STUDY OF THE EFFECTS OF COATING MATERIALS ON THE GROWTH OF BACTERIA ON EGG SHELL, YOLK AND ALBUMEN OF TABLE EGGS

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ABSTRACT

Bacteria contamination is a serious concern when it comes to food products and this includes table eggs. Table eggs are those eggs that are laid by chickens and they are unfertilized and can be consumed by people. Contamination of table eggs can cause food-borne illness to people who consume them. This study was conducted to isolate and identify the bacteria that grew on the egg shell, yolk and albumen of table eggs that have been coated with different types of coating materials. The effect of different coating materials on the growth of bacteria on egg shell, yolk and albumen of table eggs was determined. Nine chicken eggs were collected. The eggs were divided into three groups (three eggs were left uncoated in the first group, three eggs were coated with virgin coconut oil in the second group and three eggs were coated with propolis extract solution in the third group). A total of nine bacteria strain were isolated from the eggshell of the eggs that were left uncoated. A total of two bacteria strain were isolated from the eggshell of the eggs that were coated with virgin coconut oil. No bacteria strain was collected from the eggshell of the eggs that were coated with the propolis extract solution. There were no bacteria strain isolated from the contents (egg yolk and albumen) of both the coated eggs and non-coated eggs. Gram's positive bacteria (*Staphylococcus* spp.) were found predominantly on the eggshells of the eggs that were left uncoated and coated with virgin coconut oil. It was found that there were some Gram's negative bacteria found on the eggshells of the non-coated eggs. It was found that applying propolis extract solution as coating material of the eggs had entirely stopped the growth of both the Gram's positive and Gram's negative bacteria on the eggshells. It was concluded that the propolis extract solution was the best coating material to prevent bacterial contamination of table eggs.

KESAN PENGGUNAAN BAHAN PENYALUT TERHADAP PERTUMBUHAN BAKTERIA KE ATAS KULIT TELUR, KUNING TELUR DAN ALBUMIN PADA TELUR MAKAN

ABSTRAK

Pencemaran bakteria ialah satu masalah yang amat serius apabila ia berkaitan dengan produk makanan termasuk telur makan. Telur makan ialah telur yang dikeluarkan oleh ayam dan tidak disenyawakan dan biasanya dimakan oleh manusia. Pencemaran pada telur makan boleh menyebabkan penyakit makanan yang berbahaya apabila makanan tersebut dimakan oleh manusia. Kajian ini dijalankan untuk memencilkan dan menentukan jenis bakteria yang tumbuh pada kulit telur, kuning telur dan albumin pada telur makan yang disaluti oleh bahan penyalut. Daripada kajian ini, kesan penggunaaan bahan penyalut yang berbeza terhadap pertumbuhan bakteria ke atas kulit telur, kuning telur dan albumin pada telur makan dikenalpasti. Sembilan biji telur ayam telah dikumpul. Dalam kajian ini, telur-telur tersebut telah dibahagikan kepada tiga kumpulan (tiga biji telur dibiarkan tanpa sebarang bahan penyalut dalam kumpulan yang pertama, tiga biji telur disalutkan dengan minyak kelapa dara dalam kumpulan kedua dan tiga telur lagi akan disalutkan dengan cecair ekstrak propolis untuk kumpulan yang ketiga). Sembilan jenis bacteria diasingkan daripada kulit telur untuk telur-telur yang dibiarkan tanpa bahan penyalut. Dua jenis bacteria diasingkan daripada kulit telur untuk telur-telur yang disalutkan dengan minyak kelapa dara. Tiada satu jenis bakteria pun yang diasingkan daripada kulit telur untuk telur-telur yang disalutkan dengan cecair ekstrak propolis. Daripada kajian ini, ia didapati bahawa tiada satu jenis bakteria pun yang dapat diasingkan daripada kuning telur dan albumin untuk ketiga-tiga kumpulan telur-telur tersebut. Bakteria Gram positif (*Staphylococcus* spp.) dijumpai kebanyakkannya pada kulit telur untuk telur-telur yang disaluti dengan minyak kelapa dara dan telur-telur yang tidak disaluti dengan bahan penyalut. Untuk telur-telur yang tidak mempunyai bahan penyalut, sesetengah bakteria Gram negatif dijumpai pada kulit telur. Hasil eksperimen ini menunjukkan bahawa penggunaan cecair ekstrak propolis telah menyekat pertumbuhan bakteria Gram positif dan Gram negatif pada kulit telur. Kesimpulannya, ia dapat dikatakan bahawa penggunaan cecair ekstrak propolis sebagai bahan penyalut adalah kaedah yang terbaik untuk mengelakkan pencemaran bakteria pada telur makan.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

