Faculty of Resource Science and Technology

DETERMINATION OF HEAVY METALS IN CANNED SEAFOOD PRODUCTS

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DECLARATION

No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any university or institution of higher learning.

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Determination of Heavy Metals in Canned Seafood Products

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ABSTRACT

The total concentration of metals Pb, Fe, Zn, Cr, Cu and Ni were determined in different brands of sardines canned in tomato sauce and cockles canned in soy sauce, commercialized in Malaysia. The sample was dried by using freeze-dry and homogenized before digesting with a mixture of nitric acid (69%) and hydrochloric acid (37%) (ratio 1:2). The digestion solution was analyzed by using flame atomic absorption spectroscopy (FAAS). The quality of the canned sardines and cockles was evaluated among varieties, manufacturing countries and period on shelf from the date of manufacture. In general, all of the metal concentrations in canned sardines and cockles were within the maximum permitted level set by Malaysian Food Act 1983 and the Food Regulations (1996). The ANOVA test reported significant difference in Pb and Fe content in the canned sardine samples with different shelf life. The increase in Pb and Fe content with storage duration may due to leaching of the metals from the can. Other metals such as Cr, Cu, Zn and Ni did not show any significant difference with storage duration and naturally occurred in the food.

Keywords: Canned sardines and cockles, heavy metals, FAAS.

ABSTRAK

Kepekatan logam berat (Pb, Fe, Zn, Cr, Cu dan Ni) dalam makanan tin sardin dalam sos tomato dan kerang berkicap telah dianalisa. Sampel telah dikeringkan dengan menggunakan kaedah pengeringan sejukbeku and dihancurkan sebelum dicernakan dengan menggunakan campuran asid nitrik (69%) dan asid hidroklorik (37%) dengan nisbah 1:2. Larutan pencernaan telah dianalisi menggunakan Spektroskopi Serapan Atom Api (FAAS). Kualiti sardin dan kerang dalam tin telah dinilai berdasarkan jenis, negeri pengeluar and jangka hayat dari tarikh pengeluaran. Secara keseluruhan, kesemua kepekatan logam berat dalam produk sardin dan kerang didapati tidak melebihi tahap yang ditetapkan dalam Akta Makanan Malaysia 1983. Ujian ANOVA telah membuktikan bahawa terdapat perbezaan kepekatan bagi logam Pb dan Fe dengan tempoh penyimpanan. Peningkatan kepekatan Pb dan Fe ini berpunca daripada pelarutlesapan logam dari tin. Logam Cr, Cu, Zn dan Ni tidak menunjukkan perbezaan yang signifikan disebabkan logam-logam tersebut adalah wujud semulajadi di dalam makanan laut.

Kata kunci: Sardin dan kerang dalam tin, logam berat, FAAS.

CHAPTER 1

INTRODUCTION

Heavy metals are metals with density at least five times of water (5 gcm^{-3}), for example mercury, lead, nickel, zinc, arsenic, copper and cadmium (Pascow *et al.*, 1961). Heavy metals are stable. They cannot be metabolized by the body and tend to accumulate. Presence of heavy metals in large quantity in human body may lead to chronic toxicity. Contamination of heavy metals has gained concern in food industry as there are increasing incidents of agriculture and seafood sources being contaminated by heavy metals.

Many changes have occurred in the food industry since World War II. Today we use many worldwide food sources instead of depending primarily on the home-grown food. The food is mass-produced, nationally and internationally distributed. The sources of the food are subjected to environmental contamination as well as contamination during processing (Koren & Bisesi, 2003). In a study, the total concentrations of metals were determined in canned sardines commercialised in Brazil. Some metals such as zinc, iron and chromium were found to be high in the products (Tarley *et al.*, 2001). Burger & Gochfeld (2004), reported a significant level of mercury in the shop shelf canned tuna available in the central New Jersey.

The canning process is found to pose a higher risk of heavy metals contamination compare to others form of food packaging. Solder used in the manufacture of cans has been recognized as a source of lead contamination during canning. The tin-canned orange juice was found to have higher heavy metals contents than juice kept in paperboard box or laminate pouch (Ahmed Samir, 1987).

The canning also impacts the quality of food over time. The heavy metal content in the shop shelf canned products showed a relatively higher level of heavy metals compare to the immediate factory products. Oduoza (1992), revealed that shop shelf canned tomato purees and orange juices contained higher concentration of tin, iron and lead compared to immediate factory samples. Aluminium content in the beer was assessed over a period of 150 days. The study concluded that aluminium corroded over time and resulted in higher aluminium content (Vela, 1998). The contamination of heavy metals in canned food products may be attributed to the process of canning and the quality of the sources. Therefore, analytical control of heavy metals in canned food is important to ensure the quality of the products.

