ASSESSMENT OF METALS CONTENT, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF COMMERCIALLY AVAILABLE ORGANIC AND CONVENTIONAL GRAINS

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ASSESSMENT OF METALS CONTENT, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF COMMERCIALLY AVAILABLE ORGANIC AND CONVENTIONAL GRAINS

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ABSTRACT

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Nowadays, organic products are gaining more public attention than conventional products because they are free of synthetic chemical during cultivation, processing and storage. In this study, three types of grains, oats, wheat and barley from each type of cultivation technique were analyzed and compared for their metals content, total phenolic content (TPC) and antioxidant activity (AA). All grain samples were purchased at local markets. For evaluation of heavy metals (Mg, Fe, Zn, Pb and Ni), microwave acid digestion was used to digest the sample prior to analysis by using flame atomic absorption spectroscopy. TPC was determined by using Folic-Ciocalteu's reagent and gallic acid standard curve was used as standard. Quantification of AA was carried out by using 2,2-diphenyl-1-picrylhydrazyl (DPPH). From the results, it was showed that the Mg content in organic oats and organic barley was 8.69% and 17.53% higher than their respective conventional grains. Organic grains showed greater amount of Fe, which was 31.89%, 18.94%, and 70.41% higher than their respective conventional grains. Conventional grains had lower Zn concentration but higher Pb content. Ni was found absent in all grain samples. Next, the TPC of organic oats, wheat and barley were 7.83%, 0.38% and 27.78% higher than their respectively conventional grains. Organic oats, conventional wheat and conventional barley showed higher AA. As conclusion, organic grains contain higher amount of essential metals (Mg, Fe and Zn) but lower amount of non-essential metal (Pb). Organic grains contained more TPC because they tend to produce more phenolic compound in unfertilized condition. Besides, the results showed that there is no direct relationship between AA and cultivation technique. Lastly, there was no correlation between TPC and AA in grain samples.

ABSTRAK

PENILAIAN KANDUNGAN LOGAM, JUMLAH KANDUNGAN FENOLIK DAN AKTIVITI ANTIOKSIDA DALAM BIJIRIN ORGANIK DAN KONVENSIONAL KOMERSIAL

Hue Bit Kie

Kini, produk organik semakin diminati berbanding dengan produk konvensional kerana mereka adalah bebas daripada bahan kimia sintetik ketika penanaman, pemprosesan dan penyimpanan. Dalam kajian ini, tiga jenis bijirin, iaitu oat, gandum, dan barli dari setiap jenis teknik penanaman telah dianalisis dan dibanding bagi kandungan logam, jumlah kandungan fenolik dan aktiviti antioksida. Untuk penilaian logam berat (Mg, Fe, Zn, Pb and Ni), pencernaan asid mikrpgelombang telah digunakan untuk menghadam sampel sebelum dianalisis dengan menggunakan spektrometer penyerapan atom. Jumlah kandungan fenolik dikaji dengan menggunakan reagen “Folic-Ciocalteu”. Kuantifikasi aktiviti antioksida telah dijalankan dengan menggunakan DPPH. Dari keputusan yang diperolehi, kandungan Mg dalam oat organik dan barli organik adalah 8.69% dan 17.53% lebih tinggi daripada bijirin konvensional masing-masing. Bijirin organik menunjukkan lebih banyak kandungan Fe, iaitu 31.89%, 18.94%, dan 70.41%

lebih tinggi daripada bijirin konvensional masing-masing. Begitu juga, bijirin konvensional mempunyai kepekatan Zn yang lebih rendah tetapi kepekatan Pb yang lebih tinggi. Tiada Ni dikesan dalam semua sampel bijian. Jumlah kandungan fenolik dalam oat, gandum dan barli organik adalah 7.83%, 0.38% dan 27.78% lebih tinggi daripada bijirin konvensional masing-masing. Oat organik, gandum konvensional dan barli konvensional menunjukkan AA yang lebih tinggi. Sebagai kesimpulan, bijirin organik mengandungi lebih banyak logam penting (Mg, Fe dan Zn) tetapi kurang logam bukan penting (Pb). Bijirin organik mengandungi lebih tinggi TPC kerana mereka menghasilkan lebih banyak sebatian fenolik dalam keadaan tiada bahan kimia. Tambahan pula, keputusan dalam kajian ini menunjukkan bahawa tiada hubungan langsung antara AA dan teknik penanaman. Sementara iu, tiada korelasi antara TPC dan AA diamati dalam sampel gandum.

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DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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Hue Bit Kie

APPROVAL SHEET

This project report entitled "ASSESSMENT OF METALS CONTENT, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF COMMERCIALLY AVAILABLE ORGANIC AND CONVENTIONAL GRAINS" was prepared by HUE BIT KIE and submitted as partial fulfilment of the requirements for the degree of Bachelor of Science (Hons.) at Universiti Tunku Abdul Rahman.

Approved by:

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PERMISSION SHEET

It is hereby certified that **HUE BIT KIE** (ID No: **09ADB06785**) has completed this report entitled **"ASSESSMENT OF METALS CONTENT, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF COMMERCIALLY AVAILABLE ORGANIC AND CONVENTIONAL GRAINS"** under the supervision of **MS CHANG CHEW CHEEN** from the Department Science, Faculty of Science.

I understand that University will upload softcopy of my final year project in pdf format into UTAR Institutional Repository, which may be made accessible to UTAR community and public.

Yours truly,

____

(Hue Bit Kie)

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LIST OF ABBREVIATIONS

AA	Antioxidant activity
AR	Analytical reagent
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
Ca	Calcium
Cd	Cadmium
Cu	Copper
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
FAAS	Flame atomic absorption spectrometry
FCR	Folin-Ciocalteu's Reagent
Fe	Iron
GAE	Gallic acid equivalent
GFAAS	Graphite furnace atomic absorption spectrometry
H_2O_2	Hydrogen peroxide
HAT	Hydrogen atom transfer
$HClO_4$-H_2SO_4	Perchloric acid-sulphuric acid
HNO_3	Nitric acid
ICP	Inductively coupled plasma emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
K	Potassium
Mg	Magnesium
Ni	Nickel
ORAC	Oxygen radical absorption capacity
P	Phosphorus
Pb	Lead

rpm	revolution per minute
S.D	Standard deviation
Se	Selenium
SET	Single electron transfer
TOSC	Total oxyradical scavenging capacity
TPC	Total phenolic content
UV-visible	Ultraviolet-visible
v/v	Volume per volume
WHO	World Health Organization
Zn	Zinc
%	Percent
^{o}C	Degree celcius

CHAPTER 1

INTRODUCTION

1.1 Grains

1.1.1 Get to Know to Grains

Grain is the edible seed of a cereal grass, it is also known as the “fruit” of the grass. Grain is a term that refers to species of *Poaceae*, a grass family of monocotyledonous flowering plants (Whole grain definition, 2010). Grains are different with pulses, nuts and seeds structurally and biochemically. A whole grain must consist of bran, endosperm and germ, either in intact form or in the same relative proportion as the original grain while the outermost layer of grain, the inedible husk is hard and hence is usually removed during processing (Pagano, 2006). Figure 1.1 showed the structure of grain.

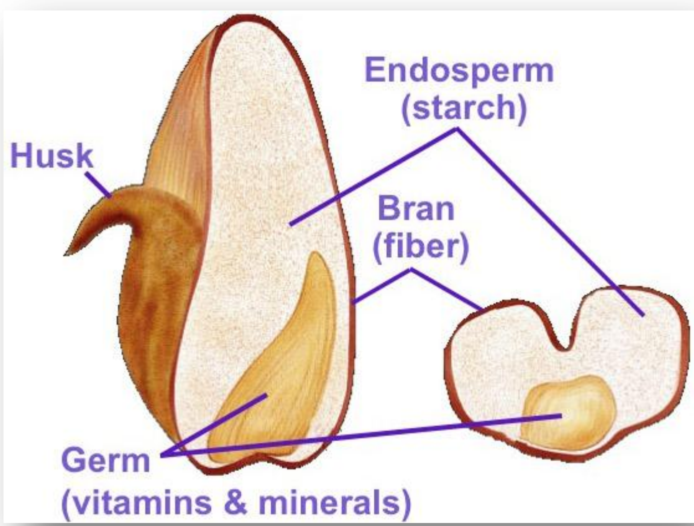


Figure 1.1: Structure of grain (Holcroft, 2013)

The inner protective covering, bran acts as a protective coat and at the same time, it is rich in insoluble fiber, B vitamins, phytonutrients and protein. Next, the major part of grain is the endosperm, which is responsible for the nourishment for germ and seed for germination. Therefore, it is full of carbohydrates and protein with some B vitamins. Lastly, the innermost layer, germ which is also embryo of the seed holds vitamin E, some proteins, minerals, phytonutirents, antioxidants, unsaturated fat and B vitamins as well. Whole grain definition (2010) also stated that typical grains contain 50-80% of starch, depend on the species, origin and growing environment.

Since 10000 years ago with the invention of farming, whole grains have already become part of the human diet (Slavin, 2004). Most of the world's population has relied upon whole grains as the staple ingredients for their diet for the last 3000-4000 years. Wheat, barley and millet are classified as the oldest form of grains that were planted. Rye and oat are grains that are cultivated later. One will first think about rice when the word of grain comes to his mind because it is the most common example especially in Asia; it is the grain with the second-highest worldwide production. Other examples of grains are flaxseed, corn, quinoa, sorghum, amaranth, buckwheat and wild rice. However, the latter three grains are not botanically true grains but they are categorized into grass family because of their similar composition (Slavin, 2004).

1.1.2 Benefits of Grain

The ability of grains to fight against oxidation stress leads to decrease of the occurrence of a number of age-related diseases such as coronary heart disease, diabetes and certain types of cancer have been proven in many epidemiological studies (Chandrasekara and Fereidoon, 2011; Ryan and Thondre, 2011; Anderson and Hanna, 1999). The presence of bioactive substances such as micronutrients and non-digestible carbohydrate in outer layer of the grains and in the germ fraction always make them be the first choice when human looking for a healthy and balance diet. The phytonutrients include vitamin E, Zn, Fe, Se, Co, Mn, S, folates, phenolic acid, carotenoids, phytic acid, lignins, lignans, which all may have significant antioxidant effects.

The non-digestible carbohydrate, which is also known as insoluble dietary fiber, gives beneficial effects in human physiology and important for normal digestive function. It is the major fiber fraction in most cereal products and made of insoluble hemicelluloses, cellulose, resistant starch and lignin. On the other hand, the soluble dietary fiber also helps to lower cholesterol and glycemic index (Sandra and Franklin, 2002). The antioxidants in grain products could act independently or synergistically with fiber for prevention of chronic diseases. Thus, grains are a very convenient way to fulfill the requirement of daily of antioxidant intake for us (Miller, Rigelhof and Kanter, 2000).

1.1.3 Grains Samples

Table 1.1: Grains picture with their respective name.

Grains	Name
	Oat *(Avena sativa)*
	Wheat *(Triticum aestivum)*
	Barley (*Hordeum vulgare)*

1.1.3.1 Oat

Among cereal grains, oats contain the highest protein content but the lowest carbohydrate content. Oats require high moisture, cool climate and fertile, well drained soil to grow healthy. Besides, it has a hard hull that is hard to remove (Grains: A growing guide, 2013). Oats are a type of crop that has many uses. One of the most important uses is acts as livestock feed, which is in the form of grazing, seed or hay. Alternately, it serves as food for human. Furthermore, it embraces significant amount of β-glucans, Se and soluble dietary fiber as ά-glucan (Ryan and Thondre, 2011). Se is an excellent element that helps to decrease asthma symptoms and repair DNA to reduce risk for cancer.

1.1.2.2 Wheat

In North America, it is the most popular and consumed grain. Neutral soil with about pH 4.6, cool and moist growing season are suitable for the growth of wheat whereas warm and dry weather is best for ripening of wheat (Grains: A growing guide, 2013). Generally, wheat is used to make breads, flour, noodles and pasta. Wheat is full of dietary antioxidants. Whole grains, including wheat, consist of some components that are able to reduce the damaging effects of oxidation reactions. Some examples included phenolics, phytate, protein, polysaccharides, lignans and tocopherols. Among the antioxidants, free and esterified phenolic acids are the most important to health (Baublis, Lu and Decker, 2000).

1.1.2.3 Barley

Barley is another important cereal crops in the world (Bicka, Karklinam and Kruma, 2011). Similarly, barley used for livestock feed, human food supply, malt and beer industry. Barley malt provides sweetening in frozen or packaged foods because it is rich in maltose. After fermentation, barley is used as the ingredient in beer and other alcoholic beverages. Barley malt contributes to 80% of phenolic compounds found in beer. They are responsible for the astringency, color and overall beer stability. On the other hand, barley is a good source of niacin, soluble dietary fiber and vitamin B. Niacin aids in lowering total cholesterol and lipoprotein level.

1.1.4 Organic and Conventional Products

1.1.4.1 Current Trend of Organic Products in Malaysia

Over these few years, the interest of consumers on organic product has raised rapidly due to enhanced awareness towards quality and healthy life. The demand for organic products is elevated dramatically especially in developed countries such as United States (Baker, cited in Dardak, Abidin and Ali, 2009, p.95). According to a study carried out in Malaysia, more than 90% of the respondents got to know organic food (Dardak, Abidin and Ali, 2009). They perceived organic products as products with free chemical, healthy food and were native. There were 16% of respondents consumed on organic products regularly because they believed that organic products were safe, healthy, with higher quality and helps to protect the environment. Malaysian consumers were willing to buy organic products if the price was not 25% higher than the conventional products. Besides, there were around 60-90% of Malaysia organic products were imported, which comprised mainly of vegetables, fruits, grains and beverages. Department of Agriculture is responsible for supervision of the production and certification of organic products in Malaysia.

