INDUSTRIAL TRAINING REPORT

CHM4901

DEPARTMENT OF CHEMISTRY

FACULTY OF SCIENCE

UNIVERSITI PUTRA MALAYSIA

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MATRIC NUMBER: 157046

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In preparing this report, I was in asking with many people especially is my supervisor's Associate Prof. Takashi Mishima at Mie University. I am also very thankful to my workmates especially in laboratory such as Toshihito Yuri, Koutatsu Sakakura, Budi Santoso and Shigetaro Arita for their guidance, advice and also motivation. They have contributed towards my understanding and thoughts.

For me it was valuable experience to be in laboratory of Mie University. During the internships I learned many new things that will help me to do my final year project and to face the working environment. Besides that, internship at the abroad is a very challenging and I learnt to be independent at the foreign country with the different lifestyle, culture and language.

1.0 EMPLOYMENT DETAILS

1.1 Employer contact details

Address: Graduate School of Regional Innovation Studies, Mie University

Address: 1577 Kurimamachiya-cho, Tsu City, Mie Prefecture, Japan 514-8507

Phone: +81-59-231-9632

Fax: +81-59-231-9634

E-mail: info (a) innov.mie-u.ac.jp

1.2 Period of employment

Started on: 24 July 2013 – 24 August 2013

1.3 Company background/history and organization structure

The history of Mie University dates back to 1648, when the Toyomiyazaki-bunko was established to the left of the Outer shrine of Ise Yamada. In August 1874, the Watarai Prefectural Normal School was established at this site. Industrial training for chemical students was placed at the section of The Graduate School of Regional Innovation fosters individuals who can assume leadership roles in the regional community, connected with regional industries centred in Mie Prefecture, and based on the regional industrial world and the academic-industry alliance model, to implement interdisciplinary research that is necessary to overcome growth constraints in the regional businesses. Based on this theory of education and research, this school also develops students into individuals who can plan and manage research and development.

