COOPERATIVE EDUCATION AND CAREER DEVELOPMENT PROGRAM BETWEEN UNIVERSITY PUTRA MALAYSIA AND MIE UNIVERSITY

130701~130824

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Table of contents page

CHAPTER1 : INTRODUCTION

As we all know, University of Putra Malaysia (UPM) has numerous linkages and collaborations with excellent and well-known international institutions and agencies around the world such as Japan, United Kingdom, the Middle East, France, Korea and others through the Memorandum of Understanding and Memorandum of Agreement (MoU/MoA). Mie University is one of the excellent agencies that have collaboration (MoU) with UPM, thus allowing us, students from UPM to participate in the International Internship program in Mie University.

As an undergraduate of Bachelor Science (Hons) in Chemistry, I am required to complete 4 credits of practical experiences in a chosen company or institution for 8 weeks. I have been accepted to do my industrial training in Mie University at the Graduate School of Regional Innovation Studies under the supervision of Associate Professor Takashi Mishima. The starting date of the internship is from July 1, 2013 until August 24, 2013.

During the internship, I have been trained using related machines such as High Performance Liquid Chromatography (HPLC), ICP, UV spectrophotometer and much more before having to complete a given project. The final project started from week 4. Later, I am required to do presentation on the completed project on August 19, 2013. The purpose of doing the presentation is the supervisor wants us to present our finding and share the results with the other students and staffs as the presentation is opened to anybody.

UPM students are required to complete the Internship in Industrial Chemistry (CHM4901) to:

i. Experience and understand real life situations in industrial organizations and their related environments.
ii. Accelerate the learning process of how student's knowledge could be used in a realistic way.
iii. Provide the exposure to practice and apply the knowledge in the working environment.
iv. Gain hands-on experience that is related to the students majoring.
v. Develop a sense of responsibility towards society.

CHAPTER 2 : THE INTERNSHIP

2.1 The training

Before starting the real project, the supervisor, Mishima Sensei trained us on how to handle the related machines such as HPLC of different types, UV Spectrophotometer, centrifuge evaporator and many more. Different experiments were given every week where each of the experiment is related and applied to the final project. The following are the description on the experiments and machines:

2.1.1 Introduction to Pipetman (P1000)

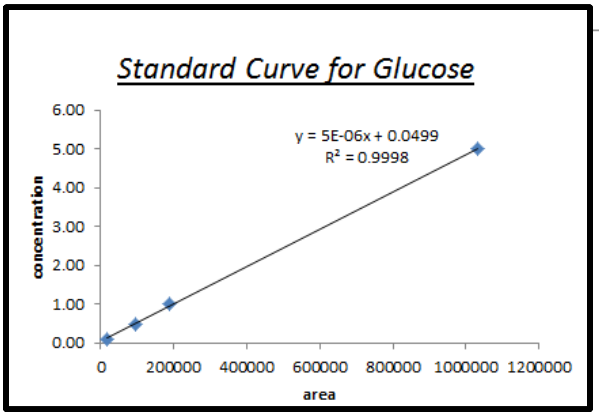
This experiment is very important as this equipment is the most basic used in the laboratory. The type of pipet use here is different from the one use in UPM. It is called Pipetman and it provides a much more accurate reading and measurement of a liquid.

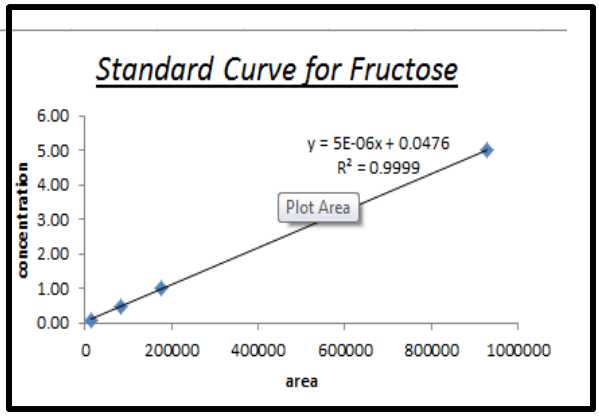
2.1.2 Measurement of UV absorbance of 0.1% Amygdalin solutions of different solvent using UV Spectrophotometer.

