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DETERMINATION OF VITAMIN D LEVELS IN FOOD SAMPLES AND EFFECT OF CALCIUM ON REGULATION OF VITAMIN D ABSORPTION

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By

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Chair: Shahrul Razid Sarbini, PhD
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Many published findings showed that vitamin D status is low across wide ranges of populations and age groups. These concerns need to be considered in the context of developing countries where dietary supplements can be very expensive. Hence, it is necessary to determine vitamin D content available in Malaysia food products rather than merely depending on nutritional supplement which is beyond affordability for certain groups of people. The purpose of this research is to sample and analyse inexpensive foods considered to be major contributors of vitamin D that are readily available in the Malaysian market. The vitamin D compounds in 13 food samples were determined by extraction process using Ultra High Performance Liquid Chromatography (UHPLC). Among these 13 food samples the local oyster mushroom (Pleurotus ostreatus) contain 1143.6 IU of vitamin D was chosen to undergo further fractionation process, as oyster mushrooms are one of the few food sources where the precursor to vitamin D occurs naturally. Since calcium is a nutrient component that needed by Vitamin D in order to be absorp by human body. It is worth to study the regulation of calcium in vitamin D absorption by in vitro methods using Caco-2 cells, which believed to be more practical and inexpensive methods compared to in vivo methods. There were two types vitamin D that have been used in this study which are 1,25-dihydroxyvitamin D2 and 1,25-dihydroxyvitamin D3. In the calcium uptake study, 1,25-dihydroxyvitamin D3 were absorbed more efficiently compared to 1,25-dihydroxyvitamin D2 after the addition of calcium. Thus, it can be concluded that the presence of calcium would help to increase the absorption of vitamin D into the intestinal cell and 1,25-dihydroxyvitamin D3 was absorbed more readily compared to 1,25-dihydroxyvitamin D2.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

PENENTUAN TAHAP VITAMIN D DI DALAM SAMPEL MAKANAN DAN KESAN KALSIUM DI DALAM PENYERAPAN VITAMIN D

Oleh

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Banyak penemuan yang diterbitkan menunjukkan status vitamin D adalah rendah di kalangan populasi dan kumpulan umur yang luas. Kebimbangan ini perlu dipertimbangkan dalam konteks negara-negara membangun di mana makanan tambahan boleh menjadi sangat mahal. Oleh itu, adalah perlu untuk menentukan kandungan vitamin D yang terdapat di Malaysia produk makanan dan bukan sekadar bergantung kepada makanan tambahan yang tidak mampu untuk golongan tertentu. Tujuan kajian ini adalah untuk sampel dan analisis makanan murah dianggap sebagai penyumbang utama vitamin D yang sedia ada di pasaran Malaysia. Sebatian vitamin D dalam 13 sampel makanan ditentukan oleh proses pengekstrakan menggunakan Kromatografi Cecair Prestasi Tinggi Ultra (UHPLC). Di antara 13 sampel makanan ini, cendawan tiram tempatan (Pleurotus ostreatus) mengandungi 1143.6 IU vitamin D dipilih untuk menjalani proses fraksionasi lagi, kerana cendawan tiram adalah salah satu daripada beberapa sumber makanan di mana prekursor untuk vitamin D berlaku secara semulajadi. Oleh kerana kalsium adalah komponen nutrien yang diperlukan oleh Vitamin D agar dapat diserap oleh tubuh manusia. Adalah penting untuk mengkaji peraturan kalsium dalam penyerapan vitamin D dengan kaedah in vitro menggunakan sel Caco-2, yang dipercayai kaedah yang lebih praktikal dan murah berbanding dengan kaedah in vivo. Terdapat dua jenis vitamin D yang telah digunakan dalam kajian ini iaitu 1,25-dihydroxyvitamin D2 dan 1,25-dihydroxyvitamin D3. Dalam kajian pengambilan kalsium, 1,25-dihydroxyvitamin D3 diserap lebih cekap berbanding dengan 1,25-dihydroxyvitamin D2 selepas penambahan kalsium. Oleh itu, dapat disimpulkan bahawa kehadiran kalsium akan membantu meningkatkan penyerapan vitamin D ke dalam sel usus dan 1,25-dihydroxyvitamin D3 diserap lebih mudah dibandingkan dengan 1,25-dihydroxyvitamin D2.