1.1.4.2 Organic Agriculture

The most distinctive different between organic and conventional agriculture is that organic agriculture does not use synthetic chemicals. Besides that, products of genetic engineering and animal cloning, synthetic food processing aids and ingredients as well as ionizing radiation are all prohibited in the planting, processing and preservation of organic products (Organic production systems permitted substance lists, 2006). The crops in organic agriculture obtain their essential nutrients via natural systems (The farming systems, 2011).

The natural system here refers to crop rotation and cover crops. Crop rotation is defined by as a series of different crops cultivated in the same area in a defined order (Thierlelder and Patrick, 2012). This can overcome the problems caused by monoculture such as growing of crop specific pests, diseases and infertility of soil. Different plants absorb nutrients in different amount, therefore by varying the type of plant cultured can make sure that fertility of soil is maintained and biodiversity is enhanced.

Undeniably, organic agriculture requires more human resource and faces more challenges. However, the benefits brought make organic agriculture worth. The reasons are the ability of organic agriculture to reduce the reliance on chemical, preserve the environment, minimize soil degradation, reduce pollution, maximize biological productivity and promote a sound state of health (Introduction to organic farming, 2009). Besides, all of the organic crops are processed carefully and eco-friendly to preserve the organic integrity and important qualities.

1.1.4.3 Conventional Agriculture

To supply sufficient requirement for crops and also increase the profit, conventional agriculture choose to buy cheap chemical. They rely heavily on pesticides, fungicides, insecticides, genetically modified organisms, antibiotics and growth hormones to ensure constant yield.

Undeniably, conventional system is an effective way to reach food demand. Pesticides can control most pests in a short time, have a long shelf life and are easily available. Some scientists from pesticides industry emphasize that pesticides would not pose major risk to farm workers and consumers if they are used in the approved regulatory manner.

The chemicals used are endangering human, animals and environment. Some of them are studied and confirmed that they bring side effects for human. For example, exposure of atrazine can lead to lower math and reading skills in children. A permitted herbicide at low level, glyphosphate-based herbicides can cause DNA damage, infertility and low sperm count when consumed in excessive amount. Moreover, pesticides are detected in breast milk and umbilical cord blood shows that accumulation of pesticide in our body (The farming systems, 2011).

In addition, some pesticides harm wildlife by decreasing the population of honeybee colonies, birds and fish. There are only less than 5% of herbicides applied on crops reach target weeds while the rest of these chemicals leach into air, surface water, groundwater, bottom sediments, food and non-targeted living organisms such as human and wildlife. Another disadvantage of conventional system is application of insecticides can kill natural predators and parasites that are helpful to control pest populations (Miller and Spoolman, 2008).

1.2 Heavy Metals

Heavy metals can be categorized into two forms: biological essential and non-biological essential metals. Biological essential metals include copper, iron, zinc, copper. Table 1.2 shows that they are needed to fulfil a wide and diverse range of functions in our body (Lippard and Mark, 1994). For instance, zinc is an essential mineral that stimulates the activity of about 100 enzymes in the body and supports healthy immune system.

Table 1.2: Biological functions of selected metal ions.

Metal	Functions
Magnesium	Structure; hydrolase; isomerase
Iron	Oxidase; dioxygen transport and storage; electron transfer; nitrogen fixation
Zinc	Structure; hydrolase

Non-biological essential metals are considered to be toxic, nevertheless, modern medicine utilized them in a diverse range of applications including diagnostics, imaging and therapeutic applications (Dyson, 2011). Some examples of non-biological essential metal are lead, mercury, cadmium and tin. For example, significant amount of lead will cause nervous disorder, high blood pressure and muscle pain. Nickel is non-biological essential because it is responsible for cancer (oral and intestinal), depression, heart attacks, haemorrhages, kidney dysfunction, low blood pressure, malaise, nausea, muscle tremors, skin problems and vomiting (Lokeshappa, Shivpuri and Dikshit, 2012). All heavy metals can be toxic when they exceed the threshold concentrations.

Heavy metals can be present in the form of gaseous, particulate, aqueous or solid. Since they are not biodegradable, they will remain in the environment. Thus, conventional methods to remove the heavy metals ions from wastewater are essential to avoid excessive intake of heavy metal. Chemical precipitation, electrodialysis, ion exchange and reverse osmosis are widely used to eliminate heavy metals. However, they have own inherent limitations such as sensitive operating conditions, efficiency, production of secondary sludge and management of secondary sludge. Adsorption onto activated carbon has been considered as highly effective adsorbents for the removal of heavy metals but its high cost and loss during regeneration restrict its application.

Natural sources of heavy metal are parent rocks and metallic minerals. Anthropogenic activities such as metal finishing, mining, industrial activities and vehicular exhaust increases concentration of heavy metal in environment such as soils, lakes, rivers, groundwater and oceans. Pesticide contains heavy metals and this causes agriculture becomes another anthropogenic source of heavy metal. Solved metals ions can be uptake by plants and adversely affect the health of human population.

Table 1.3 shows the Recommended Dietary Intake (DRI) and Adequate Intake (AI) of Mg, Fe and Zn for different life stage group (Otten, Hellwig and Meyers, 2006). Large amount of Mg is required to aid in more than 300 biochemical reactions in our body.

Table 1.3: Recommended Dietary Intake (DRI) and Adequate Intake (AI) (Otten, Hellwig and Meyers, 2006).

Life Stage Group	**Magnesium (mg / d)**	**Iron (mg / d)**	**Zinc (mg / d)**
Infants			
0 – 6 mo	30*	0.27*	2*
7 – 12 mo	75*	11	3
Children			
1 – 3 y	80	7	3
4 – 8 y	130	10	5
Males			
9 – 13 y	240	8	8
14 – 18 y	410	11	11
19 – 30 y	400	8	11
31 – 50 y	420	8	11
51 – 70 y	420	8	11
> 70 y	420	8	11
Females			
9 – 13 y	240	8	8
14 – 18 y	360	15	9
19 – 30 y	310	18	8
31 – 50 y	320	18	8
51 – 70 y	320	8	8
> 70 y	320	8	8
Pregnancy			
14 – 18 y	400	27	12
19 – 30 y	350	27	11
31 – 50 y	360	27	11
Lactation			
14 – 18 y	360	10	13
19 – 30 y	310	9	12
31 – 50 y	320	9	12

*mo = months *y = years

Number in bold represent Recommended Dietary Allowance (RDA) whereas number followed with asterisk (*) represents Adequate Intake (AI).

1.3 Antioxidant Activity

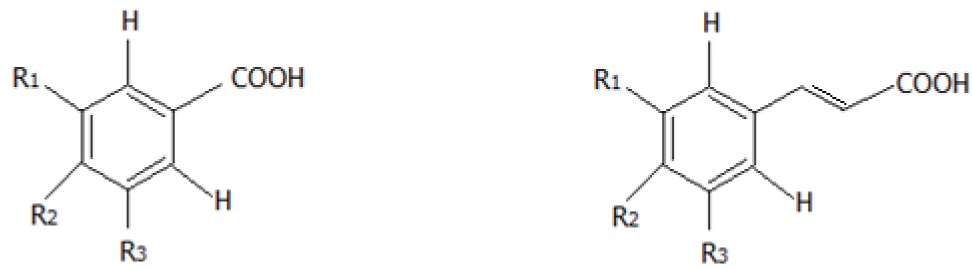
Any atomic or molecular species with unpaired electron is called free radical. They are highly stable and play an important role in biological process such as metabolic pathways, cell signaling, immune response and various kind of pathophysiological conditions (Uppu, Murthy and Parinandi, 2010). They can be both beneficial and deleterious. It is believed that the initiation phase of several diseases is triggered by free radical. Degenerative disease such as heart disease, cancer, cataracts, brain dysfunction and arthritis can occur when there is oxidation damage of biomolecules such as protein, lipids, lipid membrane and DNA. Oxidation damage is because of oxidative stress which results from imbalance of oxidants (free radicals) and antioxidants (Adom and Liu, 2002).

Antioxidants act in defense system in three main ways. It can be preventive antioxidants that suppress free radical formation, radical-scavenging antioxidants that inhibit initiation or propagation of oxidizing chain reactions as well as antioxidants that involved in repair process (Packer, Hiramatsu and Yoshikawa, 1999). There are mainly two types of antioxidant, which are natural antioxidant and synthetic antioxidant. Some natural antioxidants are vitamin C, vitamin E, β-carotene, nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, amines) and phenolic compounds. Phenolic compounds are common antioxidants, which can be found in fruits, vegetables and grains. Generally, synthetic antioxidants are phenolic compounds that have different degrees of alkyl substitution. Instances of synthetic antioxidants are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Velioglu, Mazza and Oomah, 1998).

Avenanthramides present in grains at relatively high concentration and have high antioxidant activity. Fat-soluble ferulic and caffeic acid esters of long chain mono and dialcohols serve to protect lipid membranes and spare vitamin E. These esters are similar to tocopherols as antioxidants to prevent lipid oxidation that can cause oxidation damage to cell (Miller, Rigelhof and Kanter, 2000).

1.4 Total Phenolic Content

Phenolic compounds are secondary metabolites that are created by plants during normal development and under stress conditions such as wounding, infection and ultraviolet radiation (Uppu, Murthy and Parinandi, 2010). The scientific term of “phenolics” includes around 8,000 naturally occurring compounds, in which all of them have a common phenol. Phenolic acid are a subgroup from phenolic compounds. They are phenols that own at least one carboxylic-acid functionality. Figure 1.2 shows the two main carbon frameworks of phenolic acid: hydroxybenzoic acid and hydrocinnamic structures (Uppu, Murthy and Parinandi, 2010). They can be easily found in diet, mainly in esterified forms with organic acids, sugars and lipids.



(I) (II)

Figure 1.2: General structures of naturally occurring phenolic acids. (I) hydroxybenzoic acid structure and (II) hydroxycinnamic structure. R_1, R_2 and R_3 can be chemical groups: H, OH, OCH_3 (Uppu, Murthy and Parinandi, 2010).

Phenolic antioxidants are recognized to be the most effective antioxidants from natural sources. However, unsubstituted phenol is practically inactive. Conversely, substitution of the phenolic ring with alkyl groups increases the electron density and strengthen the reactivity electron acceptors such as reactive oxygen species (ROS) (Packer, Hiramatsu and Yoshikawa, 1999). In other words, phenolics are antioxidants that have redox properties, which enable them to act as reducing agents, hydrogen donor and singlet oxygen quenchers.

Flavonoids are a type of polyphenols, which made up approximate two-thirds of the dietary phenols. Apart from grains, they also exist in vegetables, nuts, spices and beverages such as wine, beer and tea. Flavanoids have high potential in antioxidant and anticancer activities. They show a wide range of biological effects, including antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic and vasodilatory actions (Cook and Sammon, cited in Velioglu, Mazza and Oomah, 1998, p.4113).

1.5 Problem Statement

Organic products were claimed that they were a better option than conventional products. Heavy metal can pose critical risk when consumed at high level. In this study, the effect of different cultivation techniques on metals content of grains were compared. Besides, organic grains were reported by many researchers that they contained larger amount of phenolic compounds and antioxidants which are essential to prevent chronic diseases. Therefore, AA and TPC of organic and conventional grains were investigated. In addition, this study examined the correlation between AA and TPC of organic and conventional grains.

1.5 Objectives

The main objectives of this study are:

i. To determine the level of metals content (Mg, Fe, Zn, Pb and Ni) in organic and conventional grain samples.

ii. To quantify and compare the TPC and AA of organic and conventional grains samples.

iii. To evaluate the quality of organic and conventional grains based on their metal contents, TPC and AA.

iv. To investigate the relationship between TPC and AA of organic and conventional grains samples.

CHAPTER 2

LITERATURE REVIEW

2.1 Evaluation of Heavy Metals

2.1.1 Digestion of Sample Prior to Analysis

Dietary intake via contaminated food is the main source for the human intake of heavy metals. Determination of heavy metals is an important test to find out that concentration to make sure that the amount of heavy metal taken does not exceed the permissible level. In serious case, heavy metals can induce toxic effect on human blood neutrophils.

Table 2.1 describes different digestion methods prior to analysis of metals content. Generally, there are three types, which are dry ashing, wet ashing and microwave acid digestion. Drying ashing applies high temperature which is up to 550^{o}C to digest the sample. Wet ashing and microwave acid digestion utilize acid mixture to speed up the digestion process. The organic matrix of the solid samples is removed after digestion. Besides, the analytes are transferred into solutions for determination of metals content.

Table 2.1: Brief Description of the Different Digestion Methods.