1.4Position/Type of employment

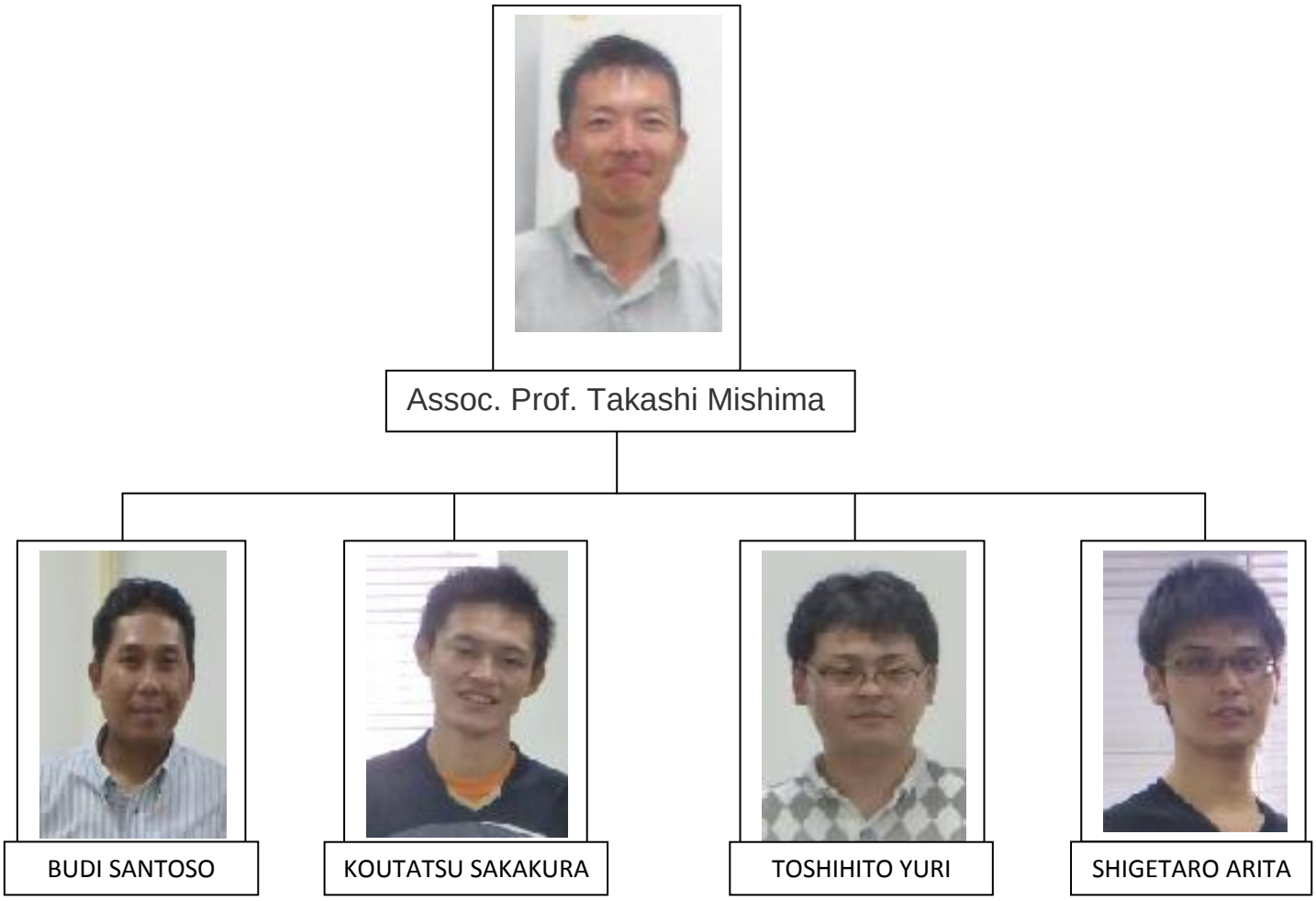
I was trained as trainee and being supervised by Associate Prof. Takashi Mishima (R&D), Food chemistry, Biomass laboratory. The description of work is on project and the scope of work is handling of equipments such as High-performance liquid chromatography (HPLC) analysis with different column, UV-1800 UV-Vis Spectrophotometer

1.5 Organization

1.5.1 President of Mie University

Atsumasa Uchida M.D.Ph.D

1.5.2 The organization in the laboratory



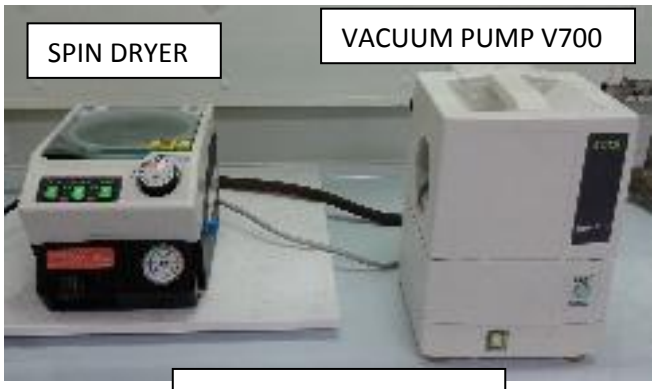
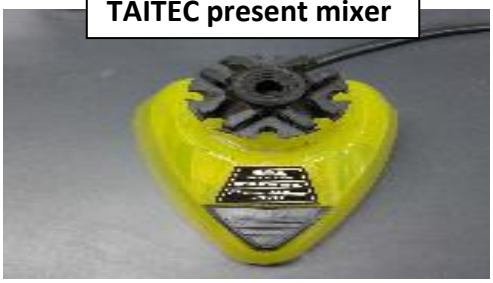
2.0 TRAINING

2.1 Activity and responsibilities

There are many activities or project that should be involved and be responsible towards the works given. The following table is a list of the types of projects which I was involved in during the course of my industrial training and the responsibilities and extent of my involvement in each. From this course I was learn to handle many instruments, how to used apparatus in laboratory and do the experiment accurately. All of the data were collected, then recorded and analyze by using the instrument depend on what type of our substances. Besides that, we also involved any outdoor activities such as science show with primary school.

