THE INFLUENCE OF NEST KEEPING AND PREPARATION METHODS ON THE MICROBIOTA ASSOCIATED WITH BACKYARD CHICKEN EGGS

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DECLARATION OF INDEPENDENT WORK

I, BOITUMELO M. MOALUSI, do hereby declare that this research project submitted to the Central University of Technology, Free State for the degree MAGISTER TECHNOLOGIAE: ENVIRONMENTAL HEALTH, is my own independent work and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

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SIGNATURE OF STUDENT DATE

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SUMMARY

In developing countries such as South Africa commercial chicken farmers produce the majority of eggs, approximately 5.8kg of eggs per capita per annum. Despite this, many people, especially in rural and marginal-urban areas, still consume eggs produced by backyard systems. Backyard systems are characterised by fragmented and small-scale production units that require minimal management and chickens are often unhoused or poorly housed. In most cases, eggs from backyard systems are laid in nests in poor hygienic condition.

Eggs are a cheap, readily available and a good source of animal protein and are consumed by the majority of the people in the community, including the young, the old and people with HIV/AIDS. With little information available regarding the microbiological quality of eggs produced by backyard chickens in Southern Africa, the risks posed by these eggs to consumers are unknown. In this study the microbiological quality of eggs from randomly selected household near Hennenman keeping backyard chickens was determined. The study was done over three seasons which included the cold-dry (May-July), mild-dry (October-February) and the warm-wet (August-September) seasons. The following organisms were isolated: *Salmonella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Escherichia coli* and Total Coliforms. *Staphylococcus* spp. was further characterised to species level. Most of the species were of human origin, with the exception of only two species, *S. hyicus* and *S. lentus,* which have previously

been associated with chickens. Furthermore, questionnaires were administered to the backyard chicken keepers to assess their knowledge regarding chicken keeping and nest hygiene, the proper method of egg collection and storage, and the preparation of eggs. The decrease of vitamins and *Staphylococcus* spp. occurring during different preparation methods (scrambling, frying and boiling) was also determined. The results obtained showed that the eggshells were more contaminated than the egg contents. This had been expected as the eggshell is more in contact with the external environment than the egg contents are. Faecal contaminants (*Salmonella* spp., *Escherichia coli* and Total Coliforms) were present in both the eggshell and the egg contents during all seasons and this could be attributed to the infrequent cleaning of chicken nests as ascertained from the questionnaires. From the vitamin analysis it was observed that backyard-produced eggs had lower concentrations of vitamins A and E compared to commercially-produced eggs. When determining the best preparation method, causing the most degradation of *Staphylococcus* spp., while on the other hand preserving vitamins, it was found that scrambling was the best method, followed by the frying and boiling methods respectively.

OPSOMMING

In ontwikkelende lande soos Suid-Afrika, word die meeste van die 5,8 kg eiers wat jaarliks per capita verbruik word, deur kommesiële hoenderboere geproduseer. Ten spyte hiervan, gebruik baie mense, veral in die platteland en die marginal stedelike gebiede, eiers wat van agterplaassisteme afkomstig is. Agterplaassistemeword gekenmerk aan gefragmenteerde en kleinskaal-produksie-eenhede wat minimale bestuur nodig en hoenders word gereeld in swak omstandighede gehuisves. In die meeste gevalle wordeiers in agterplaassisteme in onhigiëniese neste gelê.

Eiers is goedkoop, maklik verkrygbaar en ‘n goeie bron van diere proteïen. Dit word deur die meeste mense in die gemeenskap verbruik, insluitende jong kinders, bejaardes en mense met MIV/VIGS. Aangesien daar min inligting met betrekking tot die mikrobiologiese gehalte van eiers wat van agterplaashoenders afkomstig is bestaan, is die risiko’s hieraan verbonde onbekend. In hierdie navorsingsstudie is die mikrobiologiese gehalte van eiers uit ewekansig geselekteerde huishoudings naby Hennenman wat agterplaashoenders aanhou, bepaal. Die studie is oor drie seisoene uitgevoer, wat die koue/droë (Mei-Julie), gematig/droë (Okober-Februarie) en die warm/nat (Augustus-September) seisoene insluit. Die volgende organismes is geïsoleer: *Salmonella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Escherichia coli* en Totale Koliforme. *Staphylococcus* spp. is verder tot op soortvlak gekenmerk. Die meeste van die organismes se oorsprong is mense, met die uitsondering van net twee soorte,

S. hyicus en *S. lentus*, wat al van tevore met hoenders geassossieer is. Verder is vraelyste uitgedeel aan diegene wat agterplaashoenders aanhou, om hulle kennis met betrekking tot die anhou van hoenders, neshigiëne, die aangewese metode van eierinsameling en berging en die voorbereiding van eiers te bepaal. Die vermindering van vitamiene en *Staphylococcus* spp. wat tydens die verskillende voorbereidingsmetode (roer, bak en kook) plaasvind, is ook bepaal. Die resultate het bewys dat die eierdoppe meer besmet is as die inhoud van die eiers. Fekale besmetters (*Salmonella* spp., *Escherichia coli* en Totale Koliforme) is in beide die eierdoppe en die eierinhoude tydens alle seisoene gevind en dit kan toegeskryf word ann ongereelde skoonmaak van hoenerneste, soos vasgestel is aan die hand van die vraelyste. Die vitaminanalise het getoon dat agterplaasgeproduseerde eiers laer konsentrasies vitamiene A en E as kommersieel geproduseerde eiers bevat. Met die bepaling van die beste voorbereidingsmetode wat die meeste afbreking van *Staphylococcus* spp. meebring en vitamiene die beste preserver, is gevind dat roer die beste metode is, gevolg deur bak en kook onderskeidelik.

