Heat and Oxidation Stability of Bio-Pigments for Food Coloring

by

Chong Kar Yeen

13780

Dissertation submitted in partial fulfillment of the requirements for the Bachelor of Engineering (Hons) (Chemical)

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CERTIFICATION OF APPROVAL

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Approved by,

_____

(ASSOC. PROF. DR ZAKARIA BIN MAN)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

May 2014

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except in the references and the acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

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CHONG KAR YEEN

ABSTRACT

Natural pigments are becoming more important recently due to negative effects posed by synthetic colorants. However, bio-pigments are sensitive to various conditions such as temperature, pH level, storage conditions, and ultraviolet radiation. This project aims to study the heat and oxidation stability of pigments from pandan leaves (*Pandanus amaryllifolius*) and turmeric (*Curcuma longa*) by using natural stabilizers which are lye water and ascorbic acid respectively. Chlorophyll pigments from pandan leaves were extracted by blending and filtration. Lye water of different concentrations were added to these pigments. Commercial turmeric powder was mixed with different amount of ascorbic acid. In order to determine their stabilities, themogravimetric analysis was done using heating rates of 10°C/min, 20°C/min, and 30°C/min. Activation energies were then calculated. Fourier transform infrared spectroscopy was used to determine functional groups of the solutions. In both pandan solutions, it was observed that carbon dioxide is present, indicating degradation. In treated turmeric mixture, it was observed that the carbon-hydrogen stretch was less intense. Besides that, lye water was found to reduce chlorophyll degradation at 80°C and ascorbic acid was proven to be an effective oxidation stabilizer.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to the Chemical Engineering Department of Universiti Teknologi PETRONAS for providing me this opportunity to carry out final year project. This project has exposed me to new knowledge through extensive reading. I also learnt research methodologies which are useful as I plan to further my studies. In my opinion, the purpose of research work is to make an impact, be it little or large, to the society. As we have gained a lot during our lifetime, I believe in contributing back to the society and at the same time discovering the unique connection among God's creations.

A special note of thanks to my supervisor, Associate Professor Dr. Zakaria bin Man, who is always willing to guide me despite his many other academic commitments. I would also like to thank laboratory technicians for assisting in laboratory procedures. Last but not least, I would like to thank my family and friends who have provided moral support for this project. It is hoped that my peers will complete their final year projects successfully too.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS AND NOMENCLATURE

Abbreviation	Meaning
A	Pre-exponential factor
AA	Ascorbic acid
CR	Coats-Redfern method
E_a	Activation energy
FTIR	Fourier Transform Infrared spectroscopy
FWO	Flynn-Wall-Ozawa method
k	Rate constant
LW	Lye water
R	Gas constant
TGA	Thermogravimetric analysis
Greek letters	
α	Conversion
β	Heating rate

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Dyes are used extensively for coloring many consumer goods such as textiles, food, cosmetics, and toys. More specifically, food coloring is added to restore original food appearance, ensure color uniformity and intensify colors normally found in food, protect other food components such as antioxidants, and improve aesthetic appeal. Besides that, color is also linked with food safety. Inappropriate color is associated with bad processing, faulty transportation, or spoilage (Delgado-Vargas & Paredes-López, 2003).

Due to increasing negative side effects of synthetic colorants and tightened government restrictions on food coloring, alternatives of food dyes are actively being studied. Examples of natural pigments which are being used by Malaysian households for food applications are turmeric as a yellow colorant in curry dishes, butterfly pea flower to give blue color to glutinous rice cakes and pandan leaves in desserts. This project is designed to investigate the heat and oxidation stability of bio-pigments from turmeric (*Curcuma longa*) and pandan leaves (*Pandanus amaryllifolius*) for the purpose of food coloring.

Curcumin, which is the yellow, primary bioactive component of turmeric (Masek et al., 2013) has been used traditionally used to treat common ailments such as stomach upset, jaundice, and arthritis (Aggarwal & Sung, 2009). Turmeric is also used as an ingredient in many varieties of curry powders and sauces, which curcumin acts as a main coloring substance.

Pandan leaves, also known as screw pine leaves, are valued for its flavor and significant aroma. The chlorophyll pigment also colors various foods and gives them an appealing appearance.

1.2 Problem Statement

The food and beverages industries frequently use synthetic dyes in their products due to many factors including color retention. However, these artificial dyes pose some negative side effects to the consumer. Hence, industries are shifting towards natural dyes. Yet, natural dyes are found to be sensitive to various conditions such as pH level, storage conditions, temperature, and ultraviolet (UV) radiation. Thus, the application of natural dye is limited in food processing.

1.3 Objectives and Scope of Study

The objectives of this project are to:

a) To extract pandan and turmeric pigments
b) To study the heat and oxidation stability of those pigments
c) To determine the effectiveness of environmental-friendly stabilizers for the application as food coloring.

For this project, we source locally available plants which are pandan leaves and turmeric. The types of stabilizers used for this project are lye water and ascorbic acid.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Dyes and Pigments

There are two kinds of food dyes used, which are synthetic dyes and natural dyes. Synthetic food colorants are divided into five classes: the azo compounds such as amaranth and tartrazine, the chinophthalon derivatives of Quinoline Yellow, the triarylmethane group, xanthenes such as erythrosine and the indigo colorants (Sarıkaya et al., 2012). Natural food colorants are found in plants and even in microorganisms and microalgae (Delgado-Vargas et al., 2000; Dufosse et al., 2005). However, some synthetic dyes such as Allura Red and Tartrazine which have prolong history in food industry have been reported to be hazardous to health, particularly to gastrointestinal organs (Sasaki et al., 2002). Other synthetic dyes such as Brilliant Blue and Patent Blue are discovered to enter the bloodstream from the saliva through the tongue after 24 hours of diffusion (Lucova et al., 2013). In addition, natural colorants such as turmeric are commonly adulterated in India. Metanil yellow which is a non-permitted coloring is added as an adulterant to improve color because it is easily available at reasonable cost (Sudershan et al., 2009).

About 10,000 various commercial dyes and pigments are produced yearly in the world. 10% to 15% of these dyes are estimated to be lost during the dyeing process and subsequently released with the effluent (Luo et al., 2011). Synthetic dye, for example, Orange II which is commonly used in pharmaceutical, food, and cosmetics industries, is a non-biodegradable pollutant (Levec, 1997). Hence, it can be summarized that there are problems with synthetic dyes that needs further attention. With more health-conscious consumers, the search for alternative colorants is a pressing issue. It is also essential to substitute synthetic dyes with biodegradable ones. Natural pigments are known as potential biodegradable pigments.

2.2 Bio-Pigments

Many natural pigments such as butterfly pea flower (*Clitoria ternatea L.)*, roselle (*Hibiscus sabdariffa L.*), dragon fruit (*Hylocereus undatus L.*) and marigold (*Calendula officinalis L.)* have been studied for the possible use as coloring dyes (Boonsong et al., 2011). Plant pigments are also found to have active functional

components, and thus can be used as excellent materials for food supplements (Boo et al., 2012). Several plants which are commonly known by Malaysians have been studied in terms of stability.

2.2.1 Anthocyanins and Betalains

Pigment from red dragon fruit was studied by manipulating parameters such as temperature, pH, light conditions, and type of solvent used. Unfortunately, its chromatic stability was low as exposure of light cause color lost up to 50% after one week storage at room temperature (Woo et al., 2011). It was found that there was a least change in pH and pigment concentration when pigments are stored at -20°C (Rebecca et al., 2008). This is unsuitable as food processing usually involves heat treatment. Further studies and experiment are needed to confirm these initial findings. According to Herbach et al. (2006), 1% ascorbic acid managed to produce maximum pigment stability of red dragon fruit juice.

Another colorant synthesized from blue pea flower (*Clitoria ternatea*) is frequently used to dye glutinous rice cakes in Malaysia. Thermal degradation of anthocyanin extract from this flower was found to be most rapid at 37°C. The process was most likely to be catalyzed by enzymes which were most active at this temperature. To prolong color retention, a common food preservative which is benzoic acid was tested and this proved positive results (Lee et al., 2011). It was recommended that further investigation should be done using purified anthocyanins.

2.2.2 Carotenoids

β-Carotene, a red-orange pigment that is mainly present in carrots, pumpkins, and sweet potatoes, has also been studied. According to Delgado-Vargas et al. (2000), β-Carotene showed fast degradation with UV effect and visible light in the presence of oxygen. It was also observed that green tea polyphenols have stronger effects against discoloration of β-Carotene than L-ascorbate which is widely used to prevent discoloration in aqueous and oily systems.

