UNIVERSITI PUTRA MALAYSIA

REMOVAL OF BORON IMPURITIES AS BORIC ACID FROM YELLOW NOODLES BY HOT WATER LEACHING

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DEDICATED TO MY FAMILY

ABSTRACT

Yellow noodles have been reported to contain boric acid in Malaysia recently. In this study, an attempt has been made to evaluate the effectiveness of boric acid removal from locally manufactured yellow noodles by hot water leaching technique. Noodle samples were treated in boiling distilled water. Spectrophotometric assay in curcumin was used to analyze total boric acid content. Results showed that up to 33% boric acid can be removed in 40 seconds. This report also suggests that boric acid in yellow noodles can be reduced to acceptable level for consumption through hot water leaching.

ABSTRAK

Mi kuning telah dilaporkan mengandungi asid borik di Malaysia kebelakangan ini. Dalam kajian ini, penumpuan diberi untuk menilai kecekapan penyingkiran asid borik daripada mi kuning tempatan dengan menggunakan teknik pelakuan larutresap air didih. Sampel mi dirawat di dalam air suling yang mendidih. Dalam kes ini, ujian spektrofotometrik curcumin telah digunakan untuk menganalisis jumlah kandungan asid borik. Keputusan kajian ini menunjukkan bahawa asid borik boleh disingkirkan sebanyak 33% dalam 40 saat. Laporan ini juga mengusulkan asid borik dalam mi kuning dapat dikurangkan ke tahap yang selamat dimakan menurut WHO dengan menggunakan larutresapan air didih.

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Lastly, I thank my family for their encouragement and unlimited support. They have been a great source of strength and to them I dedicate this work.

I certify that this research project report entitled "Removal of Boron Impurities as Boric Acid from Yellow Noodles by Hot Water Leaching" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor of Bioindustry Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

Figure		Page

LIST OF ABBREVIATIONS

Abbreviation	Full name
AOAC	Association of Official Analytical Chemists
B	Boron
BfR	Bundesinstitut fur Risikobewerertung (Germany Federal Institute for Risk Assessment)
CAP	Consumer Association of Penang
CRD	Completely Randomized Design
DNMRT	Duncan's New Multiple Range Test
ETAAS	Electrothermal Atomic Absorption Spectrometry
EHD	2-ethyl-1,3-hexanediol
EVM	Expert Group on Vitamins and Minerals
WHO	World Health Organization
ICP	Inductively Coupled Plasma
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
NCSS	Statistical Analysis and Graphics
ppm	Part per million

CHAPTER 1

INTRODUCTION

Boric acid (H_3BO_3) or orthoboric acid is an inorganic boron compound. It is generally a white solid, but it may be clear to murky. Boric acid has been commonly used in many different commercial applications due to its pH-buffering, antiseptic, preserving, plasticizing or flame-retardant properties. It has been used as a common chemical food preservative, but is currently not listed as a permitted food additive and preservative under Food Regulation 1985 in Malaysia.

Excessive consumption of boric acid could bring harmful effects on humans such as nausea, dysentery, dermatitis and damage to the blood vessel and also may result in fatalities. In 1988, an outbreak of food poisoning related to boric acid had been recorded in Perak, Malaysia (Chao *et al.*, 1991). Epidedemiological investigations revealed that all victims ate "Loh See Fun" in which boric acid, a banned food additive and preservative, was added during production to enhance its colour, texture, flavour and crunchiness (Chao *et al.*, 1991). Since the outbreaks of boric acid food poisoning, the Consumers Association of Penang (CAP) had requested the Malaysia government to classify boric acid as a poison and restrict its sale, adding that only medical practitioners and certain food manufacturers should have access to it (Idris, 2000). However, high percentage of boric acid was still being reported in some food especially in yellow noodle (The Star, 2006), which is a very popular Chinese-style carbohydrate food in Asian countries.

Hot water extraction is currently the most widely used method and has been successfully shown to remove boron from soil in some studies (Berger and Truog, 1930; Mahler, 1984; Shiffer *et al.*, 2003). This has been proven to be one of the better predictors of total B removal in soils (Shiffler *et al.*, 2003). Beside this, hot water extraction also had been reported to be used to extract nutrients from soil (Fulkey and Czinkota, 1993).

In this project, a simple direct hot water leaching method was introduced to reduce boron impurities as boric acid from locally manufactured yellow noodles. Yellow noodles were leached with hot water to evaluate the boric acid removal efficacy and identify the relationship between boric acid removal and hot water leaching duration.