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INTRODUCTION

1.1 General background on backyard chicken egg production

Chicken and egg production systems in developing countries can be classified into two sections: 1) intensive commercial systems (broilers for meat production and layer houses for egg production), and 2) extensive/scavenger systems (also known as backyard broilers and layers). The latter system is usually found in rural communities where chickens are kept for egg and meat production (Van Marle-Köster and Nel, 2000). In areas such as Southern Asian countries, backyard chickens contribute to about 50% of the eggs produced, while in Africa, about 75% of eggs and meat produced are from this source (Van Marle-Köster and Nel, 2000; Van Marle-Köster and Webb, 2000). In Africa, chickens are also used in the performance of traditional rituals, in bartering, given as gifts and are a key source of income and employment for some of the poorest members of the society, including women (Oakeley, 2000; Thekishoe *et al.,* 2004).

Backyard chickens scavenge for food and might receive supplementary feeding consisting mainly of food scraps and left-overs from homesteads. They are kept in backyard gardens in peri-urban and even in urban areas (depending on the municipal regulations) (Mushi *et al*., 2000; Wethli, 1999). There are two types of houses used to keep backyard chickens: houses where chickens are kept during the night time and houses where they are kept 24 hours a day (Wethli, 1999) (Figure. 1.1). The night-only houses allow the chickens to sleep without being overcrowded and protect them from predators.

Figure 1.1 A photograph of a typical cage used to house backyard chickens 24 hours a day

1.2 The composition of chicken eggs

The egg is a biological structure intended by nature for reproduction. It protects and provides all the required nutrients for the developing embryo, and serves as the principal source of food for the first few days of a chick's life (Bennion, 1990). Eggs are also required by a multitude of human communities, especially by children, for normal growth when meat as a basic protein source is too expensive or unavailable (South African National Department of Agriculture, 2000). In fact, egg proteins are of excellent nutritional quality with the highest protein efficiency ratio (PER) of any of the common foods (Bennion, 1990).

Eggs are probably one of the first sources of animal protein consumed by man (Van Niekerk and Van Heerden, 1993), and they remain important in human nutrition as they are a rich and balanced source of essential amino and fatty acids and also of some minerals and vitamins (especially iron and vitamin A – Table 1.1) (Wang and Slavik, 1998; Surai and Sparks, 2001). Eggs have several other benefits which include, amongst other things, the variety they add to the diet, easy digestibility, low cost, convenience, and usefulness in food processing (Hou *et al.*, 1996; Kurtzweil, 1998).

Table 1.1 The nutritional composition of a chicken egg

Nutrient (unit)	Whole egg	Egg white	Egg yolk
Protein (g)	6.25	3.52	2.78
Total lipid (g)	5.01	0	5.12
Vitamin A (IU)	317.5	0	317
Vitamin E (mg)	0.70	0	0.70
Iron, Fe (mg)	0.72	0.01	0.59

Egg Nutrition Centre, 2001.

A typical chicken egg weighs about 60g and consists of three main parts - the shell, the white and the yolk (Gates, 1981; Grijspeerdt, 2001). The shell forms an outer protective layer and consists mainly of calcium carbonate. It may be white or brown, and variations are mostly due to the breed of the laying hen. The colour makes no difference to the nutritional value of the egg (Brownsell *et al.*, 1989). Inside the shell two thin membranes separate the shell from the white (Figure 1.2). The yolk is suspended in the white and is held in position by strands of protein called chalazae (Brownsell *et al.,* 1989; Grijspeerdt, 2001). The yolk, which contains the female germ cell, is formed in the ovaries of the hen and subsequently drops into the mouth of the oviduct. As the yolk passes slowly down the oviduct it is covered with layers of egg-white from albumen-secreting cells, then with membranous tissue from protein-secreting cells, and finally with calcium and other minerals from mineral-secreting cells near the bottom of the oviduct, which results in the eggshell (Potter, 1986).

