error sum of squares. The star points are provided for
estimating the model curvature. Pressure, temperature, extraction time and flow rate were used as the variables in this work. Each of these factors was varied at five levels, namely, pressure at 20, 30, 40, 50 and 60 MPa, temperature at 313, 333, 353, 373 and 393 K, flow rate at 1.84, 3.68, 5.52, 7.36 and 9.2 g/min and extraction time at 30, 60, 90, 120 and 150 minutes, respectively. A total of 30 experimental runs including 16 ($2^4$) factorial experiments, 8 star experiments and 6 replicates at the centre points were carried out. The experimental designs are presented in Table 3-2. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed response due to extraneous factors.

A multiple regression model (a polynomial equation of second order) was used to fit the coded variables (Liu et al., 2009). The proposed model is shown as follows:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum\sum_{i<j=1}^{4} \beta_{ij} X_i X_j , \quad (3-1)$$

where $Y$ is the response (yield of *Moringa oleifera* seed oil). $\beta_0$, $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ are constant coefficients of intercept, linear, quadratic and interaction terms, respectively. $X_i$ and $X_j$ are independent variables (pressure, temperature, CO$_2$ flow rate and extraction time). The model thus includes linear, quadratic and cross-product terms to determine the effects of process variables on the response. The fitted polynomial equation is expressed as surface and contour plots in order to visualize the relationship between the response and experimental levels of each factor and deduce the optimum conditions (Lu et al., 2008).
Table 3-2  Central composite design for the supercritical fluid extraction of *Moringa oleifera* seed oil

<table>
<thead>
<tr>
<th>Run</th>
<th>X₁ (Pressure, MPa)</th>
<th>X₂ (Temperature, K)</th>
<th>X₃ (Flow rate, g/min)</th>
<th>X₄ (Time, min)</th>
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</thead>
<tbody>
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</tr>
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</table>
In group (iii), the extractions were performed with the introduction of the modifiers. In order to determine the influence of modifiers on the extraction process, ethanol at the ratio ranging from 2.5w% to 10w% and water at the ratio of 2.5w% and 5w% were tested. In this study, the ratio of modifier was expressed as the weight of ethanol versus the weight of CO$_2$ under the processing conditions. This group of assay was performed at 353 K, 40 MPa with sample particle size under 1mm and at flow rate 3.68 g/min. The modifier remaining in the extracted oil was removed by heating the oil in a vacuum oven operating at 313 K and 0.1 MPa.

**3.4.1.2 Soxhlet extraction**

Approximately 10 grams of ground *Moringa oleifera* seed samples were placed in a cellulose thimble and transferred to a Soxhlet extractor. The extractor was filled with 150ml solvent and heated under reflux for eight hours through a water bath. The temperature of the water bath was set for each solvent such that the Soxhlet rate was at approximately 5 cycles per hour. The extraction was performed with four different solvents: n-hexane, dichloromethane, ethyl acetate and ethanol, with polarity of 0, 3.1, 4.4 and 5.2, respectively. After extraction was completed, the solvent was removed at 323 K at reduced pressure using a rotary evaporator (model N-1000S-W, EYELA, Tokyo, Japan). Each solvent extraction was performed in triplicate and the total yield of the extracted oil was obtained by the mass of the extracted oil divided by the mass of the original sample used on dry weight base.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Boiling point (K)</th>
<th>Water bath temperature (K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hexane</td>
<td>342.</td>
<td>351</td>
</tr>
<tr>
<td>dichloromethane</td>
<td>314</td>
<td>343</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>350</td>
<td>363</td>
</tr>
<tr>
<td>ethanol</td>
<td>351</td>
<td>363</td>
</tr>
</tbody>
</table>
3.4.1.3 Determination of fatty acid composition of *Moringa oleifera* seed oil

Fatty acid methyl esters were prepared according to the standard IUPAC method 2.301 (IUPAC, 1987). About 0.25 grams of the extracted oil was mixed with 6ml of 0.5N methanol sodium hydroxide solution and brought to reflux in a water bath at 363 K for 30 minutes to facilitate complete saponification of the oil. About 7ml of BF₃-CH₃OH solution was then added and continuously boiled for another 5 minutes to promote formation of methyl ester. Then, the mixture was cooled to room temperature and 5ml of heptane was added to the mixture followed by addition of a saturated NaCl solution under vigorous stirring. Finally, about 1ml of the heptane phase solution which was dried with anhydrous Na₂SO₄ was taken for the GC analysis.

The fatty acid methyl ester was analysed using an Agilent 5890 GC unit equipped with a flame ionization detector (FID) and a J&W (Folsom, CA, USA) DB-Wax capillary column (30m×0.25mm ID, fused-silica column coated with 0.25µm polyethylene glycol film). The injector and detector temperatures were set at 523 K. The oven temperature was programmed to heat from 323 K to 473 K at a rate of 298 K/min, held for 2 minutes and then increased to 503 K at a rate of 275 K/min. The carrier gas was helium with a flow rate of 1 ml/min and split ratio of 50:1. Fatty acid methyl esters were identified by comparing the GC retention times with those of a mixture of standard fatty acid methyl esters (Sigma). The compositions of the fatty acids were calculated from their respective peak areas. In order to verify whether *cis* oleic acid, *trans* oleic acid and *cis*-vaccenic acid were present in the extracted *Moringa oleifera* seed oil, the methyl ester was further analysed using a GC equipped with two columns of BPX70 and BPX90. The GC program settings were as follows: The oven temperature was held at 373 K for 5 minutes, then increased to 423 K at 275.65 K/min and held for 2 minutes, further increased to 493 K at 274.65 K/min and held for 1 minute, and finally increased to 523 K at 285 K/min and held for 12 minutes.
3.4.1.4 $^1$H NMR spectra

In addition to the GC analysis, $^1$H-NMR was employed to compare the molecular structures of the *Moringa oleifera* seed oil extracted using the supercritical fluid extraction and Soxhlet extraction. Approximately 50mg of oil was dissolved into 0.6ml of deuterated chloroform and transferred into a 5mm diameter tube. The one dimensional $^1$H analysis was performed on a Bruker Avance 500 nuclear magnetic resonance spectrometer. The Acquisition parameters were: Spectra width 7507.5 Hz, relaxation delay 30s, pulse angle 90°; acquisition time 4.36s, number of scans 32, receiver gain 48, and number of data point 65536. The deuterated chloroform chemical shift peak at 7.26 ppm was taken as internal reference. The two dimensional COSY experiment was obtained on a Bruker Avance 600 nuclear magnetic resonance spectrometer. The Acquisition parameters were: Spectra width 4807.7Hz, relaxation delay 1.5s, pulse angle 90°; acquisition time 0.11s, number of scans 2, receiver gain 322.5, and number of data point 1024. All the experiments were carried out at 298 K.

3.4.2 Extraction of *Moringa oleifera* leaf oil

3.4.2.1 Supercritical fluid extraction

About 5 grams of *Moringa oleifera* leaves were weighed and loaded into the 50ml supercritical CO$_2$ vessel. Glass wool was packed into both ends of the extractor to stop entrainment of the sample. SFE started as soon as the desired pressure and temperature had been reached. The flow rate of expanded gas CO$_2$ was approximately 3.68 g/min. The extracted oil was collected in dichloromethane in an amber bottle. In order to improve the collection efficiency, the bottle was placed in an ice bath during the dynamic extraction stage. The precipitates in the tube lines were washed out with dichloromethane and then added to the collected oil in the amber bottle. The solution was bubbled using nitrogen gas to evaporate the dichloromethane. The mass of the extracted oil was then weighed to determine the extraction yield, expressed as the
percentage of the mass of the extracted oil to the dry mass of *Moringa oleifera* leaves loaded into the extraction vessel.

Table 3-4  Three levels orthogonal design for extraction of *Moringa oleifera* leaf oil with supercritical CO\(_2\) extractions

<table>
<thead>
<tr>
<th>Run no.</th>
<th>Pressure (MPa)</th>
<th>Temperature (K)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 (30)</td>
<td>1 (313)</td>
<td>1 (60)</td>
</tr>
<tr>
<td>2</td>
<td>1 (30)</td>
<td>2 (333)</td>
<td>2 (90)</td>
</tr>
<tr>
<td>3</td>
<td>1 (30)</td>
<td>3 (353)</td>
<td>3 (120)</td>
</tr>
<tr>
<td>4</td>
<td>2 (40)</td>
<td>1 (313)</td>
<td>3 (120)</td>
</tr>
<tr>
<td>5</td>
<td>2 (40)</td>
<td>2 (333)</td>
<td>1 (60)</td>
</tr>
<tr>
<td>6</td>
<td>2 (40)</td>
<td>3 (353)</td>
<td>2 (90)</td>
</tr>
<tr>
<td>7</td>
<td>3 (50)</td>
<td>1 (313)</td>
<td>2 (90)</td>
</tr>
<tr>
<td>8</td>
<td>3 (50)</td>
<td>2 (333)</td>
<td>3 (120)</td>
</tr>
<tr>
<td>9</td>
<td>3 (50)</td>
<td>3 (353)</td>
<td>1 (60)</td>
</tr>
</tbody>
</table>

In this study, the orthogonal array design was used to investigate the effects of extraction pressure, temperature and time on the *Moringa oleifera* leaf oil yield. Orthogonal array design (OAD) is a type of factorial design in which orthogonal array is used to assign factors to a series of experimental combinations. The results can be analysed to obtain the optimum conditions as well as determine the effects of different operating parameters on the supercritical fluid extraction process (Khajeh et al., 2004). Orthogonal array design has several advantages. Firstly, it may reduce experimental numbers and time to keep the experimental cost to a minimum level. Secondly, the optimum parameters obtained from the laboratory can be utilized by a larger scale of production. An OA9 (3\(^3\)) orthogonal matrix with three factors, each factor containing three levels, was selected to arrange the experiments. Therefore, nine experiments were carried out at three levels of pressures (30, 40 50 MPa), temperatures (313, 333, 353 K),

58
and dynamic times of 60, 90, 120 minutes. The detailed experimental designs are presented in Table 3-4.

### 3.4.2.2 Soxhlet extraction

The supercritical CO$_2$ extraction experiments were bench-marked against the Soxhlet extraction method. In the Soxhlet extraction, approximately 5 grams of ground *Moringa oleifera* leaves were placed in a cellulose thimble and transferred to a Soxhlet extractor. A round bottom extraction flask was filled with 150ml n-hexane which was heated via a water bath set at 351 K. The extraction process was continuously run for 8 hours for each experimental run. After the extraction was completed, the solvent was removed at 323 K at a reduced pressure using a rotary evaporator (model N-1000S-W, EYELA, Tokyo, Japan). After the evaporation of the solvent, the oil yield was determined as percentage of the mass of extracted oil to the mass of dry *Moringa oleifera* leaves loaded in the extractor.

### 3.4.2.3 Determination of chemical composition of *Moringa oleifera* leaf oil

Although Chuang *et al.* (2007) have used GC-MS to analyse the compositions of *Moringa oleifera* leaf oil, their method could not detect the very heavy molecular weight compounds in the oil. Thus, a new method was developed in this work to analyse all compositions in the *Moringa oleifera* leaf oil. The GC settings were as follows: the oven temperature was initially set at 323 K (held for 15 minutes), then increased to 423 K at a rate of 275 K/min, held for 10 minutes at 423 K, and finally increased to 553 K at 275 K/min, held for 20 minutes. The injector temperature was 523 K. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The oil samples were diluted 10 times with dichloromethane and 0.2µl of diluted solution was injected into the GC in a split mode with a split ratio of 1:20. The temperatures of ion source and transfer line were 503 K and 523 K, respectively. The ionisation energy was 70 eV with
a scan time of 1s and mass range of 20-550 amu. The percentages of compounds were calculated from the GC peak area without considering response factors.

The components of the oil were identified by comparing their mass spectra against the NIST (National Institute of Standards and Technologies) MS spectra library. In addition, the retention indices were used to verify the components. The reproducibility and reliability of retention indices allow to verify unknown components with greater confidence. Prior to the sample analysis, the retention times of a series of n-alkane standards (C8-C40) were measured at the same chromatographic conditions described above, The retention index of the unknown compound was calculated using the equation proposed by van Den Dool and Dec. Kratz (1963) as follows:

\[ I_x = 100n + 100\left[ \frac{t_x - t_n}{t_{n+1} - t_n} \right] \]  (3-2)

Where \( I_x \) is the retention index of the interested compound, \( t_n \), \( t_{n+1} \) are the retention times of the two standard n-alkanes containing n and n+1 carbons, \( t_x \) is the retention time of the interest compound. The compounds were then confirmed by comparing their retention indices with the data published in the literature (Chuang et al., 2007, Adams 2007).

3.4.3 Extraction of *Eucalyptus* leaf oil

3.4.3.1 Supercritical fluid extraction

In this study, about 5 grams of *Eucalyptus* leaves were weighed and loaded into the 50ml supercritical CO\(_2\) vessel. About 1 gram of glass wool was packed into both ends of the extractor to stop entrainment of the substrate. Supercritical fluid extraction started as soon as the desired pressure and temperature had been reached. The flow rate of expanded gas CO\(_2\) was 3.68 g/min under every condition. The extracted oil was collected in ethanol in an amber bottle. In order to improve the collection efficiency, the bottle was placed in an ice bath during the dynamic extraction stage. Also, as the
sublimation of CO\textsubscript{2} decreased the temperature of the collection solvent, which can also act as a freezing-trap, thus the loss of volatile compounds was minimized. The precipitates in the tube lines was washed out with ethanol and then mixed with the collected oil in the amber bottle. The mixture was made up to 10ml with ethanol, and 1ml was taken for GC-MS analysis, the rest was put in a rotary evaporator to remove the ethanol solvent, and the weight of the extracted oil was measured. The extraction yield was calculated as the percent ratio of the mass of extracted oil to the dry mass of \textit{Eucalyptus} leaves loaded in the extraction vessel, as follows:

\[
\text{Extraction yield (\%)} = \frac{\text{mass of total extracts}}{\text{mass of dried material}} \times 100\%.
\] (3-3)

The SFE assays were divided into two groups, (i) pure supercritical carbon dioxide extraction and (ii) supercritical carbon dioxide extraction with the addition of modifiers. In group (i), the three-factor central composite design (CCD) combined with response surface methodology (RSM) was applied to determine the optimum conditions for the \textit{Eucalyptus} leaf oil extraction. The independent variables studied were pressure (X\textsubscript{1}:10-50 MPa), temperature (X\textsubscript{2}:313-353 K) and extraction time (X\textsubscript{3}:30-150 min), while the response variable was the extraction yield of \textit{Eucalyptus} leaf oil. Table 3-5 shows the arrangement of the CCD performed in this investigation, 20 randomized experiments including six replicates at the centre points were employed to fit the full quadratic equation model.

In group (ii), in order to determine the effects of modifier on the yield and compositions of the \textit{Eucalyptus} leaf oil extracted with supercritical carbon dioxide, ethanol at the ratio ranging from 5\textsubscript{w}\% to 15\textsubscript{w}\% were tested. In this study, the ratio of modifier was expressed as the weight of ethanol versus the weight of CO\textsubscript{2} under the processing conditions. This group of assays was performed at 333 K with two different pressures of
10 MPa and 30 MPa. The modifier contents remaining in the extracted oil were removed at 333 K at reduced pressure of 0.1 MPa using a rotary evaporator.

Table 3-5  Central composite design for the supercritical fluid extraction of *Eucalyptus* leaf oil

<table>
<thead>
<tr>
<th>Run</th>
<th>X₁</th>
<th>X₂</th>
<th>X₃</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>(Pressure, MPa)</td>
<td>(Temperature, K)</td>
<td>(Time, min)</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>343</td>
<td>60</td>
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<tr>
<td>2</td>
<td>50</td>
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<td>90</td>
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<tr>
<td>3</td>
<td>20</td>
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</tr>
<tr>
<td>20</td>
<td>30</td>
<td>313</td>
<td>90</td>
</tr>
</tbody>
</table>
3.4.3.2 Soxhlet extraction

Approximately 5 grams of ground *Eucalyptus* leaves were placed in a cellulose thimble and transferred to a Soxhlet extractor. The extractor was filled with 150ml solvent and heated under reflux for eight hours through a water bath. Two different solvents have been studied, namely hexane and ethanol. The extraction with hexane was performed at water bath temperature of 351 K for 1 hour, 2 hours and 8 hours, while the extraction with ethanol was performed at water bath temperature of 363 K for 0.5 hour, 1 hour, 2 hours and 8 hours. After the extraction was completed, the solvent was removed at 333 K at reduced pressure using a rotary evaporator (model N-1000S-W, EYELA, Tokyo, Japan). The extraction was performed in triplicate and the total yield of extracted oil was obtained by the mean value of mass of extracted oil divided by mass of raw material used on dry weight base.

3.4.3.3 Hydro-distillation

About 15 grams of *Eucalyptus* leaves immersed into 300ml water and distilled for 5 hours, the volatile distillate was collected over anhydrous sodium sulphate and refrigerated till time of analysis.

3.4.3.4 Determination of chemical composition of *Eucalyptus* leaf oil

The composition of the *Eucalyptus* leaf oil was determined using an Agilent GC-MS. The GC settings were as follows: Oven temperature was held at 313 K for 5 minutes and then increased to 523 K at 275 K/min and held at 523 K for 30 minutes. Each sample (1µL) was injected into the column at a split ratio of 20:1. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The temperature of ion source and transfer line was 503 K and 523 K, respectively. The mass spectrometer was operated in electron-impact ionization (EI) mode by the energy of 70 eV. The scanning range was 40-650 amu and the scanning rate was 0.2 s/scanning. The percentage of compound was
calculated from the GC peak area without considering response factors. The retention indices were determined relative to a homologous series of n-alkanes (C8-C40) under the same operating conditions. The components of the oil were identified by comparing their mass spectra against the NIST (National Institute of Standards and Technologies) MS spectra library. The compounds were also confirmed by comparing their retention indices with the data published in the literature (Della Porta et al., 1999).

3.5 Data analysis

The data in the thesis was presented as the average of the repeated measurements under the same conditions and the standard deviation of the measurements.

In order to determine whether the effects of different operating variables (such as pressure, temperature, flow rate and extraction time) and the interactions between variables on the extraction yield were statistically significant or not, the data collected from the SFE tests were analysed using the “Design expert” software (Design-Expert 7.1.3 Trial, State-Ease, Inc., Minneapolis MN, USA). The statistical analysis of the data was performed in the form of analysis of variance (ANOVA), values of p<0.05 were regarded as significant (Stigler, 2008).
Chapter 4 Supercritical CO\textsubscript{2} Extraction of \textit{Moringa oleifera} and \textit{Eucalyptus} Oil and Comparison with Soxhlet Extraction and Hydro-
distillation Methods

Natural products can be extracted from plants using several techniques. Soxhlet extraction is one of the most common techniques used to extract seed oil or semi-volatile solute from solid matrices. The solvent used in the Soxhlet extraction is continuously brought into contact with the sample, which improves the efficiency of the extraction process. Different solvents, such as hexane, dichloromethane, ether and ethanol can be used, because they possess distinct compounds affinities (Taveira \textit{et al.}, 2009). Hydro-distillation is the most commonly employed technique to obtain essential oil from different plant materials. Supercritical fluid extraction is a more recent technique that has received increasingly attention. This is because supercritical fluids have several major advantages (Wang and Weller, 2006). First of all, the dissolving power of a supercritical fluid depends on its density which is highly adjustable by changing the pressure and temperature. Secondly, supercritical fluid has a higher diffusion coefficient and lower viscosity and surface tension than a liquid solvent, leading to more favourable mass transfer. This new technique has been applied to extract oil and essential oil from various plants.

This chapter discussed the differences between the supercritical fluid extraction and Soxhlet extraction to extract oil and essential oil from \textit{Moringa oleifera} seeds and leaves. The effects of different solvents on Soxhlet extraction of \textit{Moringa oleifera} seed oil were studied. The results were compared in terms of the oil yield and their chemical compositions. Additionally, three extraction methods, namely, supercritical fluid extraction, hydro-distillation and Soxhlet extraction, were employed to extract oil from
Eucalyptus leaves. By doing this part of research, the advantages and disadvantages of these three methods as well as their mechanisms could be better understood.

4.1 Yield and composition differences of the Moringa oleifera seed oil obtained by supercritical fluid extraction and Soxhlet extraction with different solvents

4.1.1 Yield and composition differences of the Moringa oleifera seed oil obtained by supercritical fluid extraction and Soxhlet extraction

In order to compare the efficiency of different methods to extract oil from Moringa oleifera seeds, the supercritical carbon dioxide extraction (under conditions of pressure 50 MPa, temperature 373 K, flow rate 7.36 g/min with extraction time of 2 hours) and the Soxhlet extraction with hexane were realized, the obtained results were listed in Table 4-1, the results were compared by quantity and quality of the extracted oil.

The oil yield in the supercritical fluid extraction was slightly lower than that of Soxhlet extraction. As hexane used in the Soxhlet method and CO₂ used in the SFE are both non-polar solvents which should exhibit similar behaviours in the extraction processes. The observed difference in the oil yield between these two methods could be due to the different extraction times. As can be seen from Table 4-1, the extraction time for the Soxhlet method is four times longer than that of supercritical fluid extraction.

The fatty acid composition profiles of Moringa oleifera seed oil obtained by SFE and by the Soxhlet extraction were presented in Table 4-1 as well. As noticed from Table 4-1, there is no significant difference in the fatty acid profiles of Moringa oleifera seed oil obtained by the two methods. Similar results were found by Xu et al. (2008) who compared the oil extraction from sea buckthorn using these two methods as well.
Table 4-1 Yield and fatty acid compositions of *Moringa oleifera* seed oil obtained by supercritical fluid extraction and Soxhlet extraction

<table>
<thead>
<tr>
<th></th>
<th>Supercritical carbon dioxide extraction (under 50 MPa, 373 K, 7.36 g/min)</th>
<th>Soxhlet extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction yield</td>
<td>37.12 %</td>
<td>40.12%</td>
</tr>
<tr>
<td>Extraction time</td>
<td>2 hours</td>
<td>8 hours</td>
</tr>
<tr>
<td>Fatty acid composition (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>myristic acid (C14:0)</td>
<td>0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>palmitic acid (C16:0)</td>
<td>5.92</td>
<td>5.82</td>
</tr>
<tr>
<td>stearic acid (C18:0)</td>
<td>5.67</td>
<td>5.72</td>
</tr>
<tr>
<td>arachidic acid (C20:0)</td>
<td>3.66</td>
<td>3.85</td>
</tr>
<tr>
<td>behenic acid (C22:0)</td>
<td>6.36</td>
<td>6.85</td>
</tr>
<tr>
<td>lignoceric acid (C24:0)</td>
<td>1.13</td>
<td>1.27</td>
</tr>
<tr>
<td>total saturated fatty acids</td>
<td>22.87</td>
<td>23.64</td>
</tr>
<tr>
<td>palmitoleic acid (C16:1)</td>
<td>1.53</td>
<td>1.51</td>
</tr>
<tr>
<td>oleic acid (C18:1)</td>
<td>66.69</td>
<td>65.87</td>
</tr>
<tr>
<td>cis-vaccenic acid (C18:1)</td>
<td>6.03</td>
<td>6.06</td>
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<td>linoleic acid (C18:2)</td>
<td>0.61</td>
<td>0.60</td>
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<tr>
<td>linolenic acid (C18:3)</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>eicosenoic acid (C20:1)</td>
<td>2.10</td>
<td>2.14</td>
</tr>
<tr>
<td>total unsaturated fatty acids</td>
<td>77.14</td>
<td>76.36</td>
</tr>
</tbody>
</table>

In order to further compare the chemical structures of the oils extracted using the two methods, two oil samples were further analysed using $^1$H-NMR and their spectrums are presented in Figure 4-1.
Figure 4-1 $^1$H-NMR spectra of oils extracted by supercritical fluid extraction and Soxhlet extraction

It can be seen that total 8 spectral signals were detected. The position of the signal in the spectrum is called the chemical shift. The chemical shift is obtained in parts per million (ppm) by dividing the frequency difference between the sample signals and the deuterated chloroform signal by the deuterated chloroform frequency. These 8 spectral signals were due to the protons of the triglycerides which were the main components of the *Moringa oleifera* seed oil; the generic formula of a triglyceride is shown as below:

$$\text{CH}_2-\text{OOC-}R$$
$$\text{CH}-\text{OOC-}R'$$
$$\text{CH}_2-\text{OOC-}R''$$
Where R, R’ and R” represent the hydrocarbon chain of the fatty acid element of the triglyceride. Every signal area is proportional to the number of the hydrogen atoms of each kind present in the sample. The assignment of the various signals was accomplished by using a 2D COSY technique which is shown in Figure 4-2.

![Figure 4-2 2D COSY spectra of the oil extracted by supercritical fluid extraction](image)

From Figure 4-2, it can be deduced that signal 1 is produced by methyl group protons (\(-\text{CH}_3\)) of saturated and unsaturated acyl groups and appears between 0.86-0.89 ppm. Signal 2 is the carbonyl group (\(-\text{(CH}_2\)n\(-)\)) and the chemical shift of this methylenic proton signal is between 1.25-1.30 ppm. The protons on signal 3 are coupled to the protons on signal 5 and they are due to the methylenic protons in relation to the carboxyl group. Signal 4 (between 1.99-2.02 ppm) is called allylic proton which is due
to methylenic proton in relation to a single double bond and it is clearly coupled with signal 8 which is the olefinic proton shown in Figure 4-2. There are two peaks in signal 6 and the two peaks are coupled with each other. Moreover, signal 6 is still coupled with signal 7. Thus signal 6 and signal 7 are the protons of the glyceryl group. The chemical shifts and the assignment of these signals are listed in Table 4-2.

