INDUSTRIAL TRAINING REPORT

NORANIZA BINTI AHMAD DAUD

BACHELOR OF SCIENCE (HONS) MAJOR IN CHEMISTRY

FACULTY OF SCIENCE

UNIVERSITY OF PUTRA MALAYSIA

CENTER FOR INTERNATIONAL EDUCATION AND RESEARCH

FOOD CHEMISTRY, BIOMASS

GRADUATE SCHOOL OF REGIONAL INNOVATION STUDIES

MIE UNIVERSITY

DATE OF INDUSTRIAL TRAINING: 1st JULY till 24th AUGUST 2013

ACKNOWLEDGEMENT

It is always a pleasure to remind the fine people in the MIE University for their sincere guidance I received to uphold my practical as well as laboratory skills in Chemistry.

First of all, thanks to my parent for giving encouragement, enthusiasm and invaluable assistance to me. Without all this, I might not be able to complete this subject properly.

Second, I would like to thanks to Dr. Nor Azowa Ibrahim, Deputy Dean (Academic, SA and Alumni Division) for give us the opportunity to undergo industrial training abroad. She also gives me their guidance and support.

Thirdly, I also want to express my deepest thanks to Dr. Norhazlin Zainuddin as industry training advisory for Chemistry Department that has helped me a lot in dealing with industrial training placement. She had supported to me by showing different method of information collection about the University. She helped all time when we needed and she gave right direction toward completion of project.

Besides, I would like to thank Prof Takashi Mishima (Head and Senior Lecturer/Department of Bioresearch), Koutatsu Sakakura, Budi santoso, Toshihito Yuri, and also Shigetaro Arita (Teaching Assistant) of Bioresearch Laboratory for extending their friendship towards me and making a pleasure-training environment in the Bioresearch Laboratory.

Thanks also to Miss Mika Deguchi as a person in charge of international relations office of Mie University which has helped to manage matters related to this training industry. A paper is not enough for me too express the support and guidance I received from them almost for all the work I did there.

Finally I apologize all other unnamed who helped me in various ways to have a good training.

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INTRODUCTION

Being the only national university located within Mie Prefecture, it has provided wide variety of departments, from science to humanities. The Graduate School of Bioresources contains an independent graduate course focused on the foundation of bioresources. The Graduate School of Bioresources has a master's program for students who aspire to continue their studies after graduating from the undergraduate program and a doctoral program for those who wish to enrich their studies even further. This school also offers the worker special selection exam that engineers and researchers, who work in various institutions and businesses, can take to become eligible to enter the university. This school proactively promotes the acceptance of international exchange and foreign exchange students from counties all around the world. In collaboration with Universiti Putra Malaysia, we have go there to undergo industrial training to enhance the skills laboratory and also in preparation for the working soon.

I have done doing my industrial training at the Graduate School of Bioresources at Mie University of Japan that began from 1st July 2013 until 24th August 2013. For being there, I have been trained to analyse some ingredients in foods such as carbohydrate and amino acid. We have learned some dilution method. We also been introduced with many way to determine the total concentration of carbohydrates present in foods for example by using HPLC machine or by using phenol sulphuric acid method.

Besides that, we manage to use the HPLC and UV machine properly.
We also participate some activity that had been organised by university. At last, we had been run one project which is to analysed the food contents.

ANALYSIS IN LABORATORY

1.   DILUTION/PREPARATION SAMPLE

Generally, we are trained to prepare the solution of different type of sugar with different concentration. Usually we will use this basic formula;

$$n = \frac{MV}{1000} = \frac{mass}{molar\ mass}$$
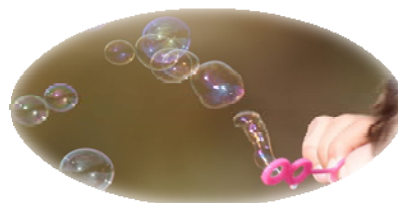
$$M_1V_1 = M_2V_2$$

OTHERS ACTIVITY

SCIENCE SHOW

1. Bubble solution

SET A

- PVA
- Distilled water
- Glycerin
- Soap

SET B

- PVA
- Distilled water
- Sucrose
- Soap

2. Liquid Nitrogen with balloon

At first, prepare the balloon. Then soak the balloon into the liquid nitrogen. The balloon will be shrink. After we take out again the balloon from the box, it will expand again as before.