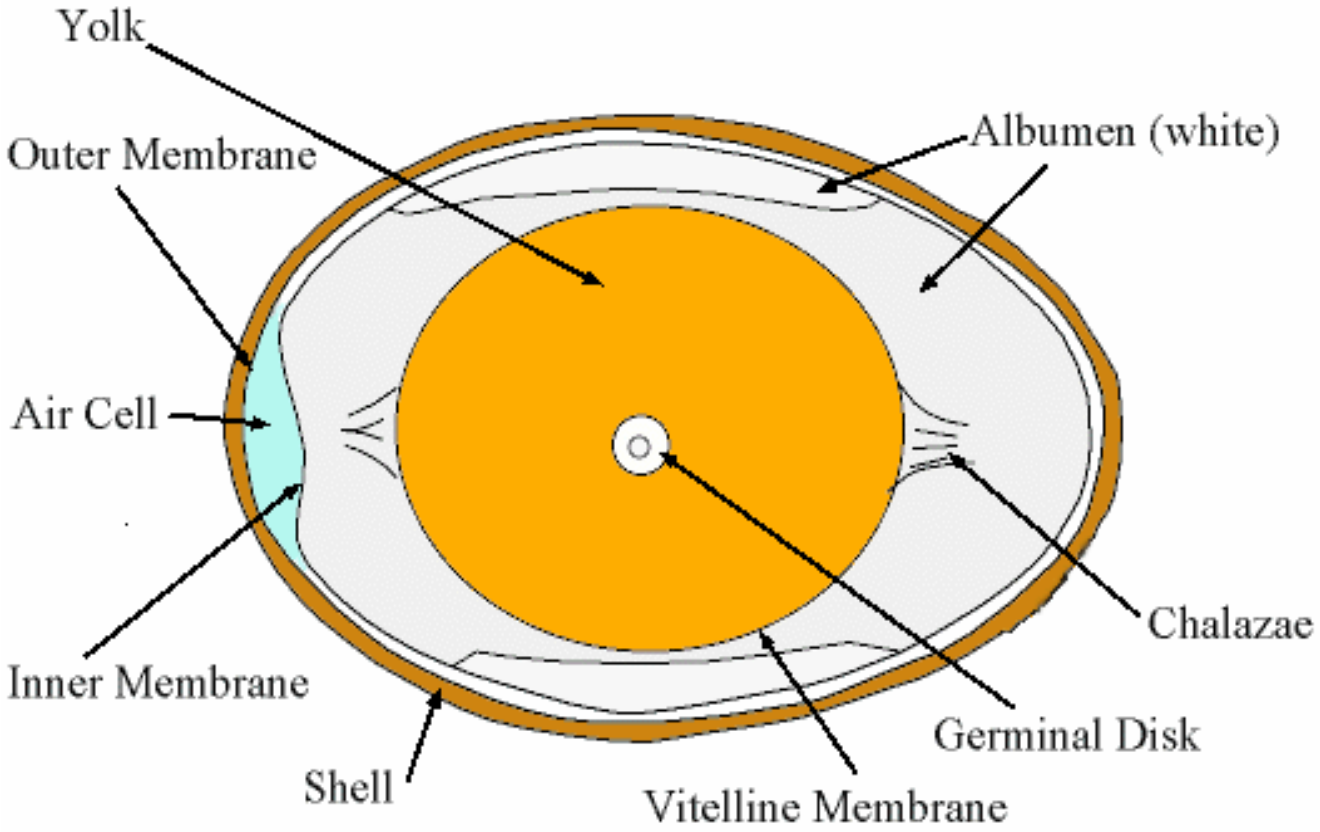

Figure 1.2 A typical hen's egg (Avian sciences homepages, 1999)

Though the contents of the egg in an unbroken shell from a healthy bird are generally free of microorganisms when freshly laid, defects do occur (Potter, 1986; Frazier and Westhoff, 1988; Jay, 2000). Defective egg formation and fertilisation usually result from ruptures in the ovary or oviduct, producing blood spots and sometimes meat specks. Egg contamination further occurs due to faecal matter from the chickens, from dirt in the cage or nest, from washing water

if the eggs are washed, by human handling, and even by the material in which eggs are packed and stored (Frazier and Westhoff, 1988).

Microorganisms typically isolated from chicken eggs include *Acinetobacter, Proteus, Aeromonas, Alcaligenes, Micrococcus, Salmonella* spp.*, Serratia, Enterobacter, Flavobacterium*, *Staphylococcus* spp., *Pseudomonas* spp. and *Escherichia coli* (Frazier and Westhoff, 1988; Jay, 2000; Theron, 2003). Of these, the organism that poses the largest threat is *Salmonella enteritidis* (Wang and Slavik, 1998; Hara-Kudo *et al.,* 2001). As with other foods, consumption of eggs contaminated with *Salmonella* spp could result in diarrhoea, abdominal cramps, nausea, vomiting, fever and headache (Child Health Alert, 1996, Yang *et al.,* 2001).

1.3 Egg production practices and related hygiene measures

Eggs are highly perishable food products (Yang *et al.,* 2001) and may lose quality rapidly if not given proper care between collection and consumption (Kamel and Diab*,* 1979). In 2000 the Centre for Disease Control (CDC) urged the public of America to use the following guidelines when using eggs in cooking: to avoid eating raw or undercooked eggs: this is especially important for people infected with the weak immune systems such as people with human immunodeficiency virus (HIV); to keep eggs refrigerated until use; and to cook them for at least 15 seconds, with the white completely set and the yolk beginning to thicken. Additional guidelines include that eggs should be eaten

soon after cooking, and the hands, utensils and cooking surfaces should be washed after contact with raw eggs (Child Health Alert, 1996; Keith, 1996).

In systems where eggs are produced in high volumes, proper management strategies are usually in place to reduce egg contamination. These include the frequent collection of eggs to minimise the time that they are exposed to high temperatures or to a contaminated environment (Patterson, 1990; Wineland and Christopher 1998) and ensuring that the egg-laying areas are kept clean, including the nest litter or pads. In systems where eggs are produced by backyard chickens it is difficult to implement such management strategies as production usually occurs in an uncontrolled environment where the producers do not employ good egg manufacturing practices (Wineland and Christopher, 1998).

In 2000, Mauldin provided a guideline for the safe production of backyard chicken eggs. Amongst other things, this guide mentions keeping the floors of the cage dry to prevent chickens from tracking mud and faeces from wet floors into the nests, that there should be one nest for every three to four hens to prevent them from laying eggs on the floor or ground, and that people should wash their hands before and after collecting eggs. When collecting the eggs, those in the nests should be collected first and put into storage. The eggs on the floor can be collected next. Collection of eggs should be done twice daily to prevent the eggs

being exposed to temperature and humidity that may accelerate microbial growth and result in egg spoilage (Frazier and Westhoff, 1988; Jay, 2000).

1.4 Rationale

In developing countries, such as South Africa, commercial chicken farmers produce the majority of eggs, approximately 5.8kg of eggs per capita per annum (South African National Department of Agriculture, 2000). Despite this, many people still consume eggs produced by backyard systems. Backyard systems are characterised by fragmented and small-scale production units that require minimal management and chickens are often unhoused or poorly housed (Wethli, 1999; Oakeley, 2000). In most cases, eggs from backyard systems are laid in nests in poor hygienic conditions.