Method	Description	Advantages	Disadvantages
Dry Ashing	• Can be completed by applying different ashing aids (for example, muffle furnace), materials of ashing vessel, ashing temperature and oxidizing gas mixtures.	• Need less reagents and hence give lower blank level	• Slow and time consuming. • Loss of volatile elements during ashing
Wet Digestion	• Supplying thermal energy and using chemical reagent such as acid to convert the components of a matrix into simple chemical forms.	• Effective on both inorganic and organic samples • Destroy or remove the sample matrix and hence reduce interference.	• Slow and time consuming. • Risk of contamination • Need large amount of required reagent and hence gave higher blank contributions • Losses of trace element.
Microwave Acid Digestion	• Extract sample by first dissolve it in acid mixture and followed by microwave heating.	• Accurate, simple and fast • Application of Teflon vessels reduce risk of sample contamination • Achieve better recovery of sample	• High cost of Teflon vessel

2.1.2 Instruments for Heavy Metals Analysis

There are a few options to analyze the metal: flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma emission spectrometry (ICP) and inductively coupled plasma mass spectrometry (ICP-MS). In FAAS, either air/acetylene or a nitrous/acetylene is used as the source of flame whereas in GFAAS, a small and electrically heated graphite tube, or cuvette is heated to very high temperature ($3000^{o}C$) to evaporate the sample solution. ICP utilizes inductively couple plasma source to excite the sample and it can measure multi-element at one time whereas in ICP-MS, the excited ions are detected instead of the light they emit (Thermo Electron, 2000).

2.1.3 Heavy Metals in Grains

With the aim of determine relationship between air pollution sources and soil variable, Bermudez, Jason and Pignata (2011) carried out a research to examine the heavy metal and trace element concentration in wheat grains and straw. They chose dry ashing method to digest their samples. The sample was ashed in a muffle furnace at $450^{o}C$ for 6 hours. Concentrated HNO_3 was added when the ashes were not completely white. Next, the mixture was heated to boiling point until no more nitrous fumes form. Then, the ashes were relocated into muffle furnace for another 2 hours, diluted with ultrapure water and analyzed by using GFAAS. Results showed that the level of Cu, Fe, Mn and Zn exceeded the tolerance limit.

Wet digestion method was used in the study of Lorenz, Farland and Maga (1977). The cereal samples were digested in HNO_3 and concentrated $HClO_4$-H_2SO_4 (1:9) mixture and then heated gently to avoid foaming. The mixture was heated until clear. After cooling, another 2 ml of concentrated HNO_3 was added for reflux purpose. The mixture was then evaporated to 2 ml diluted and measured by using FAAS.

The contents of Cd and Pb in pearled wheat fractions were determined in a study (Sovrani et al., 2012). The samples were first dissolved in nitric acid and hydrogen peroxide and then heated under reflux in a stoppered quartz vessel placed in microwave oven. Next, the solution was diluted with ultra-pure water and tested by GFAAS. Pb was absent in any of the pearled wheat fractions whereas Cd was only detected in the outermost layer of wheat. From the results, they concluded that most heavy metal were concentrated in the external layers of wheat. The external layer of the kernel should be removed although loss of high nutritional content.

Another study which also applied microwave acid digestion was carried out to find out the total elements contents in soil, soil amended with sewage sludge (SDWS) and grain samples (Jamali et al., 2009). The purpose of this study was to investigate the uptake of heavy metals from soil and SDWS to different varieties of wheat. The samples were first mixed with acid-oxidant mixture which comprised of nitric acid and hydrogen peroxide in the ratio of 2:1 (v/v). After kept at room temperature for 2 hours, the samples were digested in domestic microwave, undergo cooling, dilution and filtration and analyzed by using FAAS. Finally, the study had shown the potential accumulation of heavy metals, particularly Cd, Ni, Pb in wheat grains grown in the SDWS. The contamination of wheat with heavy metals was apparent in samples obtained from the SDWS than normal soil.

In addition, a study with objective to assess toxic metals in agriculture product was done by using microwave acid digestion as well (Lokeshappa, Shivpuri and Dikshit, 2012). The samples included vegetable, cereal and medicinal plant. At first, samples were mixed with nitric acid and hydrochloric acid in the ratio of 4:1 (v/v) and then allowed to undergo microwave digestion. After digestion was completed, the vessels were put at room temperature and supernatant was made up to 50 ml by volume with ultra-pure water, centrifuged, filtered and analyzed by using ICP. The level of macro-nutrients (Ca, Fe, K, and Mg,) and micro-nutrients (Cr, Cu, Mn and Zn) of rice were lowest among the 18 samples.

2.2 Determination of Total Phenolic Content

A research regarding the total phenolic content (TPC) of grains was done by Adom and Liu (2002). They oxidized the sample extracts with Folic-Ciocalteu's reagent (FCR) and followed by addition of sodium carbonate. Estimation of phenols with FCR was based on the reaction between phenols and an oxidizing agent phosphomolybdate in alkaline medium. A blue complex solution and its intensity of color was an important parameter in this case. The absorbance of extracts was measured by using single beam spectrophotometer and expressed as μmol of gallic acid equivalent (GAE) per gram of grain. The results showed that corn had the highest amount of TPC (15.55 $\pm$ 0.60 μmol/g of grain) followed by wheat, oats, and rice with the value of 7.99 $\pm$ 0.39, 6.53 $\pm$ 0.19 and 5.56 $\pm$ 0.17 μmol/g respectively. The main phenolic compound in grains was ferulic acid in which the majority of acid was present in bound form. Phytochemicals in the grains may survive stomach and intestinal digestion to reach the colon and this explains why a high intake of grain can reduce risk of colon cancer and other types of cancers.

Similarly, TPC of 28 samples were evaluted by Velioglu, Mazza and Oomah (1998) using the same reagent, FCR to react with antioxidants in samples. Absorbance was measured at 725 nm and converted into ferulic acid equivalents. The reported TPC values ranged from 169 to 10548 mg/100 g of dry product. As a conclusion, relationship between TPC and AA was statistically significant.

On the other hand, a research with the purpose of finding out TPC of whole kernels of 10 different colored maize genotypes was done by using FCR as well (Zilic, Serpen and Vancetovic, 2012). Appropriate amount of extra was diluted with distilled water, oxidized with addition of the reagent and followed by addition of sodium carbonate. After standing for 40 minutes, centrifugation was carried out to obtain clear supernatants for analysis at 725 nm against a blank containing an extraction solvent instead of sample. Finally, TPC was determined by means of a calibration curve prepared with gallic acid and expressed as mg of GAE per kg. The highest TPC was observed in dark-blue maize (10528.8 mg GAE/kg) whereas the lowest TPC was observed in multicolored maize (4491.1 mg GAE/kg).

2.3 Quantification of Antioxidant Activity

2.3.1 Two Main Mechanisms to Measure Antioxidant Properties

Nowadays, various methods are created to quantify antioxidant capacity of food constituents. They are classified into two different groups according to reaction mechanism, which are either hydrogen atom transfer (HAT) or single electron transfer (SET). HAT-based methods evaluate the ability of the antioxidant to quench free radicals by hydrogen donation (Charles, 2013). These reactions are not affected by solvent and pH and are quite fast. Total oxyradical scavenging capacity (TOSC), β-carotene bleaching method and oxygen radical absorption capacity (ORAC) are under category of HAT-based method.

Conversely, SET-based assays measure the ability of antioxidant to reduce a specific oxidant by donating one electron. The rate of reaction is depended on the pH because pH affects the deprotonation and ionization potential of the reactive functional group. SET-based reactions take time to react because they require multistep processes. Some examples include DPPH, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and FCR.

DPPH is widely used to determine AA in food science. The DPPH radical (DPPH·) scavenging assay is a decolorization assay which depends on the capacity of antioxidants to scavenge DPPH radicals (Yu, 2008). DPPH· is a stable organic nitrogen centered free radical. It is deep purple color and will decolorize when reduced into non-radical form by antioxidants (AH).

$$DPPH\cdot + AH \rightarrow DPPH\text{-}H + A\cdot$$

The deep purple DPPH solution absorbs optimally at 517 nm. Thus, a simple measurement of decrease in absorbance due to the reaction is used to quantify the level of antioxidants in the samples.

The results of antioxidant capacity assays are depended on factors like polarity, pH, hydrogen bond accepting ability of the solvent and the ability of the solvent to donate hydrogen atoms to free radical. Antioxidant activities vary on food composition, food structure, temperature and availability of oxygen. Therefore, the method selected should compatible with the type of food tested.

2.3.2 Common Methods to Evaluate Antioxidant Activity

The samples tested by Miller, Rigelhof and Kanter (2000) ranged from vegetables, fruits, berries to grains. After went through the grinding stage, the finely ground samples were dispersed in a 50% aqueous methanol solution of the stable free radical DPPH. Reaction between the deep purple solution of DPPH and antioxidant caused a color loss at 515 nm and this was correlated to antioxidant content, which was expressed as Trolox equivalent/100 grams. The research reported that average AA of cereal products was the same or exceeds most vegetables or fruits. Melons showed very low antioxidant activity whereas berries had relatively high activity. Therefore, they concluded that a whole grain breakfast cereals, fruits and vegetables are excellent dietary sources of antioxidants.

Alternately, in the study of Adom and Liu (2002), the total AA of four different kinds of grains were determined by using a modified TOSC assay, which measured the decrease in ethylene production caused by antioxidants. The results were measured by gas chromatography headspace analysis and expressed as micromoles of vitamin C equivalent per gram of grain. They found out that total AA of corn was the highest, which was 181.42 $\pm$ 0.86 μmol/g.

Additionally, Velioglu, Mazza and Oomah (1998) carried out an experiment to determine TPC and AA of 28 plant products, which included sunflower seeds, flaxseeds, fruits, vegetables, medicinal plant and grains such as flaxseeds, wheat germ and buckwheat. AA of methanolic extract was found out according to β-carotene bleaching method. In this method, the decrease in the rate of β-carotene decay provided by antioxidants was measured. AA was expressed in four different ways. One of the ways was as percentage inhibition relative to control. Different concentration of BHT, BHA and α-tocopherol in 80% methanol were used as standards and 80% methanol was used as the control. Among the 28 samples, it was observed that horseradish oil had the highest amount of antioxidants by showing 99.1% of AA because of its high phenolic content while the wheat germ had moderate amount of antioxidants by presenting 64.9% of AA. This indicated that wheat germ contained above an average level of phenolic compounds.

Besides that, the total AA can be determined by radical scavenging activity with ABTS. In a study, the total AA was done by both ABTS assay and DPPH assay (Zilic, Serpen and Vancetovic, 2012). The sample, maize flour was mixed by adding $ABTS^{\cdot +}$ and DPPH· working solutions respectively. Absorbance measurement was carried out at 734 and 525 nm separately. The AA was expressed as Trolox equivalent antioxidant capacity (TEAC) in mmol of Trolox per kg of flour. The results confirmed that $ABTS^{\cdot +}$ was more sensitive to phenolic-compounds than DPPH· Higher antioxidant activity observed was due to higher content of phenolic compounds in the maize kernel.

2.4 Literature Review on Organic and Conventional Products

A 21 year field experiment was done to investigate the wheat quality in organic and conventional farming (Mader et al., 2007). It was noticed that organic farming had 14% lower wheat yields. However, the baking quality and nutritional value such as protein content, amino acid decomposition, mineral contents are not differ between the two farming systems. Besides, the rats prefer organic product in the food preference test.

Another study which compared the nutritional values of organic and conventional food in the aspect of mineral concentration was completed by Ryan, Derrick and Dann (2004). Results showed that conventional grain contained lower Zn and Cu but higher Mn and P than organic grain. However, they concluded that the organic farming did not cause dramatic increases in grain mineral concentrations.

Moreover, organic and conventional rice were investigated for their difference (Sirikul, Moongngarm and Khaengkhan, 2009). In total antioxidant capacity test, organic rice bran showed higher capacity with amount of 16.35 µg GAE/mg. Finally, they concluded that the growing system posed significant effect on some chemical composition such as protein and fat in both rice bran and defatted rice bran. Besides, they claimed that extraction methods can affect their chemical composition and antioxidant activity.

Organically and conventionally grown wheat samples were examined for their differences in some nutritional quality parameters (Shivay, Prasad and Rahal, 2010). The results proved that conventional wheat had higher protein content and sedimentation value. However, organic wheat showed higher Zn concentration. Both organic and conventional wheat did not give a different in the aspect of grain hardness.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials and Reagent

Table 3.1: Different brands of grains were used. The brand names were denoted with an ID and used for the following sections.

Grain	**Category**	**Brands**	**ID**
A) **Oat**	Organic	Country Farm	OA1
		Radiant Code	OA2
		BMS	OA3
		Lohas	OA4
	Conventional	Quaker	CA1
		Captain	CA2
		Sunvill	CA3
B) **Wheat**	Organic	Country Farm	OB1
		Radiant Code	OB2
		BMS	OB3
	Conventional	Spring Food	CB1
		Mydin	CB2
		Chuan Hong	CB3
		Kijang	CB4
C) **Barley**	Organic	Country Farm	OC1
		Radiant Code	OC2
		BMS	OC3
		Good Health Long Life	OC4
	Conventional	Spring Food	CC1
		TF	CC2
		Seng Hin	CC3
		Kijang	CC4

Oats, wheat and barley were purchased commercially from hypermarket in Selangor and Perak. There were two categories of grains: organic and conventional grains. For each category of grain, few different brands were selected and investigated to obtain the average results.

Table 3.2: Chemicals used with respective manufacturer

Chemical	**Manufacturer**
Nitric Acid, HNO_3 (65%), Grade AR	Fisher Scientific
Hydrogen Peroxide, H_2O_2 (35%)	Sigma
Folin Ciocalteu's Reagent (FCR)	Merck
Sodium Carbonate, Na_2CO_3	SYSTERM®
Gallic Acid, $C_7H_6O_5$	Merck
2,2–diphenyl-1-picrylhydrazyl (DPPH), $C_{18}H_{12}N_5O_6$	Sigma
Acetone, $(CH_3)_2CO$, Grade AR	SYSTERM®
Ethanol, C_2H_5OH, Grade AR	SYSTERM®

3.2 Evaluation of Metal Content

3.2.1 Preparation of Standard Solutions for Calibration Graphs

Different concentrations of standard solutions were diluted from the stock solutions of 1000 ppm Mg, Fe, Zn, Pb and Ni in 100 ml volumetric flasks. The different concentrations of each standard solution were tabulated in Table 3.3.