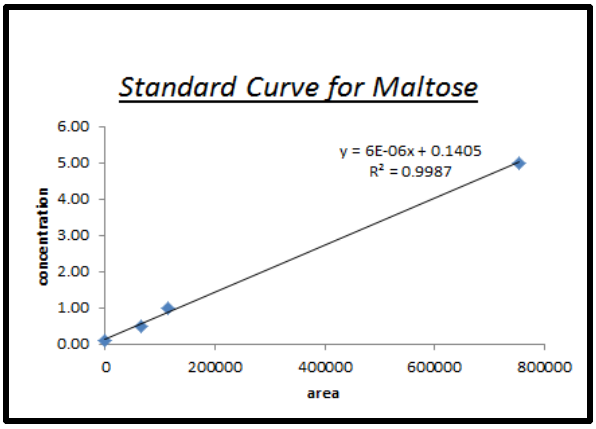
In this experiment, we use different type of solvent for dilution and measure the UV absorbance using UV spectrophotometer. The solvents are distilled water, 100% Ethanol and 50% Ethanol.

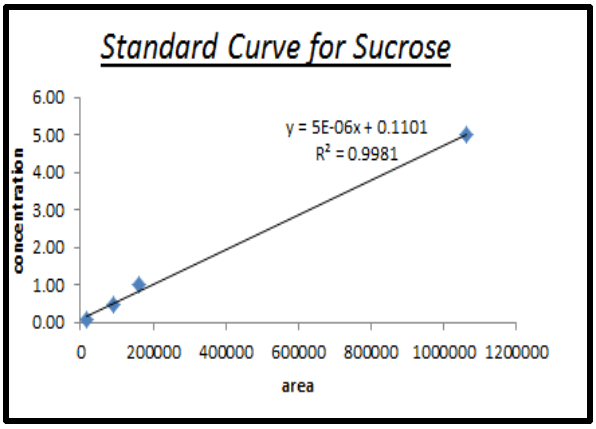
2.1.3 Analysis of five different sugar solution with different concentration prepared using two different solvent by HPLC

In this experiment, we have to analyze five different types of sugars with different concentration by using HPLC. The types of sugar that we analyzed are Glucose, Fructose, Sucrose, Maltose and Cellobiose. From the analysis of these sugars, we made a standard curve from the graph of concentration versus peak area. Later, we can use the equation of the standard curve in our project to determine the total sugar content and total amino acid content in fruit and vegetables. Below shows the standard curves of the sugar:









2.1.4 Experiments for Primary School Science Show

There are a few experiments that have been done for the Science Show. The first experiment is about air gun. Next is the experiment involving liquid nitrogen which explaining about the Charles' Law. Lastly, we did an experiment on magic color change using indigo carmine. This experiment involved the redox reaction principle.

2.1.5 Determination of reducing and non-reducing sugars using the Phenol-Sulfuric Acid Assay.

In this experiment, we use different types of sugars to measure the absorbance by using an old type UV spectrophotometer. The reason for using an old type spectrophotometer is because in this method we use a very concentrate sulfuric acid which can make the spectrophotometer to break down. Therefore, it is better to use an old and not very expensive one .There are five types of them which are glucose, maltose, galacturonic, fructose and glucosamine. This method is a common method used to determine the type of sugars.

2.1.6 Analysis of PTC-Amino Acid standard using HPLC (Wacosil PTC column)

In this analysis, we have to analyze an amino acid mixture, Glutamic acid, and Aspartic acid with different concentration. The purpose of this analysis is to determine the retention time of the amino acid. The key point this analysis is each sample have to be reacted with Phenyl Isothiocyanate (PITC) to able HPLC to detect the presence of amino acid. This is because amino acid has no chromophore group. This group is a part of a molecule responsible for its color. PITC is a type of UV detector. It has an absorbance at 260 nm. It combines its molecule with sample molecule and able the HPLc to detect the presence of amino acid. It is moderately sensitive. There is more sensitive UV detector such as fluorescence where it is 100 times or 1000 times more sensitive than PITC.