CHAPTER 2

LITERATURE REVIEW

2.1 Yellow Noodle

Yellow noodles also known as Chinese-style food, is a very popular wheat based food, especially in Asian countries. It is prepared from soft wheat and durum wheat in the ratio of 1:1, and has a bright yellow colour, fine surface formation and acceptable texture. Sometimes, alkaline water that contains sodium silicate and sodium carbonate is added in the manufacturing process. (Mohid, 1991).

2.2 Boron

Boron is a naturally occurring element which is found in the form of borates in the ocean, sedimentary rocks, coal, shale and some soils. Common borate compounds include boric acid, salts of borates, and boron oxide. It is non-metallic, with an atomic number of 5 and relative atomic mass of 10.811. Elementary boron exists in two forms; as fine crystalline (also called amorphous boron, which is brown in colour) and as crystalline boron in dark grey colour (Nemodruk and Karalova, 1969). Boron is naturally present in some foods, particularly nuts (14 mg/kg), fresh fruits (3 mg/kg) and green vegetables (2mg/kg) (EVM, 2002). It is an essential element for plants and WHO (1996) declared boron might have some beneficial effects and classified as a probable essential micronutrient for humans. Nevertheless, elevated boron concentrations are indicators of acute and possibly chronic excessive intake of boron by both animals and humans (Nielsen, 1986).

2.3 Boric Acid

2.3.1 General properties

Boric acid (H_3BO_3) or orthoboric acid is an inorganic boron compound. It is generally a white solid, but it may be clear to murky. It is also odorless and stable under normal conditions. Chemical structure of boric acid is shown in the figure below:

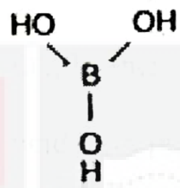

Figure 1: Chemical structure of boric acid

Figure 1 shows the $B(OH)_3$ units are linked together by hydrogen bonds to form infinite layers of nearby hexagonal symmetry. In aqueous solutions, these hydrogen bonds will be broken down and cause hydration of the boric acid ion according to the following reaction:

$$H_3BO_3 + 2H_2O \rightleftharpoons H_3O^+ + B(OH)_4^-$$

Boric acid is a water soluble compound and the percentage solubility of boric acid in water increases with temperature.

2.3.2 Usage

Boric acid has been commonly used in many different commercial applications due to its pH-buffering, antiseptic, preserving, plasticizing or flame-retardant properties (BfR, 2005). It has been used since the days of the ancient Greeks for cleaning, preserving food and other activities. Nowadays, it is found in more commercial products than ever before. Among them are in the field of pharmaceuticals, nutritional supplements, flame retardants, glass and fiber glasses, wood-processing, pest control, cosmetic, leather, textile, rubber, and paint industries. Due to the mild bactericidal and fungicidal properties of boric acid, it is also used as a disinfectant and preservative. It has been used as food preservative since 250 years ago and is used principally for preserving meat, fish and dairy products. It is one of the common chemical food preservatives which was either prohibited entirely or tolerated under certain restrictions by the Europe Federal Foods and Drugs Act (Smith, 1916). Furthermore, boric acid is not listed as a permitted food additive or preservative under the Malaysian Food Regulation 1985. In view of the cumulative toxicity, these compounds have been declared unsafe for use as food additives by the Food and Agriculture Organization-World Health Organization expert committee (Davidson, 1975). According to Idris (2000), excessive boric acid is so toxic that consumption can lead to poisoning, gastrointestinal disease, kidney damage and loss of appetite. The potential lethal doses of boric acid are 3 to 6 g for infants and 15 to 20 g for adults (WHO, 1998).

