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Diploma Programme subject in which this extended essay is registered: CHEMISTRY

(For an extended essay in the area of languages, state the language and whether it is group 1 or group 2.)

Title of the extended essay: INVESTIGATION ON THE EFFECT OF SPECIFIED DRYING TECHNIQUES IN THE COLOR STABILITY OF CAPSICUM ANNUUM

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The extended essay I am submitting is my own work (apart from guidance allowed by the International Baccalaureate).

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_____ has been highly motivated and independent in working with her extended essay topic. She has shown interest in food chemistry option and decided to do an EE on it. Her interest led her to investigate on the effect of specified drying techniques in the color stability of capsicum anuum.

At the beginning of the research process, _____ had to made lots of reading from different sources. With the effort she exerted researching and her critical mind she was able to come up of a very clear and specific research question. She did her preliminary studies independently. She is very responsible and she can work with less supervision.

_____ persevered to answer her research question. She was able to gather data from her experiment, processed and analyzed it meticulously. _____ did not only focus on the results of the experiments, but she also evaluated how did the experiment go and how can it still be improved.

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Date: 5 March 2015

Extended Essay

Subject : Chemistry

Title : Investigation on the effect of specified drying techniques to the color stability of *Capsicum annuum*

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Abstract

Chili color degradation has been a challenging problem for any food industry to solve and chemists are always in the quest to look out for efficient or better methods which can control this. Processing and storing most food or spices like *Capsicum annum* may reduce its quality and shelf life that kitchen purposes or factory manufacturers would like to minimize. In this essay, investigation was carried out to analyze which drying method would give the best color stability of *Capsicum annum*'s pigment extract through the research question: **How do different drying methods affect the color stability of *Capsicum annum*?**

This experiment was conducted by drying chili purees using traditional drying methods such as oven drying, sun-drying, microwave drying and natural convective drying to obtain chili powders. The chili powders were dissolved with propanone to obtain pigment extracts. These pigment extracts were tested for absorbance value in 4 days of storage and ASTA values were calculated.

It is found that the pigment extracts gave different color intensities according to the different drying methods. (In the latter, all pigment extracts did show degradation of absorbance values each day it being stored) Again, the degradation rate and percentage differ according to the different drying methods. The result showed that natural convective-drying to be the most effective drying method with highest color intensity and highest color stability. While, the most common and fastest way of drying, oven-drying, turned out to be the least effective in retaining color, although it did give relatively rich color at first.

(254 words)

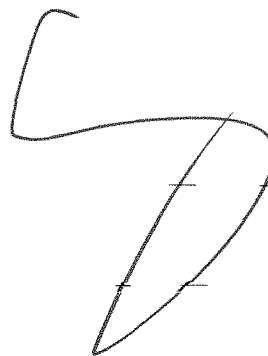
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1. Introduction:

Capsicum annum (*C. annum*) or commonly known as “chili” can be said to be one of the prime condiments in Indonesian’s dishes. There are different kinds of chili in which most give similar piquant and spicy taste that suit Indonesians’ taste buds and this reasons to the widely chili consumption in Indonesia.

Fresh chili is usually processed to give a longer shelf life for daily use as complementary condiment. Chili in Indonesia is usually served in different forms such as sauce/puree (known as “sambal”), dry chili or chili powder and even the fresh chili itself. More than home condiment purposes, *Capsicum annum* is also widely being used in the culinary and food industry for food colorings due to its high color capacity¹.

According to data from the Agricultural Ministry, Indonesia produced 855,000 tons of chilies last year to meet domestic consumption of 799,000 tons². The increasing number of demand simultaneously happen with the increment of chili prices due to economic condition of Indonesia currently³. In response to this phenomenon, former Indonesian president, Susilo Bambang Yudhoyono has suggested consumers to plant their own chilies.⁴ This may lead to more consumers, on their own, process their home grown chilies.

¹ Topuz, A., Feng, H., & Kushad, M. (2009). *The Effect of Drying Method and Storage on Color Characteristics of Paprika*. Retrieved June 30, 2014, from <http://www.journals.elsevier.com/lwt-food-science-and-technology>

² Simanjutak, T. (2014, November 16). Indonesia’s sambal love affair some like it hot. *The Jakarta Post*. Retrieved January 2, 2015, from Indonesia’s sambal love affair some like it hot

³ Afriyadi, A. (2015, January 9). Harga Cabai Makin Mahal, Ini Kata Menteri Pertanian. *Liputan6*. Retrieved from <http://bisnis.liputan6.com/read/2158552/harga-cabai-makin-mahal-ini-kata-menteri-pertanian>

⁴ Indonesians Fired up by Soaring Chili Prices. (2011, February 10). *The Jakarta Post*. Retrieved January 2, 2015.

Due to this reason, I aim to investigate which drying method of chili would be the best in retaining color, which also relates to its quality. Drying, which is one of the ways to process chili, is known as a common practice from decades ago. In storing and drying, chilies undergo reactions that degrade its quality, such as photo-oxidation causing the loss of antioxidant property which needs to be prevented by processing it with the best method. Thus, this essay will investigate on ***“How do different drying techniques affect the color stability of *Capsicum annuum*?”***

which ones?

I believe this research would be beneficial in the future not only for the food industry, but especially consumers who wish to process their own food.

2. Background Information:

2.1 *Capsicum annuum*

Capsicum annuum (Figure 2.1.1 *Capsicum annuum*⁵) is a species that consists of world's most widely used spices. It comes with extensive variety of shapes and sizes also

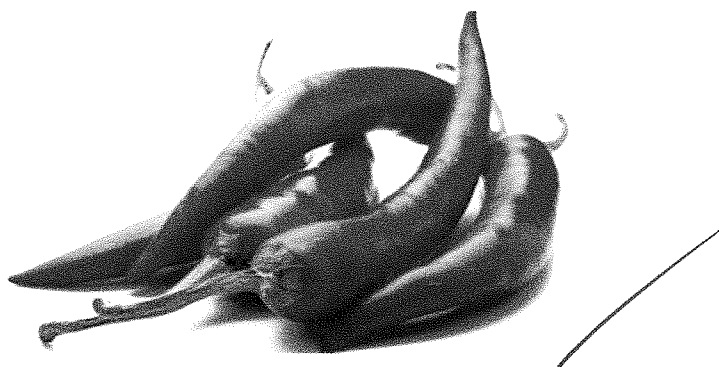


Figure 2.1.1 - *Capsicum annuum*

⁵ Griffith, P. (n.d.). Canner's Corner: Enjoying Summer's Bounty. Retrieved from http://www.wyomingextension.org/agpubs/pubs/MP119_5.pdf

taste both mild and hot ranging from vegetables such as chili, bell pepper, cayenne, jalapenos and many more⁶. The popular use of *C. annuum* throughout the world is due to its ease of cultivation, sharp taste and attractive appearance⁷.

C. annuum shows the presence of color pigment Carotenoids. It transmits the colors: yellow-orang-red⁸ as could be seen in the chilies used for this experiment. More than just giving attractive appearances, this pigment also serves some health benefits. It is an abundant supplier of provitamin A and also very well known to contain antioxidant that is highly required by the body⁹.

2.2 Carotenoids as Antioxidant

Antioxidant is a substance that delays or inhibits the process of oxidation¹⁰. Carotenoid contains antioxidant due to the arrangement of its structure in which most can be derived from a 40-carbon basal structure that includes a system of conjugated double bonds. The central chain may carry cyclic end-groups which can be substituted with oxygen-containing functional groups (see figure 2.2.1 overleaf). The double bonds in the

⁶ Sage, G., & Sidnam, B. (2006, May 21). Colors and shapes of peppers are varied. *U~T San Diego*.

⁷ Davis, S. (n.d.). *Capsicum annuum* (chilli pepper) (M. Vorontsova, Ed.). Retrieved July 8, 2014, from <http://www.kew.org/science-conservation/plants-fungi/capsicum-annuum-chilli-pepper>

⁸ Rocío Gómez-García, M., & Ochoa-Alejo, N. (2013). *Biochemistry and Molecular Biology of Carotenoid Biosynthesis in Chili Peppers (Capsicum Spp.)*. Retrieved September 27, 2014, from <http://www.mdpi.com/journal/ijms>

⁹ Higdon, J. (n.d.). Carotenoids. Retrieved July 11, 2014, from <http://pi.oregonstate.edu/infocenter/phytochemicals/carotenoids/>

¹⁰ Owen, S. (2011). Option F. In *Chemistry for the IB Diploma* (1st ed., p. 12). Cambridge University Press.

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polyene backbone also accounts for the important light absorbing and antioxidant properties of Carotenoids¹¹.

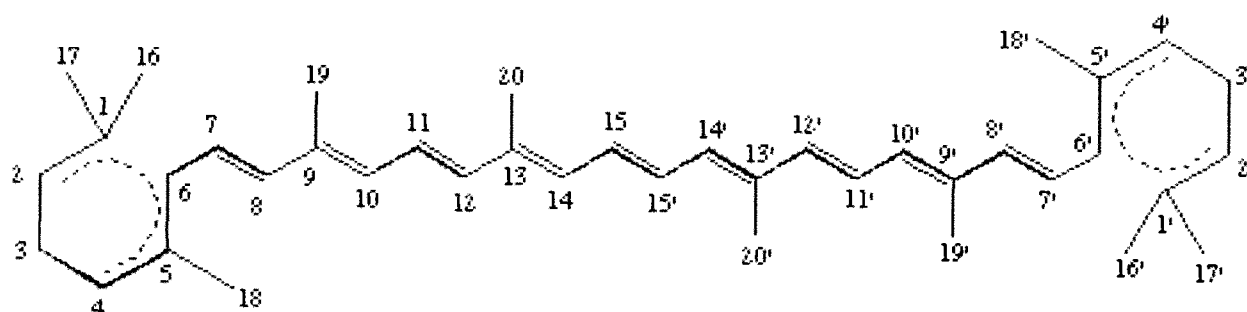


Figure 2.2.1 – basic structure of Carotenoid group¹²

Antioxidant works as quenchers or scavenger of free radicals during the initiation step of oxidation. It reacts with free radicals so that they are not available to react with lipid molecules, oxygen or other free radicals¹³ (Equation 2.2.1 and Equation 2.2.2).



