PROXIMATE ANALYSIS OF FIBRE, PROTEIN, LIPID AND CARBOHYDRATE IN PUMPKINS (*Cucurbita moschata*)

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DECLARATION

I hereby declare that this dissertation is based on my original work, except for quotations and summaries each of which have been fully acknowledged.

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ABSTRACT

Proximate analysis of moisture, ash, lipid, protein and total dietary fibre contents of Sabah local 'Golden Cushaw' pumpkin and Australian imported 'Butternut' pumpkin was carried out. Moisture, ash, lipid, protein and total dietary fibre contents were determined according to AOAC Method 952.10, Method 900.02A, Method 920.39C, Method 960.52, and Method 991.4, respectively. Total carbohydrate content was calculated according to by-difference method. The results showed that the 'Golden Cushaw' pumpkin contains 91.38 ± 0.06 g moisture, 0.75 ± 0.04 g ash, 0.15 ± 0.02 g lipid, 0.83 ± 0.06 g total dietary fibre, 1.00 ± 0.05 g protein and 6.71 ± 0.06 g total carbohydrate. Meanwhile, the 'Butternut' pumpkin contains 91.53 ± 0.09 g moisture, 0.70 ± 0.07 g ash, 0.19 ± 0.06 g lipid, 0.63 ± 0.13 g total dietary fibre, 0.99 ± 0.05 g protein and 6.63 ± 0.08 g total carbohydrate. The 'Golden Cushaw' pumpkin contains higher total dietary fibre than the 'Butternut' pumpkin. Other than that, comparisons of moisture, ash, lipid, protein and total carbohydrate content between the two cultivars do not show significant differences.

ANALISIS PROKSIMAT KANDUNGAN PELAWAS, PROTEIN, LEMAK AND KARBOHIDRAT DALAM LABU KUNING (*Cucurbita moschata*)

ABSTRAK

Analisis proksimat kandungan air, abu, lemak, protein dan pelawas labu kuning 'Golden Cushaw' yang ditanam di Sabah dan labu kuning 'Butternut' yang diimport dari Australia telah dijalankan. Kandungan air, abu, lemak, protein dan pelawas ditentukan mengikut Kaedah AOAC 952.10, Kaedah 900.02A, Kaedah 920.39C, Kaedah 960.52, dan Kaedah 991.43 masing-masing. Kandungan karbohidrat ditentukan mengikut kaedah pengiraan. Keputusan menunjukkan labu kuning 'Golden Cushaw' mengandungi 91.38 ± 0.06 g air, 0.75 ± 0.04 g abu, 0.15 ± 0.02 g lemak, 0.83 ± 0.06 g pelawas, 1.00 ± 0.05 g protein dan 6.71 ± 0.06 g karbohidrat. Sementara itu, labu kuning 'Butternut' mengandungi 91.53 ± 0.09 g air, 0.70 ± 0.07 g abu, 0.19 ± 0.06 g lemak, 0.63 ± 0.13 g pelawas, 0.99 ± 0.05 g protein dan 6.63 ± 0.08 g karbohidrat. Kandungan pelawas labu kuning 'Golden Cushaw' lebih tinggi berbanding dengan labu kuning 'Butternut'. Selain daripada itu, perbandingan kandungan air, abu, lemak, protein dan karbohidrat antara kedua-dua kultivar tidak menunjukkan perbezaan yang signifikan.

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LIST OF SYMBOLS AND ABBREVIATIONS

% v/v	volume of solute per volume of solution
ppm	parts per million
α	Alpha
MES	Morpholinoethane sulphonic acid
TRIS	*Tris*-hydroxymethyl aminomethane
AOAC	Association of Analytical Chemist
FAO	Food and Agriculture Organization of the United Nations
USDA	United States Department of Agriculture
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 PROXIMATE ANALYSIS OF FOOD PRODUCTS

Early work on the constituents of food was mainly carried out by the pharmacological and medicinal chemists; whose primary aim was the discovery of natural medicines (Southgate, 1991). Nowadays, there is an increasing tendency to examine food from a more positive viewpoint, especially the investigations in food science and technology. The food industry, government agencies or universities, often require determination of the food composition and characteristic in order to meet the quality control and quality assurance concepts, and to market safe, high quality food effectively in national and global market (Nielsen, 1998).