2.2.3 Curcumin

Various studies have been done using curcumin pigment from turmeric. Curcumin is shown to have a faster rate of degradation compared to tartrazine, its synthetic counterpart, when tested on extruded food products (Sowbhagya et al., 2005). According to Chen et al. (2014), the degradation temperature of curcumin is 190°C

when analyzed in the presence of air. Since 190°C is higher than usual frying temperature of 180°C, it can be said that curcumin is safe when used as a coloring agent for fried foods. However, the overall processing temperature of the food should not exceed 190°C. Masek et al. (2013) also reported that when nitrogen gas is used, both pure and commercial curcumin are thermally stable at temperatures from 0°C to 260°C. Moreover, it is found that commercial curcumin has slightly lower thermal stability than pure curcumin. An effort to improve curcumin's stability is the patent filed by Leshik (1981). The aforementioned author claimed the invention of an improved dry food mix comprising of ingredients such as curcumin colorant, pregelatinized tapioca starch, tetrasodium pyrophosphate, and disodium phosphate. The food mix is applied to instant pudding mixes and showed good color retention at 35°C when sealed with suitable packaging material. This mixture of colorant is claimed to be suitable for food pH ranging from 2 to 12. Besides that, tannin extract from tamarind seed coat is proven successful as a natural mordant for cotton, wool, and silk which was dyed with turmeric colorant (Prabhu & Teli, 2011). However, to my knowledge, reports on increasing curcumin's stability by using ascorbic acid are scarce.

2.2.4 Chlorophyll

Chlorophylls are green pigments from photosynthetic organisms. These organisms include plants, algae, and some bacteria (Delgado-Vargas & Paredes-López, 2003). After harvesting crops, chlorophyll degrades at a rate depending on processing conditions and plant material. It is important to preserve its greenness as consumers relates freshness to food color. Pandan leaves (*Pandanus amaryllifolius*), which are mainly cultivated in Southeast Asia, are used as food coloring and flavoring in culinary application. Current researches are mainly investigating on the aromatic compound of those leaves. The distinct aroma with a nutty and fresh hay flavor is mainly attributed to 2-acetyl-1-pyrroline volatile compound (Ooi et al., 2004). Besides that, the optimum conditions for the production of encapsulated spray-dried pandan powder as an edible coloring and flavoring powder were also studied (Loh et al., 2005). It can be acknowledged that the stability of pandan pigments has not been thoroughly researched. Table 1 shows a summary of stability studies on some bio-pigments.

TABLE 1 : Summary of stability studies on some bio-pigments

Methodology	Significant Findings	Reference
Anthocyanins and Betalains Pigments		
Spectroscopy	Source: Red dragon fruit • 0.1% ascorbic acid was able to preserve colour in all dark storage conditions • Best condition to preserve pigment is at pH 5.0 at 4°C in dark conditions. • When exposed to light, ascorbic acid was not effective	Woo et al. (2011)
Spectroscopy	Source: Red dragon fruit • Best weight:volume ratio for pigment extraction is 1:1 • Best temperature for high pigment yield is 100°C • Most stable condition for pigment storage is -20°C	Rebecca et al. (2008)
Spectroscopy	Source: Red dragon fruit • After heating at 85°C for 1 hour, optimal pigment retention occurred when 1% ascorbic acid was supplemented and adjusted to pH 4. • After thermal treatment, a 24-hour cool storage period is crucial for maximum pigment regeneration.	Herbach et al. (2006)
Spectroscopy	Source: Butterfly pea flower *(Clitoria ternatea)* • Addition of 0.02% of benzoic acid to blue CTAE enhanced stability by extending "stabilization period" from 10 to 30 days at 27°C and from 6 to 12 days at 37°C. • Further investigation should be done using purified anthocyanins	Lee et al. (2011)
β-Carotene Pigments		
-	Source: Carrots, pumpkins, sweet potatoes Green tea polyphenols have stronger effects against discoloration of β-Carotene than L-ascorbate which is widely used to prevent discoloration in aqueous and oily systems	Delgado-Vargas et al. (2000)
Curcumin Pigments from Turmeric		
Thermogravimetry in oxygen	Curcumin starts degrading at 190°C, hence processing temperature of food should not exceed this temperature.	Chen et al. (2014)
Thermogravimetry in nitrogen	• Both pure and commercial curcumin are thermally stable at temperatures from 0 to 260°C. • Commercial curcumin has slightly lower thermal stability than pure curcumin (the difference is within 10°C)	Masek et al. (2013)
-	• Invented improved dry food mix. • It is suitable for food pH ranging from 2 to 12. • It showed good color retention at 35°C when sealed with suitable packaging material	Leshik (1981)

Colorimetry	Tannin extract from tamarind seed coat is successful as a natural mordant for cotton, wool, and silk which was dyed with turmeric colorant	Prabhu et al. (2011)
Chlorophyll Pigments		
Spray-drying	Optimum conditions for production of encapsulated spray-dried pandan powder are inlet temperature of 170°C, feed rate of 6rpm, and constant outlet temperature of 90°C. These produce acceptably high color index.	Loh et al. (2005)

2.3 Potential Stabilizers

Since natural pigments are susceptible to various parameters, the addition of stabilizers is an important part of food processing.

2.3.1 Current Stabilizers and Processing Methods

Industries have used chemical stabilizers for a long time. Smith et al. (1997) states that erythorbates can prevent enzymatic browning in fruits and vegetables. For canned vegetables, additives are usually required to preserve color. These additives are classified into 2 groups which are alkalizing or alkalizing-buffering agents and metallic salts such as chlorides and acetates of zinc and copper (Segner et al., 1984). However, the United States Food and Drug Administration have imposed limits of these metallic ions in food. Another popular method to preserve green color is by blanching. The general concept of blanching is to plunge vegetables or fruits into hot boiling water, remove them quickly, and lastly, plunge them into cold water to stop the cooking process. Ihl et al. (1998) have proven that microwave blanching and boiling water blanching preserve the color of artichokes. Furthermore, for microwave blanched artichoke, ascorbic acid content is not lost. Hence, the nutrient of the food is not compromised by these pre-treatment method.

Regarding curcumin, Miuchi et al. (2009) have patented their invention on using gum ghatti as a natural stabilizer for turmeric pigments. Gum consists of polysaccharide and can be obtained by drying the sap of *Anogeissus* trees. Their turmeric pigments are reported to be dispersed at a stable rate in hydrous solution, have reduced sedimentation, have high absorbability through oral intake, and stable as a yellow colorant in aqueous products such as beverages and lotions.

2.3.2 Potential stabilizers

Since there is an increasing awareness towards healthy living, natural methods of increasing the pigments' stability are preferable. Thus, lye water and ascorbic acid will be used as stabilizers in this study after investigating the benefits of these substances.

Lye water or alkaline water is traditionally used in Malaysia as an additive for cooked vegetables to remain green for a longer time. It is also used as a tenderizer in the crust of baked moon cakes and to give a distinctive yellow color to glutinous rice dumplings. In fact, a common industrial practice is lye peeling of potatoes, tomatoes, and peaches. This kind of peeling involves bathing the produce in a caustic solution. After that, both the skin and solution are scrubbed off. Lye water is also one of the main ingredients in the production of alkaline noodles. According to Fu (2008), the degree of yellowness in alkaline noodles is related to the amount of alkaline reagent added and type of alkali used. The addition of sodium hydroxide yields noodles which are much more yellow, brighter, and discolor less with time.

For chlorophyll pigments, green chili puree is found to have better color retention when treated with lye. This is shown by a higher activation energy indicating a retarded rate of degradation (Ahmed et al., 2000). Figure 1 shows the structure of chlorophyll.

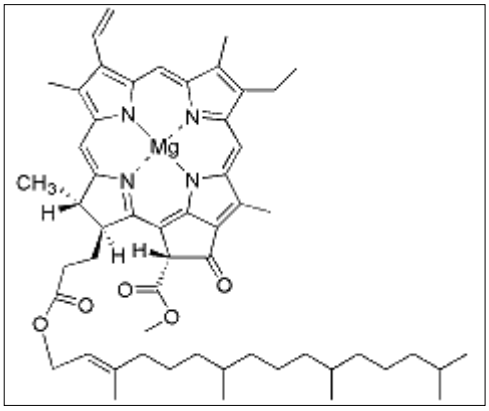


FIGURE 1: Structure of chlorophyll *(Schwartz and Lorenzo, 1990)*

According to Schwartz and Lorenzo (1990), the most common change that occurs in green vegetables is the conversion of chlorophylls to pheophytins. This causes a dramatic colour change from bright green to olive-brown. Referring to Figure 1, the

central magnesium atom is easily removed especially under acidic conditions, replacing it with hydrogen and hence forming pheophytins.

According to Delgado-Vargas and Paredes-López (2003), alkaline conditions destroy turmeric pigments and one of the approaches to prevent alkaline degradation is to add acidification agents such as citric and gallic acid. It is also reported that the stability of curcumin was improved by adding ascorbic acid which lowers the pH (Oetari et al., 1996). Besides that, the combination of ascorbic acid and curcumin has been proven to increase antifungal activities against *Candida albicans* yeast cells and also increase antioxidant activity of curcumin (Khalil et al., 2012). Ascorbic acid, which is an antioxidant, also increases retention of red beet pigments (Han et al., 1998). Hence, lye water and ascorbic acid are chosen as natural stabilizers for this project.