Table 3.3: Different concentrations of each standard solution

Elements	Concentration (ppm)				
Mg	0.4	0.8	1.2	1.6	2.0
Fe	0.4	0.8	1.2	1.6	2.0
Zn	0.05	0.50	1.00	1.50	2.00
Pb	0.02	0.05	0.07	0.10	-
Ni	0.4	0.8	1.2	1.6	2.0

3.2.2 Sample Preparation

The dried samples were blended using a kitchen blender to obtain fine and powder form grains. They were kept at 4^{o}C until the analysis started. Microwave acid digestion was applied to extract the metals from grain samples. At first, 1 g of sample was digested with 6 ml of 65% HNO_3 and 2ml 30% of H_2O_2. The digestion conditions for microwave digestion system were done as follow:

Table 3.4: Digestion conditions

Duration (minutes)	Watt (W)
2	250
2	0
6	250
5	400
8	550

After that, it was allowed to vent for 8 minutes (Soylak, Colak and Elci, 2006). Next, the samples were diluted to 50 ml with deionized water. A blank digest was carried out in the same way. All digested sample solutions were clear at the end of digestion. They were analyzed by using flame atomic absorption spectrometer (FAAS). Metals content were converted from ppm into unit of mg/kg by using the formula in Appendix A.

3.2.3 Instrument

FAAS is widely used to determine concentration of metal in samples. The instrument used in this study was AAnalyst 200 FAAS. The air-acetylene gas was used to evaporate the sample solutions and dissociate the sample into its component atoms. When light from hollow cathode lamps penetrated the clouds of atoms, the atoms of interest absorbed the light from the lamp. Each element has its own characteristic of absorption wavelength. The changes in intensity of light at this specific wavelength was measured by a detector and used to find out concentration of that element in the original sample (Thermo Electron, 2000).

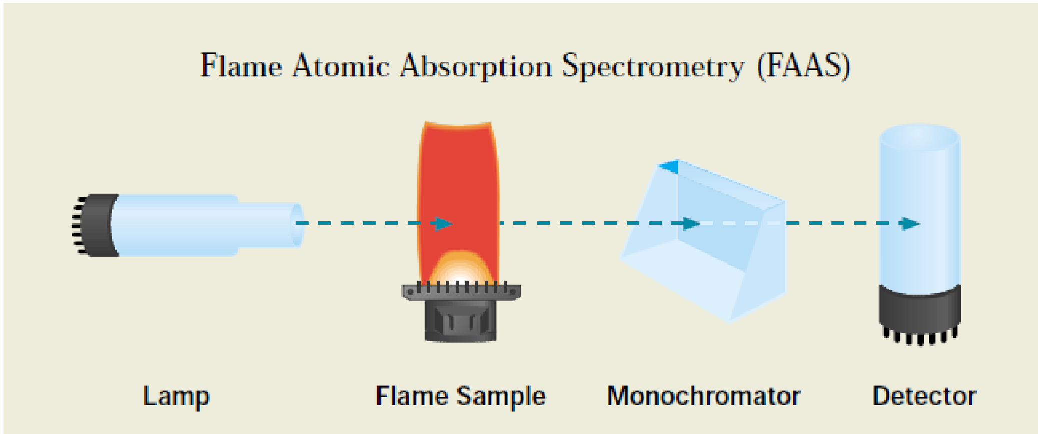


Figure 3.1: Working principle of FAAS (Thermo Electron, 2000)

3.3 Preparation of Assay for Determination of Antioxidant Activity and Total Phenolic Content

Extraction of bioactive compounds from grain sample was completed by continuous shaking 0.3 g of grain sample in 10 ml of 70% (v/v) aqueous acetone for 30 minutes at room temperature and followed by centrifugation for 20 minutes at 7000 rpm. The clear supernatant was stored at 4^{o}C until analysis.

3.4 Instrument for Analysis of Antioxidant Activity (AA) and Total Phenolic Content (TPC)

UV-visible spectrophotometer was used for both determination of antioxidant activity and total phenolic content. The wavelengths were set at 517 nm and 725 nm respectively. This instrument consists of a monochromator which placed between the source and sample, to analyze one wavelength at one time. Figure 3.2 illustrates how the instrument works. Quartz cuvette was used because it is transparent throughout UV, visible and near infrared light regions.

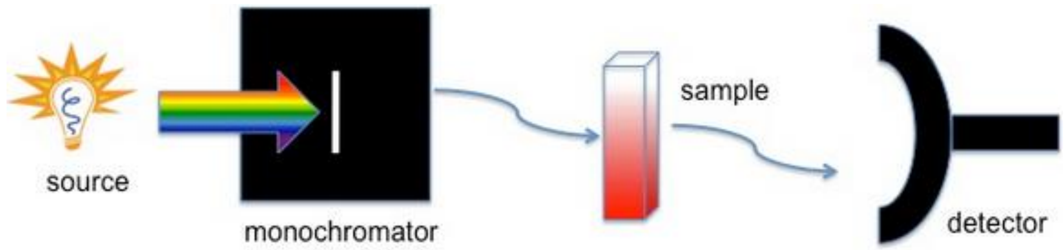


Figure 3.2: Schematic diagram of UV-visible spectrophotometer (Oliva and Barron, 2010)

3.5 Determination of Total Phenolic Content in Grain Samples

Since the total phenolic content was expressed in GAE, a gallic acid calibration graph was prepared first. 500 µg/ml stock solution of gallic acid was prepared by dissolving 0.05 g of gallic acid in 100 ml of deionized water. A series of standard gallic acid solution with concentrations of 5, 10, 20, 40, 60 and 80 µg/ml were prepared. 0.2 ml of gallic acid was first diluted with 0.3 ml of distilled water in test tube. Then, 0.25 ml of FCR and 1.25 ml of 20% sodium carbonate were added. The tubes were shaken well and kept in dark for 40 minutes. The absorbance of the resulting blue color was recorded at 725 nm against a blank containing acetone. The same procedures were repeated by using grain samples instead of gallic acid solutions. The total phenolic content in grain samples as expressed in term of µg/ml of GAE.

3.6 Quantification of Antioxidant Activity in Grain Samples

0.1 mM DPPH was prepared by dissolving 0.0039 g of DPPH in 100 ml of ethanol. Next, 2 ml of DPPH solution was mixed with 2 ml of grain sample. The reaction mixture was shaken well and incubated in dark at room temperature for 60 minutes. Finally, the absorbance of grain sample was measured at 517 nm. The control was prepared in the same way by replacing the grain sample with acetone. Antioxidant activity was expressed as the inhibition percentage by using the following formula in Appendix B.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Evaluation of Heavy Metals

Three types of grain which were oats, wheat and barleys were investigated for their metal contents. Furthermore, the effect of cultivation technique on the metal contents of grains was compared. For each category of grains, few samples from different brands were analyzed to get an average result. After went through microwave acid digestion, the diluted grain samples were analyzed by using FAAS.

In this study, standard calibration method was used to assess and determine the metals content in grains. Calibration graphs of Mg, Fe, Zn, Pb and Ni were plotted. The squared correlation coefficients, R^2 of the graphs were 0.990, 0.997, 0.996, 0.995 and 0.998 respectively (Figure 4.1-4.5). High value of R^2 is essential to make sure that a perfect agreement between the data points and best-fit line is achieved and hence important for accuracy of results. All the concentrations of metals measured were fall within the range in the standard curves to ensure accuracy of results.

