QUANTITATIVE ANALYSIS OF VITAMIN A, VITAMIN C, AND MINERALS IN PUMPKINS (*Cucurbita moschata)*

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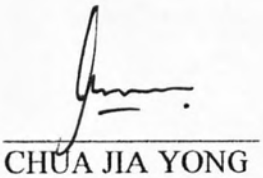

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ABSTRACT

Quantitative analysis of vitamin A, vitamin C, and mineral contents in two different cultivars of pumpkins (*Cucurbita moschata*), which were local 'Golden Cushaw' and Australian 'Butternut', were carried out. Vitamin A was analyzed using High Performance Liquid Chromatography (HPLC) while vitamin C was analyzed according to the iodometric back-titration method. Minerals were analyzed by using Atomic Absorption Spectrophotometric (AAS) and phosphorus was analyzed using UV-Vis Spectrophotometer. Based on the results obtained, 100 g of 'Golden Cushaw' flesh contained 5286 IU of vitamin A, 13.99±1.35 mg vitamin C, 6.17±0.21 mg sodium, 412.29±33.30 mg potassium, 40.16±1.74 mg magnesium, 54.46±4.17 mg calcium, 0.32±0.02 mg iron, 0.20±0.02 mg zinc, and 21.33±0.29 mg phosphorus; while 100 g of 'Butternut' contained 4533 IU of vitamin A, 15.75±1.45 mg vitamin C, 3.80±0.36 mg sodium, 291.67±1.38 mg potassium, 48.33±9.79 mg magnesium, 38.83±1.47 mg calcium, 0.29±0.01 mg iron, 0.19±0.02 mg zinc, and 48.83±0.55 mg phosphorus. The results showed that the 'Golden Cushaw' had the higher nutrition values compared to the 'Butternut'.

ANALISIS KUANTITATIF KANDUNGAN VITAMIN A, VITAMIN C, DAN GARAM MINERAL DALAM LABU KUNING (*Cucurbita moschata*)

ABSTRAK

*Kajian terhadap kandungan vitamin A, vitamin C, dan garam mineral dalam dua kultur labu (*Cucurbita moschata*) yang berlainan, iaitu labu tempatan 'Golden Cushaw' dan labu Australia 'Butternut' telah dijalankan. Kandungan vitamin A telah dianalisis dengan menggunakan kromatografi cecair tekanan tinggi (HPLC) dan kandungan vitamin C telah dianalisiskan dengan menggunakan kaedah pentitratan keterbalikan iodometrik. Kandungan mineral telah dianalisis dengan menggunakan spektroskopi serapan atom (AAS) dan kandungan fosforus dianalisis dengan menggunakan spektrofotometer UV-Vis. Berdasarkan keputusan yang diperolehi, 100 g isi 'Golden Cushaw' mengandungi 5286 IU vitamin A, 13.99±1.35 mg vitamin C, 6.17±0.21 mg natirum, 412.29±33.30 mg kalium, 40.16±1.74 mg magnesium, 54.46±4.17 mg kalsium, 0.32±0.02 mg besi, 0.20±0.02 mg zink, dan 21.33±0.29 mg fosforus; manakala 100 g isi 'Butternut' mengandungi 4533 IU of vitamin A, 15.75±1.45 mg vitamin C, 3.80±0.36 mg natrium, 291.67±1.38 mg kalium, 48.33±9.79 mg magnesium, 38.83±1.47 mg kalsium, 0.29±0.01 mg besi, 0.19±0.02 mg zink, dan 48.83±0.55 mg fosforus. Daripada keputusan yang diperolehi, labu 'Golden Cushaw' mengandungi zat nutrisi yang lebih banyak berbanding dengan labu 'Butternut'.*

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LIST OF SYMBOLS AND ABBREVIATIONS