CHAPTER 3

METHODOLOGY

3.1 Gantt Chart and Key Milestones

Figure 2 shows the Gantt chart and key milestones for Final Year Project II.

No.	Activities / Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Preparation of samples for analysis	▭	▭												
2	Analysis and Continuation of Project Work		•	•	•	▭	• •	•	▭	▭	•				
3	Submission of Progress Report								•						
4	Pre-EDX											•			
5	Submission of Draft Report												•		
6	Submission of Dissertation (Soft Bound)													•	
7	Submission of Technical Paper													•	
8	Oral Presentation														•
9	Submission of Project Dissertation (Hard Bound)														•

Legend	
Symbol	Meaning
▭	Progress
•	TGA Analysis
•	FTIR Analysis
•	Submission of documents

FIGURE 2: Gantt chart and key milestones

3.2 Chemicals and Apparatus

3.2.1 Chemicals

Ascorbic acid, lye water, 95% ethanol, acetone

3.2.2 Apparatus

Knife, grinder, oven, Soxhlet extraction set, rotary evaporator, laboratory glassware, blender, thermogravimetric analyser (TGA), Fourier transform infrared spectrometer (FTIR), freeze-dryer

3.3 Experimental Methodology

3.3.1 Sample Preparation

- **Turmeric (Trial 1)**

a) Fresh turmeric rhizomes were cleaned and washed with water.

b) The cleaned material was cut into smaller pieces.

c) Turmeric was dried in the oven with air circulation at 70°C for 24 hours.
d) It was then ground using IKA MF 10.2 Impact grinding head. The dried material, now referred as turmeric powder, is kept in plastic bags in a dark environment at 25°C to avoid photodegradation.
e) The thimble was filled with an appropriate amount of sample. The sample should not be in excessive amount to ensure effective extraction. The thimble was placed in a Soxhlet apparatus.

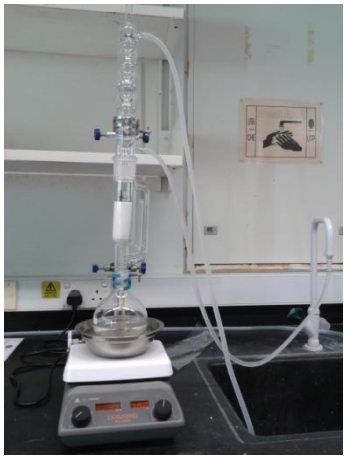


FIGURE 3: Soxhlet apparatus

f) 250ml of 95% ethanol was used as the solvent for Soxhlet extraction and it was heated to 80°C.
g) After extraction, the solvent was removed using LABCONCO freeze dryer.
h) All extracts were kept at 8°C in a dark environment after preparation.

- **Pandan (Trial 1)**

  The Soxhlet extraction procedure used with turmeric is repeated with pandan. It was found that both turmeric and pandan samples in the first trial produce inaccurate TGA curves. Hence, Trial 2 is carried out.

- **Pandan (Trial 2)**

a) Fresh pandan leaves were cut.

b) The leaves were then torn into smaller pieces and put into a food blender (Pensonic Blender PB-3203L).
c) Excess 95% ethanol was added and the blender was turned on. This was done rapidly and in dim light.
d) Then the mixture was filtered by using a Whatman glass microfiber 47mm filter, sintered glass filter, and vacuum pump to remove the solids.
e) It was found that the freeze dryer was not functioning properly as it was not cold. Thus, the pigment and ethanol mixture is used as it was due to the irregularity of the freeze dryer.

3.3.2 Characterization of Pigments

a) Characterization of extracted pigments was performed using Fourier transform infrared spectrometer (FTIR).
b) Thermogravimetric analysis (TGA) was performed for both turmeric and pandan samples. The sample amount was about 3 mg, heating rate of 10°C/min, temperature range from room temperature to 300°C, and the gases used were nitrogen to test for heat stability and oxygen to test for oxidation stability. From TGA, the thermal degradation temperature was known.

3.3.3 Analysis of Pigments

- **Trial 1**

a) Samples were prepared according to Table 1:

TABLE 2: Variables for Trial 1

Run	Acronyms[a]	Variables
1	TC Trial 1	0.1 g turmeric pigment in 100 ml ethanol
2	T+2%AA	0.1 g turmeric pigment in 98 ml ethanol + 2 ml ascorbic acid
3	T+4%AA	0.1 g turmeric pigment in 96 ml ethanol + 4 ml ascorbic acid
4	T+6%AA	0.1 g turmeric pigment in 94 ml ethanol + 6 ml ascorbic acid
5	PC	3 ml pandan leaves pigment in 100 ml distilled water
6	P+2%LW	3 ml pandan leaves pigment in 98 ml distilled water + 2 ml lye water
7	P+4%LW	3 ml pandan leaves pigment in 96 ml distilled water + 4 ml lye water
8	P+6%LW	3 ml pandan leaves pigment in 94 ml distilled water + 6 ml lye water

[a]*TC* = Turmeric Control; *T+0.01AA* = Turmeric Powder and 0.01g Ascorbic Acid; *T+0.05AA* = Turmeric Powder and 0.05g Ascorbic Acid; *T+0.1AA* = Turmeric Powder and 0.1g Ascorbic Acid; *PC* = Pandan Control; *P+2%LW* = Pandan and 2% Lye Water; *P+4%LW* = Pandan and 4% Lye Water; *P+6%LW* = Pandan and 6% Lye Water.

b) After preparing the samples, some sample were taken for FTIR analysis.
c) The samples were then analyzed with thermogravimetric analyzer at a heating rate of 5°C/min, 10°C/min, and 20°C/min.
d) For the rest of the remaining sample, they were placed in an oven with air circulation at 80°C and colour changes were monitored daily.
e) It was found that the turmeric samples in the Trial 1 (Run 1 to 4) produce inaccurate TG curves. This will be explained in Chapter 4. Run 5 to Run 8 can be used for color changes monitoring only.

- **Trial 2**

a) Focusing on turmeric for this second trial, TG analysis was done using the lab-produced turmeric powder and commercial turmeric powder widely available at any stores. In this project, Baba's brand turmeric powder was used.
b) Run 1 consisted only of commercial turmeric powder. Excess ethanol was not needed for Run 1 because the function of ethanol was to dilute ascorbic acid. Thus, these samples were prepared in a different manner according to Table 3.

TABLE 3: Variables for Trial 2

Run	Acronyms[b]	Variables
1	TC Trial 2	Commercial turmeric powder
2	T+0.01AA	10 g commercial turmeric powder + 0.01 g ascorbic acid + excess ethanol
3	T+0.05AA	10 g commercial turmeric powder + 0.05 g ascorbic acid + excess ethanol
4	T+0.1AA	10 g commercial turmeric powder + 0.1 g ascorbic acid + excess ethanol

[b]*TC* = Turmeric Control; *T+0.01AA* = Turmeric Powder and 0.01g Ascorbic Acid; *T+0.05AA* = Turmeric Powder and 0.05g Ascorbic Acid; *T+0.1AA* = Turmeric Powder and 0.1g Ascorbic Acid

FIGURE 4: Turmeric powder mixed with ascorbic acid

c) The solution for each run was mixed well using a spatula.

d) The excess ethanol is removed by a rotary evaporator.

e) The dried powder is kept at 8°C in a dark environment after preparation.

f) The samples were analyzed using FTIR and TGA. TGA analysis was performed at 10°C/min, 20°C/min, and 30°C/min from a temperature range of 30°C to 500°C.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Trial 1

4.1.1 Sample Preparation

During sample preparation, using 70°C to dry the samples, it was found that the turmeric samples were not fully dried because mould growth was observed. Hence, the temperature was increased to 80°C and drying period was extended to two days. After performing Soxhlet extraction, it was observed from Figure 5 that the pigments are extracted by looking at the colour of the solution.

FIGURE 5: Solution after Soxhlet extraction, using turmeric (left) and using pandan leaves (right)

It could be observed that a very dark greenish-black solution is obtained when pandan leaves are used and a dark orange solution is obtained from turmeric extraction. Initially, the solvent, ethanol, was removed by using rotary evaporator. Then, it was put into the freeze dryer to enable formation of solid pigments for thermogravimetric analysis. However, Figure 6 shows that the pigments did not solidify. Instead, a sticky substance was obtained.