Canned food offers a shortcut in meal preparation which is most favoured by those stretched for time. Although the canned food has reached maturity, sales growth remains positive due to increasingly busy lifestyles of consumers nowadays. Canned seafood is one of the canned food products that gains promising acceptance from the consumers. In Indonesia and Thailand, it is the most popular type of canned food. Despite the great consumption of the canned seafood products in human diet, relatively little attention has been devoted to examine the heavy metals content particularly, in relation to the shelf life of the products in our region.

The objectives of the study were:

1) To evaluate the acid digestion methods for determination of heavy metals in canned seafood products,
2) To determine the heavy metals content in the canned seafood products available locally.
3) To compare the heavy metals content in canned seafood products between the manufacturing, shelf life and the varieties of food.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metals Contamination in Canned Seafood Products

Marine organisms provide an important source of nutrient to human especially fish and shellfish. These include sardines, mackerels, cockles, oysters, clams and mussels. Consumption of fish and shellfish is believed to prevent and treat cardiovascular diseases and rheumatoid arthritis (Shahidi *et al.*, 1997). However, they are also potential cause of certain diseases in human. For example, shellfish species are filter feeders that tend to accumulate natural toxins, heavy metals and chemical contaminants from polluted environment. Presence of excess metals in the seafood sources creates concern in its canned products too as there is a direct and inevitable relationship between the quality of raw material and end products. The heavy metals are bioaccumulated and cannot be disposed from fish or shellfish during cleaning processes before canning (Koren & Bisesi, 2003). In addition to the environmental contamination, the canned products are subjected to contamination due to processing.

2.2 Processing and Canning of Seafood Products

Prior to processing and canning, seafood products harvested from sea are refrigerated to ensure the quality. Upon arrival on the processing factory, they are weighted and categorized according to size, types and quality. They are usually washed, gutted and steamed with water, oil or hot air or a combination of these. After cooling, they are filleted according to size of cans and filled into the cans. Salt solution, preservatives, additives, spices, sugar solution and permitted colouring are added into the can. The cans are then sealed and sterilized in pressure chambers heated by steam to a temperature of 121 - 125°C in which the cans can be held for a period depending on the type of food. In Malaysia, quality of the processed food is controlled with the procedures established in Hazard Analysis Critical Control Point (HACCP), Good Manufacturing Practices (GMP), Sanitation Standard Operating Procedures (SAPs), Standard and Industrial Research Institute of Malaysia (SIRIM), Quality assurance Programmes (QAP) and ISO 9000 (Jinap *et al.*, 2004).

2.3 Can-making Technology in Canned Seafood Products

Tinplate is the most frequently used packaging method for seafood products. Tinplate consists of a base plate of low-carbon mild steel coated with a layer of electrolytically deposited tin. The tin is applied to provide carbon protection of the steel base. The base plate varies depending on the size of cans and the application. It is usually between 0.15 and 0.30 mm thick. Nowadays, steel sheet is cold rolled twice prior to tin coating to obtain an extra light gauge plate feature. However, the high cost of tin has favored the production of tin-free steel (TFS) where the conventional tin and tin oxide layers are replaced by chromium and chromium oxide layers. The plain TFS cannot be used readily as it is lacking of corrosion resistance but it provides an excellent key surface for application of protective lacquers. Following the introduction of TFS, there was a change in can-making technology. A system using neither tin nor chromium but nickel as a coating material for the steel base was developed (Warne, 1998).

For canning of seafood products, sulphur resistant lacquer system is usually used to prevent formation of blue or black tin and iron sulphide. During thermal processing, zinc is likely to be released from the proteins and reacted with the sulphur compounds to form zinc sulphide precipitates (Warne, 1998).

Aluminium alloys was also employed for canning of seafood products. The aluminium alloys are lacking of chemical resistant compared to pure aluminium however they possess greater hardness. To improve its chemical resistant, small amounts of magnesium and manganese are usually introduced. Typically, the can body stock contains 0.8 – 1.3% magnesium and 1.0 – 1.5% manganese (Boustead & Hancock, 1981). Aluminium alloys for food contact materials may contain other alloying elements such as silicone, iron, copper and zinc (Baum, 2001).

2.4 Contamination of Heavy Metals from Can

2.4.1 Lead

In light of the can making technology that depends heavily on metals, contamination of heavy metals in canned food is possible. Heavy metals contamination is likely due to Pb, Sn, Cr, Fe and Al.