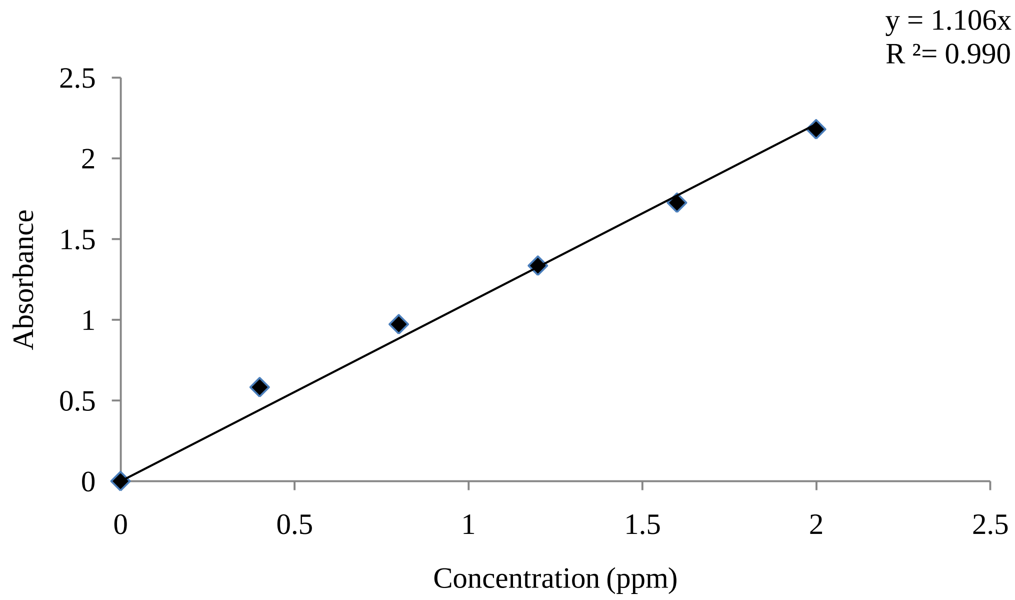


Figure 4.1: Standard calibration curve for Mg

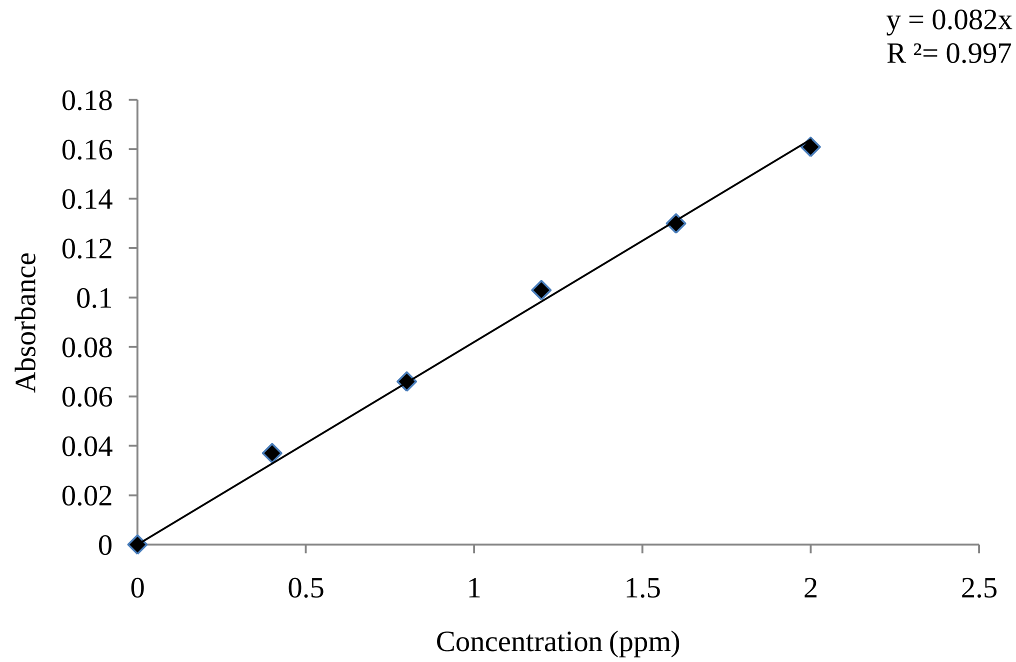


Figure 4.2: Standard calibration curve for Fe

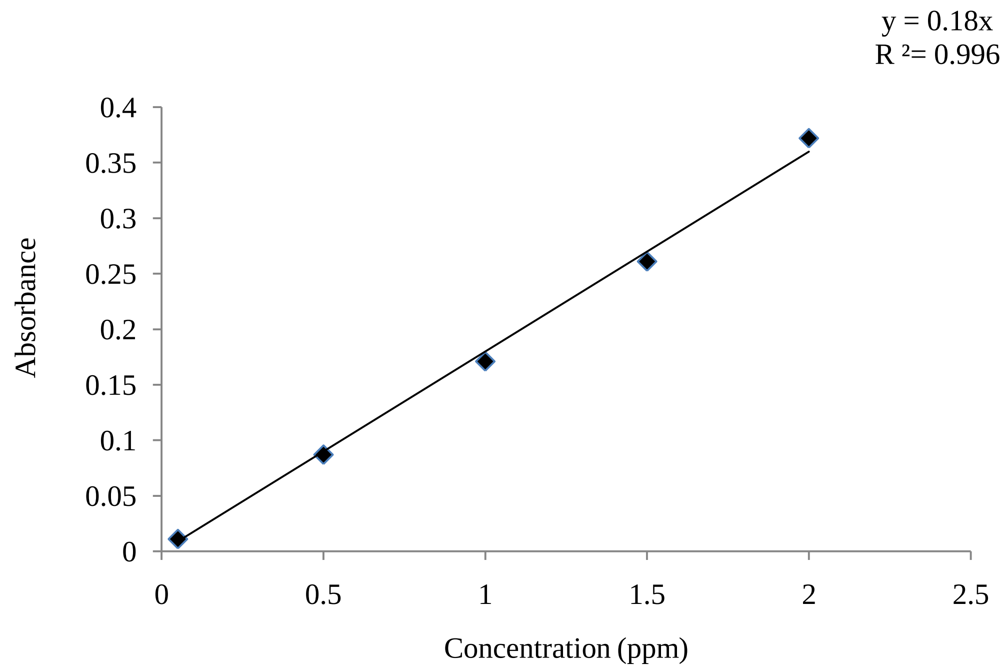


Figure 4.3: Standard calibration curve for Zn

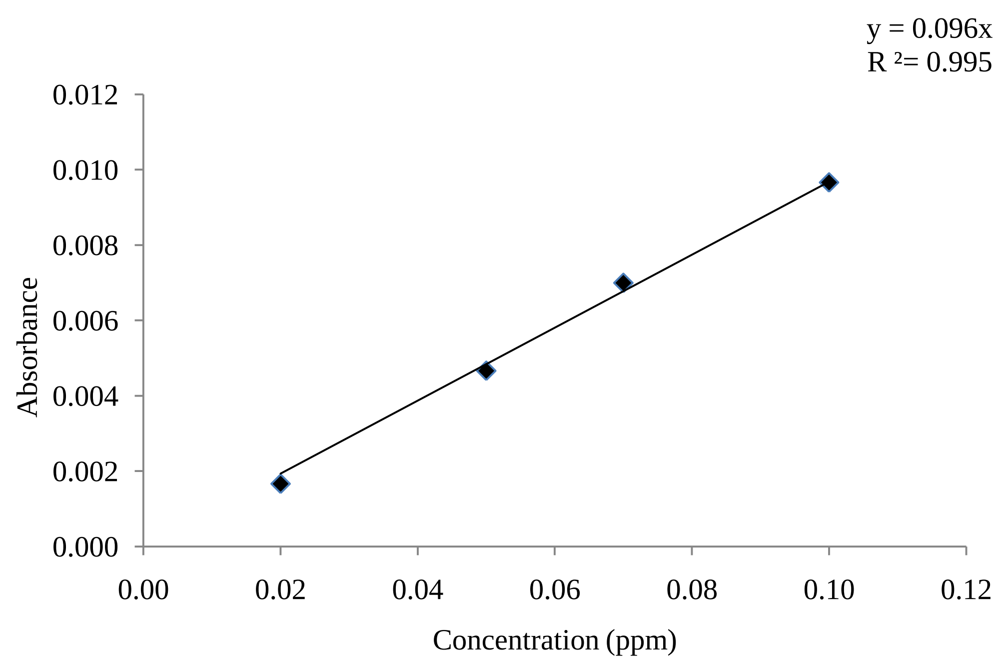


Figure 4.4: Standard calibration curve for Pb

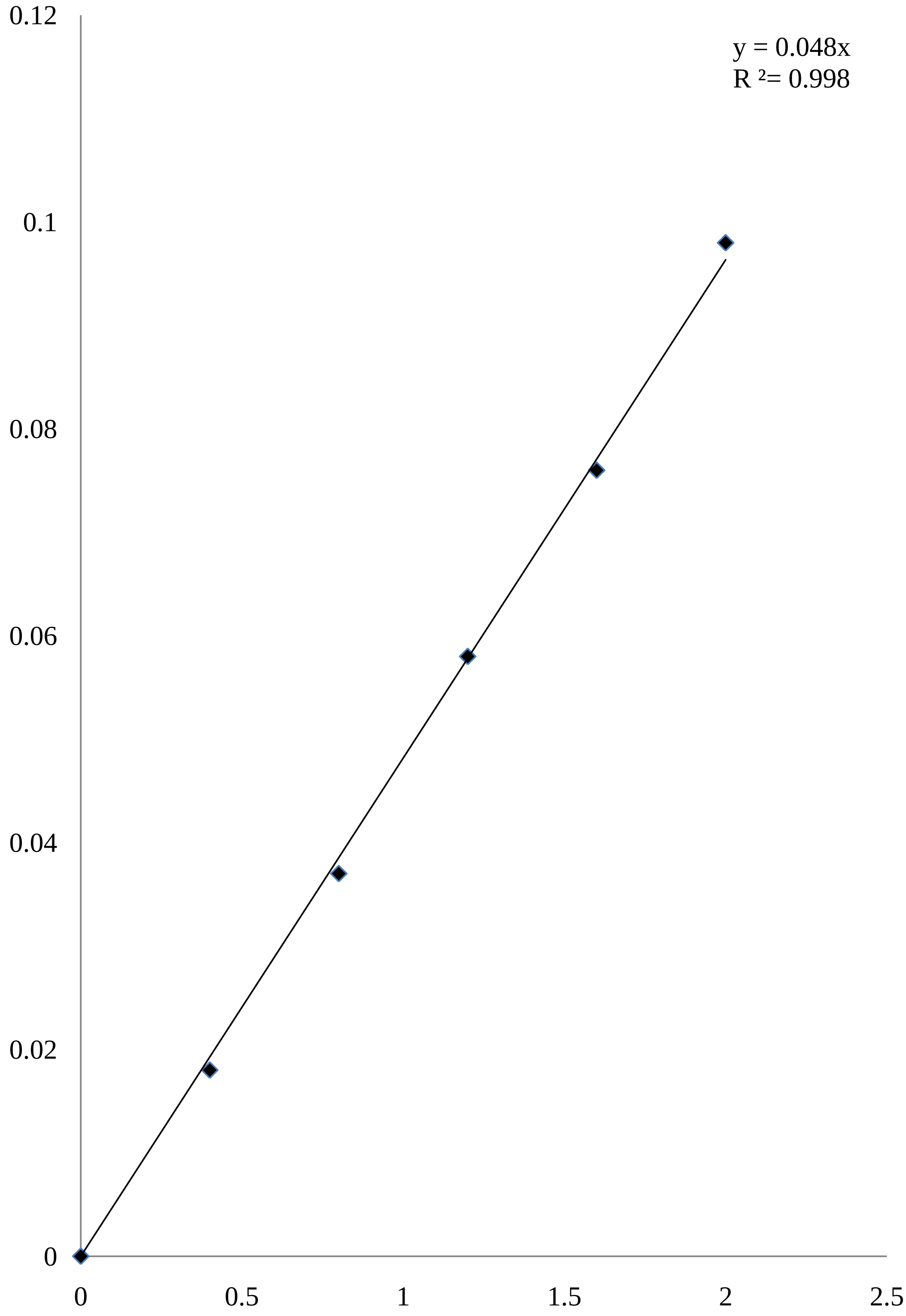


Figure 4.5: Standard calibration curve for Ni

Table 4.1: Concentration of Mg in grain samples

Grain	ID	Concentration (mg/kg)	S.D.	Average Concentration (mg/kg)	Total Average Concentration (mg/kg)
Oat	OA1	94.620	0.0121	91.003	87.36
	OA2	92.224	0.0075		
	OA3	94.801	0.0010		
	OA4	82.369	0.0115		
	CA1	92.089	0.0031	83.725	
	CA2	83.409	0.0024		
	CA3	75.678	0.0157		
Wheat	OB1	78.210	0.0191	83.288	87.03
	OB2	85.805	0.0104		
	OB3	85.850	0.0035		
	CB1	91.139	0.0129	90.766	
	CB2	95.931	0.0056		
	CB3	96.007	0.0061		
	CB4	79.973	0.0261		
Barley	OC1	91.230	0.0093	85.997	79.58
	OC2	90.732	0.0069		
	OC3	85.895	0.0099		
	OC4	76.130	0.0255		
	CC1	69.846	0.0056	73.173	
	CC2	80.892	0.0065		
	CC3	75.392	0.0143		
	CC4	66.561	0.0021		

Magnesium is a very important element in our body because it aids in more than 300 biochemical reactions, maintains normal muscle and nerve function as well as promotes normal blood pressure. Overdose of Mg can lead to adverse effects, for instances, diarrhea and abdominal cramping. As shown in Table 4.1, the highest concentration of Mg in oats was from organic sample OA3 (94.801 mg/kg) while the lowest concentration was found in conventional sample CA3 (75.678 mg/kg).

For wheat, conventional sample CB3 exhibited the highest concentration of Mg, which was 96.0067 mg/kg while organic sample exhibited the lowest concentration, which was 78.210 mg/kg. The largest amount of Mg found in barley was organic sample OC1 (91.230 mg/kg) whereas the smallest amount was detected in conventional sample CC4 (66.561 mg/kg).

The average concentration of Mg in organic oats and conventional oats were 91.0036 and 83.7251 mg/kg respectively, which showed that contents of Mg in organic oats was 8.69% higher than conventional oats. However, a greater amount of Mg was traced in conventional wheat with 8.97% higher than the organic wheat. Besides, it was observed that organic barley embraced Mg which was 17.53% higher than conventional barley. Overall, the Mg content contained in organic grains was higher. This was agreed by a study by Worthington (2001) which investigated the difference between organic crops and conventional crops. The total average concentrations of Mg in oats, wheat and barley were 87.36, 87.03 and 79.58 mg/kg respectively. These variations were closely related with irrigation water used in their production. From the results, it can be concluded that grains are important sources of Mg. In addition, all grains did not exceed the permissible WHO guideline value.

Table 4.2: Concentration of Fe in grain samples

Grain	ID	Concentration (mg/kg)	S.D.	Average Concentration (mg/kg)	Total Average Concentration (mg/kg)
Oat	OA1	23.780	0.0121	23.323	20.503
	OA2	22.561	0.0075		
	OA3	33.537	0.0010		
	OA4	13.415	0.0115		
	CA1	18.488	0.0031	17.683	
	CA2	14.024	0.0024		
	CA3	20.537	0.0157		
Wheat	OB1	31.098	0.0191	31.911	29.370
	OB2	35.366	0.0104		
	OB3	29.268	0.0035		
	CB1	29.878	0.0129	26.829	
	CB2	32.927	0.0056		
	CB3	28.659	0.0061		
	CB4	15.854	0.0261		
Barley	OC1	43.293	0.0093	25.457	20.198
	OC2	19.512	0.0069		
	OC3	12.805	0.0099		
	OC4	26.220	0.0255		
	CC1	10.976	0.0056	14.939	
	CC2	18.293	0.0065		
	CC3	17.073	0.0143		
	CC4	13.415	0.0021		

Fe is a vital component of proteins involved in oxygen transport. However, overdose of iron may cause toxicity and even death. The highest concentration of Fe in oat samples was detected in organic sample OA3 (33.537 mg/kg). On the other hand, the lowest concentration of Fe in wheat and barley were found in conventional sample CB4 and conventional sample CC1 with concentration of 15.854 and 10.976 mg/kg respectively. Besides, the iron content in wheat and barley were ranged from 15.854-35.366 mg/kg and 10.976-43.293 mg/kg respectively.

Generally, organic grains showed greater amount of Fe, which was 30.89, 18.94, and 70.41% higher than their respective conventional grains. This was supported by a research carried out by Woshington (2001) which proved that iron presented in higher amount in organic crops due to the fact that soil which was managed organically consisted of more microorganisms that helped to convert the unavailable form of iron in soil into a form that can be uptake easily by plants. Citrate and lactate were those substances synthesized by the microorganisms to combine with soil minerals and hence aid in uptake of these minerals.

Moreover, the increasing trend of concentration of Fe in grains was wheat (29.370 mg/kg) > oats (20.503 mg/kg) > barley (20.198 mg/kg). All grain samples contained Fe content below the permissible level of WHO (425 mg/kg).

Table 4.3: Concentration of Zn in grain samples

Grain	ID	Concentration (mg/kg)	S.D.	Average Concentration (mg/kg)	Total Average Concentration (mg/kg)
Oat	OA1	58.611	0.0009	54.583	60.116
	OA2	63.611	0.0015		
	OA3	60.278	0.0051		
	OA4	35.833	0.0036		
	CA1	71.667	0.0068	65.648	
	CA2	64.556	0.0054		
	CA3	60.721	0.0032		
Wheat	OB1	50.000	0.0016	53.796	45.544
	OB2	60.000	0.0064		
	OB3	51.388	0.0033		
	CB1	35.556	0.0013	37.292	
	CB2	45.833	0.0020		
	CB3	29.537	0.0021		
	CB4	38.242	0.0020		
Barley	OC1	78.889	0.0021	68.819	49.780
	OC2	67.667	0.0015		
	OC3	61.111	0.0028		
	OC4	67.609	0.0012		
	CC1	31.667	0.0009	30.741	
	CC2	30.185	0.0017		
	CC3	33.056	0.0017		
	CC4	28.056	0.0026		

Among the analyzed metals content, Zn was the second most abundant metal in grains. Zinc is essential metal which enters the food from soil and metal-based pesticides (Salama and Radwan, 2005). Table 4.3 showed the quantity of Zn in grain samples. The highest amount of Zn in oats, wheat and barley was found in conventional oat sample CA1, organic wheat sample OB2 and organic barley OC1 with concentration of 71.667, 60.000 and 78.889 mg/kg respectively.

Meanwhile, higher average Zn concentration was detected in conventional oats, organic wheat and organic barley. Zn level in conventional grains was lower because the application of soluble phosphorus in conventional agriculture tends to increase phosphorus uptake but reducing mycorrhizal colonization and hence reducing Zn uptake (Ryan, Derrick and Dann, 2004). Mycorrhizal is one type of fungi that helps for diffusion limited nutrient such as Zn and Cu (Benson and Convey, 1976). When comparing among types of grains, it was noticed that oats contained the largest amount of zinc, which is 60.116 mg/kg.