HPLC	High-Performance Liquid Chromatography
AAS	Atomic Absorption Spectrometer
UV-Vis	Ultraviolet-Visible
UV	Ultraviolet
PTFE	Polytetrafluoroethylene
AOAC	Association of Official Analytical Chemist
AACC	American Association of Cereal Chemists
USDA	United States Department of Agriculture
DRI	Dietary References Intakes
EAR	Estimate Average Requirement
RDA	Recommended Dietary Allowance
UL	Upper Intake Levels
AI	Adequate Intakes
v/v	volume of solute per volume of solution
w/v	weight of solute per volume of solution

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CHAPTER 1

INTRODUCTION

1.1 PUMPKIN AND ITS NUTRIENTS

A pumpkin is a squash fruit, most commonly green when unripe and yellow or orange on ripening. It grows as a gourd from a trailing vine of the genus *Cucurbitaceae*. Although the name pumpkin is applied to all species of *Cucurbita*, most pumpkins are *Cucurbita pepo* and *Cucurbita moschata*. Pumpkins are edible fruits that are used when ripe as forage or for human consumption, such as pies, pumpkin butters, breads, cookies, and soup. The flesh is often coarse and strong flavoured and hence is not generally served as baked vegetable. In western countries, pumpkins are traditionally used to carve Jack-o'-lanterns as part of Halloween celebrations (Fu *et al.*, 2005; Herklots, 1972; John & George, 2002).

According to Desai & Musmade (1998), the genus *Cucurbita* is indigenous to the Americas. There is good archaeological proof that *Cucurbita pepo* and *Cucurbita moschata* were widely distributed in both North and South America. As these two species tolerate hot conditions better than other cultivated species of *Cucurbita*, they are widely grown throughout the tropics of both hemispheres. Pumpkins are warm climate crops requiring a temperature range of 18-27 °C for growth and take nearly 3

to 4 months from seedling emergence to fruit maturity. Hence, pumpkins became very common foods throughout the world as they can be cultivated easily (John & George, 2002; 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. The orange-flesh of pumpkin is a source of beta carotene which is a powerful antioxidant. Beta carotene is converted to vitamin A in the body and it is essential for healthy skin, vision, bone development and many other functions. Pumpkin is also a rich source of carbohydrates and potassium. Beside these major nutrients, pumpkin contains other minerals such as calcium, iron, magnesium, phosphorus, sodium, zinc, copper and manganese; and vitamins such as thiamin (Vitamin B1), riboflavin (Vitamin B2), niacin (Vitamin B3), pantothenic acid (Vitamin B5), pyridoxine (Vitamin B6), ascorbic acid (Vitamin C), and tocopherol (Vitamin E), which are essential to our health (Barbara & Murkovic, 2004; Jung *et al.*, 2005; Desai & Musmade, 1998).

1.2 OBJECTIVES

The objectives of this study were:

a) to analyze the contents of vitamins (i.e. vitamin A and vitamins C) in two pumpkin cultivars of *C. moschata*, which are 'Golden Cushaw' and 'Butternut' pumpkins.

b) to analyze the contents of minerals in the mentioned pumpkins.

1.3 SCOPE

Two cultivars of pumpkin, *C. moschata* 'Golden Cushaw' and 'Butternut' that commercially available in the Sabah market were analyzed to determine the contents of minerals and vitamins. High Performance Liquid Chromatography (HPLC) system was used to analyze the contents of vitamin A in pumpkins according to AACC Method 86-06. Vitamin C (ascorbic acid) in pumpkins was determined by using redox titration according to the methodologies described by Kennedy (1990). The amounts of moisture and ash contents were determined using AOAC Method 925.10 and AOAC Method 940.26. Determination of minerals was carried out by using Atomic Absorption Spectrophotometer (AAS) except for phosphorus, which was carried out by using UV-Vis Spectrophotometer, according to AOAC Method 985.35 and methodologies by James (1996), respectively.