*AH is the antioxidant molecule

2.3 Color Stability

Color stability is defined as the ability of a light source to maintain its color properties over time¹⁴. Shelf life of food is defined as the period of time where the food is

¹¹ Stahl, W., & Sies, H. (2003). *Antioxidant Activity of Carotenoids*. Retrieved July 8, 2014, from <http://www.journals.elsevier.com/molecular-aspects-of-medicine>

¹² Carotenoid Rules 1 to 7. (n.d.). Retrieved July 9, 2014, from <http://www.chem.qmul.ac.uk/iupac/carot/car117.html>

¹³ Owen, S. (2011). Option F. In *Chemistry for the IB Diploma* (1st ed., p. 27). Cambridge University Press.

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still able to retain qualities of its flavor, taste, or appearance¹⁵. Thus, color stability of the pigments will also determine the shelf life of the food. The rate at which discoloration happens until its quality becomes poor may be affected by several factors such as moisture content, chemical changes, light, temperature and contact with air¹⁶.

- **Moisture Content**

The moisture content is the quantity of water contained in a substance. Moisture content in food will promote hydrolysis where the ester bonds in triglyceride is split into glycerol and the original fatty acids. The fatty acids being released will cause the food to smell unpleasant or turns rancid. The other reaction that is affected by the change in water content is the increment of non-enzymatic and enzymatic browning of food which literally causes food to turn brown. Lastly, abundance of water also increases the ability of bacteria to live and multiply, thus promoting bacterial spoilage.

- **Light**

Light can cause food to go rancid due to the process of photo-oxidation. High light intensity will also damage the chemical compounds of the nutrients and vitamins it contains, making it less useful. Besides these two, light is also responsible for the bleaching of natural dyes causing discoloration.

¹⁴ Light Sources and Color. (n.d.). Retrieved July 8, 2014, from <http://www.lrc.rpi.edu/programs/nlpip/lightinganswers/lightsources/whatisColorStability.asp>

¹⁵ Owen, S. (2011). Option F. In *Chemistry for the IB Diploma* (1st ed., p. 8). Cambridge University Press.

¹⁶ Owen, S. (2011). Option F. In *Chemistry for the IB Diploma* (1st ed., p. 9). Cambridge University Press.

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- Temperature

Chemical reactions such as microbial growth, auto-oxidation, or hydrolysis are often accelerated at higher temperature.

- Contact with Air

The atmospheric oxygen in air will trigger the spontaneous reaction of auto-oxidation

All these factors are accountable for shortening the shelf life of food.

2.4 Auto-oxidation Mechanism

Oxidation often happens to the reactive C=C double bonds in unsaturated fats. Carotenoid pigments in *C. annuum* forms conjugated double bonds of carbon chain, thus making it highly susceptible to oxidation. When this C=C bond is broken and pigment is bleached, color degradation would be observed¹⁷. The spontaneous oxidation mechanism or auto-oxidation will happen over the time when storing pigment solutions due to contact with oxygenated air.

Auto-oxidation of carotenoid will yield products with epoxy, hydroxyl and carbonyl group (Figure 2.4.1)¹⁸. The effect to each particular carotenoid pigment, depending on its

¹⁷ Owen, S. (2011). Option F. In *Chemistry for the IB Diploma* (1st ed., p. 19). Cambridge University Press.

¹⁸ Haila, K. (1999). *Effects of Carotenoids and Carotenoid-Tocopherol Interaction on Lipid Oxidation In Vitro*. Retrieved October 11, 2014, from <https://helda.helsinki.fi/bitstream/handle/10138/20834/effectso.pdf?sequence=1>

functional group, may be either pro-oxidant or antioxidant. For example, oxidation of β -carotene to epoxides would not be expected to produce antioxidant property¹⁹. Thus, this reaction eliminates one of the benefits carotenoid serves.

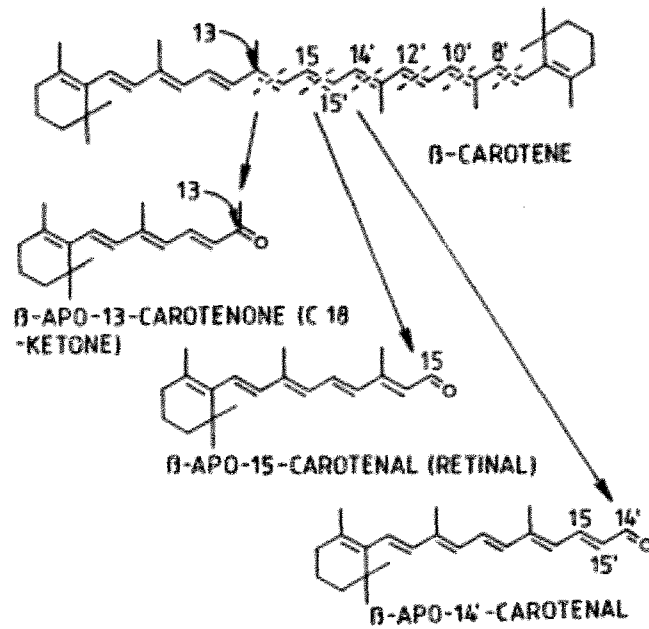


Figure 2.4.1 Auto-oxidant of beta Carotene

¹⁹ Haila, K. (1999). *Effects of Carotenoids and Carotenoid-Tocopherol Interaction on Lipid Oxidation In Vitro*. Retrieved October 11, 2014, from <https://helda.helsinki.fi/bitstream/handle/10138/20834/effectso.pdf?sequence=1>

2.5 Drying Techniques

Drying techniques are used as a mean to extend the shelf life of food by reducing its water content. However, different drying methods also promote other reactions such as:

Drying Technique	Factors Affecting Color Stability	Reactions
Sun-drying	Contact with air, light intensity, high temperature	Auto-oxidation, photo-oxidation
Oven-drying	High temperature, contact with air	Auto-oxidation
Microwave- drying	High temperature, contact with air	Auto-oxidation
Natural Convective Drying	Contact with air	Auto-oxidation

Table 2.5.1: Different factors and reactions involved in different drying techniques

2.6 Modified ASTA 20.1 Method

The whole experiment that I conducted was based on a modified ASTA 20.1 method. ASTA 20.1 is a standardized technique to measure the extractable color of spices determined by American Spice Trade Association. This method involves extracting the pigment from the spice by drying. Extractable color is measured using a spectrophotometer and presented using ASTA value. The higher the ASTA value, the greater is the effect to the richness of the pigment solution's color²⁰.

²⁰Wetzel, D., & Charalambous, G. (Eds.). (1998). *Instrumental Methods in Food and Beverage Analysis* (p. 352).

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The modified ASTA 20.1 method is adapted from a journal titled: "The Effect of Drying Method and Storage on Color Characteristics of Paprika" (Topuz, A., Feng, H., & Kushad, M., (2009)). The whole procedure would still remain the same as the original method, however data processing is going to be different.

3. Experimental Procedures:

This section elaborates on how the investigation was carried out. A more detailed procedure can be seen in Appendix B along with all the materials and reagents needed.

3.1 Assumptions and Limitations of the Study

In this experiment, the chilies used were assumed to represent the chilies in Indonesia that were cultivated in the same condition.

This study will not discuss the effect of microbial growth, as it is not intended to. Therefore, it is assumed that all chilies have the same microbial growth state.

A limitation to this experiment is this study does not investigate the color stability of each pigment, but rather Carotenoid as a whole.

Secondly, the drying techniques performed are limited to only four, while others are not considered.

3.2 Methodology

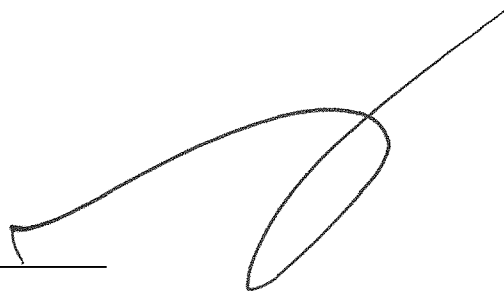
3.2.1. Preliminary Study

Before arriving at the final method, there are some trials conducted to obtain the most efficient one. In preparing the dried chilies, chili strands were cut into thin slices

(instead of blending it in the latter) before drying it. It turned out that even after 6 hours being put in the oven, the chilies weren't dry enough. This might be due to the cell walls of the chilies' strands protecting the inner tissues and cells, making it harder for water to evaporate.

3.2.2 *Preparing Chili Puree*

For the actual experiment, ten mature red chilies were rinsed using tap water. Mature red chilies were chosen as it contain the highest carotenoid content in the sweet peppers group (see Appendix E)²¹. The stems were cut and the chili strands were put inside a mixer to be blended along with its seeds. The seeds were also used because addition of seeds will give higher percentage of color retention²². The chilies were then blended until it was diluted enough, forming a puree. Five petri dishes were prepared and each was filled with 0.5 g chili puree. The puree was spread evenly, covering the entire surface of the petri dish. Each petri dish was labeled as fresh, oven-dried, microwave-dried, sun-dried and natural convection-dried. One petri dish of fresh (not dried) chili puree was also prepared to be used as a control.