AGMARK	Agricultural Marketing Adviser
CMC	Carboxymethyl Cellulose
EHEC	Enterohaemorrhagic *E. coli*
FAO	Food and Agricultural Organisation
FDA	Food and Drug Administration
FPL	Fakulti Pertanian Lestari
H_0	Null hypothesis
H_A	Alternative hypothesis
HPAI	Highly Pathogenic Avian Influenza
IEC	International Egg Commission
Ig Y	Antigen specific immunoglobulins
KOH	Potassium Hydroxide
PC	Phosphatidylcholine
SPI	Soy Protein Isolate
spp./sp.	Species
UMS	Universiti Malaysia Sabah
USDA	United States Department of Agriculture
UTI	Urinary Tract Infection
PCR	Polymerase Chain Reaction
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
MOH	Ministry of Health
%	Percent
ACN	Acetonitrile
HCCA	4-Hydroxy-α-cyanocinnamic acid
TFA	Trifluoroacetic acid
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-of-Flight
MS	Mass Spectrometry
NA	Nutrient Agar
WG	Wheat Gluten
WHO	World Health Organisation
WPI	Whey Protein Isolate

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Foodborne illness outbreaks from contaminated farm produce have been an increasing concern in many parts of the world. Foodborne illness can be caused by the ingestion of foodstuffs that are contaminated with microorganisms. The contamination can occur at any stage in the process from food production to consumption. A large proportion of foodborne disease incidents are cause by the improper preparation of foods and mishandling of foods at home, in food service establishments, markets and even farms (WHO, 2015).

Most of the fresh produce contain many kinds of bacteria that can cause foodborne illnesses. Some of the harmful bacteria are already present in foods when they are produced at the farm and purchased. These types of foods include meat, poultry, fish, eggs, unpasteurized milk and dairy products. Bacteria can contaminate food at any period during growth, harvesting or slaughtering, processing, storage and shipping and this can cause the food to be harmful to consume. Many different kinds of bacteria can cause foodborne illnesses and they are *Salmonella*, *Campylobacter jejuni*. *Shigella*, *Escherichia coli*, *Listeria monocytogens*, *Staphylococcus* spp. *Clostridium* spp. and etc. (USDA, 2013).

Eggs contain a lot of nutrients like protein, fats, carbohydrates, minerals and vitamins (Lakhotia, 2002). According to a report by the Department of Health (2013), it was stated that there are presence of various vitamins in eggs such as vitamin A, vitamin D, vitamin B2, folate, biotin, pantothenic acid and choline. However, eggs are highly susceptible to external and internal bacterial contamination at time of storage. These conditions can cause serious foodborne illness towards the consumers by the consumption of food that was contaminated by the growth of harmful bacteria

especially *Salmonella* bacteria. Baroni et al. (2013) has also reported that the consumption of contaminated food may cause serious infections and harm the health of the consumers.

Table eggs can be contaminated at both the egg shell and egg contents (yolk and albumen) by a different number of microbes with a wide range of pathogens like *Campylobacter jejuni*, *Listeria* monocytogenes, *Escherichia* coli and especially *Salmonella* (Ricke *et al.*, 2001). *Staphylococci* are the most common bacteria that contaminate the egg shells of table eggs. The eggs can also be contaminated during the formation and laying process (Abdullah, 2010). The contamination of egg shell may also cause an increase in the chances of the egg contents (yolk and albumen) to be contaminated through pathogen penetration (Messens *et al.*, 2006).

Bacterial contamination can happen at the three main parts of an egg, that is at the egg shell, egg yolk and albumen (Bahrouz, 2005). The most common foodborne pathogens that can grow on food of animal origin are *Salmonella*, *Campylobacter*, *Staphylococcous aureus* and *Escherichia coli* (Akbar and Anal, 2013; Ghasemian, 2011; Akbar and Anal, 2011). To prevent bacterial contamination and preserve the quality of eggs, a few coating materials were introduced. The coating materials include shellac, gelatin, chitosan, oils and propolis (Tariq *et al.*, 2011; Nadia *et al.*, 2012; Pujols *et al.*, 2013; Jirangrat *et al.*, 2010; Gulsen Copur *et al.*, 2007).