2.1.7 Other activities

During our internship, our supervisor has prepared some other activities for us. First is the Primary school Science Show. As mention before, we did some experiments for the Science Show. Then we went to a nearby primary school and present some interesting experiments for the students. Next is the presentation at the 3rd grade student for the 'Science English I' on the introduction of us.

2.2 The project

After the training, the supervisor gave us a final project for the presentation on August 19, 2013. The objectives of the project are:

i. To calculate the total sugar content in different vegetable samples.
ii. To determine the type of sugar present in the vegetable samples.
iii. To know the sugar distribution in the vegetable sample.
iv. To determine the type of amino acid present in samples
v. To calculate the total amino acids present in samples

The first part of the project is the collection of the vegetable samples. The vegetable samples were picked freshly from the Mie University garden ourselves. There are 21 different samples in total to be analyzed. The samples include varieties of tomatoes, eggplants, cucumbers, chilies, zucchini, and Japanese melon.

Next step, some part of the samples were extracted from the vegetables and later, by using Phenol-Sulfuric Acid assay, we determined the suitable concentration of the sample solutions before analyzing the samples using HPLC. This step is very important because HPLC cannot analyze the sample if the sample is in very high concentration. From this Phenol- Sulfuric Assay, we can measure the sample absorbance. If the absorbance is high that's means the sample is too concentrated. Thus, we only need a small sample to solvent ratio (sample : solvent).

There are two types of analysis for this project. First is the analysis of sugar content in the fruits and vegetables. The second is the analysis of amino acid presents in the fruit and vegetables. As mentioned before, this project is related to the training or the previous experiments we have done before. On the analysis of different types of sugar which are Glucose, Fructose, Sucrose, Maltose, and Cellobiose with different concentration, we analyze the retention time of the sugars. Based on the analysis, we then can determine the type of sugar present and also the total sugar in the samples by using a standard curve obtained from the graph of concentration versus peak area. This method can also be applied to the analysis of amino acid content and the total content of amino acid present in the samples.

Analysis of sugar content in fruit and vegetable

From the previous experiment, first we determine the type of solvent that is suitable for HPLC analysis. There are two types of solvents; water and 50% Acetonitrile. Based on the spectrum of HPLC analysis, we know that the most suitable solvent is 50% Acetonitrile. The difference in peaks between water and 50% Acetonitrile is shown below:

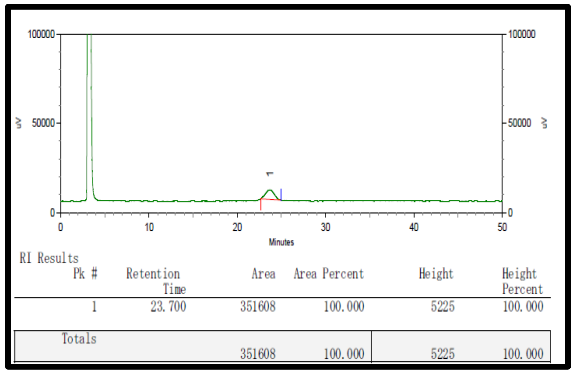


Figure 1. Dilution of Cellobiose using distilled water

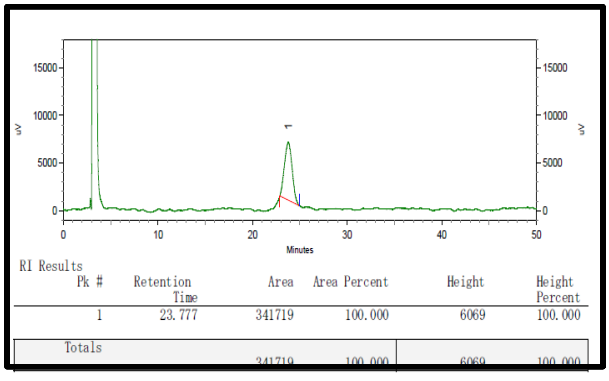


Figure 1. Dilution of Cellobiose using 50% Acetonitrile

From these two figures, we can see the differences between the peaks. The chromatograph for the dilution of Cellobiose using distilled water shows a broader and shorter peak. While the chromatograph of dilution using 50% Acetonitrile shows a sharper and narrower peak. A sharper and narrower peak shows a better result because it shows more accurate quantitation and no overlapping of the result. Thus, from this graph we know that 50% Acetonitrile is the most suitable solvent to be used in the HPLC analysis of the vegetables and fruits samples.

Next, we analyze the sample solution by using HPLC with 80% Acetonitrile as the mobile phase. From the peak area of the spectrum obtained, we substitute the peak area into the standard curve equation from the previous experiments. From there, we can then determine the sugar concentration of the fruit and vegetable and also the total sugar content in them by adding all the sugar concentration.

2.2.1 The result and calculation

Sugar content analysis

From the spectrum obtained, we determine the type of sugar present in the samples based on the retention time. Mostly of the samples contain glucose, fructose and sucrose. Below shows some of the result obtained from the HPLC analysis:

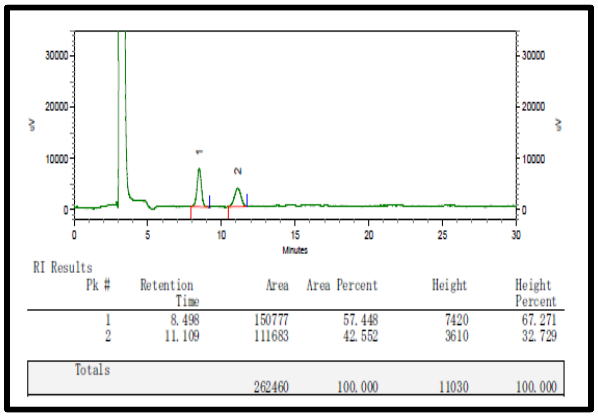


Figure 3 The chromatography of sample 13 (small, black type tomato)

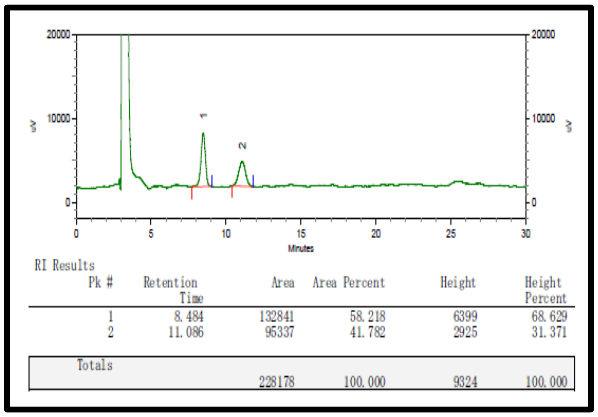
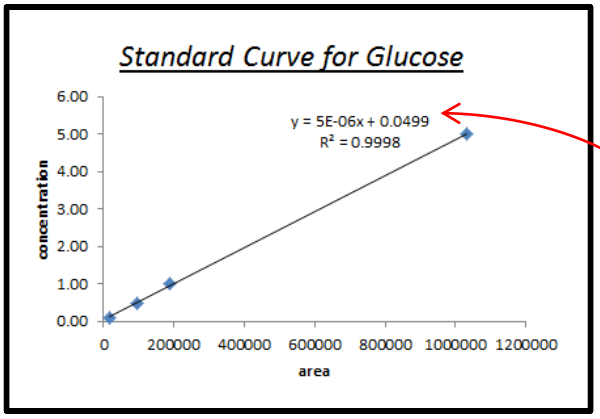
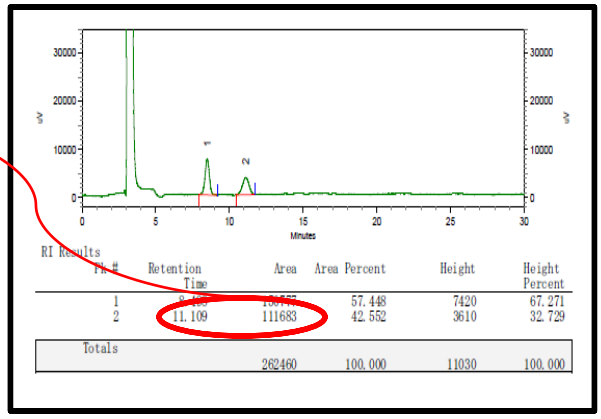