²¹ Rocío Gómez-García, M., & Ochoa-Alejo, N. (2013). *Biochemistry and Molecular Biology of Carotenoid Biosynthesis in Chili Peppers (Capsicum Spp.)*. Retrieved September 27, 2014, from <http://www.mdpi.com/journal/ijms>

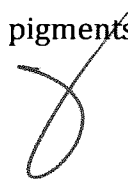
²² Markus, F., Daood, H., Kapitany, J., & Biacs, P. (1999). *Change in the Carotenoid and Antioxidant Content of Spice Red Pepper (Paprika) as a Function of Ripening and Some Technological Factors*. Retrieved October 5, 2014, from <http://lib3.dss.go.th/fulltext/Journal/J.agri.food.chem/1999/no.1p1-216/1999v47n1p100-107.pdf>

3.2.4 Preparation of Pigment Solution

The dried samples of the chili puree were grounded to powder, separately, until it became very tiny particles. The chili powder then sifted using 2mm wire mesh.

To determine the ASTA color value of the chili, the standard ASTA 20.1 procedure was followed²³. 0.1 g of the chili powder was measured and transferred to a 50 cm³ Erlenmeyer flask. This was also done for the fresh chili puree. Using measuring cylinder, 20 cm³ of propanone was measured and put into the Erlenmeyer flask. A few swirls were given to mix the chili powder/chili puree with the propanone. The use of propanone was required to extract the color pigments. The color pigments which relatively were non-polar could be separated from the other polar substances that will dissolve with polar propanone, leaving the solution with majorly the pigments alone.

Each Erlenmeyer flask filled with the propanone-chili powder/puree solution was prepared for water bath. Magnetic stir bar was dropped inside each flask and a stopper was also used to cover each flask's opening. The Erlenmeyer flasks were then placed carefully inside a water bath, quarter-filled with water, at a maintained temperature of 25°C. It was water-bathed for three hours, until the color pigments were extracted from the chili.



²³ Huisamen, D. (2006). *PROCESS USED FOR THE DETERMINATION OF EXTRACTABLE COLOUR IN CAPSICUMS*. Retrieved September 25, 2014, from http://libserv5.tut.ac.za:7780/pls/eres/wpg_docload.download_file?p_filename=F1934131062/Huisamen.pdf

After the solutions were concentrated, it was then transferred to different volumetric flasks from the Erlenmeyer flask. Each solution was then mixed with 100cm³ propanone to form 1:5 ratio of a solution.

3.2.5 Absorbance Test

The main objective of this experiment is to investigate changes of color stability of the chili pigments in different drying techniques. The color stability could therefore be analyzed for a period of time by measuring the solution's absorbance using a spectrophotometer at 460 nm.

The spectrophotometer was warmed first for 3 minutes before use. To calibrate, clear propanone blank solution was used. Using a dropper, the pigment solutions were obtained from the volumetric flask and being transferred to fill up $\frac{3}{4}$ level of the cuvette. The cuvette was placed inside and the spectrophotometer's cap was closed. Absorbance value was recorded. This was done repeatedly for five times to make sure the value was accurate and random errors could be minimized. The experiment was then continued by testing the absorbance of the other pigment solutions. This absorbance test was carried out in every twenty-four hour period for five days.

3.3 Notes on the Study

To ensure a fair and reliable test, the pigment solutions were excluded from any external factors that may affect it. This was done by storing the pigment solutions in a

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place far from exposition of light (a dark cupboard) and isolated in a volumetric flask as to avoid direct contact with atmospheric air.

*Frozen sample?
were chilis left to dry again?*

The data was also recorded for several days consecutively. This was done to observe closely the changes resulted each day so as to improve accuracy and validity of the experiment's outcome.

Moreover, it must also be taken into considerations that the results to this experiment may not be applicable to chilies cultivated in different regions or other types of *Capsicum annum*.

4. Data Summary:

Absorbance value of the pigment extracts:

Date	Average absorbance value of the pigment extracts (abs) [± 0.001 abs]				
	Fresh	Sun-dry	Microwave-dry	Oven-dry	Natural convective-dry
9/6/14	0.219	0.490	0.629	0.529	0.616
10/6/14	0.205	0.483	0.620	0.522	0.615
11/6/14	0.223	0.487	0.619	0.523	0.618
12/6/14	0.218	0.482	0.616	0.522	0.614
13/06/14	0.214	0.476	0.612	0.501	0.612

Table 4.1 Average absorbance values of the pigment extracts

5. Processing of Data and Discussion of Results:

5.1 Processing of Data

5.1.1 Calculating Color Degradation Percentage (over 4 days of storage)

$$\text{Percentage} = \left| \frac{A_4 - A_0}{A_0} \right| \times 100\% \quad \text{(Equation 5.1.1.1)}$$

A_4 = average absorbance value of the color extract on the last day

A_0 = average absorbance value of the color extract on the first day

Sample Calculation: Fresh chili pigment solution on 9th of June 2014

$$\begin{aligned} \text{Degradation Percentage} &= \left| \frac{0.214 - 0.219}{0.219} \right| \times 100\% \\ &= 2.28\% \end{aligned}$$

Table 5.1.1.1 is data summary of the processed data:

Drying Method	Color Degradation Percentage (%)
Oven-dry	5.29
Sun-dry	2.86
Microwave-dry	2.70
Fresh	2.28
Natural Convective-dry	0.65

Table 5.1.1.1: Color degradation percentage of the color extract

5.1.2 Calculating Percentage Uncertainty

$$\text{PU} = \frac{\text{uncertainty}}{\text{absorbance value}} \times 100\% \quad \text{(Equation 5.1.2.1)}$$

Sample Calculation: Fresh chili pigment solution on 9th of June 2014

$$\text{PU} = \frac{0.001}{0.629} \times 100\% = 0.16\%$$

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5.1.3 Calculating ASTA Value

Table 5.1.3.1 – 5.1.3.5 are data summary of the processed data:

Time of Storage (days)	Fresh	
	Average Absorbance Value (abs) [± 0.001 abs]	ASTA Value
0	0.219 ($\pm 0.46\%$)	508.571
1	0.205 ($\pm 0.49\%$)	476.059
2	0.223 ($\pm 0.45\%$)	517.860
3	0.218 ($\pm 0.46\%$)	506.248
4	0.214 ($\pm 0.47\%$)	496.959

Table 5.1.3.1: ASTA Value of fresh chili pigment solution

Time of Storage (days)	Sun-dry	
	Average Absorbance Value (abs) [± 0.001 abs]	ASTA Value
0	0.490 ($\pm 0.20\%$)	1138.362
1	0.483 ($\pm 0.21\%$)	1122.106
2	0.487 ($\pm 0.21\%$)	1131.395
3	0.482 ($\pm 0.21\%$)	1119.320
4	0.476 ($\pm 0.21\%$)	1104.457

Table 5.1.3.2: ASTA Value of sun-dried chili pigment solution

Time of Storage (days)	Microwave-dry	
	Average Absorbance Value (abs) [± 0.001 abs]	ASTA Value
0	0.629 ($\pm 0.16\%$)	1459.760
1	0.620 ($\pm 0.16\%$)	1439.789
2	0.619 ($\pm 0.16\%$)	1436.538
3	0.616 ($\pm 0.16\%$)	1429.571
4	0.612 ($\pm 0.16\%$)	1420.746

Table 5.1.3.3: ASTA Value of microwave-dried chili pigment solution

Time of Storage (days)	Oven-dry	
	Average Absorbance Value (abs) [± 0.001 abs]	ASTA Value
0	0.529 ($\pm 0.19\%$)	1229.394
1	0.522 ($\pm 0.19\%$)	1211.280
2	0.523 ($\pm 0.19\%$)	1214.067
3	0.522 ($\pm 0.19\%$)	1212.209
4	0.501 ($\pm 0.20\%$)	1163.907

Table 5.1.3.4: ASTA Value of oven-dried chili pigment solution

Time of Storage (days)	Natural Convective-dry	
	Average Absorbance Value (abs) [± 0.001 abs]	ASTA Value
0	0.616 ($\pm 0.16\%$)	1430.964
1	0.615 ($\pm 0.16\%$)	1427.249
2	0.618 ($\pm 0.16\%$)	1434.680
3	0.614 ($\pm 0.16\%$)	1426.320
4	0.612 ($\pm 0.16\%$)	1420.282

Table 5.1.3.5: ASTA Value of natural convective-dried chili pigment solution

The ASTA color value of the pigment solutions can be determined using ASTA unit calculated by this formula (a modified method, adapted from ASTA 20.1):

$$\text{ASTA} = A \cdot 164 \cdot if \cdot w^{-1} \quad \text{(Equation 5.1.3.1)}$$

In this equation, "A" indicates the average absorbance value of the pigment solution. *If* is the deviation factor of the spectrophotometer which was calculated by dividing the theoretical absorbance ($A_t = 0.600$) by the real absorbance (A_s) of the standard color solution (0.001 M $K_2Cr_2O_7$) and 0.09 M $(NH_4)_2Co(SO_4)_2 \cdot 6H_2O$ in 1.8 M H_2SO_4) at 460 nm, and w^{-1} is the weight (g) in dry basis²⁴.

