

**ADDIS ABABA INSTITUTE OF TECHNOLOGY**  
**SCHOOL OF CHEMICAL AND BIO ENGINEERING**

**Production of Capsaicin from Chili Pepper Using  
Experimental Work and Process Simulation**

---

By

Abdulkerim Oumer

Submitted to

The School of Chemical and Bio Engineering

Presented in Fulfilment of the Requirement for the Degree of Master of Science

Under Process Engineering Stream

Addis Ababa University

Addis Ababa, Ethiopia

October 13, 2018

## DECLARATION

I declare that this thesis entitled “*Production of Capsaicin from Chili Pepper Using Experimental Work and Process Simulation*” has not been submitted in any form for another degree, diploma or an award at any university or other institution of the tertiary education. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. Information taken from published and unpublished work of others has been acknowledged in the text and a list of references is given. The work was under the guidance of Dr. Eng. Abubeker Yimam (Associate Professor) a chair person and instructor of Addis Ababa University, School of Chemical and Bio Engineering.

Abdulkerim Oumer

_____	-----	_____
Candidate	Signature	Date

This is to certify that the above declaration made by me is corrected to the best of my knowledge and the approval of university supervisor.

Dr. Eng. Abubeker Yimam (Associate Professor)

_____	-----	_____
Advisor	Signature	Date

Date of Submission: October 13, 2018

# Addis Ababa University

## Addis Ababa Institute of Technology

### School of Chemical and Bio Engineering

This is to certify that the thesis prepared by *Abdulkerim Oumer*, entitled: *Production of Capsaicin from Chili Pepper Using Experimental Work and Process Simulation* and submitted in partial fulfilment of the requirement for the degree of Master of Science (Chemical and Bio Engineering) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Dr. Eng. Abubeker Yimam (Associate Professor)

\_\_\_\_\_

Advisor

Signature

Date

Prof. Eduardo Ojito

\_\_\_\_\_

Internal Examiner

Signature

Date

Dr. Anuradha Jabasingh (Associate Professor)

\_\_\_\_\_

External Examiner

Signature

Date

\_\_\_\_\_

School or Center of Chair Person

Signature

Date

## **ACKNOWLEDGMENTS**

To God be the glory for his love, guidance, protection and mercy upon our life.

I would like to extend my deepest gratitude and thanks to my instructor and now this thesis research advisor Dr. Eng. Abubeker Yimam (Associate Professor) for his encouragement, support, guidance, and constructive comments, for sharing his knowledge, skill, experience and fine-tuning starting from the development of proposal up to the successful completion of this thesis throughout the thesis of the studies.

I also offer my thanks to the laboratory technicians of the chemical engineering department in general and specially to Mr. Hintsu in particular for his time and help during my experimental work; Leather Industry Development Institute staffs especially Mrs. Meron, for her professional support to carry out experimental works.

My thanks also go to all the scientific and non-scientific staff of the AAiT who have directly or indirectly helped me in carrying out this work.

I record my special gratitude to my mother, grandmother, brother and sister for their constant patience and support.

This work is dedicated to my parents for all the support and motivation they have given me throughout my life. I look forward to being able to give back to all of you. Thank you all!!

## ABSTRACT

This research paper studied the development of an optimized process for the soxhlet extraction of capsaicin from chili pepper (*Capsicum Annum*), collected from local market. Experiments had performed to get maximum yield by utilization of ethanol as a solvent. Experimentation covered three factorials to evaluate significance among temperature, solvent concentration and time. The extraction temperature was varied as 85°C, 90°C, and 95°C, the solvent concentration was 79v/v, 89v/v and 99v/v and the extraction time was varied as 3hr, 4hr, and 5hr respectively. The yield of capsaicinoid was response variables. ANOVA was applied to find significance among variables and predict optimum conditions through a regression model, P-value obtained from the model was less than 0.0001, which implies that it is highly significant. The process parameters investigated in this study have a significance effect on the yield. Reduced particle sizes produced a higher yield of capsaicinoid as long as agglomeration in the extractor did not occur. The maximum yield obtained was 16.57% at a temperature of 90°C, and extraction time of 4hr and solvent concentration was 89% ethanol. The product obtained was researched for its physio-chemical properties indicated that the refractive index was 1.4967, the moisture and volatile matter was 3.5%, the density was 990Kg/m<sup>3</sup>, the specific gravity was 1.01, and the kinematic viscosity was  $3.43 \times 10^{-6} \frac{\text{m}^2}{\text{s}}$ . Process simulation in Aspen Plus<sup>TM</sup> was carried out to evaluated independent variables and to perform sensitivity analysis. The extraction process was modeled and simulated using Aspen Plus<sup>TM</sup>. The result from the simulation indicated models more or less better than the experiment process.

**Keywords:** Chili pepper, Solvent extraction, Simulation, and Characterization

# TABLE OF CONTENTS

ACKNOWLEDGMENTS .....	I
ABSTRACT.....	II
TABLE OF CONTENTS.....	III
LIST OF TABLES .....	VI
LIST OF FIGURES .....	VII
LIST OF APPENDIX .....	VIII
ACRONYMS .....	IX
1. INTRODUCTION.....	1
1.1. Statement of the Problem.....	3
1.2. Objectives of the Study .....	4
1.2.1. General Objective .....	4
1.2.2. Specific Objectives .....	4
1.3. Significance of the Study .....	5
2. LITERATURE REVIEW .....	6
2.1. Chemical Constituents of Chili Pepper.....	8
2.2. Distribution of Capsaicin in the Fruit of the Plant.....	9
2.3. Chemistry of Capsaicin.....	12
2.4. Pharmacology of Capsaicin .....	12
2.5. Methods of Extraction.....	13
2.6. Type of Extraction .....	14
2.6.1. Percolation .....	15
2.6.2. Pressurized Solvent Extraction.....	15
2.6.3. Ultrasound-assisted Solvent Extraction.....	16
2.6.4. Extraction Under Reflux and Steam Distillation.....	16
2.6.5. Extraction with Supercritical Fluids .....	17
2.6.6. Using Soxhlet Extraction.....	18
2.6.6.1. Mechanism of Mass Transfer in Solvent Extraction.....	20
2.6.7. Leaching .....	21
2.6.7.1. Selection of Solvent .....	22

2.6.7.2. Operating Conditions .....	23
2.7. Process Simulation Using Aspen Plus™ .....	23
2.7.1. Units of Measurement .....	24
2.7.2. Stream Class .....	24
2.7.3. Flash Options .....	25
2.7.3.1. Water Solubility .....	25
2.7.3.2. 4-Phase Convergence Algorithm .....	25
2.7.4. Component Types .....	25
2.7.4.1. Adding New Components .....	26
2.7.5. Property Method .....	27
2.7.6. Free-Water Method .....	28
2.7.7. Water Solubility Methods .....	29
2.7.8. Process Equipment .....	31
2.7.8.1. SWash .....	31
2.7.8.2. Mixer .....	32
2.7.8.3. Flash2 .....	32
3. MATERIALS AND METHODS .....	33
3.1. Overview .....	33
3.2. Materials and Chemicals .....	33
3.3. Equipment .....	34
3.4. Experimental procedure .....	36
3.4.1. Chili Pepper Collection and Preparation .....	36
3.4.2. Proximate Analysis of Chili Pepper .....	36
3.4.3. Moisture Content .....	36
3.5. Extraction Process .....	38
3.5.1. Separation and Purification Process .....	38
3.6. Experimental Design for Leaching Process .....	39
3.7. Using Aspen Plus™ for Process Design .....	42
3.7.1. Global Specifications .....	42
3.7.1.1. Simulation Options .....	42
3.7.2. Components .....	43

3.7.3.	Properties .....	43
3.7.4.	Process Description .....	44
3.7.5.	Leaching Unit .....	45
3.7.6.	Purification and Solvent Recovery Unit .....	46
4.	RESULTS AND DISCUSSION.....	48
4.1.	Moisture Content of the Raw Material .....	48
4.2.	Summary of the Experimental Result .....	49
4.3.	Data Analysis for Extraction of Capsaicin.....	50
4.3.1.	Analysis Using Response Surface Methodology.....	50
4.3.1.1.	Statistical Analysis on Factors Affecting Degree of Extraction .....	51
4.3.1.2.	Development of Regression Model Equation .....	52
4.3.1.3.	Model Adequacy Check.....	53
4.3.2.	Effect of Process Variables on the Yield of Capsaicin.....	54
4.3.2.1.	The Main Effect of Individual Process Variables.....	54
4.3.2.2.	Effect of Interaction between Process Variables .....	57
4.4.	Characterization of the Extracted Capsaicin.....	60
4.4.1.	Moisture and Volatile Matter Determination .....	60
4.4.2.	Refractive Index .....	60
4.4.3.	Density and Specific Gravity.....	60
4.4.4.	Kinematic Viscosity .....	61
4.5.	Process Simulation Design .....	61
4.5.1.	Purification and Solvent Recovery Unit Analysis .....	61
5.	CONCLUSION AND RECOMMENDATION .....	64
5.1.	Conclusion .....	64
5.2.	Recommendation .....	65
	REFERENCE.....	66
	APPENDIX.....	70

## LIST OF TABLES

Table 2.1: Physio-chemical properties of capsaicin .....	11
Table 2.2: Guidelines Selecting the Best Property Method.....	27
Table 2.3: Property method for the free-water phase .....	29
Table 2.4: Water solubility methods.....	30
Table 3.1: Independent variables and levels with coded and natural values used in Box- Behnken Design (BBD) .....	40
Table 3.2: The experimental run sheet design matrix.....	41
Table 4.1: Moisture content of chili pepper.....	48
Table 4.2: Experimental output.....	49
Table 4.3: ANOVA of the fitted second-order polynomial model for yield of capsaicin.	51
Table 4.4: Experimental and predicted values of capsaicin.....	52

## LIST OF FIGURES

Figure 2.1: Chili pepper samples .....	6
Figure 2.2: Product application of chili pepper .....	7
Figure 2.3: Chemical structure of different capsacinoids .....	10
Figure 2.4: Regions of the molecule of capsaicin: A-aromatic ring, B-amide bond, and C-hydrophobic side .....	12
Figure 2.5: Modes of leaching .....	22
Figure 2.6: user defined wizard for nordihydrocapsaicin .....	26
Figure 2.7: Guidelines for choosing a property method .....	28
Figure 2.8: Flowsheet Connectivity for SWash .....	31
Figure 3.1: Framework design of the research.....	35
Figure 3.2: Microwave oven used in drying of chili pepper sample .....	37
Figure 3.3: Separation and purification process .....	39
Figure 3.4: Structure of nordahydrocapsaicin.....	43
Figure 3.5 Simulation of process flowsheet .....	45
Figure 3.6: Purification and solvent recovery unit.....	47
Figure 4.1: diagnostic plots of model adequacy .....	54
Figure 4.2: percentage of yield versus temperature on the leaching.....	55
Figure 4.3: percentage of yield versus solvent concentration on the leaching .....	56
Figure 4.4: percentage of yield versus extraction time on the leaching.....	57
Figure 4.5: Surface and Contour plot of the interaction effect of temperature with time	59
Figure 4.6: Surface and Contour plot of the interaction effect of solvent concentration with time .....	59
Figure 4.7: Temperature sensitivity analysis of flash1 .....	62
Figure 4.8: Pressure sensitivity analysis of flash1 .....	63

## **List OF Appendix**

Appendix A: Formulas and Equation

Appendix B: Laboratory sample photo

Appendix C: laboratory equipment sample photo

Appendix D: Design expert output additional data

Appendix E: Aspen Plus<sup>TM</sup> additional information

## ACRONYMS

- **EtOH** Ethanol
- **GC-MS** Gas Chromatography-Mass Spectrometry
- **UV-VIS** Ultraviolet-Visible Spectro- Photometry
- **SHU** Scoville Heat unit
- **MS** Mass Spectrometer
- $\lambda_{\max}$  Maximum Wavelength Absorption
- **HPLC** High Performance Liquid Chromatography
- **PDA** Photo Diode Array Detector
- **PFE** Pressurized Fluid Extraction
- **SCFs** Supercritical fluids
- **MS** Mass Spectrometry
- **GC** Gas Chromatography
- **RP-HPLC** Reverse Phase-High Performance Liquid Chromatography
- **LC** Liquid Chromatography
- **t** Time
- **PFE** Pressurized Fluid Extraction
- **UV** Ultraviolet
- **FLD** Fluorescence Light Detection
- **LC-MS** Liquid Chromatography- Mass Spectrometry
- **PLE** Pressurized Liquid Extraction
- **TLC** Thin Layer Chromatography
- **Rpm** Revolution per Minuit
- **SLE** Solid Liquid Extraction
- **BBD** Box Behnken Design
- **NIST** National Institute Standard Technology
- **RSM** Response Surface Method
- **atm** Atmospheric
- **V** Volume
- **VLE** Vapor-Liquid Equilibrium

- **LL** Liquid - Liquid
- **S** Solvent
- **TDE** Thermodynamic Data Engine
- **SPE** Solid Phase Extraction
- **F** Feed
- **MIX** Mixer
- **RAFOUT** Raffinate Outlet Stream
- **SFC** Supercritical Fluid Chromatography
- **EXTOUT** Extract Outlet Stream
- **R** Raffinate
- **FILTOUT** Filtration Outlet
- **AAiT** Addis Ababa Institute of Technology
- **E** Extract
- **EOS** Equation of State
- **MOI** Meaning Oriented Interface
- **P** Pressure

# 1. INTRODUCTION

Chili pepper was known by humans from antiquated times as a wild crop around 7500BC, and used for more than 6000 years ago (Bharude & Techawongstien, 2011). Pepper fruits contain the chemical group of alkaloid compounds called capsaicinoids, which are responsible for the pungency of the capsaicin species. Chili peppers are generally known as ripen fruits of various species of genus capsicum. Its play an important role as one of the most commercial crops used both as condiment or culinary supplement and as vegetable. Commonly, their hot sensory taste is due to capsaicinoids as the major group of organic compounds which is closely related to the family of alkaloids and are known to be biosynthesized and accumulated in the placenta of capsicum fruits. Capsaicinoids are soluble in moderate polar organic solvents like chloroform, acetone, ethyl acetate, methylene chloride, methanol, ethanol, acetonitrile, and others. The major capsaicinoids present in most varieties of the chili pepper are capsaicin (tran-8-methyl-N-vanillyl-6-nonenamide) and dihydrocapsaicin (8-methyl-N vanillyl nonanamide). In addition, other minor ones are also found such as nordihydrocapsaicin, homocapsaicin, nornorcapsaicin, and nonivamide. (Juangsamoot, Ruangviriyachai, & Chanthai et al., 2012). The capsaicin ( $C_{18}H_{27}NO_3$ ) is a concentrated, hydrophobic liquid which contains hundreds of aromatic compounds, organic constituents, including hormones, vitamins and other natural elements (Arora, Gill, Chauhan, & Rana et al., 2011). This chemical extracted from peppers, a colorless compound used medicine flavoring.

Capsaicin has been used in neurological research to stimulate sensory nerves and also to treat bladder inflammation (Juangsamoot, 2012). Capsaicin is currently used for the treatment of diabetic neuropathy, osteoarthritis, post-herpetic neuralgia, and psoriasis, as well as there are many patents on insecticides, insect or animal repellents, and pesticides containing capsaicinoids. Appear in dental products and a wide but diminishing group of medicines. In addition, it helps to present in bakery goods, candies, confections, meat, pickles, brewery and soft drink. As well as use in cosmetics, perfumes, soaps, detergents, and miscellaneous industrial products ranging from animals feed to insecticides to paints (Olivas, Sepulveda, & Olivas et al., 2013).

Several extraction methods can be applied depending on the type of material is being used. From the fruit of hot pepper separation; like Magnetic Stirring Extraction, Solid Phase Micro Extraction, Liquid-Liquid Extraction, Pressurized Liquid Extraction, Supercritical Fluid Extraction, Microwave Assisted Extraction, Soxhlet Extraction and Ultrasonic Assisted Extraction as described in the literature. From these methods extractions by Soxhlet is more appropriate. As this method allows use of a large number of organic solvents, including benzene or ethanol which may be accepted for pharmaceutical purpose. The fundamental nature of solvent extraction was that it enables a compound or mixture of compounds to be extracted at a temperature substantially below that of the boiling point of the individual constituent. It contains components with boiling points up to 100°C or higher temperatures. Separation and purification of capsaicin and its analogues was a particularly difficult procedure because of their similar solubility, polarity and spectrometry behavior.

There are several factors that have an effect in the extraction process. One of the factor is solvent. Solvent was usually used to recover a component from either a solid or liquid. The solid was contacted with a solvent that must be dissolved the solutes of interest. So, it was related to the solubility and viscosity of the solvent itself in order to extracted the materials. The other factor was extraction time. Extraction time had its own effect on the extraction where increasing of extraction time was increased the extracts of the desired component. And also, the other factor which had been considered in this research was the temperature. The temperature contributed on the yield of required product where it was suggested concentration of capsacinoid decreased when there was increased in temperature too large. This research purpose was to determine the best solvent concentration, the best extraction time and the best temperature to produced most yield of capsacinoid.

To control dependent variable in the experiment was challenging upon acquiring a maximum and quality for optimum response. Conducted Response Surface Methodology (RSM) was suitable as it was an economical robust and widely used methods in optimization process of extraction successfully. The objective of this study was to apply Box Behnken Design (BBD) based on RSM to analyze the effects of the process parameters on capsaicinoid production from chili pepper and to search for the optimal values for attaining a higher required product yield. (Liljana, Viktorija, & Emilija et al., 2013b) in

order to offered fast and reliable methods for separation and quantification of capsaicinoid with its analogues.

The purpose of this study was determination the chemical composition as well as the content of capsaicin in pepper fruits which was growing in the Alaba and Durame, and to simulate the extraction unit operation of the capsaicin process and to proposed a realistic recovery simulation in order to reuse the solvents. Finally, evaluated experimental data compare with aspen plus<sup>TM</sup>, which used in the simulation of capsaicin extraction by applying various unit operation like SWash, filtration and flash separation those were available in Aspen Plus<sup>TM</sup> model pallet. The simulation helped to find which independent factors affect the entire process and to maximize process output put by performed sensitivity analysis.

### **1.1. Statement of the Problem**

Chili pepper (*capsicum annum*) has been cultivated in Ethiopia for long period of time. It is extensively growing in many parts of the country under warm and humid weather conditions and the best fruit is obtained in a temperature 21-27 °C during the daytime and 15-20°C at night. The major pepper producing regions in the country are, Amhara, Southern Nations and Nationality People's Regional State and Oromia. Pungency is produced by the capsaicinoids, alkaloid compounds ( $C_{18}H_{27}NO_3$ ) that are found only in the plant genus, *capsicum* which is widely used for both traditional and modern medicine to treat asthma, cancer, burning fat accumulation, coughs, a counter-irritant balm (cream), for external application of sore muscles, depression, exhaustion, labor pains and sensitive skins sore throats. Any other plants part cannot be produces capsaicinoids. It is a rich source of vitamin A, C and E. Both hot and sweet peppers contain more vitamin C than any other vegetable crops. So as to fulfill the demand of capsaicinoid for pharmaceutical industry, research about this product should be carried out to find the best technique and the cheapest way to obtain high quality yield of the product.

Even though our country produced large amount of chili pepper in various areas, it is restricted mainly for making domestic food purpose. And also, most researchers didn't

conduct to extracted high quality end product of capsaicin from chili pepper for both pharmaceutical and medical targeted. The demand of capsaicinoid increases time to time and it creates wide opportunities for global and local marketing and this leads to the requirement of competitive product in market which comes with all the advantages in terms of quality and cost.

Based on the literature review, any models have not been yet developed to simulate the process of capsaicin extraction with Aspen Plus<sup>TM</sup>. Since ethanol use as a solvent has wide interest in the field of capsaicin extraction; widely available in local market, highly affordable, recoverable and environmental friendly. Process models are necessary and solvent extraction was observed to give certain advantages like low temperature operation, high purity end products, pollution-free operation and tailored separation based on control of operating conditions. ethanol has been conventionally used for the extraction of capsaicinoid components from raw chili pepper, to check the feasibility of using ethanol in extraction units and to decide as it has a high purify for product components as compared to the other solvents.

## **1.2. Objectives of the Study**

### **1.2.1. General Objective**

Thus, this study focused on study of solvent extraction of capsaicin from chili pepper and also simulated the process by used Aspen Plus<sup>TM</sup> so as to compared with experimental result. Finally follow characterization step to qualify the final product.