Table 4-2 Assignment of signals and percentage of total hydrogen of $^1$H NMR spectra from the Moringa oleifera seed oil extracted by supercritical fluid extraction and Soxhlet extraction

<table>
<thead>
<tr>
<th>Chemical shift (ppm)</th>
<th>Functional group</th>
<th>Percentage of total hydrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Supercritical carbon dioxide extraction (under pressure 50 MPa, 373 K, 7.36 g/min, 2h)</td>
</tr>
<tr>
<td>0.86-0.89</td>
<td>-CH$_3$ (methyl proton)</td>
<td>8.58</td>
</tr>
<tr>
<td>1.25-1.30</td>
<td>(-CH$_2$)$_n$ (acyl groups)</td>
<td>62.67</td>
</tr>
<tr>
<td>1.59-1.61</td>
<td>-OCO-CH2-CH2- (acyl groups)</td>
<td>5.68</td>
</tr>
<tr>
<td>1.99-2.02</td>
<td>-CH$_2$-CH=CH- (allylic protons)</td>
<td>8.74</td>
</tr>
<tr>
<td>2.29-2.32</td>
<td>-OCO-CH$_2$ (acyl groups)</td>
<td>5.58</td>
</tr>
<tr>
<td>4.12-4.30</td>
<td>-CH$_2$OCOR (glyceryl group)</td>
<td>3.54</td>
</tr>
<tr>
<td>5.25-5.27</td>
<td>&gt;CHOCOR (glyceryl group)</td>
<td>0.84</td>
</tr>
<tr>
<td>5.32-5.36</td>
<td>-CH=CH- (olefinic proton)</td>
<td>4.37</td>
</tr>
</tbody>
</table>

The general assignment of these signals and their area values are listed in Table 4-2. It is noticed that the percentage of olefinic protons in the oil extracted by the supercritical fluid extraction was very close to those obtained using the Soxhlet extraction. Guillén et al. (2003) reported that the unsaturation degree of the oil was directly related to the proportion of the olefinic hydrogen atoms in the sample. Therefore it can be deducted that the percentage of unsaturation fatty acids in the two samples should be very similar. This is in close agreement with the results of the gas chromatography analysis. Thus, the NMR analysis further confirmed that the compositions of the oil extracted by the two
methods were very similar. This is because hexane and carbon dioxide are both non-polar solvents, thus, they have similar performance to extract chemical compounds from seeds.

4.1.2 The effects of different solvents on Soxhlet extraction of *Moringa oleifera* seed oil

To better compare SFE with Soxhlet extraction, the effect of solvent type on the oil yield obtained by Soxhlet extraction was also evaluated. Four different solvents, namely, hexane, dichloromethane, ethyl acetate and ethanol were tested for an exhaustive extraction of oil from *Moringa oleifera* seed samples. Each solvent was tested in triplicate to ensure reproducibility. The total oil yields obtained are compared in Table 4-3. The total yields of the *Moringa oleifera* seed oil determined using the Soxhlet hexane extraction was 40.1%. This result was comparable to the study from Anwar *et al.* (2003) who reported a 40.4% oil yield with the *Moringa oleifera* seeds obtained from the vicinity of the University of Sindh, Jamshoro campus in Pakistan. However, this value was a little higher as compared to the 35.7% oil yield reported by Tsaknis *et al.* (1999) who performed a ten-hour experimental run on seeds from Kenya. Such variations in the oil yield could be attributed to the possible variations in seasonal temperatures, soil conditions, nutrients and water availability as well as the maturity of the trees (Anwar *et al.*, 2006).

It can be seen that ethanol provided significantly higher oil yield, at a maximum of 45.8%, compared to other three solvents. No significant differences were observed in the total oil yields extracted by using dichloromethane and ethyl acetate. However, there was a statistical difference at the 95% level between the oil yields using hexane and ethanol, and using dichloromethane and ethanol. The polarities and boiling points of the four solvents are listed in Table 4-3. As shown in this table the solvent polarity plays an important role on the extraction process, the oil yield increases with increasing solvent...
polarity. The results indicated that *Moringa oleifera* seeds may have many intermediate to high polarity compounds which could result in high oil yields in high polarity solvents. Lalas and Tsaknis (2002) also compared the extraction of *Moringa oleifera* seed oil with hexane and a mixture of chloroform: methanol (1:1), they postulated that the mixture of chloroform and methanol had higher yield than hexane due to the increased ability of the polar solvent to overcome forces that bind lipids within the sample matrix.

Table 4-3  Solvent effect on the yield of *Moringa oleifera* seed oil extracted by the Soxhlet method

<table>
<thead>
<tr>
<th>Extraction solvent</th>
<th>Solvent polarity index</th>
<th>Boiling temperature (K)</th>
<th>Extraction yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hexane</td>
<td>0</td>
<td>342</td>
<td>40.12± 3.01 %</td>
</tr>
<tr>
<td>dichloromethane</td>
<td>3.1</td>
<td>313</td>
<td>41.30± 1.85 %</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>4.4</td>
<td>350</td>
<td>41.88± 4.16 %</td>
</tr>
<tr>
<td>ethanol</td>
<td>5.2</td>
<td>351</td>
<td>45.75± 2.30%</td>
</tr>
</tbody>
</table>

It was reported that the conditions of temperature, solvent recycle and solvent/solute interactions in the Soxhlet extraction contribute to the solubility of components from the raw materials into the solvents (Mezzomo *et al.*, 2010). In this study, the solvent cycle and the solvent to solid ratio were the same for all of the four solvents, but the operating temperatures were different. The effects of solvent temperature on the extraction process were studied by Xu and Godber (2000) who extracted γ-oryzanol from rice bran using hexane, ethyl acetate, isopropanol and different solvent combinations. They found that the concentration of γ-oryzanol in extracted oil increased with increasing extraction temperature, they thought that the solvent might alter the physical properties of the matrix and make it more penetrable by the extraction fluid, but the effect of temperature on the concentration of γ-oryzanol was not significant when using solvents with lower
polarity and viscosity. For the current study, as noticed from Table 4-3 that the boiling temperatures of ethyl acetate and ethanol are similar but ethanol extracts more oil than ethyl acetate. Therefore, it can be deduced that the effects of solvent on the extraction of *Moringa oleifera* seed oil can be attributed more significant to the solvent polarity than to the temperature effect.

Table 4-4 shows the fatty acid compositions of the *Moringa oleifera* seed oil extracted by the four different solvents. The fatty acid profile is a main determinant of the oil quality. The results indicated that *Moringa oleifera* seed oil contained mainly unsaturated fatty acids (UFA), with relative concentrations at about 77%. Oleic acid (C18:1, 9cis) which accounted for about 66% of the total fatty acids was the major UFA from all of the extracted oil samples, followed by cis-vaccenic acid. Only small amounts of palmitoleic acid (C16:1), linoleic acid (C18:2), linolenic acid (C18:3) and eicosenoic acid (C20:1) were detected. The content of the total saturated fatty acids, that were, myristic (C14:0), palmitic (C16:0), stearic (C18:0), arachidic (C20:0), behenic acids (C22:0) and lignoceric acid (C24:0) was about 23% and the most prevalent saturated fatty acid was behenic (C22:0) acid. The concentration of the oleic acid investigated in the present study was comparable to that reported by Abdulkarim *et al.* (2005), but lower than that of *Moringa oleifera* seed oil from the cultivated provenance of Sindh, Pakistan (Anwar and Bhanger, 2003) and that reported by Tsaknis *et al.* (1999) for the oil indigenous to Kenya. As reported by Sommerfeld (1983), most plant oils did not contain any trans fatty acids, and trans oleic acid were even not found in plants. *Moringa oleifera* seed oil was a natural extracted plant seed oil without any further processing, no trans oleic acid was detected and only cis oleic acid was identified in the oil extracted from the present work. Cis oleic acid was a particular important monounsaturated omega-9 fatty acid because of its palatability, stability and health-promoting properties (Abdulkarim *et al.*, 2007). Cis oleic acid has well-known benefits
in the treatment and prevention of heart disease. Recent studies also found that oleic acid content might regulate genes that trigger cancer and lower cholesterol levels which thereby could reduce the risk of cardiovascular disease (Menendez et al., 2006).

Table 4-4 Fatty acid contents of Moringa oleifera seed oil obtained by Soxhlet extraction with different solvents

<table>
<thead>
<tr>
<th>Saturated fatty acid</th>
<th>hexane</th>
<th>dichloromethane</th>
<th>ethyl acetate</th>
<th>ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>myristic acid (C14:0)</td>
<td>0.12</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>palmitic acid (C16:0)</td>
<td>5.82</td>
<td>5.90</td>
<td>5.93</td>
<td>5.94</td>
</tr>
<tr>
<td>stearic acid (C18:0)</td>
<td>5.72</td>
<td>5.66</td>
<td>5.65</td>
<td>5.64</td>
</tr>
<tr>
<td>arachidic acid (C20:0)</td>
<td>3.85</td>
<td>3.77</td>
<td>3.73</td>
<td>3.70</td>
</tr>
<tr>
<td>behenic acid (C22:0)</td>
<td>6.85</td>
<td>6.68</td>
<td>6.58</td>
<td>6.50</td>
</tr>
<tr>
<td>lignoceric acid (C24:0)</td>
<td>1.27</td>
<td>1.24</td>
<td>1.21</td>
<td>1.20</td>
</tr>
<tr>
<td>Total saturated fatty acid</td>
<td>23.64</td>
<td>23.38</td>
<td>23.23</td>
<td>23.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Unsaturated fatty acids</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>palmitoleic acid (C16:1)</td>
<td>1.51</td>
<td>1.54</td>
<td>1.56</td>
<td>1.55</td>
</tr>
<tr>
<td>oleic acid (C18:1)</td>
<td>65.87</td>
<td>66.01</td>
<td>66.13</td>
<td>66.29</td>
</tr>
<tr>
<td>cis-vaccenic acid (C18:1)</td>
<td>6.06</td>
<td>6.20</td>
<td>6.24</td>
<td>6.15</td>
</tr>
<tr>
<td>linoleic acid (C18:2)</td>
<td>0.60</td>
<td>0.61</td>
<td>0.62</td>
<td>0.62</td>
</tr>
<tr>
<td>linolenic acid (C18:3)</td>
<td>0.18</td>
<td>0.18</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>eicosenoic acid (C20:1)</td>
<td>2.14</td>
<td>2.07</td>
<td>2.02</td>
<td>2.09</td>
</tr>
<tr>
<td>Total unsaturated fatty acid</td>
<td>76.36</td>
<td>76.62</td>
<td>76.77</td>
<td>76.89</td>
</tr>
</tbody>
</table>

The effect of solvent polarity on Soxhlet extraction of saturated and unsaturated fatty acids is observed from Table 4-4 as well. There were no significant differences in the saturated fatty acids and unsaturated fatty acid contents in the oils extracted by these four different solvents. Mezzomo et al. (2010) reported that the use of solvents with
polarity indexes (PI) lower than 4.4 enhanced the extraction of oleic acid and linoleic acid among the unsaturated fatty acid. But in this present study, no relation was found between PI and fatty acid compositions. The present results were similar to Wu et al.’s (2011) study who extracted peach kernel oil using Soxhlet extraction with four different solvents (petroleum ether, ethyl ether, chloroform and hexane). They found that the fatty acid compositions differences between these four solvents were not significant.

4.2 Yield and composition differences of the Moringa oleifera leaf oil obtained using supercritical fluid extraction and Soxhlet extraction

The aim of this part research was to extract and characterize the oil from Moringa oleifera leaves using supercritical fluid extraction under conditions of 30 MPa, 353 K and extraction time 120 minutes and compare the result against that obtained using Soxhlet extraction. Table 4-5 lists the extraction yield and chemical compositions of the oil obtained by these two methods.

From this table, it can be seen that 42 compounds are identified in the Moringa oleifera leaf oil obtained by the Soxhlet extraction. In general, benzene,1-ethyl-3-methyl- (5.95%), benzene,1,2,4-trimethyl- (16.96%), heptacosane (7.45%) and nonacosane (18.65%) were the major components in the Moringa oleifera leaf oil. These compounds were different from those found by Chuang et al. (2007) whose leaf samples were from Taichung, Taiwan. In their study, it was revealed that pentacosane (17.41%), hexacosane (11.20%), (E)-phytol (7.66%) and 1-[2,3,6-trimethyl-phenyl]-2-butane (3.44%) were the major components. The presence of γ-tocopherol and dl-α-tocopherol were found in all extracted oils, accounting for a high percentage of the total extracts. These two chemicals have been recognized with medicinal properties and used against approximately 80 diseases, such as cancer, cystic fibrosis cardiovascular diseases, cell membrane, DNA damage by free radicals, oxidation of low density lipoproteins, disorders of the skin, eye, lungs, and other lipid body constituents (Jacques
et al., 2007). The finding in this study was consistent with the study of Sánchez-Machado et al. (2006) who also detected these two components from *Moringa oleifera* cultivated in the Northwest Mexico.

From Table 4-5, it is clear that there were significant differences in the oil compositions isolated by SFE and Soxhlet extractions. A lot of constituents that were identified in the oil obtained by Soxhlet extraction were not detected in the oil from supercritical CO\(_2\) extraction. Cao et al. (2007) found that there were less compounds separated by GC analyses from supercritical CO\(_2\) extracted oil than Soxhlet extracted oil when they compared the volatile components of Marchantia convolute obtained by supercritical carbon dioxide extraction and Soxhlet extraction with petrol ether. They explained that the composition differences of the two extracted oils were caused by the mechanism differences of the methods in dealing with the extracts. Other authors attempted to compare hydro-distillation with SFE, they obtained similar findings that there was a smaller number of constituents in the supercritical carbon dioxide extracted sample (Aghel et al., 2004). Probably all these missing compounds were still present in the SFE extracts but at lower contents than those in the hydro-distillation sample. This may be a consequence of the smaller quantities of plant material used in the supercritical fluid extraction and corresponding amounts of extracts. However, Safaralie et al. (2008) believed that this might be due to the differences between the two methods to trap the volatile oil: in the supercritical CO\(_2\) extraction, a part of the extracted components escaped along with CO\(_2\) from the vessel containing dichloromethane, but in the hydro-distillation, trapping the oil was simultaneously performed along with condensing the steam in the pipe. From the present experimental observations, it was believed that the composition differences was due to the different mechanisms of the two methods which would be in Section 4.3.1.
Table 4-5  Chemical compositions of the *Moringa oleifera* leaf oil obtained by Soxhlet extraction and supercritical fluid extraction

<table>
<thead>
<tr>
<th>Extraction yield</th>
<th>Soxhlet</th>
<th>SFE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.27%</td>
<td>4.87%</td>
</tr>
</tbody>
</table>

Chemical composition of the essential oil from *Moringa oleifera* leaves

<table>
<thead>
<tr>
<th>N/O</th>
<th>retention time</th>
<th>retention index</th>
<th>components</th>
<th>formula</th>
<th>composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.149</td>
<td>772.5</td>
<td>pyridine</td>
<td>C5H5N</td>
<td>0.78 ND</td>
</tr>
<tr>
<td>2</td>
<td>3.46</td>
<td>780.5</td>
<td>hepane,2-methyl-</td>
<td>C8H18</td>
<td>0.68 ND</td>
</tr>
<tr>
<td>3</td>
<td>3.532</td>
<td>782.3</td>
<td>tobuene</td>
<td>C7H8</td>
<td>1.27 ND</td>
</tr>
<tr>
<td>4</td>
<td>3.608</td>
<td>784.2</td>
<td>heptane,3-methyl-</td>
<td>C8H18</td>
<td>0.50 ND</td>
</tr>
<tr>
<td>5</td>
<td>3.734</td>
<td>787.3</td>
<td>cyclohexane,1,3-dimethyl-,cis-</td>
<td>C8H16</td>
<td>0.78 ND</td>
</tr>
<tr>
<td>6</td>
<td>4.181</td>
<td>798.7</td>
<td>octane</td>
<td>C8H18</td>
<td>1.75 ND</td>
</tr>
<tr>
<td>7</td>
<td>4.335</td>
<td>802.6</td>
<td>cyclohexane,1,4-dimethyl-,cis-</td>
<td>C8H16</td>
<td>0.14 ND</td>
</tr>
<tr>
<td>8</td>
<td>4.973</td>
<td>818.7</td>
<td>octane,2-methyl-</td>
<td>C9H20</td>
<td>0.17 ND</td>
</tr>
<tr>
<td>9</td>
<td>5.138</td>
<td>822.8</td>
<td>cyclohexane,ethyl-</td>
<td>C8H16</td>
<td>0.43 ND</td>
</tr>
<tr>
<td>10</td>
<td>6.162</td>
<td>848.6</td>
<td>ethylbenzene</td>
<td>C8H10</td>
<td>0.83 ND</td>
</tr>
<tr>
<td>11</td>
<td>6.488</td>
<td>856.8</td>
<td>benzene,1,3-dimethyl-</td>
<td>C8H10</td>
<td>4.63 ND</td>
</tr>
<tr>
<td>12</td>
<td>7.592</td>
<td>884.9</td>
<td>o-xylene</td>
<td>C8H10</td>
<td>2.82 ND</td>
</tr>
<tr>
<td>13</td>
<td>8.042</td>
<td>896.1</td>
<td>nonane</td>
<td>C9H20</td>
<td>0.14 ND</td>
</tr>
<tr>
<td>14</td>
<td>9.504</td>
<td>914</td>
<td>benzene,(1-methylethyl)-</td>
<td>C9H12</td>
<td>0.16 ND</td>
</tr>
<tr>
<td>15</td>
<td>11.722</td>
<td>937.9</td>
<td>benzene,propyl-</td>
<td>C9H12</td>
<td>1.04 ND</td>
</tr>
<tr>
<td>16</td>
<td>12.462</td>
<td>945.7</td>
<td>benzene,1-ethyl-3-methyl-</td>
<td>C9H12</td>
<td>5.95 ND</td>
</tr>
<tr>
<td>17</td>
<td>12.568</td>
<td>947</td>
<td>benzene,1-ethyl-4-methyl-</td>
<td>C9H12</td>
<td>2.39 ND</td>
</tr>
<tr>
<td>18</td>
<td>13.094</td>
<td>952.5</td>
<td>benzene,1,3,5-trimethyl-</td>
<td>C9H12</td>
<td>4.31 ND</td>
</tr>
<tr>
<td>19</td>
<td>14.225</td>
<td>964.5</td>
<td>benzene,1-ethyl-2-methyl-</td>
<td>C9H12</td>
<td>2.55 ND</td>
</tr>
<tr>
<td>20</td>
<td>15.673</td>
<td>980.1</td>
<td>benzene,1,2,4-trimethyl-</td>
<td>C9H12</td>
<td>16.96 ND</td>
</tr>
<tr>
<td>21</td>
<td>18.678</td>
<td>1011.8</td>
<td>benzene,1,2,3-trimethyl-</td>
<td>C9H12</td>
<td>4.14 ND</td>
</tr>
<tr>
<td>22</td>
<td>19.9</td>
<td>1024.2</td>
<td>indane</td>
<td>C9H10</td>
<td>0.39 ND</td>
</tr>
<tr>
<td>23</td>
<td>22.636</td>
<td>1052</td>
<td>benzene,1-ethyl-2,4-dimethyl-</td>
<td>C10H14</td>
<td>0.64 ND</td>
</tr>
<tr>
<td>24</td>
<td>25.045</td>
<td>1076.6</td>
<td>benzene,1-ethyl-2,3-dimethyl-</td>
<td>C10H14</td>
<td>0.99 ND</td>
</tr>
<tr>
<td>25</td>
<td>27.702</td>
<td>1104.2</td>
<td>benzene,1,2,3,5-tetramethyl-</td>
<td>C10H14</td>
<td>1.59 ND</td>
</tr>
<tr>
<td>No.</td>
<td>M.W.</td>
<td>R.T.</td>
<td>Chemical Name</td>
<td>Formula</td>
<td>Bob.</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
<td>-------</td>
<td>---------------</td>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>26</td>
<td>28.066</td>
<td>1108.9</td>
<td>benzene, 1,2,4,5-</td>
<td>C10H14</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tetramethyl-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>benzene, 1,2,3,4-</td>
<td>C10H14</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>tetramethyl-</td>
<td>C10H8</td>
<td>1.21</td>
</tr>
<tr>
<td>28</td>
<td>32.903</td>
<td>1163</td>
<td>naphthalene</td>
<td>C5H12NO3PS2</td>
<td>0.52</td>
</tr>
<tr>
<td>29</td>
<td>67.973</td>
<td>1708</td>
<td>dimethoate</td>
<td>C19H40</td>
<td>0.22</td>
</tr>
<tr>
<td>30</td>
<td>82.66</td>
<td>1907</td>
<td>nonadecane</td>
<td>C16H32O2</td>
<td>0.15</td>
</tr>
<tr>
<td>31</td>
<td>86.93</td>
<td>1966</td>
<td>n-hexadecanoic</td>
<td>C20H42</td>
<td>0.31</td>
</tr>
<tr>
<td>32</td>
<td>89.137</td>
<td>1997.4</td>
<td>eicosane</td>
<td>C25H52</td>
<td>2.14</td>
</tr>
<tr>
<td>33</td>
<td>112.38</td>
<td>2510</td>
<td>pentacosane</td>
<td>C16H22O4</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,2-benzenedicarboxylic</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>acid, mono(2-ethylhexyl)ester</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>114.005</td>
<td>2558</td>
<td>heptacosane</td>
<td>C27H56</td>
<td>7.45</td>
</tr>
<tr>
<td>35</td>
<td>119.844</td>
<td>2725.9</td>
<td>nonacosane</td>
<td>C29H60</td>
<td>18.65</td>
</tr>
<tr>
<td>36</td>
<td>126.724</td>
<td>2924.3</td>
<td>γ-tocopherol</td>
<td>C28h48O2</td>
<td>0.75</td>
</tr>
<tr>
<td>37</td>
<td>131.169</td>
<td>3052.3</td>
<td>sulfurous acid,</td>
<td>C21H44O3S</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>hexyl pentadecyl ester</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>133.089</td>
<td>3108</td>
<td>dl-α-tocopherol</td>
<td>C29H50O2</td>
<td>1.05</td>
</tr>
<tr>
<td>39</td>
<td>133.73</td>
<td>3126.7</td>
<td>1,3-triacontanediol</td>
<td>C30H62O2</td>
<td>3.06</td>
</tr>
<tr>
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<td>3232.8</td>
<td>γ-sitosterol</td>
<td>C29H50O</td>
<td>0.86</td>
</tr>
<tr>
<td>41</td>
<td>138.639</td>
<td>3267.8</td>
<td>β-amyrin</td>
<td>C30H50O</td>
<td>4.60</td>
</tr>
</tbody>
</table>

Note: ND stands for “not detected”.

Furthermore, Soxhlet extraction using hexane produced a higher yield (9.27%) than supercritical fluid extraction (4.87%). This was in agreement with the results obtained by some other authors (Danh et al., 2009) who compared the vetiver oil extracted by hydro-distillation, Soxhlet extraction with hexane and supercritical carbon dioxide extraction, they found that the oil yield by Soxhlet extraction was the highest among the three methods and deducted that vetiver hexane-extract can be defined as 100% recovery of essential oil from the roots.
4.3 Yield and composition differences of the Eucalyptus leaf oil obtained by supercritical fluid extraction, Soxhlet extraction with different solvents and hydro-distillation

4.3.1 Comparison of SFE, Soxhlet extraction and hydro-distillation

The yields of *Eucalyptus* leaf oil obtained by SFE, hydro-distillation and Soxhlet extraction with ethanol and hexane are summarised in Figure 4-3. It can be seen that Soxhlet extraction with ethanol brought out the highest oil yield of 36.33%, while hydro-distillation had the lowest yield of 3.77% and SFE showed an intermediate yield. The SFE yield was up to 4.78%, comparable in magnitude to the hexane Soxhlet extraction result. Similar results were reported by other authors (Glisic *et al.*, 2010, Guan *et al.*, 2007). Glisic *et al.* (2010) investigated the supercritical carbon dioxide extraction of Dalmatian sage essential oil and compared that with Soxhlet extraction using ethanol-water (70:30) mixture and hydro-distillation. Their results revealed that Soxhlet extraction achieved the highest yield of 26.5wt%, while supercritical carbon dioxide extraction at 30 MPa resulted in a yield of 4.82wt% and hydro-distillation only had a yield of 0.5% (v/w) of essential oil. They explained that SFE allowed a wide range of chemicals to be isolated, while hydro-distillation was limited to volatile compounds and Soxhlet extraction was limited to high molecular compounds. Vági *et al.* (2005) also compared the essential oil extraction from marjoram using supercritical carbon dioxide extraction (323 K and 45 MPa), ethanol Soxhlet extraction and hydro-distillation, the oil yields were 3.8 g/100g, 9.1 g/100g, and 0.8 ml/100g, respectively. However, the SFE extract comprised 21% essential oil, while the ethanol Soxhlet extract only contained 9% volatile oil substance.
Figure 4-3 Extraction yields of *Eucalyptus* leaf oil obtained by different methods: hydro-distillation (HD), Soxhlet extraction with hexane at different times (Hexane), Soxhlet extraction with ethanol at different times (Ethanol) and supercritical fluid extraction (SFE) under different extraction conditions.