HPLC

Liquid chromatography (LC) is a method of a separation which employs a liquid mobile phases and either a solid or liquid immobilized on a solid. A liquid may be immobilized on the solid by chemical reaction with the solid, by reaction to form an insoluble polymer deposited on the surface or it may be a liquid that is insoluble in the mobile phases to be used. Liquid-chromatography was usually to separate and isolate variety of compounds. HPLC have several types of detector which are based on Refractive index measurement and UV detectors.

Mobile Phases

Common mobile phase uses include any miscible combination of water with various organic solvents for example acetonitrile and methanol. The composition of mobile phases may be kept constant (isocratic mode) or varied (gradient mode) during the analysis. When changing new mobile phase, it is hard to get the same concentration as before and it may change the retention time. So as the precaution step, we need to analyse using the same mobile phase.

The UV Detector

There are several ways to detect substances when it passed through the column. One of it is by uses ultra-violet absorption. Generally, most organic compounds absorb UV light of different wavelengths. If we have a beam of UV light shining through the stream of liquid that coming out from the column, and the UV detector is located in front of the stream, easily we can get a direct reading of how much of the light had been absorbed. In addition, the solvent or mobile phases will also absorb the UV light but it differ as the other compounds as the different compound will absorb more strongly in different parts of the UV spectrum. We had used this detector to measure the amino acid present in the sample. We cannot use this detector to analyse sugar because sugar don't absorb UV light due to don't possess chromophore in their structure. So for measuring the sugar, usually we used the refractive index detector.

The Refractive Index Detector

This detector's sensitivity is limited. This detector can be very useful for detecting those compounds that are non-ionic, do not adsorb in the UV, and do not fluoresce. This is a property of all liquids and solids through which light can pass. As the light passes through the material, the beam is refracted and the extent of this refraction is measured by the Refractive Index.

PHENOL SULPHURIC ACID METHOD

The phenol-sulphuric acid method is an example of a colorimetric method that had been used to determine the total concentration of sugar present in food. First, the clear solution of sugar that wants to analyse was placed in a test tube. After that, phenol and sulphuric acid are added. Let the solution react for about 30 minutes and the solution will change the colour into yellow-brownish colour as a result of interaction between the carbohydrates and the phenol. The present of sulphuric acid will causes all the non-reducing sugar to be converted to reducing sugars and from that we can determines the total sugars content in solution. Usually we used the absorbance at 420nm which is proportional to the carbohydrate concentration initially in the sample. After we get the reading, we need to make the calibration standards of sugar.

AMINO ACID DETECTION

Amino acid analysis is an important application area in protein chemistry and food analysis. We had learned how to analyse the amino acid components by using HPLC. By using the UV detection, its means that it required the absorption of the carboxyl group (-COOH) which is in range 200~210nm. Some amino acid that content benzene rings also can be detect in 250~280nm range but in general, they are difficult to analyse.

The pre column derivatization method had been applied here. In this method, the amino acids are derivative by reacting the free amino acids, under basic conditions, with phenylisothiocyanate (PITC) to produce phenylthiocarbanyl (PTC) amino acid derivatives. An amino acid mixture was also has been derivatised to

generate a calibration file for references. The benefits of this method are the consumption rate of reagent can be minimized by specifying a small reaction system.

ANALYSIS OF FOOD CONTENTS

OBJECTIVE:

1. To analyse the sugar content in some type of fruit and vegetables.
2. To analyse the amino acid content in some type of fruit and vegetables.
3. To increase the proficiency of using HPLC machine.

INTRODUCTION:

Investigation in food content, whether by the food industry, governmental agencies, or universities, often required determination of food composition and characteristics. As we know, different kind of food has various compositions. Therefore, consumers need to know the composition of food to ensure food safety and food qualities guaranteed.

Combination of amino acids with their respective tastes is a key determinant for the taste of food. Relationship between amino acids and taste has been explored since the discovery of glutamate as umami ingredient. Determination of amino acids contained in food revealed that the taste we feel mostly depends on the kinds and amount of the amino acids.

In this project, we will use the HPLC machine with Refractive Index Detector to detect the present of sugar inside the all extraction. Different type of sugar will have different retention time. So from the result we get, based on retention time, we can know what type of sugar present in the sample.

METHOD

EXTRACTION OF SAMPLE SOLUTION FROM FRUIT

1. The fruit had been cut about 2~3g and transfer into the PP tube.
2. Then, distilled water was added into the tube as much as 3 times the weight of the fruit that had been cut before.
3. The sample solution had been blended by using polytron until get the homogenize solution.