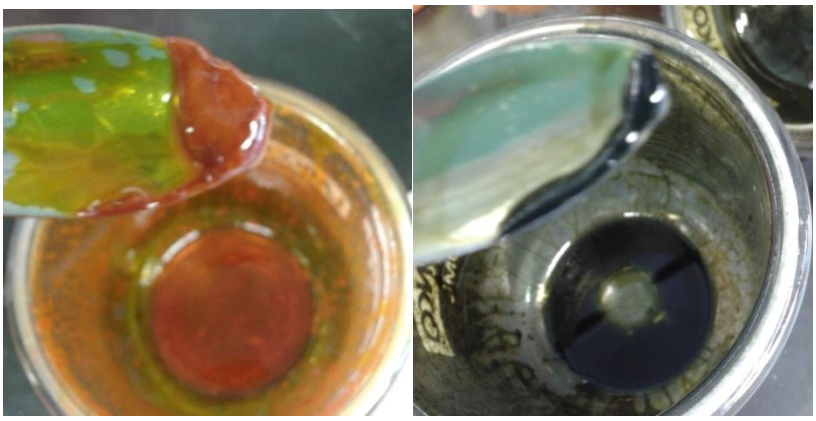


FIGURE 6: Sticky substance after freeze drying, using turmeric (left) and using pandan leaves (right)

Since solid pigments could not be obtained, the methodology was changed to use only freeze drying. Rotary evaporating was not used hereafter. Figure 7 shows the low yield of pigments.

FIGURE 7: Pigment obtained during second batch of extraction, using only freeze drying technique, using turmeric (left) and using pandan leaves (right)

According to Delgado-Vargas and Paredes-López (2003), turmeric rhizomes become hard, brittle, and of uniform yellow colour after drying. Solvents approved for the oil industry include hexane, heptanes, acetone, alcohol, and ethylene dichloride. However, acetone is reported to be the better solvent to extract an oleoresin of good quality due to the polarity of turmeric. The Soxhlet extraction yield is about 5%, containing 42% curcuminoids, in 4 to 5 hours. Curcuminoids are natural phenol compounds that produce the significant yellow colour. The sticky substance obtained is in accordance with the findings of Delgado-Vargas and Paredes-López (2003), which states that the final product is a highly viscous oil with 4.5 to 5% curcuminoids, a deep brownish-orange product, 30 to 40%

curcumin, and 15 to 20% volatile oil. The viscous product is often mixed with permitted diluents such as propylene glycol or polysorbate to obtain homogenous product for easier handling.

For chlorophyll, Schwartz and Lorenzo (1990) reported that a very slow rate of pheophytin formation is observed in freeze-dried spinach at water activity values less than 0.32. Water activity is defined as the ratio between vapour pressure of the food itself and the vapour pressure of distilled water under identical conditions (U.S. Food and Drug Administration, 2010). With regards to the dark greenish-black substance obtained, Hicks and Panisset (1934) found that it was impossible to obtain dry crystals of chlorophyll even after frequent purifications. A sticky, inconstant product was always produced. To overcome amorphous chlorophyll and its instability when dried, Germille et al. (1960) suggested precipitating chlorophyll with methanol-petroleum ether solution.

According to Schwartz and Lorenzo (1990), to remove chlorophyll from plant tissue, the leaves need to be grinded or blended with excess solvent. Then, the mixture is filtered or centrifuged to remove solids from the solvent. Filtration is preferred as not all solids are removed by centrifugation. The process of grinding and filtration should be carried out in dim light to prevent photobleaching of pigments. Thus, the pandan leaves are not suitable to be pre-dried since a very low yield of pigments is observed. Trial 2 uses this altered methodology.

4.1.2 TGA Analysis

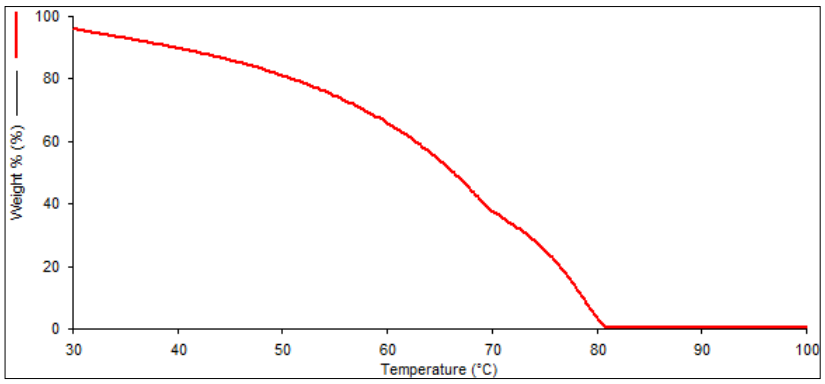


FIGURE 8: Graph of weight loss against temperature for liquid T+2%AA in nitrogen at 20°C/min

From Figure 8, it is observed that weight loss was very rapid and it reached to almost zero at 81°C. It then showed a constant weight loss for higher temperatures. Analysis

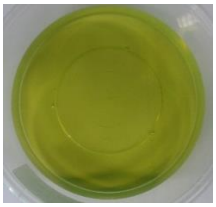
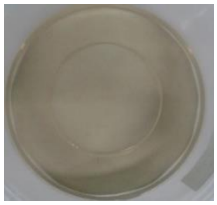
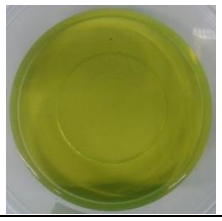
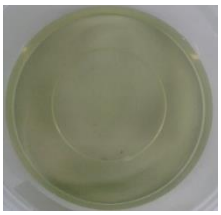
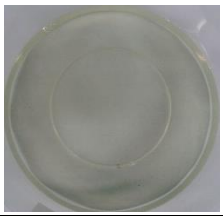
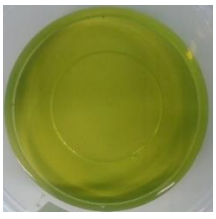
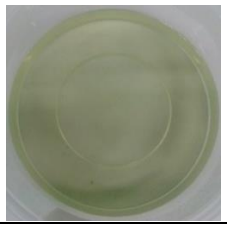
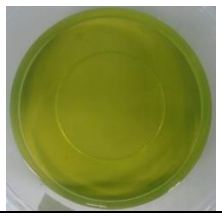
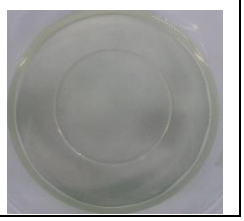
was stopped before the intended temperature of 300°C because it was of no purpose for further heating as all of the sample has already reacted. This occurred because in the sample, it mainly consisted of ethanol which has a boiling point of 78.37°C. Ethanol will evaporate rapidly and hence, the degradation temperature could not be obtained. Gabbott (2008) states that this curve shape indicates volatile melt in which the liquid sample evaporates. All other samples in Trial 1 produced similar results. These data could not be used and Trial 2 commenced.

4.2 Trial 2

4.2.1 Color Change Analysis

Table 4 shows the changes in color of treated and untreated pandan solution over a 6-day period.

TABLE 4: Monitoring color change of pandan mixture

Name[c]	Day 1	Day 2	Day 3	Day 6
PC				
P+2%LW				
P+4%LW				
P+6%LW				

[c]*PC* = Pandan Control; *P+2%LW* = Pandan and 2% Lye Water; *P+4%LW* = Pandan and 4% Lye Water; *P+6%LW* = Pandan and 6% Lye Water.

On Day 2 of exposing the pandan solution to heat at 80°C, it was clearly seen that the color for all solutions faded to a lighter hue. However, Pandan Control solution turned brownish while the other three solutions which had added lye were comparatively greener than Pandan Control solution. On Day 3, the color for pandan solution with 2% lye shows slight browning. On Day 6, all solutions faded quite drastically but it was obvious that the Pandan Control solution showed browning. Hence, it can be said that lye water helps to retain some greenness from chlorophyll pigments.

4.2.2 TGA Analysis for Thermal Stability

Table 5 shows the results of thermogravimetric analysis for commercial turmeric powder and self-produced turmeric powder. Onset temperature is determined from the tangents of the weight loss curve by using auxiliary TG software.

TABLE 5: Onset temperature for untreated turmeric powder using nitrogen

Heating Rate (°C/min)	Onset Temperature (°C)	
	Commercial turmeric powder	Self-made turmeric powder
10	261.13	261.08
20	273.38	274.70
30	269.04	275.73

Since the onset temperature for commercial and self-made turmeric powder was similar, commercial turmeric powder was used hereafter as it is widely available. Table 6 shows the onset temperature for turmeric powder that had been mixed with ascorbic acid as stabilizer.

TABLE 6: Onset temperature for treated turmeric powder using nitrogen

Heating Rate (°C/min)	Onset Temperature (°C)		
	T+0.01AA[d]	T+0.05AA[d]	T+0.1AA[d]
10	254.05	262.79	261.67
20	270.66	268.25	266.05
30	271.95	270.86	275.99

[d] *T+0.01AA* = Turmeric Powder and 0.01g Ascorbic Acid; *T+0.05AA* = Turmeric Powder and 0.05g Ascorbic Acid; *T+0.1AA* = Turmeric Powder and 0.1g Ascorbic Acid

Figure 9, 10, and 11 show the plot of weight loss against temperature for all heating rates.