Sample Calculation: Fresh chili pigment solution on 9th of June 2014

$$A = 0.219$$

$$If = \frac{0.600}{0.4236} = 1.416$$

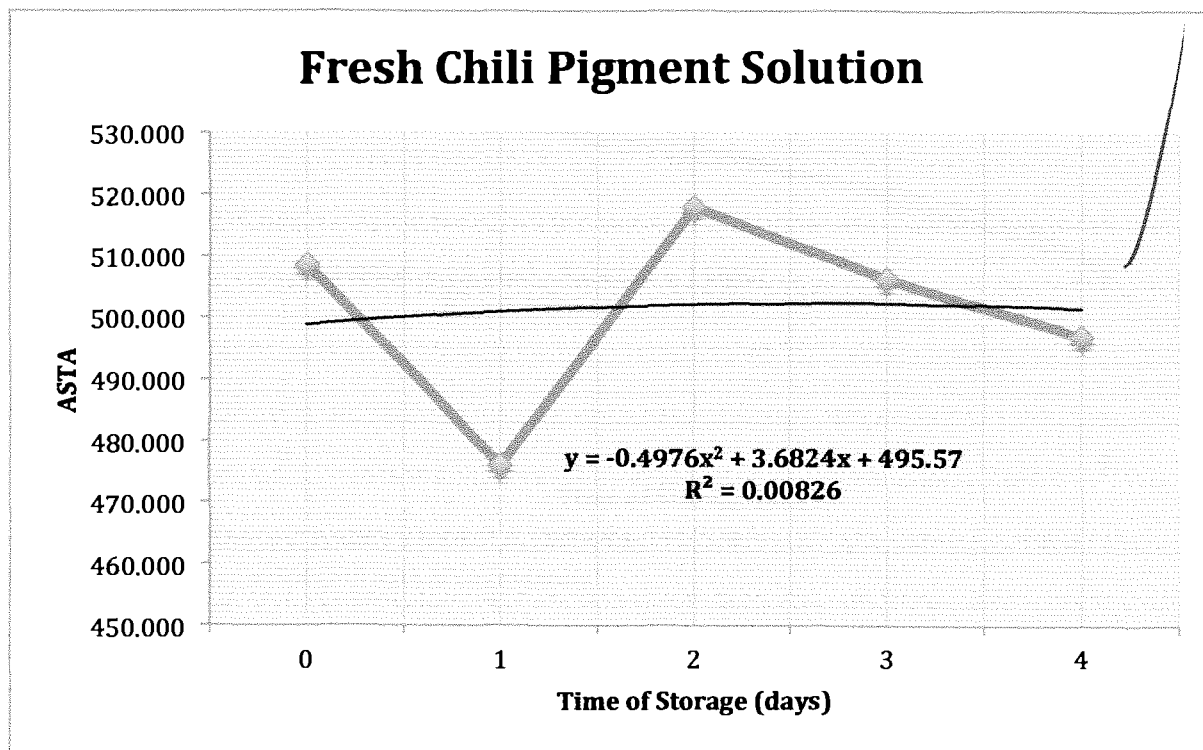
$$w^{-1} = 0.1^{-1} = 10$$

$$\text{ASTA} = 0.219 \times 164 \times 1.416 \times 10 = 508.571$$

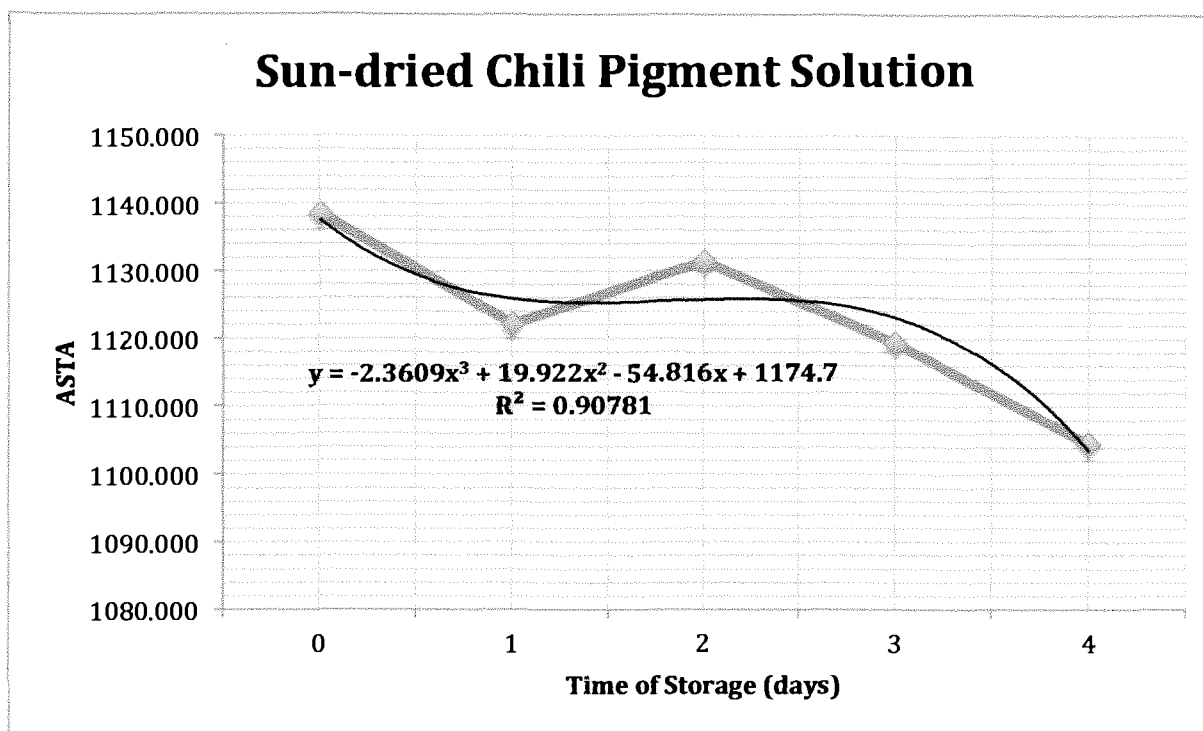
²⁴ Topuz, A., Feng, H., & Kushad, M. (2009). *The Effect of Drying Method and Storage on Color Characteristics of Paprika*. Retrieved June 30, 2014, from <http://www.journals.elsevier.com/food-science-and-technology>

5.1.4 Presentation of Graphs

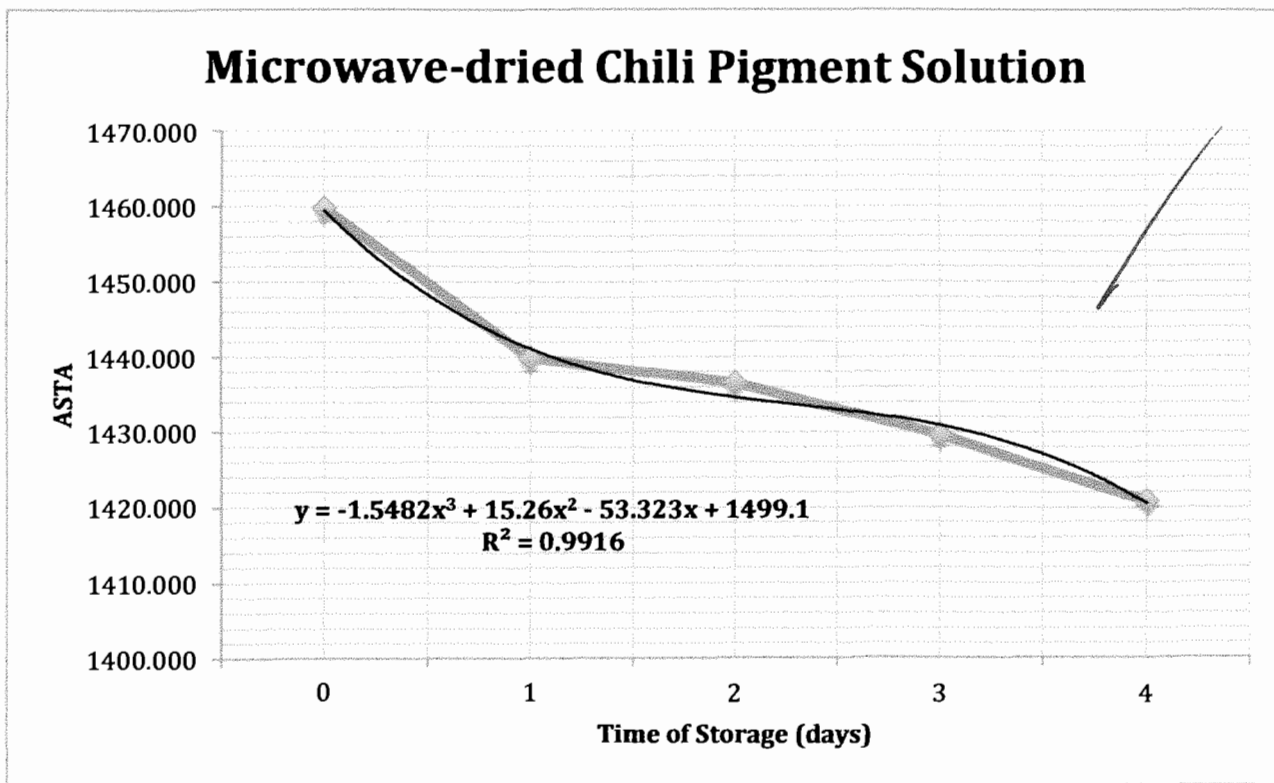
The following graphs are presented to show the changes in ASTA Value of each pigment extract for a period of time:



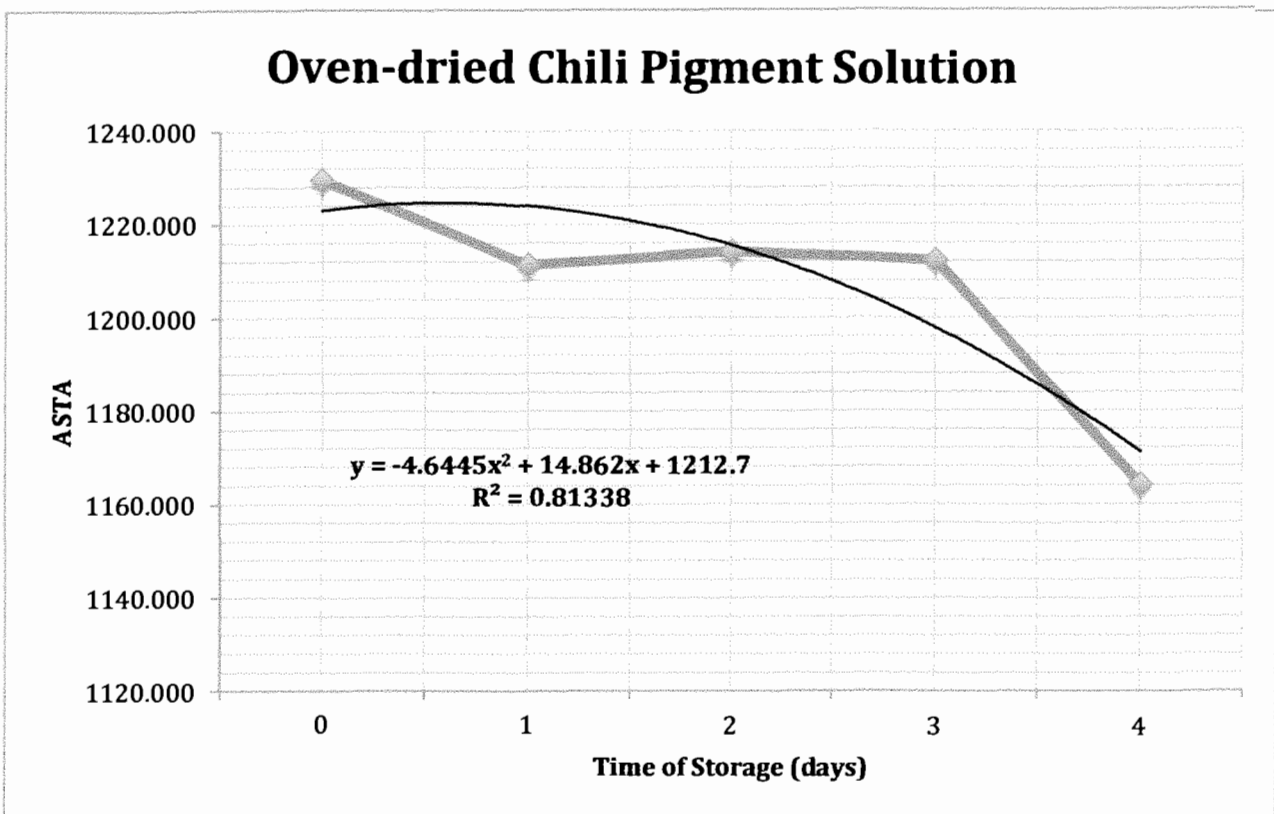
Graph 5.1.4.1 Fresh Chili Rate of Degradation



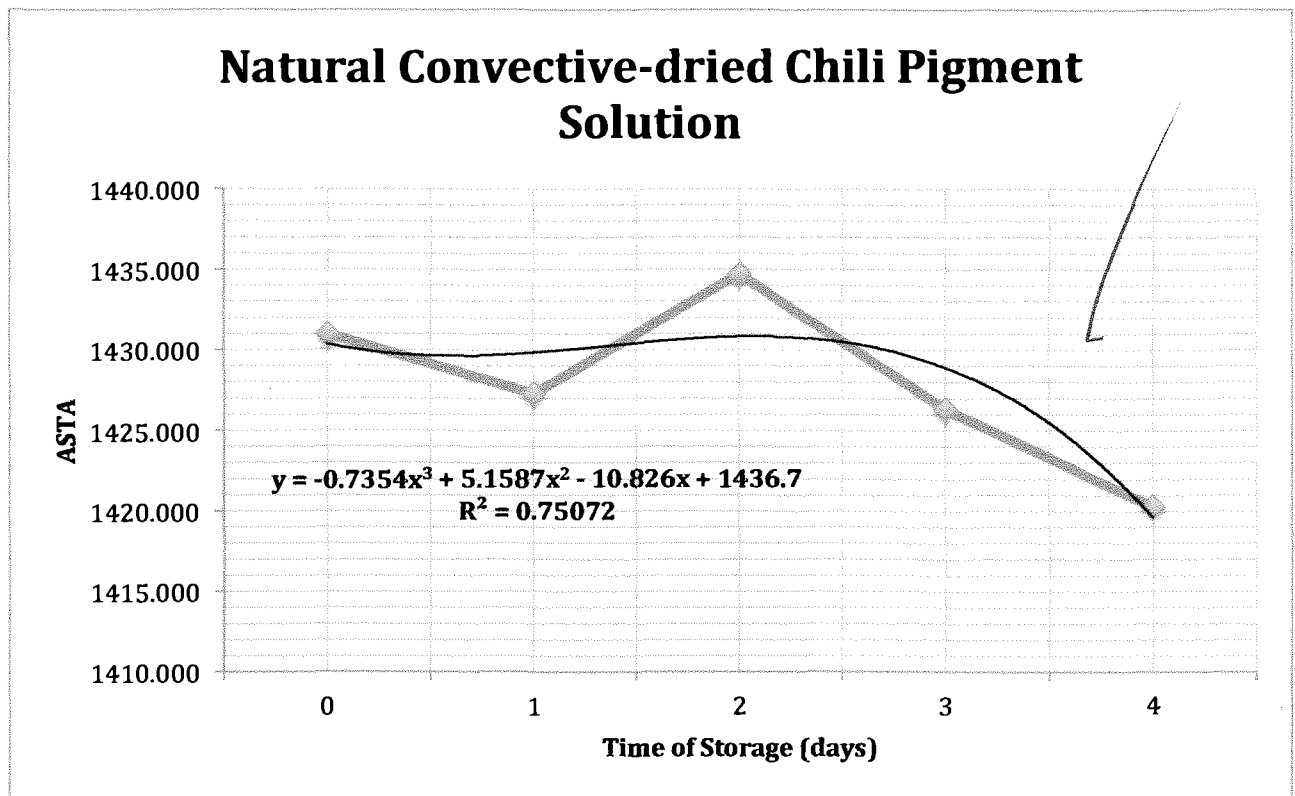
Graph 5.1.4.2 Sun-dried Chili Rate of Degradation



Graph 5.1.4.3 Microwave-dried Chili Rate of Degradation



Graph 5.1.4.4 Oven-dried Chili Rate of Degradation



Graph 5.1.4.5 Natural-convective dried Chili Rate of Degradation

From these graphs, rate of color degradation of each chili by different drying methods could be deduced. This can be done by taking the gradient value of the curve equation.

Drying Methods	Rate of Degradation
Oven-dried	4.6445
Sun-dried	2.3609
Microwave-dried	1.5482
Natural convective-dried	0.7354
Fresh Chili	0.4970

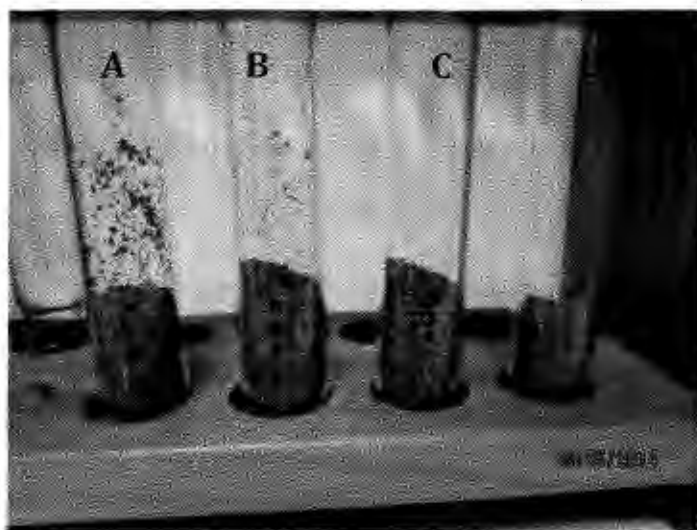
Table 5.1.4.1 - Rate of Degradation

5.2 Discussion of Results

5.2.1 Level of Dryness

As the chili purees were treated with different drying methods it became dry. However, the wet basis or dryness of each chili puree was not the same. This could be analyzed from the grounded dried chili obtained.

How else could this have been verified?



- A = Microwave-dried
- B = Natural convective-dried
- C = Sun-dried
- D = Oven-dried

Figure 5.2.1.1 Grounded Chili

From figure 4.2.1.1, it could be seen that Microwave-dried has the largest size of particle. The grounded chilies that stick to the walls of the test tube is due to the water molecules that it still contained. Despite of having it crushed several times using the mixer, it was the smallest size of particle that can be obtained from the microwave-dried chili. In contrast, grounded oven-dried chili has the tiniest particle with least moisture; proven by how none of the particles stuck to the walls.

The state of dryness indicates the effectiveness of each drying method to dry the chilies. Oven-drying resulted to grounded chili with lowest moisture content is the most effective method in terms of reaching dryness followed by sun-drying, natural convective-drying, and lastly microwave-drying method.