In order to explain the yield differences of the three extraction methods, the compositions of the extracted oils were analysed using GC-MS. The chemical profiles of the oils extracted by ethanol and hexane Soxhlet extraction, SFE (under conditions of 40 MPa, 343 K and time 60 minutes) and hydro-distillation are shown in Figure 4-4. The major compounds identified are listed in details in the Appendix. The fractions of all compounds were calculated using the area normalization method (Zhao and Zhang, 2013). Clearly, the chemical compositions of the oils extracted by the three methods differed both qualitatively and quantitatively. In order to appreciate the chemical compositions of the *Eucalyptus* leaf oil, the identified compounds are grouped in Figure 4-5 into five families: monoterpenes, oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes and other compounds.
Figure 4-4 Total ion compound (TIC) chromatograms of the *Eucalyptus* leaf oil obtained by Soxhlet extraction with ethanol and hexane, supercritical fluid extraction and hydro-distillation
Figure 4-5 Composition profiles of the *Eucalyptus* leaf oil extracted by SFE (under conditions of 40 MPa, 343 K and time of 60 minutes), hydro-distillation, and Soxhlet extraction.

Figure 4-5 shows that the common compounds present in the *Eucalyptus* leaf oil obtained by the three methods are oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes. The main oxygenated monoterpenes, 1,8-cineole, the key and most beneficial ingredient of *Eucalyptus* leaf oil, was consistently the most abundant compound in every sample. The proportions of this constituent in the extracted oils were 46.19% for SFE, 70.03% for hydro-distillation, 29.85% for ethanol Soxhlet, and 56.4% for hexane Soxhlet. Other major oxygenated monoterpenes present in all the oil samples, but at much lower concentrations, were L-pinocarveol, pinocarvone, 4-terpineol, p-cymen-8-ol, α-terpinol, verbenone, cis-carveol, cuminal, pipertitone, L-perillaldehyde and thymol. Oxygenated sesquiterpenes was the second most significant group of compounds in all the oil samples, including epiglobulol, palustrol, (-)-spathulenol, caryophyllene oxide, globulol, germacrene D-4-ol, ledol, cubenol, tau.-muutolol, δ-cadinol, isoaromadendrene epoxide and longiverbenone. The
third major group was the sesquiterpenes substances such as copaene, β-cubebene, (+)-aromadendrene and cadalene. Although some monoterpene hydrocarbons like α-cymol were also detected, but only in trace amounts found in the SFE and Soxhlet extracted oil and in small amounts in the hydro-distillation oil.

The GC-MS results showed that the number of compounds found in the hydro-distillation oil was significantly less than those in the SFE and Soxhlet extraction oil. This was because the Eucalyptus leaf oil extracted by hydro-distillation contained mostly volatile compounds, with the majority appearing in the first 66 minutes on the GC separation chromatograph as shown in Figure 4-4. In contrast, The SFE and Soxhlet extraction oils contained not only the volatile components, but also heavier compounds appearing after 66 minutes on the GC separation chromatograph (Figure 4-4). Similar results have been reported in the literature (da Cruz Francisco et al., 2001).

The reason for the observed composition differences could be due to their different isolation mechanisms as shown in Figure 4-6. In the hydro-distillation process, water does not dissolve organic components. The substrate sample is immersed in water and heated to ca 373 K. The essential oil components desorb from the substrate matrix and then diffuse into the liquid phase. These components form an azeotropic mixture with water, then the volatile constituents gradually evaporate from the liquid into the steam phase, which then condense in the condenser. The oil separates from water to form an oil phase. The water is returned to the flask to hydro-distillate the sample again. In this process, only volatile compounds can be evaporated and carried away by steam. The heavier compounds with higher boiling points are hard to evaporate with water and thus are not detected in the hydro-distillation oil as observed in this study.
Hydro-distillation process:
1. the desorption of the substrate
2. the internal diffusion of the substrate
3. Azeotropic (water-essential oil) distillation

Soxhlet extraction process:
1. the diffusion of the solvent
2. the dissolution of extracted substance
3. the internal diffusion of the substrate and the solvent
4. the external diffusion of the substrate and the solvent

Supercritical extraction process:
1. the diffusion of the supercritical fluid
2. the dissolution of extracted substance
3. the diffusion of the substrate and the supercritical fluid
4. flow away of the supercritical fluid and the substrate mixture

Figure 4-6 Schematic diagrams of the hydro-distillation process, Soxhlet extraction process and supercritical fluid extraction process
In conventional Soxhlet extraction, an organic solvent of hexane or ethanol is used. The solvent type and its properties have a strong influence on the compositions of the extract from the substrate. Hexane Soxhlet achieved higher oil yields than SFE and hydrodistillation in this work. On the other hand, ethanol Soxhlet achieved the highest oil yield, by an order of magnitude higher than those of the other methods studied in this work. As aforementioned, this is because ethanol, owing to its polar nature, can extract not only polar compounds that are not dissolvable in hexane, but also non-polar components. Moreover, ethanol Soxhlet also extracts very heavy components such as waxes, chlorophyll and others that are not detected in the GC-MS analysis (Danielski et al., 2007a). Furthermore, the contact between the solvent and the substrate in Soxhlet extraction is different from those in hydro-distillation and supercritical fluid extraction. In Soxhlet extraction, the solvent is heated to reflux, the vapour passes through a bypass arm to reach the condenser. The solvent drips down into the thimble and diffuses into the substrate matrix. The extractable substances then dissolve into the solvent. The mixture of the solvent and extracted substances diffuses from the substrate matrix back to the bulk solvent. Once the mixture level in the extractor reaches the top of the siphon arm, the solvent and the extracted substances are siphoned back into the lower flask. Thus, Soxhlet extraction is a batch extraction process. Supercritical carbon dioxide was used in the supercritical fluid extraction in the present study. The oil yields in SFE and hexane Soxhlet were of the same magnitude. This is because both supercritical carbon dioxide and hexane are non-polar solvents and they only preferably solubilise non-polar components. However, the molecular weight of hexane is greater than that of CO₂, enabling it to dissolve some long chain alkanes or heavy molecular compounds more easily than supercritical carbon dioxide. Therefore the compositions of the oil extracted were quite different when using hexane Soxhlet extraction and supercritical fluid extraction. In the supercritical fluid extraction process, once supercritical carbon dioxide
diffuses into the substrate matrix and solubilises the extractable compounds, the mixture of the compounds and supercritical carbon dioxide diffuses out of the substrate matrix and flows away with the bulk fluid phase. Therefore supercritical fluid extraction is a continuous process, involving very different transport phenomena from hydro-distillation and Soxhlet extraction processes, as shown in Figure 4-6. Comparing these three techniques, it can be seen that although the oil extracted by hydro-distillation had the highest content of 1,8-cineole, this method produced the lowest yield and it took a long extraction time to achieve its maximum yield. The oil yield obtained by ethanol Soxhlet extraction was the highest but had a very low 1,8-cineole content. The SFE is therefore considered the best alternative method for *Eucalyptus* leaf oil extraction.

### 4.3.2 The effect of operating parameters on Soxhlet extraction

To compare SFE with other common extraction methods, the effects of solvent type and extraction time on the oil yield obtained by Soxhlet extraction were also evaluated and the results are shown in Figure 4-7. The experimental results were the average of three measurements, with error bars showing the standard deviation of these measurements. Ethanol and hexane were chosen in this study as the extraction solvent. Figure 4-7 shows that the oil yield increased rapidly with increasing extraction time till ca. 2 hours, but levelled off with further increasing from 2 hours to 8 hours. However, the solvent type had a more significant effect on the oil yield, with ethanol showing much higher oil yield than hexane over the same extraction time. For example, the oil yield extracted by ethanol at 8 hours was 36.33% which was nearly four times than that by hexane at 7.90%. In order to explain the difference, the total ion compound chromatograms of the *Eucalyptus* leaf oil obtained by the two solvents were acquired using a GC-MS. The results are shown in Figure 4-4 and the detailed compounds information is listed in the Appendix. Figure 4-4 indicates that the compositions of the extracted oil obtained by the two solvents were quite different; the hexane extracted oil contained a lot of alkanes and
other hydrocarbons that were not detected in the ethanol extracted oil, such as octane, undecane, dodecane, heptadecane, nonadecane, eicosane, docosane, toluene, benzene, 1,3-dimethyl- and etc. On the other hand, there were a number of polar components that were identified in the ethanol extracted oil were not present in the hexane extracted oil, such as myrtenol, (-)-carvone, 1,2,3-benzenetriol, cadinene, γ-muurolene, p-menthane-1,2,3-triol, δ-cadinene, tetradecanoic acid, n-hexadecanoic acid, 9,12,15-octadecatrienoic acid, (Z,Z,Z)-γ-sitosterol and etc. It is well known that hexane is a non-polar solvent while ethanol is a strong polar solvent. Based on the basic principle of “like dissolves like” (Bicking, 2000), the alkane compounds are non-polar and therefore more easily extracted by hexane. For the polar compounds such as myrtenol, 1,2,3-benzenetriol et al., as they contain the O-H ends, they readily interact with the hydrogen bond of ethanol and thus make them more easily separated by ethanol. This observation in the composition details did not only explains the huge difference between the ethanol and hexane Soxhlet extraction yields, it also implies that essential oil often extracted using such methods is not always the same.

Figure 4-7  Effect of solvent type and extraction time on the yield of Soxhlet extraction of *Eucalyptus* leaf oil
4.3.3 Hydro-distillation

Figure 4-8 shows *Eucalyptus* leaf oil yield as a function of extraction time in hydro-distillation. As expected, the oil yield increased with increasing time, from 3.1% at 1 hour to 3.8% at 5 hours of extraction, indicating a rapid extraction rate in the first 1 hour. This is probably because the *Eucalyptus* leaves grinding process liberated the essential oil onto the surface of the plant particles (Gavahian *et al*., 2012), allowing more ready extraction by the water vapour. Therefore, most of oil was extracted during the first hour (Gavahian *et al*., 2012).

![Figure 4-8](image)

**Figure 4-8**  Effect of extraction time on the yield of hydro-distillation of *Eucalyptus* leaf oil

4.4 Conclusions

The extraction of *Moringa oleifera* seed oil obtained by Soxhlet extraction with four different solvents of hexane, dichloromethane, ethyl acetate and ethanol were compared. The experimental results indicated that the oil yield obtained by Soxhlet extraction increased with increasing solvent polarity index, while ethanol provided the highest oil yield of 45.75%, compared to the other solvents. Based on the fatty acid composition,
there were no significant differences in the fatty acid profiles of *Moringa oleifera* seed oil obtained with four different solvents. The oil obtained contained mainly oleic acid and eicosenoic acid. Additionally, supercritical fluid extraction was also applied to extract oil from *Moringa oleifera* seeds. And the oil was characterized using GC and $^1$H NMR and compared against the oil extracted using Soxhlet extraction with hexane. Although the oil yield of the supercritical fluid extraction was slightly lower than that of the Soxhlet extraction, the GC and $^1$H NMR analysis indicated that the chemical compositions of the oils extracted by these two methods were very similar.

The extraction and characterisation of the essential oil extracted from *Moringa oleifera* leaves using supercritical carbon dioxide were discussed in this chapter. The results were compared against those obtained using the Soxhlet extraction method. The yield of the SFE process (6.34%) was lower than that of the Soxhlet extraction (9.27%) and there were great differences in the oil compositions by these two methods, as some components identified in the Soxhlet extracted oil were not extracted by supercritical fluid extraction.

Finally, the *Eucalyptus* leaf oil was extracted by Soxhlet extraction, hydro-distillation and supercritical fluid extraction. The highest overall yield of 36.33% was achieved with the 8 hours Soxhlet extraction using ethanol as solvent, while hydro-distillation produced the lowest yield of 3.77%. Furthermore, the chemical compositions of the extracted oil were analysed with a gas chromatography-mass spectrometry (GC-MS). The main component in all the extracted oil was 1,8-cineole. Chemical compositions of the *Eucalyptus* leaf oil extracted by the three methods were quite different; the oil extracted by hydro-distillation contained only volatile compounds while the SFE and Soxhlet extraction provided isolation of both the volatile and the higher molecular weight compounds.
Chapter 5 Solubility of *Moringa oleifera* Seed Oil in Supercritical Carbon Dioxide

Supercritical fluid extraction rate of oil from solid material is often limited by the solubility of the oil in the solvent. As such, the optimization of the SFE process requires the knowledge of solubility data, which allows the selection of the most adequate operational conditions, such as temperature, pressure, extraction time and solvent flow rate (Danielski *et al.*, 2007b). Besides, the solubility information is important for the process design as well as analytical applications. Oil solubility can be measured using either static or dynamic method, both by contacting supercritical CO\(_2\) with the previously obtained oil directly or with the ground substrate materials (Sovová *et al.*, 2001). In the dynamic method, the supercritical fluid flows continuously through an equilibrium vessel containing the oil being studied or with a solid material of large surface wetted with the oil. The operation conditions are chosen so that the outlet stream can be assumed to have reached the equilibrium. The dissolved or extracted oil is usually precipitated out and weighed, and the gaseous flow out of the restrictor is measured by using a wet or dry flow meter to determine the amount of supercritical fluid used to dissolve the oil (Graham *et al.*, 1998, del Valle and de la Fuente, 2006). A number of researchers have used the dynamic method to investigate the solubility of different oils in supercritical carbon dioxide, such as Sunflower oil (Soares *et al.*, 2007), Corn oil (Soares *et al.*, 2007), Rosehip oil (Machmudah *et al.*, 2007) and Borage oil (Gaspar *et al.*, 2002). In the static method, on the other hand, the oil and supercritical carbon dioxide are contacted in a high pressure vessel and allowed to reach equilibrium under controlled pressure and temperature. The samples from both the vapour phase and the liquid phase are withdrawn and analysed. Both the dynamic and static methods have been applied to measure the solubility of oil in supercritical CO\(_2\) (del Valle and de la Fuente, 2006).
As it is usually difficult and expensive to obtain equilibrium data at high pressures and temperatures, correlations and predictive models of solute solubility in supercritical carbon dioxide are essential. Different equations have been developed for mathematical modelling of solubility data in supercritical carbon dioxide, such as theoretical equations of state (EoS) and semi-empirical correlation equations. The equations of state models have proven to be useful tools for the correlation of experimental data and prediction of the phase equilibrium. The Peng-Robinson equation of state with the van der Waals mixing rules has been shown to give good result for the description of the vapour-liquid equilibrium of complex mixtures with supercritical carbon dioxide (Araujo et al., 2001). Rodrigues et al. (2005) have successfully used the Peng-Robinson equation to describe the solubility of Brazil nut oil in supercritical carbon dioxide. The phase equilibrium of Sacha Inchi oil in supercritical CO₂ have been studied and correlated with the Peng-Robinson equation of state by do Prado et al. (2011). On the other hand, the density-based models provide very simple and efficient means to correlate the solubility of substances in a wide range of temperatures and pressures; since they only use readily available independent variables (pressure, temperature and density of pure supercritical carbon dioxide). These equations include three or more parameters that are obtained on the basis of simple error minimisation using data fitting (Hezave et al., 2012). By far and large, numerous studies have been performed to investigate and predict the solubility of oil in supercritical carbon dioxide using the density based models (Sovová et al., 2001, Chen et al., 2011, Jokić et al., 2011).

The aim of this chapter was to experimentally obtain the solubility data of *Moringa oleifera* seed oil in supercritical carbon dioxide in a pressure range between 20 and 50 MPa and at temperatures of 333 K, 353 K and 373 K. The experimental solubility data was analysed using the Peng-Robinson equation of state with the conventional van der Waals mixing rules. Three density based models, proposed by Chrastil (1982), del Valle
and Aguilera (1988), and Adachi and Lu (1983) were also used to correlate the solubility data in this study.

5.1 Solubility calculations

Normally an extraction curve is divided into three stages (Min et al., 2010). In the first stage, the yield of the extract increases rapidly and linearly with solvent consumption or extraction time and then enters into a slow declining second stage. In the third stage, the extraction yield remains almost invariant, indicating the completion of extraction process. According to Reverchon and Marrone (2001), the solubility can be calculated from the experimental plot of the oil yield as a function of the mass of the solvent flow. Accordingly, the solubility of Moringa oleifera seed oil in supercritical CO\textsubscript{2} was determined from the slope of the linear part of extraction curve in this work, as described by the following equation:

\[
S = \frac{\text{mass of oil (g)}}{\text{mass of supercritical carbon dioxide (g)}},
\]

where \(S\) is the solubility of Moringa oleifera seed oil in supercritical CO\textsubscript{2}.

5.2 Effect of flow rate on solubility

The dynamic method to determine the solubility of vegetable oils in supercritical fluids has been increasingly widely accepted as it is relatively simple and easy to conduct and the results are repeatable and reliable (Gaspar et al., 2002, Machmudah et al., 2007, Soares et al., 2007). However, the validity of the dynamic method depends on the solvent flow rate; usually, a very small flow rate is required in order to ensure the saturation of the solute (the oil) in the flowing supercritical fluid. In order to validly use the dynamic method to assess experimental solubility values, the influence of the solvent flow rate on the solubility of Moringa oleifera seed oil in CO\textsubscript{2} must be evaluated.
Figure 5-1 Overall extraction yield curves obtained at different flow rates at pressure 40 MPa and temperature 353 K

Figure 5-1 shows the overall extraction curves obtained at pressure 40 MPa, temperature 353 K and four different flow rates ranging from 1.84 g/min to 7.36 g/min. The x-axis for the mass of CO$_2$ shown in Figure 5-1 was obtained by multiplying the CO$_2$ flow rate with extraction time. Similar to the discussion in Section 5.1, the extraction curves in Figure 5-1 divides into two stages. In the first stage, the yield of the oil increases rapidly with solvent consumption then enters into a slow declining second stage. The curves in the first stage lie on nearly the same line, indicating that the exit concentration of solute was independent of the carbon dioxide flow rate and therefore equilibrium solubility was achieved under the four flow rates investigated. The solubility values calculated using equation (5-1) at flow rates from 1.84 g/min to 7.36 g/min were almost the same, further confirming that the solubility was not a function of the supercritical carbon dioxide flow rate within the present experimental conditions. Therefore, supercritical carbon dioxide flow rate of 3.68 g/min was considered to be applicable to assure saturation during all the experimental solubility measurements.
reproducibility of the data is discussed further in the following Section 5.3 along with a comparison with the solubility data of other plant oils reported in the literature.

5.3 Comparison of Moringa oleifera seed oil with other vegetable oil

To the best of the authors’ knowledge, there is no previously reported solubility data of Moringa oleifera seed oil in supercritical CO$_2$ in the literature. The solubility of Moringa oleifera seed oil measured at pressures 20-50 MPa and temperatures 333-373 K are presented in Table 5-1, where the density values of pure CO$_2$ were obtained from the NIST on-line chemistry web book (NIST). To ensure the validity of the measured results, each experiment was repeated at least three times. The results presented in Table 5-1 were the average of three measurements under the same conditions and the standard deviations of the measurements. Table 5-1 indicates that the discrepancies between measurements are reasonable, since the standard deviation among the data is under 5.35%.

<table>
<thead>
<tr>
<th>T (K)</th>
<th>P (MPa)</th>
<th>$\rho$ (g/L)</th>
<th>$S$ (g oil/L CO$_2$)</th>
<th>$S$ (g oil/kg CO$_2$)</th>
<th>$y_2 \times 10^4$</th>
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<tbody>
<tr>
<td>333</td>
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<td>723.68</td>
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<td>1.61±0.12</td>
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<td>10.38±0.26</td>
<td>12.68±0.32</td>
<td>6.3±0.16</td>
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</table>

The solubility values of Sunflower oil (Soares et al., 2007), Corn oil (Soares et al., 2007), Rosehip seed oil (Machmudah et al., 2007), Borage oil (Gaspar et al., 2002), and Lunaria oil (Gaspar et al., 2002) are also provided in Table 5-2 for the purpose of
comparing with and validating the magnitude of the solubility data of *Moringa oleifera* seed oil of the present work.

Table 5-2  Solubility values of *Moringa oleifera* seed oil and other plant seed oil in supercritical carbon dioxide

<table>
<thead>
<tr>
<th>T(K)</th>
<th>P (MPa)</th>
<th>Solubility (g oil/kg CO₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Moringa oleifera</em> seed oil</td>
</tr>
<tr>
<td>333</td>
<td>20</td>
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<td></td>
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<td>8.32</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>12.68</td>
</tr>
</tbody>
</table>

Note: Symbol* represent experimental data taken from the reference stated for each sample, the remaining data were calculated using the Chrustil equation. Note that all the Chrastil equation constants were provided by the original authors and thus were used to calculate the data in this table.

Note that the solubility data of the Sunflower oil and Corn oil was experimental value determined using the same dynamic method (Soares et al., 2007) under conditions of 333 K, 30 MPa and 353 K, 30 MPa, respectively. The other values were calculated using the Chrastil equation of each individual oil under our experimental conditions, the Chrastil equation of each oil was developed by fitting to the experimental data determined with the dynamic method from the literature for the Sunflower oil (Soares et al., 2007), Corn oil (Soares et al., 2007), Rosehip seed oil (Machmudah et al., 2007), Borage oil (Gaspar et al., 2002) and Lunaria oil (Gaspar et al., 2002). As can be seen, the solubility data of *Moringa oleifera* seed oil was of a similar magnitude to those of Lunaria oil, but higher than those of Rosehip seed oil and lower than those of Sunflower.
oil, Corn oil and Borage oil. Soares et al. (2007) compared the solubility values of Babassu, Ucuuba, Sunflower, Corn, Blackcurrant and Canola oil. They found that the values obtained for the Sunflower, Corn, Blackcurrant and Canola oils were of a similar magnitude. However, the solubility values of Babassu fat were about five times greater than the values obtained for the above four oils, while the Ucuuba fat showed intermediate values among the six oils. They revealed that the oils composed of shorter chain fatty acids are more soluble than those with longer chain fatty acid. Follegatti-Romero et al. (2009) considered that the functional groups and the glyceride structure of the oil might have affected the vapour pressure, which in turn affected the solubility as well.

5.4 Fatty acid composition

Vegetable oils consist mainly of triglycerides. It has been reported that the compositions of vegetable oils influence their solubilities in supercritical CO$_2$ (Sovová et al., 2001). Thus, the fatty acid compositions of the Moringa oleifera seed oil were analysed using the GC. The fatty acid compositions of the Moringa oleifera seed oil extracted by supercritical CO$_2$ at 40 MPa and 333 K are summarised in Table 5-3. The results indicated that Moringa oleifera seed oil contained mainly unsaturated fatty acids up to 77.39%. Oleic acid, accounting for 66.96% of the total fatty acids, was the predominant fatty acid, followed by cis-vaccenic acid (6.03%). The contents of oleic acid and cis-vaccenic acid in the Moringa oleifera seed oil were similar to those reported by Vlahov et al. (2002). A small amount of palmitoleic acid, linoleic acid, linolenic acid and eicosenoic acid, was also detected. The contents of the total saturates, that is, myristic, palmitic, stearic, arachidic, behenic and lignoceric acid, in the oil were 0.13%, 5.95%, 5.78%, 3.77%, 6.05% and 0.94%, respectively, of which behenic acid was dominant. Therefore, Moringa oleifera seed oil is high in oleic acid, and is equivalent to olive oil in terms of its chemical properties (Abdulkarim et al., 2005). For comparison, the fatty
acid compositions of Sunflower, Corn, Rosehip, Borage and Lunaria are also listed in Table 5-3. It is clear that the fatty acid compositions of these oils were quite different which could have contributed to the differences in the solubility of these oils as compared to that of the *Moringa oleifera* seed oil.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Moringa oleifera seed Oil</th>
<th>Sunflower Oil (Soares et al., 2007)</th>
<th>Corn Oil (Soares et al., 2007)</th>
<th>Rosehip Oil (Machmudah et al., 2007)</th>
<th>Borage Oil (Gaspar et al., 2002)</th>
<th>Lunaria Oil (Gaspar et al., 2002)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric acid (C12:0)</td>
<td>--</td>
<td>--</td>
<td>0.13</td>
<td>NG</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Myristic acid (C14:0)</td>
<td>0.13±0.00</td>
<td>--</td>
<td>0.13</td>
<td>NG</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Palmitic acid (C16:0)</td>
<td>5.95±0.05</td>
<td>5.76</td>
<td>13.1</td>
<td>47.02-50.25</td>
<td>9-12</td>
<td>1-3</td>
</tr>
<tr>
<td>Palmitoleic acid (C16:1)</td>
<td>1.51±0.02</td>
<td>--</td>
<td>--</td>
<td>NG</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Stearic acid (C18:0)</td>
<td>5.78±0.02</td>
<td>4.05</td>
<td>2.98</td>
<td>0-2.88</td>
<td>3-5</td>
<td>1-2</td>
</tr>
<tr>
<td>Oleic acid (C18:1, 9cis)</td>
<td>66.96±0.14</td>
<td>29.6</td>
<td>32.5</td>
<td>NG</td>
<td>15-20</td>
<td>16-20</td>
</tr>
<tr>
<td>cis-Vaccenic acid (C18:1, 11cis)</td>
<td>6.03±0.03</td>
<td>--</td>
<td>--</td>
<td>NG</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Linoleic acid (C18:2)</td>
<td>0.59±0.01</td>
<td>58.9</td>
<td>48.4</td>
<td>0.05-0.87</td>
<td>30-40</td>
<td>8-10</td>
</tr>
<tr>
<td>Linolenic acid (C18:3)</td>
<td>0.17±0.01</td>
<td>0.17</td>
<td>1.20</td>
<td>0.05-0.94</td>
<td>18-25</td>
<td>2-4</td>
</tr>
<tr>
<td>Arachidic acid (C20:0)</td>
<td>3.77±0.04</td>
<td>0.30</td>
<td>0.65</td>
<td>NG</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Eicosenoic acid (C20:1, 11cis)</td>
<td>2.12±0.02</td>
<td>0.18</td>
<td>0.32</td>
<td>NG</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Behenic acid (C22:0)</td>
<td>6.05±0.11</td>
<td>0.80</td>
<td>0.25</td>
<td>NG</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Erucic acid (C22:1)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>NG</td>
<td>--</td>
<td>38-48</td>
</tr>
<tr>
<td>Lignoceric acid (C24:0)</td>
<td>0.94±0.02</td>
<td>0.28</td>
<td>0.31</td>
<td>NG</td>
<td>--</td>
<td>22-25</td>
</tr>
</tbody>
</table>

Note: NG means information is not given from the reference cited.
5.5 Effect of pressure and temperature on solubility

Statistical analysis (ANOVA) of the solubility measurements of the *Moringa oleifera* seed oil in supercritical carbon dioxide was performed. It was found that both pressure (p<0.05) and temperature (p<0.05) had significant effects on the solubility of the *Moringa oleifera* seed oil in supercritical carbon dioxide. The solubility data is graphically shown in Figure 5-2. It is clear that the effect of pressure on the solubility indicated the expected trends, that was, the solubility increased with increasing pressure at constant temperature due to the increase in solvent density which led to an increase in the solvating power of supercritical CO\(_2\). The temperature effect, however, was more complex as can be observed in Figure 5-2. At 20 and 30 MPa, the solubility showed a slight decrease with temperature. However, at 40 MPa and 50 MPa, the solubility increased with increasing temperature. These phenomena resulted from the competing effects of reducing solvent density and increasing solute vapour pressure.
with increasing temperature (Danielski et al., 2007a). In the retrograde region, the increase in the solvent density and dissolving power with a decrease in temperature was more pronounced than the decrease in solute vapour pressure. This was consistent with the findings that were reported for supercritical CO$_2$ extraction of hazelnut seed oil (Özkal et al., 2005).