Microorganisms from various sources are able to infect these eggs. The outside of the shell normally carries the highest number of bacteria as a result of contamination from faeces and dust. This situation cannot be controlled in backyard chickens. Eggs produced by backyard chickens are consumed by the people in most households in developing countries. Despite the increased production of broilers and layers in the developing world, it is estimated that backyard production contributes up to 75% of eggs and meat produced in Africa (Van Marle-Köster and Nel 2000). With little information available on the microbiological quality of the eggs produced by backyard chickens in Southern Africa, the risks posed by these eggs to consumers are currently unknown.

This study, was conducted to identify the presence of potential microbial hazards such as *Salmonella* spp*., Staphylococcus* spp*., Pseudomona*s spp., and *Escherichia coli* on or in backyard chicken eggs. The specific aims for this study were:

- to assess the knowledge of the people in the township of Phomolong, near Hennenman, in the Free State Province, regarding aspects of chicken-keeping and nest hygiene, as well as collection, storage and the preparation of eggs (Chapter 2);
- to quantify the microbiota which might be hazardous for human consumption on backyard chicken eggs during different seasons with specific emphasis on *Salmonella* spp*., Staphylococcus* spp,*.* which was also characterised to species level*, Pseudomonas* spp*.,* and *Escherichia* spp. (Chapter 3); and
- to determine the best common egg preparation method that would reduce the Staphylococcal load on the egg without influencing the vitamin content (Chapters 4 and 5).

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CHAPTER 2

CHICKEN-KEEPING AND EGG PRODUCTION ASSOCIATED WITH THE PRODUCTION OF BACKYARD CHICKEN EGGS

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2.1 ABSTRACT

A structured questionnaire was used to interview people who keep backyard chickens, regarding various aspects related to chicken-keeping and egg production in the township of Phomolong, near Hennenman. The questions included in the questionnaire were intended to elicit responses regarding the interviewees' personal information, chicken-keeping and nest hygiene, collection of eggs, storage of eggs and preparation of eggs. The study revealed that there were respondents who did not understand the importance of nest cleaning (50%), hand washing before and after egg collection (67%), proper egg storage (60%) or the influence of egg washing (77%) on egg safety. Some of the practices, which included the tendency to not wash hands before egg collection (67%), the storage of eggs uncovered (60%) and the washing of eggs which were collected from the nests (77%), clearly indicated that the respondents were uninformed on the basic aspects of proper egg production. It was thus recommended that the community in Phomolong be taught the importance of proper hand washing practices prior to and after egg collection. The microbiological content on/in the eggs, as well as the degradation of vitamins and microorganisms after the various preparation methods used, was also determined.

Keywords: backyard chickens, chicken-keeping.

2.2 INTRODUCTION

Broiler chicken and egg production may be classified into two groups in the developing countries. These two groups are intensive commercial systems and extensive/backyard systems. The backyard system is common in rural communities where chickens roam freely and scavenge for food in backyards (confined spaces) or in the village and its surroundings (The South African National Department of Agriculture, 2000; Van Marle-Köster and Nel, 2000). Eggs produced from this system are, however, produced in an uncontrolled environment with no proper egg manufacturing and storage management (Wineland and Christopher, 1998). Since eggs are highly perishable and lose quality rapidly if not handled properly between collection and consumption, poor management of backyard egg production generally leads to a poor quality product (Kamel and Diab, 1980; Yang *et al*., 2001).

The bacteria that proliferate on and can contaminate chicken eggs are ubiquitous in nature and have been isolated from soil, manure, and even from airborne dust particles (Pienaar *et al.,* 1994; Desmarchelier *et al*., 1999; Venter *et al.,* 2004). Eggshells still moist after laying easily become soiled with dirt from the environment and consequently may be contaminated with microorganisms (Pienaar *et al.,* 1994). De Reu *et al.* (2004) noted this to be the most common way that eggs become contaminated.

Guidelines for the storage and cooking of eggs to ensure their safety at the time of consumption generally include avoiding eating raw or undercooked eggs, the refrigeration of eggs until use and the cooking of eggs until the white is completely set and the yolk beginning to set (Centre for Disease Control, 2000). With these guidelines as background this study was conducted to assess the knowledge and practices of people who keep backyard chickens regarding chicken-keeping and egg production in a typical developing township.

2.3 MATERIALS AND METHODS

2.3.1 Questionnaire survey (Appendix A)

This study was conducted during the cold-dry (May-July), mild-dry (August-September) and warm-wet (October-February) seasonal periods in the township of Phomolong, near Hennenman in the Free State Province, South Africa. Sixty questionnaires were administered to people who keep backyard chickens. The interview method was used. The questionnaires were compiled in English but during the interviews the questions were translated into the respondents' preferred language. These languages included Sesotho and Xhosa. Both closed and open-ended questions were included in the questionnaire (Coggon, 1995) and the questions were presented in a simple and concise manner which prevented the respondent from own interpretation (Nel, 2003; Walker *et al*., 2003; Rasephei, 2005). In this way the interviewer also had more control over the interview and could clarify unclear questions. The questionnaire included various questions which elicited information regarding 1) each respondent's personal

information; 2) chicken-keeping and nest hygiene; 3) collection of eggs; 4) storage of eggs and 5) preparation of eggs.

2.3.2 Pilot study

In order to assess the clarity and acceptability of the questions, a pilot study was conducted using five people who kept backyard chickens, and who were not included in the final sample.

2.3.3 Data analysis of completed questionnaires

The questionnaires were pre-coded and a code list was subsequently drawn up. The analysis of the questionnaire was done by hand using the code list. Finally, the data was described using frequencies and percentages.

2.4 Results and discussion

2.4.1 Respondents' personal information

From the respondents' personal information (Table 2.1) it was reflected that chickens in the township of Phomolong were mostly kept by females (77%). This supports the findings of Oakeley (2000) and Thekishoe *et al.* (2004) that most women in Africa keep chickens as their source of income and employment. 48% of the respondents (both female and male) were above the age of 50 with 47% educated up to primary school level and 35% educated up to secondary school level.