4. After that, the homogenize solution had been centrifuge for about 10 minutes with 3000rpm speed.

5. The supernatant has been filtered by using filter paper no.2 and these products had been kept in fridge.

SUGAR MEASUREMENT

1. 1.0µl of the extracted solution has been filtered by using membrane filter (0.45µm **Ø)**

2. This solution has been kept in eppendolf tube.

3. The mobile phase had been prepared which is 80% acetonitrile.

4. The condition for HPLC had been set up and the sugar content for each fruit had been analysed.

AMINO ACID MEASUREMENT

1. The mobile phase was prepared from acetonitrile and 60mM of sodium acetate of pH6.00 with ratio 6/94 (v/v).

2. (Ethanol/Distilled water/Triethylamine(TEA)) had been mixed with ratio of (2/2/1) and this solution was labelled as solution A.

3. (Ethanol/Distilled water/TEA/PITC) had been mixed with ratio of (7/1/1/1) and this solution was labelled as solution B.

4. 50µl of extracted solution was put inside the eppen tube.

5. The solution had been dried by centrifuge evaporator for 15 minutes.

6. Then, 100µl of solution A was added into the eppen tube and mixed the solution before dried again for 15 minutes.

7. 100µl of solution B was added into the eppen tube and mixed the solution before let it to react for 20 minutes in room temperature.

8. Then, the solution was dried again by centrifuge evaporator for 15 minutes.

9. The solution was mixed with 200µl of mobile phase that have been prepared before.

10. 20µl of the solution was inject for analysed with HPLC using Wacosil PTC column.

   The condition of HPLC are as stated below:

   Column oven 40^oC

   Flow rate 0.8ml/min

   UV 254nm detection

   The data had been recorded.

RESULT & DISCUSSION

Most vegetables and fruits are taste exceptionally sweet due to high levels of sugars they contain. Not all fruits contain the same amount or type of sugar and amino acid. Some fruit contain high level of sugar like tomato and eggplant, others hardly has little bit. Some fruits contain mostly glucose and fructose while other high-sugar fruits contain no sucrose at all. From graph 1, it shows the amount of sugar present in the sample. We can see that all fruit and vegetables contain glucose and fructose but only green gourd and striped eggplant have sucrose. Next, graph 2 shows the percentage of sugar content in sample. This is the ratio of sugar content. We can see that small tomato has the highest sugar content with about 1.11% followed by the striped eggplant with 1.04%. The lowest percentage of sugar content is white eggplant and yellow gourd with 0.31% and 0.34% respectively. As conclusion from this project we can said that all type of fruit and vegetables contains sugar but in different amount.

For amino acid measurement, we only analysed two type of amino acid which is glutamic acid and aspartic acid. Based on the result in Graph 3, the highest content of amino acid is long tomato followed by chilli paprika and small yellow tomato with amount 3.405%, 2.901% and 2.924% respectively. For the Graph 4, it show the concentration of each glutamic acid and aspartic acid. We can see that the highest concentration of glutamic acid inside the long tomato with 5.55mg/L followed by yellow tomato with 5.28mg/L. on the other hand, the aspartic acid is highest inside the chilli paprika with 5.37mg/L.

The results that we get may not be accurate because the amount of sugar and amino acids in fruits and vegetables are different based on their maturity levels. Maybe some fruits and vegetables that we collect have achieve a maturity and some of them are not and still young.

SUGESSTION

Based on experience for 2 months in Laboratory of Bioresearch, this suggestion can be taken into consideration to upgrade future practical training in this place:

1. The duration of training may can be longer as it will be more beneficial, giving more exposure to the trainers to allow them to understand the flow of project. The shorter duration was not viable for practitioners to let the trainee handle a project more comprehensively. The longer duration would give the students more time to explore and experience on the project.

CONCLUSION

Japan is one of the countries that have the advanced technology besides diligent and good attitude of people in doing work. By training under the laboratory of this University country, a lot of knowledge and experience can be gained and built. Moreover, this laboratory is suitable for undergraduate students who will work soon or further their study in research field. From this industrial training, student can improve their laboratory skills and also can increase the science knowledge.

Besides, I also can train myself to be good in attitude while doing my work. In addition, the knowledge and research skills can be improved as when I was doing project of analysis food content. This is important things for our chemist or science student that working on laboratory.