5.6 Thermodynamic analysis of experimental data

The solubility of a solid in compressed gas can be expressed by Equation (5-2):

$$y_2 = \frac{p_{2,\text{sub}}^s}{\varphi_2^{\text{SCF}}} \exp \left( \frac{v_2^s (P - p_{2,\text{sub}}^s)}{RT} \right), \quad (5-2)$$

This equation is obtained from the equifugacity condition between the solid and the solvent fluid phase, assuming that the fugacity of the solute in the supercritical phase is equal to the solute in the solid state. It also considers that: (1) the solubility of the solvent in the solute is negligible; (2) the solute is incompressible; and (3) the pure solute saturated vapour at sublimation behaves like an ideal gas (de Sousa et al., 2012). Here, $y_2$ is the mole fraction of the solute in the supercritical fluid. $T$ is the temperature (K) and $R$ is the gas constant. $p_{2,\text{sub}}^s$ is the sublimation pressure of solute (Pa), $v_2^s$ is the molar volume of the solute (m$^3$/mol), and $\varphi_2^{\text{SCF}}$ is the fugacity coefficient of the solute in the supercritical phase. The fugacity coefficient $\varphi_2^{\text{SCF}}$ is obtained from an equation of state and mixing rules using an exactly thermodynamic relationship (de Sousa et al., 2012). In this work, the Peng-Robinson equation of state (PR-EoS) with classical van der Waals mixing rules was used to calculate the fugacity coefficient (Peng and Robinson, 1976). The expression of the Peng-Robinson equation of state is shown below:

$$p = \frac{RT}{v-b_m} - \frac{a_m}{v^2 + 2b_m v - b_m^2}, \quad (5-3)$$

This equation provides a mathematical relationship of pressure versus temperature and volume. It describes the pressure as the sum of two terms, a repulsion pressure...
expressed as $\frac{RT}{v-b_m}$ and an attraction pressure expressed as $\frac{a_m}{v^2 + 2b_mv - b_m^2}$. $a_m$ and $b_m$ are parameters calculated from a mixing rule (Peng and Robinson, 1976). In this study, the classical van der Waals mixing rules were applied which are described as follows:

$$a_m = \sum_1^n \sum_1^n y_i y_j a_{ij}$$ (5-4)

$$b_m = \sum_1^n \sum_1^n y_i y_j b_{ij}$$ (5-5)

In these equations, $a_{ij}$ and $b_{ij}$, $(i=j)$ are parameters corresponding to pure component $(i)$ while $a_{ij}$ and $b_{ij}$, $(i\neq j)$ are called the unlike-interaction parameters. It is customary to relate the unlike-interaction parameters to the pure-component parameters by the following equations (Kwak and Mansoori, 1986):

$$a_{ij} = (1 - k_{ij})\sqrt{a_i a_j}$$ (5-6)

$$b_{ij} = (1 - l_{ij})\frac{b_i + b_j}{2}$$ (5-7)

where $k_{ij}$ and $l_{ij}$ are binary interaction parameters which characterise the binary mixture formed by components $i$ and $j$. The binary interaction parameters take into account the differences in the strength of intermolecular interactions during the interaction of the two substances, such as carbon dioxide and *Moringa Oleifera* seed oil in this study (Reid, 1987). The binary interaction parameters are estimated from the measured solubility data that enables the Peng-Robinson equation of state to better predict the desired phase behaviour.

$$a_i = \frac{0.45724R^{2/3} \sqrt{P_{ci}^2}}{P_{ci}} [1 + (0.37464 + 1.54226\omega - 0.26992\omega^2)(1 - \sqrt{\frac{T}{T_{ci}}})^2]$$ (5-8a)

$$b_i = \frac{0.07779RT_{ci}}{P_{ci}}$$ (5-8b)

where $a_i$ and $b_i$ are the PR-EoS parameters for pure component $i$, $T_{ci}$ is the critical temperature for pure component $i$ ($K$), $P_{ci}$ is the critical pressure for component $i$ (Pa), $\omega$
is the acentric factor. The fugacity coefficient of the solute in the supercritical fluid \( \phi_{SCF} \) is given by the following equation:

\[
\ln(\phi_{2 \text{SCF}}) = \frac{2 \sum_i y_i b_i - b_m}{b_m} (Z - 1) - \ln(Z - B) + \frac{A}{2 \sqrt{2} B} \left( \frac{2 \sum_i y_i^{a/2} b_{i/2} - b_m}{b_m} - \right.
\]

\[
\frac{2 \sum_i y_i^{a/2}}{a_m} \ln \left[ \frac{Z + B (1 + \sqrt{2})}{Z + B (1 - \sqrt{2})} \right],
\]

(5-9)

where

\[
Z = \frac{PV}{RT},
\]

(5-10a)

\[
A = \frac{p a_m}{R^2 T^2},
\]

(5-10b)

\[
B = \frac{p b_m}{RT}.
\]

(5-10c)

To apply the Peng-Robinson equation of state to analyse the phase equilibrium of the \( \text{CO}_2 \) and \textit{Moringa oleifera} seed oil system, the critical parameters and acentric factors of \( \text{CO}_2 \) and \textit{Moringa oleifera} seed oil are required. The parameters for pure carbon dioxide were taken from the literature (Weber et al., 1999) as listed in Table 5-4. However, it is very difficult to obtain the experimental values of these properties for oils due to their chemically unstable nature at high temperatures (Weber et al., 1999). As the most abundant group of compounds in \textit{Moringa oleifera} seed oil are the triglycerides, triolein which is the principle triglyceride was selected to represent the oil. Similar assumptions were also reported by other authors (Yu et al., 1994) who studied the phase equilibrium of oils in supercritical \( \text{CO}_2 \). The critical temperature, critical pressure, acentric factor and molar volume estimated by Tang et al. (2006) were used in this work and are listed in Table 5-4. The vapour pressure data of triolein was available in the literature (Perry et al., 1949). Extrapolative method was used to estimate the vapour pressure at the low temperature as the data was not available due to the difficulty to carry out vapour pressure measurement (Tang et al., 2006).
Table 5-4  Estimated critical parameters andacentric factors used in the equation of state for calculations

<table>
<thead>
<tr>
<th>Substance</th>
<th>$T_c$ (K)</th>
<th>$P_c$ (Pa)</th>
<th>$\omega$</th>
<th>Molar volume $v_2^s$ (m$^3$/mol)</th>
<th>Molecular weight (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$</td>
<td>304.1</td>
<td>7.38×10$^6$</td>
<td>0.225</td>
<td></td>
<td>44.01</td>
</tr>
<tr>
<td>triolein</td>
<td>860.46</td>
<td>1.70×10$^6$</td>
<td>0.73</td>
<td>0.97×10$^{-3}$</td>
<td>885.43</td>
</tr>
</tbody>
</table>

The binary interaction parameters of triolein reported by Weber et al. (1999) were taken to calculate the oil solubility using the Peng-Robinson equation of state. Table 5-5 lists the calculated binary interaction parameters of triolein. By using the binary interaction parameters of the triolein–supercritical CO$_2$ system, the calculated solubility of the *Moringa oleifera* seed oil in supercritical CO$_2$ was compared with our experimental data, as shown in Figure 5-3. It can be seen that the model prediction with the triolein’s binary parameters did not describe the experimental data quantitatively. This was suspected to be because *Moringa oleifera* seed oil is a mixture of different compounds such as triglycerides, fatty acid and tocopherols, et al. and triolein is only one component of the oil. The oil and the triolein could have different solubility behaviour.

Table 5-5  The optimal fitted binary parameters of triolein

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$k_{ij}$</th>
<th>$l_{ij}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>333</td>
<td>0.104</td>
<td>-0.449</td>
</tr>
<tr>
<td>353</td>
<td>0.272</td>
<td>-0.297</td>
</tr>
</tbody>
</table>
In order to find the optimal binary interaction parameters of *Moringa oleifera* seed oil, the experimental solubility data was fitted with the Peng-Robinson equation of state with artificially varying the binary interaction parameters of $k_{ij}$ and $l_{ij}$ for each temperature. The calculation process was done by minimising the objective function of the average absolute relative deviation (AARD) defined as:

$$AARD = \frac{100}{n} \sum \left| \frac{y_{cal} - y_{exp}}{y_{exp}} \right|.$$  (5-11)

In this equation, $n$ is the number of experimental data points for each isotherm, $y_{cal}$ is the calculated solubility and $y_{exp}$ corresponds to the experimental solubility data points. The resultant optimal fitted binary parameters of *Moringa oleifera* seed oil are listed in Table 5-6.

<table>
<thead>
<tr>
<th>$T$ (K)</th>
<th>$k_{ij}$</th>
<th>$l_{ij}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>333</td>
<td>0.017</td>
<td>-0.615</td>
</tr>
<tr>
<td>353</td>
<td>0.147</td>
<td>-0.468</td>
</tr>
<tr>
<td>373</td>
<td>0.261</td>
<td>-0.342</td>
</tr>
</tbody>
</table>
Figure 5-4 compares the experimental solubility data and the model calculated data based on the parameters in Table 5-6. It is clear that the predicted solubility was in much better agreement with the experimental data. In addition, the experimental solubility data followed the same trend as the presentations from this thermodynamic model. Furthermore, as can be seen from Table 5-6, the two interaction parameters of $k_{ij}$ and $l_{ij}$ increase linearly with increasing temperature, indicating that it is possible to regress the binary interaction parameters of $k_{ij}$ and $l_{ij}$ as a liner function of temperature. The regression equations for $k_{ij}$ and $l_{ij}$ were obtained as shown in equation (5-12) and (5-13), with coefficients of determination being 0.999 and 0.998, respectively, indicating these two equations fitted the data well. This approach effectively provided a new method to estimate the binary interaction parameters of $k_{ij}$ and $l_{ij}$ at other temperatures and enables the application of the Peng-Robinson equation of state to other previous unknown oils.

\[ k_{ij}(T) = 0.0061T - 2.0067, \quad (5-12) \]

\[ l_{ij}(T) = 0.0068T - 2.8869, \quad (5-13) \]

where $T$ is the temperature (K).
Figure 5-4  Solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide. Lines are predicted solubility using PR-EoS with \( k_{ij} \) and \( l_{ij} \) of the *Moringa oleifera* seed oil
5.7 Correlation of the experimental solubility data with density based model

Although the Peng-Robinson equation of state is often used to predict the solubility of a solute in supercritical carbon dioxide, it has some limitations in its use. For example, the absence of the required thermophysical data of the extracted solute such as critical properties, acentric factor, solute molar volume, and sublimation pressures. In this case, the density based models may provide alternatives. This is because the density based models use only available variables such as pressure, temperature and density of pure supercritical fluid instead of solute properties. The calculation process is much simpler than that of the equations of state models and the parameters of the density based models can be easily determined by fitting to the experimental data.

5.7.1 Chrastil Equation

The density based equation commonly used for correlating the solubility of oils is that proposed by Chrastil (1982) who considered a solvate-complex between the solute and supercritical CO$_2$ at equilibrium. Chrastil thought that under ideal conditions, one molecule of a solute $A$ associates with $k$ molecules of a gas $B$ would form one molecule of a solvate complex $AB_k$ in equilibrium with the system, which can be written:

$$A + kB \rightleftharpoons AB_k,$$

$$k = \frac{[AB_k]}{[A][B]^k},$$

$$lnk + ln[A] + kln[B] = ln[AB_k],$$

where $[A]$ is the molar vapour concentration of a solute, $[B]$ is the molar concentration of a gas and $[AB_k]$ is the molar concentration of the solute in a gas, $k$ is the equilibrium constant.

According to the Clapeyron-Clausium equation, $ln[A] = \frac{\Delta H_{vap}}{RT} + q_v$, where $\Delta H_{vap}$ is the heat of vapourization of the solute and $q_v$ is a constant.
According to the Van’t Hoff equation, \( \ln k = \frac{\Delta H_{\text{solv}}}{RT} + q_s \), where \( \Delta H_{\text{solv}} \) is the heat of solvation and \( q_s \) is a constant. Putting these two expressions into equation (5-16), we get

\[
\frac{\Delta H}{RT} + q + k\ln M_B = \ln [AB_k], \tag{5-17}
\]

where \( \Delta H \) is the total reaction heat \( \Delta H = \Delta H_{\text{vap}} + \Delta H_{\text{solv}} \), and \( q = q_s + q_v \).

As \( [B] = \rho/M_B \), and \( [AB_k] = S/(M_A + kM_B) \), where \( S \) is the concentration of the solute in a gas (g/L), \( \rho \) is the density of a gas (g/L), \( M_A \) and \( M_B \) are the molecular weights of the solute and the gas, correspondingly. Then we can get:

\[
\frac{\Delta H}{RT} + q + k\ln d - k\ln M_B = \ln S - \ln (M_A + kM_B), \tag{5-18}
\]

thus \( \ln S = k \ln \rho + \frac{a}{T} + b \), \tag{5-19}

where \( S \) is the solubility of a solute (g/L), \( \rho \) is the density of supercritical \( \text{CO}_2 \) (g/L) under the operating condition. The association number \( k \) is the slope of the linear correlation and represents the average number of \( \text{CO}_2 \) molecules in the solvated complex. The constant \( a = \Delta H/R \), where \( R \) is the gas constant (8.314 J mol\(^{-1}\) K\(^{-1}\)). The constant \( b = \ln (M_A + kM_B) + q - k \ln (M_B) \), According to the Chrastil equation, a log-log graph of \( S \) versus \( \rho \) should give a straight line.

### 5.7.2 del Valle and Aguilera Equation

However, the Chrastil equation has some limitations. It is not valid over a wide range of temperature and for solubility higher than 100-200 kg/m\(^3\) (Garlapati and Madras, 2010, Tabernero et al., 2010). In addition, deviation from the linear relation is expected at high solute concentrations in fluid phase as the solvent density starts to deviate from the density of pure solvent. The solute polarity has also been reported to affect the accuracy of the model. The model is not accurate for highly polar solid solutes up to a reduced density of 1.5, indicating density dependence of the association number (Rincón et al.,...
Therefore, several modified forms of Chrastil equation have been developed to take account of different parameters’ effect on solubility.

The model proposed by del Valle and Aguilera (1988) is a modification of the Chrastil equation and takes into account the enthalpy change of vaporisation with the temperature, as shown below:

\[ \ln S = k \ln \rho + \frac{a}{T} + \frac{a_1}{T^2} + b, \]  
\[ (5-20) \]

where \( S \) is the solubility of a solute (g/L), \( \rho \) is the density of supercritical CO\(_2\) (g/L) and \( T \) is the temperature (K). Constants \( a \) and \( a_1 \) represent the thermal effects involved in the solubilisation process, while the constant \( b \) is related to the solute and the solvent molecular weights, and \( k \) represents the same parameter proposed by Chrastil (1982).

### 5.7.3 Solubility correlation with the Adachi and Lu Equation

Note that both Chrastil and del Valle and Aguilera have assumed the association number \( k \) to be constant and independent of density or temperature. Adachi and Lu (1983) changed the association number to a second-order polynomial of the supercritical fluid density, therefore, they proposed another equation as expressed below:

\[ \ln S = (e_0 + e_1 \rho + e_2 \rho^2) \ln \rho + \frac{a}{T} + b. \]  
\[ (5-21) \]

The association number is the exponent of the density (referred to \( k \) in the original Chrastil equation) and shows the dependence of the solubility on density. In the Adachi-Lu version of the model, \( k \) is a function of the density as seen in equation 5-22:

\[ k = e_0 + e_1 \rho + e_2 \rho^2. \]  
\[ (5-22) \]
5.7.4 Correlation results with the three density based models.

In the present work, the parameters of all models were calculated using the nonlinear regression method using MATLAB 7.9.0. In order to provide a reliable accuracy criterion to compare the accuracy of the models which possess different numbers of curve-fitting parameters, the average absolute relative deviations (AARD) were calculated using equation (5-23).

\[
AARD = \frac{100}{n} \sum \left| \frac{S_{cal} - S_{exp}}{S_{exp}} \right|,
\]

(5-23)

where \( n \) is the number of solubility experimental data measurements, \( S_{cal} \) is the calculated solubility while \( S_{exp} \) is the experimental solubility of *Moringa oleifera* seed oil.

Figure 5-5 compares the calculated solubility of *Moringa oleifera* seed oil using the Chrastil and modified Chrastil models against the experimentally measured values. It can be seen that the experimental results were satisfactorily regressed as a linear function. The solubility curves of the three density based models were highly consistent with the experimental data. The constants for the Chrastil, del Valle and Aguilera, Adachi and Lu equations, are given in Table 5-7 with their AARD for the temperature and pressure range investigated. As shown in this table, the AARD values range from 3.71% to 5.16%, indicating that all of the three empirical models can fit the experimental data well. The comparison also showed that the del Valle and Aguilera model achieved the best fit with the experimental data with an overall AARD of 3.71%.

The values for the association constant \( k \) were about 7 in both Chrastil and del Valle and Aguilera models. This indicated that there were about seven molecules of CO\(_2\) clustered for one molecule of *Moringa oleifera* seed oil to form a quasi-solvato complex (Chrastil, 1982). However, the parameter \( b \) for Chrastil and del Valle and Aguilera
models were different (-32.52 for Chrastil model and -8.17 for del Valle and Aguilera model). As explained in Section 5.7.1, the adjustable parameter $b$ is defined as: $b = \ln (M_A + kM_B) + q - k \ln (M_B)$, This value difference of parameter $b$ between these two models was probably because del Valle and Aguilera equation includes a new adjustable parameter $a1$ in equation 5-20. This new parameter $a1$ leads to a higher magnitude constant of $q$ in del Valle and Aguilera equation than that in Chrastil equation. (-13.72 for Chrastil and 12.06 for del Valle and Aguilera).

Slightly better correlation results were obtained for the three density based models than the Peng-Robinson equation of state model. This indicated that the assumption underpinning the density based models were more application than those underpinning the Peng–Robinson equation of state. As discussed in Section 5.7.1, the Chrastil equation was derived using the chemical equilibrium consideration, it assumes the molecules of a solute associate with the molecules of a gas with the formation of a solvato complex, which is in equilibrium with the gas. Both the del Valle and Aguilera model and the Adachi and Lu model are modified Chrastil models. The difference between these two models is that the del Valle and Aguilera model takes into account the enthalpy change of vaporisation with temperature, and the Adachi and Lu model considers $k$ to be density dependent. There are several assumptions applied in the Peng–Robinson equation of state model as mentioned in Section 5.6 and the van der Waals mixing rules were used in the calculation. Some authors (Martín z et al., 2009, Lee et al., 2005) found that the correlation results were affected by different mixing rules, the mixing rule applied in this study was probably not the best for the Moringa oleifera seed oil solubility calculation. In conclusion, although density based models have less physical significance than the equation of state model, these models provide a simple way to correlate the solubility of oil in supercritical fluid and they don’t require the critical or thermophysical properties of the oil to be estimated.
Table 5-7  The obtained fitting constants for three density based correlations

<table>
<thead>
<tr>
<th></th>
<th>Chrastil</th>
<th>del Valle and Aguilera</th>
<th>Adachi and Lu</th>
</tr>
</thead>
<tbody>
<tr>
<td>k</td>
<td>6.84</td>
<td>7.22</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>-4.11×10³</td>
<td>-2.30×10⁻⁴</td>
<td>-4.21×10³</td>
</tr>
<tr>
<td>a1</td>
<td></td>
<td>3.31×10⁶</td>
<td></td>
</tr>
<tr>
<td>b</td>
<td>-32.52</td>
<td>-8.17</td>
<td>-81.12</td>
</tr>
<tr>
<td>e0</td>
<td></td>
<td></td>
<td>16.20</td>
</tr>
<tr>
<td>e1</td>
<td></td>
<td></td>
<td>-3.83×10⁻³</td>
</tr>
<tr>
<td>e2</td>
<td></td>
<td></td>
<td>1.58×10⁶</td>
</tr>
<tr>
<td>AARD (%)</td>
<td>5.16</td>
<td>3.71</td>
<td>4.95</td>
</tr>
</tbody>
</table>

Note: k, a and b are the constants for the Chrastil equation
k, a, b and c are the constants for the del Valle and Aguilera equation
a, b, e0, e1 and e2 are the constants for the Adachi and Lu equation
AARD is the average absolute relative deviation
Figure 5-5  Comparison of the experimental and correlated solubility data with the three density based models. ($\rho$, density of supercritical CO$_2$; $S$, solubility of *Moring oleifera* seed oil in supercritical CO$_2$)
5.8 Conclusions

For the first time, the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide was measured at pressures of 20-50 MPa and temperatures of 333-373 K. Within the experimental conditions, the solubility increased with increasing pressure at a given temperature. However, the effect of temperature showed different trends under different pressures. The solubility decreased with increasing temperature at 20 and 30 MPa but increased at 40 and 50 MPa. The experimental solubility data was analysed with the Peng-Robinson equation of state and three density based models (Chrastil model, del Valle and Aguilera model, and Adachi and Lu models). By comparing these four models, it was found that the del Valle and Aguilera models gave the best correlation. In addition, the solubility trend of *Moringa oleifera* seed oil in supercritical CO₂ was also successfully described and explained using the Peng-Robinson equation of state and a new method to estimate the binary interaction parameters of \( k_{ij} \) and \( l_{ij} \) in the thermodynamic model was proposed. These two parameters varied linearly with temperature.
Chapter 6 The Effects of Different Parameters on Supercritical Carbon Dioxide Extraction of Oils from *Moringa oleifera* and *Eucalyptus*

There are a large number of experimental variables that may potentially affect the efficiency of SFE, such as operation temperature, pressure, extraction time, solvent flow rate, moisture content of sample and modifier effects, the traditional one-factor-at-a-time method is time consuming and neglects the interactions of these complex factors. Currently there is an increasing trend to use experimental design to optimize the extraction process, reduce cost, enhance convenience and save time and labour. In this chapter, the response surface methodology was used to evaluate the effect of different parameters on the oil extraction from *Moringa oleifera* seeds and *Eucalyptus* leaves, and the orthogonal array design was used to analyse the pressure, temperature and time effect on the *Moringa oleifera* leaf oil extraction process.

6.1 Parametric study of *Moringa oleifera* seed oil extraction

6.1.1. Response surface analysis

The response surface methodology (RSM) has been demonstrated to be a powerful tool to determine the interactions between the factors. The RSM is a collection of mathematical and statistical technique useful for the modelling and analysing problems in which a response of interest is influenced by several variables and the objective is to optimize this response (Yim *et al*., 2012). This procedure involves fitting a function to the experimental data and using optimization techniques to obtain the optimum parameters (Ramalhosa *et al*., 2012). It is much faster and more economical for gathering research results than the classic one variable-at-a-time or full-factors design method (Li *et al*., 2012). Therefore, in order to investigate the effect of four operating conditions, namely pressure, temperature, flow rate and extraction time, on the
supercritical fluid extraction of *Moringa oleifera* seed oil, the central composition design of the response surface methodology was employed in this study, the experimental responses in terms of the oil yield are summarized in Table 6-1. As seen from this table, the oil yield ranged from 4.57% to 37.12%. The maximum yield was obtained under pressure 50 MPa, temperature 373 K, flow rate 7.36 g/min and extraction time of 120 minutes.

The experimental data was analysed using the analysis of variance to assess the “goodness of fit”, as listed in Table 6-2. The probability (p) value of the quadratic model was less than 0.0001 which showed an excellent fit and appeared to reasonably represent the data. The “lack of fit” measures how well the model fits the data. If the p value of the “lack of fit” is smaller than 0.05, it indicates that the model does not fit the data well. The probability of the "lack of fit" in Table 6-2 was 0.0524, which suggested that the model was acceptable. Moreover, the coefficient of determination (R$^2$) should also be taken into consideration when validating the model. The R$^2$ value at 0.9734 was considered very good, indicating that 97.34% of the variables in the extraction yield of oil could be explained by the quadratic model. Therefore, the R$^2$ value of the response model was sufficiently high so that the response surface model was workable.