Activity	Responsibility
1. Used pipetman correctly PIPETMAN ELECTRONIC BALANCE	• Measure the volume of distilled water by using pipetman. • Pipetman have many types with different measurement. • Weight the mass of distilled water by using electronic balance. • Decimal place is 0.0001g • Calculate the weight average and coefficient of variation by using excel
2. Determination of reducing and nonreducing sugar using phenol-sulfuric acid	• The standard solution was prepared for Glucose, Maltose, Galacturonic acid, Fructose and Glucosamine • Measure the absorbance when react with the phenol-sulphuric acid

3. Solubility Amygdalin with different solvent 	• Solvent: Distilled water,100% Ethanol and 50% Ethanol • Solute : Amygdalin in powder form • The solubility of Amygdalin was observe • observe the absorbance by using UV-1800 UV-Vis Spectrophotometer • Plot graph absorbance versus wavelength
4. Run HPLC by using AsahiPak NH_2P_5O-4E column for all standard sugar 	• Mobile phase: 70% and 80% of Acetonitrile. • Kept for 1 day before run the HPLC. • Make the dilution of all Standard sugar for Glucose, Fructose, Sucrose, Maltose and Cellobiose. • Dilute standard sugar with different concentration : 10.0,0.1,0.01,0.00 mg/mL • Sample was putted in glass bottle. • Run the HPLC 30 minutes for each sample • Collect the data • Sketch the graph concentration sugar versus Area of peak by using excel

5. Prepare PTC Amino acid CENTRIFUGE EVAPORATION   	• PTC Amino acid need to dry • Use centrifuge evaporation to dry the sample. • Sample was putted in the eppen tube. • Handle the experiment in the fume chamber. • Make sure all the solution totally mixed before add the mobile phase. • Shake the solution by using blender
6. Run HPLC by using Wakosil –PTC column for amino acid 	• Set up theHPLC • HPLC must be stabilize before analyze the sample • Run the sample. • Analyze for each sample- 47 minutes. • Collect the data and the peak of the sample. • Compare the area of peak for each sample. • Repeat the step above for Glutamic acid and Aspartic acid. • Dilute both samples with the different concentration.

3.0 Highlight on project

3.1 Objective

The objectives for this project are

i. Analysis the Standard sugar content in the vegetables and fruits
ii. Analysis the Amino acid content in the vegetables and fruits

3.2 Methodology

3.2.1 Extracted the fruits and vegetables for sugar and Amino acid measurement.

The fruit and vegetables got from the Mie University Farm near the Tsu City. The fruits and vegetables that would use must be fresh for measure the standard sugar and Amino acid content. All fruits and vegetables contain the different concentration of sugar and Amino acid. There are five standard sugar will be measure are Glucose, Fructose, Sucrose, Maltose and Cellobiose. While for Amino acid content are Glutamic acid and Aspartic acid. Firstly, the fruits and vegetables must be extracted before do the analysing.

i. Make sure all the samples must be weight and record.
ii. The sample was cut into small pieces about 2~3g and then weight of each sample must be record.
iii. Cut's sample was put in the eppen tube.
iv. Distilled water was added three time of sample's weight.
v. Use polyron to homogenize the samples and it take long time to blend the sample.
vi. Handle the equipment carefully because it is dangerous.
vii. The samples need to centrifuge 3000 rpm for 10 minutes to separate residue and extracted solution
viii. Supernatant was filter by using filter paper number two for HPLC
ix. 1.0mL of extracted solution was collected and put in the eppen tube.
x. Keep the solution in the refrigerator.