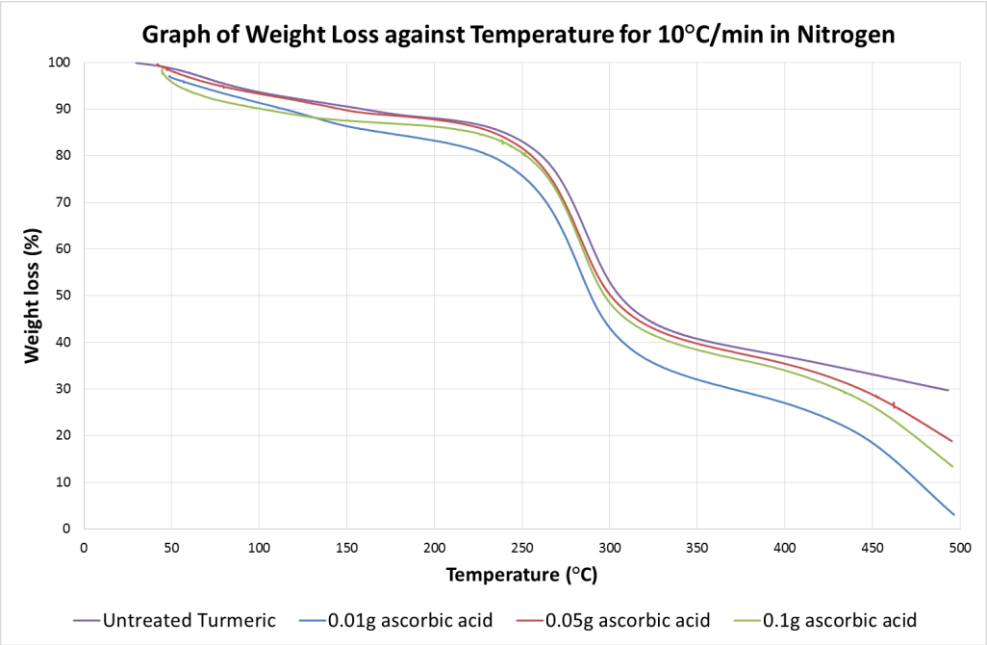


FIGURE 9: Thermogravimetric curve obtained with 10°C/min heating rate using nitrogen

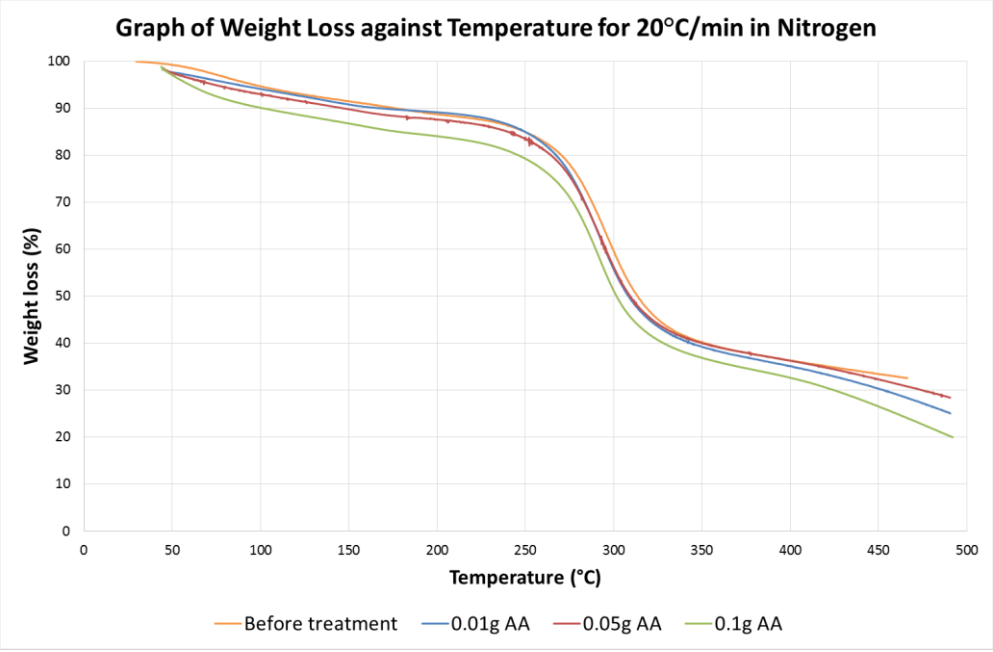


FIGURE 10: Thermogravimetric curve obtained with 20°C/min heating rate using nitrogen

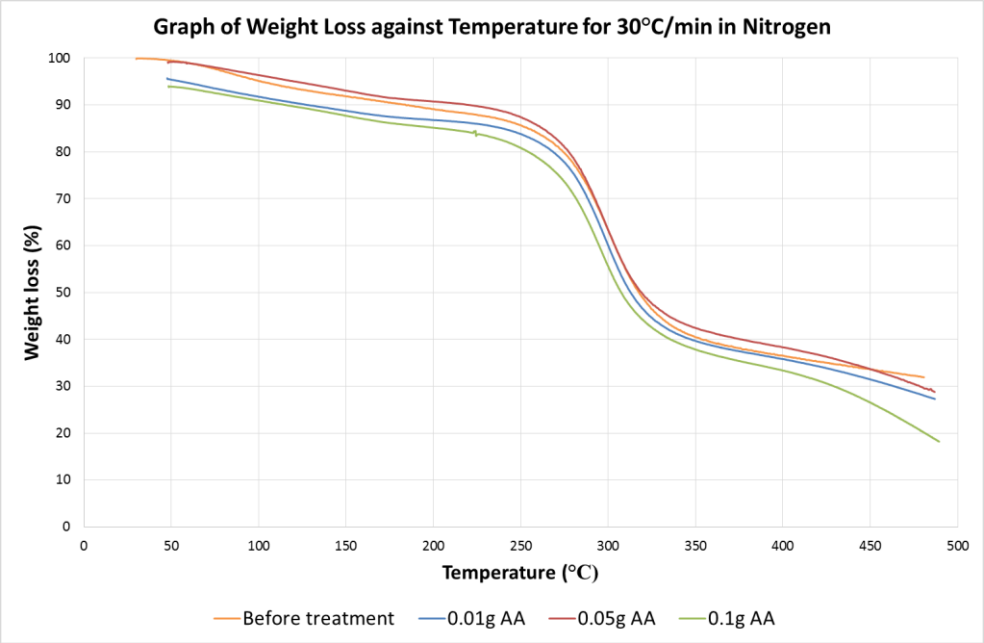


FIGURE 11: Thermogravimetric curve obtained with 30°C/min heating rate using nitrogen

As shown above, the degradation curves for untreated and treated turmeric powder are similar.

4.2.3 TGA Analysis for Oxidation Stability

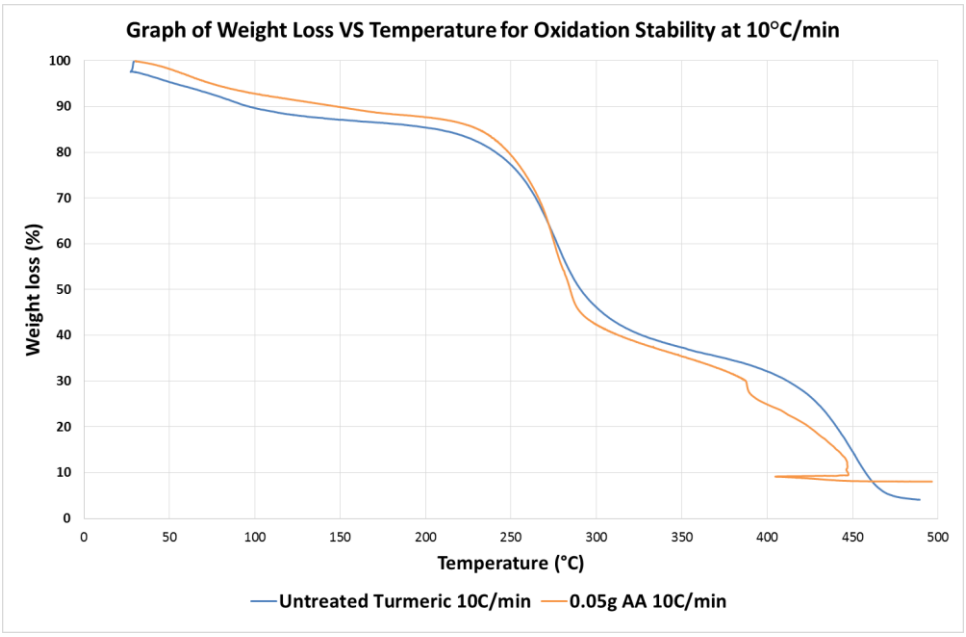


FIGURE 12: Thermogravimetric curve obtained with 10°C/min heating rate using oxygen