Figure 4 The chromatography of sample 20 (Japanese melon)

Then, we calculate the sugar concentration in each sample by substituting the peak area value into the standard curve equation.





The analyzed retention time of Glucose based on the previous experiment is 11.2 minutes. While the retention time for fructose is at 8.6 minutes. Therefore, we can identify the presence of Glucose and Fructose in the sample around 11.109 minutes and 8.498 minutes respectively. While the peak area value for Glucose and fructose are 111683 and 150777. Below is the calculation of the sugar concentration in sample 13 which are a small, black type tomato:

<u>Sugar concentration in sample 13:</u>

Glucose peak area= 111683

$$y = 5 \times 10^{-6}x + 0.0499$$
$$= 5 \times 10^{-6}(111683) + 0.0499$$
$$= 0.6083\ mg/mL$$

Glucose ratio in the sample 13:

$$\text{Ratio} = \text{sugar concentration} \times \text{mass of total fruit extracted} + \text{mass of water}$$

= 0.608315 mg/Ml x 10.136 Ml

= 0.00243

Fructose peak area=150777

$$y = 5 \times 10^{-6}x + 0.0476$$
$$= 5 \times 10^{-6}(150777) + 0.0476$$
$$= 0.8015\ mg/mL$$

Fructose ratio in the sample 13:

$$\text{Ratio} = \text{sugar concentration} \times \text{mass of total fruit extracted} + \text{mass of water}$$

= 0.801485mg/Ml x 10.136 Ml

= 0.00321

Total sugar content in sample 13:

$$Total\ sugar\ content = (glucose\ ratio + fructose\ ratio)\ \text{x}100\%$$

$$= (0.00243 + 0.00321)\ \text{x}\ 100\%$$

$$= 0.564\ \%$$

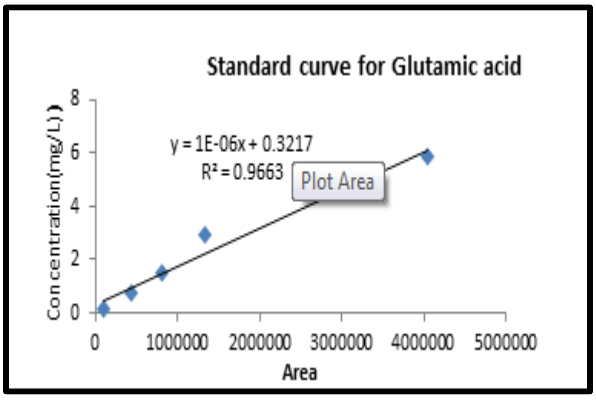
For Amino acid analysis too, we analyze the presence of Glutamic and Aspartic acid in the fruit and vegetables based on the chromatograph of amino acid mixture from the previous experiment. As the result shown, all fruit and vegetables shows the presence of Glutamic and Aspartic acid. The analyzed retention time for Glutamic acid and Aspartic acid are around 4.2 minutes and 3.9 minutes respectively. Below shows the result obtained from the HPLC analysis:

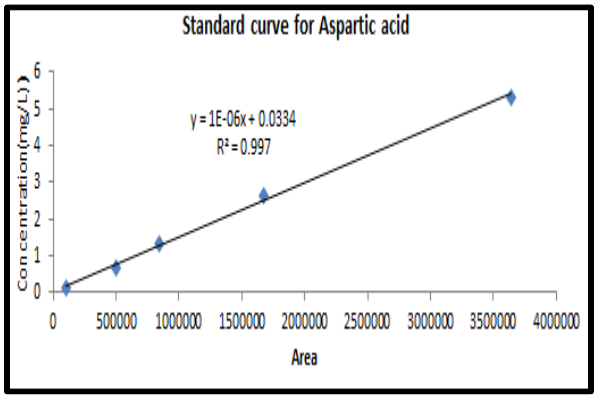
Glutamic acid

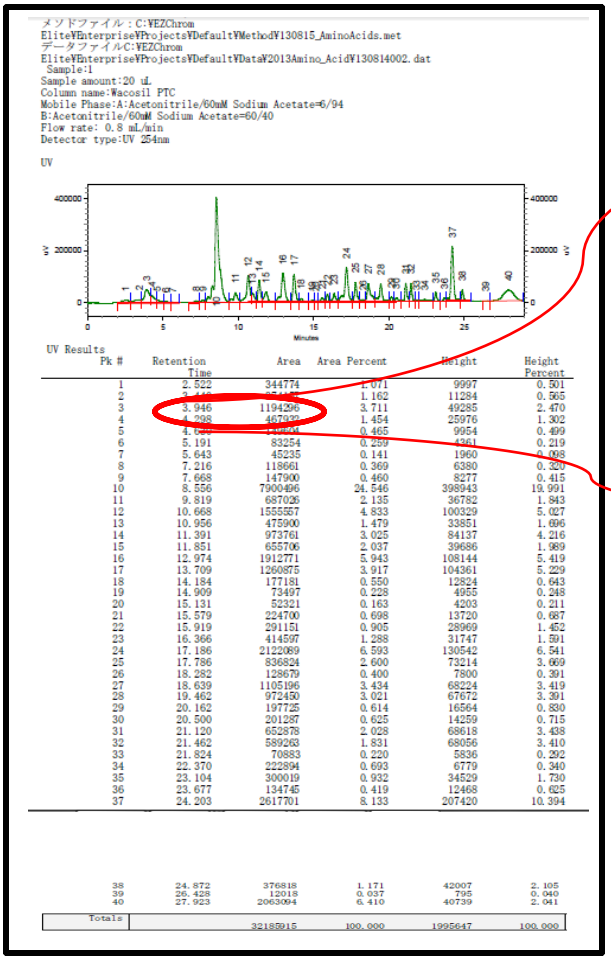
Peak Area	Concentration (mM/L)	Concentration(mg/L)
101690	0.001	0.14713
424993	0.005	0.73565
809859	0.010	1.4713
1330366	0.020	2.9426
4050285	0.040	5.8852

Aspartic acid

Peak Area	Concentration (mM/L)	Concentration(mg/L)
100855	0.001	0.13311
499618	0.005	0.66555
841735	0.010	1.3311
1669303	0.020	2.6622
3643993	0.040	5.3244