### **1.2.2. Specific Objectives**

- To extract capsaicin from chili pepper using ethanol as a solvent
- To simulate a model for extraction process using Aspen Plus<sup>TM</sup>
- To determine the effects of temperature, time and solvent concentration exploit sensitivity analysis
- To investigate the main and interaction effect of Soxhlet extraction parameters (extraction temperature, extraction time and solvent concentration) using response surface methodology RSM.

- To find optimum process condition to maximizing the yield
- To characterize the chili pepper and capsaicinoid composition to qualify the final product.

### **1.3. Significance of the Study**

As we know Ethiopia is an importer of capsaicin for different medicine. Our government spends a substantial amount of its foreign currency reserves on these capsaicin products. Hence by producing this product from locally available raw materials we can save this currency for our country. In general, the significance of this research can be seen from different perspectives, based on experimental studies of solvent extraction (using ethanol). In order to achieve the objectives mentioned above, significance has been identified; Solvent extraction method is economical and also relatively pure than other methods, it helps to provide an economically feasible option to produce capsaicin locally, which will play a major role to substitute import and promote export capsaicin chemical which save hard currency and create job opportunity, to compare amount of capsaicin extracted from belonging to different place or country product, properties of capsaicin produced by solvent extraction method are not altered, from the output of the project investors, farmers, researchers and higher learning institution will be benefited, to determine optimum operating conditions in producing highest quality and substantial yield of capsaicin, and To encourage the use of ethanol as suitable solvents for producing highest quality of capsaicin.

## 2. LITERATURE REVIEW

Chili peppers, which belong to the plant genus *capsicum*, are widely grown for their fruits (Olivas, 2013). Capsaicin and several related compounds are called capsaicinoids. Capsaicin is the active element in pepper, which accounts for its prominent pharmaceutical and antioxidant properties. Research has shown that the more the capsaicin, the hotter the pepper, and the higher the antioxidant level. Pure capsaicin is a hydrophobic, colorless, highly pungent, and crystalline to waxy compound. This may be eaten fresh or cooked, used as a dried powder, or processed into oleoresins. Paprika oleoresin, a viscous, dark red liquid, is prepared industrially by solvent extraction of the dried fruit and the subsequent removal of the solvent (Wesołowska, Jadczak, & Grzeszczuk et al., 2011).



Red chili

Green chili

Green pepper

**Figure 2.1:** Chili pepper samples (Source: Al-snafi, 2015)

Several types of capsaicinoids can be present in the oleoresin extracted from hot peppers. A major component of this group of compounds is capsaicin and its dihydro derivative-dihydrocapsaicin. The great diversity of the genus *capsicum*, always complicated the taxonomic classification of peppers, and thus the extraction and identification of the exact number of isomers of capsaicin that can be found in them. From biogenetic point of view, capsaicin is acylated degraded phenylpropene, whereby the aromatic ring and acyl radicals are the result of the unique metabolic processes that take place only in the placenta of the fruit of hot pepper.

Chili peppers have been used for a long time ago in the food industry, in traditional medicine, in agricultural industry and for many other aims. These fruits contain large group of alkaloids capsacinoids, which are characteristic only for the genus capsicum. These alkaloids are responsible for the pungency of hot peppers (Sethuraman & Ch, 1997). The great diversity of the genus capsicum always complicated the taxonomic classification of peppers, and thus the extraction and identification of the exact number of capsacinoids. Capsaicin was first crystallized in 1876 by Tresh, who named it and capsaicin's molecular structure, was resolved by Nelson and dawson in 1919.

The extract of these indigenous herbs is highly effective in rheumatism, stiff joints, bronchitis and chest colds with cough and headache. Also effective in wasting of muscles in paresis. It may be used as a cream for the temporary relief of minor aches and pains of muscles and joints associated with arthritis, simple backache, strains and sprains (Bharude & Techawongstien, 2011)



**Figure 2.2:** Product application of chili pepper (Source: Sethuraman & Ch, 1997)

For centuries capsaicin was used unknowingly in the form of chili peppers in foods in order to enhance their taste, aroma, color and hotness.(Al-snafi, 2015) Besides it was used in food industry, capsaicin has found its application in pharmaceutical industry as well providing many health benefits and treatment strategies for medical conditions. (Gudeva, Maksimova, & Spasov et al., 2001) Capsaicin is also known to be active against neurogenic inflammation which causes burning and stinging sensation in hands mouth and eyes. These properties make capsaicin an active ingredient in different pepper sprays.

## **2.1. Chemical Constituents of Chili Pepper**

Capsicum contains ‘capsaicin’ which gives pungent taste to the capsicum. Capsicum also contains pigments like ‘capsanthin’ and ‘carotene which gives red color to the fruit. Capsicum also contains fixed oils, proteins, ascorbic acid and thiamine. Chillies are high in vitamin C (about twice that of citrus fruits) even after cooking it only loses 30 percent of its vitamin C, dried chillies are very high in vitamin A, red chillies are a great source of  $\beta$ -carotene, it is effective in protecting against cancer, chillies have antibacterial qualities, and contain bio-flavinoids, anti-oxidants most common in apple juice (Haanpää & Treede, 2012). The pungency of capsicum can be destroyed by oxidizing agents like potassium permanganate. IUPAC NAME (Chili): 8-Methyl-N-vanillyl-trans-6-nonenamide. It uses are the following:

- ✓ Chillies used as a condiment under the name of cayenne pepper.
- ✓ The drug given internally in atonic dyspepsia and flatulence.
- ✓ It is used as counter-irritant, in the form of ointment, plaster, medicated wool etc. for the relief of rheumatism, lumbago, etc.
- ✓ Capsaicin creams are available for the relief of pain in osteoarthritis, post-herpetic neuralgia painful diabetic neuropathy.
- ✓ If powdered red chili is applied to the part affected by a dog bite, immediately it minimizes the effect of the poison. It also acts as an antiseptic by preventing the formation of puss in the wound.
- ✓ Boil water, mixed with one spoonful of powdered chili and one spoonful of salt in it. If this hot solution is drunk, it is beneficial in cholera.

- ✓ Boil water, in which powdered red chili has been mixed, sprinkle this water on those areas where bed bugs are present, bed bugs will be eliminated.
- ✓ When seeds of chilies are swallowed with hot water, then the stomachache due to cold, gets vanished.

Property of capsaicin is an odorless, colorless, hydrophilic, crystalline- waxy compound with molecular mass 305.4g/mol, melting point 62-65°C boiling point at 0.01mmHg 210-220°C and sublimate at 115°C. It has  $UV_{max}$  227,281nm ( $\epsilon=7000, 2500$ ). Capsaicin is highly soluble in alcohol, ether, benzene and chloroform, slightly soluble in carbon disulfide and hot water (Al-snafi, 2015). It is fairly resistant to acids and alkali solutions at room temperatures.

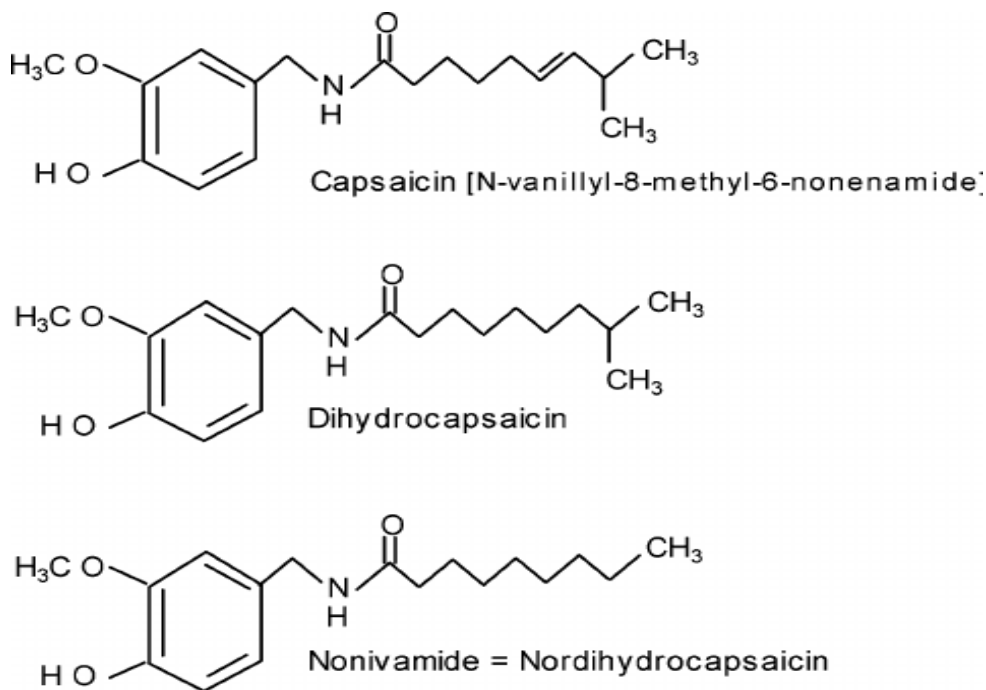
Pepper pungency is measured in Scoville Heat Units (SHU). This measurement is the highest dilution of a chili pepper extract at which heat can be detected by a taste panel. It is represented by 69%, dihydrocapsacin by 22%, nordihydrocapsacin by 7% homocapsaicin and homodihydrocapsaicin takes only 1% in the group of capsaicinoids (Gudeva, Mitrev, & Treede et al., 2001).

## **2.2. Distribution of Capsaicin in the Fruit of the Plant**

Recent studies indicate that the highest concentrations of capsaicin are found in the ovary and in the lower flesh (tip) and the lowest content of capsaicin can be found in the seeds. The gland on the of the fruit produces capsaicinoids. The seeds are not the source of pungency but they occasionally absorb capsaicin because they are in close proximity to the placenta. No other plant part produces capsaicinoids. The majority, about 89%, of the capsaicin is associated with the placental partition of the fruit and nearly 5-6% in the pericarp and the seed. Composition of capsaicin may vary among different varieties of same species and with fruit of a single variety. The pungency is influenced with the weather conditions such as heat wave and it increases with the growth of the maturity of fruit (Arora, Gill, and Chuhan et al., 2011).

According to most reports, capsaicinoids accumulate in the epidermis of the placenta stored in vesicles on the surface of this tissue, while seeds occasionally absorb capsaicinoids

because of their proximity to the placenta (Botanicae Horti Agrobotanici Cluj-Napoca & Sharma et al., 2008). Recent studies showed that the concentration of capsaicin varies with taxa and genotype, the geographical origin and the climatic conditions (Delelegn, n.d.). The only *Capsicum* which doesn't contain capsaicin is the sweet pepper.



**Figure 2.3:** Chemical structure of different capsaicinoids (Source: (Botanicae Horti Agrobotanici Cluj-Napoca & Sharma et al., 2008))

The structural characteristics of capsaicinoids that determine their spicy properties are associated with (Liljana, Viktorija, & Emilija et al., 2013b) the presence of an amide bond connecting a vanillyl ring and an acyl chain. The physio-chemical properties of capsaicin are listed below in the table 2.1. The placenta of the fruit is the unique part of capsicum plant which produces capsaicin. The highest capsaicin concentrations are found in the ovary and in the lower flesh (Gudeva, Maksimova, & Spasov et al., 2001). The seeds are not the source of capsaicin, they contain the lower capsaicin concentrations, (Zahra, Arshad, & Inam et al., 2016) they get their pungency through contact with the placenta. The production of capsaicin is controlled by a single dominant gene.

**Table 2.1:** Physio-chemical properties of capsaicin

<b>properties</b>	<b>Value</b>
Molecular weight	305.41 g/ mol
Melting point	62 to 65 °C (335 to 338K)
Boiling point	210 to 220 °C (483 to 493K)
Flash point	113°C
Stability	Stable. Incompatible with strong oxidizing agents.
Solubility	H <sub>2</sub> O insoluble; alcohols and organic solvents soluble
UVmax	227- 281mm

(Source: Liljana, Viktorija, & Emilija et al., 2013a)

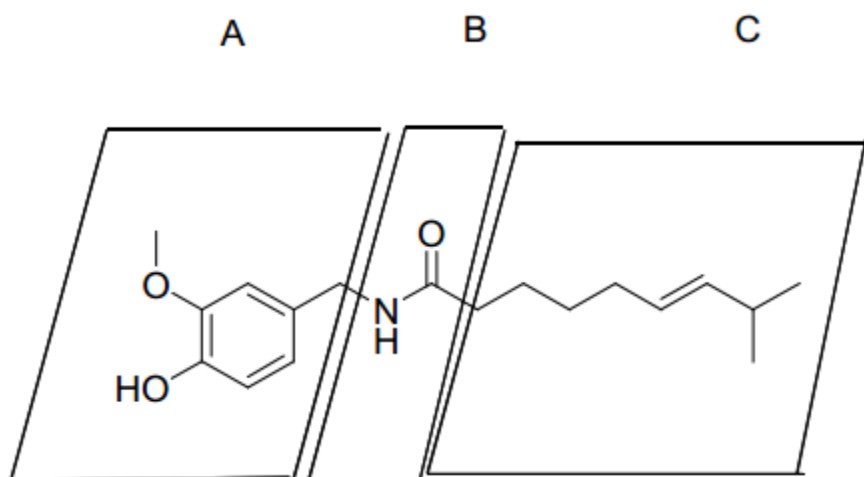
The capsaicin content of peppers is one of the parameters that determine their commercial quality. According to (Eda, 2012) the amount of capsaicin can vary, depending on the light intensity and temperature at which the plant is grown, the age of the fruit, and the position of the fruit in the plant.

The main component in this group is capsaicin. Capsaicin is presented with 69-72 % of the content of capsaicinoids, and is the most responsible component for pungency of the peppers together with dihydrocapsaicin. (Isobe et al., 2003) The degree of pungency depends on the capsicum species and cultivars, because capsaicin and dihydrocapsaicin

content can be affected by different factors such as developmental stage of the fruit, and the environmental growth conditions. The first test developed to measure pungency was the Scoville test, first developed in 1912 by Wilbur Scoville and it was an organoleptic test. Nowadays, however, this test has been largely replaced by (Man, Wilhad, & Reuter, n.d.) spectroscopic and chromatographic methods which are considered to be more reliable and accurate.

### 2.3. Chemistry of Capsaicin

Capsaicin molecular structure has been first resolved by Nelson and Dawson in 1919. Since the double bond seems to prevent the internal rotation, thus capsaicin shows trans/cis isomerism. But mostly it is found in trans isomeric form because in cis form the  $\text{CH}(\text{CH}_3)_2$  and the longer chain on other side of the double bond will be close together causing steric hindrance due to slight repulsion between them (Arora, Gill, & Chauhan et al., 2011)



**Figure 2.4:** Regions of the molecule of capsaicin: A-aromatic ring, B-amide bond, and C-hydrophobic side (Source: Arora, Gill, & Chauhan et al., 2011)

### 2.4. Pharmacology of Capsaicin

As (Ada, Omer, & الفلفل et al., 2016) reported that chili pepper is one of the traditional plant that has so many pharmacological effects. Capsaicin is the active principle which accounts for the pharmaceutical properties of peppers. It has been used as an analgesic against arthritis pain and inflammation. It has been reported to show anticancer effect, a capsaicin

cream is used to lower the sensation of pain in such conditions as arthritis, helps to chronic back pain, relieve chronic soft tissue pain, provide relief for neuropathic pain, it plays some great roles in suppressing prostate cancer cells, and other painful chronic condition and neurogenic inflammation which is the basis for the use of capsaicin as defensive pepper sprays. Capsaicin has also been reported to show protective effects against cholesterol and obesity (Eghdami, Salehi, & Babakhani et al, 2014).

Capsaicin enhances digestion because it increases stomach acid secretion and stimulates the mucous membrane and (Haanpää & Treede, 2012) mentioned that capsaicin is used medicinally as analgesic relieve the pain, when it causes the brain to release endorphins. Among the pains which treated by capsaicin arthritis, cluster headaches, and neuropathic pain. Capsaicin also has antimicrobial effects for food preservation. Capsaicin has both internal and external medicinal uses; it is used internally for various conditions such as colic and improving peripheral circulation, externally it is used for unbroken chilblains. A cream for topical application has been used to relieve the pain of post herpetic neuralgia and other pain syndromes.

## **2.5. Methods of Extraction**

When extracted, Capsaicin ( $C_{18}H_{27}NO_3$ ) (Al-snafi, 2015) is an odorless, white crystal having harsh flaring pungency. Its melting point is  $65^{\circ}C$  and boiling point is  $210^{\circ}C$  to  $220^{\circ}C$ . It is slightly soluble in water and adequately soluble in ether, benzene, alcohol and chloroform. Its molecular structure shows (De Lourdes Reyes-Escogido, Gonzalez-Mondragon, & Vazquez-Tzompantzi, 2011) cis-trans isomerism and doesn't allow internal rotation due to presence of double bond. Its molecule is most active in trans form. As mentioned above, capsaicinoids are mostly soluble in organic solvents such as methanol, ethanol, acetonitrile, n-butyl chloride, ethyl acetate, acetone, propanol and hexane.

Using these solvents, capsaicinoids are extracted by a number of methods such as (Zahra, Arshad, & Inam et al., 2016) Magnetic Stirring Extraction, Solid Phase Microextraction, Liquid Liquid Extraction, Pressurized Liquid Extraction, Supercritical Fluid Extraction,

Microwave Assisted Extraction, Soxhlet Extraction and Ultrasonic Assisted Extraction. Generally, the fruit from which the capsaicinoids are to be extracted are first dried and smashed then further dehydrated using desiccator. According to (Rafael Rocha-Herrera & Rafael, n.d.) this material is then treated with organic solvents like methanol, ethanol or acetonitrile and is incubated at temperature of 40°C. Organic extraction of capsaicin is obtained by filtration or centrifugation. (Zahra, Arshad, & Inam et al., 2016) This extract needs to be ultra-filtered before its estimation by HPLC or spectrometric analysis. Techniques like SPE are further used to purify the extract. Although there are many techniques for extraction, this review focuses on some of the modern techniques used for extraction like (Resources, 2012) Accelerated Solvent Extraction, Solid Phase Extraction and Magnetic Stirring Extraction.

One of the most common hot pepper products is the pungent capsicum oleoresin that presents an organic oily resin derived from dried ripe fruits of different pungent varieties of capsicum by means of solid - liquid extraction (Arora et al., 2011). Generally, the most commonly employed and a preferred method for extraction of compounds present in plant matrices the conventional solid – liquid extraction using organic solvents. In later studies, these conventional methods were improved, modified or rationalized by varying different operating parameters. (Liljana et al., 2013b) There are several techniques that can be used for solid – liquid extraction of capsaicin like vacuum filtration, percolation, soxhlet technique, supercritical fluid extraction etc. (Stoica, Moscovici, Tomulescu, & Băbeanu, n.d.). Other factors include toxicity, flammability, interfacial tension, density, viscosity, boiling point, availability and cost.

## **2.6. Type of Extraction**

Several approaches can be employed to extract the plant material. Although water is used as an extract and in many traditional protocols, organic solvents of varying polarities are generally selected in modern methods of extraction to exploit the various solubilities of plant constituents.

### **2.6.1. Percolation**

The powdered plant material is soaked initially in a solvent in a percolator. Additional solvent is then poured on top of the plant material and allowed to percolate slowly (dropwise) out of the bottom of the percolator. Additional filtration of the extract is not required because there is a filter at the outlet of the percolator. Percolation is adequate for both initial and large-scale extraction. The main disadvantages are :

- ✓ Fine powders and materials such as resins and plants that swell excessively (e.g., those containing mucilages) can clog the percolator.
- ✓ If the material is not distributed homogeneously in the container, the solvent may not reach all areas and the extraction will be incomplete.

### **2.6.2. Pressurized Solvent Extraction**

Pressurized Fluid Extraction (PFE) or Pressurized Liquid Extraction (PLE) is a new sample extraction method that employs liquid solvents at elevated temperatures and pressures to prepare samples for analysis by either gas chromatography or liquid chromatography. Pressurized liquid extraction is similar to Soxhlet extraction, except that during the extraction process the solvent condition inside the PLE cell approaches the supercritical region which results in more efficient extractions. The elevated temperature allows the sample to become more soluble and achieve a higher diffusion rate while the elevated pressure keeps the solvent below its boiling point. At elevated pressures and temperatures solvents can penetrate solid samples more efficiently which reduces solvent usage.

The powdered plant material is loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time. The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to solubilize the remaining components. A final purge with nitrogen gas is performed to dry the material. Offers a more economical and environment-friendly alternative to conventional approaches.