Lead is one of the most possible metal contaminants in canned food due to soldering process and tin coating on tinplate. Contribution of lead from tin coating is considered insignificant (Weedon, 1983). However, contamination attribute to soldering process should not be overlooked.

Lead is heavily used for soldering of the canned food for example the side seam on most of the tinplate cans is soldered with an alloy containing 98% lead / 2% tin. Although non-lead soldering has been developed, lead soldering remains popular in canned food industry as the technology of non lead soldering is costly (Baum, 2001). The nature of food stored in can further determine the extent of Pb contamination in the canned food. Study evidenced that cans containing food rich of mallic acids are more likely to subject to lead dissolution although the contamination is not significant. Oxygen that presents in the can headspace after sealed may also contribute to the lead dissolution.

2.4.2 Tin

The use of tinplate for canned food will result in tin dissolution. Fresh food usually contain little amount of tin however, canning process increases the level of tin especially for foods store in unlacquered can (Weedon, 1983). The rate of detinnng depends on presence of oxidizing agents or corrosion accelerators such as oxygen and nitrates (Weedon, 1983; Baum, 2001; Blunden & Wallace, 2003), the acidity of foods store in cans (Blunden & Wallace, 2003), the presence of anthocyanins compounds, storage duration and conditions of the cans (Blunden & Wallace, 2003). A study of canned US Military rations showed that tin content in canned food was higher at higher temperature (Blunden & Wallace, 2003).

Presence of oxygen that is usually found in the can headspace, nitrate that is the nature of the ingredients and anthocyanin compounds may accelerate tin dissolution in the canned food (Weedon, 1983; Blunden & Wallace, 2003). However, addition of oxidizing agents in the canned food is found to be most significant to the detinning rate as this will lead to rapid corrosion of unprotected steel with vigorous evolution of hydrogen (Blunden & Wallace, 2003). The dissolution of tin protects the can from perforations and the contents from degradation in flavour and colour during storage (Blunden & Wallace, 2003).

2.4.3 Iron

Food contained in cans may expose to iron contamination under certain circumstances. The iron in the mild steel may dissolve into the food when the lacquer coating is damaged. The rate of iron dissolution depends on the storage duration (Blunden & Wallace, 2003). Iron is an essential element for human that present naturally in food. It is more concern as a deficiency problem rather than contamination. Therefore, no maximum permitted level of iron was established in the Food Act 1983 and the Food Regulations (1996). Nevertheless, the recommended intake of iron is drawn at 10 mg/day for men and 12 mg/day for women (Weedon, 1983).

2.4.4 Chromium or Chromium (III) Oxide

Chromium is also a potential contaminant in canned food. During can making, a light film of chromium and chromium (III) oxide is usually employed in passivation treatment. Although the thin film generally contains very little Cr (~ 1μg Cr/cm^2), the passivation film may dissolve into food when the tin coating is attacked (Weedon, 1983). Acidic canned food in non-lacquered cans especially, showed a higher Cr content than the fresh food. The dissolved chromium was found as Cr (III) rather than Cr (VI) as under acidic condition only Cr (III) is susceptible to dissolution (Guglhofer & Bianchi, 1991).

2.4.5 Aluminium

Most of the carbonated beverages cans are made from aluminium and fitted with an 'easy open' aluminium end. The dissolution of aluminium into the food occurs when the lacquer of the can is damaged (Weedon, 1983). Dissolution of aluminium is accelerated when the food is stored under acidic condition and containing a high salt concentration of over 3.5% of sodium chloride. The content of aluminium is in addition dependent on the storage time and temperature (Baum, 2001).

CHAPTER 3

METHODOLOGY

3.1 Samples

Two different types of shop shelf canned seafood products were obtained from the local market. These include sardines (*Sardinops* sp. and *Sardinellas* sp.) and cockle (*Anadaras* sp.) products as shown in Appendix 1. They were categorized according to manufacturing countries and period on shelf from date of manufacture. The heavy metals concentrations of the flesh and food gravy were analysed. Table 1 summarizes the details of the samples analysed.

Table 3.1: The details of canned seafood products analysed.