3.2.2 Standard sugar measurement.

All the standard sugar with different concentration is already measure. The standard curve of concentration (mg/mL) versus area will use to measure the concentration standard sugar for each sample. The concentrations for each standard sugar are 0.1mg/mL,0.5mg/mL,1.0mg/mL

and 5.0mg/mL. The concentration of extracted solution for each sample is unknowns. Before run the HPLC , the concentration of each sample must be know because if the sample have a high concentration ,the HPLC could not analyse the sugar content in the sample.

a) Thus, the sample must be reacting with Phenol-sulphuric acid to know the absorbance each sample.
    i. Prepare 10 times and 100 time dilution for each sample.
    ii. 500µL of 5% phenol and 2500µL of 96% sulphuric acid was added.
    iii. Handle the experiment in the fume cupboard
    iv. The solution will turn to brown color and boil vigorously.
    v. Leave the solution for 30 minutes until cold.
    vi. Measure the absorbance by using the Biochrom WPA CO7500 Colorimeter
    vii. Record all data
b) Dilution for HPLC
    viii. 100 time dilution is most suitable concentration for run the HPLC.
    ix. Dilute the sample with 50% of Acetonitrile for HPLC and fill in the small glass bottle.
    x. Before run the sample make sure that temperature and pressure is stable.
    xi. The condition of HPLC are
        a. Flow rate is 1min/mL
        b. Use the AsahiPak NH_2P_5O column
        c. Analyse for 30 minutes each sample
        d. Use 80% of Acetonitrile as mobile phase
        e. Detector type is RI
    xii. Collect the data and calculate the concentration of standard sugar for each sample from the peak area.
c) Preparation of mobile phase
    i. 1L of 80% of Acetonitrile will be prepare
    ii. 800mL of Acetonitrile is adding into 200mL of distilled water.
    iii. The solution must be shake to homogenize
    iv. Kept the solution in bottle for 1 day before use because Acetonitrile difficult to mix with distilled water.

3.2.3. Amino acid measurement

There are many types of Amino acid but for this project only Glutamic acid and Aspartic acid content will be measure. Both Amino acid is important for our taste but not for healthy because people will prefer to eat something that have taste. Tasty food is a main role in quality of life and it will make people enjoyment during eating. From the standard curve (concentration of Amino acid versus peak area) that has been plotted, the concentration for both Amino acids can be calculated for all samples. PTC Amino acid must be prepare before analyse the Amino acid contents by using HPLC.

a) Prepare solution A and B

- 3mL of solution A
    i. Ethanol: Deionized water:Triethylamine(TEA)= (800µL:800µL:400µL)
    ii. The solution was mixed by using TAITEC present mixer.
- 3mL of solution B
    i. Ethanol: Deionized water: TEA: Phenyl Isothiocyanate (PITC) = 2100 µL:300 µL:300µL:300 µL
    ii. Mixed up the mixture.

b) Preparation of mobile phase A and B

- 1000mL of mobile phase A
    i. 960mL of Sodium Acetate was prepared (pH6.0)
    ii. 60mL of Acetonitrile was added into Sodium Acetate
    iii. Kept the mixture solution in the bottle 1 day before use.
- 1000mL of mobile phase B
    i. 400mL of Sodium Acetate was prepared (pH6.0)
    ii. 600mL of Acetonitrile was added into Sodium Acetate.
    iii. Kept the mixture solution in the bottle 1 day before use.

c) Preparation of PTC Amino acid

i. 50µL of extracted solution was taken and put in the eppen tube.
ii. The sample must be dry by using the a centrifugal evaporator until the solution is dried.

iii. 100µL of solution A was added and mixed
iv. The mixture sample was dried by using centrifugal evaporator
v. 100µL of solution was added in the same eppen tube and dry it
vi. The solution must be totally dry before adding 200µL of mobile phase A
vii. the mixture was mixed and filtrate by using membrane filter (0.45µm)
viii. the sample solution was kept in the freezer