Table 2.1 Personal information of respondents in the township of Phomolong, near Hennenman

Parameter	Frequency (n= 60)	Occurrence (%)
Gender		
Female	46	77
Male	14	23
Age		
20-30	6	10
31-40	8	13
41-50	18	30
50 and above	28	47
Education level		
None	3	5
Primary school	28	47
Secondary school	21	35
Tertiary	8	13

2.4.2 Chicken-keeping and nest hygiene

The respondents' practices regarding chicken-keeping and nest hygiene are summarised in Table 2.2. Though 63% of the respondents indicated that their chickens roam freely within a confined area, 37% kept the chickens in cages. It was gathered however, that even the caged chickens are allowed to run freely in a confined space from time to time to scratch around in the backyard, as the chicken-keepers believe that this practice increases egg production. In addition to managing the chickens' movement, 50% of the respondents indicated that they clean the chicken nests daily, whereas 22% admitted that they cleaned the nests once a month. This practice will directly influence the quality of eggs as cleaner nests will minimise contamination on the eggshells that could penetrate into the egg contents and subsequently spoil the egg (Frazier and Westhoff, 1988).

Another factor that contributes to the quality of the egg is the fodder provided to the hen (The South African National Department of Agriculture, 2000). Fodder should include nutrients such as vitamins, minerals, and carbohydrates that are essential for proper growth and development of the egg. The majority (58%) of the respondents interviewed in this study fed their chickens whole or crushed maize, and some (3%) fed the chickens wheat. Two percent of the respondents acknowledged that they fed their chickens left-over food from the homestead, crushed mealies (17%) and sunflower seeds (20%). In addition to fodder, providing clean (potable) water to the hens is just as important for egg

Table 2.2 Chicken-keeping and nest hygiene practised by the respondents in the township of Phomolong, near Hennenman

Parameter	Frequency (n= 60)	Occurrence (%)
Place where chickens are kept		
Cage	22	37
Walk around in a confined space	38	63
Walk around anywhere	0	0
Confinement of chickens		
24 hours a day	9	41
During night time only	6	27
24 hours, but not every day	7	32
Cleaning of nests		
Once a day	11	50
Once in three days	3	14
Once a week	3	14
Once a month	5	22
Chicken feed		
Mealies	35	58
Wheat	2	3
Other: Sunflower seeds	12	20
Crushed mealies	10	17
Left-over porridge	1	2
Frequency of changing water and feed		
Once a day	23	38
Twice a day	27	45
Once in two days	10	17
Specific construction of water/ feed trough		
Yes	39	65
No	21	35

development (The South African National Department of Agriculture, 2000). The respondents adhered in general to this principle although 65% did not provide water in properly constructed troughs.

2.4.3 Collection of eggs

Table 2.3 summarises the practices of the respondents pertaining to egg collection. It is well known that humans could harbour and shed bacteria at rates of 10^3 to 10^4 cfu.min^{-1} viable microorganisms per minute (Frazier and Westhoff, 1988), which increases the probability of chicken-keepers being a source of contamination of the eggs they produce. Therefore a personal hygiene-related question was included. 67% of the respondents did not wash their hands before egg collection. Of the 33% who did wash their hands, 55% did so with cold water and disinfectant soap, and 45% with water only. This could encourage cross-contamination from the majority of the respondents to the eggs. Egg collection is further also conducted in a manner that does not promote quality in the eggs produced (Table 2.3). The largest proportion (77%) of the respondents further mentioned that they wash soiled or dirty eggs, in the process removing the cuticle which acts as a barrier inhibiting bacterial penetration by closing the pores within the shell (Wang and Slavik, 1998). Almost half (57%) of the respondents reported that they seldom remove cracked eggs from the chicken nests, while 20% of them consumed the cracked eggs regardless (Table 2.3). A cracked egg left in the nest for a week will encourage the penetration and subsequent

Table 2.3 The practices of egg collection exhibited by the respondents in the township of Phomolong, near Hennenman

Parameter	Frequency (n= 60)	Occurrence (%)
Hand washing		
Yes	20	33
No	40	67
Method of hand washing		
With cold water and disinfectant soap	11	55
With hot water and disinfectant soap	0	0
With running tap water only	9	45
Frequency of egg collection		
Once a day	33	55
Twice a day	2	3
Once a week	18	30
Other	7	12
Method of collection		
Collect clean eggs first	29	48
Collect both clean and soiled eggs at once	31	52
Separate bucket for egg collection		
Always	29	48
Sometimes	9	14
Never	22	38
Washing of eggs after collection		
Yes	46	77
No	14	23
Cracked or broken eggs in the nest		
Yes	34	57
No	26	43
What do you do with cracked eggs?		
Use	7	20
Discard	28	80

proliferation of bacteria in the egg (Frazier and Westhoff, 1988; Jay, 2000). An already contaminated egg (possibly with cracks) may therefore be introduced into the homestead.

2.4.4 Storage of eggs

In 90% of cases, eggs are stored at room temperature for up to three days (Table 2.4). Storage of eggs under these conditions is known to degrade the quality of the eggs. Due to moisture loss, the content of the egg shrinks and consequently bacteria are drawn in through the shell pores, introducing them into an environment where they can proliferate with ease (Frazier and Westhoff, 1988; Theron, 2003).

2.4.5 Preparation of eggs

The respondents in this study were generally aware that eggs are nutritious. They further indicated that all members of the family consume the eggs on a frequent basis (Table 2.5). The eggs are, to a limited extent, consumed raw, while of those who do further process the eggs (92%), 78% preferred to cook them thoroughly. It was further noted from the results that the majority of the respondents preferred the eggs scrambled or boiled.