2.3.3 Issues of Food Poisoning by Boric Acid in Malaysia

Some Asian-style food products contain high amounts of boron due to deliberate addition of these compounds. Since 1989, up to 1500 mg/kg of boron levels have been found in some Asian-style products such as pickled fruit, meat, noodle, beef, pork, chicken and seafood. (Ziaziaris and Kacprzak, 1995). The issue of boric acid in food was first brought up by the Consumer Association of Penang (CAP) 22 years ago. Their surveys showed that boric acid was popularly used by food producers and fishmongers to hide the staleness and preserve the freshness of fish, prawn and meat (Idris, 2000). An outbreak of food poisoning resulting in 13 deaths in children occurred in Perak, Malaysia during the Chinese Festival of the Nine-Emperor Gods in 1988 (Chao et al., 1991). Epidedemiological investigations revealed that all victims ate "Loh See Fun" in which boric acid, a banned food additive and preservative, had been added to enhance its colour, texture, flavour and crunchiness. (Chao *et al*., 1991). In a statement released by Health Minister Datuk Dr. Chua Soi Lek, there was a high percentage of boric acid misuse as a food preservative in peninsular Malaysia. (The Star, 1 March 2006). In the report, yellow noodles contained the highest amount of boric acid at 70.4 %, followed by wantan noodles with 14.8% boric acid content, followed by koay teow, laksa, loh see fun and spring roll with 3.7% each. A random survey carried out in April 2006 showed that Selangor topped the list with 19% of noodle samples containing dangerously high levels of boric acid, followed by Penang (16%) (The Star, 1 March 2006). This deliberate addition of boric acid in yellow noodle is still happening as there were subsequent incidences of boric acid poisoning.

2.4 Hot Water Extraction

Currently, the most widely used soil B test to estimate plant available B is a hot water extractable method (Berger and Truog, 1939; Mahler, 1984). This method was found to be successful in removing boron from soils in some studies. According to Bruce *et al.* (2002), extractable boron using boiling hot water method can range from 0.04 to 2.45 mg/kg from arid zone soils. Hot water extraction method was proven as one of the better predictors of total B removal in soils (Shiffler *et al.*, 2003). A hot water percolation method to extract both macro and micro nutrients was reported for the acidic soils of Hungary by Fulkey and Czinkota (1993). In the present study, a simple and direct treatment was introduced to remove boron impurities as boric acid from locally manufactured yellow noodles. Yellow noodles were leached in boiling hot water to evaluate removal efficiency.

2.5 Analytical Techniques for Boron

The analytical techniques used for determination of boron range from simple electrochemical methods to complex nuclear methods (Sah and Brown, 1997). The most common methods for the boron determination are spectrophotometric methods, application of inductively coupled plasma atomic emission spectrometry (ICP-AES), electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma mass spectrometry (ICP-MS) method (Li and Zhang, 2006). However, ICP-AES and ETAAS methods had limited use because these methods had poor sensitivity and they suffer from serious memory effects and interferences (Sah and Brown, 1997; Burguera et al., 2001). In a study of boron determination in raw noodles, a rapid microwave digestion followed by inductively coupled plasma atomic emission spectrometric (ICP-AES) method was used due to its relative simplicity and rapidity (Ziaziaris and Kacprzak, 1995). Though ICP-mass spectrometry (ICP-MS) provide high sensitivity and low detection limit, it requires sophisticated and expensive analytical equipment, which may be prohibitive especially for smaller laboratories (Monika and Heiner, 1999). While, older and less expensive colorimetric techniques based on changes in the colour and intensity of certain dyes when boron is present is sometimes still used. In this case, spectrophotometric assays using curcumin (Dible *et al.*, 1954; Yamada and Hattori, 1986), Alizarin Red S (Garcia-Campana *et al.*, 1992) and Azomethine H (Wolf, 1974; Javor *et al.*, 1984) have been used satisfactory for biological samples. These methods are sensitive and generally used in laboratories which do not have inductively coupled plasma facilities. Moreover, atomic absorption spectrometry, adopted by the Association of Official Analytical Chemists (AOAC), was replaced by colorimetry with curcumin to increase the sensitivity and lower the detection limit (Ogawa *et al.*, 1979).

In the present study, a colorimetric technique described by Grinstead and Snider (1967) and Presley (1971) was used with minor modification to determine the boric acid levels in noodle samples. Levels were measured according to the formation of a boron-curcumin complex in acidic conditions, which shows an orange-red color (JOIDES, 1991). Interferences in this method can be avoided by extracting the sample solution with a mixture of 2-ethyl-1,3-hexanediol/chloroform (10% v/v) (Wikner and Uppstorm, 1980). Considerable work has been reported on chelate extraction of boron with 2-ethyl-1,3-hexanediol (EHD) (Agazzi, 1967;

Weger, 1969; Pau *et al.*, 1972; Horta, 1978), and it has been adopted as an official method by the Association of Official Analytical Chemists (1975).

CHAPTER 3

METHODOLOGY

Yellow noodle samples were collected from a local noodles manufacturer in Bintulu. All chemicals used in this study were analytical grade reagents and the use of borosilicate glassware was completely avoided.