Types	Manufacturing Country	Brand	Period on shelf from date of manufacture (year)
***Sardinops* sp.**	Malaysia	King Cup	Less than 1
			1 – 2
			2 – 3
***Sardinellas* sp.**	Thailand	ES	Less than 1
			1 – 2
			2 – 3
***Anadaras* sp.**	Malaysia	Wee	Less than 1
			1 – 2
			2 – 3

3.2 Metal Analysis

3.2.1 Evaluation on the Acid Digestion Methods

In this study, five hot plate digestion methods were evaluated for determination of Pb, Fe and Zn. The digestion methods include HNO_3, HNO_3 : HCl (1:1), HNO_3 : HCl (1:2), HNO_3 : HCl (1:3) and HNO_3 : H_2SO_4 (1:1). Approximately 1.00 g of finely powdered samples were spiked with a known concentration of lead, iron and zinc and homogenized. The concentrations of Pb spiked were 0.5 mg/kg and 1.0 mg/kg and the concentrations of Fe and Zn spiked were 5 mg/kg and 10 mg/kg. They were digested with 5 ml of nitric acid (69%), 2 ml nitric acid - 2 ml hydrochloric acid (37%), 2 ml nitric acid – 4 ml hydrochloric acid, 2 ml nitric acid – 6 ml hydrochloric acid, and 2 ml nitric acid – 2 ml sulfuric acid (96%).

Each of the samples was swirled gently, covered with watch glass and left at room temperature for about one hour until most of the samples were dissolved. The solutions were heated on the hot plate for 90 minutes until yellow fume was released and the solution was clear. After cooling, the acid solutions were filtered through 0.45 μm glass micofibre filters paper into 50 ml volumetric flask and made up to mark with deionized water. Three replicates were prepared for each digestion and subjected to analysis of Pb, Fe and Zn using Flame Atomic Absorption Spectrophotometry (FAAS).

In the stage of digestion, the blank samples and non-spiked samples were prepared simultaneously for quality assurance. For digestion solution that favoured the formation of white precipitate due to fatty residue, 2 ml of ethanol (98%) was added for digestion at room temperature.

3.2.2 Determination of Metal Concentrations in Dry Sample and Recovery Percentage

The dry sample metal concentration in mg/kg can be calculated by using the formula:

$$\text{Metal concentration of sample, mg/kg} = \frac{\text{Metal concentration of sample (mg/L)} \times 0.050\text{L}}{\text{Sample Weight (kg)}}$$

The percentage of recovery test can be calculated by using the formula below:

$$\text{\% of Recovery} = \frac{\text{Metals in spiked samples (mg/kg)} - \text{metals in non-spiked samples (mg/kg)}}{\text{Metals in non-spiked samples (mg/kg)}} \times 100$$

3.2.3 Detection Limit

The detection limit is defined as the concentration of element which produces a signal / noise ratio of 3 (Perkin Elmer, 1982). Hence, the detection limit considers both of the signal amplitude and the baseline noise. It is the lowest concentration that can be clearly differentiated from zero. The detection limit was calculated based on the following equation.

$$\text{Detection Limit} = \frac{\text{Standard Concentration} \times 3\ \text{Standard Deviation}}{\text{Mean}}$$

The detection limits of Pb, Fe and Zn analyzed were 0.03 mg/L, 0.03 mg/L and 0.002 mg/L, respectively.

3.3 Determination of Heavy Metals in *Sardinops* sp., *Sardinellas* sp., and *Anadaras* sp.

The digestion method that produced good recovery percentage was chosen for analysis of heavy metals in canned sardines and canned cockles from different shelf life. Thirty samples of canned sardines in tomato sauce representing two manufacturing countries and three categories of shelf life were analyzed for Pb. Fe, Zn, Ni, Cr and Cu. The shelf life concerned was less than 1 year, 1 - 2 years and 2 - 3 years.

The sardine samples from Malaysia were identified as *Sardinops* sp. and the sardine samples from Thailand were *Sardinellas* sp. Fifteen samples of canned cockles in soy sauce manufactured in Malaysia representing three different shelf lives were also analysed for the heavy metals content. The content of the can was drained and the samples were washed and freeze-dried. The freeze-dried samples were pulverized and homogenized prior to metal analysis.

Approximately 5.00 g of finely powdered samples were digested with 10 ml of nitric acid (69% HNO_3) for 10 minutes. Twenty millilitres hydrochloric acid (37% HCl) of was later added slowly. The samples were swirled gently, covered with watch glass and left at room temperature for about one hour until most of the samples were dissolved. The solutions were heated on the hot plate for 1½ hour until yellow fume was released and the solution was clear. After cooling, the acid solutions were filtered through 0.45 μm glass microfibre filters into 50 ml volumetric flask and made up to mark with deionized water (Voegborlo *et al.*, 2003). The samples were analyzed for the metals content using Flame Atomic Absorption Spectrophotometry (FAAS).