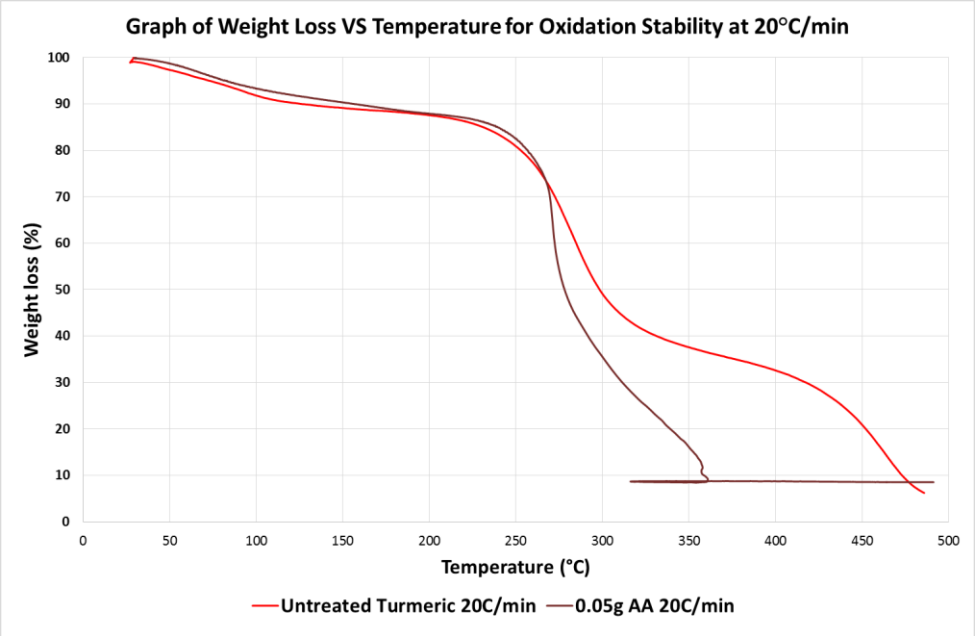


FIGURE 13: Thermogravimetric curve obtained with 20°C/min heating rate using oxygen

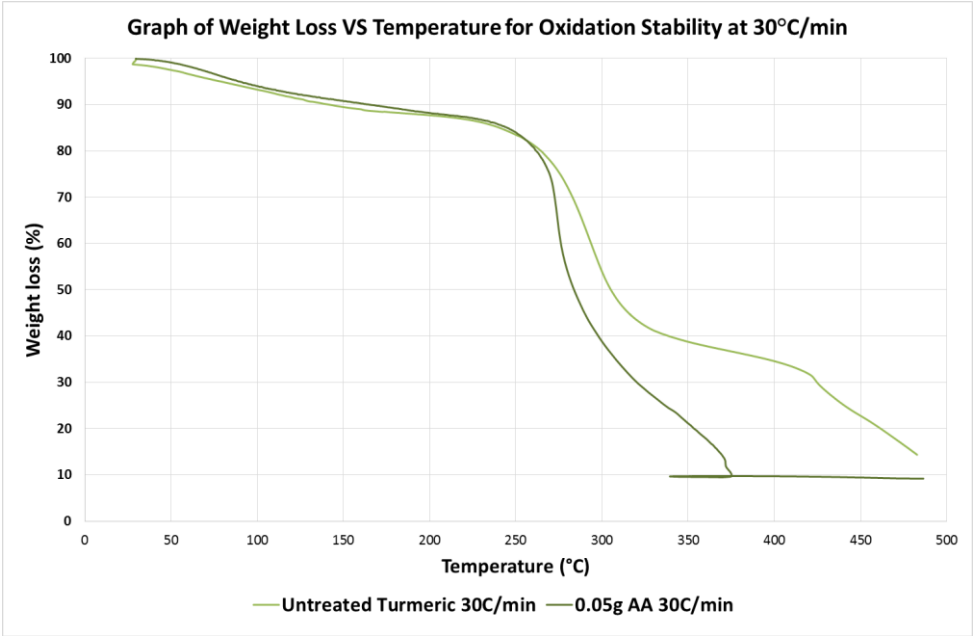


FIGURE 14: Thermogravimetric curve obtained with 30°C/min heating rate using oxygen

From Figure 12 to Figure 14, it is obvious that there is constant weight loss at certain temperatures when ascorbic acid is added. This indicates that there is an increase of weight. Weight increase can result from either chemical reactions with the purge gas or physical transitions such as adsorption of gaseous substances on samples. It is

reported that the major degradation products of curcumin decomposition are vanillin, ferulic acid, and feruloyl methane (Wang et al., 1997). This may contribute to the weight increase.

4.2.4 Calculation of Activation Energy

The rate equation commonly used in kinetic studies is generally described as:

$$\frac{d\propto}{dt} = kf(\propto) \tag{1}$$

where k is the rate constant, f(α) is the function depending on the actual reaction mechanism, and α is the conversion rate which is defined as:

$$\propto = \frac{w_0 - w_t}{w_0 - w_\infty} \tag{2}$$

where w_0, w_t, and w_∞ are the initial weight, weight at time t during thermal analysis, and final weight of samples, respectively. The rate constant, k, is generally given by Arrhenius equation,

$$k = A \exp\left(\frac{-E_a}{RT}\right) \tag{3}$$

where E_a is the apparent activation energy (kJ/mol), R is the gas constant (8.314J/K.mol), A is the pre-exponential factor (min-1), and T is the absolute temperature (K). When Equation (1) and Equation (3) are combined:

$$\frac{d\propto}{dt} = A \exp\left(\frac{-E_a}{RT}\right) f(\propto) \tag{4}$$

For dynamic TGA process, the heating rate β=dT/dt, is introduced to Equation (4) to obtain the following Equation (5).

$$\frac{d\propto}{dT} = \left(\frac{A}{\beta}\right) \exp\left(\frac{-E_a}{RT}\right) f(\propto) \tag{5}$$

Equation (4) and (5) are the basic expression of analytical methods used to calculate kinetic parameters using TGA data. For this project, two methods are being used to determine the activation energy, which are Flynn-Wall-Ozawa method and Coats-Redfern (modified) method (Liu et al., 2013). Table 7 shows an overview of these two methods.

TABLE 7: Flynn-Wall-Ozawa and Coats-Redfern (modified) method

Method	Expression	Eq.	Plot
Flynn-Wall-Ozawa (FWO)	$\log\beta = \log\left(\frac{AE_a}{RG(\alpha)}\right) - 2.315 - 0.4567\frac{E_a}{RT}$	(6)[c]	logβ against 1/T
Coats-Redfern (modified) CR	$\ln\left(\frac{\beta}{T^2\left(1-\frac{2RT}{E_a}\right)}\right) = \ln\left(\frac{-AR}{E_a \ln(1-\propto)}\right) - \frac{E_a}{RT}$	(7)	$\ln(\beta/T^2)$ against 1/T

[c] *G(α)* in Equation (6) is the integral function of conversion.

The activation energy was calculated by plotting Equation (6) and (7) at 10°C/min, 20°C/min, and 30°C/min heating rates for both untreated and treated turmeric samples. For each heating rate, five conversion rates which are 10%, 20%, 30%, 40%, and 50% are used. The values of activation energies are calculated from the slope of the graph. Figure 15 and 16 show the example plot using untreated turmeric powder.