The permissible level of Zn in food is 100 mg/kg (WHO, 2001). Therefore, no grain sample exceeded the maximum level. Physical factors such as soil pH and concentration of Zn in soil can contributed to Zn level in grains (Salama and Radwan, 2005).

Table 4.4: Concentration of Pb in grain samples

Grain	ID	Concentration (mg/kg)	S.D.	Average Concentration (mg/kg)	Total Average Concentration (mg/kg)
Oat	OA1	0.0000	0.0029	1.302	1.866
	OA2	1.5625	0.0010		
	OA3	1.5625	0.0012		
	OA4	2.0833	0.0020		
	CA1	2.0833	0.0015	2.431	
	CA2	1.5625	0.0032		
	CA3	3.6458	0.0021		
Wheat	OB1	0.1736	0.0015	0.926	2.156
	OB2	1.0417	0.0017		
	OB3	1.5625	0.0015		
	CB1	4.1667	0.0012	3.385	
	CB2	3.1250	0.0006		
	CB3	4.1667	0.0006		
	CB4	2.0833	0.0006		
Barley	OC1	4.8611	0.0006	2.487	3.088
	OC2	2.3090	0.0048		
	OC3	2.2569	0.0006		
	OC4	0.5208	0.0000		
	CC1	2.9514	0.0006	3.689	
	CC2	3.8194	0.0012		
	CC3	3.4722	0.0015		
	CC4	4.5139	0.0025		

Pb is an endocrine-disrupting metal which is also claimed as human carcinogen (Huang, Zhou and Zhao, 2008). Pb is accumulated in the skeleton and may contribute to kidney damage (Salama and Radwan, 2005)**.** In this study, the concentration of Pb was found higher than those of Ni in grain. This matched with the result obtained by Huang, Zhou and Zhao (2008). Among the oats samples, organic sample OA4 and conventional sample CA1 exhibited the highest amount of Pb, which are 2.0833 mg/kg. For wheat and barley, the highest levels of Pb were observed in CB1, CB3 and OA1 (4.1667, 4.1667 and 4.8611 mg/kg). Accumulation of heavy metals is affected by environment. The difference in the lead content was due to different sources of grain, for example rural areas and industrial area.

The average concentrations of Pb in all organic grains were lower than conventional grains. This can be explained by organic agriculture which did not use synthetic chemicals and fertilizer produced from industrial waste which were most contaminated to maintain yield, and hence the growing condition contained less toxic heavy metal (Worthington, 2001). Conversely, the environment for conventional agriculture was contaminated by Pb. The possible sources of Pb include irrigation with contaminated water, application of fertilizer and metal-based pesticides, industrial emissions, transportation as well as method of harvesting and storage (Maleki and Zarasvand, 2008).

The total average concentration of Pb is in the order of: Barley > Wheat > Oats (3.088, 2.156 and 1.866 mg/kg). The same trend was observed in a study by (Baxter and Salmon, 2006). According to WHO standard, the permissible level for Pb was 5 mg/kg. Thus, none of the grain samples exceeded the permissible limit and all of the grains are safe to be consumed.

Table 4.5: Concentration of Ni in grain samples

Grain	ID	Concentration (mg/kg)	S.D.	Average Concentration (mg/kg)	Total Average Concentration (mg/kg)
Oat	OA1	*n.d.	0.0010	*n.d.	*n.d.
	OA2	*n.d.	0.0021		
	OA3	*n.d.	0.0014		
	OA4	*n.d.	0.0031		
	CA1	*n.d.	0.0014	*n.d.	
	CA2	*n.d.	0.0020		
	CA3	*n.d.	0.0015		
Wheat	OB1	*n.d.	0.0012	*n.d.	*n.d.
	OB2	*n.d.	0.0012		
	OB3	*n.d.	0.0034		
	CB1	*n.d.	0.0000	*n.d.	
	CB2	*n.d.	0.0004		
	CB3	*n.d.	0.0019		
	CB4	*n.d.	0.0014		
Barley	OC1	*n.d.	0.0044	*n.d.	*n.d.
	OC2	*n.d.	0.0030		
	OC3	*n.d.	0.0018		
	OC4	*n.d.	0.0012		
	CC1	*n.d.	0.0007	*n.d.	
	CC2	*n.d.	0.0022		
	CC3	*n.d.	0.0008		
	CC4	*n.d.	0.0007		

*n.d. = not detected

Ni is a non-essential element because it can give rise to many health problems such as cancer, nausea and depression. No Ni was detected in any grain sample. This indicated that the soil in which grains grown was least contaminated with Ni. Besides, it can be deduced that the soil has pH values over 6.5. This is because at this pH value, the mobility of Ni was very low and hence its concentration in grain was negligible (Bermudez, Jason and Pignata, 2011). It can be concluded that all grain samples were free from Ni and safe for human consumption.

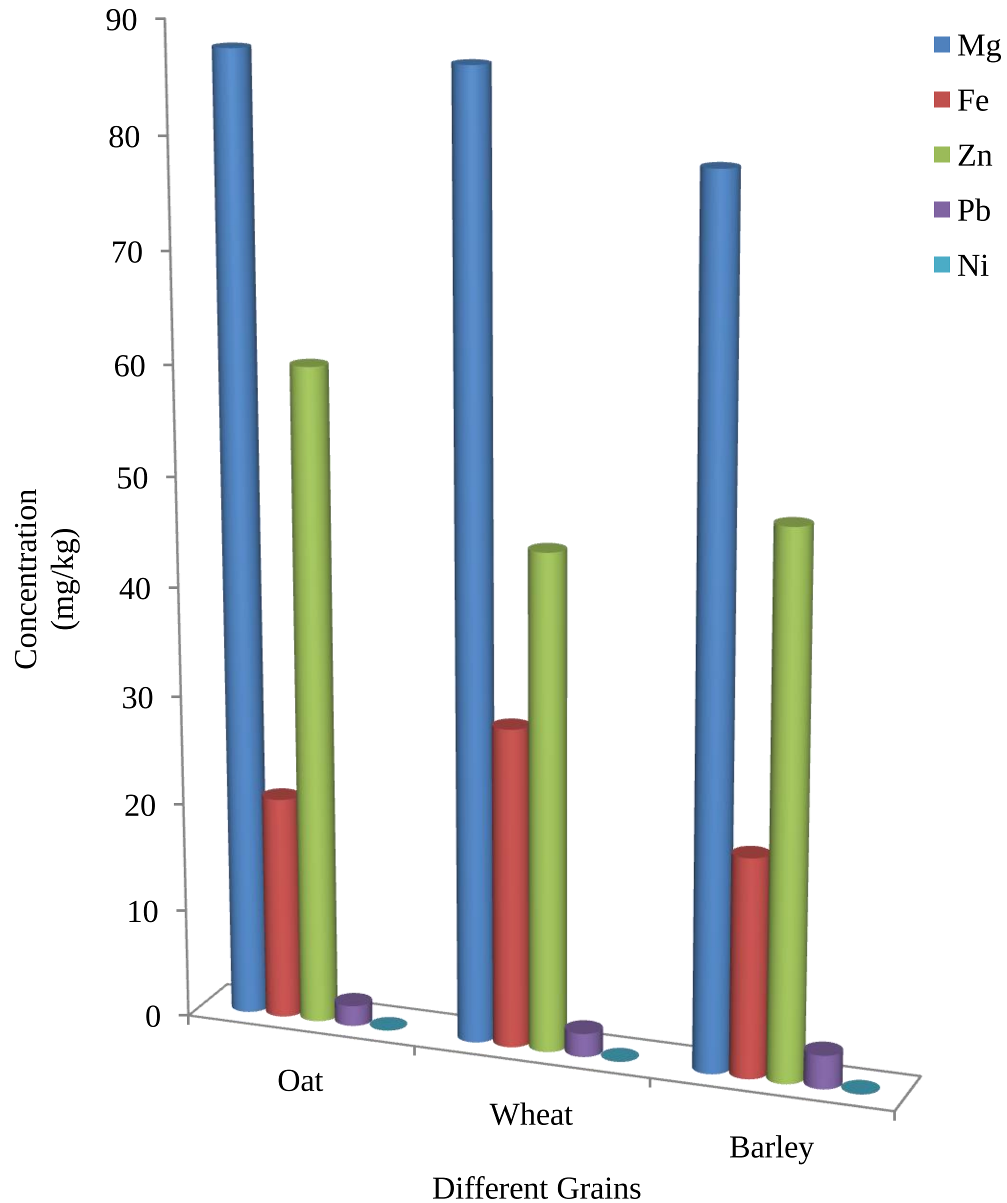


Figure 4.6: Concentration of metals in oats, wheat and barley

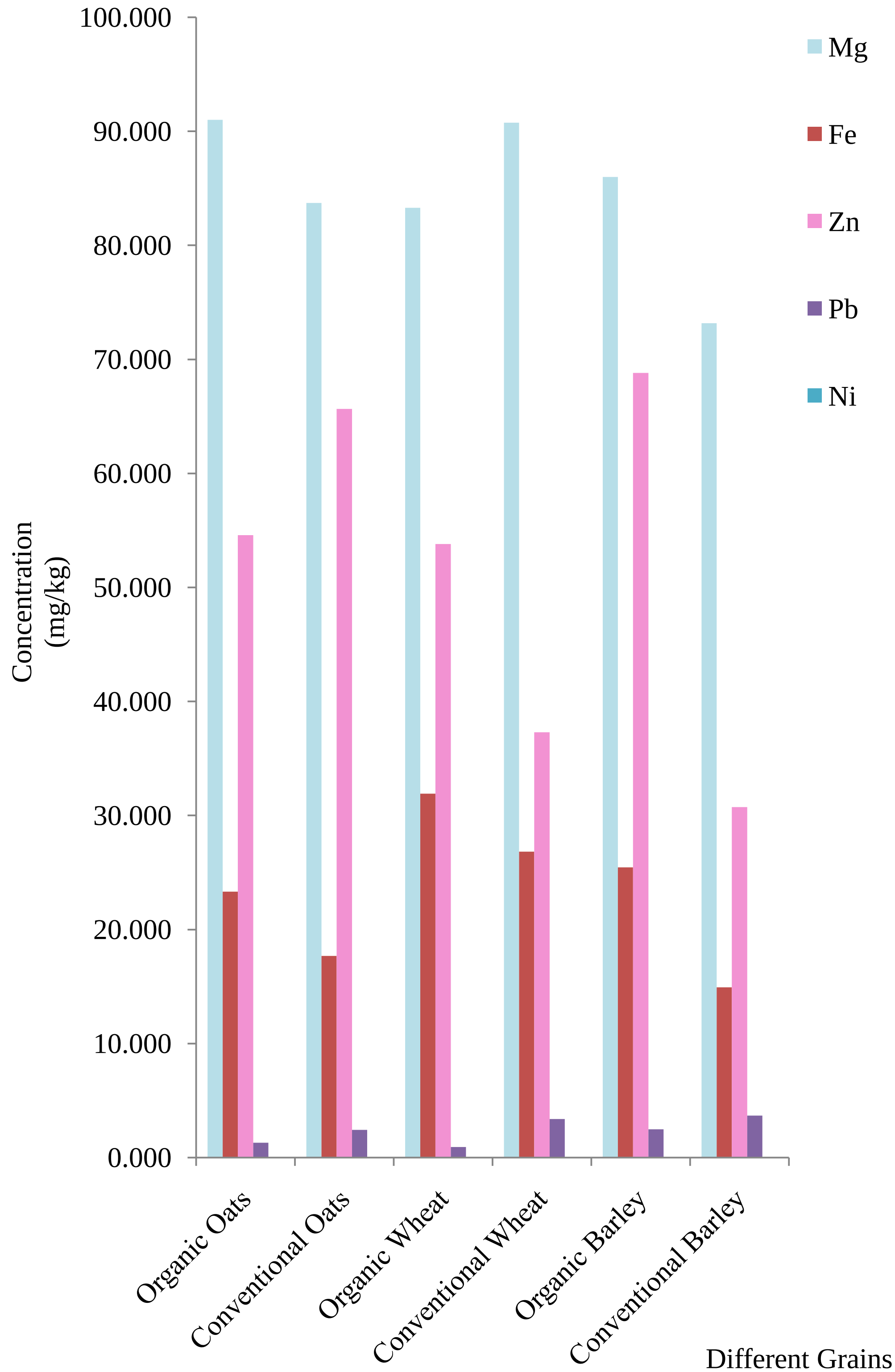


Figure 4.7: Average metals content in organic and conventional grains

Table 4.6: Overall Metals Content in Grain Samples

Grain	Category	Average Metal Contents (mg/kg)				
		Essential Metal			Non-essential Metal	
		Mg	Fe	Zn	Pb	Ni
Oats	Org	91.004	23.323	54.583	1.302	*n.d.
	Cvt	83.725	17.683	65.648	2.431	*n.d.
	Higher content	Org	Org	Cvt	Cvt	-
	Difference (%)	8.69	31.89	20.27	86.71	-
Wheat	Org	83.288	31.911	53.796	0.926	*n.d.
	Cvt	90.763	26.829	37.292	3.385	*n.d.
	Higher content	Cvt	Org	Org	Cvt	-
	Difference (%)	8.97	18.94	44.26	265.55	-
Barley	Org	85.997	25.457	68.819	2.487	*n.d.
	Cvt	73.173	14.939	30.741	3.689	*n.d.
	Higher content	Org	Org	Org	Cvt	-
	Difference (%)	17.53	70.41	123.87	48.33	-

*n.d. = not detected
Org = Organic
Cvt = Conventional

Table 4.7: The limitation of heavy metal set by WHO

Metal	Sample Range (mg/kg)	Concentration (mg/kg)
Mg	66.561 - 96.007	1000
Fe	10.976 - 43.293	425
Zn	28.056 - 78.889	100
Pb	0.000 - 4.8611	5
Ni	*n.d.	10

*n.d. = not detected

The metals content in grain samples is summarized in Table 4.6. It was observed that the essential elements such as Mg, Fe and Zn were present in higher concentration in organic grains. This was associated with better organic cultivation technique which managed to impose more of these elements into grains. However, the non-essential element, Pb was present in lower concentration in organic grains. This result showed that the absence of chemical pesticide can decrease the lead content. Another reason was due to organic grains which grown in less contaminated soil. Besides, no Ni was found in all grain samples. From the result, it can be deduced that the order of decreasing metals content found in all grains samples was Mg > Zn > Fe > Pb > Ni. In addition, oat contained the greatest amount of Mg and Zn which is 87.36 and 60.12 mg/kg respectively whereas wheat contained highest amount of Fe (29.37 mg/kg). When comparing the result with the heavy metal limitation set by WHO, none of the grain samples exceeded the permissible level. Therefore, all of them are considered safe to be consumed.