Furthermore, as each individual experiment was repeated and the statistical analysis of each individual experiment was included during the data analysis process, it is reasonable to use to experimental results to valid the model. Several other authors have also used the significance level of 0.05 to analyse the significance of “lack of fit” in their research study (Cui et al., 2011, Cai et al., 2012, Tabarak et al., 2012).
Table 6-1 Experimental results for the supercritical fluid extraction of *Moringa oleifera* seed oil using central composite design

<table>
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<tr>
<th>Run</th>
<th>Factors</th>
<th>Oil yield (%)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>X&lt;sub&gt;1&lt;/sub&gt; (Pressure, MPa)</td>
<td>X&lt;sub&gt;2&lt;/sub&gt; (Temperature, K)</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
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</tr>
<tr>
<td>2</td>
<td>40</td>
<td>353</td>
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<tr>
<td>3</td>
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<td>Degree of freedom</td>
<td>Regression coefficient</td>
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<td>------------------------</td>
</tr>
<tr>
<td>Model</td>
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<td>Lack of fit</td>
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<tr>
<td>R² = 0.9734</td>
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<td>X₁ pressure</td>
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<td>X₂ temperature</td>
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</tr>
<tr>
<td>X₃ flow rate</td>
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</tr>
<tr>
<td>X₄ time</td>
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</tr>
<tr>
<td>X₁X₂</td>
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</tr>
<tr>
<td>X₁X₃</td>
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</tr>
<tr>
<td>X₁X₄</td>
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<td>-0.056</td>
</tr>
<tr>
<td>X₂X₃</td>
<td>1</td>
<td>-0.48</td>
</tr>
<tr>
<td>X₂X₄</td>
<td>1</td>
<td>-0.69</td>
</tr>
<tr>
<td>X₃X₄</td>
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<td>-1.28</td>
</tr>
<tr>
<td>X₁²</td>
<td>1</td>
<td>-2.27</td>
</tr>
<tr>
<td>X₂²</td>
<td>1</td>
<td>-0.26</td>
</tr>
<tr>
<td>X₃²</td>
<td>1</td>
<td>-1.11</td>
</tr>
<tr>
<td>X₄²</td>
<td>1</td>
<td>-1.36</td>
</tr>
</tbody>
</table>

The coefficients of the regression equation and their p values are shown in Table 6-2. Fitting the constants and coefficients into equation 6-1, the regression model as a function of pressure, temperature, flow rate and time thus could be obtained as follows (in terms of coded levels):

\[
Y = 27.74 + 6.33X_1 + 0.61X_2 + 5.23X_3 + 4.12X_4 + 1.25X_1X_2 + 0.77X_1X_3 - 0.056X_1X_4 - 0.48X_2X_3 - 0.69X_2X_4 - 1.28X_3X_4 - 2.27X_1^2 - 0.26X_2^2 - 1.11X_3^2 - 1.36X_4^2
\]

(6-1)
The significance of each coefficient was also determined by the F values and p values as listed in Table 6-2. Value of p<0.05 indicated that the model term was significant, the larger the magnitude of the F value and the smaller the p value mean a greater significance of the corresponding coefficient. As can be seen from Table 6-2, the linear terms of pressure, flow rate and time had significant (p<0.0001) effects on the recovery of *Moringa oleifera* seed oil, while the effect of temperature (p=0.0826) on the oil yield was small. Although temperature did not have a significant linear effect, its interaction with pressure (p=0.0420) was important. It was also noticed that all the second order terms of the four processing parameters had negative effects on the oil yield. The prediction model was rearranged by eliminating the terms that were not significant in the second order polynomial model. Finally, the following equation was obtained:

\[
Y = 27.74 + 6.33X_1 + 0.61X_2 + 5.23X_3 + 4.12X_4 + 1.25X_1X_2 - 0.69X_2X_4 - 1.28X_3X_4 - 2.27X_1^2 - 1.11X_3^2 - 1.36X_4^2
\]

(6-2)

Figure 6-1 Effect of particle size on extraction yield of the *Moringa oleifera* seed oil (40 MPa, 353 K and 3.68 g/min)
As the oil yield extracted by supercritical CO₂ depends on not only the pressure, temperature, flow rate and extraction time, but also the particle size of the samples. Therefore, initial experiments were conducted to investigate the effect of particle size on the oil extraction from Moringa oleifera seeds. The extractions were performed at various particle sizes (1000-2000µm, 710-1000µm, 500-710µm and <500µm) at 40 MPa, 353 K and a constant CO₂ flow rate of 3.68 g/min. The variations in the oil yield with operating time for these four different particle size ranges are shown in Figure 6-1. It can be seen that the extraction curves can be characterized by two periods (Follegatti-Romero et al., 2009). The first period of constant extraction rate was characterized by the extraction of the solute more accessible to the solvent, which was found in the superficial layer of the particles. In the second period of falling extraction rate, the amount of the solute available on the surface decreased and the solute inside the particles had to diffuse into the surface. Therefore, the extraction rate was limited by the intra-particle diffusion of the solute. During the initial extraction period, the slopes of the four particle size extraction curves were almost the same, showing that in this stage only the temperature and pressure were important and the flow rate employed was sufficiently low to promote solvent saturation in this stage. In the second period, it can be seen that the yield of Moringa oleifera seed oil increased when the particle size decreased from 1000-2000µm to less than 500µm. This was because at this stage, the intra-particle diffusion controlled mass transfer from the interior to the surface of the particles. The intra-particle diffusion resistance was smaller for smaller particles due to the shorter diffusion path and greater specific surface areas. Additionally, the grinding process could also have disrupted the cell walls and other inner barriers to mass transfer. Thus the mass transfer resistance was lower for smaller particles, making the sample more easily accessible to the supercritical fluid (Zuo et al., 2008).
The best way to visualize the influence of the independent variables on the dependent variable is to generate response surface plots of the model, which was done by varying two variables within the experimental range and holding the other two constant. Figure 6-2 shows the effects of pressure and temperature on the oil yield at the flow rate of 5.52 g/min and extraction time of 90 minutes. It can be seen that the oil yield significantly increased with increasing pressure at a given temperature. This can be explained by the increase in the solvent density of the supercritical CO$_2$ with increasing pressure. As the density increased, the distance between molecules decreased and the interaction between oil and CO$_2$ increased, leading to a greater oil solubility in CO$_2$ (Molero Gómez and Martinez de la Ossa, 2002).

![Diagram of response surface plot](image)

Figure 6-2 The three-dimensional plot of the response surface for the oil yield as related to pressure and temperature at the flow rate of 5.52 g/min and extraction time of 90 minutes

The influence of temperature on oil yield was more difficult to predict than that of pressure, because of its two counter effects on the oil yield. Firstly, increasing the temperature reduces the solvent density and thus its solvation power at constant
pressure. On the other hand, increasing temperature increases the vapour pressure of the solute, therefore increasing its solubility in the supercritical solvent (Couto et al., 2009). Furthermore, high temperatures could increase the mass transfer. Therefore, the increase in temperature could have either a positive or a negative effect, which is dependent on whether the solvent density or the solute vapour pressure was predominant (Krichnavaruk et al., 2008). From Figure 6-2, it can be seen that the oil yield decreased with the rise of temperature at low pressures, due to the reduced density of CO\textsubscript{2} with increased temperature. However, in the present experiments at high pressures above 30 MPa, the oil yield increased with the rise of temperature. The pressure at which the effect of temperature on the performance is reversed is called the crossover pressure. Similar behaviour was also reported for supercritical CO\textsubscript{2} extraction of silkworm pupal oil (Wei et al., 2009). Since the solubility of the oil in supercritical CO\textsubscript{2} depends largely on the balance between the fluid density and solute vapour pressure which are both controlled by fluid pressure and temperature, it is important to choose the suitable conditions for the extraction process (Krichnavaruk et al., 2008).

Figure 6-3 shows the effect of pressure and flow rate on the oil yield at the fixed temperature of 353 K and extraction time of 150 minutes. Figure 6-4 demonstrates the interactions between temperature and flow rate with pressure and time set at 40 MPa and 150 minutes. There were also interactions between pressure and flow rate, extraction temperature and flow rate as shown in these figures. At high pressures and/or low temperatures, the oil yield always increased with increasing the flow rate. However, at low-pressures and/or high temperatures, the oil yield increased firstly and then decreased with increasing flow rate. Some other authors also found that increasing the flow rate of supercritical carbon dioxide led to a higher yield, but further increasing the flow rate beyond an optimal point could lead to a lower yield (Kumoro and Hasan, 2007, Rozzi et al., 2002). Rozzi et al. (2002) examined the effect of supercritical carbon
dioxide flow rate on lycopene extraction from tomato seeds and skins and they found that the yield of lycopene decreased as the flow rate increased from 2.5 ml/min to 15 ml/min. They interpreted that the lower amount of lycopene extracted at higher flow rate could be attributed to the reduced contact time between solvent and the samples. They also believed that this phenomenon should be attributed to the channelling effect, where the supercritical carbon dioxide solvent at high flow rates would just flow around the samples and was not able to diffuse through the pores within the samples. Furthermore, the increased flow rate could cause the sample to compact and thus limit CO$_2$ movement into and out of the sample matrix. As a result, the amount of CO$_2$ in contact with samples could be reduced. Kumoro et al. (2007) also observed that a too high flow rate could decrease the yield of the Andrographolide extracted from *Andrographic paniculata*. They explained that increasing the flow rate above its optimum value would reduce the residence time and cause the system to deviate from its equilibrium state; as a result, the solvent exited the extractor unsaturated. This was because there was intra-particle diffusion or an external film resistance in the extraction process, as a result the excess solvent just simply bypassed the samples and did not penetrate through the pores of the sample particles. In the present work, it was believed that the flow rate effect on the oil yield was combination effects of a mass transfer process and a thermodynamic equilibrium state. This is because the extraction process normally involves four steps as shown in Figure 6-5: (1) the diffusion of the supercritical fluid into the matrix; (2) the dissolution of the extracted substance into the supercritical fluid; (3) the diffusion of the substance and the supercritical fluid into the outside of the solid surface; and (4) the flow away of the supercritical fluid and substance mixture from the matrix. Supercritical CO$_2$ had a high density at high pressures or low temperatures, it was easy for the oil to be dissolved into the solvent and reached its equilibrium state. Thus the mass transfer resistance, not the solubility,
controlled the extraction process over the range of experimental conditions investigated here. Therefore, the oil yield always increased with increasing the flow rate at high pressures or low temperatures. However, when extracted at low pressures or high temperatures, the solubility power of the supercritical CO\textsubscript{2} was not enough to dissolve the oil and a longer residence time would be required for the solvent to become saturated with oil. However, due to the intra-particle diffusion resistance in the extraction process, a further increase in the flow rate could easily cause the channelling effect and result in decreased yields.

Figure 6-3 The three-dimensional plot of the response surface for the oil yield as related to flow rate and pressure at the fixed temperature of 353 K and extraction time of 150 minutes
Figure 6-4 The three-dimensional plot of the response surface for the oil yield as related to flow rate and temperature at 40 MPa and 150 minutes

Figure 6-5 A schematic diagram of the supercritical fluid extraction process

Figure 6-6 shows the three-dimensional plot of the response surface for the oil yield as related to extraction pressure and time. As can be seen, extraction time exhibited an important effect on extraction efficiency. This might be due to the time required for supercritical CO$_2$ to penetrate into the sample, dissolve the oil and subsequently diffuse...
A longer extraction time presented a positive effect on the oil yield. However, it did not continue to increase significantly until the extraction time was over 120 minutes. Although prolonging the contact time led to a slight increase in the oil yield, further increasing the extraction time up to 150 minutes resulted in only a little change in the oil yield. From Figure 6-6, it can be seen that at lower-pressure levels, extraction time was long when the oil yield reached a high value, however, at higher-pressure levels, extraction time was short when the oil reached a high value.

Figure 6-6 The three-dimensional plot of the response surface for the oil yield as related to time and pressure at temperature of 353 K and flow rate of 5.52 g/min

6.1.2 Chemical composition of Moringa oleifera seed oil

The fatty acid compositions of the Moringa oleifera seed oil at different operation conditions are present in Table 6-3. It can be seen that Moringa oleifera seed oil contained mainly unsaturated fatty acids (UFA). Of the identified components in the analysed extracts, oleic acid was a particular important monounsaturated omega-9 fatty acid.
acid because of its palatability, stability and health-promoting properties (Abdulkarim et al., 2007, Eduardo, 2010). Oleic acid has well-known benefits in the treatment and prevention of heart disease. Recent studies also found that oleic acid content might regulate genes that trigger cancer and lower cholesterol levels which thereby could reduce the risk of cardiovascular disease (Menendez et al., 2006). On the other hand, the total saturated fatty acids were the main undesired components in the extracted oil, as too much of these saturated fatty acids could increase the cholesterol level and risk of heart disease (Uffe, 1998). As noticed from Table 6-3, the fatty acid contents varied under different operation conditions, therefore the effects of operating conditions on the contents of oleic acid and total saturated fatty acids were investigated and the results are illustrated in Figure 6-7.

As shown in Figure 6-7, at low pressures and temperatures, more oleic acid and total unsaturated fatty acids were extracted. As the pressure and temperature increased, the content of saturated fatty acid increased as shown by an increase in the saturated/unsaturated ratios. Yu et al. (1994) suggested that the molecular weight of the fatty acids (carbon chain length) was a more important factor than the degree of unsaturation affecting their solubility in supercritical CO₂. As most of the saturated fatty acids in Moringa oleifera seed oil were long carbon chain fatty acids, such as stearic acid (C18:0), arachidic acid (C20:0), behenic acids (C22:0) and lignoceric acid (C24:0), thus, as the temperature and pressure increased, the solubility of these long chain saturated fatty acids increased. On the other hand, the solubility of the shorter chain of oleic acid decreased simultaneously. It was also noticed from Figure 6-7 that the total amount of saturated fatty acids in the extract increased with extraction time and flow rate, while the percentage of oleic acid and total unsaturated fatty acids decreased with increasing extraction time and flow rate.
Table 6-3  Fatty acid composition (percent fatty acid) of *Moringa oleifera* seed oil extracted by supercritical fluid extraction (SFE) under different conditions

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<th>C16:0</th>
<th>C16:1</th>
<th>C18:0</th>
<th>C18:1 9cis</th>
<th>C18:1 11cis</th>
<th>C18:2</th>
<th>C18:3</th>
<th>C20:0</th>
<th>C20:1</th>
<th>C22:0</th>
<th>C24:0</th>
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<th>UFA</th>
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<td>1.76</td>
<td>5.50</td>
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*C14:0, myristic acid; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1(9cis), oleic acid; C18:1(11cis), cis-vaccenic acid; C18:2, linoleic acid; C18:3, linolenic acid; C20:0, arachidic acid; C20:1, eicosenoic acid; C22:0, behenic acids; C24:0, lignoceric acid; SFA, saturated fatty acids; UFA, unsaturated fatty acids; Ω9, oleic acid + eicosenoic acid*
Figure 6-7  Contour plot showing the effects of operating parameter on fatty acid composition (A) Oleic acid composition vs. pressure and temperature at extraction time of 90 min, flow rate of 5.52 g/min (B) Total saturated fatty acid composition vs. pressure and temperature at extraction time of 90 min, flow rate of 5.52 g/min (C) Oleic acid composition vs. time and flow rate at temperature of 353 K, pressure of 40 MPa (D) Total saturated fatty acid composition vs. time and flow rate at temperature of 353 K, pressure of 40 MPa
6.2 Parametric study of Moringa oleifera leaf oil extraction

6.2.1 Yields of Moringa oleifera leaf oil

In order to study the effects of multiple variables on the extraction yield of *Moringa oleifera* leaf oil, an orthogonal array design was used to arrange the experiments and optimise the extraction process. The extraction yields at different sets of pressure, temperature and time were studied under an OA9 ($3^3$) design. Nine experiments were carried out at three levels of pressures (30, 40 50 MPa), temperatures (313, 333, 353 K), and dynamic times of 60, 90, 120 minutes. The results presented in Table 6-4 are the average of two measurements under the same conditions as well as the standard deviation of the measurements. The results shown in Table 6-4 indicate that there were substantial differences in the oil yield obtained under different SFE conditions. The oil yield of Run 1 to Run 9 ranged from 4.0% to 6.3%. A further orthogonal analysis was conducted; the K, k and R values were calculated and also listed in Table 6-4. The K value is the sum of the evaluation indices of all levels in each factor and k (the mean value of K) determines the optimal level and the optimal combination of factors. The optimal level for each factor could be obtained when k is the largest. The R value indicates the range between the maximum and minimum values of k and evaluates the importance of the factor. A larger R means a greater importance of the factor (Wu and Leung, 2011). According to the R values presented in Table 6-4, the most significant parameter affecting the extraction yield was temperature, followed by pressure and time. The highest yield of 6.34% was obtained under the conditions of pressure 50 MPa, temperature 333 K and time of 120 minutes.
Table 6-4  Experimental results for supercritical fluid extraction of *Moringa oleifera* leaf oil by three level orthogonal design

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<th>Time (min)</th>
<th>Yield (%)</th>
<th>Standard deviation</th>
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K values

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^a K_1 = \sum_{i=1}^{3} \text{extraction yield of oil at A}_i, \text{A stands for pressure, temperature and time, } i \text{ is from 1 to 3}

^b k_1 = K_1/3

^c R = \max\{k_1\} - \min\{k_1\}
6.2.2 Effect of SFE conditions on extraction yield

To evaluate the influence of operating factors, namely, pressure, temperature and time, on the extraction yield, the variation in the oil yield as a function of the changes in the different levels of the factors is depicted in Figure 6-8, with the error bars showing the standard deviation of the two measurements. As expected, the extraction yield was enhanced significantly with increasing pressure. This was because raising the extraction pressure at a constant temperature led to a higher fluid density thus increased the solubility of the extracted oil.

Temperature affects the density of the fluid, the volatility of the extract components and desorption of the extracts from the matrix. It can be observed from Figure 6-8b that increasing temperature from 313 K to 333 K resulted in a significant increase in the extraction yield, while further increasing temperature from 333 K to 353 K resulted in only a small increase in the oil yield. This can be explained as that at a higher temperature, although the fluid density decreased, the solubility power of the fluid still increased. The extraction time had a positive influence on the extraction yield as shown in Figure 6-8c. The extraction yield gradually increased with increasing time, and reached a maximum at 120 minutes within the tested range.

Table 6-5 shows that there were 12 components detected in all the SFE extracted oils and the oils obtained by SFE under different conditions had similar compositions. However, the quantitative compositions of the products were quite different, especially for the SFE run 8 under conditions of 50 MPa, 333 K and 120 minutes. Under this condition, the main components identified in the extracted oil were 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester (76.23%), followed by nonacosane (13.37%), heptacosane (4.98%) and β-amyrin (1.50%). However, in the other SFE experiments, the major constituent in the extracted oil was nonacosane, which accounted for nearly 50%. Other main components identified in those samples were
pentacosane, heptacosane, dimethoate, n-hexadecanoic acid, γ-tocopherol, sulfurous acid, hexyl pentadecyl ester, dl-α-tocopherol, 1,30-triacontanediol, γ-sitosetrol and β-amyrin. It is interesting to note that the extractions of cuticular waxes, represented by the long chain alkanes, were verified in all the experiments. The most abundant alkanes were nonacosane (13.37%-60.06%), pentacosane (1.00%-6.32%), and heptacosane (4.98%-22.66%). This could be explained by the fact that cuticular waxes were located on the surface of the plant and were readily available for extraction and faced little mass transfer resistance, while the oil compounds might be in the internal part of the leaves and the mass transfer mechanism was related to the internal diffusion stages (Aghel et al., 2004). However, no general tendency was observed regarding the effect of extraction conditions on the percentage of these alkenes, except in the case of experimental 8. This was probably because the mass-transfer mechanisms of the cuticular waxes and the oil components were quite different. Under the SFE run 8 condition, the fluid with a very high pressure and a mild temperature, the extraction time was very long, thus the dissolution power of the supercritical CO₂ under this condition was very high and more readily able to overcome the larger mass transfer resistances of the components in the internal parts of the leaves, therefore, it increased the extraction of the oil components and the extraction under this condition produced the highest yield of Moringa oleifera leaf oil (Carlson et al., 2001).
Figure 6-8  Effect of pressure (a), temperature (b) and extraction time (c) on the yield of the extracted oil from *Moringa oleifera* leaves
Table 6-5  The compositions (wt%) of compounds from *Moringa oleifera* leaf oil obtained by supercritical CO$_2$ extractions from three level orthogonal design

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Retention index</th>
<th>Component</th>
<th>SFE1</th>
<th>SFE2</th>
<th>SFE3</th>
<th>SFE4</th>
<th>SFE5</th>
<th>SFE6</th>
<th>SFE7</th>
<th>SFE8</th>
<th>SFE9</th>
</tr>
</thead>
<tbody>
<tr>
<td>67.973</td>
<td>1708</td>
<td>dimethoate</td>
<td>1.48</td>
<td>0.66</td>
<td>0.90</td>
<td>1.63</td>
<td>1.03</td>
<td>0.94</td>
<td>1.77</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>86.93</td>
<td>1966</td>
<td>n-hexadecanoic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.31</td>
<td></td>
<td>0.62</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>112.38</td>
<td>2510</td>
<td>pentacosane</td>
<td>5.09</td>
<td>3.88</td>
<td>4.28</td>
<td>5.53</td>
<td>3.57</td>
<td>4.32</td>
<td>6.32</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>114.0047</td>
<td>2558</td>
<td>1,2-benzenedicarboxylic acid, mono(2-ethylhexyl)ester</td>
<td>0.51</td>
<td>0.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>76.23</td>
<td></td>
</tr>
<tr>
<td>119.844</td>
<td>2725.9</td>
<td>heptacosane</td>
<td>20.00</td>
<td>21.35</td>
<td>20.86</td>
<td>21.86</td>
<td>21.80</td>
<td>19.72</td>
<td>22.66</td>
<td>4.98</td>
<td>20.52</td>
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<tr>
<td>126.724</td>
<td>2924.3</td>
<td>nonacosane</td>
<td>54.98</td>
<td>60.06</td>
<td>54.80</td>
<td>51.99</td>
<td>55.51</td>
<td>49.74</td>
<td>49.71</td>
<td>13.37</td>
<td>48.97</td>
</tr>
<tr>
<td>131.169</td>
<td>3052.3</td>
<td>γ-tocopherol</td>
<td>1.34</td>
<td>1.55</td>
<td>2.16</td>
<td>1.87</td>
<td>2.35</td>
<td>3.38</td>
<td>1.84</td>
<td>0.41</td>
<td>3.54</td>
</tr>
<tr>
<td>133.089</td>
<td>3108</td>
<td>sulfurous acid, hexyl pentadecyl ester</td>
<td>1.44</td>
<td>0.95</td>
<td>0.84</td>
<td>1.40</td>
<td>0.93</td>
<td>1.18</td>
<td>0.79</td>
<td>0.24</td>
<td>0.96</td>
</tr>
<tr>
<td>133.73</td>
<td>3126.7</td>
<td>dl-α-tocopherol</td>
<td>2.67</td>
<td>1.42</td>
<td>1.83</td>
<td>2.97</td>
<td>1.89</td>
<td>2.40</td>
<td>3.52</td>
<td>0.57</td>
<td>3.00</td>
</tr>
<tr>
<td>137.421</td>
<td>3232.8</td>
<td>1,30-triacontanediol</td>
<td>2.82</td>
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<td>6.03</td>
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<td>6.14</td>
<td>10.05</td>
<td>3.20</td>
<td>0.86</td>
<td>7.61</td>
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<tr>
<td>138.639</td>
<td>3267.8</td>
<td>γ-sitosterol</td>
<td>1.54</td>
<td>0.97</td>
<td>1.70</td>
<td>2.77</td>
<td>1.31</td>
<td>2.74</td>
<td>2.85</td>
<td>0.47</td>
<td>2.95</td>
</tr>
<tr>
<td>139.232</td>
<td>3283.9</td>
<td>β-amyrin</td>
<td>8.14</td>
<td>4.83</td>
<td>5.51</td>
<td>6.47</td>
<td>5.48</td>
<td>5.53</td>
<td>6.72</td>
<td>1.5</td>
<td>6.18</td>
</tr>
</tbody>
</table>
6.3 Parametric study of Eucalyptus leaf oil extraction

6.3.1. Response surface analysis

To study the effect of pressure, temperature and extraction time on the yield of the Eucalyptus leaf oil; the response surface methodology was used to arrange the experiments and to optimize the extraction process. This design includes 20 experimental runs including 6 replicated of the central points. The pressure ranging from 10-50 MPa, temperature ranging from 313-353 K and extraction time 30-150 minutes were chosen in this study. Table 6-6 shows the complete design matrix together with the response variable (the yield of oil) obtained from the experiments. The oil yield ranged from 0.99% to 4.78%. The maximum yield was obtained under pressure 40 MPa, temperature 343 K and extraction time of 120 minutes.

The SFE experimental data was analysed using the analysis of variance (ANOVA) to assess the “goodness of fit”, which is listed in Table 6-7. As shown in this table, the probability (p) value of the quadratic equation was less than 0.0001 which implied that the equation was an excellent fit and was very suitable for the present experimental data. A statistically significant confidence level at 99.999% was obtained. A “lack of a fit” measures the failure of the equation to represent data in the experimental domain at points which are not included in the regression. If the p-value of the “lack of fit” was smaller than 0.05, the model did not fit the data well (Stigler, 2008). The probability value of “lack of fit” was 0.0507, suggesting that the model was acceptable (Stigler, 2008). The coefficient of determination (R²) obtained from the calculated quadratic equation should also be taken into consideration when validating the model. The R² value at 0.9537 was considered very good, indicating that this equation adequately represented the real relationship among the parameters chosen. Furthermore, each experiment was repeated and the statistical analysis was performed so that the
experimental results could be used to valid the model with confidence which was consistent with the report from the literature (Cui et al., 2011).