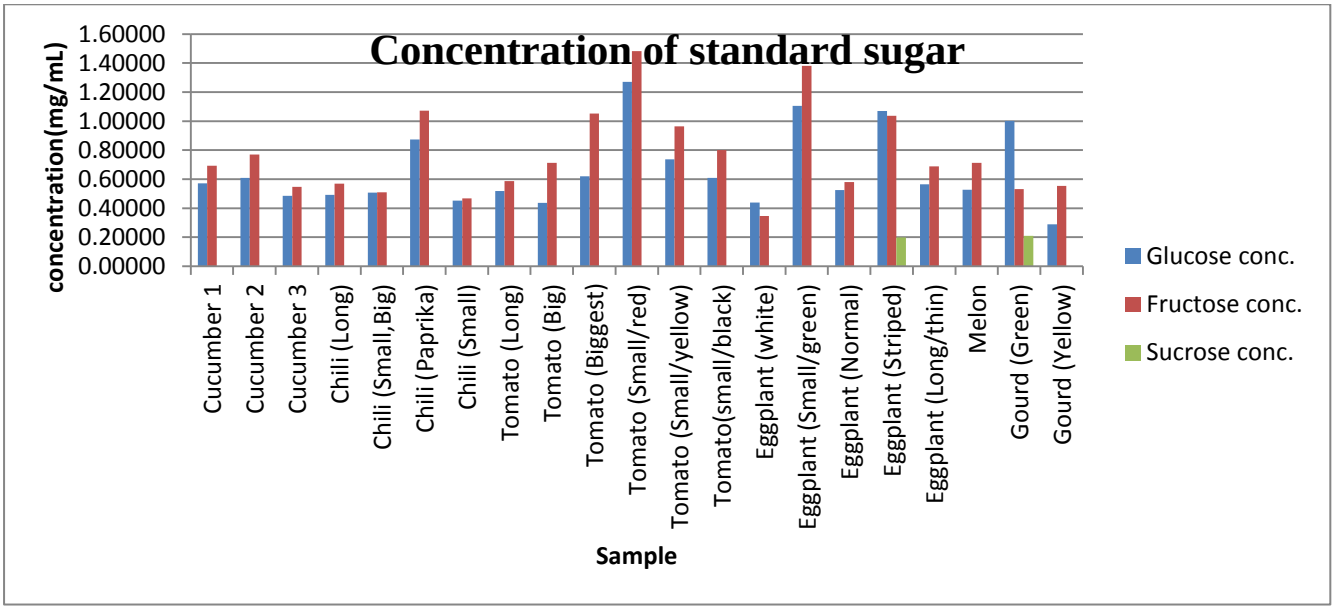
d) Run the HPLC

- The condition of HPLC:
  i. Use wakosil-PTC column
  ii. Flow rate is 0.8mL/min
  iii. Detector type is 254nm
  iv. Analyse 47 min for each sample
- Collect the data and measure the concentration of Glutamic acid and Aspartic acid.