1.2 Justification

The applications of coating materials on eggs are able to reduce or prevent the growth of bacteria especially pathogenic bacteria on eggs. Thus, the purpose of this study is to isolate and determine the total number of bacteria on egg shell, yolk and albumen from eggs that have been treated with different coating materials and also to compare the effectiveness of different coating materials in the reduction of bacterial growth on egg shell, yolk and albumen. To limit the growth of bacteria on eggs, a few studies have been conducted by the application of certain coating materials on eggs. From a study conducted by Kim *et al.* (2008), it was found that the application of chitosan as the coating material of eggs was able to preserve the internal quality of the eggs by reducing the growth of bacteria within the eggs (yolk and albumen) as it acts as an antimicrobial agent. From a study of the effect of the application of propolis on

eggshell microbial activity, it was found that the propolis applied has effectively reduced the microbial activity on the surface of the quail eggs during storage (Ali Aygun and Durmus Sert, 2013). Since the effectiveness of coating materials have been reported in the reduction of bacterial growth on table eggs, it is proposed to conduct similar study on the effects of different coating materials on the growth of bacteria on egg shell, yolk and albumen of table eggs.

1.3 Objectives

To determine the effects of the application of different coating materials like virgin coconut oil and propolis extract solution on the growth of bacteria on egg shell, yolk and albumen of table eggs.

1.4 Hypothesis

H_0 : There is no significant difference in the effect of coating materials on the growth of various bacteria on egg shell, yolk and albumen of table eggs.

H_A : There is a significant difference in the effect of coating materials on the growth of various bacteria on egg shell, yolk and albumen of table eggs.

CHAPTER 2

LITERATURE REVIEW

2.1 Foodborne Illness

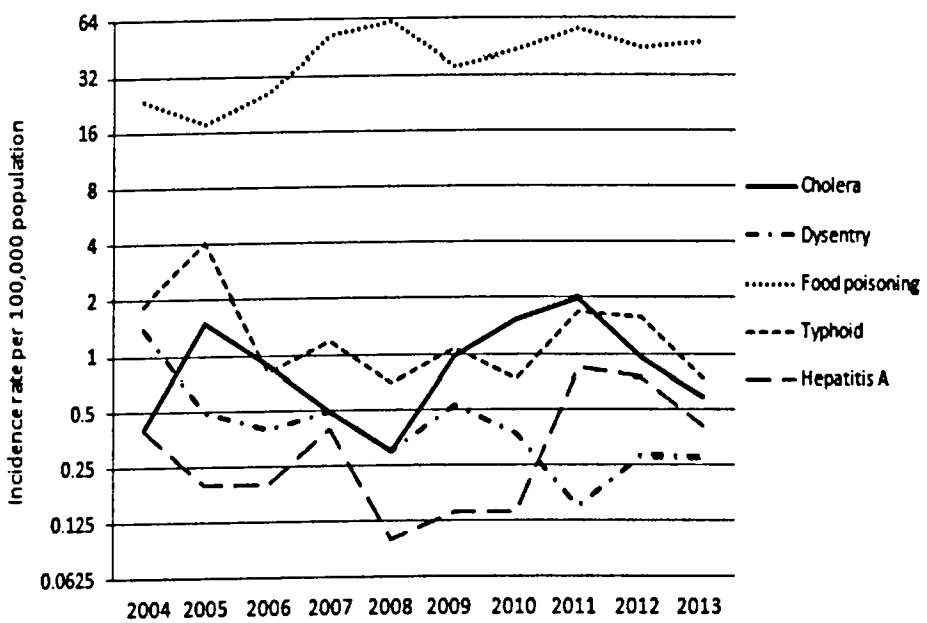
Foodborne diseases or illnesses have been a serious issue for all humans. As reported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) (2014), foodborne illnesses or foodborne diseases are infections of the gastrointestinal (GI) tract which are caused by the food that are contaminated with harmful bacteria, parasites, viruses or chemicals. The outbreak of foodborne illnesses is defined as the happening of two or more cases of similar illnesses caused by the consumption of food (Soon *et al.*, 2011).