Pressurized solvent extraction or “accelerated solvent extraction, ’employs temperatures that are higher than those used in other methods of extraction and requires high pressures to maintain the solvent in a liquid state at high temperatures. It is best suited for the rapid and reproducible initial extraction of a number of samples. The solid biomass sample is loaded into an extraction cell, which is placed in an oven. The solvent is then pumped from a reservoir to fill the cell, which is heated and pressurized at programmed levels for a set period of time. The cell is flushed with nitrogen gas, and the extract, which is automatically filtered, is collected in a flask. Fresh solvent is used to rinse the cell and to solubilize the remaining components. A final purge with nitrogen gas is performed to dry the material. High temperatures and pressures increase the penetration of solvent into the material and improve metabolite solubilization, enhancing extraction speed and yield. Moreover, with low solvent requirements, pressurized solvent extraction offers a more economical and environment-friendly alternative to conventional approaches. As the material is dried thoroughly after extraction, it is possible to perform repeated extractions with the same solvent or successive extractions with solvents of increasing polarity. An additional advantage is that the technique can be programmable, which will offer increased reproducibility. However, variable factors, e.g., the optimal extraction temperature, extraction time, and most suitable solvent, have to be determined for each sample (Kaufmann, 2002; Tsubaki, 2010; Sarkar, 2006).

### **2.6.3. Ultrasound-assisted Solvent Extraction**

This is a modified maceration method where the extraction is facilitated by the use of ultrasound . The plant powder is placed in a vial. The vial is placed in an ultrasonic bath, and ultrasound is used to induce a mechanical stress on the cells through the production of cavitations in the sample. The cellular breakdown increases the solubilization of metabolites in the solvent and improves extraction yields.It is mostly used for the initial extraction of a small amount of material.

### **2.6.4. Extraction Under Reflux and Steam Distillation**

plant material is immersed in a solvent in a round-bottomed flask, which is connected to a condenser. The solvent is heated until it reaches its boiling point. As the vapor is

condensed, the solvent is recycled to the flask. It is commonly applied to the extraction of plant essential oils. The main disadvantage is that thermolabile components risk being degraded.

### **2.6.5. Extraction with Supercritical Fluids**

Supercritical fluid extraction (SFE) is one of the relatively new efficient separation methods for the extraction of essential oils from different plant materials. The new products, extracts, can be used as a good base for the production of pharmaceutical drugs and additives in the perfume, cosmetic, and food industries. Use of SFE under different conditions can allow selecting the extraction of different constituents. The main reason for the interest in SFE was the possibility of carrying out extractions at temperature near to ambient, thus preventing the substance of interest from incurring in thermal denaturation. Supercritical fluid extraction has proved effective in the separation of essential oils and its derivatives for use in the food, cosmetics, pharmaceutical and other related industries, producing high-quality essential oils with commercially more satisfactory compositions Biomass Now – Sustainable Growth and Use 392 (lower monoterpenes) than obtained with conventional hydro. Also, extraction with supercritical fluids requires higher investment but can be highly selective and more suitable for food products. This plays a mechanistic role in supercritical fluid chromatography (SFC), where it contributes to the separation of the solutes that are injected into the chromatographic system. Supercritical fluid extraction is an interesting technique for the extraction of flavoring compounds from vegetable material. It can constitute an industrial alternative to solvent extraction and steam distillation processes (Stahl and Gerard, 1985). SFE allows a continuous modification of solvent power and selectivity by changing the solvent density (Nykanen, 1991). Nevertheless, the simple SFE process, consisting of supercritical CO<sub>2</sub> extraction and a one-stage subcritical separation, in many cases do not allow a selective extraction because of the simultaneous extraction of many unwanted compounds.

Supercritical fluids (SCFs) are increasingly replacing organic solvents, e.g., n-hexane, dichloromethane, chloroform, and so on, that are conventionally used in industrial extraction operations because of regulatory and environmental pressures on hydrocarbon

and ozone-depleting emissions. Most of the currently available Solvent Free Extraction systems utilize CO<sub>2</sub>, which is generally considered as safe for solvent-free extraction processes. The fundamental steps involved in SFE are as follows:

- a) Liquid CO<sub>2</sub> is forced into supercritical state by regulating its temperature and pressure.
- b) Supercritical CO<sub>2</sub> has solvent power and extracts predominantly lipophilic and volatile compounds.
- c) Gaseous CO<sub>2</sub> returns to CO<sub>2</sub> tank. After a full round, the new extraction starts with circulating CO<sub>2</sub>.

#### **2.6.6. Using Soxhlet Extraction**

This method is adequate for both initial and bulk extraction. In the solvent-extraction method, a hydrocarbon solvent is used for extraction. It repeatedly washed the solute with the solvent. All the extractable material from the plant is dissolved in the solvent. This includes highly volatile aroma molecules as well as non-aroma waxes and pigments. (Rafael Rocha-Herrera & Rafael, n.d.). The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the set up is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath. The solvent is heated to reflux. The solvent vapor travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound dissolves in the warm solvent. When the Soxhlet chamber is almost full, the chamber is emptied by the siphon. The solvent is returned to the conical flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle may be allowed to repeat many times, over hours or days. The concentrated concretes are further processed to remove the waxy materials. To prepare the absolute from the concrete, the waxy concrete is warmed and stirred with alcohol (ethanol). (Arora et al., 2011) During the heating and stirring process the concrete

breaks up into minute globules. Since the aroma molecules are more soluble in alcohol than the waxes, an efficient separation of the two results.

Capsaicin oil ( $C_{18}H_{27}NO_3$ ) extraction is a special type of solid-liquid extraction or a separation process for temperature sensitive materials like oils, resins, hydrocarbons, etc. which are slightly soluble in water and may decompose at their boiling point. The fundamental nature of is that soxhlet extraction it enables a compound or mixture of compounds to be distilled at a temperature substantially below that of the boiling point(s) of the individual constituents. It contains substances with boiling points up to  $105^{\circ}C$  or higher temperatures. In the presence of steam or boiling water, however, these substances are volatilized at a temperature close to  $100^{\circ}C$ , at atmospheric pressure. During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

Fresh, or sometimes dried, botanical material is placed in the plant chamber of the still and the steam is allowing to pass through the herb material under pressure which softens the cells and allows the chemical to escape in liquid form. The temperature of the solvent must be high enough to dissolve the capsaicinoid present, yet not so high that it destroys the plants or burns the desired chemicals.

A number of factors determine the final quality of a soxhlet extraction product. Apart from the plant material, most important are time, temperature and pressure, and the quality of the extraction equipment. It comprises very complex products. Each is made up of many, sometimes hundreds, of distinct molecules which come together to form the product's aroma and therapeutic properties. Some of these molecules are fairly delicate structures which can be altered or destroyed by adverse environmental conditions. It is possible that longer extraction times may give more complete desired product. (Liljana, 2013a)

The main advantage of soxhlet extraction is that it is an effective process. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This technique is particularly useful in cases

when the pure compound is partially soluble in a solvent and the impurity is not soluble in that solvent and vice versa. Also, the working principle of mechanism is so simple that we can obtain more desired compounds without any difficulty. It is the most useful apparatus for solid–liquid extraction in various fields such as pharmaceuticals, Environment & also foodstuffs nowadays, Soxhlet apparatus is still common and widely used as a reference and standard method in many laboratories for the extraction of oil from various materials. solvent extraction method is that it is a relatively cheap process to operate at a basic level, and the properties of capsaicin produced by this method are not altered. As solvent reduces the boiling point of a particular component of the capsaicin, it never decomposes in this method. This method apart from being economical, it is also relatively faster than other methods. Due to different purpose and advantages we select solvent extraction method because it’s one of the best methods to extract capsaicin from pepper.

**2.6.6.1. Mechanism of Mass Transfer in Solvent Extraction**

The transfer of soluble material from a particle by the actions of a solvent or utilization of EtOH is termed leaching or percolation. Leaching is a complex mechanism involving the transfer of the solvent to the surface of the solid particles, penetration of the solvent into the solid, dissolution of the solute into the solvent, diffusion of the solute into the solvent and transfer of the solute to the bulk solvent. Based on the different phenomena for the leaching process, it becomes virtually impractical to use any one theory to explain or describe the leaching activity. The dissolution of a sample from the solid to the liquid phase depends on the rate of mass transfer from the solid surface to the solvent as the controlling factor.(Liljana, Viktorija, & Marija et al., 2009)

The mass transfer rate of a solute A being dissolved in a solvent of volume V (m<sup>3</sup>) is

$$N_A = K_L\alpha(C_{AS} - C_A) \dots\dots\dots 2.1$$

Where, N<sub>A</sub> is the kg/mol of A dissolving to the solution per second,

$\alpha$  is inter-surface area of the particles (m<sup>2</sup>),

K<sub>L</sub> is mass transfer coefficient (m/s),

$C_{AS}$  is saturation solubility of the solute (kg/mol/m<sup>3</sup>)

$C_A$  is time dependent concentration of the solute.

The rate of accumulation of A into the solvent by material balance is:

$$\frac{VdC_A}{dt} = K_L\alpha(C_{AS} - C_A) \dots\dots\dots 2.2$$

By integration of equation (2.2) for  $t = 0, C_A = C_{A0}=0, t = t_f, C_A = C_{AS}$

$$\frac{C_{AS}-C_A}{C_{AS}-C_{A0}} = 1^{-(K_L)t} \text{ or } \ln \frac{C_{AS}-C_A}{C_{AS}-C_{A0}} = -(K_L)t \dots\dots\dots 2.3$$

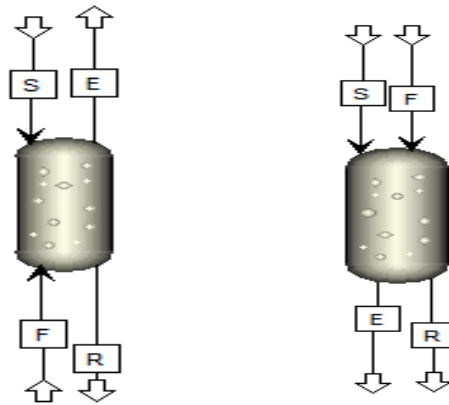
These equations aid in the understanding and calculation of the process of leaching of solute or liquid from a solid substance using a solvent.

### 2.6.7. Leaching

Leaching is a mass transfer operation to separate components distributed between two insoluble phases of a mixture. When both the phases are liquid it is known as liquid-liquid extraction (LLE) whereas when one of them is solid it is called leaching or solid-liquid extraction (SLE). The mass transfer operations fall into two categories, direct and indirect. Direct operations like distillation, evaporation and zone refining are those which do not utilize added substances and indirect operations like solid-liquid extraction, liquid-liquid extraction, extractive distillation, absorption and adsorption involve a foreign substance like solvent. Solid-liquid extraction uses a solvent to remove a soluble component from an insoluble component stream.

The solution containing the components to be separated is the feed sent to the SWash process. The major component in this solution is called the feed solvent and the other components are called solutes. The solvent strips the solutes from the feed by absorbing them. Of the two streams produced after extraction, the solvent rich stream containing the desired solute is called extract and the residual stream rich in feed solvent is called raffinate. The yield and economics of the extraction process strongly depend on the solvent used, operating conditions, mode of operation and equipment.

The leaching process is carried out either in cross-current or counter-current mode. In the cross-current mode, both solid and liquid phases are feed in the same direction and they are separated before leaving each stage. It is used for low capacity multi product batch operations like pharmaceutical and agro-chemical processes. It is practical and economical for washing and neutralization operations and also offers good flexibility. In the counter-current extraction scheme, feed (F) and solvent (S) enter the leacher from opposite ends and pass each other counter currently. This mode is used for large volume operations and for an effective use of the solvent. The configurations are explained in figure 2.5. The solvent is then recovered from the extract stream leaving the solid-liquid contactor. Evaporation, pervaporation, distillation and flash separation are the methods generally used to recover solvent. The recovered solvent is recycled for reuse.



**Figure 2.5:** Modes of leaching

### 2.6.7.1. Selection of Solvent

The desirable characteristics of a solvent for solid-liquid extraction are

- a) **Selectivity:** This is defined as the ability of the solvent to preferentially dissolve more of one component than the other.
- b) **Distribution coefficient:** This is also known as Partition ratio and is defined as the ratio of a certain component in extract phase to raffinate phase. The partition ratio of solute should be fairly large.

- c) **Recoverability:** This stands for the ease of separation of the solvent from the extract and raffinate phases.
- d) **Cost:** It should be low price and available in local market.
- e) **Capacity:** This represents the amount of solute loaded per weight of solvent in the extract at the solubility limit.
- f) **Toxicity:** It should be non-hazardous and un reactive
- g) **Flammability:** Should not be flammable

#### 2.6.7.2. Operating Conditions

The yield of the solid-liquid extraction process depends on the temperature, solvent concentration and retention time of the process. The effect of pressure on the leaching process was negligible and hence operating pressure is usually governed by vapor pressure considerations. High temperatures may sometimes be used to minimize mass transfer resistance. Solubility, yield and vapor pressure are other conditions to be considered.

### 2.7. Process Simulation Using Aspen Plus™

Aspen Plus™ is a very powerful and global process simulation tool. It is extensively used to design a new process, troubleshoot an existing process unit and/or optimize operations in a process. (Plus & Guide, n.d.) Using Aspen Plus, the behavior of the process can be predicted based on basic relations like mass and energy balances and phase equilibrium. Primary purposes of this study were to design a process to extract capsaicin and develop a method to use Aspen Plus™ for leaching or solid-liquid extraction. (Aspen Plus, n.d.) Each step involved in developing both steady state and dynamic model using Aspen Plus™ is explained in detail.

Simulation is a process of designing an operational model of a system and conducting experiments with this model for the purpose either of understanding the behavior of the system or of evaluating alternative strategies for the development or operation of the system. It has to be able to reproduce selected aspects of the behavior of the system modeled to an accepted degree of accuracy. (Magnusson, n.d.) Process simulation can guide and minimize the experimental research, but not eliminate it. Actually, the calibration

of models requires accurate experimental data. It is the experiment that proves the model. Statistical planning of experiments is nowadays obsolete. Instead the experimental research should take profit from rigorous models incorporated in simulation packages, particularly in the field of thermodynamics. (Technology, Park, Systems, Technology, & Park, n.d.) For instance, simple vapor liquid equilibrium (VLE) experiments in laboratory can be used to increase the reliability of the feasibility study in innovative processes

### **2.7.1. Units of Measurement**

Specify global information before entering any engineering specifications for Aspen Plus<sup>TM</sup> run. The input and output units are specified on the Setup | Global | Specifications sheet. The units of any property in each set can be modified on the Setup | Units-sets form according to convenience (Guide, Aspen Plus n.d.).

### **2.7.2. Stream Class**

The default option for stream class is conventional, stated as CONVENTIONAL in Aspen Plus<sup>TM</sup>. This stream class is used when either no solids are present in the simulation or the present solids are electrolyte salts. This stream class is used with MIXED sub-stream and is specified on Setup | Specifications | Global form or Setup | Streamclass | Global. The stream class MIXNC is used when the simulation contains non-conventional solids without particle size distribution. For solids with particle size distribution, MIXNCPSD is used. For conventional solids with and without particle size distribution, MIXCISLD and MIXCIPSD (Method & Assistant, n.d.) are used respectively. When both conventional and non-conventional solids are present MIXCINC and MCINCPSD are used while the latter is used for particle size distribution. For each stream class, a respective sub stream is selected. (Plus & Guide, n.d.)

The flow basis can be mass or mole. (Guide, n.d.) The valid phases are vapor-liquid-liquid or liquid-only for solvent extraction. It is always a good practice to set the valid phase option to vapor-liquid-liquid when not sure about the presence of vapor. Either yes, no or dirty water is selected for the free water option on the Setup | Specifications | Global

### **2.7.3. Flash Options**

According to (Technology et al., n.d.) flash option can be specified as the Setup | Simulation options | Flash convergence, the upper and lower limits for temperature and pressure are usually left as default values. They can be changed if needed.

There are two algorithms for flash convergence. Either Inside-out or Gibbs can be used for sequential modular calculations. The default algorithm used in Aspen Plus<sup>TM</sup> is Inside-out for all flash calculations except three-phase true-species electrolyte calculations. Gibbs algorithm is preferred for three-phase calculations and when convergence problems arise with the inside-out algorithm.

#### **2.7.3.1. Water Solubility**

The option limit water solubility for hydrocarbon phase allows to override the water solubility calculated by the specified physical property method and limit the water solubility in the organic phase. This option is used when water is highly soluble in the organic phase. Checking or unchecking the box for this option makes no difference in the results if the water solubility is not significant.

#### **2.7.3.2. 4-Phase Convergence Algorithm**

As Aspen user guide stated (Plus, n.d.) the 4-phase convergence algorithm is more rigorous than the 3-phase and so is preferred for three phase calculations. Vapor, liquid and liquid are the three phases while water being the fourth phase in this algorithm.

### **2.7.4. Component Types**

In general, all the components are conventional. Non-conventional components are not pure chemical species but are complex mixtures. They cannot be characterized by molecular weight (Plus et al., n.d.). The properties of conventional components are already present in the built-in databanks of Aspen Plus<sup>TM</sup> but those of non-conventional components are calculated. Methods for calculating enthalpy, density and component attributes are specified in the Properties/ Advanced/ NC Props form. Property Method The property method for the process is specified on properties | Specifications | Global

form as shown in the figure 2.7. The general methods used to model the solid - Liquid equilibrium in Aspen Plus™ are given (Plus et al., n.d.)

#### 2.7.4.1. Adding New Components

According to (Plus & Guide, n.d.) a new component not present in the built-in databanks is specified using the user-defined component wizard shown in the figure 2.6. Molecular weight, chemical formula and structure of the component are required while other properties like normal boiling point and specific gravity are specified if available.

User-Defined Component Wizard

Welcome to the User-Defined Component Wizard, the quickest way to enter properties for user-defined component. This wizard will lead you through the steps to enter the required physical properties for the user-defined component based on its type.

Component ID:  Type:

Alias

Required properties for conventional components include  
Molecular weight, normal boiling point, molecular structure, vapor pressure and ideal gas heat capacity

**Figure 2.6:** user defined wizard for nordihydrocapsaicin

So as to draw structure of components enter molecular structure on Draw | Import Structure | single click of the left mouse button produces a carbon atom on the left menu, right click on the atom erases it and double click allows the user to change the carbon atom to another atom. Connecting the atoms produces a single bond and clicking on the bond allows the user to change it to double or triple bond on the conventional component additional data form, if available, further information is specified and the properties are evaluated using NIST Thermodynamic Data Engine (TDE).

### 2.7.5. Property Method

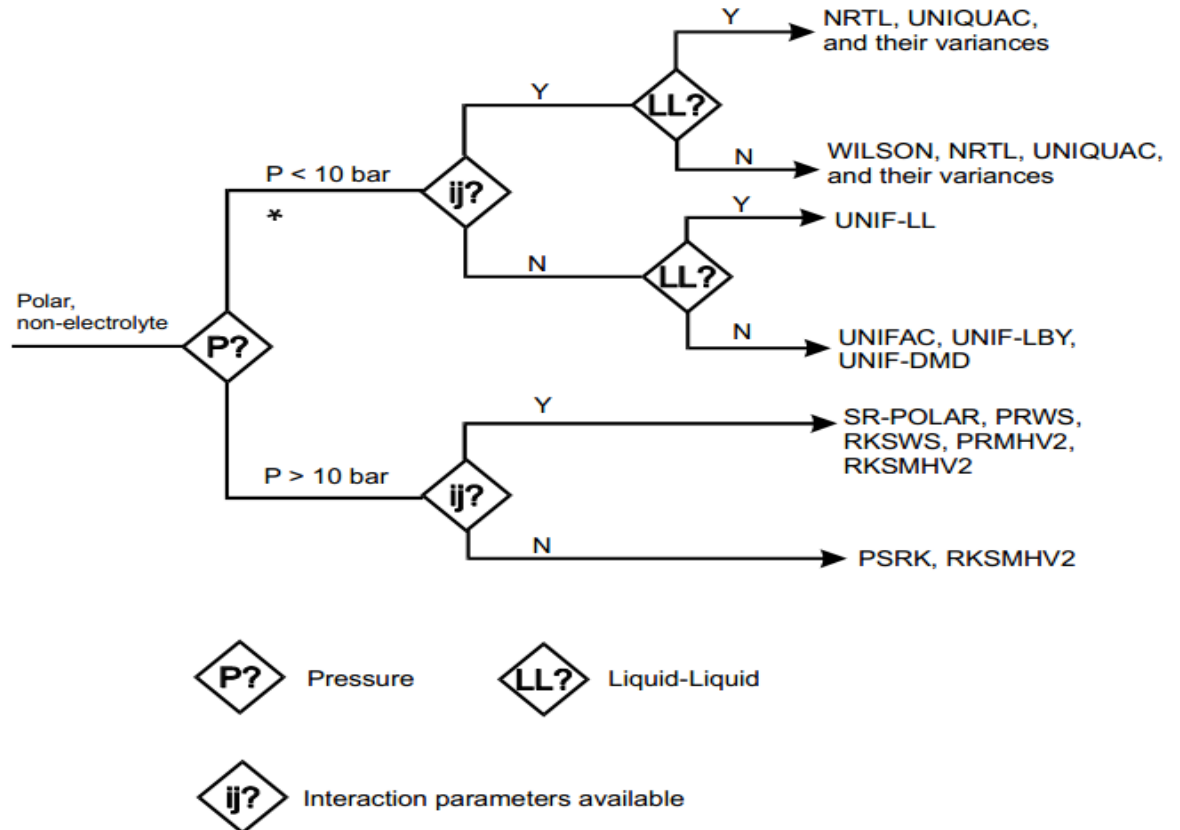
A property method is a collection of methods and models that Aspen Plus™ used to compute thermodynamic and transport properties. The property method for the process is specified on Properties | Specifications | Global form. The general methods used to model the solid-Liquid equilibrium in Aspen Plus™ are given in the table 2.2.