4.1.1 Recommended Grain Intakes

According to Otten, Hellwig and Meyers (2006), the recommended dietary intake of Mg, Fe and Zn for 19-30 years old males are 400, 11 and 11 mg/day while for 19-30 females are 320, 8 and 8 mg/day. From the result, it was observed that grain did not contain sufficient amount of Mg to meet the daily requirement. However, it is a excellent source of Fe and Zn. Therefore, consumers can rely on grain to fulfill their demand of minerals.

4.2 Determination of Total Phenolic Content in Grain Samples

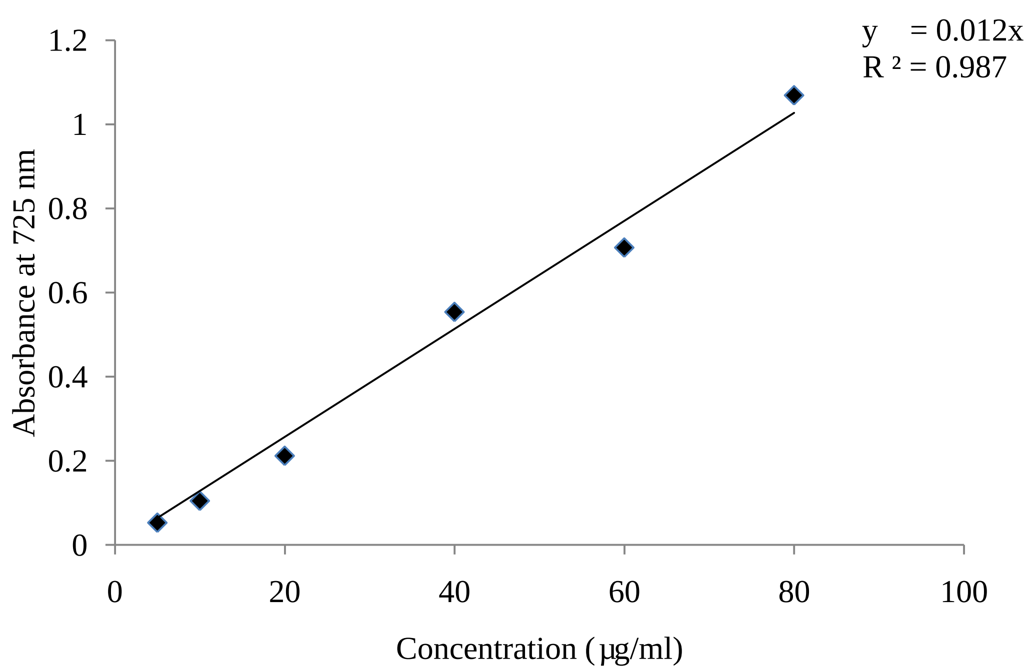


Figure 4.8: Calibration graph of gallic acid

The standard curve of gallic acid was plotted and showed in Figure 4.8. The straight line has square correlation coefficient of 0.987.

Table 4.8: TPC expressed as Gallic Acid Equivalent, GAE (µg/ml)

Grain	**ID**	**TPC in GAE (µg/ml)**	**Average TPC in GAE (µg/ml)**	**Percentage difference between organic and conventional (%)**	**Total Average TPC in GAE (µg/ml)**
Oat	OA1	21.056	25.799	7.83	24.789
	OA2	18.167			
	OA3	29.750			
	OA4	34.222			
	CA1	25.333	23.778		
	CA2	25.500			
	CA3	20.500			
Wheat	OB1	33.889	34.389	0.38	34.323
	OB2	34.306			
	OB3	34.972			
	CB1	31.000	34.257		
	CB2	43.444			
	CB3	31.944			
	CB4	30.639			
Barley	OC1	56.083	47.090	27.78	40.549
	OC2	44.972			
	OC3	51.444			
	OC4	35.861			
	CC1	35.944	34.007		
	CC2	23.528			
	CC3	23.500			
	CC4	53.056			

Phenolic acids are common antioxidants present in fruits, vegetables, legumes as well as grains. The major forms of phenolic acid are substituted benzoic and cinnamic acid compounds (Miller, Rigelhof and Kanter, 2000). The total phenolic acid was expressed in gallic acid equivalent, GAE in the unit of µg/ml. The TPC of the grain samples were tabulated in Table 4.8. The TPC of the oat samples ranged from 18.167 to 34.222 µg/ml, whereas for wheat samples, the TPC ranged from 30.639 to 43.444 µg/ml. The average TPC in organic oat samples and conventional oat samples were 25.799 and 23.778 µg/ml. This indicated that the TPC in organic oats was 7.83% higher than those in conventional oats. Meanwhile, the average TPC in organic wheat is slightly higher than conventional wheat's by 0.38%. Among the barley samples, organic barley sample OC1 had the greatest TPC (56.083 µg/ml) while the conventional barley sample CC3 had the smallest TPC (23.500 µg/ml). In addition, all organic barley samples exhibited high TPC and hence average TPC calculated was 47.090, which is 27.78% higher than average TPC in conventional barley.

An obvious trend was observed when determining TPC. All organic grains had higher average amount of TPC than their conventional grains as shown in Figure 4.9. This result agreed with the study by Vaher, Matso and Kaljurand (2010) which examined TPC of the spring wheat varieties grown in organic and conventional condition. TPC of the former was sparingly higher than that of the latter. They concluded that cultivation techniques have a certain extent of effect on the biosynthesis and accumulation of phenolic compounds (Vaher, Matso and Kaljurand, 2010). As there is no synthetic chemical applied, the organic plant is more susceptible to the infection and hence they will produce more phenolic compounds.

The order of total average TPC was Barley < Wheat < Oats with TPC of 24.789, 34.323 and 40.549 μg/ml respectively (Figure 4.10). Antioxidant compounds existed in barley samples were complex and their activity and mechanism were greatly affected by composition and conditions of the test systems. The application of 70% acetone (v/v) as extraction solvent in this study managed to extract the antioxidants in barley efficiently since the recommended solvent suggested by Zhao et al. was 80% acetone (v/v) (Vaher, Matso and Kaljurand, 2010). Therefore, it was not surprise that barley showed the highest TPC in this study. Another reason was a number of classes of compounds in barley that have a phenolic structure, which included benzoic and cinnamic acid derivatives, proanthocyanidins, flavonols, flavones and many other phenolic compounds (Bicka, Karklinam and Kruma, 2011).

The second highest amount of TPC was observed in wheat grains (34.232 μg/ml). In fact, wheat contained miscellaneous phenolic acids in which ferulic, *p*-coumaric and vanillic acids present in higher proportion. However, most of them were existed in the bound form with other grain components, for instances, starch, cellulose, β-glucan and pentosane (Vaher, Matso and Kaljurand, 2010). This explains why the TPC of wheat is not as high as expected. From the result, it was observed that wheat had higher TPC than oats. This finding was confirmed by a reaseach carried out by Adom and Liu (2002) which found that TPC in wheat and oats were 7.99 and 6.53 µmol/g of grain.

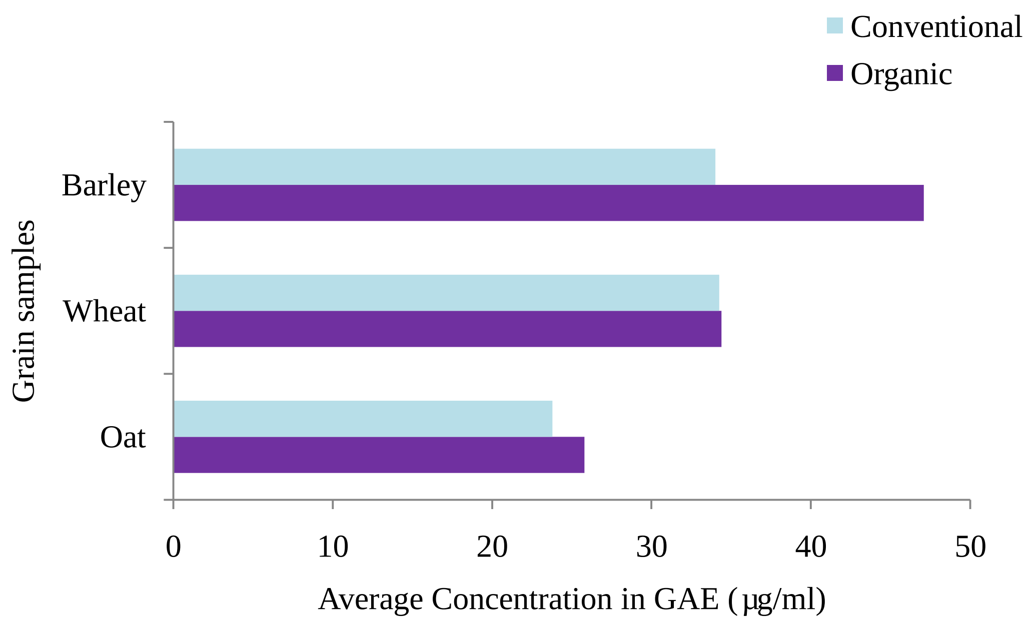


Figure 4.9: Average TPC in organic and conventional grains

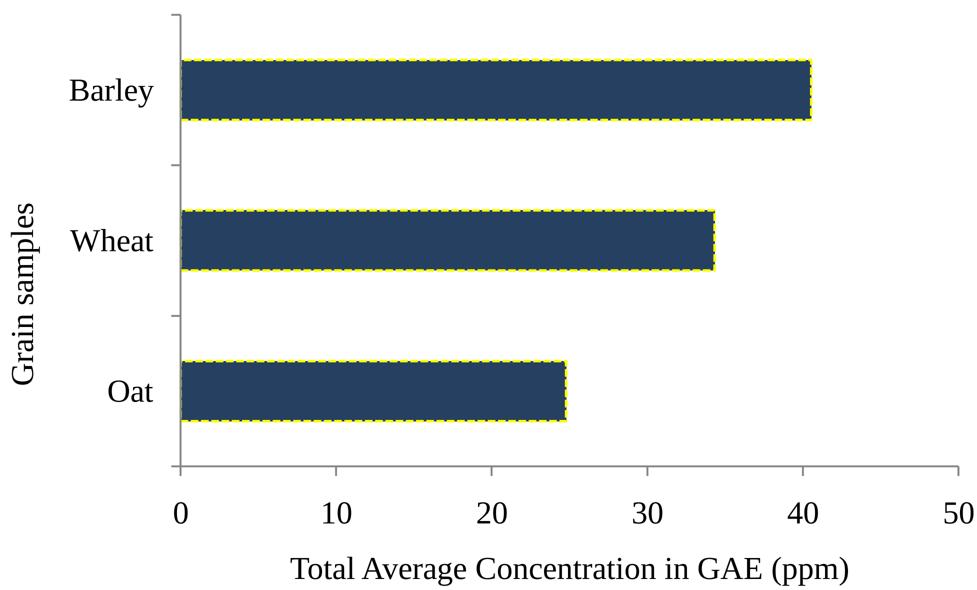


Figure 4.10: Comparison in total average TPC in grains

4.3 Quantification of Antioxidant Activity in Grain Samples

Antioxidant activity (AA) of whole grains contributes to mechanisms that fight against chronic diseases (Miller, Rigelhof and Kanter, 2000). The AA of grains is contributed majorly by total flavonoid contents. The presences of polyhydroxyl groups in flavonoids make them become good radical scavengers for antiinflammation and other chronic diseases (Zilic et al., 2011). The antioxidant activity on DPPH by grain samples was presented in percentage (%) in Table 4.9. Figure 4.11 illustrates how antioxidant scavenges the DPPH radical and reduces its amount.