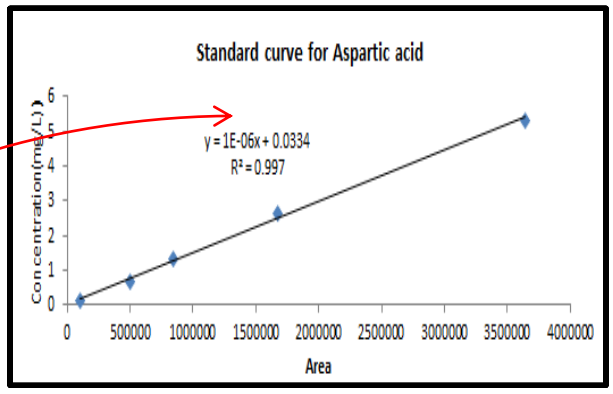


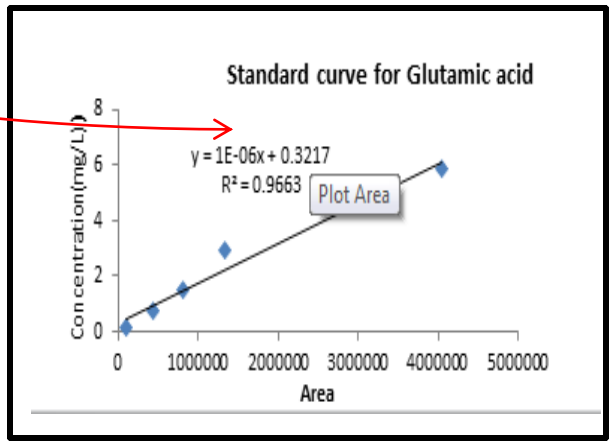
メソドファイル：C:¥EZChrom
Elite¥Enterprise¥Projects¥Default¥Method¥130815_AminoAcids.met
データファイルC:¥EZChrom
Elite¥Enterprise¥Projects¥Default¥Data¥2013Amino_Acid¥130814002.dat
Sample:1
Sample amount:20 uL
Column name:¥Wacosil PTC
Mobile Phase:A:Acetonitrile/60mM Sodium Acetate=6/94
B:Acetonitrile/60mM Sodium Acetate=60/40
Flow rate: 0.8 mL/min
Detector type:UV 254nm

UV

UV Results

Pk #	Retention Time	Area	Area Percent	Height	Height Percent
1	2.522	344774	1.071	9997	0.501
2	3.446	374452	1.162	11284	0.565
3	3.946	1194296	3.711	49285	2.470
4	4.298	467332	1.454	25976	1.302
5	4.685	149514	0.465	9954	0.499
6	5.191	83254	0.259	4361	0.219
7	5.643	45235	0.141	1960	0.098
8	7.216	118661	0.369	6380	0.320
9	7.668	147900	0.460	8277	0.415
10	8.556	7900496	24.546	398943	19.991
11	9.819	687026	2.135	36782	1.843
12	10.668	1555657	4.833	100329	5.027
13	10.956	475900	1.479	33851	1.696
14	11.391	973761	3.025	84137	4.216
15	11.851	655706	2.037	39686	1.989
16	12.974	1912771	5.943	108144	5.419
17	13.709	1260875	3.917	104361	5.229
18	14.184	177181	0.550	12824	0.643
19	14.909	73497	0.228	4955	0.248
20	15.131	52321	0.163	4203	0.211
21	15.579	224700	0.698	13720	0.687
22	15.919	291151	0.905	28969	1.452
23	16.366	414597	1.288	31747	1.591
24	17.186	2122089	6.593	130542	6.541
25	17.786	836824	2.600	73214	3.669
26	18.282	128679	0.400	7800	0.391
27	18.639	1105196	3.434	68224	3.419
28	19.462	972450	3.021	67672	3.391
29	20.162	197725	0.614	16564	0.830
30	20.500	201287	0.625	14259	0.715
31	21.120	652878	2.028	68618	3.438
32	21.462	589263	1.831	68056	3.410
33	21.824	70883	0.220	5836	0.292
34	22.370	222894	0.693	6779	0.340
35	23.104	300019	0.932	34529	1.730
36	23.677	134745	0.419	12468	0.625
37	24.203	2617701	8.133	207420	10.394
38	24.872	376818	1.171	42007	2.105
39	26.428	12018	0.037	795	0.040
40	27.923	2063094	6.410	40739	2.041
Totals		32185915	100.000	1995647	100.000