5.2.2 Pigment Extract in the beginning

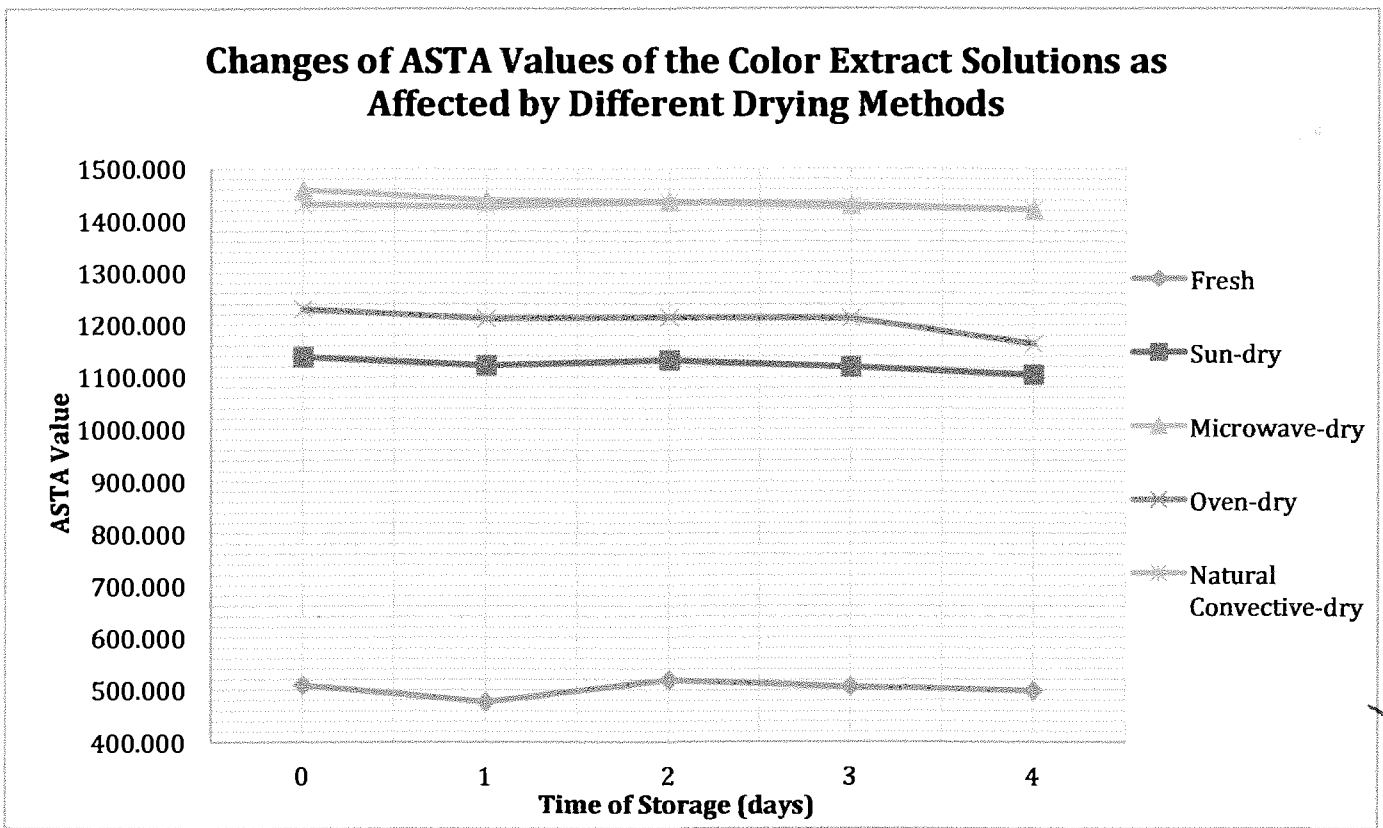


In the beginning, it could be found that the absorbance of the color extract solution from fresh chili had the lowest value, whilst microwave-dried chili's color extract solution has the highest value. Low absorbance value of the fresh chili indicates that color extract is light in color which is due to the water that the fresh chili still contained. The chili purees that had been dried first were exposed to heat and it will produce color extract solutions that are darker in color. This is because heating had made the cells to be dehydrated and their cell walls to be damaged, as a result, leaching of water-soluble compounds such as the color pigments are unavoidable. As this color pigment is no longer bounded by the cell walls, it could easily be released to the medium. This is because diffusion of cellular fluid from the plant cells to the medium is highly accelerated by heating.

The difference to what certain extent these chilies were heated also affected its extract solution's color intensity. The damage to cell walls differed accordingly and it determined how readily the water-soluble compounds inside to diffuse out.

5.2.3 Color Degradation

The color extract solutions were stored, and it was observed that the ASTA value decreased over time. It could be linked back to the auto-oxidation mechanism that might have happened and caused slight discoloration. However, the rate at which these solutions decrease in value was different according to the different drying methods as can be seen in the following graph:



Graph 5.2.3.1 – Changes of ASTA Values over 5 days of storage

The solution from the different drying methods resulted to oven-dried chili to have the highest ASTA value change from when it was first extracted until the 4th day of storage. The oven-dried chili's color extract solution experienced the most discoloration compared to the other solutions (5.29% degradation). Natural convective drying

method, in the other hand, gives the least discoloration. It is because this method deals with the least interference of heat, high temperature, light or direct air which all contributed to the degradation color of the extract as it has been discussed earlier in the introduction. Fresh chili that was left in normal condition also gave similar result of color degradation percentage as natural convective dried-chili's color extract.

Oven-dried chili's color extract has the highest discoloration percentage. This significant change could have been the consequence of drying method that involved very high temperature above 50°C. Carotenoids when heated above 50°C will result to abundant naturally occurring all-*trans* form rearranges to yield a variety of *cis* isomers in which causes color degradation or more color instability²⁵.

Looking at the rate of color degradation of the pigment extracts, it is found out that there is a significant fluctuation in the trend of absorbance value of the extracts from natural convective-dried chilies and fresh chilies. It was expected that a gradual decrease in the color degradation to be obtained, but as can be seen (Graph 5.1.4.1 and Graph 5.1.4.5) the ASTA values fluctuated greatly. The experiment has been repeated twice, and yet the same results were obtained. This is one of the unanswered questions in the experiment which might be answered if the extracts were stored for longer time. By storing it longer, a bigger picture of the trend could be obtained. Analysis regarding any outlier can therefore be deduced with wider range of possibilities.

²⁵ Owen, S. (2011). Option F. In *Chemistry for the IB Diploma* (1st ed., p. 7). Cambridge University Press.

6. Conclusion:

In this experiment, it is found out that the drying method that involved the least extreme heat such as natural convective dry gave the highest color quality at first and the least degradation. This indicates that drying through heating method, which involves not only high temperature, but also light triggering oxidation to happen, greatly affected the chemical structure, and thus the characteristic of the pigments, in which its color could easily degrade.

Other methods of drying that involves heating such as oven-drying, microwave-drying and sun-drying also gave good color quality of the extracts although ASTA value is not as high as the ones obtained from natural convective-dry's pigment extract. However, amongst the three heating methods, oven-drying technique gave the least stable extracts color.

This is due to the theory that states, *heating above 50°C will result to lesser ability for the color to retain*²⁶, while sun-drying and microwave-drying does not involve heating up until that level.

Although, oven-drying method might be the most common and easiest way to dry food, it is proven to be the least effective one in maintaining color stability.

²⁶ Owen, S. (2011). Option F. In *Chemistry for the IB Diploma* (1st ed., p. 19). Cambridge University Press.

It seems that not all of them were dried to the same extent.

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All in all, the best method to process chili would be drying with natural convective method. This is very advantageous, as it only requires the chili puree to be stored in an isolated place for a week or more. The result will give an appealing color extract that could retain the longest compared to the other drying methods.



7. Evaluation and Improvement:

As it has been discussed before, the dryness of each puree at the end of drying was proven to be different from one another. This was due to some drying methods reducing the moisture content of the chili only to certain extent. The drying time was also different for each drying technique. Therefore, the chilies dried through heating methods might have not achieved the same level of dryness as the natural convective dried chilies. This could be improved by setting a standard to the final percentage moisture content by measuring the mass of the chili before and after drying. ✓

On top of that, the thin layer of chili puree spread on the petri dish might be uneven throughout the whole surface. Although a ruler was used to measure its thickness (1 mm for all), this doesn't guarantee for the whole surface. The uneven layer can cause some parts of the puree to be drier than the other, thus reducing its dryness level for some regions.

Further improvements that could be done for this experiment is prolonging the days of storage and absorbance data recording. This will allow a more accurate observation regarding the changes in the color intensity of the pigments since chemical reactions inside the pigments will take time to cause changes. It is also more applicable in daily life when foods are stored longer than one or two weeks.

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Futhermore, in my experiment, the color stability of the pigments were only measured by absorbance/ASTA value. There are different aspects in which color stability can be measured such as through red/yellow pigment ratio and browning index. The results to these can be compared to one another, allowing a more thorough analysis to be drawn regarding individual pigments found in the chilies.

If feasible, in the future I would also like to test out other drying techniques such as freeze drying that does not involve high temperature, but instead extreme low temperature. This is a method of drying food under the temperature of -70°C . Unfortunately, this method is only possible with more advanced equipment and tools which I didn't have access to.



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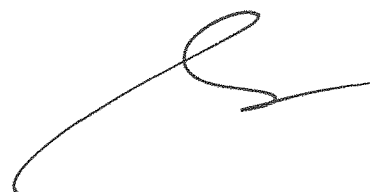
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9. Appendix:

9.1 Appendix A: Pictures

The following pictures show the color degradation of the pigment extracts over 5 days:

A	Fresh
B	Sun-dry
C	Microwave-dry
D	Oven-dry
E	Natural convective-dry



Figure 9.1.1 - Date: 9/7/14

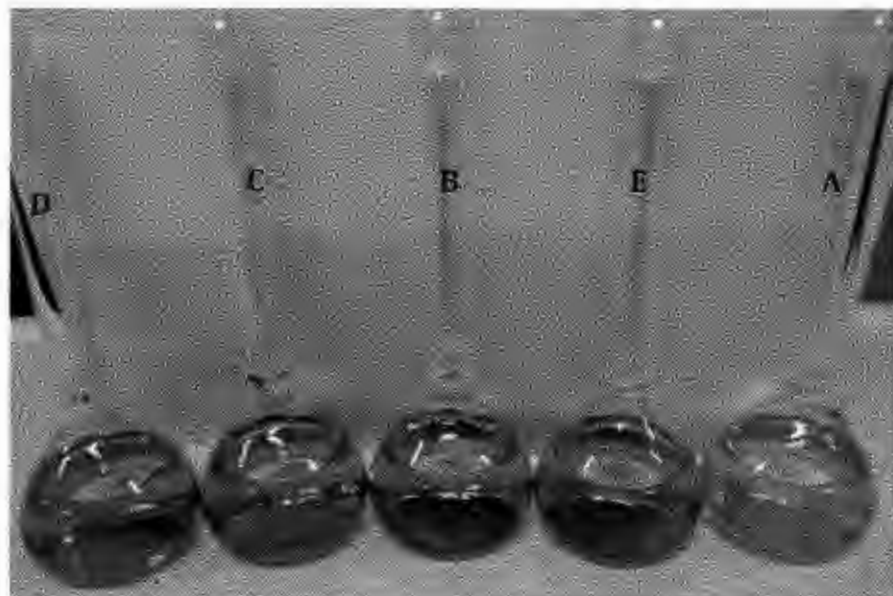


Figure 9.1.2 - Date: 10/7/14

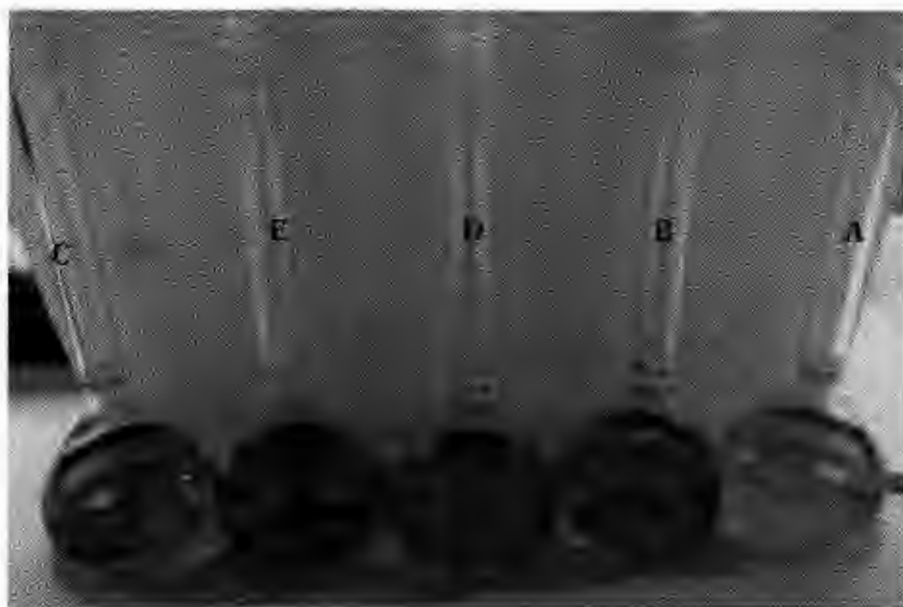


Figure 9.1.3 - Date: 11/7/14 1

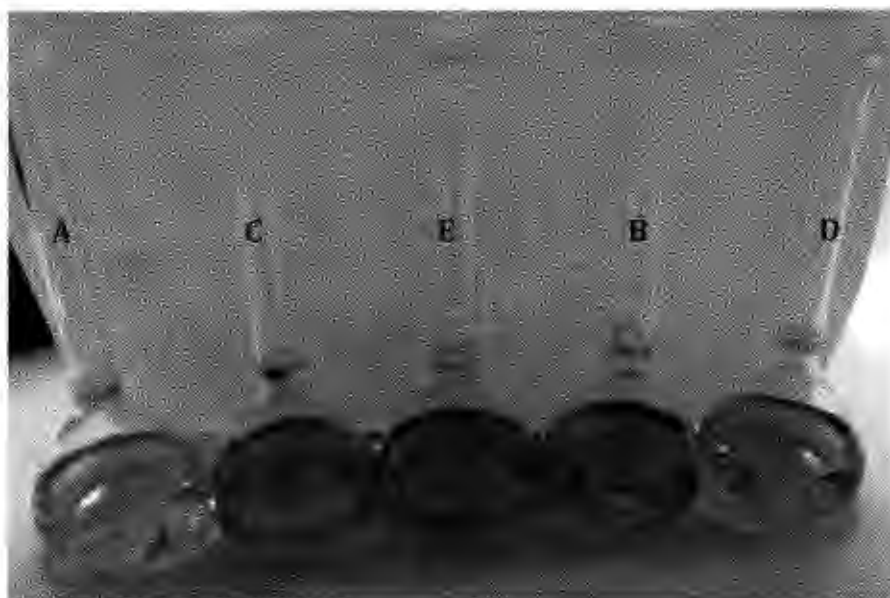


Figure 9.1.4 - Date: 12/7/14

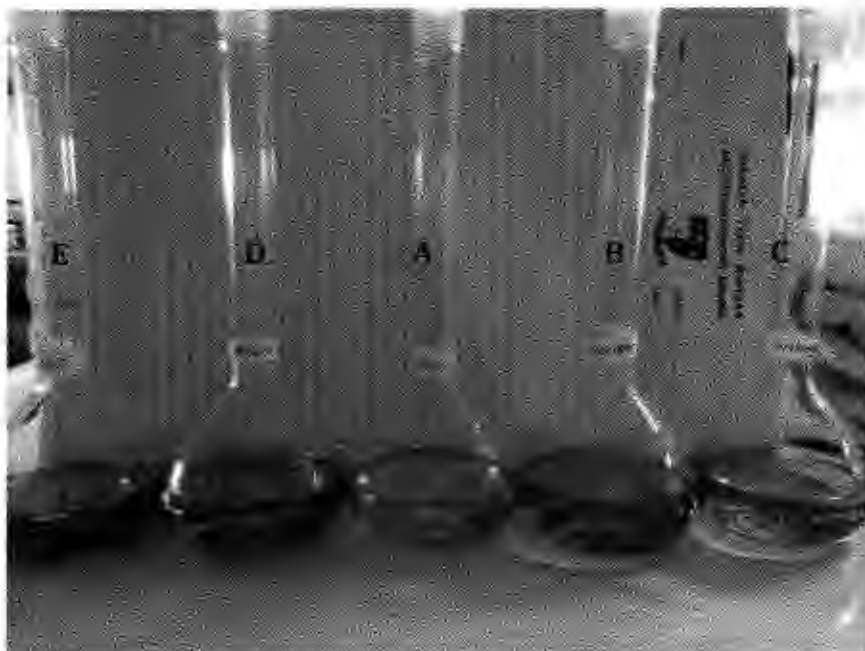


Figure 9.1.5 - Date 13/7/14

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9.2 Appendix B: Materials and Procedure

A. Materials

I. Apparatus

- Weighing Balance ($\pm 0.01\text{g}$)
- Petri dish (x5)
- Spatula
- Wire mesh (2 mm)
- 50 cm³ Erlenmeyer flask (x5)
- 100 cm³ Volumetric flask (x5)
- 20 cm³ Measuring cylinder [$\pm 0.01\text{ cm}^3$]
- Magnetic stir bar (x5)
- Rubber Stopper for conical flasks (x5)
- Filter funnel
- Dropper
- Blender/Mixer
- Blast-drying Oven
- Microwave (700 W)
- Spectrophotometer (460 nm)
- Water bath
- Ruler (cm) [$\pm 0.1\text{ cm}$]

II. Reagents

- 10 Mature red chillies (*Capsicum annuum*)
- 700 cm³ Propanone solution

B. Procedure (adapted from ASTA 20.1)

I. Preparing Chili Puree

1. Pick 10 strands of red, mature chilies (*Capsicum annuum*)
2. Rinse with tap water and remove stems
3. Along with its seeds and skin, put the chilies inside the mixer. Press pulse button several times to crush the chilies. After the chilies were crushed into pieces, proceed with continuous mix until it becomes soft textured puree.
4. Using weighing balance, weigh 0.50 g of the puree. Spread it on a petri dish and measure its thickness using a ruler into 1mm thickness. Repeat this step for 4 times and label each petri dish as oven-dried, microwave-dried, sun-dried, and natural convective-dried.

II. Drying

1. Oven drying: put the petri dish inside a blast-drying oven with temperature of 60°C for 2 hours.
2. Microwave drying: put a petri dish inside the oven at 60°C for 1 hour. Continued with heating it inside a microwave for 1 minute at high heat.
3. Sun-drying: Place the petri dish in an open space with direct heat from sunlight. This should be done during the day with surrounding temperature >30°C for 2 hours.
4. Natural-convective drying: Place the petri dish inside a dark, isolated cupboard for 10 days.

III. Preparation of Pigment Solution

1. Using a spatula, scrape the dried chili from each petri dish
2. Separately, ground the dried chili using mixer until it becomes very tiny particles (powder)

3. Using weighing balance, weigh 0.1g of the chili powder and place it in a 50 cm³ Erlenmeyer flask. For the control, 0.1 g chili puree was also weighed and put into a 50 cm³ Erlenmeyer flask.
4. Using 20 cm³ measuring cylinder, measure propanone solution and dilute it with the 0.1 g chili powder in each Erlenmeyer flask. Give a few swirls to mix the solution.
5. Prepare a water bath, quarter-filled with water.
6. Drop a magnetic stir bar in each Erlenmeyer flask. Place the Erlenmeyer flask in water-baths.
7. Water-bath the solutions at a maintained temperature of 25° C for 3 hours.
8. After the pigment is concentrated, transfer each solution in Erlenmeyer flask to different 100cm³ volumetric flasks.
9. Make 1:5 ratio solution by adding 100 cm³ propanone to the each volumetric flask.

IV. Absorbance Test

1. Warm the spectrophotometer up for 3 minutes
2. Calibrate the spectrophotometer using clear propanone blank.
3. Using a dropper to transfer a portion of clear propanone blank to half-filled the cuvette. Put the cuvette inside the spectrophotometer and close the cap. Set to zero.
4. After the spectrophotometer has been calibrated, draw a portion of the pigment extract using a pipette to half-filled the cuvette. Put the cuvette inside the spectrophotometer and close the cap. Measure the absorbance.
5. Repeat step 4 for each extract solution with the different drying methods. 5 readings should be taken for each.