3.3 Result

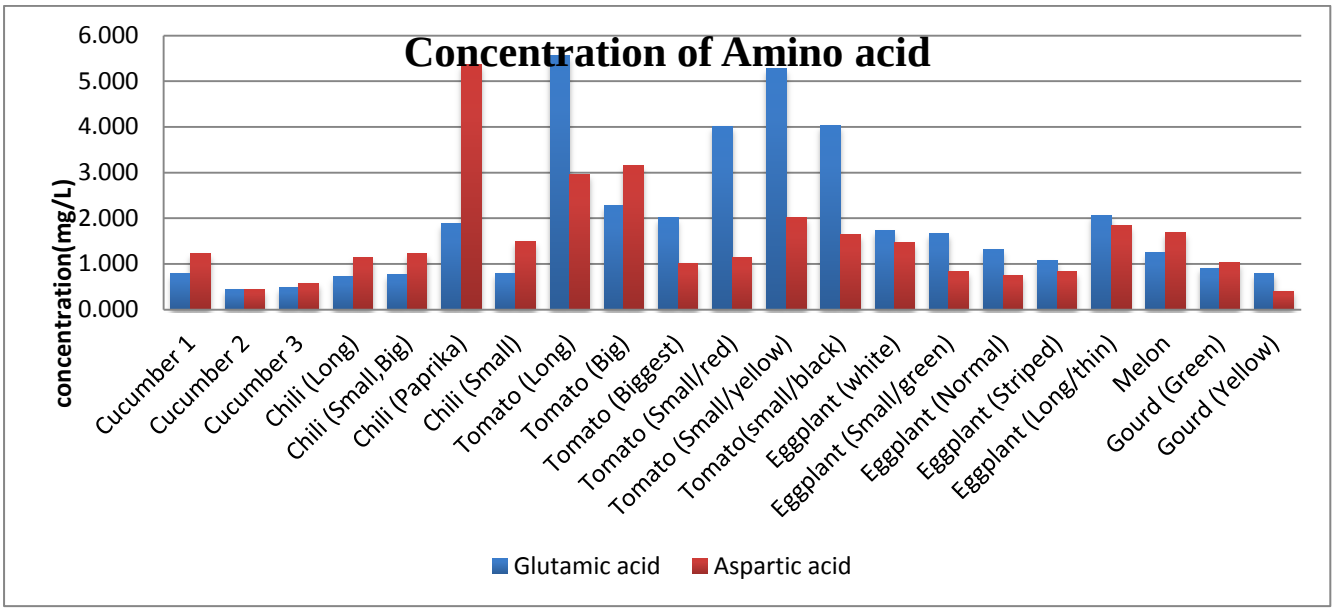
3.3.1 Standard sugar analysis

a) Concentration of standard sugar



3.3.2 Amino acid analysis

a) The result of concentration of Amino acid for each simple.



3.4 Calculations

3.4.1 Standard sugar content

The concentration of standard sugar will calculate from the standard curve of standard sugar. From the graph of concentration standard sugar versus area of peak, the concentration of sugar content for vegetables and fruit can be calculated.

Standard sugar	Standard curve
Glucose	y = 5E-06x + 0.0499
Fructose	y = 5E-06x + 0.0476
Sucrose	y = 5E-06x + 0.1101
Maltose	y = 6E-06x + 0.1405
Cellobiose	y = 1E-05x - 0.4109

<u>Sample 1: cucumber 1</u>

The area of peak from chromatogram of sample 1 is

Glucose: 104307 and Sucrose: 129218

The concentration of Glucose y = 5E-06X + 0.0499

*X is the value of area of peak.

y = 5E-06(104307) + 0.0499

= 0.57144 mg/ mL

The formula for ratio of concentration is

Ratio of concentration:

$$= \frac{\text{concentration of sugar x (total mass extracted sample + mass of distilled water)}}{\text{mass of extracted sample}}$$

$=\frac{0.57144X(10.364)}{2.591g}$mg/mL

=0.22857mg/mL

*calculations for standard sugar and Amino acid are same for all samples.

3.5 Discussion

This project we need to analyse the standard sugar and Amino acid content in the vegetables and fruits by using High-performance liquid chromatography (HPLC). The fruit and vegetable had been collected at the Mie University Farm. The sample must be extract to get their supernatant. HPLC formerly referred to as high-pressure liquid chromatography is a chromatographic technique used to separate the components in a mixture, to identify each component, and to quantify each component. In general, the method involves a liquid sample being passed over a solid adsorbent material packed into a column using a flow of liquid solvent.