**Table 2.2:** Guidelines Selecting the Best Property Method

Application	Recommended property methods
Solvent reovery	WILSON, NRTL, UNIQUAC
hydrocarbon stripping	WILSON, NRTL, UNIQUAC
Acid gas stripping from methanol NMP(PURISOL)(RECTISOL) .	PRWS,RKSWs,PRMHV2,RKSMHV2, PSRK, SR-POLAR

(Source: Plus & Guide, n.d.)

The property method selection assistant is available on the Specifications | Global form. A list of suitable property methods is generated based on either component type or process type. Once component type is selected, further options, like pressure conditions, are selected to obtain a suggested property method (Plus & Guide, n.d.).



**Figure 2.7:** Guidelines for choosing a property method (Source: Plus, guide, n.d)

### 2.7.6. Free-Water Method

In three-phase calculations, Aspen Plus<sup>TM</sup> provides an option free water, which can be set to yes, no or dirty water. Setting this option to yes allows Aspen Plus<sup>TM</sup> to assume and treat the second liquid phase in the vapor-liquid-liquid phase system as pure water. Free water is the pure water layer in the two liquid phases. Solubility of organics in water is treated as zero. This option is generally used when solubility of organic phase in water is insignificant like refining applications. Any of the four water solubility methods (0 1 2 3) is used to calculate the solubility of water in the organic phase.

**Table 2.3:** Property method for the free-water phase

Property method	Description	Merits
STEAM-TA	1967 ASME steam table correlation (default)	-
STEAMNBS	NBS/NRC steam table correlations	More accurate than the ASME steam table
IDEAL or SYSOPO	For systems at low or moderate pressure	More efficient calculation than STEAM-TA or STEAMNBS

(Source: Aspen Physical Property System, 2011)

If a free-water method is specified, a free-water property method is used for stream properties; else a primary property method is used. When a free-water method is used, either water is specified as a component or water basis is selected as dry in the Properties | Prop-Set | qualifiers sheet. Free water calculations are rigorous, except for the assumption of pure water, but faster than the three phase calculations and also require less property data interpretation. Free water is generally used for a water-hydrocarbon system with insignificant solubility of water in the hydrocarbon phase.

### 2.7.7. Water Solubility Methods

According to (Plus & Guide, n.d.) the various water solubility methods are used to calculate the organic phase properties. These methods help determine the liquid fugacity in the organic phase. The k-value of water in the organic phase is calculated as

$$K_w = \frac{\gamma_w \phi_w^{*L}}{\phi_w^v} \dots\dots\dots 2.3$$

Where,  $\gamma_w$ , the activity coefficient of water in organic phase and  $\phi_w^v$ , the fugacity coefficient of water in vapor phase mixture are now calculated accordingly as given in the table 2.4.

The limiting solubility of water in the organic phase is the mole fraction weighted average of the solubilities of water in the individual organic species.

$$X_W^{SOL} = \frac{\sum_i X_i \frac{X_W^{SOL}}{1-X_W^{SOL}}}{1 + \sum_i X_i \frac{X_W^{SOL}}{1-X_W^{SOL}}} \dots\dots\dots 2.5$$

Where,  $X_i$  is the water free mole fraction of the  $i^{th}$  organic species.

$X_W^{SOL}$  is the mole fraction of soluble water in the  $i^{th}$  organic species and calculated from water solubility method.

**Table 2.4:** Water solubility methods

Sole-water option	$\gamma_w$ calculation	$\phi_w^v$ calculation	Assumptions
0	$\gamma_w = \frac{1}{X_W^{SOL}}$	Free water property method	Organic phase saturated vapor phase mostly water
1	$\gamma_w = \frac{1}{X_W^{SOL}}$	Primary property method	Organic phase saturated vapor phase mostly organic
2	$\gamma_w = f(T, X_w)$ $\gamma_w = \frac{1}{X_W^{SOL}}$ when $X_w = X_W^{SOL}$	Primary property method	Not enough to form a second liquid phase
3	Primary property method	Primary property method	None
4	$\gamma_w = 1$	Primary property method	Water solubility is 1
5	$\gamma_w = f(T, X_w)$ $\gamma_w = \frac{1}{X_W^{SOL}}$ when $X_w = X_W^{SOL}$	Free water property method	Ideal vapor

(Source: Aspen Plus Guide 7 10)

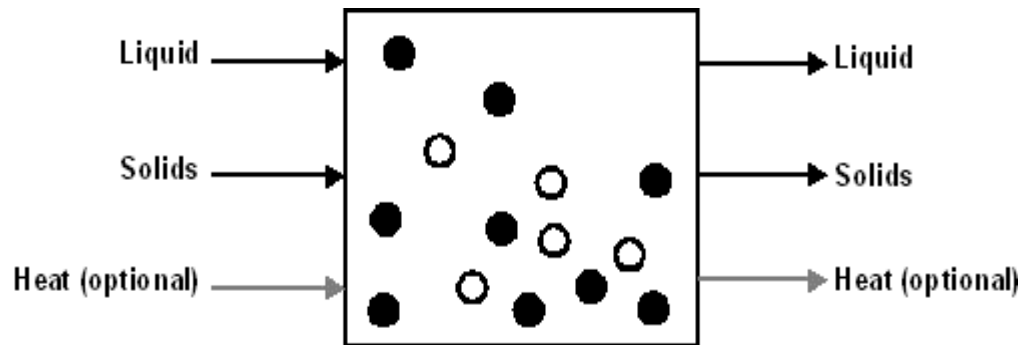
Methods 0, 1, 2 and 3 are generally used when free water option is specified. Method 2 is good for unsaturated systems. Method 4 is used in VLE systems when liquid phase is mostly water. For rigorous three-phase calculations, method 3 is used as default but only when regressed binary interaction parameters are available from liquid-liquid equilibrium data. (Plus & Guide, n.d.)

### 2.7.8. Process Equipment

Several extractors are available in Aspen Plus™ model library for solid-liquid extraction. They are broadly classified as

#### 2.7.8.1. SWash

To simulate solids washers in which dissolved components in the entrained liquid of a solids stream are recovered by a washing liquid. SWash simulates a single-stage solids washer; it does not consider the presence of a vapor phase.



**Figure 2.8:** Flowsheet Connectivity for SWash

SWash calculates the flow rates and compositions of the outlet solids and liquid streams from a user-specified liquid-to-solid mass ratio of the outlet solids stream and the mixing efficiency of the washer. For non-adiabatic operations, SWash determines the outlet temperature when outlet pressure and heat duty are given. Alternatively, SWash calculates the required heat duty when outlet temperature and pressure are specified.

The mixing efficiency of the washer,  $E$ , is defined as

$$E = \frac{X_{IN}^S - X_{OUT}^S}{X_{IN}^S - X_{OUT}^L} \dots\dots\dots 2.1$$

Where,

$X_{IN}^S$  = Mass fraction of dissolved components in the entrained liquid of the inlet solids stream

$X_{OUT}^S$  = Mass fraction of dissolved components in the entrained liquid of the outlet solid stream

$X_{OUT}^L$  = Mass fraction of dissolved components in the outlet liquid stream

when mixing efficiency is less than 1 apply bypass fraction. It is the fraction of liquid in the feed that bypasses the mixing. It is calculated as:

$$\text{Bypass fraction} = (1 - \text{mixing efficiency}) \frac{\text{liquid-to solid ratio specified for SWash}}{\text{Liquid-to solid ratio inlet solid stream}} \dots\dots 2.2$$

**2.7.8.2. Mixer**

Combines material streams (or heat streams or work streams) into one outlet stream. If material streams are mixed, you can use an optional water decant stream to decant free water from the outlet. You can specify an outlet pressure or pressure drop for material streams. The mixer model determines the combined outlet stream temperature and phase condition by performing an adiabatic phase equilibrium flash calculation on the composite feed streams.

**2.7.8.3. Flash2**

Performs rigorous 2 (vapor liquid) or 3 (vapor liquid liquid) phase equilibrium calculations. Flash2 produces one vapor outlet stream, one liquid outlet stream, and an optional water decant stream. It helps to model flashes, evaporators, knock-out drums, and other single-stage separators. Flash2 performs vapor-liquid or vapor-liquid-liquid equilibrium calculations. When you specify the outlet conditions, Flash2 determines the thermal and phase conditions of a mixture of one or more inlet streams.

### **3. MATERIALS AND METHODS**

#### **3.1. Overview**

All the experimental works start from proximate analysis, mechanical treatment of the raw material, preparation of the raw materials, the extraction process and purification processes were done at School of Chemical and Bio Engineering Laboratory, AAiT. Characterization and qualification of the final product through Refractive index, Viscosity, Density and Specific gravity were carried out at 5 Killo Leather Industry Development Institute. Finally, the simulation of experimental process was done by the help of Aspen Plus<sup>TM</sup>, Chemical Engineering dynamic software.

This chapter will discuss the experimental work on the study of chili pepper extraction using soxhlet extraction method. There are five stages involved in completing the experiment including sample preparation process, extraction process, separation process, simulation and lastly analysis process. In the sample preparation process, chili pepper samples were prepared by constant drying time for 2 hrs, at 50 °C. Then, extraction is made among seventeen samples with different parameters in extraction process. The extracted capsaicin was separated from solvent at separation process. Finally, the extracted capsaicin yield is determined. The extraction yield is compared in order to determine the optimum process parameter variables for the production processes.

#### **3.2. Materials and Chemicals**

The main raw material used throughout the experimental work was chili pepper and its traditional name known by most Ethiopian people is Mareko which was collected from local market at Alaba and Durame which found in southern part of the country.

The main chemical used in this research are Ethanol. Ethanol was absolute anhydrous grade and bought from Scilab Trading PLC and Heparin Treading PLC, Addis Ababa.

### 3.3. Equipment

During the experimental work the following equipments are used in this study

- ✓ Goggles: - for eyes protection
- ✓ Protective gloves: - for hand safety
- ✓ Electrical grinder: - used to reduce the size of chili pepper
- ✓ Dry oven: - to dry the raw material as well as performed final product analysis
- ✓ Graduated test tube: - to measure the liquid volume
- ✓ Cotton ball: - to hold paper thimble
- ✓ Sharp knife: - to remove the ribs from the flash
- ✓ Electronic balance: - to measures the weight of sample
- ✓ Baking tray: - to hold the chili pepper for drying purpose
- ✓ Sieve: - to screening the desired size
- ✓ Thermometer: - to measure the temperature
- ✓ Desiccator: - to hold dried sample for prevent the moisture from the environment
- ✓ Graduated cylinder: - to measure the decanted product and the required solvent
- ✓ Filter paper: - to separate solid from liquid
- ✓ Digital micro pipette: - to sack products from decanter
- ✓ Plastic bags: - to store the sample
- ✓ Soxhlet extractor: - leaching process carried out in this section
- ✓ Condenser: - to liquefy the vapor product and to recovered the solvent
- ✓ Water bath (stove): - for heating the solvent it operates below 90°C
- ✓ Oil bath: - to heated the solvent above 90°C
- ✓ Chiller: - to cool the Soxhlet extractor
- ✓ Round balloon flask: - to hold the solvent and during extraction the solution
- ✓ Viscometer: - to measure viscosity of the capsaicin
- ✓ Density meter: - to measure the density of capsaicin
- ✓ Refractometer: - to measure the ability passing the light of capsaicin

In this research used different softwares accustomed for this thesis are Design Expert V-6.08, Edraw, Mendeley, Matlab and Aspen Plus<sup>TM</sup> V- 8.8. The overall framework design of this research is shown as below in figure 3.1.



### **3.4. Experimental procedure**

#### **3.4.1. Chili Pepper Collection and Preparation**

The fruits of chili pepper plant used for this experiment was obtained from Alaba and Durame. The moisture content of the pepper was determined before dried. The dried pepper was then reduced into the desired particle sizes using the laboratory crusher. The reduced chili pepper was then stored in air impenetrable plastic bags.

After the sample was prepared for run, soxhlet extraction of the pepper samples were performed on the extractor. The factors investigatee in the extraction were temperature, concentration of solvent and the extraction time. Each run used 10grams of pepper with moderately fine particle size, which was determined in the preliminary experiment. The solvents used, was 79% ethanol, 89% ethanol, and 99% ethanol (v/v) with a constant volume of 200ml. The solvent was heated with different temperatures at 85°C, 90°C, and 95°C. The extraction process times were 3hours, 4hours and 5hours.

The percentage yield of capsaicin is taken as the response variable. The Extraction temperatures, the solvent concentration and the extraction time are varied. This study used the interpretation of Box Behnken Design. The numbers of runs were 17 (seventeen) with 5 (five) center points. For the first run the values that had been used for the temperature was 85°C, solvent concentration was 97% ethanol and extraction time was 3hours.

#### **3.4.2. Proximate Analysis of Chili Pepper**

The proximate analysis of moderately dried chili pepper has carried out, extraction process needs to determine the moisture content of the raw chili pepper which is purchased from local market. The measurement was done three times and the average has been taken to increase the accuracy.

#### **3.4.3. Moisture Content**

To determine the moisture content of chili pepper, the tray was weighed with and without the presence of prepared chili pepper. The tray with sample is placed in the oven for drying process at temperature of 50°C. The chili pepper was staid in the oven for two hours

respectively. During drying process, the dried chili pepper was removed after two hours from the oven and re-weighed till constant weight was obtained. And then it inserted in the airtight containers or desiccator and kept in a dry place. Finally, the weight was taken and compared with the initially recorded weight.



**Figure 3.2:** Microwave oven used in drying of chili pepper sample

The percentage weight in the sample was calculated using the following formula:

$$\text{Moisture content, \%} = \frac{w_1 - w_2}{w_1} 100 \dots \dots \dots 3.1$$

Where,  $W_1$  is weight of sample before drying and

$W_2$  is weight of sample after drying

After determining the moisture content, soxhlet extraction of the pepper samples were performed. The factors observed in the extraction were temperature, concentration of solvent and the extraction time. The capsaicin extracts obtained were then analyzed in the physio-chemical analyzer. After acquiring the results of the percentage yields of each were calculated and the data were analyzed using the Design Expert Program.

### 3.5. Extraction Process

The capsaicin extraction was performed by using Soxhlet extraction method. The 10grams of chili pepper moderately powdered was placed in paper thimble (packed in filter paper). The thimble was placed in extraction chamber of the soxhlet extractor, which is located between the boiling flask at bottom and condenser at the top. The round boiling flask was filled with 200ml of solvent ethanol respectively.

Each run used 10grams of chili pepper with moderate particle size which was determined in the preliminary experiment. The solvents used, 79%v/v ethanol, 89%v/v ethanol, and 99% ethanol (v/v) with a constant volume of 200ml. The solvent was heated with different temperatures at 85°C, 90°C, and 95 °C. The extraction process was conducted for 3 hours, 4 hours and 5 hours respectively.

The extraction temperatures, the solvent concentration used and the extraction time were varied. The response obtained was the percentage yield. The yield of product extracted will be calculated using the equation given below

$$\text{Yield of Capsaicinoid} = \frac{\text{weight of capsacinoid extracted}}{\text{weight of sample}} 100\% \dots\dots\dots 3.2$$

The numbers of runs were 17 with 5 center points. The interpretation of the Box Behnken Design was that for example for the first run the values that will be used for the temperature, solvent concentration and extraction time were 85°C, 79%ethanol and 4hours. Also, the response of the design was the percentage yield.

#### 3.5.1. Separation and Purification Process

The separation of capsaicin from solvent was performed after the extraction method. The capsaicin and solvent were separated by using pilot distillation. The mixture was placed in the conical flask before it is attached to the condenser equipment. The temperature used onto this equipment was from 85°C up to 90°C which was based on the boiling point of the EtOH. Lastly, purification process was carried out by decantation for throughout a day by gravity difference.



**Figure 3.3:** Separation and purification process

### **3.6. Experimental Design for Leaching Process**

In this task the capsaicin was produced through leaching extraction considered three independent variables which was temperature, ethanol concentration and extraction time in order to examine the ANOVA. Temperature represented as factor A, ethanol concentration represented as factor B and retention time (extraction time) represented as factor C, in this case the response variable measured was the percentage of yield. As well as it has been three dissimilar retention time, solvent concentration, and extraction temperature ranges will be used. Totally, it was conducted seventeen experimental sittings. The experimental design and multiple linear regression analysis were performed using

Design-Expert 6.0.8 software to obtain a suitable model equation for the percentage extraction of capsaicin as a function of the independent variables.

The response surface method (RSM) was applied to evaluate the effects of three different parameters and optimize conditions for the response. Box-Behnken experimental design (BBD) with three numeric factors on three levels was used. This design consisted of seventeen randomized runs with five replicates at the central point to minimize the error, identification of curvature in the system and its curvature effects with its importance, and estimation of the pure error.

The range and levels of the three independent variables studied in experimental design shows in Table 3.1 below. The lower and higher levels are chosen by considering the operating limits of extraction (leaching) process conditions.

**Table 3.1:** Independent variables and levels with coded and natural values used in Box-Behnken Design (BBD)

Factor	unit	Symbol	Coded variable level		
			Level 1	Level 2	Level 3
			-1	0	1
Temperature	<sup>o</sup> C	A	85	90	95
Solvent concentration	V/V	B	79	89	99
Extraction time	hrs	C	3	4	5

Table 3.2 lists the run sheet of all experimental design matrix of BBD for the factorial design was shown. The order in which the runs were made was randomized to minimize systematic errors. Each variable was coded variables in dimensionless numbers from -1 to 1 (Selvaraj & Sivakumar, 2013).

**Table 3.2:** The experimental run sheet design matrix

Run	Real factors		
	Temperature, °C	Solvent concentration, v/v	Extraction time, hrs
1	-1.00	-1.00	0.00
9	1.00	-1.00	0.00
16	-1.00	1.00	0.00
2	1.00	1.00	0.00
4	-1.00	0.00	-1.00
14	1.00	0.00	-1.00
6	-1.00	0.00	1.00
17	1.00	0.00	1.00
5	0.00	-1.00	-1.00
8	0.00	1.00	-1.00
3	0.00	-1.00	1.00
11	0.00	1.00	1.00
13	0.00	0.00	0.00
12	0.00	0.00	0.00
10	0.00	0.00	0.00
15	0.00	0.00	0.00
7	0.00	0.00	0.00

The response variable was fitted to the following second-order polynomial model (Eq.3.3) which was generally able to describe relationship between the responses and the

independent variables (Leivisk, 2013; Tekindal, Science, Of, Engineering, & Science, 2012).

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 AC + \beta_6 BC + \beta_7 A^2 + \beta_8 B^2 + \beta_9 C^2 \dots \dots \dots 3.3$$

Where, Y represents the response variable, A, B and C are the independent variables affecting the dependent response, and  $\beta_0, \beta_1, \beta_2, \beta_3, \beta_4, \beta_5, \beta_6, \beta_7, \beta_8,$  and  $\beta_9$  are the regression coefficients for intercept, linear, quadratic and interaction terms.