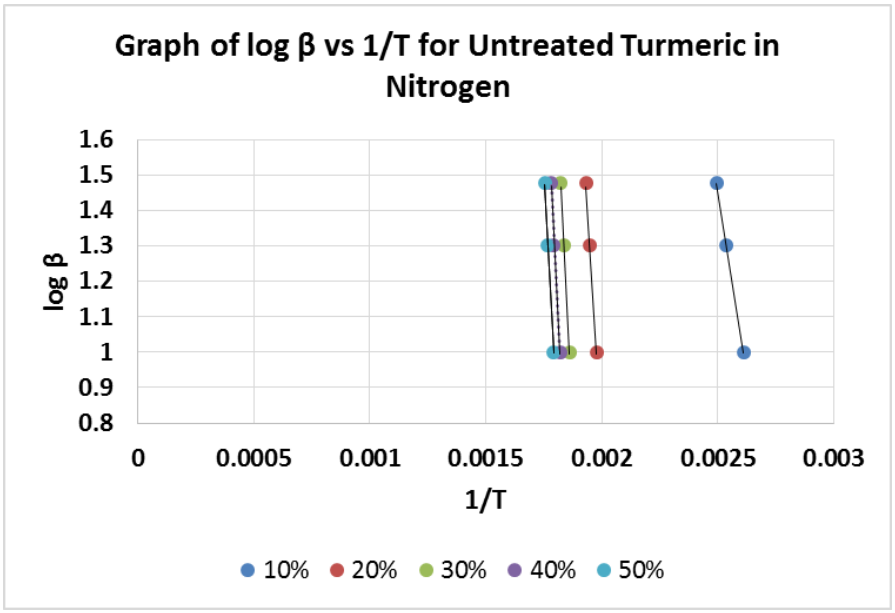


FIGURE 15: Plot to determine activation energy using Flynn-Wall-Ozawa method

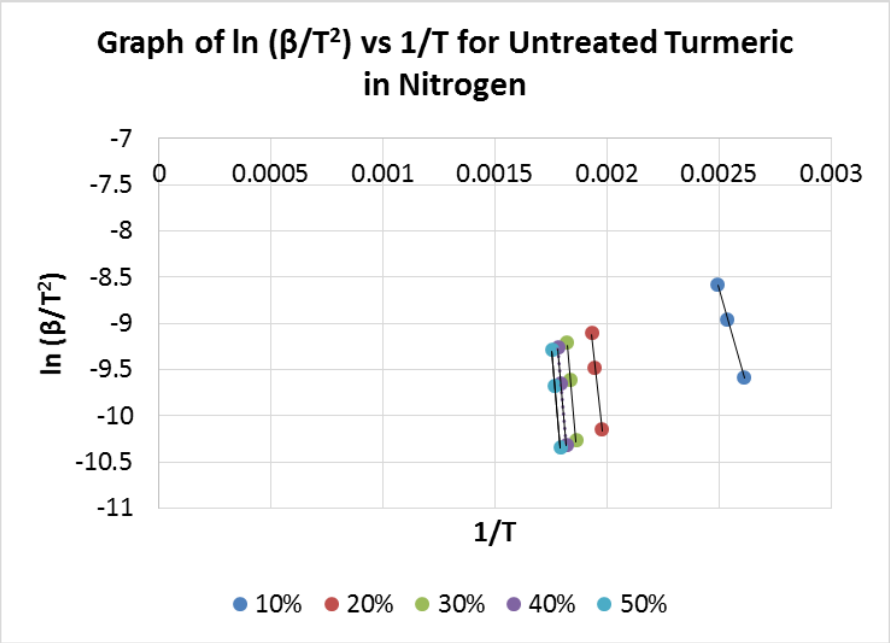


FIGURE 16: Plot to determine activation energy using Coats-Redfern method

Table 8 shows the activation energies which were calculated from the plot's slope.

TABLE 8: Activation energies using two different methods

Thermal Stability			
Sample	Conversion Rate (%)	E_a (kJ/mol)	
		Flynn-Wall-Ozawa method	Coats-Redfern (modified) method
Untreated Turmeric Powder	10	73.89	71.20
	20	192.39	193.82
	30	216.94	219.16
	40	223.82	226.15
	50	230.47	232.98
Average		187.50	188.65
Turmeric and 0.01g Ascorbic Acid	10	69.77	66.44
	20	109.31	106.26
	30	177.93	178.04
	40	228.10	230.61
	50	298.83	304.82
Average		176.79	177.23
Turmeric and 0.05g Ascorbic Acid	10	23.93	18.24
	20	91.62	87.60
	30	163.37	162.70
	40	194.44	195.20
	50	210.39	211.82
Average		136.75	135.12
Turmeric and 0.1g Ascorbic Acid	10	11.08	9.97
	20	76.24	69.41

	30	108.52	105.04
	40	196.99	197.86
	50	219.47	221.35
Average		122.46	120.73
Oxidation Stability			
Sample	Conversion Rate (%)	E_a (kJ/mol)	
		Flynn-Wall-Ozawa method	Coats-Redfern (modified) method
Untreated Turmeric Powder	10	77.32	74.86
	20	156.82	156.28
	30	183.36	183.80
	40	216.31	218.23
	50	311.08	317.74
Average		188.98	190.18
Turmeric and 0.05g Ascorbic Acid	10	105.73	104.32
	20	175.33	175.67
	30	305.58	312.37
	40	508.36	525.50
	50	509.91	565.77
Average		320.98	336.73

Based on Table 8, untreated turmeric powder which is the commercial turmeric powder has higher activation energy compared to a combination of turmeric powder and ascorbic acid when tested in nitrogen. Activation energy is the minimum kinetic energy that reactants must have in order to form products. An increase in activation energy means an increase in the powder's stability. Hence, the thermal stability can be arranged in decreasing order: Commercial turmeric powder > Turmeric and 0.01g ascorbic acid > Turmeric and 0.05g ascorbic acid > Turmeric and 0.1g ascorbic acid.

According to Juhász et al. (2012), ascorbic acid starts decomposing at 191°C and 188°C in nitrogen and oxygen respectively. Thus, the ascorbic acid used in this experiment may have degraded since the temperature used was from 30°C to 500°C. Ascorbic acid degradation is reported to occur in three stages (Jingyan et al., 2013). In the first stage decarboxylation and dehydration were the main decomposition reactions, and in the second stage decarboxylation and decarbonylation were the main decomposition. At the third stage, only a slow carbonization process happened. Furthermore, Delgado-Vargas and Paredes-López (2003) states that turmeric pigment is stable for up to 12 hours at high ambient temperatures which are 25°C to 32°C. When using rotary evaporator to remove excess solvent, the temperature of 60°C used may have degraded the pigment. Previously, freeze drying have been tried and it

produced sticky pigments in small amounts. Moreover, according to Zebib et al. (2010), curcumin has poor light stability and decomposes when exposed to sunlight. While transferring samples for analysis, the samples are wrapped in black plastic bags. However, some light may penetrate through. This may cause inaccurate readings. It is recommended that curcumin should be stored in amber-colored bottle to prevent photo oxidation.

For oxidation stability, the combination of turmeric powder and 0.05g ascorbic acid is more stable than untreated turmeric as shown from the activation energies calculated. The untreated turmeric powder has slightly higher activation energy in oxygen when compared to when exposed to nitrogen. This is different from the hypothesis that turmeric is more stable in nitrogen conditions compared to oxygen conditions. This inconsistency may be attributed to different thermogravimetric equipment used for analysis due to equipment limitations.

4.2.5 Fourier Transform Infrared Spectroscopy (FTIR) Analysis

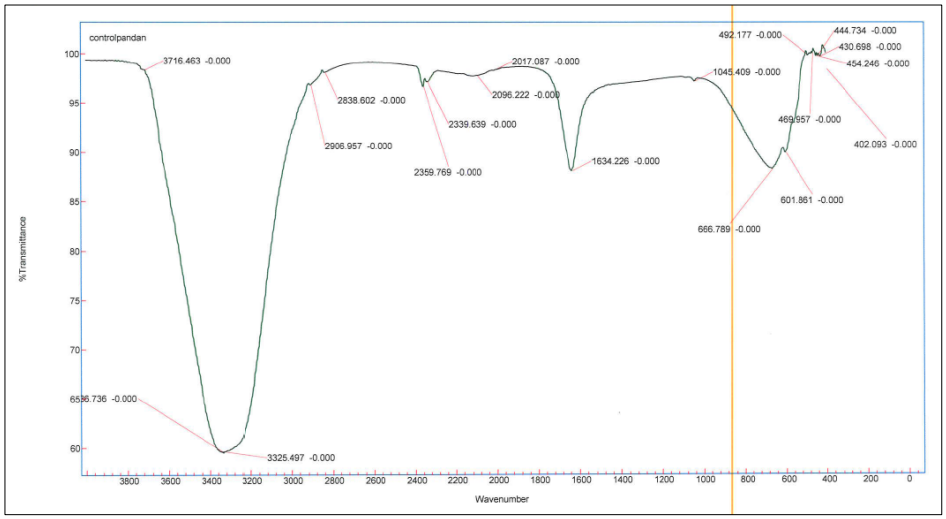


FIGURE 17: FTIR spectrum for untreated pandan solution

Figure 17 shows the FTIR spectrum for untreated pandan solution, which is chlorophyll and water solution. It can be seen from Figure 17 that the stretch at 2339.639 cm^{-1} and 666.789 cm^{-1} shows an obvious characteristic of carbon dioxide (Gerakines et al., 1995). This shows that the solution is already degrading and emitted carbon dioxide. The broad peak at 3325.497cm^{-1} is attributed to the N-H functional group. It is deduced that this is an amine group present in the new symmetrical-

structure alkaloid compound named *Pandamine* with a molecular formula of $C_{18}H_{23}NO_4$ (Abdullah et al. 2012). This position and shape of band also indicates the presence of O-H group which is due to water in the solution. A medium peak is noted at 1634.226cm^{-1} which indicates C=O functional group. Figure 18 shows the chemical structure of *Pandamine*.