Table 6-6  Experimental results for supercritical fluid extraction of *Eucalyptus* leaf oil by central composite design

<table>
<thead>
<tr>
<th>Run</th>
<th>Factors</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$X_1$ (Pressure, MPa)</td>
<td>$X_2$ (Temperature, K)</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>343</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>333</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>323</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>333</td>
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<tr>
<td>5</td>
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<td>333</td>
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<tr>
<td>6</td>
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<td>333</td>
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<td>7</td>
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<td>8</td>
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<td>323</td>
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<td>333</td>
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<td>18</td>
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<td>333</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>313</td>
</tr>
</tbody>
</table>
Table 6-7 Analyses of variance of the regression parameters

<table>
<thead>
<tr>
<th>Source</th>
<th>Degree of freedom</th>
<th>Regression coefficient</th>
<th>F-value</th>
<th>Probability(p)</th>
<th>significances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>9</td>
<td></td>
<td>22.91</td>
<td>&lt;0.0001</td>
<td>significant</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>5</td>
<td></td>
<td></td>
<td>0.0507</td>
<td>Not significant</td>
</tr>
<tr>
<td>$R^2$=0.9537</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| $X_1$ pressure | 1                 | 0.78                   | 127.30  | <0.0001        |               |
| $X_2$ temperature | 1             | 0.18                   | 7.06    | 0.0240         |               |
| $X_3$ time     | 1                 | 0.35                   | 25.27   | 0.0005         |               |
| $X_1X_2$       | 1                 | 0.32                   | 10.38   | 0.0091         |               |
| $X_1X_3$       | 1                 | 0.01                   | 0.02    | 0.8813         |               |
| $X_2X_3$       | 1                 | -0.11                  | 1.32    | 0.2767         |               |
| $X_1^2$        | 1                 | -0.32                  | 33.26   | 0.0002         |               |
| $X_2^2$        | 1                 | -0.05                  | 0.84    | 0.3807         |               |
| $X_3^2$        | 1                 | -0.13                  | 5.50    | 0.0410         |               |

The coefficients of the regression equation for the *Eucalyptus* leaf oil yield data are shown in Table 6-7. Fitting the constants and coefficients into equation (6-3), the regression equation as a function of pressure, temperature and time thus could be obtained as follows (in terms of coded levels):

$$Y = 4.12 + 0.78X_1 + 0.18X_2 + 0.35X_3 + 0.32X_1X_2 + 0.01X_1X_3 - 0.11X_2X_3$$

$$-0.32X_1^2 - 0.05X_2^2 - 0.13X_3^2,$$

(6-3)

where $Y$ is the extraction yield of *Eucalyptus* leaf oil (%, dry weight of plant materials), $X_1$ is the extraction pressure, $X_2$ is the extraction temperature and $X_3$ is the extraction time.
The significance of each coefficient was also determined by F value and p value as listed in Table 6-7. A value of p<0.05 indicates that the equation term is significant. In this case, $X_1$, $X_2$, $X_3$, $X_1X_2$, $X_1^2$, and $X_3^2$ were all significant equation terms. The larger magnitude of F-values and the smaller p-values mean a higher significance of the corresponding coefficient. The influence on the oil yield decreased in the order of pressure > extraction time > temperature based on the F-values and p-values as shown in Table 6-7. Eliminating the terms that were not significant in the second order polynomial equation, the prediction equation might be rearranged to the following equation (in terms of coded levels):

$$Y = 4.12 + 0.78X_1 + 0.18X_2 + 0.35X_3 + 0.32X_1X_2 - 0.32X_1^2 - 0.13X_3^2.$$  \hspace{1cm} (6-4)

The best way to visualize the influence of the independent variables on the oil yield is to generate 3-dimensional response surface plots by varying two variables while holding the other one constant, as shown in Figure 6-9 and Figure 6-10.

![Figure 6-9](image-url)  
**Figure 6-9** Response surface for the oil yield as related to temperature and pressure at fixed 90 minutes extraction
Figure 6-9 describes the interactive effect of pressure and temperature on the oil yield. The pressure and temperature are the main parameters that influence the extraction efficiency. It was observed that at a given temperature, the oil yield increased significantly with increasing pressure, especially at low pressure levels. This is because raising the extraction pressure at a constant temperature led to a higher fluid density thus increased the solubility of the *Eucalyptus* leaf oil. It is also noted that the increase in oil yield with increasing pressure became more apparent as the temperature increased. For example, as pressure increased from 20 MPa to 40 MPa, the oil yield increased from 2.99% to 3.39% at 323 K and from 2.51% to 4.66% at 343 K. Such increments in yields were due to the interactions between the pressure and temperature. However, there was a negative quadratic effect at high pressures as shown in Table 6-7, This was probably a reflection of the increased repulsive solute-solvent interactions resulted from the highly compressed CO$_2$ at high pressures (Liu *et al.*, 2009). Furthermore, although at a constant temperature, the increase in pressure would cause an increase in fluid density, the fluid diffusion coefficient would decrease. These counteracting effects result in higher pressures having little effect on the oil yield (Wang *et al.*, 2012, Şanal *et al.*, 2005). Thus, a very high pressure is not always recommended for SFE.

It was very interesting to note that the effect of temperature on the oil yield showed different trends at different pressures. On one hand, increasing temperature reduces the density of CO$_2$ and thus its solvation power at a constant pressure. On the other hand, raising temperature increases the solute vapour pressure, resulting in enhanced supercritical carbon dioxide solubility. Therefore, the solubility of supercritical carbon dioxide may increase, remain constant, or decrease with rising temperature at a constant pressure, depending on whether solute vapour pressure or solvent density dominates at the particular pressure (Wang *et al.*, 2012). From Figure 6-9, it can be seen that at low
pressures, increasing temperature resulted in a decrease in the oil yield. However at higher pressures the oil yield increased with temperature. The oil yield decreased from 3.50% to 3.06% when temperature increased from 323 K to 343 K at 20 MPa. However at 40 MPa the same temperature increment corresponded to a change in the oil yield from 4.45% to 4.78%. This was because the solvent density reduction at lower pressures was more influential than that at higher pressures. For example, the density of supercritical \( \text{CO}_2 \) reduced 0.13 g/ml from 323 to 343 K at 20 MPa, while the reduction was only 0.07 g/ml at 40 MPa. A similar trend was also reported in the literature on supercritical fluid extraction of \textit{Cyperus rotundus} Linn essential oil using the response surface method (Wang et al., 2012).

Figure 6-10 Response surface for the oil yield as related to pressure and time and temperature of 333K

Figure 6-10 presents the response surface plots showing the effect of pressure and extraction time on the oil yield at a fixed temperature of 333 K. How pressure affected the oil yield was described in Figure 6-9. As can be seen from Figure 6-10, the
extraction time exhibited an important effect on the extraction efficiency, clearly due to the time required for supercritical CO\textsubscript{2} to penetrate into the substrate matrix, dissolve the oil and subsequently diffuse out from the material. A longer extraction time had a positive effect on the oil yield. However, the extraction yield did not increase further with longer extraction time. As shown in Figure 6-10, a further increase in the extraction time from 120 minutes to 150 minutes resulted in little change in the oil yield. As discussed in Section 6.1.1, there are two periods in the extraction process; the extraction rate was limited by the intra-particle diffusion of the solute in the second periods. Therefore, the extraction yield increased very slow even with longer extraction time.

6.3.2 Chemical compositions of *Eucalyptus* leaf oil and selectivity of 1,8-cineole

In order to evaluate the quality of *Eucalyptus* leaf oil and the selectively of the 1,8-cineole obtained under different conditions, the chromatographic analysis of the oil obtained under each condition was carried out. Table 6-8 shows the comparative compositions of the *Eucalyptus* leaf oil obtained under different conditions. It can be observed from Table 6-8 that *Eucalyptus* leaf oil was comprised of many chemical compounds, most of them in small quantities. The compounds present in higher quantities were the same for all the samples which were: 1,8-cineole, verbenone, piperitone, (+)-carvone, α-terpinyl acetate, 1,2-cyclohexanediol, 1-methyl-4-(1-methylethenyl)-, (+)-ledene, butylated hydroxytoluene, (-)-spathulenol, caryophyllene oxide, globulol, cubenol, alloaromadendrene oxide, squalene, 1,1'-bicyclopentyl, 2-hexadecyl-, (Z)-14-Tricosenyl formate, vitamin E, 1,30-triacontanediol and γ-sitosterol.
Table 6-8  The compositions (wt%) of compounds from *Eucalyptus* leaf oil obtained by supercritical CO₂ extraction by centre composite design

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Compound</th>
<th>SFE1</th>
<th>SFE2</th>
<th>SFE3</th>
<th>SFE5</th>
<th>SFE6</th>
<th>SFE7</th>
<th>SFE8</th>
<th>SFE9</th>
<th>SFE11</th>
<th>SFE12</th>
<th>SFE14</th>
<th>SFE17</th>
<th>SFE18</th>
<th>SFE20</th>
<th>Central point</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.186</td>
<td>α-pinene</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01±0.00</td>
<td></td>
</tr>
<tr>
<td>14.833</td>
<td>β-pinene</td>
<td>0.03</td>
<td>0.02</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.05</td>
<td>0.05</td>
<td>0.03</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
<td>0.03</td>
<td>0.02±0.01</td>
<td></td>
</tr>
<tr>
<td>16.009</td>
<td>2,3-dehydro-1,8-cineole</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02±0.00</td>
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</tr>
<tr>
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<td>18.884</td>
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<td>46.19</td>
<td>44.72</td>
<td>57.55</td>
<td>55.16</td>
<td>49.41</td>
<td>71.17</td>
<td>63.14</td>
<td>56.38</td>
<td>56.94</td>
<td>36.48</td>
<td>60.49</td>
<td>54.64</td>
<td>41.50</td>
<td>53.85</td>
<td>49.95±3.5</td>
</tr>
<tr>
<td>26.168</td>
<td>L-pinocarveol</td>
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<td>0.23</td>
<td>0.20</td>
<td>0.19</td>
<td>0.20</td>
<td>0.27</td>
<td>0.20</td>
<td>0.21</td>
<td>0.25</td>
<td>0.19</td>
<td>0.20</td>
<td>0.18</td>
<td>0.22</td>
<td>0.24</td>
<td>0.22±0.04</td>
</tr>
<tr>
<td>26.746</td>
<td>cis-verbenol</td>
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<td>0.17</td>
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<td>0.17</td>
<td>0.18</td>
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<td>0.15</td>
<td>0.16</td>
<td>0.16</td>
<td>0.18</td>
<td>0.19</td>
<td>0.17±0.01</td>
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| 1,4-methanoazulen-7-ol, decahydro-1,5,5,8a-tetramethyl-,[1s-(1α,3αβ,4α,7β,8aβ)]-tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl- 
<p>| 1,5,5,8a-1,5,δ-utenal, 2-dimethylol, 2-hydroxymethylvinyl | 0.24   | 0.28   | 0.11   | 0.24   | 0.24   | 0.24   | 0.16   | 0.25   | 0.29   | 0.23   | 0.24   | 0.15   | 0.27   | 0.28   | 0.25±0.03|
| 61.952 | 0.55   | 0.60   | 0.61   | 0.47   | 0.55   | 0.18   | 0.38   | 0.51   | 0.62   | 0.55   | 0.49   | 0.52   | 0.63   | 0.58   | 0.57±0.08|
| 62.338 | 0.23   | 0.22   | 0.27   | 0.22   | 0.21   | 0.23   | 0.19   | 0.22   | 0.25   | 0.20   | 0.22   | 0.20   | 0.23   | 0.25   | 0.22±0.02|
| 62.917 | 0.30   | 0.29   | 0.38   | 0.26   | 0.27   | 0.03   | 0.18   | 0.28   | 0.31   | 0.26   | 0.25   | 0.24   | 0.30   | 0.29   | 0.28±0.04|
| 65.399 | 0.26   | 0.32   | 0.33   | 0.24   | 0.27   | 0.06   | 0.20   | 0.25   | 0.29   | 0.27   | 0.25   | 0.25   | 0.30   | 0.31   | 0.28±0.04|
| 65.800 | 0.10   | 0.32   | 0.22   | 0.11   | 0.19   | 0.03   | 0.06   | 0.04   | 0.04   | 0.28   | 0.07   | 0.16   | 0.08   | 0.15   | 0.22±0.14|
| 66.409 | 0.44   | 0.51   | 0.36   | 0.24   | 0.40   | 0.06   | 0.20   | 0.30   | 0.42   | 0.44   | 0.30   | 0.34   | 0.47   | 0.45   | 0.42±0.10|
| 66.601 | 0.41   | 0.42   | 0.38   | 0.34   | 0.40   | 0.08   | 0.27   | 0.38   | 0.47   | 0.39   | 0.36   | 0.32   | 0.42   | 0.44   | 0.41±0.05|
| 66.968 | 0.33   | 0.37   | 0.33   | 0.27   | 0.32   | 0.08   | 0.21   | 0.28   | 0.34   | 0.34   | 0.28   | 0.27   | 0.37   | 0.35   | 0.34±0.06|
| 68.918 | 0.24   | 0.32   | 0.35   | 0.25   | 0.30   | 0.07   | 0.19   | 0.24   | 0.36   | 0.33   | 0.26   | 0.30   | 0.29   | 0.26   | 0.31±0.09|</p>
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<td>3.41</td>
<td>0.80</td>
<td>1.02</td>
<td>1.87</td>
<td>0.91</td>
<td>6.51</td>
<td>1.57</td>
<td>2.74</td>
<td>3.90</td>
<td>1.39</td>
<td>3.13±0.70</td>
</tr>
<tr>
<td>132.214</td>
<td>γ-sitosterol</td>
<td>1.04</td>
<td>1.28</td>
<td>0.25</td>
<td>0.25</td>
<td>1.09</td>
<td>0.31</td>
<td>0.75</td>
<td>0.75</td>
<td>1.22</td>
<td>0.33</td>
<td>1.22</td>
<td>1.42</td>
<td>0.99</td>
<td>0.99±0.11</td>
<td></td>
</tr>
</tbody>
</table>
Among all the compounds, 1,8-cineole was the main and most important oxygenated monoterpenes in the *Eucalyptus* leaf oil, as it determined the oil quality. Therefore, the selectivity of 1,8-cineole compared to all the other compounds present in the samples was studied. The selectivity of 1,8-cineole was defined and calculated as follows:

\[
S = \frac{\text{concentration of 1,8-cineole}}{\text{total concentration of all the other compounds}}.
\]  

(6-5)

<table>
<thead>
<tr>
<th>Run</th>
<th>Factors</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X₁ (Pressure, MPa)</td>
<td>X₂ (Temperature, K)</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>343</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>333</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>323</td>
</tr>
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<td>4</td>
<td>30</td>
<td>333</td>
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<td>5</td>
<td>30</td>
<td>333</td>
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<td>6</td>
<td>30</td>
<td>333</td>
</tr>
<tr>
<td>7</td>
<td>10</td>
<td>333</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>323</td>
</tr>
<tr>
<td>9</td>
<td>40</td>
<td>323</td>
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<td>15</td>
<td>30</td>
<td>333</td>
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<td>16</td>
<td>30</td>
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<td>40</td>
<td>323</td>
</tr>
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<td>18</td>
<td>40</td>
<td>343</td>
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<td>30</td>
<td>333</td>
</tr>
<tr>
<td>20</td>
<td>30</td>
<td>313</td>
</tr>
</tbody>
</table>
Table 6-10 Regression coefficients and corresponding t and p-values for the selectivity of 1,8-cineole

<table>
<thead>
<tr>
<th>source</th>
<th>Degree of freedom</th>
<th>Regression coefficient</th>
<th>F-value</th>
<th>Probability(p)</th>
<th>significances</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>9</td>
<td></td>
<td>13.15</td>
<td>0.0002</td>
<td>significant</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>5</td>
<td></td>
<td>2.74</td>
<td>0.1464</td>
<td>Not significant</td>
</tr>
<tr>
<td>( R^2 = 0.922 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( X_1 \) pressure 1 -0.32 65.18 <0.0001
\( X_2 \) temperature 1 -0.15 13.20 0.0046
\( X_3 \) time 1 -0.082 4.17 0.0683
\( X_1 X_2 \) 1 -0.089 2.48 0.1461
\( X_1 X_3 \) 1 0.041 0.53 0.4845
\( X_2 X_3 \) 1 0.011 0.037 0.8515
\( X_1^2 \) 1 0.17 29.43 0.0003
\( X_2^2 \) 1 -0.019 0.34 0.5715
\( X_3^2 \) 1 0.04 1.53 0.2447
The selectivity of 1,8-cineole under every condition is listed in Table 6-9. The extraction performed at 10 MPa and 333 K was considered the best condition since it had the highest selectivity of 1,8-cineole, which meant a good quality of *Eucalyptus* leaf oil with large amount of 1,8-cineole. At conditions of pressure 40 MPa, temperature 343 K and extraction time 120 minutes, although the yield was high, the extraction had a lower selectivity of 1,8-cineole.

From the multiple regression analysis as shown in Table 6-10, both pressure and temperature had significant effect on the selectivity while the extraction time had less effect on 1,8-cineole’s selectivity. The effect of pressure and temperature on the selectivity of 1,8-cineole is shown in Figure 6-11. It can be observed that an increase in temperature resulted in a decrease in the selectivity of 1,8-cineole. Although the increase in pressure promoted an increase in yield, this increase associated with the reduction in selectivity. This can be highlighted by the fact that at 323 K, the increase in pressure from 20 MPa to 40 MPa led to an decrease in the selectivity of 1,8-cineole.
from 1.7126 to 1.2926. Moreover, high levels of 1,8-cineole’s selectivity were obtained at low temperatures. This was because heavier compounds, with retention time longer than 66 minutes (under the chromatographic conditions employed), were found in all the extracted samples. The extraction of heavy compounds was favoured by the larger density values of carbon dioxide under high pressures, correspondingly, the content of the relatively lighter compound of 1,8-cineole reduced.

Similar findings have been reported for the selectivity of some terpenes and oxygenated terpenes. It was reported (Reverchon, 1997) that at low CO$_2$ densities (T=313-323 K, P=8-9 MPa), terpenes were one or more orders of magnitude more soluble than heavy paraffins. The other families of compounds showed negligible solubilities. However, at high CO$_2$ densities (T=313-323 K, P=10-20 MPa), although terpenes and oxygenated terpenes were completely miscible in supercritical CO$_2$, the solubilities of many other compound families increased from appreciable level to very high level. Therefore, the selectivity of the terpenes reduced with the CO$_2$ density increased. Reverchon et al. (1994) also conducted a comparison of the supercritical fluid extraction of peppermint leaves at two different conditions: 9 MPa and 323 K, and 12 MPa and 313 K. On increasing the CO$_2$ density, oxygenated monoterpenes in the extracts decreased from 84.4% to 72.7%, whereas sesquiterpenes increased from 4.6% to 13.2%. Moreover, the co-extraction of waxes increased from about 10% to 30% by weight compared to the yield of peppermint essential oil. In conclusion, as the high molecular weight compounds increased significantly with extraction pressure, high pressure was not favourable for the selectivity of lighter compounds.

**6.4 Conclusions**

The parametric studies of supercritical fluid extraction of *Moringa oleifera* seed oil and *Eucalyptus* leaf oil were conducted using response surface methodology. The orthogonal array design was used to study the effect of operating parameters on the
Moringa oleifera leaf oil extraction. It was found that pressure and temperature were the most significant parameters that influence the extraction efficiency. Increasing pressure increased the oil yield under any conditions. However for the temperature effect, increasing temperature could have either a positive or negative effect on the oil yield, depending on the levels of pressure investigated. It was observed that at low pressures, increasing temperature resulted in a decrease in the oil yield. However the oil yield increased with temperature at higher pressures. Furthermore, the extraction time also had a positive effect on the extraction yield and the oil yield increased with increasing extraction time.

On the other hand, the compositions of the extracted oil could be changed under different conditions. It was found that for Moringa oleifera seed oil, oleic acid and total unsaturated fatty acids decreased with increasing pressure and temperature, while for the Eucalyptus leaf oil, low CO$_2$ densities should be a good choice to extract volatile compounds selectively as compared to higher molecular-weight compounds. Therefore it was possible to fractionate different compounds under different extraction conditions. Also it was noticeable that the co-extraction of undesired high molecular-weight compounds was found for both the Moringa oleifera leaf oil and Eucalyptus leaf oil, this problem may be solved by introducing fractional separation of the extracted oil in the future.
Chapter 7 The Effect of Modifiers on Supercritical Fluid Extraction as Applied to *Moringa oleifera* Seeds and *Eucalyptus* Leaves

A disadvantage of supercritical carbon dioxide extraction is the low polarity of CO₂, which makes the extraction of polar compounds very difficult. However, the selectivity and solubility of polar compounds in supercritical CO₂ can be improved with the addition of co-solvents or modifiers such as ethanol or water at low concentrations. In this chapter, the effect of the use of a co-solvent or modifier on the supercritical fluid extraction process was investigated. Ethanol and water were chosen as the modifiers. The effect of the modifier on the yield and compositions of the oil extracted from *Moringa oleifera* seeds and *Eucalyptus* leaves was studied.

7.1 Effect of modifiers on the *Moringa oleifera* seed oil extraction

7.1.1 Effect of modifiers on the yield of *Moringa oleifera* seed oil

The introduction of a modifier can increase the extraction efficiency and consequently, reduce the extraction time and improve the recovery of different types of natural products from plant materials. But due to different interactions of the modifiers with the sample matrix, choosing a suitable modifier for a specific sample without experimentation is impossible. It was reported that a modifier might influence the extraction in three different ways depending on the types of the sample matrix and the affinity of the solute for the matrix: (1) increasing solvating power of the supercritical fluid as a result of the increased solvent density. (2) there were specific intermolecular interactions between the modifier and the solutes, such as H-bonding; and (3) that modifiers could swell the cellular structure of the matrix and thus distort the matrix-solute diffusion process and favour the penetration of the supercritical fluid inside the matrix (Casas *et al.*, 2007).
In this work, two different modifiers, namely, ethanol and water, were selected to evaluate their effects on the Moringa oleifera seed oil yield under conditions of 40 MPa, 353 K, CO$_2$ flow rate of 3.68 g/min and particle size below 1000µm. Ethanol and water were chosen as the modifiers as both of them can be used without concern for food safety. Four different ratios of ethanol ranging from 2.5w% to 10w% while two ratios of water at 2.5w% and 5w% were studied. Figure 7-1 shows the Moringa oleifera seed oil yields with different ethanol concentrations as well as with pure supercritical CO$_2$ fluid.

It can be seen from Figure 7-1 that under the same extraction conditions, the yield increased with increasing ethanol ratio. The extraction yield obtained using 10w% ethanol was improved by 29.8% compared with the extraction yield obtained using only supercritical carbon dioxide at an extraction time of 150 minutes.

In order to explain why the ethanol modified supercritical carbon dioxide could increase the yield of Moringa oleifera seed oil, the solubility parameter of the supercritical carbon dioxide with and without modifier was studied. The solubility
parameter can be quantitatively represented by a commonly applied concept, such as the Hildebrand solubility parameter (Hildebrand et al., 1970). The Hildebrand solubility parameter is a measure of the cohesive energy density of a substance and is useful for indicating the solvency behaviour of a specific solvent. Marcus (2006) established a useful correlation to estimate the solvent power of the supercritical solvents, which can be written as follows.

\[
\delta = 3.02 \times P_c^{1/2} \times \rho_r ,
\]  

(7-1)

where \(\delta\) is the Hildebrand solubility parameter, \(P_c\) is the critical pressure (MPa) and \(\rho_r\) is the reduced density (=\(\rho/\rho_r\)). For a mixture consisting of supercritical carbon dioxide and a modifier, the solubility parameter for the mixed fluid is determined as follows (Gaspar, 2002):

\[
\delta_{mix} = v_{co2}\delta_{co2} + v_m\delta_m ,
\]  

(7-2)

where \(v_{co2}\) and \(v_m\) are the volume fractions of supercritical carbon dioxide and modifier, \(\delta_{co2}\) and \(\delta_m\) are the Hildebrand solubilities of supercritical fluid and modifier.