In most food laboratories, the main bulk of the routine work comprises the methods of proximate analysis and the study of additives and contaminants (Pearson, 1976; Omaye, 2004). Proximate analysis is the determination of a group of closely related components together, such as the amount of water, protein, fat or ether extract, ash, fibre, with nitrogen-free extract being estimated by subtracting the sum of these five percentages from 100 (Hart & Fisher, 1971; Nitisewojo, 1995). The procedures of proximate analysis are based on a system that were introduced about 100 years ago by two German scientists, Henneberg and Stohmann, for the analysis of animal feedstuffs and described as the Proximate Analysis of Foods. It involves the estimation of the main components of food using procedures that allow a reasonably rapid and acceptable measurement of various food fractions without the need for sophisticated equipment or chemicals (James, 1995).

Subsequently, several nonprofit scientific organizations such as AOAC International, which formerly known as the Association of Official Analytical Chemists, The American Association of Cereal Chemists (AACC) and The American Oil Chemists' Society (AOCS) have compiled and published methods of analysis for food products, which have been carefully developed and standardized. Each of the proximate tests has testing variations that are applied to specific food types. The chemical composition and physical properties of foods are used to determine the nutritive value, functional characteristics and the acceptability of the food product (Nielsen, 1998).

In contrast, pumpkin has been widely accepted as a dietary constituent. It is a squash vegetable from the genus of *Cucurbita*, which belong to the Cucurbitaceae family. *Cucurbita* species include *Curcurbita pepo*, *Cucurbita maxima*, *Cucurbita mixta*, *Cucurbita moschata* and *Cucurbita ficifolia*. Cultivated pumpkins varied greatly in size, shape and colour. The surface of immature pumpkin is usually green, becoming yellow or brownish upon maturity (Whitaker and Davis, 1962; Desai & Musmade, 1998).

Pumpkin has received considerable attention in recent years because of the nutritional and health protective value of the proteins and oil from the seeds as well as the polysaccharides from the fruits (Barbara & Michael, 2004; Murkovic *et al.*, 2004). The protein-bound polysaccharides from the pumpkin can increase the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose (Li *et al.*, 2005). It is rich in carotene, pectin, mineral salts, vitamins and other substances beneficial to health, resulting in various processed food products being developed (Jun *et al.*, 2006).

Food scientist and technologist are concerned with many aspects of food, not the least being their nutritional value since last decade. Hence, an accurate and specific determination of the nutrient content of food is important to understand the relationship of dietary intake and human health (Nielsen, 1998).

1.2 OBJECTIVES

The objectives of this study are:

a) to determine the content of moisture, ash, lipid, protein, and total dietary fibre in two pumpkin cultivars, *C. moschata* 'Butternut' and *C. moschata* 'Golden Cushaw', and
b) to determine the content of total carbohydrate, in the mentioned pumpkin cultivars, by calculation.

1.3 SCOPE

This study is to determine the level of content of moisture, ash, lipid, protein, total dietary fibre and total carbohydrate in pumpkins (*C. moschata* 'Golden Cushaw' and *C. moschata* 'Butternut'). Soxhlet Extraction method (AOAC: 920.39C) was used to determine the lipid content of the sample while protein was calculated from the total nitrogen according to the Kjeldahl method (AOAC: 960.52). The moisture content of the fresh samples was determined by drying the sample to a constant weight at 103 °C in an oven (AOAC: 952.10). Ash content was determined by ignition in a muffle furnace overnight at 550 °C (AOAC: 900.02A). Dietary fibre content was estimated from the loss in weight of the crucible and its content on ignition (AOAC: 991.43). Total carbohydrates content was determined when the sum of the percentages of moisture, ash, lipid and protein were subtracted from 100 (James, 1995).

CHAPTER 2

LITERATURE REVIEW

2.1 NUTRITION AND NUTRIENTS

Nutrition is a science that studies all the interactions that occur between living organisms and food. It is a key element to any strategy to reduce the global burden of disease. A good nutrition will translate into large improvements in health but inadequate food and malnutrition may leads to a downward spiral of increased susceptibility to illness, sickness and loss of livelihood ending in death (WHO, 2002). Indeed, food, which includes plant and animal, is vital to provide energy and nutrients that needed to maintain life and allow growth and reproduction (Grosvenor & Smolin, 2002).