### **3.7. Using Aspen Plus™ for Process Design**

The process design of input specifications for each form in Aspen Plus™ and the thermodynamic model are stated in detail as follows.

#### **3.7.1. Global Specifications**

The input and output units were specified as MET with temperature in degree centigrade and pressure in bar. The stream class was specified both SOLID and CONVEN as the simulation contains both solid and liquids. The sub-stream was specified as MIXCISLD. The run type was flow sheet and the flow basis were specified as mass. Free water was selected as No. Since, the solubility of organic phase in EtOH is significant. The Gibbs method was selected for the flash convergence algorithm as the simulation involves three phase rigorous calculations.

##### **3.7.1.1. Simulation Options**

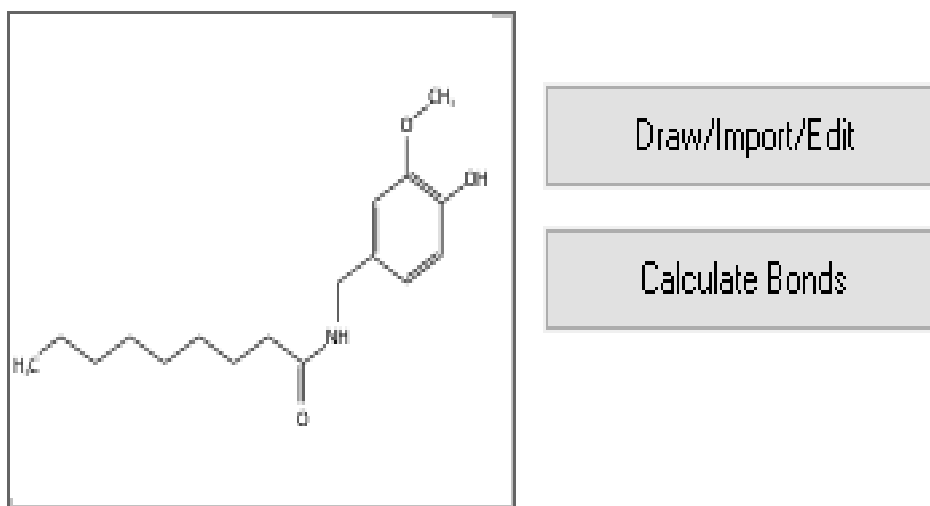
The flow basis was specified as mass. The valid phases are vapor-liquid-liquid as the presence of vapor in the simulation is uncertain. Free water was selected as No since the solubility of organic phase in water is significant in the simulation. If Free-water method is selected, Aspen Plus™ gives 100 % separation of water and organic phase which is not true in reality.

On the Setup | Simulation Options | Flash Convergence form the upper and lower limits for temperature and pressure were left as default values. The Gibbs method was selected for the flash convergence algorithm as the simulation involves three phases rigorous calculations. The option Limit water solubility in the hydrocarbon phase was not selected,

as the solubility data calculated from the primary property method was preferred and the 4-phase convergence algorithm was used to obtain better output.

### 3.7.2. Components

All the components were specified as conventional and those are found in Aspen Plus<sup>TM</sup> database except for nordahydrocapsaicin. To define nordahydrocapsaicin was specified through a user defined wizard as described in the previous chapter. The structure of nordahydrocapsaicin (figure 3.5) was imported as a Meaning Oriented Interface (MOI) file downloaded from the NIST database. The properties of nordahydrocapsaicin were evaluated from TDE after inserted the drawn molecular structure in the wizard. Pure component properties must be calculated using group contribution methods from the NIST database.



**Figure 3.4:** Structure of nordahydrocapsaicin

### 3.7.3. Properties

Accuracy of a process design greatly depends on the thermodynamic model used. Then selection of thermodynamic model is the crucial step in process simulation. The selection of a property model was based on type of molecules, type of mixture, operating condition and availability of data. The activity coefficient method can model at low pressures and binary interaction parameters must be obtained either from a database or experimental data.

In activity coefficient models, vapor phase properties are derived from EOS and the liquid phase properties are derived from pure component properties.

In capsaicinoid separation process containing both polar and non-polar components at a pressure less than 10atm. Hence, a flexible activity coefficient method should be used. The system was modeled using the UNIQUAC equation.

Water solubility is calculated using method 2. Though method 3 is the default option for three-phase systems, it was not opted due to lack of binary interaction parameters regressed from liquid-liquid equilibrium data. Method 5 does not use a primary property method or vapor phase calculations. Method 1 was not preferred as it does not have correction for unsaturated systems like method 2.

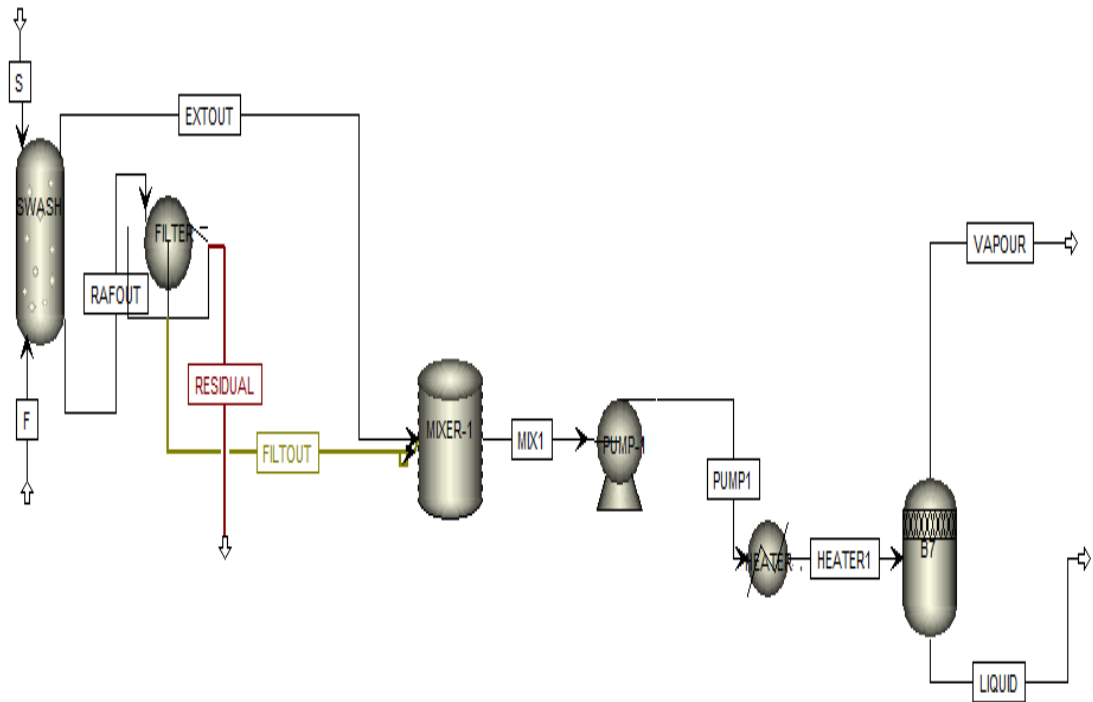
In the free water property method, no free water was selected. It is changed from STEAM-TA, the default option, to STEAMNBS. As state in earlier chapter, STEAMNBS is the preferred method for certain equations of state like UNIQUAC due to its extrapolation capability.

#### **3.7.4. Process Description**

The whole process was divided into 2 sub-sections those are leaching and purification or solvent recovery unit.

The following assumptions were made for the process simulation.

- Flowrate of solvent was 500kg/hr
- Flowrate of fine chili pepper was 100kg/hr
- Steady state process



**Figure 3.5** Simulation of process flowsheet

### 3.7.5. Leaching Unit

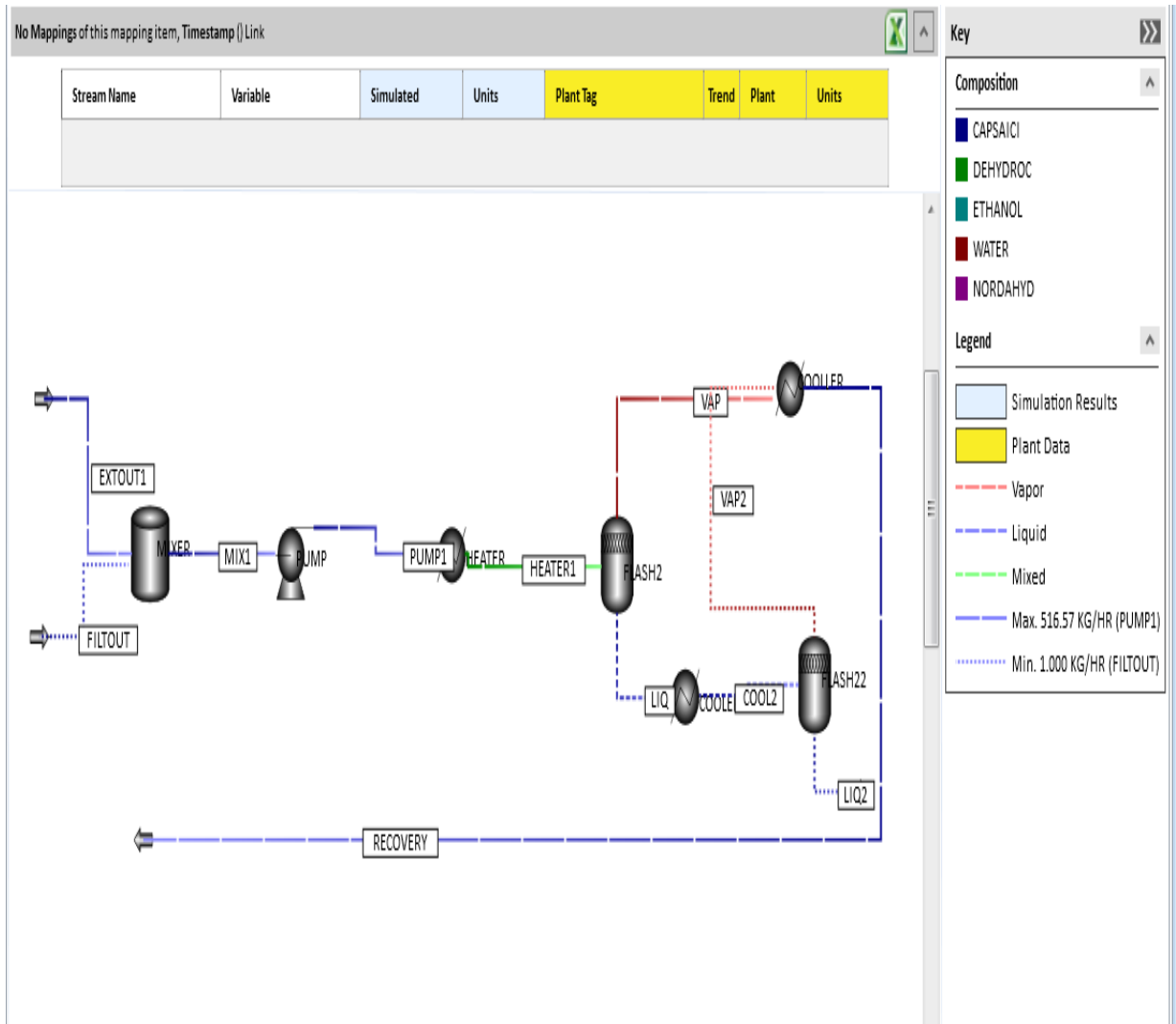
The first step in the leaching process, capsaicinoid was separated from chili pepper (*capsicum annume*). In Aspen Plus<sup>TM</sup> database cannot recognized with *capsicum annume* as a solid then it is better to assumed based on experimental output. This solid-liquid process carried out through SWasher in countercurrent mode. Moderately fined chili pepper is feed at bottom with a mass flowrate of 100kg/hr at 25°C and atmospheric pressure, and at the top, the solvent feed with a mass flowrate of 500kg/hr at 47°C and atmospheric pressure, based on solid to solvent ratio. The feed flowrate and solvent flowrate were charged based on optimum experimental operating conditions. For both at ambient temperature and pressure is selected with negligible vapor fraction. The dissolved components in the entrained liquid of a solid stream are recovered by washing liquid. It doesn't consider the presence of vapor phase.

The leaching SWasher column is modeled as a single staged counter-current solid-liquid washer operating adiabatically at 47°C and atm pressure. The raffinate phase from the leaching was solid with 5% solution. It should be treated on the filtration so as to separate solution from solid residue. On the other hand, the extract phase contained 95% solution. All the leached stream from this section are then mixed and sent to purification and solvent recovery unit.

### **3.7.6. Purification and Solvent Recovery Unit**

The EXTRACT stream from the leaching process and FILTOUT streams were purified by flash separation process which was based on the difference in the boiling points of capsaicinoid and solvent concentration. The vapor-liquid flash drum was modeled as a FLASH 2 which is available in the model library palate of Aspen Plus™. The input stream MIX1 is flashed at 321K and 1atm. At these conditions of temperature and pressure, ethanol vaporizes while capsaicinoid remains liquid enabling separation of solvent from the extract.

In the solvent recovery unit, ethanol and water were recovered from extract stream. The recovery process was carried out in a vapor-liquid flash drum, the mixture was then heated to the operating condition of 78.8°C and atmospheric pressure and then sent to a heater and followed to a flash drum operating at 85°C and 1atm in order to vaporize the EtOH with water while capsaicin remains liquid enabling separation of solvent from the extract. Both VAP and LIQ streams were cooled down to 47.5°C. The LIQ stream flashed again for another purification of the desired product. Flash22 specification was used at constant pressure and operating temperature at 103°C to get highly purified capsaicinoid. The product of first solvent VAP stream from flash1 and the second solvent VAP2 stream from flash22 cooled down to cooler1 at a temperature of 47.5°C for reuse.



**Figure 3.6:** Purification and solvent recovery unit

## 4. RESULTS AND DISCUSSION

### 4.1. Moisture Content of the Raw Material

The fruit of the chili pepper plant was collected from local market at Alaba and durama. In this process, after reduced the size of chili pepper the desirable size was weighed three sample (30gram) at constant drying temperature of 50°C respectively. Chili pepper was left in the oven for 2 hours respectively. During drying process, the dried chili pepper was removed after 2 hours from the oven and re-weighed till constant weight is obtained. And then the moisture content of samples was determined as shown in table 4.1.

**Table 4.1:** Moisture content of chili pepper

	Sample weight (grams)		
	Sample 1	Sample2	Sample3
Initial mass (t=0)	30	30	30
Mass after (t=2)	24.6	23.9	24.1
Mass after (t=4)	22.1	21.8	21.7
Mass after (t=6)	21.4	21.2	21.3
Mass after (t=8)	21.4	21.2	21.3
Total moisture content	31.32	31.85	31.45

Based on equation 3.1, the total moisture content of the three samples have been 31.32%, 31.85% and 31.45% respectively. The mean moisture content of the three samples gives 31.54% respectively.

## 4.2. Summary of the Experimental Result

The result from the experiment has indicated that the optimum process condition for the extraction of capsaicin from chili pepper are shown in table 4.2.

**Table 4.2:** Experimental output

Run	Independent Variable			Yield of Capsaicin, %
	Temperature, °C	Solvent Concentration, v/v	Extraction Time, hrs.	
1	85	79	4	7.32
2	95	79	4	6.49
3	85	99	4	10.49
4	95	99	4	7.59
5	85	89	3	9.25
6	95	89	3	7.59
7	85	89	5	14.36
8	95	89	5	8.84
9	90	79	3	7.04
10	90	99	3	5.94
11	90	79	5	7.46
12	90	99	5	11.05
13	90	89	4	16.30
14	90	89	4	15.60
15	90	89	4	16.57
16	90	89	4	15.05
17	90	89	4	16.43

Under these conditions a maximum yield of 16.57% capsaicinoid is obtained.

### **4.3. Data Analysis for Extraction of Capsaicin**

The statistical analysis of extraction of capsaicin from chili pepper is discussed in the following succeeded sections.

#### **4.3.1. Analysis Using Response Surface Methodology**

The Design Expert Program was used in order to analyze the data acquired. A probability of less than 0.05 shows that the lack of fit is significant. An insignificant lack of fit means that there is no need to look for a higher order type of model. In this case, the only model with an insignificant lack of fit is quadratic with a probability value of 0.0500 or based on a 95% confidence level.

Analysis of Variance (ANOVA) was done to test the validity of the model as well as whether the factors; temperature, solvent and time of leaching, observed have a significant effect on the percentage yield of capsaicin. It is summarized in table 4.1 respectively.

The F-value of the model is 53.97 which implies that it is highly significant. Also, the probability of it occurring due to personal error, noise or disturbance is only 0.01%. The values of the "Prob > F" should be less than 0.05 for the model terms to be considered as significant. Since the values are less than 0.0001, the model terms (A, B, C, A<sup>2</sup>, B<sup>2</sup>, C<sup>2</sup>, AC and BC) are significant model terms.

The "Lack of Fit F - value" of 1.58 implies that of it is not significant compared to the pure error. There are 32.63% chances that a "Lack of Fit F - value" this large could occur due to noise. Non-significant lack of fit is good because we want the model to fit.

**Table 4.3:** ANOVA of the fitted second-order polynomial model for yield of capsaicin

<b>ANOVA for Response Surface Quadratic Model</b>						
<b>Analysis of Variance Table [Partial sum of squares]</b>						
<b>Source</b>	<b>Sum of Squars</b>	<b>DF</b>	<b>Mean Square</b>	<b>F Value</b>	<b>P-value Prob&gt;F</b>	<b>Remark</b>
Model	251.99	9	28.00	53.97	<0.0001	a
A - extraction temperature	14.88	1	14.88	28.68	0.0011	b
B - solvent concentration	5.71	1	5.71	11.01	0.0128	b
C- extraction time	17.67	1	17.67	34.06	0.0006	b
A <sup>2</sup>	36.39	1	36.39	70.15	<0.0001	a
B <sup>2</sup>	108.55	1	108.55	209.23	<0.0001	a
C <sup>2</sup>	38.91	1	38.91	75.00	<0.0001	a
AB	1.07	1	1.07	2.06	0.1939	c
AC	3.72	1	3.72	7.18	0.0316	b
BC	5.50	1	5.50	10.60	0.0139	b
Residual	3.63	7	0.52	-	-	-
Lack of Fit	1.97	3	0.66	1.58	0.3263	c
Pure Error	1.66	4	0.42	-	-	-
Cor Total	255.62	16		-	-	-

Where, a, indicates highly significant

b, indicates significant

c, indicates not significant

#### **4.3.1.1. Statistical Analysis on Factors Affecting Degree of Extraction**

The statistical software program is used to generate the model equation, interaction effects of the independent variables and surface plots using the fitted equation obtained from the

regression analysis holding one of the independent variable constants. Their respective responses are summarized in table 4.2 respectively.

**Table 4.4:** Experimental and predicted values of capsaicin

Run Order	Independent Variable			Yield of Capsaicin		Residual
	Temperatu, °C	Solvent Concentrati, v/v	Extraction Time, hrs.	Actual Result	Predictd Result	
1	-1.00	-1.00	0.00	7.32	7.97	-0.65
2	1.00	-1.00	0.00	6.49	6.28	0.21
3	-1.00	1.00	0.00	10.49	10.70	-0.21
4	1.00	1.00	0.00	7.59	6.94	0.65
5	-1.00	0.00	-1.00	9.25	8.92	0.33
6	1.00	0.00	-1.00	7.59	8.13	-0.54
7	-1.00	0.00	1.00	14.36	13.83	0.53
8	1.00	0.00	1.00	8.84	9.17	-0.33
9	0.00	-1.00	-1.00	7.04	6.71	0.33
10	0.00	1.00	-1.00	5.94	6.06	-0.12
11	0.00	-1.00	1.00	7.46	7.34	0.12
12	0.00	1.00	1.00	11.05	11.38	-0.33
13	0.00	0.00	0.00	16.30	15.99	0.31
14	0.00	0.00	0.00	15.60	15.99	-0.39
15	0.00	0.00	0.00	16.57	15.99	0.58
16	0.00	0.00	0.00	15.05	15.99	-0.94
17	0.00	0.00	0.00	16.43	15.99	0.44

#### 4.3.1.2. Development of Regression Model Equation

Experimental values were fitted to a second-order polynomial model and the model equation that correlates the response (yield of capsaicinoid) to the extraction process variables in terms of actual value after excluding the insignificant terms was given below.