2.1.1 Status of Foodborne Illnesses in the World and Malaysia

Foodborne disease outbreaks have been reported to a relationship with human morbidity worldwide and pose a threat towards the human population. At the same time, diarrhoeal diseases are the main reason for mortality in less developed country (Schlundt *et al.*, 2004). In a worldwide scale, diarrhoeal disease has caused 3% mortality and this should be of a great concern (World Health Organization, 2014). Meanwhile, in industrialized country, foodborne diseases are not rare as 30% of the global population suffered from foodborne illnesses each year. Teisl and Roe (2010) have reported on the incidence cases of foodborne diseases in France, United Kingdom, Australia and the United States. From these incidence cases, it was found that there were 1210 cases of foodborne illnesses per 100,000 inhabitants in France, 2600 cases of foodborne illnesses per 100,000 in the United Kingdom and more than 25,000 cases of foodborne illnesses per 100,000 inhabitants in Australia and the United States.

Cases of foodborne diseases in Malaysia is much lower than these countries because most of the cases went unreported and a chain of events must be addressed first before it was brought to the authority (Soon *et al.*, 2011). In Malaysia, foodborne diseases are not rare. Not all the cases of food poisoning are reported as most of the affected people do not seek treatment at hospital, especially when the cases are not serious. Besides that, before a case of food poisoning can be reported to the authority, a complex chain of events called population exposure must happen first (Soon *et al.*, 2011).

The trends of food poisoning and foodborne and waterborne diseases change over the years from 2004 to 2013 (Figure 2.1). Based on Figure 2.1, it was found that there was a rise in food poisoning and hepatitis A from 2009 to 2011, but a decrease of dysentery between those years. Moreover, food poisoning cases reduced in 2012 but slightly rose in 2013.



Diseases	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Cholera	0.4	1.5	0.9	0.5	0.3	0.98	1.56	2.02	0.96	0.58
Dysentry	1.4	0.5	0.4	0.5	0.3	0.54	0.37	0.15	0.29	0.28
Food poisoning	23.89	17.8	26	53.2	62.5	36.17	44.18	56.25	44.93	47.79
Typhoid	1.9	4.1	0.8	1.2	0.7	1.07	0.74	1.71	1.58	0.73
Hepatitis A	0.4	0.2	0.2	0.4	0.1	0.14	0.14	0.84	0.75	0.41

Figure 2.1 Incidence rate of food and waterborne diseases
Source: MOH, 2014

2.1.2 Common Causes and Effects of Foodborne Illness

According to a report by World Health Organization (WHO) (2015), foodborne illnesses can be caused by bacteria, viruses, parasites or even chemical substance that enter the body through contaminated food or water. Some harmful bacteria or bacteria that can cause foodborne illnesses are already present in foods when they are purchased and they include meat, poultry, fish and shellfish, eggs, unpasteurized milk and dairy products (NIDDK, 2014). The contamination of food can also be caused at any stage in food production to consumption and they can also result from environmental pollution (WHO, 2015).

Foodborne pathogens can cause severe diarrhoea or food poisoning to humans that consume the contaminated food. Chemical contamination of food can cause acute poisoning or long-term severe diseases like cancer. Foodborne illness can also lead to death and disability (WHO, 2015).

2.2 Egg Production

Eggs are one of the food products that are widely consumed around the world. Hence, eggs represent a vital segment of the world food industry and an important aspect in international trades (Stadelman, 1995). The Food and Agriculture Organisation (FAO) (2014) has released a report on the global egg production and also the trend of the egg production in Asia between 2000 and 2013. The production of egg worldwide in 2013 totalled 68.3 million tonnes that represented an increase in the production of 3.02% compared with that of the previous year (2012) (FAO, 2014). Of this total egg production in 2013, Asia comprised about 58.57%, making it the largest egg producer worldwide (Table 2.1).

Table 2.1 World egg production (million tonnes)

Region	2000	2005	2006	2007	2008	2009	2010	2011	2012	2013
Africa	1.9	2.2	2.3	2.5	2.6	2.5	2.8	2.9	3.0	3.1
America	10.5	11.7	12.3	12.3	12.5	12.9	13.1	13.5	13.2	14.0
Asia	29.0	32.6	32.9	34.5	36.2	37.0	37.5	38.1	39.2	40.0
Europe	9.5	9.9	10.1	10.1	10.2	10.3	10.5	10.7	10.6	10.9
Oceania	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3
World	51.1	56.6	57.9	59.6	61.8	62.9	64.2	65.4	66.3	68.3