Table 2.4 Egg storage practices of the respondents in the township of Phomolong, near Hennenman

Parameters	Frequency (n= 60)	Occurrence (%)
Storage of eggs after collection		
Yes	54	90
No	0	0
Sometimes	6	10
Where are the eggs stored?		
In the refrigerator	2	4
In a cupboard	32	60
In a maize meal bin	3	5
Under the table or cupboard	14	26
In an egg carton in the cupboard	3	5
How are the eggs stored?		
In an uncovered egg carton	30	56
Covered in a bowl	7	13
Uncovered in a bowl	17	31
Period of storage before consumption		
One day	21	35
Two days	25	42
More than three days	14	23

Table 2.5 Preparation method of eggs applied by the respondents in the township of Phomolong, near Hennenman

Parameters	Frequency (n= 60)	Occurrence (%)
Are eggs nutritious?		
Yes	57	95
No	3	5
Do you drink or eat raw egg yolk		
Yes	5	8
No	55	92
Consumption of eggs		
One per day	5	8
Two per day	36	60
Three per day	15	25
More, specify	4	7
Who consumes most eggs?		
The children	23	38
The elderly	34	57
Both	3	5
Most commonly used preparation method		
Fried	10	17
Scrambled	44	73
Boiled	35	58
In baking	12	20
How do you prefer to eat the eggs?		
With the yolk not solid and still flowing	13	22
With the yolk solid and cooked through	47	78
Cleaning of working surfaces and equipment after preparation		
Yes	59	98
No	1	2

In conclusion, the following recommendations are made to improve the quality of eggs produced in backyard systems. The majority of the chicken-keepers were elderly people above the age of 50 and were not educated, therefore not informed regarding the importance of nest hygiene and proper egg collection and storage. It is thus required that the chicken-keepers in Phomolong be taught about the importance of proper hand washing before and after egg collection as it was indicated from the questionnaires that these eggs were consumed by the most susceptible groups in the community such as the elderly, the young and probably people with HIV/AIDS. Proper hand washing will limit the transfer of pathogenic organisms such as *Staphylococcus* spp. from the eggs onto other food that is consumed by such people.

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CHAPTER 3

MICROBIOTA ASSOCIATED WITH BACKYARD CHICKEN EGGS

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3.1 Abstract

Backyard produced eggs were collected from randomly selected households during the cold-dry (May-July), mild-dry (August-September) and warm-wet (October-February) seasons for microbial analysis. Samples of two eggs each were collected from households selected for the study, tallying 150 samples. The microbial content on the eggshells and in the egg contents was determined using various selective and differential propagation methods. The isolated Staphylococci were further characterised and the following species were identified: *S. capitis; S. epidermidis; S. homonis; S. hyicus; S. lentus* and *S. xylosus.* Questionnaires were administered in order to determine the practices of the respondents and to establish any relationship between the organisms identified and the respondents' practices. The findings of the study revealed that though the backyard chicken eggs were produced under unrestricted conditions, their microbial quality was generally better than that of eggs produced commercially in the same region. Total Coliforms ($1 – 5 \times 10^1$ cfu. 2 eggs) and *Escherichia coli* ($1 – 5 \times 10^2$ cfu. 2 eggs) were present in both the contents as well as on the surface of the eggs during all seasons mentioned. With the exception of the Total Coliforms the presence of these organisms on the shells was not affected by seasonal changes.The irregular cleaning of nests explained the prevalence of faecal contaminants such as *Salmonella* spp. and *Escherichia coli* in and on the eggs. The presence of *Staphylococcus* spp. on the eggshells was attributed to extensive handling of eggs by the keepers.

Keywords: Backyard chickens, bacterial contamination, eggs.

3.2 Introduction

Although commercial chicken farmers in South Africa produce the majority of the approximately 5.8kg of eggs per capita per annum, there are many households that still produce and consume eggs from backyard systems. Backyard systems, producing what are also referred to as traditional or indigenous chickens, are characterised by fragmented and small-scale production units that require minimal management (Moreki and Masupu, 2000; Oakeley, 2000; Thekishoe *et al*, 2004). In rural Southern Africa, backyard chickens are found everywhere, providing, amongst other things, highly nutritious, readily available and inexpensive eggs (Van Niekerk and Van Heerden, 1993; The South African National Department of Agriculture (NDA), 2000; Furusawa, 2003). These chickens are known to feed on a variety of macro-vertebrates from the soil surface, including spiders and earthworms, as they roam around unrestricted (Clark and Gage, 1997)

As a result the eggs produced by these chickens usually become contaminated when laid in dirty nests or on dirty floors (Gentry and Quarles, 1972; Pienaar *et al*., 1994; De Reu *et al*., 2004). De Reu *et al*. (2004) and Pienaar *et al.* (1994) also reported that high moisture excreta can directly increase the microbial contamination of the eggshell and consequently increase the risk of microbial contamination of the internal contents of intact eggs. Eggs are known to be highly perishable products that lose quality rapidly if not given proper care between collection and consumption (Kamel and Diab, 1980). Cox *et al.* (2000) noted that

contamination of eggs can occur in two ways: 1) vertical transmission, in which bacteria from an infected hen can infect the egg before laying, and 2) horizontal transmission, which is the invasion of the shell by bacteria after the egg has been laid.

In theory, the incidence of egg contamination should vary based on the ability of the egg to withstand bacterial invasion and the kind of bacteria on or in the egg. Previous studies addressing egg spoilage generally focused on eggs being produced commercially. In a typical South African township, however, the eggs that are consumed are in general not commercially produced and are exposed to unique challenges regarding microbial contamination and the health of the hen. In addition, the population consuming the eggs also face unique challenges as the majority are poorly educated, and often not working due to old age. They therefore rely profoundly on the availability of cheap nutritious foods such as eggs and cannot afford to fall ill as a result of food poisoning brought on by the consumption of a poor quality product produced by themselves.