The predicted model for percentage of yield of capsaicinoid in terms of the actual factors is given as.

$$\text{yield of capsaicinoid (\%)} = +15.99000 - 1.36375 * A + 0.84500 * B + 1.48625 * C - 2.94000 * A^2 - 5.07750 * B^2 - 3.04000 * C^2 - 0.96500 * A * C + 1.17250 * B * C \dots\dots\dots 4.1$$

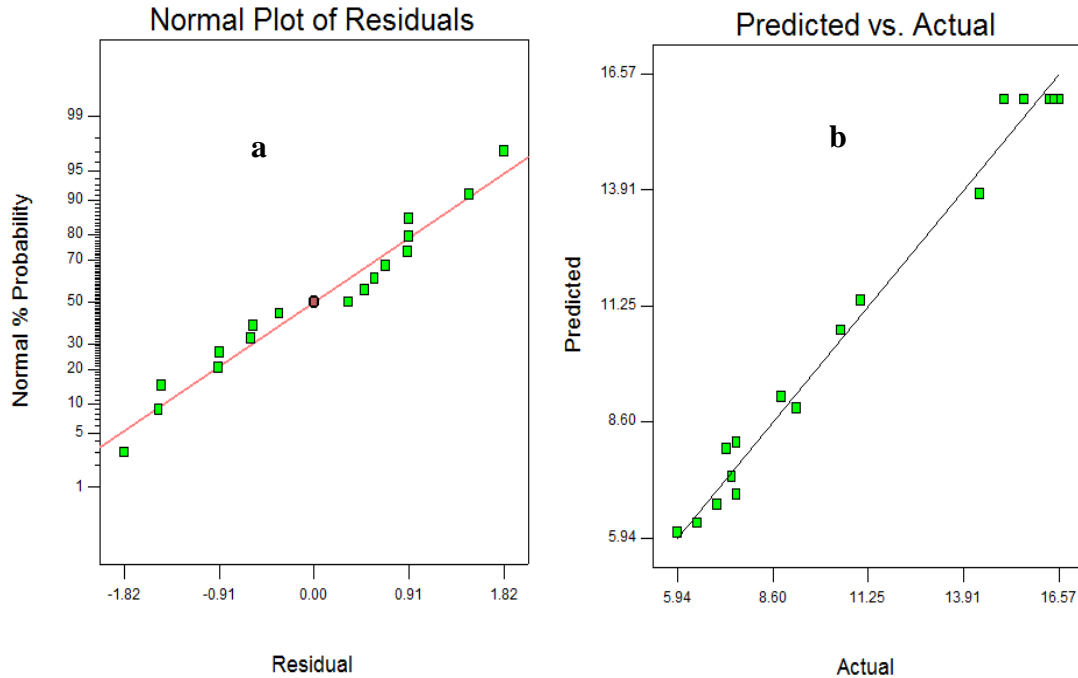
where, A – extraction temperature  
B – solvent concentration difference  
C – extraction time

#### **4.3.1.3. Model Adequacy Check**

The model adequacy is tested by analysis of variance. The regression model was found to be highly significant with the correlation coefficients of determination of R-Squared, adjusted R-Squared and predicted R-Squared with having a value of 0.9858, 0.9675, and 0.8665 respectively. The Predicted R-Squared for the developed correlation of 0.8665 is in reasonable agreement with the Adjusted R-Squared of 0.9675; i.e. the difference is less than 0.101, then the model is fitting the data and can reliably be used to interpolate.

The adequacy precision measures the signal to noise ratio. A ratio greater than 4 is desirable i.e. the model has a strong enough signal to be used for optimization. This ratio of 17.977 indicates an adequate signal. This model can be used to navigate the design space. The adequacy of the model was further checked with analysis of variance as shown in Table 4.1. P-value was calculated by model mean square divided by residual mean square. Figure 4.1 shows below do not give any indication of serious problems and that demonstrated the regression model equation provided accurate description of the experimental data.

The normal plot of residuals can be seen in Figure 4.1.a. The points on the graph represent the normal percentage probability of the percentage yields with respect to the residuals. It can be seen that the points are approximately linear. Since the plot of residuals fit the expected pattern, it shows that the residuals are distributed normally. On the other hand, Figure 4.1.b. shows, the plot of externally standardized predicted versus actual values. Since the points are in random and show pattern, the model is suitable to the data. It also satisfies the independent normally distributed residuals that are usually assumed.



**Figure 4.1:** diagnostic plots of model adequacy

This result indicates that it had the correct result in capturing the correlation between the three extraction separation process variables to the percentage of yield.

#### 4.3.2. Effect of Process Variables on the Yield of Capsaicin

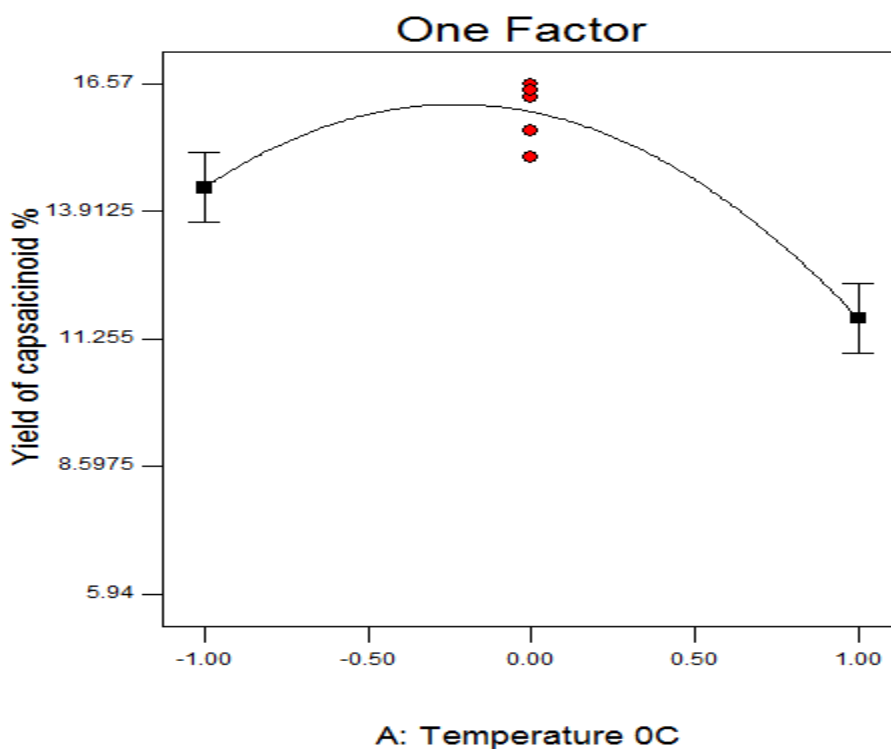
Depend on the analysis of variance, extraction process was significantly affected by various quality parameters. This result demonstrated that the advantage of using BBD surface response for experimental data analysis in capturing the interaction between independent variables that affects the extraction process. Furthermore, the interaction effect, significant individual process variables that affect the extraction process were temperature, duration and concentration difference. From acquired experimental data and developed model the single factor plots and three-dimensional response surface were constructed to illustrate the main and interactive effect of independent variable on the response.

##### 4.3.2.1. The Main Effect of Individual Process Variables

As shown in Figure 4.2 below, the percentage of extraction is significantly affected by extraction temperature on the yield of capsaicin. It can be seen from the figure that with increasing temperature until it reaches its center value would result increasing in the

percentage of extraction, then it starts to drop as the temperature tend to increase above the center limit. Such behavior could be attributed to the following reasons.

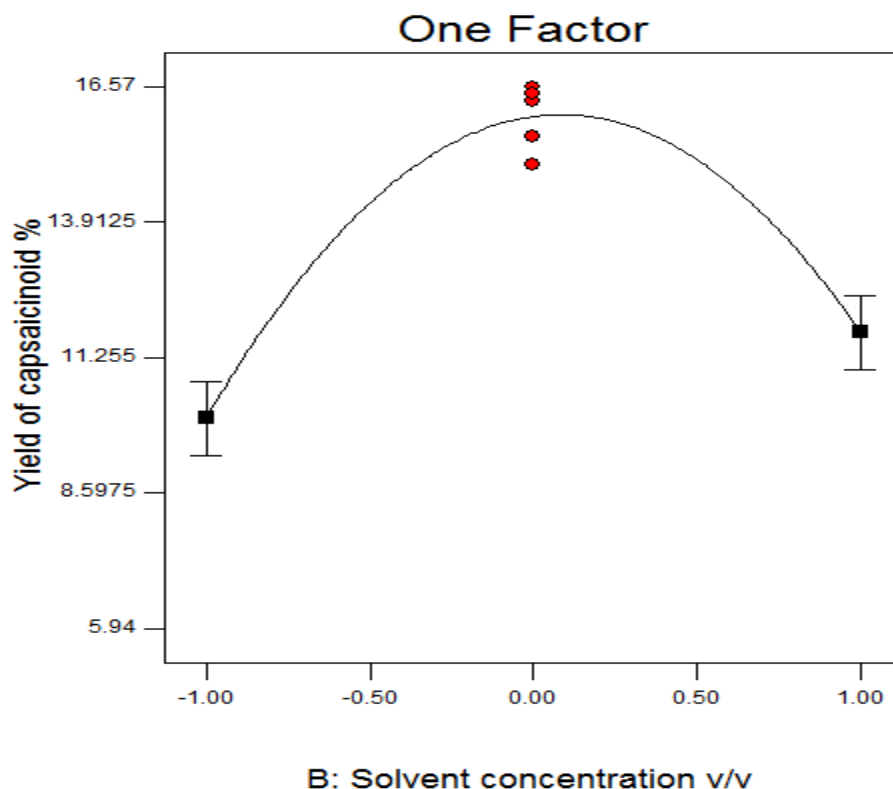
Increasing temperature favored the yield of extraction linearly. The yield of extracted capsaicin increased remarkably with increasing leaching temperature from 85°C to 95°C at a given extraction time before it reached a maximum value. When the leaching is conducted at optimum condition of 90°C, capsaicinoids yield of 16.57% is obtained at 4hrs with 89 solvent concentration. From the results, it can be seen that temperature has a significant effect on the extraction of capsaicin. However, the results actually affirm the theory that extracting at high temperatures could degrade and lose some organic compounds.



**Figure 4.2:** percentage of yield versus temperature on the leaching

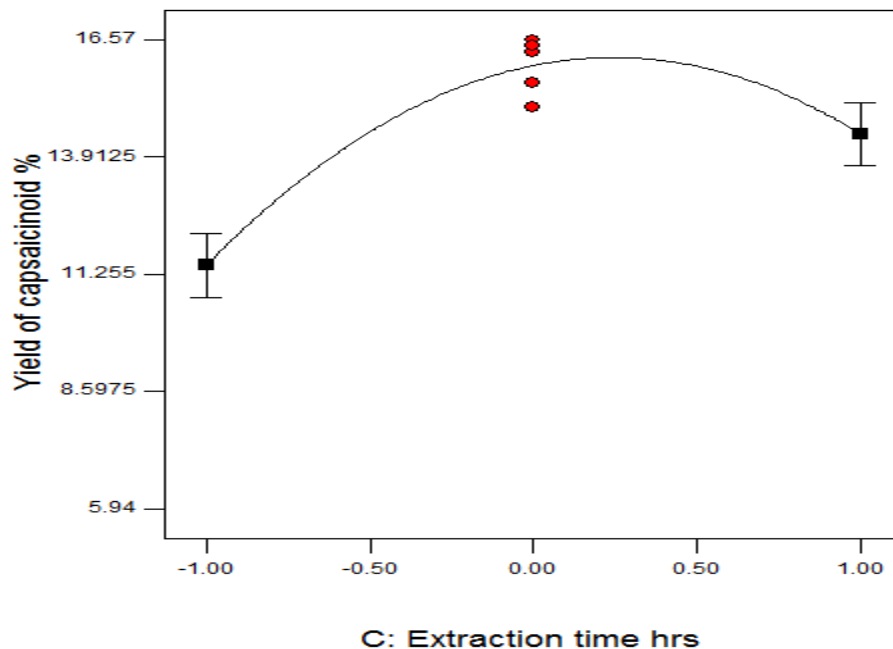
The results of trial, as shown on Figure 4.3, determined 89% ethanol as the solvent ratio that gave the highest percentage yields for the extraction times of 3, 4, and 5 hours with 9.25%, 16.57%, and 14.36%, respectively. On the other hand, extraction with lower concentration resulted to the lowest percentage yields of 7.04%, 7.32%, and 7.46% for 3, 4, and 5 hours. Moreover, a higher percentage yield was also observed for solvents that

had higher percentages of ethanol. Although chili pepper is slightly soluble in low concentration of ethanol, low concentration of ethanol proved to be an ineffective extraction solvent for capsaicin. The effectiveness of ethanol as an extraction solvent for capsaicin could be due to it being an organic polar solvent. The polarity of ethanol makes it able to have strong interactions with polar substances such as capsaicin.



**Figure 4.3:** percentage of yield versus solvent concentration on the leaching

As expected, a longer extraction time generally led to a higher percentage yield of capsaicin. The yield of capsaicin showed a trend of gradual increased as the time extended to 4 hr. This could have been due to the longer amount of time the solute and solvent were in contact with each other. Longer contact time favored the system to have more mass transfer. However, excessive extraction time would be unnecessary as the solvent and sample would be in final equilibrium after certain duration. This is based on Fick's second law of diffusion. By then, the rate of extraction of compounds would decelerate. From the results, it can be seen that time has a significant effect on the leaching process.



**Figure 4.4:** percentage of yield versus extraction time on the leaching

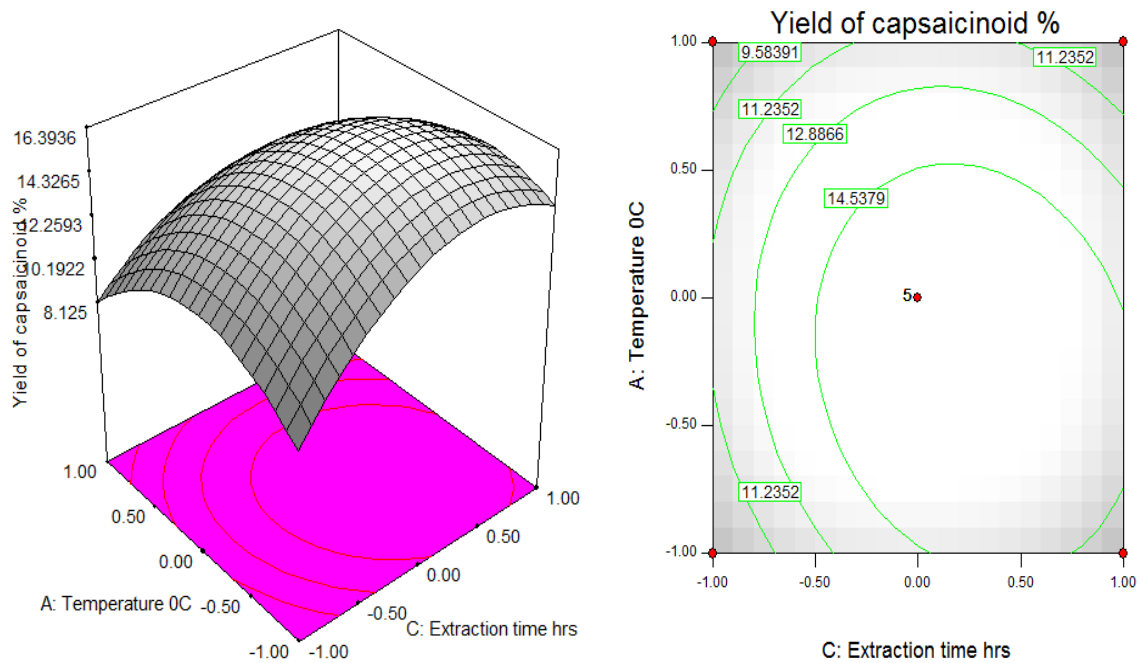
#### 4.3.2.2. Effect of Interaction between Process Variables

The most common way to summarize the results of a box behnken design experiment is in the form of three-dimensional response surface plot and via response contours plot. The process variables were found to have significant interaction effects but less interaction effect of extraction temperature with solvent concentration. Figure 4.5 and Figure 4.6 shows the interaction between extraction temperature with extraction time, and solvent concentration with extraction time on percentage of yield respectively. The lower extraction temperature could not extract the desired product efficiently and thereby reduce the yield of capsaicin. On the other way higher extraction temperature increases the leaching that can be increases the yield of capsaicin, but if extraction temperature increases too much beyond optimum value it will degrade the capsaicin or desired product.

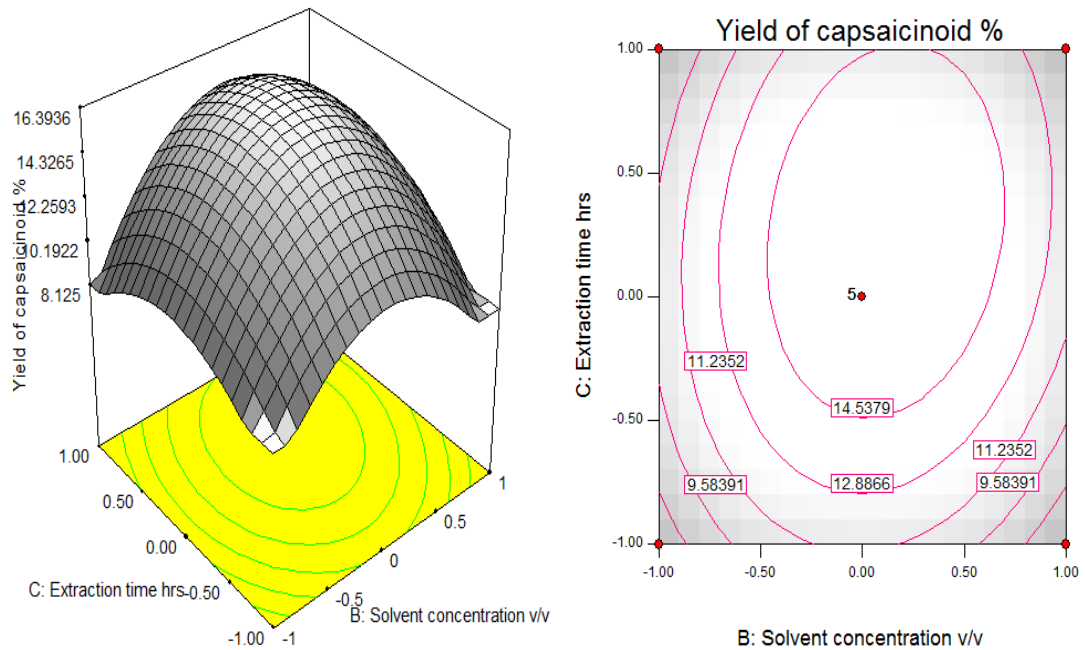
Figure 4.5 shows, the effect of extraction temperature with extraction time on yield of capsaicinoid. It shown the figure both temperature and extraction time have increased up to optimum so as to increase the yield of extraction. This similar explanation given in the previous section.

From the three interaction effects shown in the figures and contours shown as below, at lower range of extraction time, extraction temperature at the center point and solvent concentration at the center point, always resulted in the percentage of yield higher than when using lower or higher leaching temperature and higher or lower solvent concentration.

As long as this process conditions, long leaching time was already sufficient to push the extraction forward. This phenomenon is further supported by the fact that extraction time was the most significant process variable that affect the percentage of conversion as indicated by the highest F – value in the analysis of variance as shown in Table 4.1. The Response Surface Methodology (RSM) was used to optimize the conditions of yield for extraction chili pepper and to understand the interaction of the factors affecting the extraction of capsaicin. Figure 4.5 shown the concentration difference was 89 percent and Figure 4.6 shown the leaching temperature was 90°C surface plots between the independent and dependent variable for fixed parameters.



**Figure 4.5:** Surface and Contour plot of the interaction effect of temperature with time



**Figure 4.6:** Surface and Contour plot of the interaction effect of solvent concentration with time

#### 4.4. Characterization of the Extracted Capsaicin

Played process parameter when the leaching was conducted at optimum condition of 90°C, capsaicinoids yield of 16.57% is obtained at 4hr with 89v/v solvent concentration physical and chemical properties were deliberated. Several indices are used for the determination of product quality. For the purpose of this experiment, the following parameters were analyzed.

##### 4.4.1. Moisture and Volatile Matter Determination

Determination of moisture and volatile matter by used oven 2grams of capsaicinoid was taken and set in oven for 1hr. Then, weighted the sample and reduces to 1.98gm and also put in to oven for another 1hr. Finally, weighted gives the same result of 1.98gm.