There are 21 types of sample had been analyse. We used AsahiPak NH2P5O column and 80% of Acetonitrile as a mobile phase to measure the standard sugar. Mobile phase is the most important parameter in reversed-phase HPLC. Type of mobile phase used may have a big effect on the retention time. Retention time is the amount of time elapsed from the injection of a sample into the chromatographic system to the recording of the peak maximum of the component in the chromatogram. It can promote or suppress an ionization of the analyse molecules, and it also can shield an accessible residual silanol or any other active adsorption canters on the adsorbent surface. Proper selection of the mobile phase is the second most important step in the development of the separation method. In this project we choose 80% of Acetonitrile because the peak of standard sugar found is sharper and narrow compare 70% of Acetonitrile. The shape of peak is an important because it will shows the

concentration of each standard sugar. The flow rate is 1 mL /min and the detector for analyse sugar contents is ultraviolet (UV). Besides that, the solvent that use for dilution also play a main role in this analysis. The peak sample with solvent 50% of Acetonitrile is more sharp and narrow. Thus this peak is more accurate quantisation.

The concentration of standard sugar of each had been calculated from standard curve of Glucose, Fructose, Sucrose, Maltose and Cellobiose. All the sample contain of Glucose and Fructose while for Sucrose only for long and thin Eggplant and green gourd. The highest concentration of Glucose contents is red small tomato and the lowest contents are yellow gourd. For the highest Fructose concentration is small red tomato and the lowest is white eggplant. If the vegetables and fruit have the high total sugar content, the taste will be sweet. From the result of total concentration of sugar, the small green tomato the highest concentration of sugar and the white eggplant show the lowest sugar content. For striped eggplant and green gourd is also show the high sugar content because it contains Sucrose comparing the other sample only contains Glucose and Fructose. The retention time for Glucose, Sucrose, Frucrose, Maltose and cellobiose is 11.2 min.8.6 min, 17.8 min, 21.5 min and 24.5 min. For all samples do not have peak on 21.5 min and 24.5 min.

The second part of our analysis is measure the Amino acid content. There a lot of Amino acid but in this project we only measure Glutamic acid and Aspartic acid content. From the result both the Amino acid had found in the all sample but their concentration is different. We need to prepare the PTC Amino acid before analysis all the sample by using the HPLC. Each sample must react with Phenyl Isothiocyanate (PITC) to be able to detect by HPLC.In this analysis wacosil column will be use to analyse the Amino acid content. The sample must be analysing for 47 minutes each sample. The flow rate is 0.8ml/min and the detector is reflective index (RI). The RI is a measure of molecule's ability to deflect lighting a flowing mobile phase in a flow cell relative to a static mobile phase contained in a reference flow cell. We need to analyse Amino acid mixture at first and at the end of the experiment because to detect the retention time of Amino acid is change or not. The wacosil column are use two mobile phases and it has the different concentration. From the result, the peak of Aspartic acid will be appearing at time 3.99 minutes and Glutamic acid is at 4.22 minutes. The retention time of Amino acid will be same if we not change their mobile phase. All samples contain both of Amino acid but their concentration is different for all the samples. The

highest concentration of Glutamic acid and Aspartic acid is chilli paprika and long tomato. Therefore, both this sample tastier compares the other sample. While the lowest this Amino acid is cucumber number two. As we know Glutamic acid is usually used for flavour in the food and it is no side effects have been noted with this amino acid. There are few type of tomato that had been analyzed and the result show that for the small tomato the Concentration of Glutamic acid and Aspartic acid is more higher compare to the biggest tomato. This is because of the biggest tomato has a higher volume of water. That's why the concentration of both Amino acids is lower compare to the smaller size. The highest total concentration of Amino acid is chili paprika. People will be interested to eat chili paprika because it is tastier compare cucumber number two because is tasteless.

3.6 Conclusion

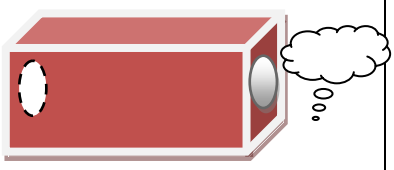
From the analysis of sugar and Amino acid content by using the HPLC, their concentration will be known for each sample. For the sample that has a higher sugar content the taste will be sweeter and for Amino acid the sample is tastier and suitable used as flavor in the food.