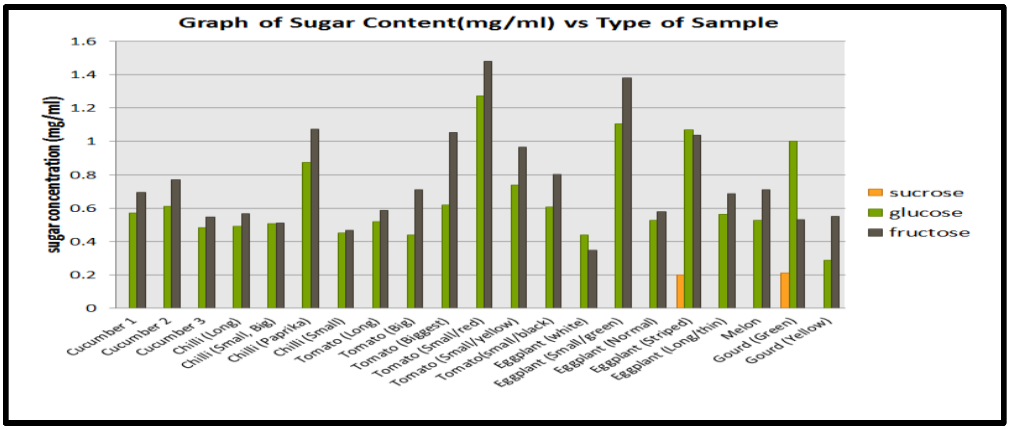


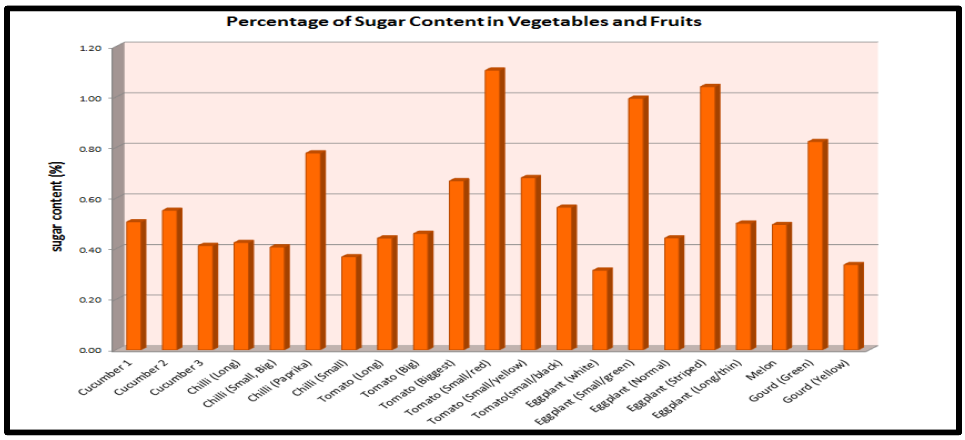


2.2.2 Discussion

From the analysis, the sample that shows the highest sugar content is the small red tomato, followed by the striped type eggplant and the green typed eggplant. While the lowest sugar

content is white type eggplant followed by yellow bitter gourd. Below is the graph of sugar content (mg/Ml) versus type of samples:





This result may explain why people more prefer to eat the small red tomato as in salad without cooking it as it is already sweet and no need to add any flavor to make it tasty.

2.2.3 Conclusion

Therefore, we can conclude that among all the samples, small red tomato has the highest sugar content and the lowest sugar content is in the white type eggplant.

CHAPTER 3 CONCLUSION

As for the conclusion, for these two months I have been followed the training course in Mie University, it has taught me a lot of things. The training has improved my laboratory skills and introduced me to a lot of related machines which will help me in the future. It also gives me the chance to experience and understand real life situations in industrial organizations and thus allowed me to apply the knowledge that I have learnt before in university. I also gain hands-on experience that is related to my majoring that is Chemistry.

But on top of that, the most important thing is this practical experience has developed a sense of responsibility towards the society inside of me. It encourages me to want to contribute something beneficial to the society when I graduate later. Other than that, the program has given me a once in lifetime chance that is absolutely precious and valuable for me. I am able to know and learn a lot about japan and meet with all the kind and beautiful people here in Japan. Therefore, I would like to say that this internship program is a really good and beneficial program for the students to experience. I am really glad to be one of the lucky participants. Once again, thank you very much for the given opportunity.