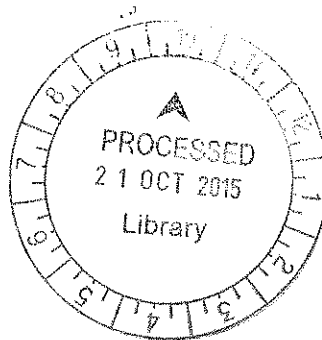


**GLUCOSE AND ALCOHOL CONTENT AND  
DIGESTIVE ACTIVITY OF ENZYMATIC DRINKS**

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**KOH ZHI JIE**



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**FACULTY OF SCIENCE, TECHNOLOGY,  
ENGINEERING AND MATHEMATICS  
INTI INTERNATIONAL UNIVERSITY**

**JUNE 2015**

GLUCOSE AND ALCOHOL CONTENT AND  
DIGESTIVE ACTIVITY OF ENZYMATIC DRINKS

KOH ZHI JIE

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF  
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## ABSTRACT

Many experiments had been done to determine the glucose and ethanol levels in foods and beverages, such as wine, energy drinks, bread, fruit juices and etc. To date, no research had been conducted on 'fruit enzyme'. The first aim of this research was to determine the glucose level using Glucose Hexokinase (HK) Assay kit and spectrophotometric determination. The second aim of this research was to determine the alcoholic level in each of the tested sample using spectrophotometry. The third aim of this research was to analyse the effect of 'fruit enzyme' in digestion of carbohydrate, lipid and protein. Glucose (HK) Assay kit and spectrophotometry were used to determine the glucose concentrations in each 'fruit enzyme', whereas the alcohol concentrations were determined using spectrophotometry. Benedict's test and Iodine test were performed to confirm starch digestion; Phenol red was used as pH indicator in fat digestion; Biuret test was performed to confirm protein digestion. By using Glucose (HK) Assay kit and spectrophotometry, the glucose content in each sample resulted the highest glucose content was found in 'Commercial' sample, whereas the lowest glucose content was found in 'Fresh' sample. Under the assay of alcoholic determination, the highest alcohol content was found in 'Fresh' sample, whereas the lowest alcohol content was found in '1 week' sample. Besides, for the assay on digestion of carbohydrate, lipid and protein by various samples, the results showed that there were no any enzyme activity occurred in each home-made 'fruit enzyme' in general. In conclusion, the 'fruit enzyme' or enzymatic drink' was not suitable to be termed as 'enzyme' since they had no pure enzyme but a mixture of organic compounds.

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## LIST OF ABBREVIATIONS

4-AP	4-aminiantipyrine
Abs	Absorbance
AOX	Alcohol oxidase
°C	Degree Celsius
cm	Centimeter
CO <sub>2</sub>	Carbon dioxide
dH <sub>2</sub> O	Deionized water
g	Gram
GOX	Glucose oxidase
GSS	Glucose stock solution
H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
HCl	Hydrochloric acid
HK	Hexokinase
HRP	Horseradish peroxidase
min	Minutes
mL	Mililiter
mM	Milimolar
nm	Nanometer

## 1.0 CHAPTER 1:

### INTRODUCTION

What actually is a real enzyme? As we all know, enzyme is the protein that produced by cells in the human body and all other living organisms (Aehle, 2007). Enzymes act as protein catalysts and they can catalyze biological reactions under certain conditions that maintain life (Aehle, 2007). Numerous sorts of enzymes exist and each of them function separately and significantly. Exploration on enzymes has now entered another stage with the combination of thoughts from molecular biology, protein chemistry and molecular biophysics (Aehle, 2007). Undeniably, without enzyme, without life.

In the market, some products are referred as 'fruit enzymes' or 'enzymatic drinks'. They are produced from fermentation processes. The producer claimed that the product contain enzymes from vegetables and fruits. During fermentation process, the enzyme activity in vegetables and fruits might decrease as time passed (Hui, 2004). After fermentation, the final product so call 'fruit enzyme' is produced, alcohol will be produced as by-product during fermentation because microorganisms utilize sugars such as glucose and fructose, then convert the sugars into alcohol and carbon dioxide (CO<sub>2</sub>) (Choo, 2015). Most people believed that drinking this particular 'fruit enzyme' can nourish human body immune system, relieve digestive discomfort, detoxification and revitalization of human body cells (Huang & Huang, 2007).

Enzymes usually are sensitive to pH and temperature. All of the enzymes have their optimum temperature and pH range for their particular activity (Aehle, 2007). The optimum temperature for most of the enzymes typically between 40 and 60°C whereas the pH range 5 to 7 is the optimum value for most enzymes (Aehle, 2007). Changes of those values influence the collaborations of protein chain and cause enzymes to denature (Aehle, 2007). Since they are sensitive to pH and temperature, so how do they be conserved in the enzymatic drinks? During fermentation, the processes

also produce alcohol, so it also related to religious issues. However, none of the commercialize products indicate the alcoholic content in most of the market currently.

Public are consuming this “fruit enzymes” without proper knowledge about the content of this “fruit enzymes”. Hence, it is very important to clarify the doubts and myths on “fruit enzymes”, therefore, the objectives of this study are (a) to determine the glucose level using Glucose Hexokinase (HK) Assay Kit (b) to determine the glucose level using spectrophotometry by enzymatic reactions (c) to determine the alcoholic level using spectrophotometry by enzymatic reactions (d) to determine the effect of “fruit enzyme” in digestion of carbohydrate, lipid and protein.