Some reagents were prepared prior to analysis. Acid reagent was prepared from sulfuric acid (J.T.Baker) and glacial acetic acid in a ratio of 1:1. The curcumin reagent was a 0.125 % (w/v), solution of curcumin 98 % (Acros Organic) in glacial acetic acid (R&M). EHD-Chloroform was prepared from 2-ethyl-1,3-hexanediol (Merck) and chloroform (R&M) in a ratio of 1:4. Buffer solution (ammonium acetate-acetic acid) was prepared by dissolving 250 g of ammonium acetate (Techno Pharmchen) in 300 ml of glacial acetic acid and the volume was made up to 1 liter with distilled water.

Yellow noodles were leached in 2.5 liter boiling distilled water at 100 °C for 40 seconds, and 20 g of the noodles were sampled out from the treatment basin at 20, 30 and 40 seconds. They were dried overnight in an oven (ULM500, Memmert) at 80 °C.

After the leaching was completed samples was analyzed by uv-vis spectrophotometer (Cary, WinUV) using polystyrene plastic microcuvette (Bibby Sterilin). In addition to analysis, leached samples were grounded and ashed in a muffle furnance (L3/12/C6, Naberthem) for 4 hours at 500 °C and then left overnight. Ashed samples were transfered into polypropylene tube containing 2 ml of 6 M HCl (R&M) and filtered through Whatman filter paper no. 2. prior to spectrophotometric analysis for boron.

Spectrophotometric analytical conditions were as follow: sample extraction with 1 ml EHD-Chloroform reagent (1:4) for 30 minutes, colour development by adding curcumin reagent (100 µL) and acid reagent (400 µL) to 25 µL of organic phase, addition of 1.85 ml ammonium acetate-acetic acid buffer for 1.5 hours, and absorbance measurement at 555 nm with uv-vis spectrophotometer. Standard solutions were run at the same time as the samples.

The data that obtained was analyzed using Completely Randomized Design (CRD) with Statistical Analysis and Graphics (NCSS) program and the means were separated by Duncan's New Multiple Range Test (DNMRT) at P=0.05.

CHAPTER 4

RESULTS

In this study, the boric acid level in yellow noodles decreased after hot water leaching (Figure 2). Table 1 shows the percentage removal and average concentration of boric acid in yellow noodles before and after hot water leaching.

A regression curve with the equation $y = 0.016x^2 + 0.2005x - 0.2091$ (y is percentages of boric acid removal and x is duration of hot water leaching; $r^2 = 0.9924$) was plotted for the data collected (Figure 3). This regression curve allowed for conversion of amount boric acid removed to values for which yield correlation were available. The plot shows a positive relationship between hot water leaching duration and the amount of B removed in yellow noodles. The percentage of boric acid removal increased dramatically from 20 seconds until 40 seconds. There was around 10 % increment in boric acid removal for every 10 seconds after 20 seconds of leaching process. Within 40 seconds, the amount of boric acid removed was 33 %.

Table 1: Mean concentration and percentage removal of boric acid from yellow noodles with different hot water leaching duration.

Leaching Duration (s)	Boric Acid Concentration (ppm)	Boric Acid Removal (%)
0	0.797 **a**	0
20	0.722 **b**	9
30	0.620 **b**	22
40	0.536 **c**	33

Means within columns with the same letters are not significantly different at P=0.05 by DNMRT.