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This thesis was submitted to the Senate of University Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of Supervisory Committee were as follows:

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LIST OF ABREVIATIONS

UHPLC	Ultra High Performance Liquid Chromatography
RPHPLC	Recycling preparative High Performance Liquid Chromatography
Nm	Nanometer
IU	International unit
µg	Microgram
µm	Micrometer
mL	Mililiter
ppm	Part per million
rpm	Rotation per minute
UV	Ultra violet
DHC	Dehydrocholesterol
PTH	Parathyroid hormone
CYP	Cytochrome p450 mixed-function oxidase
DBP	Vitamin D binding protein
1,25(OH)2D	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
MS	Mass spectrometry
DAD	Diode array
LC-MS	Liquid Chromatography mass spectrometer
VDR	Vitamin D receptor
SD	Standard deviation
fw	Fresh weight
min	Minute

CHAPTER 1

INTRODUCTION

Vitamin D is an essential fat-soluble vitamin that helps regulate levels of calcium and phosphate in the body in order to promote healthy bone development. There are two major forms of vitamin D: vitamins D2 and D3. Vitamin D2 or ergocalciferol (made from ergosterol) is produced by fungus and plants in response to sunlight (UV irradiation), whereas vitamin D3 or cholecalciferol (made from 7-dehydrocholesterol) is made in the skin when 7-dehydrocholesterol reacts with ultraviolet light at 270–300 nm wavelengths-peak vitamin D3 production occurs between 295 and 297 nm. Deficiency of vitamin D in children can cause rickets in which the bones soften, becoming weakened and deformed. In adults, the deficiency disease of vitamin D is called osteomalacia; it can cause the bones to become brittle. There may be a preamble at the beginning of a chapter. The purpose may be to introduce the themes of the main headings.

Vitamin D is a sunshine vitamin that can be recognised as a steroid hormone. Despite the fact that a large number of tropical countries lie in zones that have sufficient sunlight for vitamin D synthesis for most, if not all, of the year, recent studies have demonstrated that vitamin D insufficiency is common in tropical countries like Vietnam, Malaysia, and Indonesia (Nimitphong & Holick, 2013; Chin et al., 2014). Thus, vitamin D deficiency is becoming a global health problem. As such, vitamin supplements and foods containing vitamin D are important sources for vitamin D. However, the number of foods that are high in vitamin D is limited.

Human body usually needs about 1,000 to 2,000 IU/d (25 –50µg) of vitamin D, but a glass of milk only supplies 100 IU while a multivitamin only contains 400 IU of vitamin D. Most people need the sun to avoid deficiency. Generally, people who are exposed to normal quantities of sunlight do not need vitamin D supplements because sunlight promotes sufficient vitamin D synthesis in the skin. However, despite abundant sun exposure, a vast majority of military dependents in Hawaii have less than optimal vitamin D levels at birth, supporting the recommendation for supplementation in this population (Palmer et al., 2013). Furthermore, people who live in tropical climates who always wear sunscreen or avoid any direct sun exposure will be at risk of developing vitamin D deficiency.

Vitamin D is created by the body after exposure to UV rays, or it can be obtained through some foods or supplements. Based on a study of young adults in Hawaii that was presented at the annual meeting of the American Society for Bone and Mineral Research, abundant sun exposure did not guarantee adequate serum levels of vitamin D (Binkley, 2007). The amount of vitamin D made when the skin is exposed to sunlight depends on several factors, including age, skin colour, length of exposure, geographic location, time of year, time of day, cloud cover, smog, dust, or haze. Darker-skinned people need somewhat longer UV exposures to trigger their bodies to make vitamin D, and older people do not make as much vitamin D as younger people in response to

sunlight. Sunscreen also blocks some UV rays, which reduces the body's ability to produce vitamin D. Even in a sunny climate, sunlight effects can be hard to predict (Harris, 2006).

One of the recommended priority areas in the Malaysia by the National Coordinating Committee on Food and Nutrition from the Ministry of Health is to include vitamin D in Malaysian food composition table. Many published findings showed that vitamin D status is low across wide ranges of populations and age groups even at very moderate latitudes; these concerns need to be considered in the context of developing countries where dietary supplements can be very expensive. Hence, it is necessary to determine vitamin D content available in Malaysia food products rather than merely depending on nutritional supplement which is beyond affordability for certain groups of people. Since nutrient components have been known for many years to have a variable effect on vitamin absorption in human body, it is well worth studying their direct and indirect effect on vitamin D absorption by utilising in vitro human cell culture i.e. Caco-2 cells which is more practical and inexpensive compared to in vivo methods.

Vitamin D plays an important role in maintaining the concentration of calcium and phosphorus homeostasis in extracellular fluid to be within the normal range. As an important nutrient for strengthening bones, this vitamin helps to prevent rickets in children and osteoporosis in adult, reduce tumour growth and lower the risk of cancer. This fat-soluble vitamin is taken for granted as it is assumed to be plentiful in daily food. Unfortunately, only few foods naturally contain vitamin D; the rest are fortification foods (Holick, 2004).

This study's aims are as follows:

1. To determine vitamin D content available in Malaysia selected food products.
2. To increase the fractionation amount of vitamin D compounds that extract from food samples.
3. To determine the effect of calcium on vitamin D absorption by in vitro study.

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