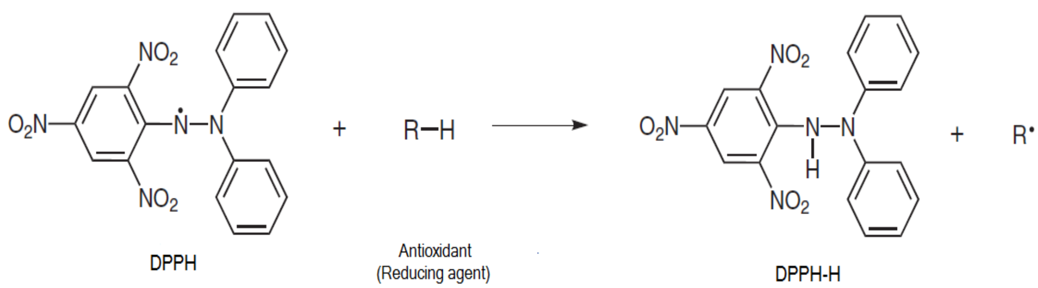


Figure 4.11: A simple illustration on how antioxidants scavenge DPPH radical (Gressler et al., 2010)

The higher the AA, the more DPPH radicals are able to be scavenged by the antioxidants. Hence, this antioxidant is excellent in prevent cell damage by reduce the oxidation damage caused by radicals in human body. A narrow range of AA was observed in oat samples (77.55-82.60%). In addition, average AA of organic oats and conventional oats were high, which were 82.23% and 80.92% respectively. In contrast, the range of AA obtained from wheat samples was 40.35 -68.57% only. Organic wheat and conventional wheat showed the lowest AA among other samples (51.22% and 56.44%). Among the barley samples, organic barley sample OC1 seems to be able to quench the highest amount of DPPH free radicals since it showed the highest AA, 82.51%.

Table 4.9: Antioxidant Activity (AA) on DPPH

Grain	ID	AA (%)	Average AA (%)	Percentage difference between AA of two categories	Total Average AA (%)
Oat	OA1	82.38	82.23	0.16	81.57
	OA2	81.82			
	OA3	82.77			
	OA4	81.93			
	CA1	82.60	80.92		
	CA2	82.60			
	CA3	77.55			
Wheat	OB1	52.30	51.22	10.19	53.83
	OB2	53.03			
	OB3	48.34			
	CB1	65.13	56.44		
	CB2	51.72			
	CB3	68.57			
	CB4	40.35			
Barley	OC1	82.51	72.79	10.51	76.61
	OC2	59.77			
	OC3	76.27			
	OC4	72.59			
	CC1	81.63	80.44		
	CC2	81.81			
	CC3	78.66			
	CC4	79.65			

From Figure 4.13, it was observed that organic oats, conventional wheat and conventional barley showed higher AA. This was further confirmed by Bartosova, Kosik and Kobida (2013) who demonstrated that there was no significant difference of antioxidant activity detected between wheat samples from different farming systems. There was no direct relationship between cultivation techniques and antioxidant activity. Other factors such as cropping year and forecrop had greater affect on bioactive components in crops.

As shown in Figure 4.14, among the three types of grains, oats gave the highest AA (81.58%), followed by barley (76.61%) and then wheat (53.83%). This was due to the fact that oats contained avenanthramides, which was found in oats only. These compounds were cinnamoyl conjugates that able to give rise to high AA (Adom and Liu, 2002). Anvenanthramides-c had higher antioxidant activity than a and b. The structure of anvenanthramides-c is shown in Figure 4.12. Other flavonoids such as apigenin and quercetin can be found in oats as well.

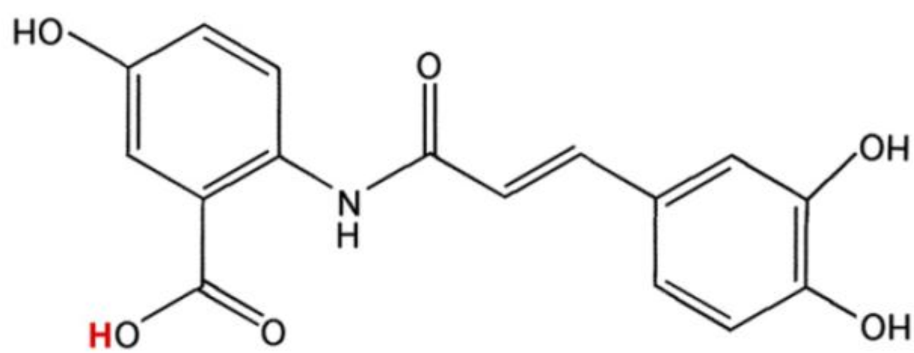


Figure 4.12: The chemical structure of anvenanthramides-c (Guo, Wise and Meydani, 2008)

Barley exhibited the second highest value of AA. It consists of a subclass of flavonoids, flavan-3-ols which was absent in oats and wheat. The extractable of this compound contribute to moderate AA of barley by react actively in DPPH radical scavenging and aids in reduction of Fe^{3+} (Zilic et al., 2011).

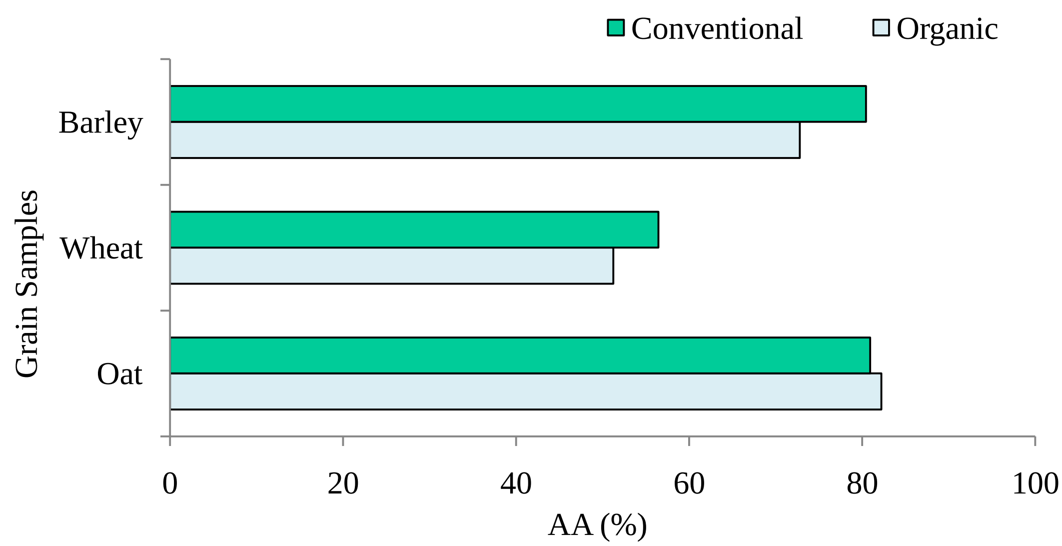


Figure 4.13: Average AA of Grain Samples from Different Cultivation Techniques

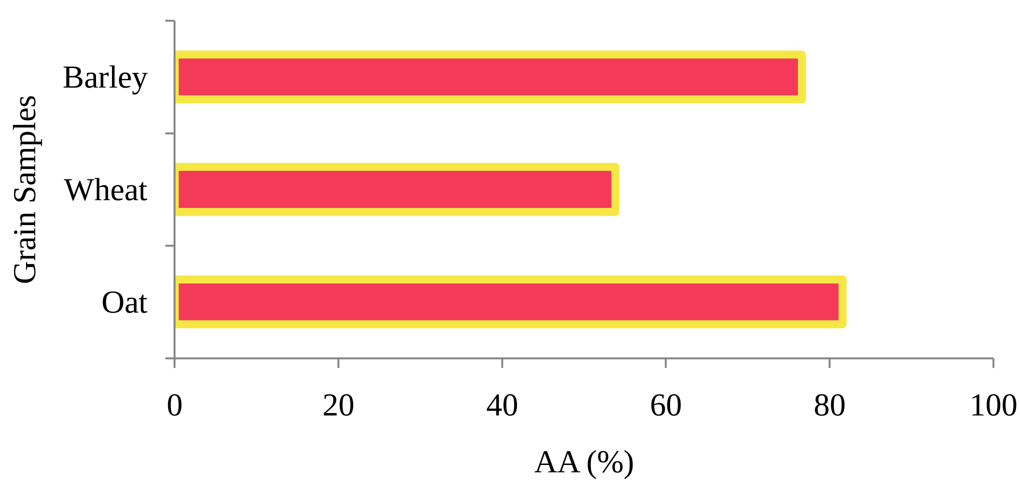


Figure 4.14: Total average AA among grain samples

4.4 The Correlation between Total Phenolic Content and Antioxidant Activity

From the results, there was no correlation between TPC and AA among organic grain and conventional grain samples. For example, the TPC of organic barley sample, OC1 was the highest among other organic grains, but it only ranked third best antioxidant ability in quantification of AA. Besides, organic oats sample, OA2 had the lowest TPC but its AA was quite high, which is 81.82%.

Similarly, Velioglu, Mazza and Oomah (1998) and Miller, Rigelhof and Kanter (2000) found that the TPC were inconsistent with the AA of their grain samples. This showed that other factors except total phenolic compounds can affect the antioxidant activity of grains. For example, microchemicals such as vitamin and tocopherol exhibit significant antioxidant activity as well. Hence, they claimed that further research has to be carried out to find out specific composition to explain the underlying mechanism.

In addition, solubility of different antioxidant compound varies in the different reagents in the TPC and AA analysis. This can result in different responses initiated and lead to lack of correlation between TPC and AA. Besides, evaluation of TPC by using FCR might overestimate the TPC. The reason was that this reagent not only reacted with phenolic compounds, but also sugars and ascorbic acid in sample (Mariko, Hassimotto and Genovese, 2005).

CHAPTER 5

CONCLUSION

As a conclusion, organic grains contained higher level of essential elements (Mg, Fe and Zn) and lower non-essential element (Pb) than conventional grains. These were because of organic cultivation technique which did not use synthetic chemicals and have organically managed soil that consisted of more helpful microorganisms. Besides, no nickel was detected in any grain samples. All grain samples did not exceed the limitation of heavy metal concentration set by WHO.

Organic grains contained more TPC because they tend to produce more phenolic compound in unfertilized condition. Among grain types, barley exhibited the highest TPC because it contained many phenolic compounds such as benzoic and cinnamic acid derivatives. Besides, organic oats, conventional wheat and conventional barley showed higher AA. These showed that there was no direct relationship between cultivation techniques and antioxidant activity. Other factors such as cropping year and forecrop had greater affect on bioactive components in crops. Lack of correlation between TPC and AA was because of contribution of other compounds to AA other than phenolic compounds. In short, grains are food rich in essential metal and antioxidant that good for human.

FURTHER STUDIES

Apart from the three kinds of grains examined in this study (oats, wheat and barley), other kinds of grains such as flaxseed, millet, rye, buckwheat, amaranth, corn, quinoa and sorghum should be assessed to further compare the difference between organic and conventional grains. Besides, comparison can be made among different kinds of grains. For evaluation of metals content, other metallic elements such as arsenic, aluminium, mercury, cadmium, copper, manganese and molybdenum should be examined to make sure their content do not exceed the permissible level. Excessive mercury and cadmium are lethal. Furthermore, instrument such as ICP can be used to evaluate the metals content because this method is more time-saving and able to provide more accurate results.

Different solvents such as methanol, ethanol, water and petroleum-ether are other choices to extract the antioxidants and phenolic compounds in grain samples. The results will vary due to different solubility of these compounds in different solvent. TOSC assay, β-carotene bleaching method and ABTS assay are other efficient method to quantify antioxidant capacity. The results can be compared to get a more consistent result regarding differences between organic and conventional grains. Since grains are full of soluble and insoluble fiber, further studies regarding these compounds should be proposed to compare their amount in organic and conventional grains.

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APPENDIX A

Evaluation of Metals Content

$$C_{metal} = c_{metal} \times Vm$$

i.C_{metal} is concentration of metal in (mg/kg)

ii. c_{metal} is concentration of metal in (mg/l)

iii. V is total volume of the dilution sample (l)

iv. m is mass of sample used (kg)

APPENDIX B

Quantification of Antioxidant Activity in Grain Samples

$$\text{AA (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100\%$$

$\text{Abs}_{\text{control}}$ = Absorbance of the control reaction

$\text{Abs}_{\text{sample}}$ = Absorbance of the grain sample

APPENDIX C

Table of absorbance measured at known concentrations of Mg standard solutions, ppm

Concentration (ppm)	**Average Absorbance Value (n = 3)**	**S.D**
0.4	0.582	0.0015
0.8	0.972	0.0017
1.2	1.335	0.0006
1.6	1.725	0.0004
2.0	2.180	0.0080

APPENDIX D

Table of absorbance measured at known concentrations of Fe standard solutions, ppm

Concentration (ppm)	**Average Absorbance Value (n = 3)**	**S.D**
0.4	0.037	0.0005
0.8	0.066	0.0005
1.2	0.103	0.0015
1.6	0.130	0.0011
2.0	0.161	0.0001

APPENDIX E

Table of absorbance measured at known concentrations of Zn standard solutions, ppm

Concentration (ppm)	Average Absorbance Value (n = 3)	S.D
0.05	0.011	0.0006
0.50	0.087	0.0006
1.00	0.171	0.0015
1.50	0.261	0.0020
2.00	0.372	0.0015

APPENDIX F

Table of absorbance measured at known concentrations of Pb standard solutions, ppm

Concentration (ppm)	**Average Absorbance Value (n = 3)**	**S.D**
0.02	0.002	0.0006
0.05	0.005	0.0006
0.07	0.007	0.0010
0.10	0.010	0.0006

APPENDIX G

Table of absorbance measured at known concentrations of Ni standard solution, ppm

Concentration (ppm)	Average Absorbance Value (n = 3)	S.D
0.4	0.018	0.0006
0.8	0.037	0.0010
1.2	0.058	0.0017
1.6	0.076	0.0006
2.0	0.098	0.0006

APPENDIX H

Table of absorbance for different concentrations of gallic acid, µg/ml

Concentration	Absorbance at λ = 725 nm
5	0.053
10	0.105
20	0.212
40	0.554
60	0.707
80	1.069