Many nutrients are required for growth and health. Human body can make some nutrients, but it cannot make all of them; and it makes some in insufficient quantities to meet its needs (Whitney & Rolfes, 2005). Humans require at least 50 known essential nutrients, in adequate amounts, consistently, to live healthy and productive lives (Welch & Graham, 2005). Essential nutrients are those substances necessary to support life that must be supplied in the diet because they are either cannot be made by the body or cannot be made in large enough quantities to meet needs (Grosvenor & Smolin, 2002). The amount of essential nutrients in which human need each day varies a great deal, from amounts measured in cups to micrograms. It varies among people based on age, sex, growth status, body size and genetic traits. It also based on the presence of conditions such as pregnancy, breastfeeding, illness, drug use and exposure to environmental contaminants (Brown, 2005).

Nutritional experts have recently drawn up some specific nutritional goals in an attempt to decrease the incidence of chronic nutritional diseases and to improve public health. This has led to the definition of dietary guidelines by each country (WHO, 1990; Eurodiet Core Report, 2001; SENC & Aranceta, 2001). The dietary guidelines generally recommend increasing or decreasing the consumption of a particular food group to improve the nutritional profile of the population in question. It is important to assess the benefits produced by a single, specific food recommendation, and also to observe the beneficial or harmful effects on the rest of the dietary profile (Brown, 2005).

Chemically, there are six classes of nutrients in food, namely carbohydrates, proteins, lipids, vitamins, minerals and water (Insel *et al*., 2003). Carbohydrates, proteins and lipids supply calories and are called the energy nutrients. Although each of these three types of nutrients performs a variety of functions, they share the property of being the body's only sources of fuel (Brown, 2005). These energy-yielding nutrients, along with water, constitute the major portion of most food, and are required in relatively large amounts in the diet. Therefore, they are referred as macronutrients (Grosvenor & Smolin, 2002). Vitamins and minerals are classified as micronutrients because the amounts the body needs are comparatively small (Insel *et al.*, 2003). Table 2.1 illustrates 50 known essential nutrients for sustaining human life.

Table 2.1 The known 50 essential nutrients for human.

Water and energy	**Protein (amino acids)**	**Lipids (fatty acids)**	**Macro minerals**	**Microelements**	**Vitamins**
Water	Histidine	Linoleic acid	Na	Fe	B_1 (thiamine)
Carbohydrates	Isoleucine	Linolenic acid	K	Zn	B_2 (riboflavin)
	Leucine		Ca	Cu	B_3 (pantothenic acid)
	Lysine		Mg	Mn	B_6
	Methionine		S	I	Folic acid
	Phenylalanine		P	F	Biotin
	Threonine		Cl	B	Niacin
	Tryptophan			Se	B_{12} (cobalamin)
	Valine			Mo	
				Ni	
				Cr	
				Si	
				As	
				Li	
				Sn	
				V	
				Co (in B_{12})	

(Source: Welch & Graham, 2005)

2.1.1 Carbohydrates

Carbohydrates are the most abundant and diverse class of organic compounds occurring in the nature. They are also one of the most versatile materials available.

They contain only carbon, hydrogen and oxygen, with the ratio of 2 hydrogen atoms to 1 oxygen atom, the same as in water. Hence, the name carbohydrates, or hydrates of carbon was given (Cui, 2005).

Carbohydrates are the major source of energy for people throughout the world. They are the primary ingredient of staple food (Brown, 2005). They act as ballast materials in a balanced daily nutrition. They act as sweetening, gel-forming and thickening agents, stabilizers and are precursors for aroma and colouring substances (Belitz & Grosch, 1999). Carbohydrates represent the most valuable of the food component from an energy point of view, where daily adult intake should contain about 500 g carbohydrates (Charrondiere *et al*., 2004).

The carbohydrates in food have been classified in a number of different ways but one of the more important is the system used by McCance and Lawrence, who considered that was appropriate to divide them into two classes, namely available carbohydrates and unavailable carbohydrates (Southgate, 1991). Carbohydrates can also be grouped, chemically or structurally, according to the degree of polymerization into sugars, oligosaccharides and polysaccharides (Charrondiere *et al*., 2004). Figure 2.1 illustrates the classification of carbohydrates in food.