CHAPTER 2

LITERATURE REVIEW

2.1 PUMPKIN

Pumpkin, also known as winter squash, is a member of the Cucurbitaceae family and relative of the melon and the cucumber, come in many different varieties. Genus *Cucurbita* consists of pumpkins, squashes and gourds. Although the name pumpkin is applied to all species of *Cucurbita*, most pumpkins are *Cucurbita pepo* and *Cucurbita moschata* (John & George, 2002; Rubatzky & Yamaguchi, 1997). The classification of different *Cucurbita* species and pumpkins are shown in Table 2.1.

Table 2.1 Classification of different *Cucurbita* species, descriptions, and their origin.

***Cucurbita* Species**	**Descriptions**	**Geographic Origins**
C. pepo	Field and pie pumpkins, naked-seeded pumpkins, and miniatures.	Northern Mexico, Eastern United State.
C. moschata	Large cheese and crookneck pumpkins.	Central America, Northern South America.
C. maxima	Decorative or jumbo pumpkins.	Bolivia, Chile, Argentina.
C. mixta	Cushaw	Southern Mexico, Central America.

(Source: John & George, 2002, Whitaker & Davis, 1962)

The name 'pumpkin' was originated from the Greek word for 'large melon' which is 'pepon'. 'Pepon' was nasalized by the French into 'pompon'. The English changed 'pompon' to 'pumpion'. Finally, American colonists changed 'pumpion' into 'pumpkin' (Herklots, 1972).

The genus *Cucurbita* is indigenous to the Americas. Most of the pumpkin species are originated from North America, except *C. maxima* which are from South America. The centre of distribution of the genus seems to be central or southern Mexico or perhaps the northern portion of Central America. As the species spread northwards, a large number of perennial xerophily types evolved and adapted to the desert and plateau regions of northern Mexico and the southwestern United States. In addition to the xerophily types, species adapted to humid forest or savannah conditions also migrated to north (Whitaker & Davis, 1962). The origins of different species of pumpkins are shown in Table 2.1.

There are large diversities in size, shape, and color exists among the four pumpkin species. The small type (4 to 6 pounds) are grown mostly for cooking and for baking in pies; the naked-seeded types for their seeds, which can be roasted and used for snacks; the intermediate (8 to 15 pounds) and large (15 to 25 pounds) cultivars for cooking and jack-o'-lanterns; the jumbo or mammoth types (50 to 100 pounds) for exhibit purposes; and the miniature cultivars for specialty markets. The largest pumpkin ever grown weighed to 1,140 pounds (John & George, 2002).

2.2 NUTRITION AND NUTRIENTS

Nutrition is a science that studies all the interactions that occur between living organisms and food to promote and maintain health. It is a combination of processes by which all parts of the body receive and utilize the materials necessary for the performance of their functions and for the growth and renewal of all the components (Grosvenor & Smolin, 2002; Joshi, 2002).

In fact, people in different parts of the world eat very different foods and can still remain fit and healthy. The reason indicates that the foods contain a mixture of materials which are called nutrients. Nutrients are chemical components of the foods that supply nourishment to human body (Joshi, 2002; Muller, 1998). From the dietitian point of view, good nutrition involves an accounting of calories and other essential nutrients, such as amino acids, vitamins, and minerals. There are six categories of nutrients and each category, except water, consists of a number of different substances used by body for growth and health. Among the six categories, the carbohydrate category includes simple sugars and complex carbohydrates (starch and dietary fiber). The protein category includes 20 amino acids, the chemical units that serve as the "building blocks" for protein. Several different types of fat are included in the fat category. Of primary concern are the saturated fats, unsaturated fats, essential fatty acids, and cholesterol. The vitamin category consists of 13 vitamins, and the mineral category includes 15 minerals. Water makes up a nutrient category by itself (Desai, 2000; Judith, 2005).

In contrast, carbohydrates, proteins, and fats 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. Vitamins, minerals, and water are chemicals that body needed for converting carbohydrates, proteins, and fats into energy and for building and maintaining muscles, blood components, bones, and other parts of the body (Judith, 2005). Water, indispensable and abundant, provides the environment in which nearly all the body's activities are conducted. It participates in many metabolic reactions and supplies the medium for transporting vital materials to cells and waste products away from them (Whitney & Rolfes, 2005).