Then, from eqn (Appendix A.1)

$W_1$  is loss in gm in material on drying =  $(2 - 1.93)$  gm = 0.07gm

W is weight in gm of oil capsaicinoids taken for the check = 2gm

Substituting the above values in the equation 3.2.

$$\begin{aligned}\text{Moisture and volatile matter} &= \frac{0.02}{2} * 100\% \\ &= \underline{3.5\%}\end{aligned}$$

##### 4.4.2. Refractive Index

Refractometer was used to determine the refractive index of the capsaicinoid and A.O.C.S official method 921.0 was implemented. A refractive index of the product was 1.4967 at a temperature of 23°C was obtained. Refractive index indicates the purity of capsaicin. The lower the refractive index showed the higher the quality of capsaicinoid. The result obtained indicated that the product was of high quality.

##### 4.4.3. Density and Specific Gravity

Both density and specific gravity of capsaicinoid, which was measured simultaneously from density meter output displayed as,

Density of capsaicinoid = 990Kg/m<sup>3</sup>

Specific gravity of capsaicinoid = 1.01

#### 4.4.4. Kinematic Viscosity

Dynamic viscosity of capsaicinoids, which was read from rotational spindle speed 200-3000 rpm viscometer was 340.mpa.s at a temperature of 22.6°C.

Replacing the dynamic viscosity of capsaicinoids to kinematic viscosity

$$340\text{mlpa.s} = \frac{34 \cdot 10^{-4} \text{kg.s}}{\text{m}}, \text{ density of capsaicinoids is } \frac{1.01 \text{g}}{\text{cm}^3} = \frac{990 \text{kg}}{\text{m}^3} \text{ in equation}$$

(Appendix A.2)

$$\text{Kinematic viscosity} = \frac{\frac{34 \cdot 10^{-4} \text{kg.s}}{\text{m}}}{\frac{990 \text{kg}}{\text{m}^3}} = 3.43 \cdot 10^{-6} \frac{\text{m}^2}{\text{s}}$$

Then, the kinematic viscosity of capsaicinoids is =  $3.43 \cdot 10^{-6} \frac{\text{m}^2}{\text{s}}$

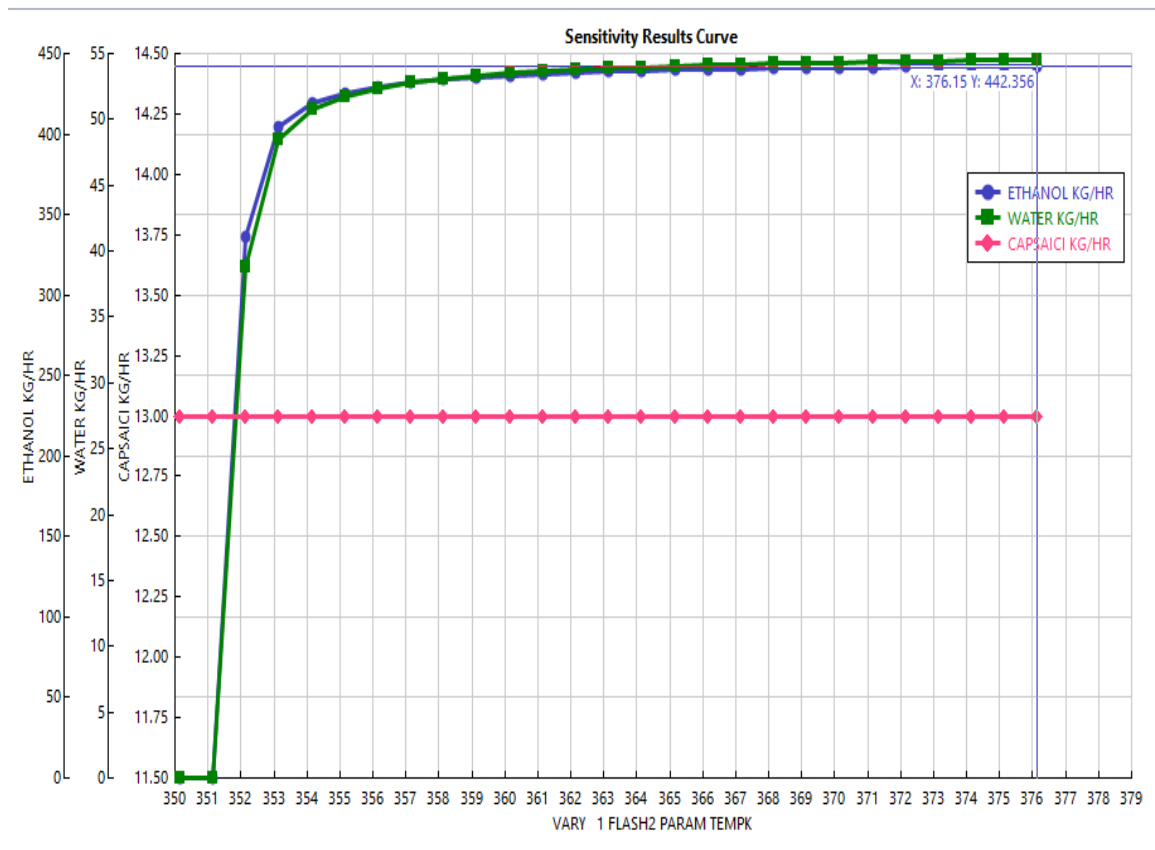
### 4.5. Process Simulation Design

The aim of simulation in Aspen Plus™ is an approximate representation of the solid - liquid extraction which was carried out in AAiT research lab. SWash unit operation was not performed as well to achieve maximum possible removal of solute from solid chili pepper by utilization of EtOH because of unrecognition of capsicum annum with Aspen Plus™ data base. So, in the first unit operation Swash cannot apply the sensitivity study of relationships amongst process variables simulation to determine the optimum operating conditions.

#### 4.5.1. Purification and Solvent Recovery Unit Analysis

A number of sensitivity analysis were run prior to the final simulation to determine the optimum operating conditions. The extract and raffinate from the leaching process containing 16.57% capsaicinoid. Total capsaicinoid was purified by flash separation at

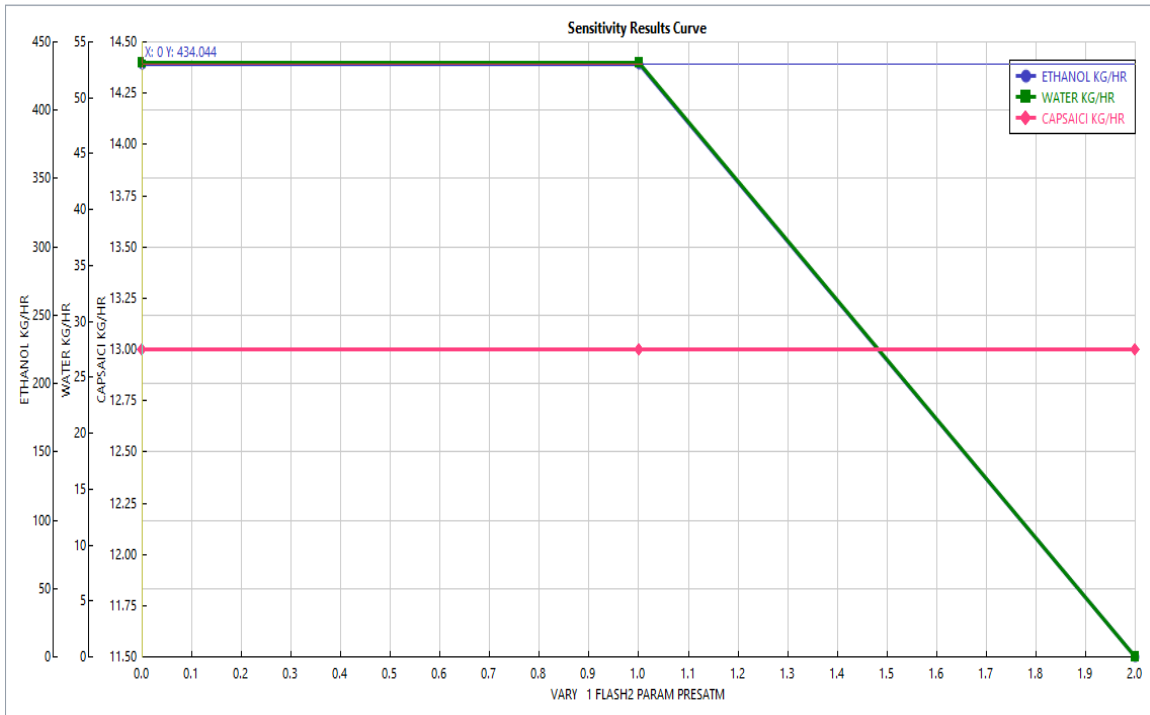
353.15K and atmospheric pressure, 97.53% EtOH and 96.54% water reached at top of the column. For a purification model to examined a sensitivity effect of change in temperature and pressure of a flash drum on the purity of EtOH and water at vapor product and capsaicin at the bottom product. The present of product plotted for wide temperature range from 350K to 379K. The effect of temperature and pressure on the flash drum is shown in the figure below. Along with ethanol, most of water started to vaporize. On the other hand, capsaicin stayed at the bottom of the column because it doesn't start to vaporize at the specified temperature. Maximum solvents were recovered when the temperature increases from 353K to 364.692K the recovery of ethanol and water were 440.11kg/hr and 54.033Kg/hr respectively. The temperature above 376K no solvent recovered.



**Figure 4.7:** Temperature sensitivity analysis of flash1

From pressure sensitivity analysis showed in figure in figure 4.8 each pressure increment above atmospheric pressure both EtOH and water mass flowrate at top of the column

decreases. The optimum condition is found to be below 1atm with gives maximum recovery of solvents.



**Figure 4.8:** Pressure sensitivity analysis of flash1

Hence it can be seen that higher temperature and low pressure are suitable to obtain maximum recovery.

The mass flowrate of 29kg/hr LIQ stream from flash1 was sent to a second flash22 after being cooled to 321K for further purification of the desired product. In this flash, the input stream was vaporized and the capsaicinoid was separated as a liquid stream. Maximum solvents were recovered at 373.5K the recovery of ethanol and water were 8kg/hr and 1.22Kg/hr respectively. Totally, 2.954021kg/hr ethanol and 0.6802063kg/hr water out of 20kg/hr desired product stream. From total capsaicinoid product stream 1.24% water and 0.66% ethanol presented. Based on Aspen Plus<sup>TM</sup> output moisture content was 1.9%.

The recovered solvent stream VAP from Flash2 was mixed with the solvent stream VAP2 recovered from Flash22 cooled down at 321K in Cooler2 and recycled as a fresh solvent.

## 5. CONCLUSION AND RECOMMENDATION

### 5.1. Conclusion

The proximate result of the sample prior to the extraction process indicated the sample had all the compositional requirements of chili pepper outlined in the literature. The results of the study showed that various parameters contributed to the extraction of capsaicinoid. In this study temperature; 85°C, 90°C and 95°C, solvent concentration; 79% v/v, 89% v/v 99% v/v and extraction time; 3hr, 4hr and 5hr were used.

From the experimental result it is shown that the optimum condition of temperature 90°C, extraction time of 4hr and solvent concentration of 89%. Under this condition the highest percentage yield of 16.57% capsaicinoids was obtained.

Analysis based on experimental design software the analysis of ANOVA, P- value <0.0001. It indicated which operating parameters have significant effect on the yield of capsaicinoid. Investigation based on the extraction duration showed that temperature and time were highly significant in the capsaicinoid extraction process. As expected, a longer extraction time generally led to a higher percentage yield of capsaicin. This could have been due to the longer amount of time the solute and solvent were in contact with each other. Longer contact time favored the system to have more mass transfer. Moreover, a higher percentage yield was also observed for solvents that had higher percentages of ethanol. Although capsaicinoid is slightly soluble in low concentration of ethanol, low concentration of ethanol proved to be an ineffective extraction solvent for capsaicinoid. The effectiveness of ethanol as an extraction solvent for capsaicin could be due to it being an organic polar solvent.

Based on optimum operating condition extracted yield determined physicochemical properties like, moisture and volatile matter 3.5%, kinematic viscosity  $3.43 \times 10^{-6} \frac{\text{m}^2}{\text{s}}$ , density 990Kg/m<sup>3</sup>, specific gravity 1.01 and refractive index 1.4967 were determined for characterization and qualitative analysis. their value were within the standards.

AspenPlus™ was used to generate the process models and simulations for leaching process. The design parameters were obtained based on the optimum experimental output. The sensitivity analyses carried out for purification and recovery unit. The operating conditions were set to provide maximum product purity and solvent recovery.

Percentage recovery of solvent increases with an increase in temperature. It can be carried out at 364.692K was 98.9% EtOH and 98.24% water achieved. Due to the big boiling point difference along with solvent capsaicinoid was not vaporized to top of the column.

## **5.2. Recommendation**

Even though capsaicin has been used all over the world for pharmaceutical industry as medicines, in our country peoples didn't conduct on this area to done researches rather than exporting the raw chili pepper. So, I recommended further studies would be performed as much as possible on the performance of capsaicin to be used in pharmaceutical industry for production of different medicines.

The study could not consider certain parameters such as extraction with more solvents to aid in the search for a suitable replacement of ethanol.

The quantification of final product was not analyzed by GC/MS or spectrometric analysis due to lack of reagents, standards and optimum operation method.

During experimental work the oil bath is not properly working, its temperature controller was not working as well when adjusted at 95°C.

This model doesn't consider Swash to analyze the sensitivity analysis due to lack of integration capsicum annum to aspen plus™ database as a solid.

A further study should be considered by using other pharmaceutical grade solvents and operating mechanism.

## REFERENCE

- Al-snafi, A. E. (2015). THE PHARMACOLOGICAL IMPORTANCE OF CAPSICUM, 5(3), 124–142.
- Arora, R., Gill, N. S., Chauhan, G., & Rana, A. C. (2011). Review Article An Overview about Versatile Molecule Capsaicin, 3(4), 280–286.
- Bharude, N. V, & Article, R. (2011). Chillies as food, spice and medicine: a perspective, 1(3).
- Botanicae Horti Agrobotanici Cluj-Napoca, N., & Sharma, G. J. (2008). Capsaicin Content and Pungency of Diierent Capsicum spp. Cultivars. Not. Bot. Hort. Agrobot. Cluj, 36(2), 89–90.
- De Lourdes Reyes-Escogido, M., Gonzalez-Mondragon, E. G., & Vazquez-Tzompantzi, E. (2011). Chemical and pharmacological aspects of capsaicin. *Molecules*. <https://doi.org/10.3390/molecules>
- Delelegn, S. (n.d.). EVALUATION OF ELITE HOT PEPPER VARIETIES ( Capsicum species ) FOR GROWTH , DRY POD YIELD AND QUALITY UNDER JIMMA CONDITION , SOUTH WEST.
- Eda, W. (2012). 1 . Exploratory Data Analysis 1 . Exploratory Data Analysis - Detailed Table of Contents [ 1 .].
- EFFECT OF DIFFERENT SOLVENTS ON THE EXTRACTION OF SOYA BEAN. (2009).
- Eghdami, A., Salehi, M. A., & Babakhani, M. (2014). Determination of physicochemical properties of capsaicin and cytotoxic effect of capsicum extract in breast cancer (MCF7) cell line. *Int. J. Biosci.* <https://doi.org/10.12692/ijb/>
- Elasheed, A., & Abdelmajid, E. (2016). Extraction and Characterization of Capsaicin from Capsicum frutescens and Capsicum annum, (300), 90–92.

- Gudeva, L. K., Mitrev, S., Maksimova, V., & Spasov, D. (2001). Content of capsaicin extracted from hot pepper ( *Capsicum annum* ssp . *microcarpum* L .) and its use as an ecopesticide, 671–675. <https://doi.org>
- Guide, U. (n.d.). Aspen Plus 7 10, 2.
- Haanpää, M., & Treede, R. D. (2012). Capsaicin for neuropathic pain: Linking traditional medicine and molecular biology. *European Neurology*. <https://doi.org>
- Isobe, T., Shiraishi, H., Yasuda, M., Shinoda, A., Suzuki, H., & Morita, M. (2003). Determination of estrogens and their conjugates in water using solid-phase extraction followed by liquid chromatography – tandem mass spectrometry, 984, 195–202.
- Juangsamoot, J., Ruangviriyachai, C., Techawongstien, S., & Chanthai, S. (2012). Determination of capsaicin and dihydrocapsaicin in some hot chilli varieties by RP-HPLC-PDA after magnetic stirring extraction and clean up with C 18 cartridge. *International Food Research Journal*. <https://doi.org/qww2>
- Leivisk, K. (2013). Introduction to Experiment Design Kauko Leiviskä University of Oulu Control Engineering Laboratory Table of Contents.
- Liljana, K. G., Viktorija, M., Marija, S. D., Rubin, G., & Emilija, I. J. (2013a). THE EFFECT OF DIFFERENT METHODS OF EXTRACTIONS OF CAPSAICIN ON ITS CONTENT IN THE CAPSICUM OLEORESINS.
- Liljana, K. G., Viktorija, M., Marija, S. D., Rubin, G., & Emilija, I. J. (2013b). THE EFFECT OF DIFFERENT METHODS OF EXTRACTIONS OF CAPSAICIN ON ITS CONTENT IN THE CAPSICUM OLEORESINS Koleva G . Liljana \*, Maksimova Viktorija \*\*, Serafimovska D . Marija \*\*, Gulabovski \* Faculty of Agriculture , University “ Goce Delcev ”, Stip , R . of. Food Science, Engineering and Technology, LX, 917–922.
- Magnusson, H. (n.d.). Process Simulation in Aspen Plus of an Integrated Ethanol and CHP plant.

- Man, C., Wilhad, N., & Reuter, M. (n.d.). Analysis of Capsaicin and Dihydrocapsaicin in Chili Peppers.
- Method, P., & Assistant, S. (n.d.). Guidelines for Choosing a Property Method Guidelines for Choosing an Activity Coefficient Property Method Guidelines for Choosing a Property Method for Polar Non-Electrolyte Systems.
- Olivas, G. I., Sepulveda, D. R., & Olivas, G. I. (2013). Capsaicinoids content and proximate composition of Mexican chili peppers ( *Capsicum spp.* ) cultivated in the State of Chihuahua, 6337(December 2017). <https://doi.org>
- Aspen Plus, Steady State Simulation, A. (n.d.). Aspen Plus 7 10 September 2001.
- Aspen plus, Unit Operation Model, A., & Guide, U. (n.d.). Aspen Plus ®.
- Plus, A., Properties, A., Suite, A. E., Technology, A., Park, T. C., Systems, O., ... Park, T. C. (n.d.). Part Number : Aspen Physical Property System 11 . 1 September 2001.
- Rafael Rocha-Herrera, A., & Rafael, A. (n.d.). Influence of solvent extraction, maturity stage, and thermal treatment on the determination of capsaicin in capsicums (*Capsicum annum spp.*) and their products Recommended Citation. Retrieved from <http://lib.dr.iastate.edu/rtd>
- Resources, A. (2012). Determination of Capsaicin and Dihydrocapsaicin in Some Chilli Varieties using Accelerated Solvent Extraction Associated with Solid-Phase Extraction Methods and RP-HPLC-Fluorescence, 9(3), 1550–1561.
- Selvaraj, T., & Sivakumar, D. (2013). Application of Box Behnken design to optimize the parameters for turning Inconel 718 using coated carbide tools, 4(4).
- Sethuraman, R., & Ch, B. E. E. (1997). SUPERCRITICAL FLUID EXTRACTION OF CAPSAICIN FROM PEPPERS.
- Stoica, R.-M., Moscovici, M., Tomulescu, C., & Băbeanu, N. (n.d.). EXTRACTION AND ANALYTICAL METHODS OF CAPSAICINOIDS - A REVIEW.