## 2.0 CHAPTER 2:

### LITERATURE REVIEW

#### 2.1 ENZYME

In the early 19<sup>th</sup> century, the presence of living microorganisms was a matter of verbal confrontation. Louis Pasteur, was the first scientist to study the enzyme reaction (Barnett, 2007). He presumed that the fermentation was catalyzed by a crucial power contained inside the yeast cells called ferments, which he thought only worked inside living life forms (Manchester, 1995).

As we know, microorganisms utilize the enzymes to operate various functions (Agarwal, 2006). But, actually how really we understand what a real enzyme is? In fact, enzymes are the proteins produced by cells in the human body or other living organisms. Most of the enzymes are in charge of catalyzing reactions of biological systems in whole living cells (Agarwal, 2006; Alberts, 2002). It is extraordinary that enzymes are very useful catalysts because they able to accelerate many chemical reactions (Agarwal, 2006). It's been a long time showed that the enzyme activity related to their structure had been described as a "lock-and-key" and "induced-fit". The theory suggested that the structural collaborations between enzymes and substrates play an important role in protein catalysis (Berg, Tymoczko, Stryer & Stryer, 2002).

For those people without scientific background, they define enzyme as fermented drink that produced from fermentation processes. Many people believe that this kind of enzyme drink has high nutritional value and able to enhance the immunity and health of human. However, the real definition for enzyme is a kind of protein but not a beverage. This issue was caused by misunderstanding and misconception of the production process and has misleading many people.

## 2.2 FERMENTATION

In food and beverage processing, fermentation is the conversion of carbohydrates into alcohols and carbon dioxide (CO<sub>2</sub>) using bacteria or yeast. Foods were preserved traditionally through naturally occurring fermentation, nevertheless, modern large scale generation currently exploits the utilization of characterized microorganisms strain systems to guarantee quality and consistency of the final fermented product (Paul, Morgan & Hill, 2002). The alcoholic fermentation including brewing and winemaking had been accidentally discovered during 2000-4000 BC by the Sumerians and Egyptians (Paul *et al.*, 2002). The Egyptians additionally developed dough fermentation used as part of leavened bread production. Despite the fact that fermentations have been exploited as a system for preservation of food and beverage for a large number of years, it has just been in the more recent past that microbes were discovered as responsible in the fermentation process (Paul *et al.*, 2002).

The term 'Fruit Enzyme' has been known by the public. It was a collective term for the liquid solution made by different fermentation processes, to declare that this fruit enzyme drink contains enzymes from those natural products. But, there is no any scientific investigation proof to show what is really in this solution other than high level of alcohol and fruit residue. Fruit enzyme is cited on the Internet as made by adding sugar and lemon with pieces of fruits and keep in glass container at room temperature for two to three weeks before serving. The 30 mL of the resulting beverage is mixed with 10 times of water and it is ready to be served (Huang & Huang, 2007). According to "Healthy Enzyme DIY", the enzyme drinks able to perform detoxification, regulation, tonification and medication (Huang & Huang, 2007). Detoxification involves cleansing of blood, body and intestine, body heatiness; regulation involves the body's internal balancing; tonification involves enzyme remedies that nourishing body and enhance body function; last but not least, medication involving prevention of some common diseases such as fever, common flu, cough and etc. (Huang & Huang, 2007).

Nowadays, most of the sugar found in the market is cane sugar, a common disaccharide. This particular disaccharide is digested into monosaccharide such as glucose and fructose and over consumption of these sugars will lead to chronic diseases, especially diabetes (Huang & Huang, 2007). In addition, yeast and living microorganisms utilize the sugars as a source of food and will release alcohol and also carbon dioxide as a by-product. If a lot of sugars added, the final product of enzyme drink will taste like wine (Huang & Huang, 2007). The lethal dose of alcohol is 5 to 8g/kg for adult and 3g/kg for children (Lohr, 2005). The alcohol will damage the human nerve system and neurotransmission channel. For those who having liver problem and hepatitis B carrier, they also should avoid consuming any alcoholic beverage (Huang & Huang, 2007).

### **2.3 UTILIZATION OF ENZYMES IN FOOD PRODUCTION**

Today, bread and baked products are among the major nutritional sources. Europeans are assessed to get roughly half their required carbohydrates and around 33% of their protein from bread (Capra, 2006).

From the beginning to end of the history of bread making, enzymes have constantly played a critical role. First application of the enzymes in baked merchandise was supplementation of  $\alpha$ -amylase by addition of malt to redress the concentration of endogenous  $\alpha$ -amylase in flour. After that, malt was replaced by microbial  $\alpha$ -amylase that having more suitable thermostability for baking (Aehle, 2007). This was the primary enzyme that industrially produced as a tool to facilitate bread making using natural flour.

In the 1930s, the main utilization of enzymes in fruit juice industry was the use of pectinases for apple juice extraction (Cantarelli & Lanzarini, 1989). The quick extraction of juice after breakdown of pectin by pectinases and lower in juice viscosity brought about a shorter procedure and significantly enhanced the quality of apple juice (Aehle, 2007).