2.2.1 Vitamins

Vitamins are organic compounds that are essential in the diet where only small amounts are sufficient to promote and regulate body functions for growth, reproduction, and the maintenance of health. An organic compound is classified as a vitamin if a lack of the compound in the diet results in deficiency symptoms that are relieved by its addition to the diet. Although vitamins do not provide energy, but they aid in the chemical reactions that produce energy from carbohydrates, fat, protein, and alcohol (Grosvenor & Smolin, 2002).

A number of vitamins play important roles as coenzymes in the conversion of proteins, carbohydrates, and fats into energy. These coenzymes also involved in the reactions that build and maintain body tissues such as bone, muscles, and red blood cells. Furthermore, vitamins participate in reactions that affect behaviors where

alteration in behaviors such as reduced attention span, poor appetite, irritability, depression, or paranoia often precedes the physical signs of vitamin deficiency (Judith, 2005). Indeed, vitamins can function only if they are intact. But because they are complex organic molecules, they are vulnerable to destruction by heat, light, and chemical agents (Whitney & Rolfes, 2005).

Traditionally, vitamins have been grouped based on their solubility in water or fat. This chemical characteristic allows generalizations to be made about how they are absorbed, transported, excreted and stored in the body. Those water-soluble vitamins are vitamin B-complex and vitamin C. Meanwhile, fat-soluble vitamins consist of vitamin A, D, E, and K (Grosvenor & Smolin, 2006; Potter & Hotchikiss, 1995). However, human can have deficient, adequate, or toxic intakes of any essential nutrient (Driskell, 2004). Over few decades, vitamins deficiencies remain a major public health problem throughout the world. In industrialized countries, the risks of consuming toxic amounts from fortified foods and supplements are being concerned (Grosvenor & Smolin, 2002).

Group at risk of deficiency include those whose requirements are increased, such as pregnant women and children; those whose intake is limited by financial or dietary restrictions; and those whose absorption or utilization is limited by a disease state. For years it was thought only the fat-soluble vitamins could build up to toxic levels in the body and excesses of water-soluble vitamins were merely excreted in the urine and could not reach toxic levels. However, as nutrient supplements became more popular, reports of toxicities due to water-soluble vitamins began to appear (Grosvenor & Smolin, 2002).

a. Water-Soluble Vitamins

Water-soluble vitamins are found in the watery compartments of foods. Once being absorbed, the water soluble vitamins move directly into blood and travel freely. Upon reaching the cells, the water-soluble vitamins freely circulate in the water-filled compartments of the body. The water soluble vitamins are retained for varying periods in the body and unstable since they are more easily affected by factors like heat, light oxidation and radiation, therefore they must be eaten more regularly than the fat soluble vitamins (Whitney & Rolfes, 2005; Potter & Hotchkiss, 1995). Table 2.2 shows the chief functions, deficiencies, and excesses of water-soluble vitamins.

Table 2.2 Chief functions, deficiencies, and excesses of water-soluble vitamins.

Vitamins	Chief Functions	Deficiencies	Excesses
B_1 (Thiamin)	Release energy from carbohydrates; facilitates growth and maintenance of nerve and muscle tissues; promotes normal appetite.	Fatigue, weakness; nerve disorders, mental confusion, apathy; impaired growth; swelling; heart irregularity and failure.	None known. Excesses are rapidly excreted.
B_2 (Riboflavin)	Capture and use energy released from carbohydrates, proteins, and fats; aids in cell division; promotes growth and tissue repair; promotes normal vision.	Reddened lips, cracks at both corners of the mouth; fatigue.	None known. Excesses are rapidly excreted.
B_3 (Niacin)	Capture and use energy released from carbohydrates, proteins, and fats; assists in manufacture of body fats; maintain normal nervous system function.	Skin disorders; nervous and mental disorders, diarrhea, indigestion; fatigue.	Flushing, headache, cramps, rapid heartbeat, nausea, diarrhea, decreases liver function.