Therefore, the solubility parameters of the modified CO\(_2\) mixture was calculated using equation 7-2 and listed in Table 7-1

<table>
<thead>
<tr>
<th>Pure CO(_2)</th>
<th>CO(_2) + 2.5w% ethanol</th>
<th>CO(_2) + 5w% ethanol</th>
<th>CO(_2) + 7.5w% ethanol</th>
<th>CO(_2) + 10w% ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility Parameter (\delta) ((MPa(^{1/2}))</td>
<td>14.41</td>
<td>14.59</td>
<td>14.76</td>
<td>14.93</td>
</tr>
</tbody>
</table>

As can be seen from Table 7-1, there is little difference in the solubility parameter of pure and modified supercritical carbon dioxide solvent. Thus the increased yield of
Moringa oleifera seed oil with ethanol could not be attributed to the increased solvating power of the supercritical fluid. The increased yield caused by the addition of ethanol could be mainly due to the improved polarity of the solvent and structure alteration of the matrix. On one hand, the polarity of the supercritical fluid changed by the addition of ethanol, thus some polar compounds such as phospholipids and glycolipids, which could not be extracted by CO₂ alone, became soluble. Similar results were reported by Sánchez-Camargo et al. (2012) who studied the effect of using supercritical carbon dioxide and ethanol on the extraction of lipids from Brazilian redspotted shrimp waste. On the other hand, the hydrogen bond interactions amongst ethanol and the oil components such as fatty acids in the Moringa oleifera seed oil also contributed to the higher oil solubility. This is because fatty acid (−COOH) is capable of participating in H-bonding interactions both as an H-bond acceptor and as a donor due to its carboxylic group; and ethanol also has both H-bond donor and acceptor properties; therefore, H-bonding between ethanol and these fatty acids could contribute to the higher oil yield. The H-bonding of acetic and palmitic acids in supercritical carbon dioxide with ethanol and stearic acid with ethanol have been demonstrated by using FT-IR spectroscopy (Güçlü-Üstündağ and Temelli, 2005). Secondly, it could also be due to the swelling of the cellular structure of the ground seeds which could facilitate supercritical carbon dioxide penetration to the oil rich area of the cell as reported by Nguyen et al. (2011). In their study, initially ethanol was added directly to the extractor and blended with supercritical carbon dioxide. As the amount of ethanol in the extractor was nearly exhausted within the first 30 minutes, they believed that the enhanced extraction yield was mainly due to the swelling of the structure since the increase in local density and polarity was minor in their experiments.
Figure 7-2 shows the effect of water as a modifier on the extraction yields of *Moringa oleifera* seed oil. It was found that as the ratio of water increased from 2.5w% to 5w%, the oil yield decreased. This was probably because higher water content caused excessive polarity of the supercritical solvent which was not suitable for *Moringa oleifera* seed oil extraction, thus the yield decreased. Additionally, according to the study of Lehotay (1997), the CO$_2$/water mixture at 353 K at 40 MPa exists as two separate phases of water and CO$_2$, respectively, because water is only slightly soluble in supercritical CO$_2$. Any attempt to create a higher water concentration than 0.3 (vol%) in supercritical CO$_2$ will result in two phases. In this study, as the water concentration 3.9 vol% was too high, the existence of the liquid water phase could be attributed to the low yield of the *Moringa oleifera* seed oil. On one hand, oil is hardly dissolved in the water phase and on the other hand, excess water remaining in the vessel could have plugged the material pores and even could have acted as a barrier to the transfer of the oil to the fluid (Lee *et al*., 2010).
7.1.2 Effect of modifiers on seed oil composition

The composition profiles of saturated and unsaturated fatty acids of *Moringa oleifera* seed oil obtained by the supercritical fluid extraction with different modifiers are presented in Table 7-2 and Figure 7-3.

It was observed that the saturated fatty acids and unsaturated fatty acid profiles of the oil extracted by using supercritical CO$_2$ and modified CO$_2$ with ethanol and water were very similar. The findings were similar to the results of the supercritical fluid extraction of peach seed oil using carbon dioxide and ethanol reported by Sánchez-Vicente *et al.* (2009). However, in Sánchez-Camargo *et al.’s* (2012) report about the extraction of omega-3 fatty acids from Brazilian redspotted shrimp waste using mixtures of supercritical CO$_2$ and ethanol, they indicated that the saturated fatty acid content of the lipid extracted decreased with increasing the percentage of ethanol. The opposite behaviour was observed with respect to the unsaturated fatty acids, in that their contents increased with increasing the amount of co-solvent used. The different observations between their study and the present results were probably because *Moringa oleifera* seed oil contains more monounsaturated fatty acids like oleic acid while shrimp residue contains more polyunsaturated fatty acids such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. Supercritical CO$_2$ was not selective in extracting the monounsaturated fatty acids with the different ethanol and supercritical carbon dioxide proportions. However, the effect of ethanol on the compositions of EPA and DHA was significant and enhanced as the ethanol concentration (more polar) increased because they contain more than one double bonds and have a higher polarity (Sánchez-Camargo *et al.*, 2012, Mezzomo *et al.*, 2010).
Table 7-2  Fatty acid contents of *Moringa oleifera* seed oil obtained by supercritical fluid extraction (SFE) with different concentration of modifiers

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Percentage of total fatty acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pure CO₂</td>
</tr>
<tr>
<td>saturated fatty acids</td>
<td>myristic acid (C14:0)</td>
</tr>
<tr>
<td></td>
<td>palmitic acid (C16:0)</td>
</tr>
<tr>
<td></td>
<td>stearic acid (C18:0)</td>
</tr>
<tr>
<td></td>
<td>arachidic acid (C20:0)</td>
</tr>
<tr>
<td></td>
<td>behenic acid (C22:0)</td>
</tr>
<tr>
<td></td>
<td>lignoceric acid (C24:0)</td>
</tr>
<tr>
<td></td>
<td>total saturated fatty acid</td>
</tr>
<tr>
<td>unsaturated fatty acids</td>
<td>palmitoleic acid (C16:1)</td>
</tr>
<tr>
<td></td>
<td>oleic acid (C18:1)</td>
</tr>
<tr>
<td></td>
<td>cis-vaccenic acid (C18:1)</td>
</tr>
<tr>
<td></td>
<td>linoleic acid (C18:2)</td>
</tr>
<tr>
<td></td>
<td>linolenic acid (C18:3)</td>
</tr>
<tr>
<td></td>
<td>eicosenoic acid (C20:1)</td>
</tr>
<tr>
<td></td>
<td>total unsaturated fatty acid</td>
</tr>
</tbody>
</table>
Figure 7-3 Fatty acid compositions of the *Moringa oleifera* seed oil extracted using supercritical fluid extraction with different co-solvents

### 7.2 The effects of modifier on the Eucalyptus leaf oil extraction

The *Eucalyptus* leaf oil is valued because of its main component, 1,8-cineole, which is an antiseptic used in the treatment of respiratory tract infection. In order to improve the yield of *Eucalyptus* leaf oil and the selectivity of 1,8-cineole, ethanol was added in the extraction process. Ethanol was chosen as the modifier since it doesn’t present any safety issues for the environment and people’s health.

#### 7.2.1 Effect of modifier on extraction yield

The effect of ethanol on the yield of *Eucalyptus* leaf oil was evaluated at a temperature of 333 K, CO$_2$ flow rate of 3.68 g/min, extraction time of 90 minutes and two pressures, namely 10 MPa and 30 MPa, with the concentration of ethanol varied from 5w% to 15w%. All the experimental results are shown in Figure 7-4. As per the discussions in Chapter 6, the experiments conducted at higher pressures resulted in a higher yield for all ethanol concentrations. With the pressure increased from 10 MPa to 30 MPa, the oil
yields increased from 0.94% to 6.23% at 5% ethanol concentrations, and from 6.44% to 9.64% at 15% ethanol concentrations. This can be attributed to the increased solvent density caused by the increased pressure.

It also can be seen from Figure 7-4 that the yield of the extracted oil increased with increasing ethanol concentration under both high and low pressures. This is because most of the compounds in *Eucalyptus* leaves are polar compounds and thus they are more likely to be dissolved at high polar solvents. Some other researchers have also observed similar findings. Michielen *et al.* (2009) studied the extraction of Cordia verbenacea using pure CO$_2$ and CO$_2$ with modifiers of ethanol and ethyl acetate, and the yield increased up to 54% using 5% ethanol compared with pure CO$_2$. They concluded that these increases were related to the extraction of the more polar solutes from the starting material. It was interesting to note that there was approximately two times yield increase at 10 MPa than at 30 MPa when the ethanol concentration increased from 5% to 15%. When the ethanol concentration increased from 5% to 15%, the oil yields increased from 0.94% to 6.44% at 10 MPa, and from 6.23% to 9.64% at 30 MPa. The supercritical solvent solubility parameter was calculated and is listed in Table 7-3. The solubility parameter of pure CO$_2$ at 10 MPa was only 5.08, hence it didn’t have sufficient solubility power to extract *Eucalyptus* leaf oil from *Eucalyptus* leaves. The introduction of the ethanol significantly increased the solubility parameter of the modified CO$_2$ solvent, the solubility parameter increased approximately 50% with the addition of 15% ethanol compared with the pure CO$_2$ solvent. However, the difference of the solubility parameter at 30 MPa was small and thus might contribute less to the distinct extraction efficiency. Therefore, the degree of the oil increased was higher at 10 MPa than that at 30 MPa with the addition of ethanol.
Table 7-3  The solubility parameter of the pure and modifier CO₂

<table>
<thead>
<tr>
<th>Solubility Parameter</th>
<th>Pure CO₂</th>
<th>CO₂ + 2.5w% ethanol</th>
<th>CO₂ + 5w% ethanol</th>
<th>CO₂ + 7.5w% ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter at 10 MPa</td>
<td>5.08</td>
<td>5.89</td>
<td>6.63</td>
<td>7.3</td>
</tr>
<tr>
<td>Parameter at 30 MPa</td>
<td>14.41</td>
<td>14.88</td>
<td>15.20</td>
<td>15.49</td>
</tr>
</tbody>
</table>

Figure 7-4  Effect of ethanol’s concentration on the yield of Eucalyptus leaf oil at 333 K

7.2.2 Effect of modifiers on composition and selectivity of Eucalyptus leaf oil

Figure 7-5 shows the content of 1,8-cineole under different ethanol concentrations ranging from 5w% to 15w% at pressures of 10 MPa and 30 MPa. While Figure 7-6 shows the selectivity of 1,8-cineole compared to all the other compounds present in the sample under the same conditions. The selectivity of 1,8-cineole was defined as the same in Chapter 6 as:

\[ S = \frac{\text{concentration of 1,8-cineole (\%)}}{\text{total concentration of all the other compounds (\%)}}. \]  

(7-3)
As can be seen from Figure 7-5 and 7-6, increasing ethanol content had a significant impact on the content and selectivity of 1,8-cineole in the *Eucalyptus* leaf oil extracted at 10 MPa, and there was a significant decrease in the content and selectivity of 1,8-cineole with increasing ethanol concentration from 5w% to 15w%. The content of 1,8-cineole decreased from 76.97% to 46.09% while the selectivity of 1,8-cineole changed from 3.34 at 5w% ethanol to 0.85 at 15w% ethanol. This was because the solubility of 1,8-cineole decreased with increasing ethanol concentration at 10 MPa. The effects of modifiers including ethanol, water, acetone and hexane on the solubility and selectivity of α-pinene and 1,8-cineole in supercritical CO$_2$ were investigated by Shimoyama et al. at 323 K and 8.0 MPa (Shimoyama et al., 2010). They found that the solubility of 1,8-cineole decreased from 0.00853 mol/L to 0.00736 mol/L with ethanol concentration increased from 0.00221 mol/L to 0.00562 mol/L at this low pressure.

![Figure 7-5 Effect of ethanol concentrations on the content of 1,8-cineole](image)
Figure 7-6 Effect of ethanol concentrations on the selectivity of 1,8-cineole

However, on the other hand, there was no significant difference in the selectivity of 1,8-
cineole under 30 MPa with increasing ethanol concentration. The 1,8-cineole content
under different ethanol concentrations remained almost the same at 49%. No
information about the solubility of 1,8-cineole in ethanol modified supercritical carbon
dioxide under high pressures were encountered in the literature. It is thus surmised that
the unchanged selectivity could be attributed to the greater solvating power of
supercritical carbon dioxide under the high pressures. At the pressure of 30 MPa, the
density of supercritical carbon dioxide is very high and the carbon dioxide density effect
exceeded the ethanol effect, hence the selectivity of 1,8-cineole did not change
significantly with the introduction of ethanol.

7.3 Conclusions

In this chapter, the effects of modifiers on the extraction of Moringa oleifera seed oil
and Eucalyptus leaf oil were investigated. Ethanol and water were chosen as the
modifiers for the Moringa oleifera seed oil extraction, and it was found that under a
given extraction condition, the extraction yield increased as the ratio of ethanol rose due to the interactions among the alcohol group and the oil components. However, for the modifier of water, the extraction yield decreased as the ratio of water increased due to the low solubility of water in supercritical carbon dioxide. Based on the fatty acid compositions, there were no significant differences in the fatty acid profiles of *Moringa oleifera* seed oil obtained by the supercritical fluid extraction with or without modifiers.

With ethanol introduced to the supercritical CO$_2$ extraction of *Eucalyptus* leaf oil, the results indicated that the yield of *Eucalyptus* leaf oil increased with increasing ethanol concentration under both the low pressure of 10 MPa and the high pressure of 30 MPa. However, the selectivity of 1,8-cineole decreased with increasing ethanol concentration at the low pressure of 10 MPa but stayed unchanged under the high pressure of 30 MPa.
Chapter 8 Evaluation and Implication

In this chapter, experimental results from Chapter 4 to Chapter 7 were evaluated against the objectives that were set up in Chapter 2 and the usefulness of the research findings was assessed. In order to evaluate the findings of the present work, the present results were compared with the available literature data. In addition, the contributions of the study were discussed and new gaps were identified which led to the recommendations for future research.

8.1 Experimental results evaluation

In this study, supercritical fluid extraction was used to extract oils from *Moringa oleifera* seeds. The experiments were conducted in the pressure range of 10-50 MPa, temperature range of 313-393 K, CO\(_2\) flow rate of 1.84-9.2 g/min and extraction time of 30-150 minutes. The experimental results in Section 6.1.1 indicated that the oil yield ranged from 4.57% to 37.12%. The maximum yield was obtained under pressure 50 MPa, temperature of 373 K, CO\(_2\) flow rate of 7.36 g/min and extraction time of 120 minutes. Until the present thesis work, very little research had been conducted to investigate the oil extraction from *Moringa oleifera* seeds using supercritical carbon dioxide extraction. Only one paper (Nguyen et al. 2011) was found and used to compare with this present work. Table 8-1 shows a comparison of the results from the present study and those from the literature. It can be seen that the experimental methodology and the operating conditions of the present study were quite different from the study reported by Nguyen et al. (2011). The operational conditions studied in this thesis were much wider than those from the literature. In addition, supercritical carbon dioxide was modified by 10% ethanol in all the experiments from that literature which made it very hard to separate the ethanol effect from that of other operational conditions. However, the experiments performed in this thesis were grouped into two sections. Firstly, the effects of different operational conditions on *Moringa oleifera* seed oil extraction were
investigated using pure supercritical carbon dioxide. Secondly, the modifiers were introduced at given operating conditions (40 MPa, 353 K, 3.68 g/min, 150 minutes and with particle size under 1000µm), therefore, the modifier effect can be distinguished from other conditions. As can be seen from Table 8-1, the oil yield of the current study is similar to the results published by Nguyen et al. (2011), but the optimum condition of the current study is quite different from the literature due to different operating conditions.

Table 8-1 Comparison between the present work and the literature results on the *Moringa oleifera* seed oil extraction

<table>
<thead>
<tr>
<th></th>
<th>Present Study</th>
<th>Literature research (Nguyen et al., 2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Materials</strong></td>
<td>The seeds were from the Department of Agriculture and Food of Western Australia</td>
<td>The seeds were from Ilocos Norte and Ilocos Sur</td>
</tr>
</tbody>
</table>
| **Operating conditions** | Pressure: 15-50 MPa  
Temperature: 313-393 K  
Flow rate: 1.94-9.2 g/min  
Extraction time: 30-150 minutes  
Particle size: 0-2 mm  
Ethanol concentration: 0-10% | Pressure: 15-30 MPa  
Temperature: 308-333 K  
Flow rate: 0.5 m³/h  
Extraction time: 7 hours  
Particle size: 0.16-1.12 mm  
Ethanol concentration: 10% |
| **Extraction yield** | 4.57-37.12% without modifiers, reach to 39.5% with 10% ethanol (40 MPa, 353 K, 3.68 g/min, time 150 minutes and with particle size under 1000µm) | 6.71-37.82% with 10% ethanol |
| **Optimum conditions** | Pressure 50 MPa, Temperature 373 K, Flow rate 7.36 g/min, Extraction time: 120 minutes without ethanol | Pressure: 28.97 MPa  
Temperature: 317.5 K  
Particle size: 0.54 mm with ethanol addition |
Table 8-2  Comparison between the present work and the literature results on the
*Eucalyptus* leaf oil extraction

<table>
<thead>
<tr>
<th>Materials</th>
<th>Present Study</th>
<th>Herzi et al’s study (Herzi et al., 2013)</th>
<th>Della Porta et al’s study (Della Porta et al., 1999)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus loxophleba subspecies lissophloia leaves were collected from trees in the south west region of Western Australia</td>
<td>Eucalyptus Camaldulensis and Eucalyptus Cineria were from the region of Korbos</td>
<td>Eucalyptus Camaldulensis Dehn. leaves are supplied from Maputo, Mozambique</td>
<td></td>
</tr>
<tr>
<td>Operating conditions</td>
<td>Pressure: 10-50 MPa, Temperature: 313-353 K, Flow rate: 3.68 g/min, Extraction time: 30-150 minutes</td>
<td>Pressure: 9 MPa, Temperature: 313 K, Flow rate: 20 g/min, Extraction time: 30 minutes</td>
<td>Pressure: 8-25 MPa, Temperature: 313 K, Flow rate: 250 ml/min, Extraction time: 2 hours</td>
</tr>
<tr>
<td>Extraction yield</td>
<td>0.99-4.78%</td>
<td>E. cinerea: 2.75%</td>
<td>1.4-2.0%</td>
</tr>
<tr>
<td>Optimum conditions</td>
<td>Pressure: 40 MPa, temperature: 343 K, extraction time: 120 minutes.</td>
<td>Not studied</td>
<td>Not studied</td>
</tr>
</tbody>
</table>

The supercritical CO₂ extraction of *Eucalyptus* leaf oil was studied as well. A few studies have been published to explore the possible use of supercritical CO₂ to extract essential oil from *Eucalyptus* leaves (da Cruz Francisco et al., 2001, Herzi et al., 2013). The results from present study in Section 6.3.1 were compared with those from literatures as shown in Table 8-2. The oil yield of current study ranged from 0.99% to 4.78%. The extraction yield of present study under conditions of 10 MPa, 333 K and extraction time of 90 minutes was 0.99%. This yield was comparable to the yield of *Eucalyptus* camaldulensis leaf oil (0.88%) reported by Herzi et al. (2013) and the *Eucalyptus* camaldulensis leaf oil (1.4%) reported by Della Porta et al. (1999). However,
higher yield was obtained for the species of *E. cinerea* (about 2.75%) under similar extraction conditions. Such variations in the oil yield could be attributed to the possible variations in the sample structures and compositions of the extracted oil. Note that the optimum extraction condition was studied in Section 6.3.1 and the maximum yield of *Eucalyptus* leaf oil was obtained under pressure 40 MPa, temperature 343 K and extraction time of 120 minutes. However; the operating conditions were not optimized in the other studies.

The solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide was investigated. As discussed in Section 5.3, the solubility of *Moringa oleifera* seed oil was compared with those of Sunflower oil (Soares *et al*., 2007), Corn oil (Soares *et al*., 2007), Rosehip seed oil (Machmudah *et al*., 2007), Borage oil (Gaspar *et al*., 2002) and Lunaria oil (Gaspar *et al*., 2002) as listed in Table 5-2. It was found that the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide was of a similar magnitude to that of Lunaria oil, but higher than that of Rosehip seed oil and lower than those of Sunflower oil, Corn oil and Borage oil due to the different compositions of these oils. Moreover, the experimental results revealed that the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide was a function of temperature and pressure. Increased pressure would increase the oil solubility. However, the effect of temperature on the solubility is complex due to the combination of two factors: an increase in temperature could increase the vapour pressure of the solute, enhancing its solubility in supercritical fluid, but an increase in temperature also decreases the solvent density. Our experimental results showed that at low pressures of 20 and 30 MPa, the solubility of *Moringa oleifera* seed oil decreased with increasing temperature which indicated that the density was the dominant effect on the solubility, while at high pressures of 40 MPa and 50 MPa, the solubility increased with increasing temperature indicating that the
vapour pressure was the dominant effect on the solubility. This was in agreement with other peoples’ research (Follegatti-Romero et al., 2009, Özkal et al., 2005)

8.2 Contributions to science and technology

First of all, the performance of the supercritical fluid extraction was evaluated and compared with the Soxhlet extraction and hydro-distillation methods. The comparison of the three methods were summarized in Table 8-3 (Sahena et al., 2009). The study in Chapter 4 indicated that supercritical fluid extraction could be a good alternative to replace Soxhlet extraction and hydro-distillation to extract oil and essential oil from plant materials. The results discussed in Section 4.1.1 indicated that the oil yield of supercritical fluid extraction can represent 92.52% of the yield obtained by Soxhlet hexane extraction, and the fatty acid compositions of the oils extracted by these two methods were similar. Several researchers (Sahena et al., 2009, Cheung et al., 1998) have also compared the performance of supercritical fluid extraction with the conventional Soxhlet extraction method. It was found that supercritical fluid extraction obtained from 97% to 100% of a Soxhlet extraction in seeds, seed meals and several other food products (Sahena et al., 2009). For example, Cheung et al. (1998) had significantly higher recoveries of lipid and fatty acid contents using SFE than using a chloroform and methanol Soxhlet extraction from seaweeds. In fact, SFE has been included in the recommended methods from the Association of Official Analytical Chemists (AOAC) to extract oil from oil seeds (AOAC, 1997).
Table 8-3 Comparison of supercritical fluid extraction with Soxhlet extraction and hydro-distillation methods

<table>
<thead>
<tr>
<th></th>
<th>Supercritical extraction</th>
<th>Soxhlet extraction</th>
<th>Hydro-distillation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total free of solvent and therefore the sample is clean</td>
<td>Have solvent residues in the sample</td>
<td>Have water content in the sample</td>
</tr>
<tr>
<td>2</td>
<td>Can be performed at lower temperatures (thus avoiding both losses and degradation of volatile and thermolabile compounds)</td>
<td>Performed at high temperatures (could destroy some thermolabile compounds)</td>
<td>Performed at high temperature (could destroy thermolabile compounds)</td>
</tr>
<tr>
<td>3</td>
<td>Extraction is fast</td>
<td>Extraction is slow</td>
<td>Extraction is slow</td>
</tr>
<tr>
<td>4</td>
<td>High level of automation</td>
<td>Low level of automation</td>
<td>Low level of automation</td>
</tr>
<tr>
<td>5</td>
<td>Easy turning of solvency and selectivity (by changing temperature and pressure)</td>
<td>Hard to change the solvent selectivity</td>
<td>Hard to change the solvent selectivity</td>
</tr>
<tr>
<td>6</td>
<td>Medium yield, co-extraction of unwanted compounds (can be improved by employing fractionation technique)</td>
<td>Highest extraction yield, very high concentration of co-extracted unwanted compounds</td>
<td>Lowest extraction yield Very pure essential oil</td>
</tr>
<tr>
<td>7</td>
<td>No extra operation required</td>
<td>Solvent removal required extra unit operation, this could increase the capital cost</td>
<td>Water removal is required</td>
</tr>
<tr>
<td>8</td>
<td>The system is very expensive and need a skilled operator</td>
<td>The system is cheap and easy to use</td>
<td>The system is cheap and easy to use</td>
</tr>
</tbody>
</table>

The *Eucalyptus* leaf oil extraction using supercritical carbon dioxide was compared with that obtained by hydro-distillation in Section 4.3.1, it was found that the oil yield obtained by supercritical fluid extraction was higher than that obtained by hydro-distillation. However, the 1,8-cineole content in supercritical fluid extracted oil was lower than that in the hydro-distillation oil. This was because some high molecular weight compounds were also extracted by the supercritical carbon dioxide. Similar
results were observed by da Cruz Francisco et al. (2001) who studied the extraction of *Eucalyptus* camaldulensis Dehn leaf oil using hydro-distillation and supercritical carbon dioxide extraction. The oil obtained by hydro-distillation had higher concentrations of 1,8-cineole (43%), α-pinene (5.5%), β-pinene (3.4%), p-cymene (5.2%), terpinen-4-ol (3.1%), and globulol (4.1%). The oil extracted by supercritical carbon dioxide had lower amounts of 1,8-cineole, α-pinene, β-pinene, and terpinen-4-ol, but had higher amounts of *allo*-aromadendrene and globulol. However, when the *Eucalyptus* leaf oil was extracted using supercritical CO$_2$ with a fractional separation technique, a higher 1,8-cineole content and a higher oil yield was achieved in supercritical carbon dioxide extraction than that was achieved by hydro-distillation (Della Porta et al., 1999). Therefore it is possible to use supercritical fluid extraction to obtain high yield and high quality oil with a separation technique applied.

Secondly, while most literature reports only focused on the performance differences between supercritical fluid extraction, Soxhlet extraction and hydro-distillation methods, this study have investigated and compared the mechanism differences between the three methods. The extraction mechanisms of these three methods are shown in Figure 8-1 and discussed comprehensively in Section 4.3.1. The biggest difference between hydro-distillation, Soxhlet extraction and supercritical fluid extraction is that the water used in hydro-distillation method does not dissolve organic components, while the common solvents used in Soxhlet extraction and supercritical fluid extraction have strong solvation power which can dissolve a variety of components from plant materials. Therefore, hydro-distillation does not have the solvent dissolution step, while the dissolution of the components into the solvent is a very crucial step for the Soxhlet extraction and supercritical fluid extraction. Therefore, the types and properties of the solvent used in Soxhlet extraction and supercritical fluid extraction have a strong influence on the compositions of the extracts from the sample matrix. The effect of
solvent type on *Eucalyptus* leaf oil compositions was discussed in Section 4.3.2. The major difference between Soxhlet extraction and supercritical fluid extraction is that Soxhlet extraction is a batch process normally operated under atmosphere pressure while supercritical fluid extraction can be either a batch process or a continuous process operated under high pressure. Changing operating conditions such as temperature, pressure *et al.* will have more influential effects for the supercritical fluid extraction than that for the Soxhlet extraction.