This study aimed at quantifying the specific microbiota present on/in eggs produced by backyard chickens in a typical South African township. Nest-keeping, egg collection and storage practices were also assessed to establish the routes of contamination in this scenario.

3.3 Materials and methods

3.3.1 Sampling

The study was conducted in the township of Phomolong (a marginal-urban area) in close proximity to the town of Hennenman, in the Free State Province, South Africa. All eggs were collected over a period of nine months that included the cold-dry (May-July), mild-dry (August-September) and warm-wet (October-February) seasonal periods from randomly selected households which kept backyard chickens. The eggs were collected from the nests as well as from areas where eggs were kept after collection, such as in egg cartons in or under cupboards. In total 150 eggs (two per household) were collected during the mentioned seasons. Theron (2003a) proposed that the analysis of the eggs should consist of two main steps, namely 1) the analysis of the microbial contaminants on the eggshell (egg intact); and 2) the analysis of the contaminants associated with the egg contents.

3.3.2 Eggshell

Two eggs were washed in 50ml Nutrient Broth, each in a sterile plastic bag, by rubbing the surface of the eggs through the plastic bag for 30 seconds. After the egg washing procedure, 50ml of Nutrient Broth (from each egg washing) was mixed together in a sterile Schott bottle and shaken until evenly mixed.

3.3.3 Egg contents

After the egg washing, the eggshells were sterilised by submerging them briefly in alcohol, and then dried in a sterile cabinet. The contents of the two eggs were obtained by cracking and breaking the eggs aseptically. The liquid contents were emptied into 200ml sterile Nutrient Broth and the liquid was shaken until evenly mixed.

For dilutions, 1ml of the Nutrient Broth from each of the eggshell and the egg contents was used to prepare serial dilutions using saline solution. One hundred microlitres were subsequently transferred to the agar plates mentioned below (Biolab-RSA) and the streak plate method was applied to distribute the suspension evenly. Plate Count Agar (PCA) (MERCK-RSA, Martley *et al*., 1970) was used, followed by incubation at 25ºC for 48 hours, for the enumeration of the Total Viable Counts (TVC); Cetrimide Agar with 5ml added glycerol (MERCK-RSA, Goto and Enomoto, 1970) was used, followed by incubation at 25ºC for 18-48 hours for *Pseudomonas* spp.*;* Baird-Parker Agar with added 50ml egg-yolk tellurite emulsion (MERCK-RSA, Nikanen and Aalto, 1978) was incubated at 36ºC for 48 hours for *Staphylococcus* spp*.* enumeration*;* and Violet-Red-Bile-MUG Agar (Biolab-RSA) was incubated at 36ºC for 48 hours for Total Coliforms and *Escherichia coli. Staphylococcus* spp. were further purified on Nutrient Agar with 50ml added blood and characterised to species level using the API-Staph system (Omnimed-RSA).

3.3.4 Questionnaire design

See section on questionnaire survey (Chapter 2; pages 23-24).

3.4 Results and discussion

In general the microbial quality of the eggs evaluated in this study was better than those produced commercially in the same region as indicated in the study done by Theron (2003a). This was not expected as free-roaming chickens are more exposed to adverse and ever-changing environmental conditions, uncontrolled diets, other breeds of chicken and other animals. The inability to control the nesting area, nesting material and chicken health further generated the expectation that the eggs would have a poor microbial quality (Table 2.2).

At the same time, it was anticipated that the practices of the chicken keepers pertaining to the keeping of the chickens, egg collection, egg storage and the preparation of eggs would play a role in the egg quality (see Chapter 2, Tables 2.2 and 2.3). Only half of the respondents cleaned the nests frequently which gave the first clue as to the probable source of faecal contamination on the eggs (Table 2.2). Though in low quantities, Total Coliforms ($1 – 5 \times 10^1$ cfu per two eggs) and *Escherichia coli* ($1 – 5 \times 10^2$ cfu per two eggs) (Figure 3.1) were present both in the contents as well as on the surfaces of the eggs during all seasons mentioned. Similarly, *Salmonella* spp. present in the same range. With the exception of the Total Coliforms the presence of these organisms on the shells was not affected by seasonal changes. The presence of these organisms in the contents of the eggs, however, suggested otherwise. During the cold-dry

season lower quantities of faecal contaminants were present in the contents of the eggs compared to the other two seasons. The highest quantities were detectable during the mild-dry season (August-September). This phenomenon is not easily quantifiable and possibly results from the effects of seasonal change on chicken health.

In this community rotten eggs occurred frequently (Table 2.3). In this case the organism responsible is probably *Pseudomonas* spp. In the contents and on the shells the quantity of this organism ranged from $8 \times 10^{-1} – 8 \times 10^{0}$ cfu per two eggs. Though this organism has the ability to survive and proliferate at low temperatures, seasonal changes had little influence on its presence. This implies that either the eggs were not exposed to lower temperatures during winter due to frequent collection from the nests, or that season-associated temperature fluctuations did not influence the ability of the egg to resist bacterial growth. The information assimilated by means of the questionnaire did not provide the answer, although work done by Theron (2003b) on the influence of cold shock on the ability of bacteria to proliferate in or on eggs would have us believe that seasonal change has a negligible influence on egg health and therefore its ability to have a bacteriostatic effect.