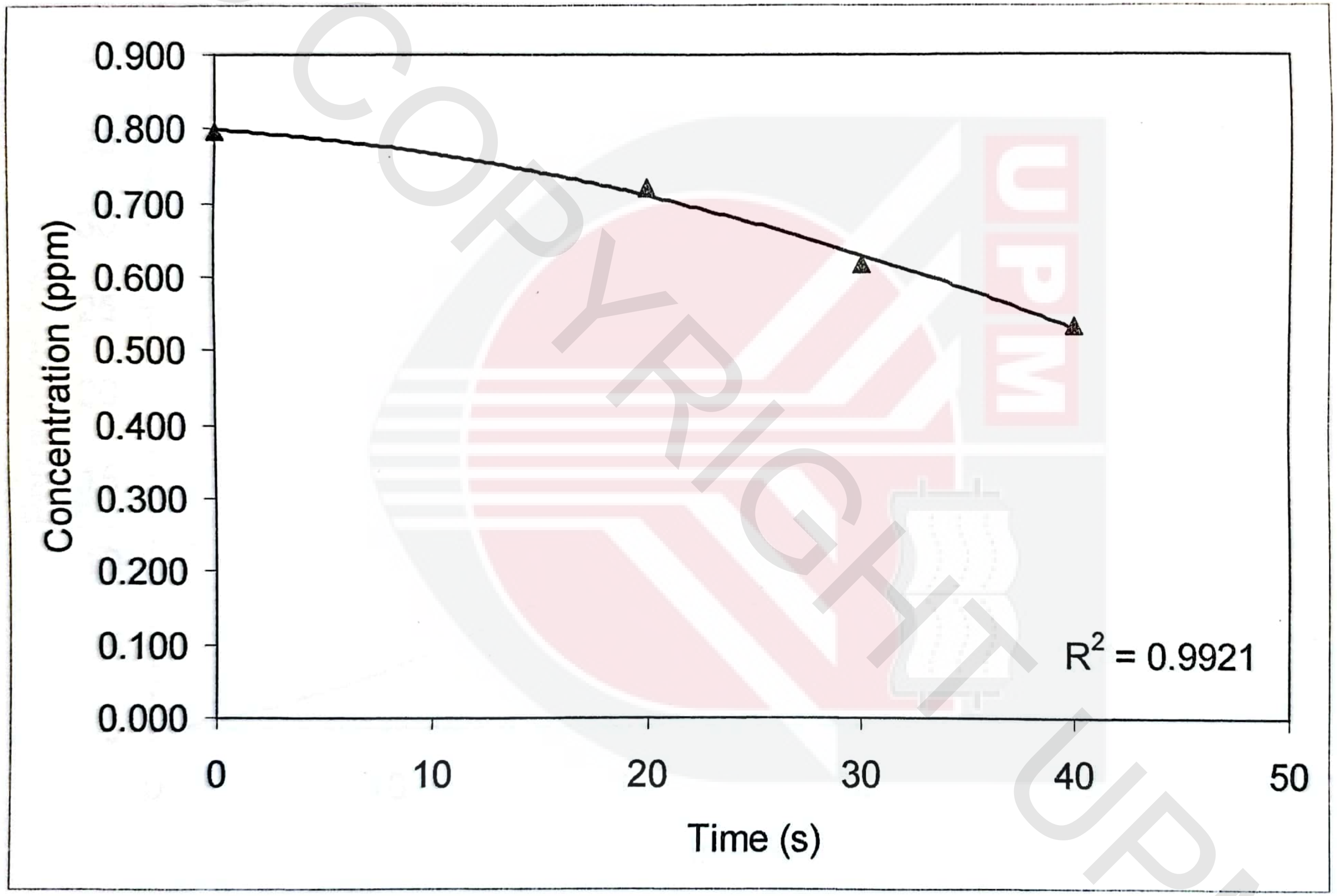

Figure 2: Boric acid concentration in yellow noodles in relation to hot water leaching duration.