Available carbohydrates may be defined as those which are susceptible to the endogenous enzymes of the upper digestive systems of humans, and are characterized as those carbohydrates which produce energy in the human body (James, 1995). They include the monosaccharides glucose and fructose, the disaccharides sucrose, lactose and maltose, oligosaccharides and complex carbohydrates such as dextrins, starch and glycogen (Food Standards Agency, 2002). These are the carbohydrates which are digested and absorbed, and are glucogenic in human. They are termed as glycaemic carbohydrates in the FAO/WHO report on Carbohydrates in Human Nutrition (FAO, 1998).

Total carbohydrates 'by difference'	Sugars Dextrins Starches	Available carbohydrates
	Pectins Hemicelluloses Celluloses Lignin	Unavailable carbohydrates ≡ dietary fibre

(Source: Southgate, 1991)

Figure 2.1 Classification of carbohydrates in food.

Unavailable carbohydrates, often described alternatively as dietary fibre or non-starch polysaccharides, or by the older term roughage, are those which are resistant to the endogenous enzymes of the human upper digestive system but which may be either resistant or susceptible to bacterial enzymes in the larger intestine (James, 1995).

a. Monosaccharides

Monosaccharides, which are free sugars, are classified according to the number of carbon atoms in a molecule (Eleanor & Sharon, 2002). The three most common monosaccharides in the diet are glucose, fructose and galactose. Each contains 6 carbon, 12 hydrogen, and 6 oxygen atoms. They differ in the positions of oxygen and hydrogen around the ring (Hotchkiss & Potter, 1999). The differences in the arrangement of the elements result in differences in the solubility and sweetness of these monosaccharides (Whitney & Rolfes, 2005).

Glucose, commonly referred to as blood sugar, is the most important carbohydrate fuel in the body. It is produced in plants by the process of photosynthesis, which uses energy from the sun to combine carbon dioxide and water (Cataldo *et al.*, 1999). Glucose rarely occurs as monosaccharide in food. It is most often found as part of a disaccharide or starch (Grosvenor & Smolin, 2002). The arrangement of the atoms in fructose stimulates the taste buds on the tongue to produce the sweet sensation. It tastes the sweetest of all the sugars (Insel *et al.*, 2003). Galactose occurs most often as part of lactose, the disaccharide in milk, and is rarely present as monosaccharide in the food (Grosvenor & Smolin, 2002).

b. Disaccharides

The disaccharides are pairs of the three monosaccharides. Disaccharides consists of two molecules, the combination of a glucose molecule and a fructose molecule makes sucrose; maltose is made from two glucose molecules; and lactose consists of a glucose molecule plus a galactose molecule (Brown, 2005).

Sucrose is the most familiar of the three disaccharides. It provides some of the natural sweetness of honey, maple syrup, fruits and vegetables. When a food label lists sugar as an ingredient, the term refers to sucrose (Insel *et al.*, 2003). Maltose is produced whenever starch breaks down, as happen in plants when seeds germinate and in human being during carbohydrate digestion (Whitney & Rolfes, 2005). Lactose is the principal carbohydrate of milk. It contributes about 30% of the energy in whole cow's milk and about 40% of the energy in human milk (Grosvenor & Smolin, 2002).

c. Polysaccharides

In contrast, a few other monosaccharides strung together as polysaccharides. Three types of polysaccharides are important in nutrition: glycogen, starches and fibres. Glycogen is a storage form of energy in the animal body; starches play that role in plants; and fibres provide structure in stems, trunks, roots, leaves and skins of plants (Whitney & Rolfes, 2005).

Dietary fibre, which is one of the polysaccharides, is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fibre includes polysaccharides, oligosaccharides, lignin and associated plant substances (AACC, 2000). Dietary fibre has no calories. It prevents constipation and may lower the risk of heart disease, obesity and diabetes (Brown, 2005).

Some fibres dissolve in water and form viscous solutions in the intestine. These are called viscous or soluble fibres. Soluble fibres are found around and inside plant cells. They include pectin, gums and some hemicelluloses. Fibres that cannot be digested by bacteria in the large intestine are called insoluble fibres because they do not dissolve in water. Insoluble fibres are primarily derived from the structural parts of

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