B_5 (Pantothenic Acid)	Releases energy from fat and carbohydrates.	Fatigue, sleep disturbance, impaired coordination; vomiting, nausea.	None known. Excesses are rapidly excreted.
B_6 (Pyridoxine)	Needed for reaction that build proteins; assists in the conversion of tryptophan to niacin; needed for red blood cell formation; promotes normal functioning of the nervous system.	Irritability, depression; convulsions, twitching; muscular weakness; dermatitis near the eyes; anemia; kidney stones.	Bone pain, loss of feeling in fingers and toes, muscular weakness, numbness, loss of balance (mimicking multiple sclerosis).
B_7 (Biotin)	Needed for the body's manufacture of fats, proteins, and glycogen.	Depression, fatigue, nausea; hair loss, dry and scaly skin; muscular pain.	None known. Excesses are rapidly excreted.
B_9 (Folic acid)	Needed for reactions that utilize amino acids for protein tissue formation; promotes the normal formation of red blood cells.	Megaloblastic anemia; diarrhea; red, sore tongue; neural tube defects, low birth weight (pregnancy); increased risk of heart disease and stroke; elevated blood levels of homocysteine.	May cover up signs of vitamin B_{12} deficiency (pernicious anemia)
B_{12}	Maintain nerve tissues; aids in reaction that build up protein tissues; needed for normal red blood cell development.	Neurological disorders; pernicious anemia; fatigue; elevated blood levels of homocysteine.	Vitamin B_{12} injections may cause a temporary feeling of heightened energy.
C (Ascorbic acid)	Needed for manufacture of collagen; fight infections, repair wound; as an antioxidant; enhances iron absorption.	Bleeding and bruising easily; slow recovery from infections and poor wound healing; fatigue, depression.	Nausea, cramps, and diarrhea, and may increase the risk of kidney stones.

(Sources: Brian & Allan, 1977; Brown, 2005; Judith, 2005;Grosvenor & Smolin, 2002; Grosvenor & Smolin, 2006; Food and Nutrition Board, 2005; Whitney & Rolfes, 2005)

b. Fat-Soluble Vitamins

Fat-soluble vitamins are stored in body fat, the liver, and other parts of the body. Being insoluble in body, these vitamins require bile for their absorption. Upon absorption, fat-soluble vitamins travel through the lymphatic system within chylomicrons before entering the bloodstream, where many of them require protein carriers for transport. The fat-soluble vitamins participate in numerous activities all over the body, but excesses are stored primarily in the liver and adipose tissue. The body maintains blood concentration by retrieving these vitamins from storage as needed. By the same token, because fat-soluble vitamins are not readily excreted, the risk of toxicity is greater than it is for the water-soluble vitamins (Grosvenor & Smolin, 2002; Whitney & Rolfes, 2005). The chief functions, deficiencies, and excesses of fat-soluble vitamins are shown in Table 2.3.

Table 2.3 Chief functions, deficiencies, and excesses of fat-soluble vitamins.

Vitamins	Chief Functions	Deficiencies	Excesses
A	Helps in the formation and maintenance of healthy teeth, skeletal and soft tissue, mucous membranes; essential for night vision; β-carotene is the most important and active precursors of vitamin A which helping the body deal with free radicals.	Night blindness, diarrhea; intestinal infections, impaired vision, inflammation of eyes, keratinization of skin and eyes, blindness in children.	Nausea, blurred vision, growth retardation, irritability, enlargement of liver and spleen, loss of hair, bone pain, increased pressure in skull, skin changes.
D	Needed for the absorption of calcium and phosphorus, and for their utilization in bone formation, nerve and muscle activity.	Weak, deformed bones (children); loss of calcium from bones (adults), osteoporosis.	Nausea, mental retardation, weight loss, irritability, kidney damage, deposition of calcium.

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