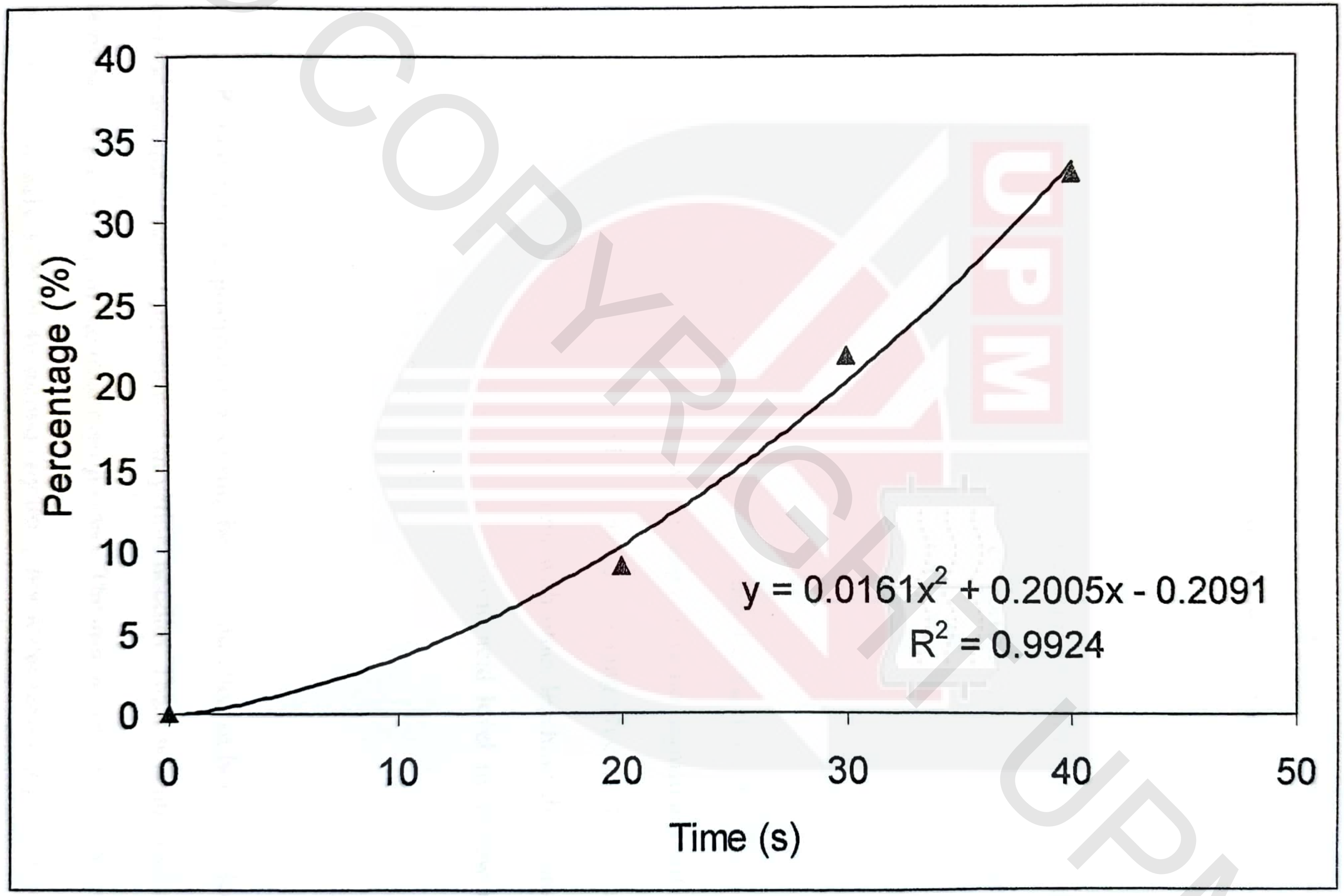

Figure 3: Percentage boric acid removal in relation to hot water leaching duration.

CHAPTER 5

DISCUSSION

This study revealed that the locally manufactured yellow noodles contained relatively low levels of boric acid as compared to those reported recently.

Figure 2 indicates the reduction of boric acid levels in yellow noodles in relation to hot water leaching duration. However, boric acid was still present in the yellow noodles after the 40 seconds leaching process.

In this study, there was a significant difference between the boric acid levels before and after hot water leaching. While, there was no significant difference between the boric acid level at 20 and 30 seconds leaching process, the boric acid level at 40 seconds was significantly different with other leaching durations. This showed that the most significant reduction of boric acid level in yellow noodle happened at 40 seconds of hot water leaching.

Results reveal a positive relationship between duration of hot water leaching and the amount of boric acid removed in yellow noodles. There was an increment in boric acid removal within the treatment process. The amount of boric acid removed was approximately 33 % in 40 seconds by using hot water leaching procedure. This increment is due to the solubility of the boric acid in water. During the leaching process, boric acid in the yellow noodles was leached into water and may evaporate to the atmosphere.

However, this method has not been tested on yellow noodles that contain high level of boric acid. Several experimental parameters will need to be evaluated to see the potential of this method in boric acid removal. This includes water to sample ratio, reaction pressure and addition of organic chemicals like alcohols.

The results in this study are in agreement with the observations of other researchers such as Berger and Truog (1939), Mahler *et al.* (1984), and Shiffler *et al.* (2003). They have published reports on hot water extraction of boron from soil samples. According to Odom (1980), boron removal from soil increased with boiling time from 0 to 15 minutes.

CHAPTER 6

CONCLUSION

Boric acid was found to be present in yellow noodles from selected manufacturer in Bintulu with a mean concentration of 0.797 ppm. The results of this study showed that boric acid levels in yellow noodle were significantly reduced by hot water leaching. There was a positive relationship between boric acid removal and hot water leaching duration, where up to 33% of boric acid removal can be achieved through hot water leaching technique.

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Removal of Boron Impurities as Boric Acid from Yellow Noodles by Hot Water Leaching" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, University Putra Malaysia Bintulu Campus and published in the form approved by the Faculty.

THEN SHEUE YANN

Date: 04/05/2007