Thirdly, solubility is the most important criterion affecting the efficiency of most of the supercritical fluid extraction, for example, solubility can have a direct impact on the extraction rate and yield. In this respect, the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide has been investigated. To the best of the author’s knowledge, there has been no previously reported solubility data of *Moringa oleifera* seed oil in supercritical carbon dioxide in the literature. This solubility study is fundamental to quantify the ability of supercritical carbon dioxide to extract oil from *Moringa oleifera* seeds.
Figure 8-1 Mechanism comparisons of hydro-distillation, Soxhlet extraction and supercritical fluid extraction
Due to the strong dependence of solubility on temperature and pressure of supercritical fluids, extensive amount of data are need at different conditions. However, sometimes it is difficult and time consuming to obtain large amounts of experimental solubility data, for this reason it is desirable to have a tool to predict the solubility in supercritical fluids based on the available experimental data. In this study, the Peng-Robinson equation of state and three density based models, namely, Chrastil, del Valle and Aguilera, Adachi and Lu models were applied to analyse the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide. All the models were validated by the present experimental work in Chapter 5 and a good agreement was achieved at various operating pressures and temperatures. Therefore, these models are anticipated to be able to predict the *Moringa oleifera* seed oil solubility in supercritical carbon dioxide under other operation conditions.

Finally, the effects of different operating conditions including pressure, temperature, flow rate, extraction time, and sample particle size on the supercritical fluid extraction process have been extensively studied in Chapter 6. In general, pressure and temperature are the most important parameters in SFE, and extraction yield increases with increasing pressure due to an increase in CO\textsubscript{2} density, however, the impact of temperature is dependent on the extraction pressure and a well-known cross-over phenomenon has been observed both in the *Moringa oleifera* seed oil extraction and *Eucalyptus* leaf oil extraction.

As expected, increasing extraction time increased the extraction yield for all the experiments. A very interesting finding was observed when studying the effect of flow rate on supercritical fluid extraction of *Moringa oleifera* seed oil in Section 6.1.1. At low-pressures and/or high temperature conditions, the *Moringa oleifera* seed oil yield firstly increased and then decreased with increasing flow rate. This was not as expected, as initially it was thought that the oil yield would always increase with increasing flow rate.
rate. Different opinions were found in the literatures about the effect of flow rate on the extraction yield. Some authors (Pourmortazavi and Hajimirsadeghi, 2007) thought that higher flow rates resulted in a decrease in the yield either because of an elevated pressure drop through the extraction cell or because of increasing analyte loss during decompression of the fluid. Some other authors (Rozzi et al., 2002) thought that the decreased yield could be attributed to the reduced contact time between solvent and sample, the increased flow rate could also cause the sample to compact and restrict CO\textsubscript{2} movement into and out of the sample thus reducing the amount of CO\textsubscript{2} that came into contact with samples. As discussed in Section 6.1.1, there was intra-particle diffusion resistance in the extraction process, the current study deduced that a further increase in flow rate could easily cause the channelling effect and thus resulted in decreased yield.

The sample particle size has a direct effect on the supercritical fluid extraction process. Our experimental results in Section 6.1.1 revealed that the yield of *Moringa oleifera* seed oil increased when the particle size decreased from 1000-2000µm to less than 500µm, but much smaller particles were not studied in this thesis. It has been reported that very small particles would produce smaller volume voids in the sample matrix, providing resistance to fluid flow with the extraction (Pronyk and Mazza, 2009). Therefore further studies should be conducted to see whether smaller particles could produce a lower yield or not.

### 8.3 Implications and research gap identifications

The results from the current research have important implications in the application of supercritical fluid extraction in the industry and other plant materials. First of all, this thesis has investigated the optimum conditions to extract oils from *Moringa oleifera* and *Eucalyptus* in Chapter 6. The research methodology applied in the optimisation study in Section 3.4 provided a good guidance for extraction of other similar plant materials. For example, this study can help other researchers to identify the range of operating
condition to start their experiments. Most importantly, the discussion in Chapter 6 has provided a step by step guidance to find out the optimum conditions for other plant materials.

Secondly, the selectivity property of supercritical fluid extraction makes it a promising technique in the fractional extraction process. The selectivity of supercritical fluid extraction under different conditions has been evaluated in Chapter 6. It was found that the compositions of the oil extracted from *Moringa oleifera* seeds, *Moringa oleifera* leaves and *Eucalyptus* leaves varied under different extraction conditions. Similarly, the selectivity of supercritical carbon dioxide in the fractionation of fish oil was investigated by Lopes et al. (2012), they found that under the pressure conditions of 10 and 20 MPa, supercritical carbon dioxide was selective in fractionating the triacylglycerol that contained EPA and DHA fatty acids. The selectivity of diterpenes extracted from sage by supercritical fluid extraction was also studied (Glisic et al., 2010), the authors found that the selectivity of diterpenes was the highest at 10-15 MPa compared to heavier compounds (triterpenes, esters and waxes). Therefore, supercritical fluid extraction offers possibilities for selective extractions and fractionations by changing the extraction conditions.

However, there are still several research gaps identified in this study. To begin with, although the solubility of *Moringa oleifera* seed oil was successfully correlated using both the Peng-Robinson equation of state and the density based models in Chapter 5; it was found that both of these two approaches had their limitations. For the Peng-Robinson equation of state, it is impossible to obtain thermodynamic properties of the *Moringa oleifera* seed oil since the oil is a complex multi-components mixture. Therefore the oil was treated as pure compound of triolein characterized by definite molecular parameter (molar volume, critical temperature and pressure). However, this method could bring modelling errors and reduce the quality of the supercritical fluid
extraction prediction. For examples, some authors (Vafai et al., 1993) have pointed out the importance of accurate values of the solid molar volume for modelling supercritical fluid extraction, they demonstrated that an 11% error in the solid molar volume of naphthalene would correspond to a 21% error in the enhancement factor at 40 MPa and 310 K. Therefore some other thermodynamic models such as the group contribution equation of state (GC-EoS) could be studied and correlated with our experimental data in the future study. Furthermore, it was found that the solubility behaviour of triolein (Weber et al., 1999) was different from the Moringa oleifera seed oil. Therefore, more experimental work should be carried out in the future to investigate the solubility of pure triolein in supercritical carbon dioxide. For the density based models, they are lack of physical meanings and therefore could not provide a thermodynamic explanation of the observed experimental results.

Secondly, some other factors such as the moisture content of the samples, the drying process for the plant samples, the collection methods, the amount of samples loaded into the vessel et al. could also affect the extraction process, but theses parameters have not been studied in this thesis, therefore more research should be carried out to understand these parameter effects on the extraction process. In addition, this thesis only studied the fatty acid compositions of the Moringa oleifera seed oil, more research is needed to investigate the quality attributes of supercritical carbon dioxide extracted oil such as antioxidant compositions and stability of bioactive components throughout extraction.

Thirdly, in order to improve the selectivity of the fatty acid in Moringa oleifera seed oil extraction and 1,8-cineole in Eucalyptus leaf oil extraction, water and ethanol were introduced as the modifiers during the extraction process in Chapter 7. Although the yields of Moringa oleifera seed oil and Eucalyptus leaf oil increased with the introduction of ethanol, the selectivities of the fatty acid and 1,8-cineole were not improved. Also, it was unsuccessful to use water as the modifier to extract Moringa
oleifera seed oil, as the yield of Moringa oleifera seed oil decreased with increasing water concentration. Therefore, some other solvents or methods should be investigated to improve the yield as well as the selectivity of the supercritical fluid extraction towards target compounds.

8.4 General discussion of the supercritical fluid extraction performance

![Diagram showing interactions of fluid properties, process conditions, and sample matrix characteristics](image)

Figure 8-2 The interactions of the fluid properties, process conditions and sample matrix characteristics and their effect on the performance of a SFE process

The performance of the supercritical fluid extraction is affected by three groups of factors, the properties of the supercritical fluid, the extraction process conditions and the characteristics of the materials to be extracted. The interactions amongst the three groups are discussed with the aid of a triangle “phase diagram” shown in Figure 8-2.

First of all, the properties of a supercritical fluid including density, viscosity, diffusivity, dipole moment, and dielectric constant et al. have an important effect on the extraction process, as they greatly influence the solvent strength and the solubility of target compounds in the fluid. Density is one of the most significant properties as it determines the solvation power of the supercritical fluid to dissolve the extracted
substance. In general, the fluid with higher density is easier to dissolve components from sample matrix. This can explain why the oil yield always increased with increasing pressure as discussed in Chapter 6, as the solvent density increased with increasing pressure.

The polarity of the solvent will decide the solvent’s ability to dissolve “polar” or “non-polar” components from the sample matrix. Water is the most polar solvent and can dissolve all kinds of compounds that can be ionized or that contains hydrophilic molecules such as sugars, proteins or amino-acids. On the contrary, supercritical carbon dioxide or other organic solvents like hexane do not dissolve these compounds, but only dissolve hydrophobic molecules that are not soluble in water like fats, oils, hydrocarbons or essential oil. Other solvents like alcohols exhibit intermediate behaviour. Although supercritical carbon dioxide behave like non-polar solvents, it is possible to tune it polarity by adding a polar solvent (such as ethanol or water). It should be emphasized that the addition of co-solvent can change other properties of the solvent; for example, the critical parameters (critical pressure, critical temperature) may differ considerably from that of pure carbon dioxide. For example, the critical temperature and pressure for a 5 mol% of ethanol modified carbon dioxide are 315 K and 8.6 MPa, while the critical parameters for pure carbon dioxide are 304 K and 7.4 MPa (Francisco and Sivik, 2002). Therefore, it is essential to understand the properties of the supercritical fluid or the modified supercritical fluid before any extraction was performed.

The properties of a supercritical fluid can be controlled by varying the operating conditions of pressure and temperature, or both in combination. For example, the density of carbon dioxide at the critical point (313 K, 7.4 MPa) is in the region of 0.4 g/ml. However, this value increases to 0.88 g/ml when the pressure increases to 25 MPa which is comparable to that of liquid carbon dioxide. Therefore, the extraction process

180
can be affected by simply changing the operating pressure and temperature to alter the solvating power of the solvent.

Secondly, it is also very important to maximize the contact between the supercritical fluid solvent and the sample matrix in order to enhance the efficiency of the supercritical fluid extraction. Several variables could influence the solvent contact with sample materials such as solvent flow rate and extraction time. The effects of extraction time and flow rate on supercritical fluid extraction have been explained comprehensively in Chapter 6.

Thirdly, the characteristics of the sample matrix are also very important because the solute must be released from matrix, and then transport by diffusion from the interior of the matrix pores to the surface. Maksimovic et al. (2012) and Zizovic et al. (2007) reported that the yield of an SFE process was influenced by the particle size and physical structure of the oil seeds. They found that oil seeds had to be ground to assure complete extraction of the oil. The present study also indicated that the oil yield increased with decreasing particle size. Furthermore, it was reported that the shape of the ground seeds also affected the supercritical fluid extraction process (Sovová et al., 2001).

The moisture content also plays an important role in the supercritical fluid extraction process. The current study indicated that the introduction of water might provide an aqueous barrier inhibiting diffusion of supercritical carbon dioxide into the matrix as well as diffusion of oil out of the matrix. Nagy et al. (2008) also reported that moisture content between 7% and 18% had a negligible effect on the extractability of paprika oil. However, when the moisture level was above 18%, the increase in moisture content decreased the extraction efficiency. That is why the sample matrix must be dried before any extraction is performed. However, in some other cases, the presence of water in the matrix may act as a modifier and improve the selectivity of the supercritical fluid.
extraction for a solute. For example, Sovová et al. (2001) reported that the polar component solubilities in supercritical carbon dioxide were improved at higher moisture content sample matrix. Swelling of plant materials in the presence of water may also be quite significant for extraction because it widens plant cell capillaries, thereby increasing the matrix porosity with subsequent improvement in solute diffusion. Therefore, how to dry the samples and keep the optimum moisture content in the sample is a very important factor to be considered for the extraction process.

As discussed in Chapter 4 and Chapter 6, there are four steps involved in the supercritical fluid extraction, but the limiting step could be different for different plant materials, therefore it is very hard to come to a conclusion as to which part plays the most important role in the extraction process. If the rate-limiting step or the largest mass transfer resistance of the extraction process is the diffusion of the supercritical fluid into the matrix, then increasing the diffusivity of the supercritical fluid will speed up the extraction. However, if the solubilisation of the extracted substance in supercritical fluid determined the extraction rate, then changing the operating conditions or the introduction of a modifier will be helpful. In another case, if the internal solid diffusion controls the mass transfer rate, then reducing the particle size will be favourable. The general consideration to design an extraction process is to firstly choose the proper supercritical fluid solvent. Ideally the components of interest should be highly soluble in the selected solvent, whereas the matrix or other major components should have little solubility in it. Then the samples should be pre-treated to reduce the particle size as well as the moisture if necessary. Finally, the operating conditions can be manipulated to achieve the desired outcomes. Each part of the process has to be carefully optimized in order to obtain qualitative and quantitative recoveries.
Chapter 9 Conclusions and Recommendations

Based on the preceding evaluation of the findings of the present thesis work, this chapter summarised the conclusions drawn from the present study. This thesis has significantly advanced the understanding of the properties of the supercritical fluid and supercritical fluid extraction process by comparing with the conventional Soxhlet extraction and hydro-distillation methods. The effects of various operating conditions (such as temperature, pressure, CO$_2$ flow rate and particle size) and the modifiers on the supercritical carbon dioxide extraction of oils from *Moringa oleifera* and *Eucalyptus* were systematically investigated and discussed. The evaluation and implications of this thesis work will lead to the recommendations for the future work in this area of research.

9.1 Summary of conclusions

9.1.1 Supercritical CO$_2$ extraction of *Moringa oleifera* and *Eucalyptus* leaf oil and comparison with Soxhlet extraction and hydro-distillation methods

The potential application of supercritical fluid extraction to extract natural compounds from *Moringa oleifera* and *Eucalyptus* plants was extensively studied in this thesis. Firstly, the *Moringa oleifera* seed oil was extracted by supercritical carbon dioxide and compared with the oil extracted by Soxhlet extraction using hexane. The results revealed that the oil yield of the supercritical fluid extraction under pressure 50 MPa, temperature 373 K, flow rate 7.36 g/min and extraction time of 120 minutes can represent 92.52% of the 8 hours hexane extraction, and the fatty acid compositions of the oil extracted by the two methods were quite similar.

The yield and compositions of the oil extracted from *Moringa oleifera* leaves and *Eucalyptus* leaves obtained by SFE and Soxhlet extraction were compared. The results showed that both the yield of *Moringa oleifera* leaf oil and *Eucalyptus* leaf oil extracted
by SFE process was lower than that of the Soxhlet extraction. There were great differences in the oil compositions obtained using these two methods, and more components were identified in the Soxhlet extracted samples than the SFE samples.

Hydro-distillation was also applied to the *Eucalyptus* leaf oil extraction and compared with the Soxhlet extraction and supercritical carbon dioxide extraction, it was found that hydro-distillation gave the lowest yield of 3.77%, while the ethanol Soxhlet extraction provided the highest yield of *Eucalyptus* leaf oil at about 36.33%. It was also noted that due to the different mechanisms of the three methods, hydro-distillation only extracted the volatile compounds, while Soxhlet extraction and SFE extracted a wide range of components including not only the volatile but also the high molecular weight compounds. The content of 1,8-cineole in the extracted oils were 46.19% for SFE, 70.03% for hydro-distillation sample and 29.85% for ethanol Soxhlet sample.

**9.1.2 Solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide**

As supercritical fluid extraction is often limited to the solubility of the target extracts in the supercritical fluids employed, the solubility of *Moringa oleifera* seed oil in supercritical carbon dioxide was measured at pressures of 20-50 MPa and temperatures of 333-373 K. It was found that the solubility increased with increasing pressure at constant temperature. However, the solubility decreased with increasing temperature at low pressures of 20 MPa and 30 MPa but increased with increasing temperature at high pressures of 40 MPa and 50 MPa. The experimental results were correlated with four models which included three density based models, namely the Chrastil, del Valle and Aguilera, Adachi and Lu models, and the Peng-Robinson equation of state with the conventional van del Waals mixing rules. The solubility trend of *Moringa oleifera* seed oil in supercritical carbon dioxide was successfully described and explained using the Peng-Robinson equation of state. The binary interaction parameters of $k_{ij}$ and $l_{ij}$ in the thermodynamic model were estimated and it was found that these two parameters varied
linearly with temperature. The best correlation results, in terms of AARD, were obtained with the three employed density-based models. This clearly indicated the utility of these kinds of simple-to-use models to correlate the solubility of solids in supercritical fluids, without the necessity of estimating any of the solid’s critical or thermo-physical properties.

9.1.3 The effects of different parameters on supercritical carbon dioxide extraction of *Moringa oleifera* and *Eucalyptus*

The effects of extraction pressure, temperature, CO$_2$ flow rate and extraction time on the supercritical fluid extraction of *Moringa oleifera* seed oil were investigated using the response surface methodology. The results indicated that extraction pressure, flow rate, extraction time had the greatest impact on the oil yield with p<0.05. The oil yield increased with increasing time and pressure, but increasing temperature and flow rate could have either a positive or negative effect on the oil yield, depending on the levels of pressure investigated. A simplified regression equation was developed in this thesis to predict the *Moringa oleifera* seed oil yield and was shown to be sufficient to describe and predict the extraction process. A decrease in particle size increased the yield of *Moringa Oleifera* seed oil. The chemical compositions and molecular structures of the oils extracted were determined using the GC-FID and $^1$H NMR analysis. The oil obtained contained mainly oleic acid and eicosenoic acid. These oils may be considered an important source of oleic acid. The fatty acid compositions of the oils varied with extraction conditions, more specifically, the oleic acid and total unsaturated fatty acids decreased with increasing pressure and temperature.

Supercritical fluid extraction was applied to the extraction of oil from *Moringa oleifera* leaves and the effects of pressure, temperature and extraction time on the oil yield were investigated. The results indicated that temperature had the most significant effect on the oil yield followed by pressure and extraction time. The oil yield increased with an
increase in any of these three factors. The compositions of the *Moringa oleifera* leaf oil obtained by the SFE were analysed by GC-MS. The major components were 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester, nonacosane, heptacosane and β-amyrrin. The chemical analysis revealed that although the components in the SFE extracts were roughly the same, their quantities were quite different, thus it is possible to manipulate the compositions of the oil by changing the parameters of the SFE process.

In addition, the *Eucalyptus* leaves were also extracted by supercritical carbon dioxide and the central composite design was employed to determine the operating parameters effects and their interactions. The results showed that pressure had the most significant positive effect on the oil yield, while temperature and time had a lesser impact. There was also pronounced interaction between temperature and pressure on the yield in which the oil yield increased with increasing temperature at high pressures but decreased at low pressures. Furthermore, 1,8-cineole, which has anti-inflammatory, antimicrobial, nematicidal and pain release properties, was the main component in the *Eucalyptus* leaf oil. It was found that the selectivity of 1,8-cineole increased with decreasing pressure and temperature.

9.1.4 The effects of modifiers on supercritical fluid extraction as applied to *Moringa oleifera* and *Eucalyptus*

The addition of ethanol in supercritical carbon dioxide improved the efficiency of supercritical fluid extraction. It was found that both the yields of *Moringa oleifera* seed oil and *Eucalyptus* leaf oil increased with increasing ethanol concentrations. Interestingly, there were no significant differences in the fatty acid compositions of the *Moringa oleifera* seed oil extracted by supercritical carbon dioxide with and without ethanol. But for the *Eucalyptus* leaf oil, the compositions of the oil differed quantitatively under different ethanol concentrations. There was a significant decrease
in the content and selectivity of 1,8-cineole with increasing ethanol concentration from 5% to 15% at 10 MPa. The effect of water on the supercritical fluid extraction process was also evaluated on the *Moringa oleifera* seed oil extraction, unfortunately, the addition of water had negative effect on the extraction process, and the yield of *Moringa oleifera* seed oil decreased with increasing water concentration.

### 9.2 Future works

Only the fatty acid compositions of the *Moringa oleifera* seed oil were analysed, further tests of these oils, such as the vitamin E content of the oils, the antioxidant capacity of the oils and other biological activities can be carried out to evaluate the oil’s applicability to pharmaceutical and food industries. It is also very useful to investigate if the addition of modifiers can change the properties of the oil.

The solubility of *Moringa oleifera* seed oil was correlated by the thermodynamic model of the Peng-Robinson equation of state. In the calculation process, the oil was simplified as pure triolein. However, the oil and pure triolein had different solubility behaviours in supercritical carbon dioxide, thus the solubility of pure triolein in supercritical carbon dioxide should be measured experimentally in the future. This will provide additional information which can further help us understand the oil solubility behaviour in supercritical carbon dioxide. Additionally, the performance of some other thermodynamic models such as the group contribution equation of state can be evaluated to analyse the oil solubility in carbon dioxide.

To better understand the supercritical fluid extraction process, the effects of some other parameters such as the collection methods, the amount of samples loaded into the vessels, the moisture content of the samples and the drying process for the plant samples *et al* can be further investigated.
Unfortunately, the selectivity of fatty acid in *Moringa oleifera* seed oil was not improved with the addition of ethanol and water. The selectivity of 1,8-cineole in *Eucalyptus* leaf oil was not improved as well with introducing ethanol, the co-extraction of wax in the *Eucalyptus* leaf oil was a problem. In order to improve the selectivity of 1,8-cineole and separate the wax from the oil, it is suggested to modify the current supercritical fluid system, two or more separators can be installed after the restrictor. The separators should be able to set at different pressures and temperatures so that the oil with high content of 1,8-cineole and wax can be precipitated selectively in different separators.
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Appendix

Composition of *Eucalyptus* leaf oil extracted by SFE, hydro-distillation and Soxhlet extraction with ethanol and hexane

<table>
<thead>
<tr>
<th>Retention time</th>
<th>Compound</th>
<th>SFE (40MPa 70 60 minutes)</th>
<th>hydro-distillation (5 hours)</th>
<th>Ethanol extraction (8 hours)</th>
<th>Hexane extraction (8 hours)</th>
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</thead>
<tbody>
<tr>
<td>3.367</td>
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<p>| 43.6552  | β-phenylethyl butyrate | 0.090 | 0.157 | 0.060 | 0.111 |
| 44.087  | tetradecane            |       |       |       | 0.346 |
| 45.447  | s-(+)-5-(1-hydroxy-1-methylene)-2-methyl-2-cyclohexen-1-one | 0.302 | 0.010 | 0.280 | 0.175 |
| 45.798  | unidentified           |       |       |       | 0.139 |
| 45.9859 | (+)-aromadendrene      | 0.289 | 0.090 | 0.237 | 0.215 |
| 46.736  | a-elemene              |       | 0.236 |       | 0.314 |
| 47.314  | alloaromadendren       |       |       |       | 0.048 |
| 47.525  | 1-methyl-4-(1-acetoxy-1-methylene)-cyclohex-2-enol | 0.474 |       | 0.610 | 0.195 |
| 47.874  | tetradecane, 2,6,10-trimethyl-cadinene |       |       |       | 0.290 |
| 48.2371 | γ-murolene             | 0.384 | 0.043 |       | 0.246 |
| 48.856  | p-menthane-1,2,3-triol |       |       |       | 0.513 |
| 49.206  | (+)-epi-bicyclosesquiphellandrene |       | 0.377 |       | 0.206 |
| 49.482  | (+)-ledene             | 1.514 | 0.122 |       | 0.234 |
| 49.589  | unidentified           |       |       |       | 0.324 | 1.195 |
| 49.8923 | α-murolene             | 0.051 | 0.096 |       | 0.195 |
| 50.183  | unidentified           |       |       |       | 0.084 | 0.540 | 0.535 |
| 50.7938 | butylatedHydroxytoluene | 1.527 | 0.295 | 1.256 | 1.134 |
| 51.192  | calamene               | 0.395 |       |       |       | 0.428 |
| 51.2998 | δ-cadinene             |       | 3.121 |       | 1.622 |
| 51.4275 | hydroxy-α-terpenyl acetate | 0.301 |       | 0.304 | 0.258 |
| 51.7129 | naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-1(2H)-naphthalenone,5-ethyl-3,4-dihydro-α-calacorene | 0.064 | 0.285 |       | 0.314 |
| 52.1569 | 1-methyl-4-(1-acetoxy-1-methylene)-cyclohex-2-enol | 0.153 | 0.382 | 0.527 | 0.175 |
| 52.2915 | unidentified           |       | 0.477 |       | 0.162 |
| 52.7382 | cis-Z-α-bisabolene epoxide |       | 0.139 | 0.318 | 0.116 |
| 52.944  | 2(3H)-furanone,dihydro-5,5-dimethyl-4(3-oxobutyl)-epiglobulol | 0.385 |       |       | 0.246 |
| 53.138  | 2(3H)-furanone,dihydro-5,5-dimethyl-4(3-oxobutyl)-epiglobulol | 0.095 | 0.035 | 0.377 | 0.060 |
| 53.463  | cadala-1(10),3,8-triene |       |       |       | 0.128 |
| 53.5806 | palustrol              | 0.039 | 0.130 | 0.545 | 0.040 |
| 53.8565 | (S)-(+)carvone acetate | 0.744 | 0.135 | 0.655 | 0.665 |
| 54.2387 | (-)-spathulenol        | 0.602 | 0.985 | 0.381 | 0.509 |
| 54.4647 | caryophyllene oxide    | 0.896 | 1.175 | 0.383 | 0.798 |
| 54.5872 | globulol               | 1.571 | 2.531 | 0.891 | 1.251 |
| 54.8106 | germacrene D-4-ol      | 0.564 | 0.845 | 0.250 | 0.316 |
| 55.6166 | ledol                  | 0.411 | 0.495 | 0.299 | 0.347 |</p>
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