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9.3 Appendix C: Raw Data and Calculations

*PU: Percentage Uncertainty

Fresh								
Date	Absorbance (abs) [± 0.001 abs]						Average	PU (%)
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5			
9/6/14	0.219	0.219	0.220	0.22	0.218	0.219	0.46	
10/6/14	0.207	0.208	0.200	0.205	0.205	0.205	0.49	
11/6/14	0.225	0.223	0.227	0.221	0.221	0.223	0.45	
12/6/14	0.222	0.215	0.219	0.215	0.218	0.218	0.46	
13/06/14	0.214	0.214	0.214	0.214	0.214	0.214	0.47	

Table 9.3.1 Absorbance of Fresh Pigment Extract

Sun-dry								
Date	Absorbance (abs) [± 0.001 abs]						Average	PU (%)
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5			
9/6/14	0.498	0.489	0.491	0.488	0.485	0.490	0.20	
10/6/14	0.484	0.485	0.482	0.484	0.481	0.483	0.21	
11/6/14	0.487	0.488	0.488	0.485	0.488	0.487	0.21	
12/6/14	0.483	0.485	0.480	0.480	0.482	0.482	0.21	
13/06/14	0.476	0.479	0.474	0.474	0.475	0.476	0.21	

Table 9.3.2 Absorbance of Sun-dried Pigment Extract

Microwave-dry								
Date	Absorbance (abs) [± 0.001 abs]						Average	PU (%)
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5			
9/6/14	0.631	0.629	0.629	0.627	0.627	0.629	0.16	
10/6/14	0.62	0.624	0.618	0.620	0.618	0.620	0.16	
11/6/14	0.619	0.619	0.615	0.621	0.619	0.619	0.16	
12/6/14	0.617	0.612	0.617	0.617	0.615	0.616	0.16	
13/06/14	0.609	0.612	0.613	0.613	0.612	0.612	0.16	

Table 9.3.3 Absorbance of Microwave-dried Pigment Extract

Oven-dry								
Date	Absorbance (abs) [± 0.001 abs]						Average	PU (%)
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5			
9/6/14	0.529	0.531	0.529	0.529	0.529	0.529	0.19	
10/6/14	0.520	0.522	0.523	0.521	0.522	0.522	0.19	
11/6/14	0.521	0.522	0.523	0.525	0.523	0.523	0.19	
12/6/14	0.521	0.522	0.522	0.525	0.520	0.522	0.19	
13/06/14	0.503	0.500	0.502	0.501	0.500	0.501	0.20	

Table 9.3.4 Absorbance of Oven-dried Pigment Extract

Natural Convective-dry							
Date	Absorbance (abs) [± 0.001 abs]					Average	PU (%)
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5		
9/6/14	0.618	0.617	0.616	0.615	0.615	0.616	0.16
10/6/14	0.618	0.612	0.613	0.615	0.615	0.615	0.16
11/6/14	0.619	0.62	0.618	0.616	0.616	0.618	0.16
12/6/14	0.613	0.616	0.619	0.614	0.609	0.614	0.16
13/06/14	0.611	0.611	0.608	0.608	0.620	0.612	0.16

Table 9.3.5 Absorbance of Natural convective-dried Pigment Extract

$K_2Cr_2O_7$ (aq)						
Absorbance (abs) [± 0.001 abs]						
Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average	
0.423	0.425	0.422	0.424	0.424	0.4236	

Table 9.3.6 Absorbance of $K_2Cr_2O_7$

9.4 Appendix D: The Standard ASTA 20.1 Method²⁷

9.4.1 Description

This is the method for determining extractable colour in Capsicums (American Spice Trade Association, 1985:43).

9.4.2 Purpose

To determine the extractable colour in Capsicums by measuring the absorbance of an acetone extract with a spectrophotometer at 460 nm.

9.4.3 Apparatus

- Spectrophotometer – capable of accurately measuring the absorbance at 460 nm.
- Absorption cells (cuvette) – 1 cm square matched quarts cells.
- Standard glass filter – (Standard reference material 2030 or 930D [30% T] from the SA Bureau of Standards) (see Annexure C).
- Volumetric flasks – 100 ml with ground glass stoppers.

9.4.4 Reagent

- Acetone: reagent grade: purity = 100.0 ± 2.0%

²⁷ Huisamen, D. (2006). *PROCESS USED FOR THE DETERMINATION OF EXTRACTABLE COLOUR IN CAPSICUMS*. Retrieved September 25, 2014, from http://libserv5.tut.ac.za:7780/pls/eres/wpg_docload.download_file?p_filename=F1934131062/Huisamen.pdf

9.4.5 Procedure for Capsicums

Ungrounded Capsicums (paprika) was grounded to pass a 1 mm sieve. A mass of 70 to 100 mg of sample was accurately weighed and transferred to a 100 ml volumetric flask. The flask was filled to the 100 ml mark with acetone and stopper tightly.

- The flask was shaken and allowed to stand for 16 hours at room temperature in the dark.
- The flask was shaken and allowed to stand for 2 minutes for the particles to settle.

A portion of the extract was transferred to the spectrophotometer cell (cuvette) and the absorbance was measured at 460 nm with an acetone blank.

The absorbance of a glass filter (purchased from the SA Bureau of Standards) was determined at 465 nm.

9.4.6 Calculations for Capsicums

9.4.6.1 Determination of the instrument correction factor (If)

Measure the absorbance of the standard glass filter (purchased from the SA Bureau of Standards), and compare this value to the certified value of the SA Bureau of Standards (SABS). The instrument correction factor is calculated according to the following formula:

$$I_f = \frac{\text{SABS Absorbance at 465nm}}{\text{Laboratory absorbance at 465nm}} \quad (\text{Eq. 3.1})$$

The instrument correction factor (I_f) is part of system suitability testing, and is an indication of the reliability of the instrument (spectrophotometer).

9.4.6.2 Determination of extractable colour

An ASTA value is a scientific measurement of the colour quality of paprika and is a measure of the level of Capsanthin. ASTA colour is calculated according to the following formula (American Spice Trade Association, 1985:43):

$$\text{ASTA colour} = \frac{\text{Absorbance of acetone extract} \times 16.4 \times I_f}{\text{Sample mass in grams}} \quad (\text{Eq.3.2})$$

Notes:

The absorbance of the glass filter needs only be determined once per day. The recommended range of absorbance values is between 0.30 and 0.70 absorbance units.

Extracts having absorbances greater than 0.70 should be diluted with acetone to one-half the original concentration. Extracts having absorbances less than 0.30 should be discarded and the extraction performed with a larger sample (American Spice Trade Association, 1985:1).

9.5 Appendix E: Carotenoid contents in fruits from several *Capsicum annum* varieties²⁸

Chili pepper type	Ripening stage	Main Carotenoids	Total Carotenoids
Red bell pepper	Mature (red)	Capsanthin (34.7%), β -carotene (11.6%), violaxanthin (9.9%)	284 and 127 mg per kg f.wt. (as β -carotene) (two lots of peppers)
Green bell pepper	Immature (Green)	Lutein (40.8%), neoxanthin (15.1%), violaxanthin (13.8%), β -carotene (13.4%)	10.6, 11.2 and 9.0 mg per kg f.wt. (as β -carotene) (three lots of green peppers)
Yellow pepper	Immature (Green)	Violaxanthin (34%), antheraxanthin (10.5%), lutein (9.2%), zeaxanthin (8.5%)	13.2 mg/100 g d.wt.
	Mature (orange)	Lutein (37.8%), β -carotene (19.8%), neoxanthin (5.5%)	488.6 mg/100 g d.wt.
Black Paprika	Immature (Black)	Lutein (28.5%), zeaxanthin (11.96%)	48.5 mg/100 g of d.wt.
	Mature (Red)	Capsanthin (42%), zeaxanthin (8%), capsorubin (3.2%), β -carotene (7%)	3211 mg/100 g d.wt.
Szentesi Kosszarvú	Immature (Green)	Lutein (31.9%), β -carotene (11.3%)	11.5 mg/100 g d.wt.
	Mature (Red)	Capsanthin (29%), zeaxanthin (15%)	994.7 mg/100 g d.wt.
Capsicum annuum var. lycopersiciforme rubrum	Immature (Green)	Lutein (31.6%) and β -carotene (13.7%)	19.6 mg/100 g d.wt.
	Mature (Red)	Capsanthin (37%), zeaxanthin (8%), β -carotene (9%)	1297.1 mg/100 g d.wt.
Sweet peppers	Immature (Green)	Lutein (2.3 mg/100g f.wt.), β -carotene (1.7 mg/100 g f.wt.)	5.1 mg/100 g f.wt.

²⁸ Rocío Gómez-García, M., & Ochoa-Alejo, N. (2013). *Biochemistry and Molecular Biology of Carotenoid Biosynthesis in Chili Peppers (Capsicum Spp.)*. Retrieved September 27, 2014, from <http://www.mdpi.com/journal/ijms>

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	Green	Lutein (1.4 mg/100g f.wt.), β -carotene (2.1 mg/100 g f.wt.)	4.9 mg/100 g f.wt.
	Immature (Red)	β -carotene (1.9 mg/100 g f.wt.), zeaxanthin (2.9 mg/100 g f.wt.)	9.5 mg/100 g f.wt.
	Red	β -carotene (4.3 mg/100 g f.wt.), capsanthin (19.9 mg/100 g f.wt.)	45.6 mg/100 g f.wt.
Ancho, guajillo and mulato	Ancho (Mature)	β -carotene (20.9%), violaxanthin (14.5%)	7.5 mg/100 g d.wt
	Guajillo (Mature)	β -carotene (17.9%), violaxanthin (13.2%)	6.8 mg/100 g d.wt.
	Mulato (Mature)	Violaxanthin (22%) β -carotene (14.9%)	7.2 mg/100 g d.wt.