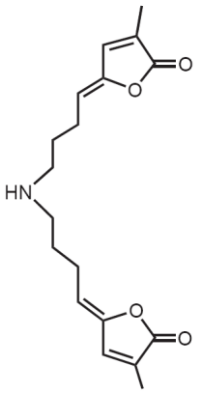


FIGURE 18: Chemical structure of *Pandamine (Abdullah et al. 2012)*

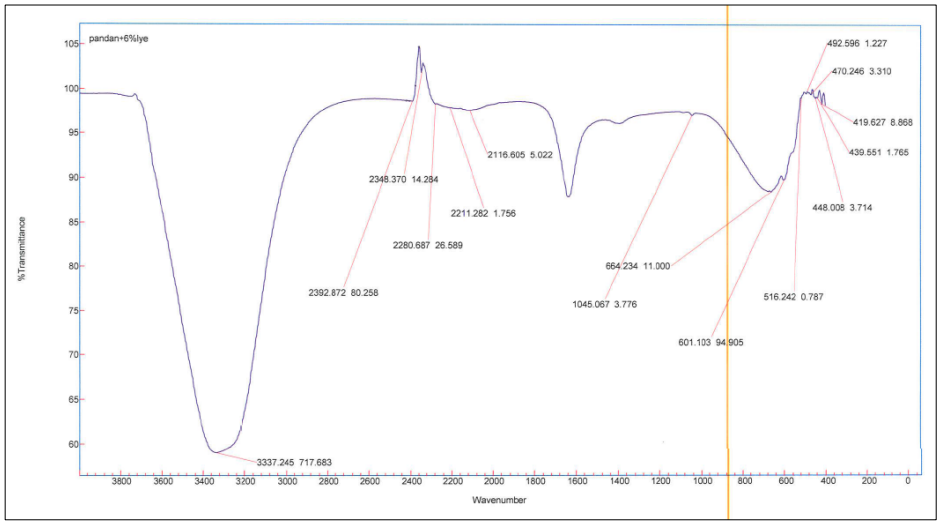


FIGURE 19: FTIR spectrum for mixture of pandan and 6% lye

Figure 19 shows the FTIR spectrum for 6% lye solution. It has similar shape with the untreated pandan solution spectrum. The only notable difference is the presence of a weak peak at 2348.370cm^{-1} which has more than 100% transmittance. This error may be due to noise.

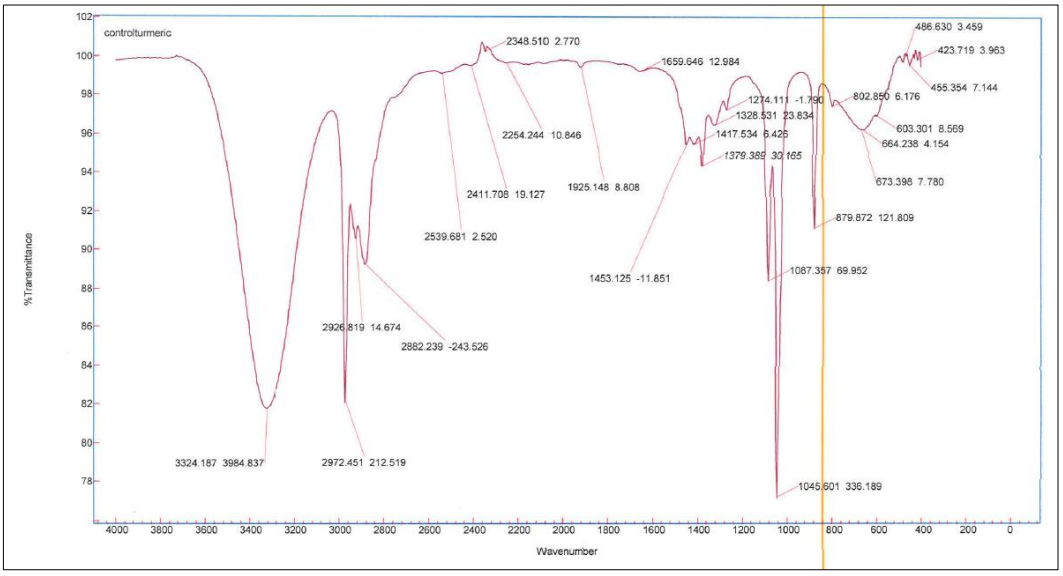


FIGURE 20: FTIR spectrum for untreated turmeric

Figure 20 shows the FTIR spectrum for untreated turmeric. A broad peak at 3324.187cm^{-1} indicates the presence of O-H stretch. A sharp peak at 2972.451 cm^{-1} and a medium peak at 2882.239 cm^{-1} show that C-H stretch is present and an alkane group may be present. A sharp peak at 1045.601 cm^{-1} indicates possibility of C-O stretch which indicates enol group.

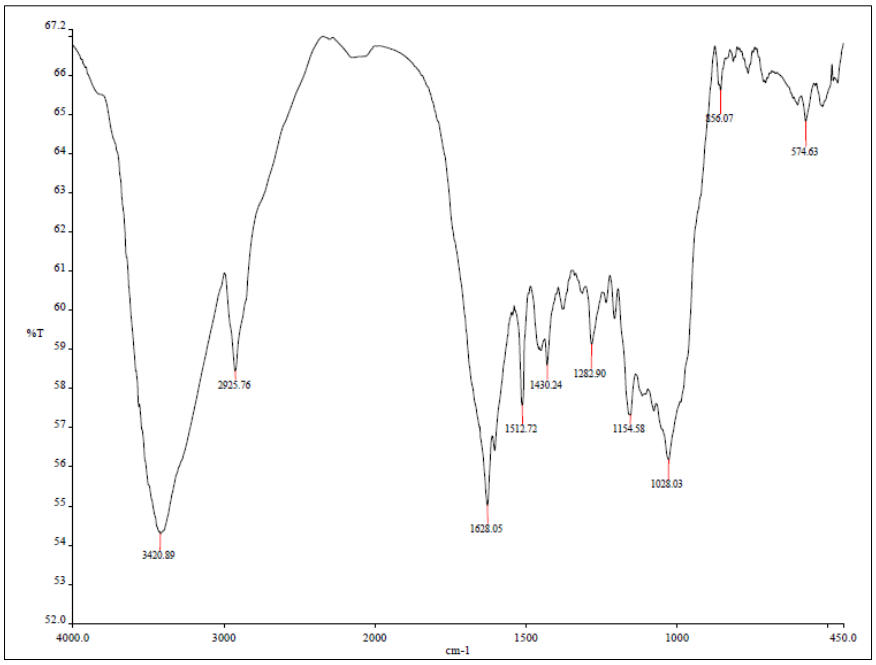


FIGURE 21: FTIR spectrum for mixture of turmeric and 0.01g ascorbic acid

Figure 21 shows the FTIR spectrum for the mixture of turmeric powder and 0.01g ascorbic acid. A broad peak at 3420.89 cm^{-1} and a medium peak at 2925.76 cm^{-1}

indicate the presence of O-H stretch. A sharp peak at 1628.05 cm^{-1} shows a possibility of C=O stretch which indicates carboxylic acid. Another peak at 1028.03 cm^{-1} indicates C-O stretch. A weak peak at 574.63 cm^{-1} indicates presence of alkyl halide group.

CHAPTER 5

CONCLUSION

Lye water is able to reduce chlorophyll degradation at 80°C. Hence, it can be for food processing at similar conditions. The thermal stability of turmeric is arranged as follows in descending order. Commercial turmeric powder > Turmeric and 0.01g ascorbic acid > Turmeric and 0.05g ascorbic acid > Turmeric and 0.1g ascorbic acid. It is shown that ascorbic acid has degraded at high temperatures, causing it to be unsuitable to be used in high temperature food processing. Under oxygen conditions, the addition of 0.05g ascorbic acid is proven to be more stable than untreated turmeric. This shows that ascorbic acid is an effective oxidation stabilizer and can be used in similar food processing conditions.

To have a better understanding in thermal and oxidation stability of turmeric at various temperatures, further research using purified curcumin at lower temperatures is recommended. Moreover, it is recommended to include pH value as a parameter so that the actual value of acidity or alkalinity is known. Usually, the study of pigments involves utilization of Hunterlab colorimeter or spectrophotometer. It works on the basis of three variables, which are *L* which represents lightness (100 for white and 0 for black), *a* which represents greenness and redness (-80 for green and 100 for red), and *b* which signifies blueness and yellowness (-80 for blue and 70 for yellow). This equipment would give us more accurate colour analysis. Besides that, it is recommended to have more analytical units in our university because after the sample is ready, we have to wait for a period of time before the sample can be analyzed due to long booking queues. This will affect the accuracy of the results obtained, particularly for sensitive pigments. During Soxhlet extraction, a stainless steel container for water bath was provided by the lab technician. It is recommended to use round-bottom flask heaters so that water does not need to be added at intervals. This is because addition of water reduces temperature of water bath. Inefficient heating also occurs as the container absorbs heat.

To sum up, this project contributes to deeper understanding of bio-pigments, particularly turmeric and pandan leaf pigments. Pandan and turmeric pigments were extracted and oxidation stability was studied with and without the presence of

stabilizer. For heat stability, only turmeric is tested due to time and equipment limitations. Hence, the objectives are mostly achieved. Natural colorants are gaining importance as consumers emphasize on health these days. Research in this area is critical as improving the stability of these natural pigments will surely promote their usage in food and beverage industries.

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