Source: FAO, 2014

Table 2.1 shows that America is the second largest egg producer, and represents about 20.50% (14.0 million tonnes) of the total world production in 2013, followed by Europe (15.96%), Africa (4.54%) and Oceania (0.44%) (FAO, 2014). A huge proportion of the egg production market belongs to the developing countries (above 50%), and this can well be explained by the necessity for these countries to meet their protein needs due to their increasing population. However, limited technology, feed supplies and low production of native chickens have caused some issues in the process of meeting the protein needs of the rising population in the developing countries (Stadelman, 1995). From Table 2.1, it was found that there is an increase in the amount of egg produced around the world from 2000 to 2013. During those years, egg production in Asia grew at 2.5% per year from 29 million tonnes to 40 million tonnes. Africa and America saw a steady rise in egg production. Egg production in Africa has increased from 1.9 million tonnes in 2000 to 3.1 million tonnes in 2013. The increase of egg production in Africa was 63.16% between 2000 and 2013. America has increased their egg production from 10.5 million tonnes to 14.0 million tonnes between 2000 and 2013. The increase of egg production in America was 33.33% between 2000 and 2013. The amount of egg produced in Europe showed a trend of slight increase from year 2000 to 2013, that was from 9.5 million tonnes to 10.9 million tonnes. The increase of egg production in Europe was 14.74% between 2000 and 2013.

Based on Table 2.2 below, the number of layers in the world during the review period has rose from 4976 million to 7035 million with the total in Asia increasing from 3055 million to 4494 million.

Table 2.2 Layer numbers in Asia and World (millions)

Region	2000	2005	2006	2007	2008	2009	2010	2011	2012	2013
Asia	3,055	3,557	3,702	3,852	3,983	4,086	4,187	4,246	4,412	4,494
World	4,976	5,690	5,909	6,062	6,229	6,349	6,520	6,605	6,825	7,035