- Technology, A., Park, T. C., Systems, O., Technology, A., & Park, T. C. (n.d.).  
September 2001.
- Tekindal, M. A., Science, B. M., Of, D., Engineering, F., & Science, B. M. (2012). BOX-BEHNKEN EXPERIMENTAL DESIGN IN FACTORIAL EXPERIMENTS : THE IMPORTANCE OF BREAD FOR NUTRITION, 17(2), 115–123.
- Usman, M. G., Rafii, M. Y., Ismail, M. R., Malek, A., & Latif, M. A. (2014). Capsaicin and Dihydrocapsaicin Determination in Chili Pepper Genotypes Using Ultra-Fast Liquid Chromatography, 6474–6488. <https://doi.org>
- Wesołowska, A., Jadczyk, D., & Grzeszczuk, M. (2011). CHEMICAL COMPOSITION OF THE PEPPER FRUIT EXTRACTS OF HOT CULTIVARS *Capsicum annuum* L. *Acta Sci. Pol. Hortorum Cultus*.
- Zahra, N., Kalim, I., Hina, S., Javed, A., Manzar Inam, S., Muhammad Malik, S., & Arshad, F. (2016). Estimation of capsaicin in different chilli varieties using different Extraction Techniques and HPLC method: A Review. *Pakistan Journal of Food Sciences*, 26(1s), 54–60.
- الحار الحلو, والفلفل, Elrasheed, A., Ada, O., Omer, S., Gibla, A. M., & Annum, C. (2016). Extraction and Characterization of Capsaicin *Capsicum frutescens* Extraction and Characterization of Capsaicin from.

# APPENDIX

## Appendix A: Formulas and Equation

$$\text{Moisture content, \%} = \frac{w_1 - w_2}{w_1} 100 \dots \dots \dots (\text{A.1})$$

Where:  $W_1$  = Original weight of the sample before drying

$W_2$  = Weight of the sample after drying

$$\text{Kinematic viscosity, } \nu = \frac{\mu}{\rho} \dots \dots \dots (\text{A.2})$$

Where:  $\mu$  = dynamic viscosity

$\rho$  = density of oil

$$\text{Moisture and volatile matter} = \frac{W_1}{W_2} * 100 \dots \dots \dots (\text{A.3})$$

Where:  $W_1$  = loss in gm of the material on drying

$W_2$  = weight in gm of oil taken for the test

## Appendix B: Laboratory sample photo



**Raw material**



**Powdered form**

## Appendix C: Laboratory equipment sample photo



**Crusher of raw material**



**During extraction**



**After extraction (solution)**



**During recovery of solvent**



**Final product after decantation (capsaicinoids)**



**Viscometer**

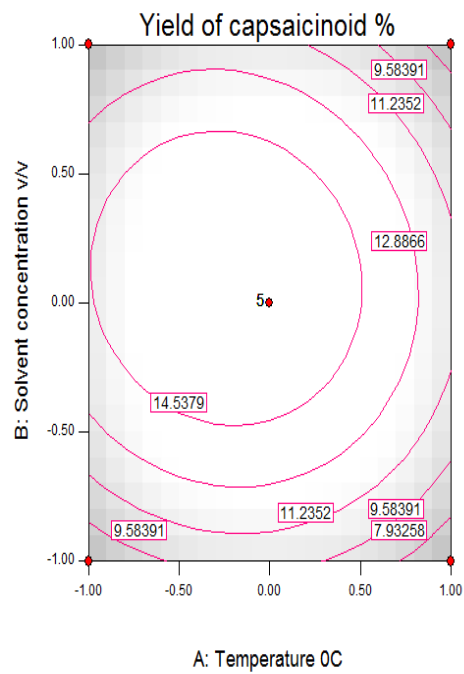
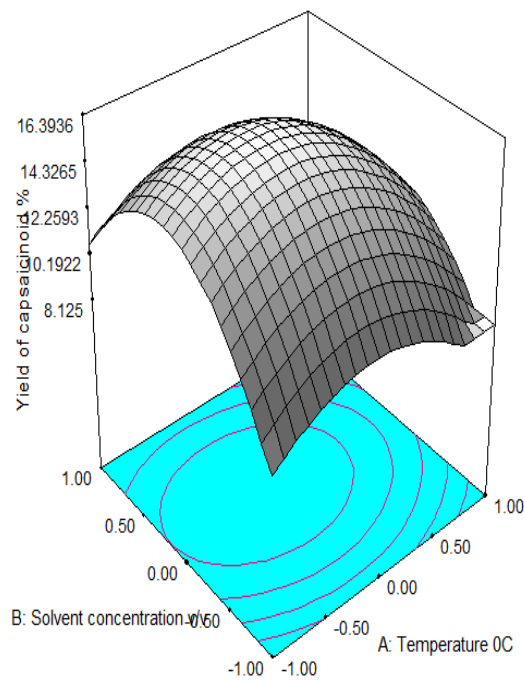


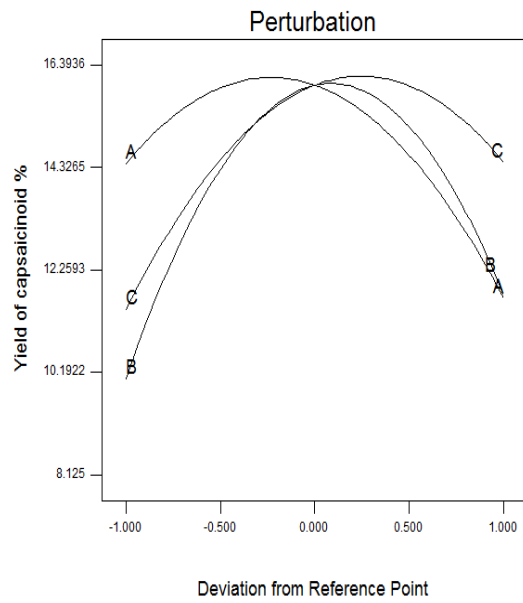
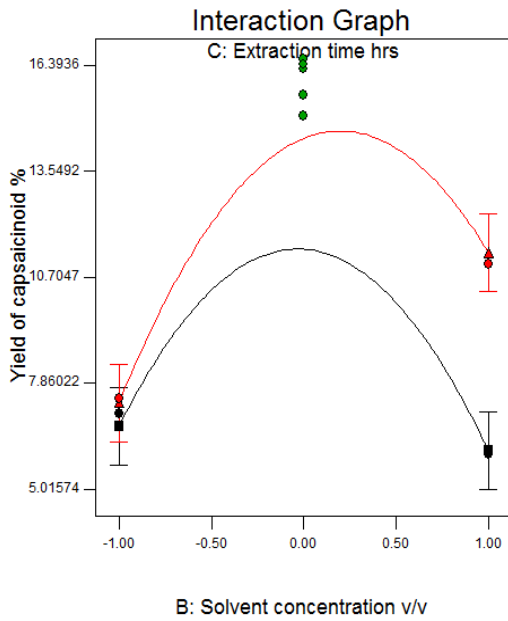
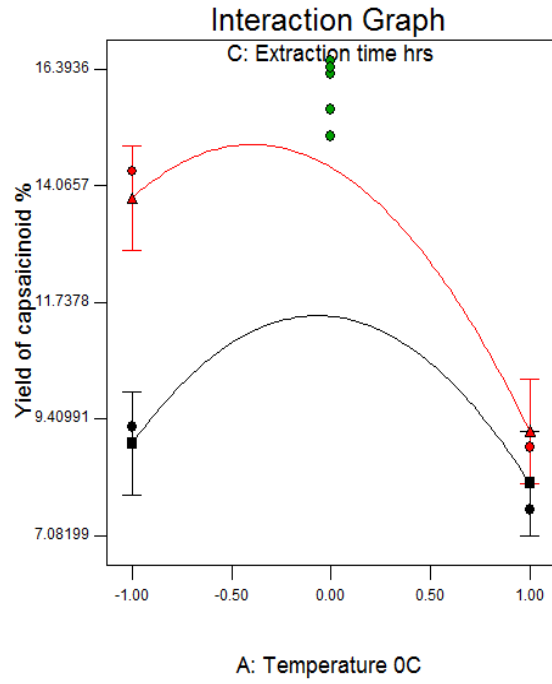
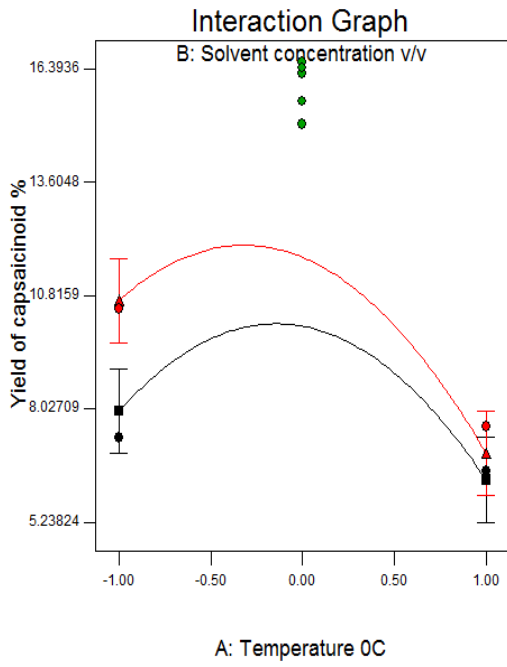
**Refractometer**



Density and specific gravity meter

**Appendix D: Design expert output additional data**





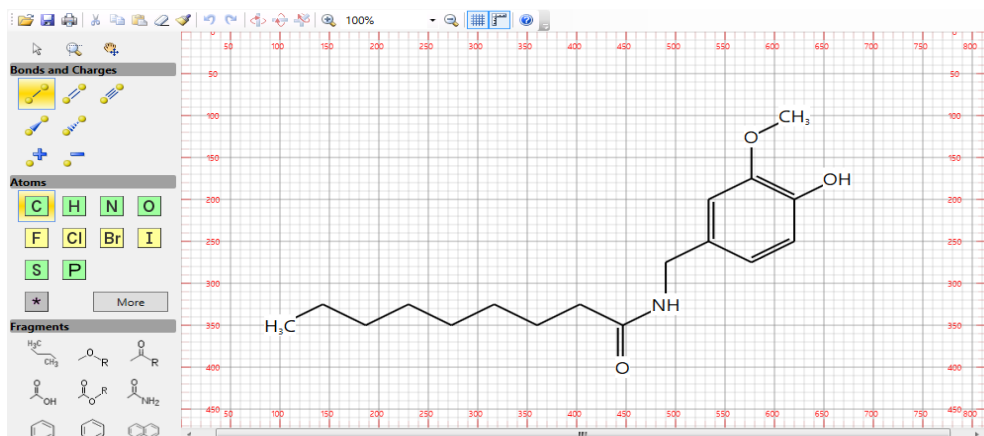
Design Summary							
<b>Study Type</b>	Response Surface		<b>Experiments</b>	17			
<b>Initial Design</b>	Box Behnken		<b>Blocks</b>	No Blocks			
<b>Design Model</b>	Quadratic						
Response	Name	Units	Obs	Minimum	Maximum	Trans	Model
Y1	y		17	5.94	16.57	None	Quadratic
Factor	Name	Units	Type	Low Actual	High Actual	Low Coded	High Coded
A	A		Numeric	-1.00	1.00	-1.000	1.000
B	B		Numeric	-1.00	1.00	-1.000	1.000
C	C		Numeric	-1.00	1.00	-1.000	1.000

## Appendix E: Aspen Plus™ additional information

Properties for NORDAHYD		Parameters			
Name	Description	Value	Units	Uncertainty	
OMEGA	Pitzer acentric factor	1.1377		0	
ZC	Critical compressibility factor	0.207		0.207	
VC	Critical volume	0.9339	cum/kmol	-0.0607	
PC	Critical pressure	1708517	N/sqm	1708517	
TC	Critical temperature	929	K	32.9	
DNLEXSAT	TDE expansion for liquid molar density	+	kg/cum		
DHVLTDEW	TDE Watson equation for heat of vaporization	+	J/kmol		
CPIALEE	TDE Aly-Lee ideal gas Cp	+	J/kmol-K		
MW	Molecular weight	293.41		0	
TB	Normal boiling point	740.4	K	68.2	
DELTA	Solubility parameter @ 25 C	25023.2	(J/cum)**.5	25023.2	
SG	Specific gravity	1.221		0.158	
VLSTD	API standard liquid molar volume	0.2406	cum/kmol	0.0312	
KVTMLPO	ThermoML polynomials for vapor thermal conductivity	+	Watt/m-K		
KLTMLPO	ThermoML polynomials for liquid thermal conductivity	+	Watt/m-K		
WAGNER25	TDE Wagner 25 liquid vapor pressure	+	N/sqm		
MUVTMLPO	ThermoML polynomials for vapor viscosity	+	N-sec/sqm		
MULPPDS9	PPDS9 equation for liquid viscosity	+	N-sec/sqm		
FAMILY	Compound family name	POLYFUNCTIONAL AMIDES/			
SUB FAMILY	Compound sub family name	hydroxyamine			

Re-evaluate Save Parameters Help TDE version: 8.2, Database version: 8.2

Estimated pure component parameter of nordahydrocapsaicin



Structural formula of nordihydrocapsaicin ( $C_{17}H_{27}NO_3$ )

Stream summary for purification and recovery process

	FEED	MIX1	PUMP1	HEATER1	VAP
<b>Substream: MIXED</b>					
<b>Mole Flow kmol/hr</b>					
<b>CAPSAICI</b>	0.0425647	0.0425647	0.0425647	0.0425647	4.45E-10
<b>DEHYDROC</b>	0.0116122	0.0116122	0.0116122	0.0116122	1.40E-10
<b>ETHANOL</b>	9.659416	9.659416	9.659416	9.659416	9.421589
<b>WATER</b>	3.052964	3.052964	3.052964	3.052964	2.947336
<b>NORDAHDYD</b>	0.001403	0.001403	0.001403	0.001403	6.32E-10
<b>Mole Frac</b>					
<b>CAPSAICI</b>	3.33E-03	3.33E-03	3.33E-03	3.33E-03	3.60E-11
<b>DEHYDROC</b>	9.10E-04	9.10E-04	9.10E-04	9.10E-04	1.13E-11
<b>ETHANOL</b>	0.7566187	0.7566187	0.7566187	0.7566187	0.7617144
<b>WATER</b>	0.2391376	0.2391376	0.2391376	0.2391376	0.2382856
<b>NORDAHDYD</b>	4.11E-05	4.11E-05	4.11E-05	4.11E-05	5.40E-11
<b>Mass Flow kg/hr</b>					
<b>CAPSAICI</b>	13	13	13	13	1.36E-07
<b>DEHYDROC</b>	2.5	2.5	2.5	2.5	4.30E-08
<b>ETHANOL</b>	445	445	445	445	434.0436
<b>WATER</b>	55	55	55	55	53.09709
<b>NORDAHDYD</b>	1.07	1.07	1.07	1.07	5.27E-09
<b>Mass Frac</b>					

<b>CAPSAICI</b>	0.025166	0.025166	0.025166	0.025166	2.79E-10
<b>DEHYDROC</b>	6.91E-03	6.91E-03	6.91E-03	6.91E-03	8.83E-11
<b>ETHANOL</b>	0.8614515	0.8614515	0.8614515	0.8614515	0.8910026
<b>WATER</b>	0.1064715	0.1064715	0.1064715	0.1064715	0.1089974
<b>NORDAHYD</b>	3.71E-5	3.71E-5	3.71E-5	3.71E-5	2.32E-14
<b>Total Flow kmol/hr</b>	12.76656	12.76656	12.76656	12.76656	12.36893
<b>Total Flow kg/hr</b>	516.57	516.57	516.57	516.57	487.1406
<b>Total Flow l/min</b>	10.47623	10.47623	10.60615	3776.147	6058.377
<b>Temperature K</b>	298.15	298.15	307	351.95	358.15
<b>Pressure atm</b>	1	1	1	1	1
<b>Vapor Frac</b>	0	0	0	0.6137963	1
<b>Liquid Frac</b>	1	1	1	0.3862037	0
<b>Solid Frac</b>	0	0	0	0	0
<b>Enthalpy cal/mol</b>	-67093.55	-67093.55	-66826.3	-59625.3	-55628.05
<b>Enthalpy cal/gm</b>	-1658.156	-1658.156	-1651.551	-1473.585	-1412.445
<b>Enthalpy cal/sec</b>	-2.38E+05	-2.38E+05	-2.37E+05	-2.11E+05	-1.91E+05
<b>Entropy cal/mol-K</b>	-72.89277	-72.89277	-72.01791	-51.33936	-39.69967
<b>Entropy cal/gm-K</b>	-1.801478	-1.801478	-1.779857	-1.268806	-1.008009
<b>Density mol/cc</b>	0.0203103	0.0203103	0.0200615	5.63E-05	3.40E-05
<b>Density gm/cc</b>	0.8218126	0.8218126	0.8117459	2.28E-03	1.34E-03
<b>Average MW</b>	40.46275	40.46275	40.46275	40.46275	39.38423
<b>Liq Vol 60F l/min</b>	10.45336	10.45336	10.45336	10.45336	10.02131

	<b>LIQ</b>	<b>COOL2</b>	<b>VAP2</b>	<b>LIQ2</b>	<b>RECOVERY</b>
<b>Substream: MIXED</b>					
<b>Mole Flow kmol/hr</b>					
<b>CAPSAICI</b>	0.0425647	0.0425647	0.0425647	0.0425647	4.45E-10
<b>DEHYDROC</b>	0.0116122	0.0116122	0.0116122	0.0116122	1.40E-10
<b>ETHANOL</b>	9.659416	9.659416	9.659416	9.659416	9.421589
<b>WATER</b>	3.052964	3.052964	3.052964	3.052964	2.947336
<b>NORDAHYD</b>	0.001206	0.001206	0.001206	0.001206	2.51E-10
<b>Mole Frac</b>					
<b>CAPSAICI</b>	3.33E-03	3.33E-03	3.33E-03	3.33E-03	3.60E-11
<b>DEHYDROC</b>	9.10E-04	9.10E-04	9.10E-04	9.10E-04	1.13E-11
<b>ETHANOL</b>	0.7566187	0.7566187	0.7566187	0.7566187	0.7617144
<b>WATER</b>	0.2391376	0.2391376	0.2391376	0.2391376	0.2382856
<b>NORDAHYD</b>	6.14E-05	6.14E-05	6.14E-05	6.14E-05	2.15E-11
<b>Mass Flow kg/hr</b>					
<b>CAPSAICI</b>	13	13	13	13	1.36E-07
<b>DEHYDROC</b>	2.5	2.5	2.5	2.5	4.30E-08
<b>ETHANOL</b>	445	445	445	445	434.0436
<b>WATER</b>	55	55	55	55	53.09709
<b>NORDAHYD</b>	1.07	1.07	1.07	107	1.04E-10
<b>Mass Frac</b>					
<b>CAPSAICI</b>	0.025166	0.025166	0.025166	0.025166	2.79E-10
<b>DEHYDROC</b>	6.91E-03	6.91E-03	6.91E-03	6.91E-03	8.83E-11
<b>ETHANOL</b>	0.8614515	0.8614515	0.8614515	0.8614515	0.8910026
<b>WATER</b>	0.1064715	0.1064715	0.1064715	0.1064715	0.1089974
<b>NORDAHYD</b>	2.13E-04	2.13E-04	2.13E-04	2.13E-04	2.13E-04
<b>Total Flow kmol/hr</b>	12.76656	12.76656	12.76656	12.76656	12.36893
<b>Total Flow kg/hr</b>	516.57	516.57	516.57	516.57	487.1406
<b>Total Flow l/min</b>	10.47623	10.47623	10.60615	3776.147	6058.377
<b>Temperature K</b>	298.15	298.15	307	351.95	358.15
<b>Pressure atm</b>	1	1	1	1	1
<b>Vapor Frac</b>	0	0	0	0.6137963	1
<b>Liquid Frac</b>	1	1	1	0.3862037	0

<b>Solid Frac</b>	0	0	0	0	0
<b>Enthalpy cal/mol</b>	-67093.55	-67093.55	-66826.3	-59625.3	-55628.05
<b>Enthalpy cal/gm</b>	-1658.156	-1658.156	-1651.551	-1473.585	-1412.445
<b>Enthalpy cal/sec</b>	-2.38E+05	-2.38E+05	-2.37E+05	-2.11E+05	-1.91E+05
<b>Entropy cal/mol-K</b>	-72.89277	-72.89277	-72.01791	-51.33936	-39.69967
<b>Entropy cal/gm-K</b>	-1.801478	-1.801478	-1.779857	-1.268806	-1.008009
<b>Density mol/cc</b>	0.0203103	0.0203103	0.0200615	5.63E-05	3.40E-05
<b>Density gm/cc</b>	0.8218126	0.8218126	0.8117459	2.28E-03	1.34E-03
<b>Average MW</b>	40.46275	40.46275	40.46275	40.46275	39.38423
<b>Liq Vol 60F l/min</b>	10.45336	10.45336	10.45336	10.45336	10.02131