4.0 Other Activity

During in an industrial training, the others work that I done is join the science show with primary school. We had done three experiments to show to them

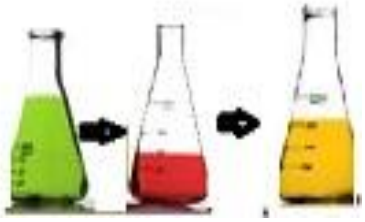
a. Air box gun with dry ice.
    i. Make a hole at the box and dry ice was put in the box. All side of box was cover by scotch tape and tap at the both side of the box. Then, we can see the blow smoke will come out from the hole of the box
b. Balloon in the liquid nitrogen
    i. Make a puppy from the balloon and the balloon was put into the liquid nitrogen and then balloon will be compress immediately. It will turn to normal shape when put at outside.
c. Color change

i. Mix the Glucose, distilled water, Sodium hydroxide and indigo carmine and shake the solution. It will change from green to red and to yellow. The solution will turn back to green color when it static.



Air box gun with dry ice

Balloon in liquid nitrogen



Colour change

5.0 New Tools and Technologies

There are many new tools and high technologies that I used during the internships. The equipment is more accurate and easy to handle. For example at the below:

1. Eppendoft tube

There are many sizes of eppendoft tube and capped plastic tubes. It is usually use for non toxicity reagent. For the sizes range about 0.5-1.5mL it will thrown way after use. It is easy to used compare put in the test tube and easy to keep because has a cap. For eppendoft tube with yellow caps usually the size around 15mL until 50mL and it will reuse.

2. Pipetman

Pipetman is used for measure the small volume of liquid around 10µL to 1000µL. it is more accurate and easy to use compare with pipette. Besides that, it will avoid the solution become contaminate because after use the tube it will thrown out. Furthermore, the pipetman are durable because it made from plastic.

3. Distilled water equipment

Distilled water is water that has many of its impurities removed through distillation. Distillation involves boiling the water and then condensing the steam into a clean container. We used distilled water because it does not contain any ion compare tap water. When used the HPLC, distilled water also play a main role. We need to filter the distilled water before used for HPLC. In our laboratory it provides the equipment that produces distilled water and already filters the distilled water.

4. Syringe's filter

Supernatants are difficult to filter by using normal filter paper. Thus, by using syringe's filter the solution that will get from filtration is totally clear and suitable for HPLC. It will filter the entire residue in the supernatants.

6.0 Suggestions for the Improvement of Training

The suggestion is to extend the industrial training more than three month because some of company more prefer to accept the student will do the industrial more than three month. Besides that, student also will learn many things and more experience that we will get from the internship. Especially for the student do the internship at the abroad two month is not enough. We only handle one project because we do not enough time to do another project. If we do more than three month probability to get the scholarship during the internship is high because internship at the abroad use more money compare at our country. Besides that, student should be providing to do internship at the abroad because they will expose to different and high technologies, management styles and achieve more knowledge. Furthermore, it will gain career experience while gaining international experience that will be helpful when applied work in the country. They also can improve their language skill and self confidents. Besides that, student should be preparing their knowledge before entering the laboratory.

7.0 Conclusion.

The experience and knowledge during the internship at Graduate School of Regional Innovation Studies, Mie University it was great. This organization has a superb work culture,

great minds and very high quality of work. At the laboratory, they provide many instrument and their student will conduct the entire instrument by their own. During the internship, I was introduced and learned to handle equipment such as HPLC for measure the standard sugar and Amino acid contents, UV-1800 UV-Vis Spectrophotometer and Biochrom WPA CO7500 Colorimeter for measure the absorbance and other equipment. The knowledge and skills get from the internship will be use to apply a work and do the final year project. Working with people from different country was a rare chance and it was another opportunity to make friends and share ideas. During the internship I saw that Japanese people really hardworking and very helpful. They will help us until the problem is solved. Besides that, when student do the internship at the abroad they not only get the knowledge during working but they also will learnt how to be independent and survive at the foreign country with different culture, lifestyle and language.

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