Source: FAO, 2014

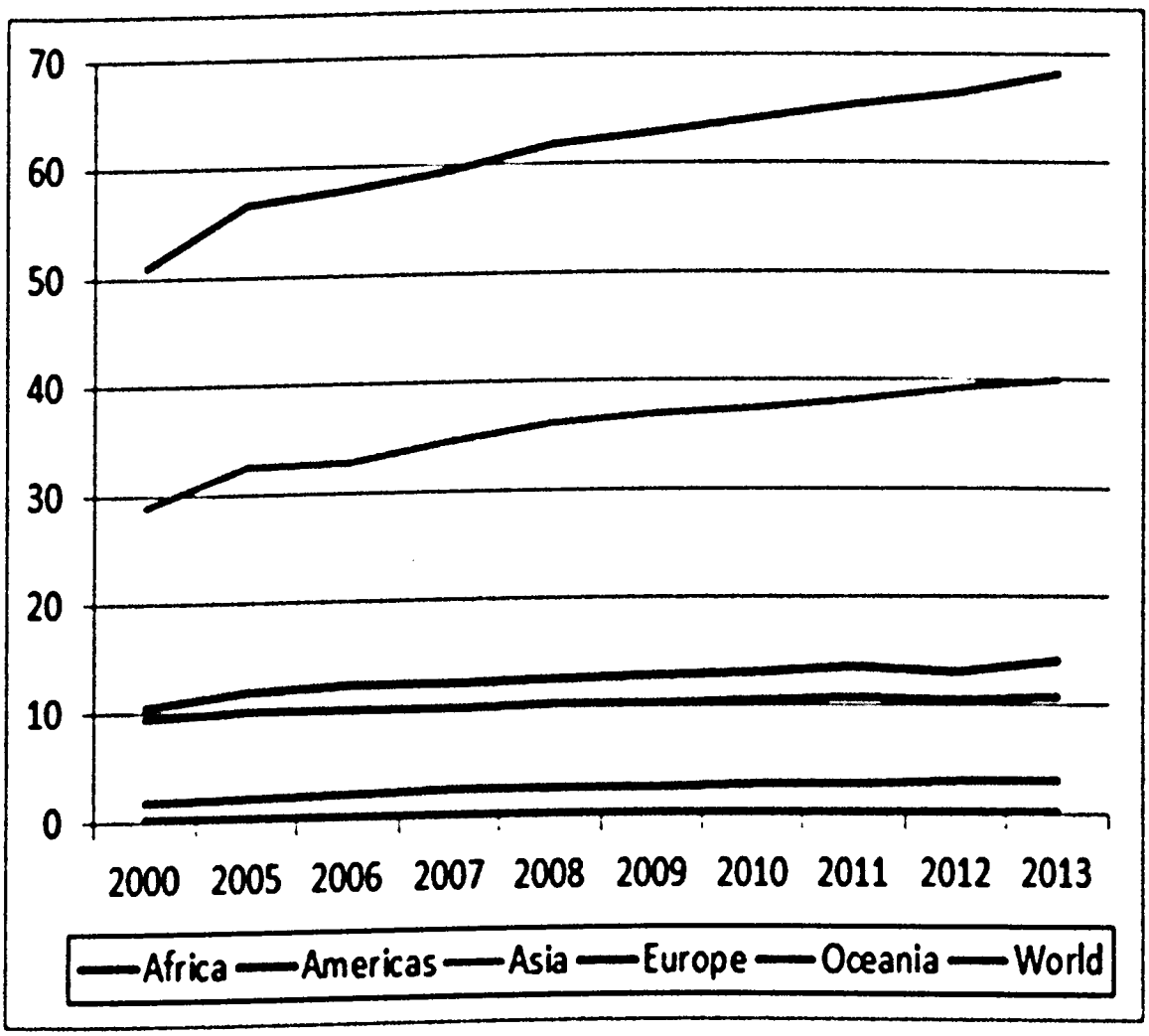


Figure 2.2 World egg production (million tonnes)
Source: FAO, 2014

China dominates the Asian egg production industry, making up for 67.31% of the regional total egg production in 2013 (Table 2.3). With yearly growth of just over two per cent, production of eggs in mainland China increased drastically from 18.6 million tonnes in 2000 to 24.5 million tonnes in 2013.

Table 2.3 Leading egg producers in Asia ('000 tonnes)

Country	2000	2005	2009	2010	2011	2012	2013
China, mainland	18,547	20,724	23,311	23,483	23,897	24,320	24,446
India	2,035	2,568	3,230	3,378	3,466	3,655	3,835
Japan	2,535	2,481	2,507	2,515	2,483	2,507	2,522
Indonesia	642	857	1,072	1,121	1,028	1,140	1,224
Turkey	810	753	865	740	810	932	1,031
Iran Isl. Rep.	579	758	725	687	559	625	665
Malaysia	391	442	510	587	622	643	664
Pakistan	344	401	529	556	604	618	649
Thailand	515	469	577	585	601	659	668
Korea Rep.	479	515	602	590	595	600	615
Total of above	26,877	29,967	33,928	34,242	34,664	35,698	36,320

Source: FAO, 2014

India has gained five per cent per annum increase, pushing the production to 3.8 million tonnes in 2013. However, according to the data given by the International Egg Commission (IEC), production of eggs reached almost 4.2 million tonnes in 2013. The egg production in Indonesia has shown a similar growth to India, as the total egg produced in Indonesia doubled between 2000 and 2013 to achieve 1.2 million tonnes. Egg production in Turkey has shown a strong growth from 2010 to 2013. High outbreaks of Highly Pathogenic Avian Influenza (HPAI) has reduced the production of eggs drastically in 2011 (FAO, 2014). Each of the remaining four countries, Malaysia, Pakistan, Thailand and Republic of Korea produced more than 600,000 tonnes of eggs in 2013. Among these four countries, Malaysia is the second biggest egg producer with 664 000 tonnes.

Figure 2.3 below shows the leading egg producers in Asia, excluding China (´000 tonnes). From the figure, it is found that Japan is the only country that does not show much increase or growth during the review period compared to the other major egg producers in Asia.

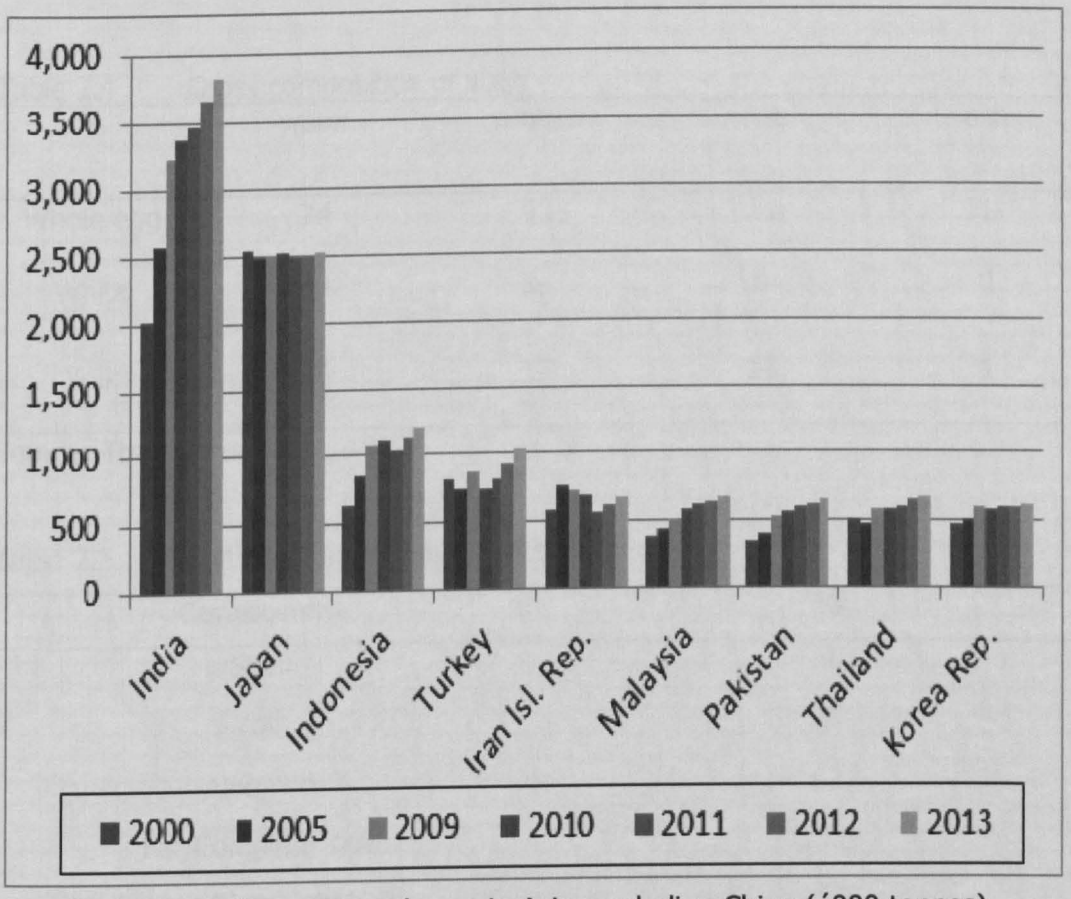


Figure 2.3 Leading egg producers in Asia, excluding China (´000 tonnes)
Source: FAO, 2014

2.3 Table Eggs

According to Kuepper, Born and Fanatico (2009), table eggs or shell eggs are eggs that are in the form that it is most familiar to consumers. AGMARK (1968) has also reported that table eggs are those edible eggs that are derived as a product of poultry husbandry.

2.3.1 Table Eggs Structure and Composition

The structure of the hen's egg is shown in Figure 2.3 and Figure 2.4. Generally, a hen can only lay one egg per day and there are some days when it does not lay even a

single egg at all (Jacob, 2015). According to Jacob (2015), it will take 26 hours for an egg to form fully in a hen's body. The hen egg is composed of three distinct structures: egg shell, egg white and yolk (Kovacs-Nolan *et al.*, 2004). The composition of the egg is given in Table 2.4 and Table 2.5. However, the composition of eggs does differ with breed, age of layer and even nutrition.

Table 2.4 Gross composition of eggs

%	water	protein	Fat	ash
Whole egg	74	13	11	1
White	88	11	0	0
Yolk	48	17	13	1

Source: Tharrington *et al.*, 1999

Table 2.5 Composition of components in the egg

Component	**%**
Shell	9
Yolk	29
Albumen	62
Fat/Complete Yolk	33.0
Protein/Complete Yolk	15.7
Yolk solids	51.0
Albumen Protein	10.5
Albumen Solids	11.8

Source: Tharrington *et al.*, 1999

2.3.1.1 Egg Shell

The egg shell is formed in the last 20-22 hours before the egg is laid by the hen. The egg shell acts as a protective layer and protects the contents (yolk and albumen) from damage or spoilage. The chicken egg shell consists of calcified shell and shell membranes that includes the inner and outer membranes. These membranes plays a role in the retaining of albumen and the prevention of penetration of bacteria (Nakano

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