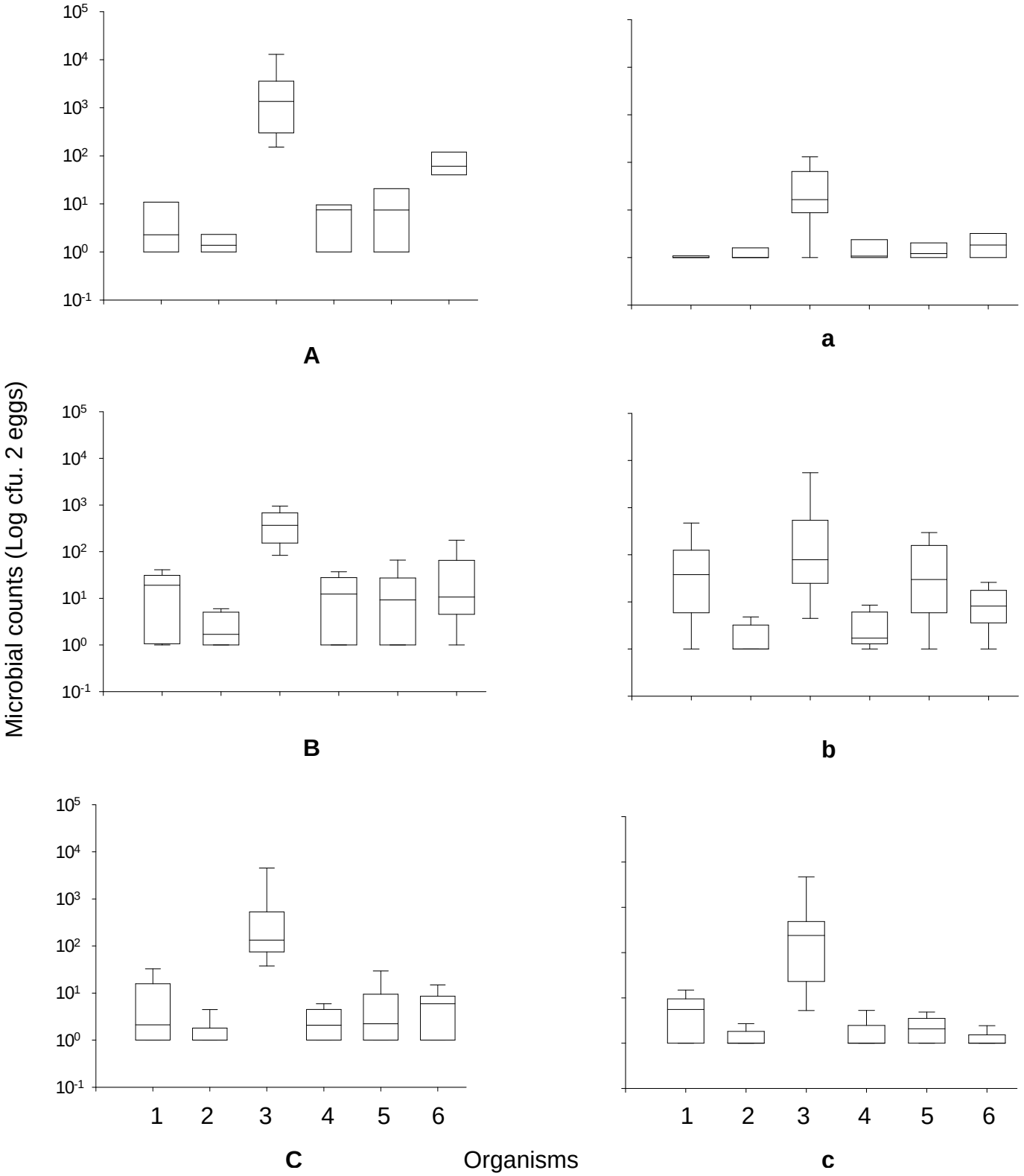

Figure 3.1 Microbial counts on/in eggs from backyard-produced chickens in Phomolong, near Hennenman, where 1 = *Salmonella* spp., 2 = *Pseudomonas* spp., 3 = Total Viable Counts (TVC), 4 = Total Coliforms, 5 = *Escherichia coli* and 6 = *Staphylococcus* spp. A = shell (cold-dry season), a = contents (cold-dry season); B = shell (mild-dry season), b = contents (mild-dry season) and C = shell (warm-wet season), c = contents (warm-wet season)

The increased presence of *Staphylococcus* spp. (usually related to humans) on the surface of the eggs indicated extensive handling of the eggs by the keepers. Cox *et al*. (2000) mentioned that the presence of this organism inside the eggs could be due to horizontal transmission. In this case chicken keepers rarely (Table 2.3) washed their hands prior to egg collection. They further indicated that of the eggs collected, 57% were broken or cracked, a situation that could favour penetration of organisms into the egg. Subsequent characterisation of the *Staphylococcus* spp. (Table 3.2) revealed only two species, *S. hyicus* and *S. lentus,* which have previously been associated with chickens (Takeuchi *et al.*, 2000; Nagase *et al.,* 2001). The rest of the identified species are frequently associated with humans (Nagase *et al*., 2001; Euzéby, 2003; Le Loir *et al.,* 2004).

The presence of the staphylococci, similarly to *Pseudomonas* spp., was not notably affected by seasonal changes. Finally, as indicator of the general microbial quality of the eggs, the Total Viable Counts (TVC) were much lower than previously reported for commercially produced eggs (Theron, 2003a). Seasonal changes did, however, appear to impact on the quantity of these organisms present on and in the eggs ($1 – 10^4$ cfu per two eggs). The cold-dry season (May-July) produced higher counts than the warm-wet (Oct-Feb) season on the surface of the eggs. This probably occurred due to higher levels of dust that are present during this season, which would facilitate increased microbial presence both outside and inside the house. Except for during the warm-wet

Table 3.1 *Staphylococcus* spp. identified on backyard-produced chicken eggs in the township of Phomolong, near Hennenman in the Free State

Staphylococcus spp.	(%) of contamination on shells (n=150)	(%) of contamination in egg contents (n=150)
S. capitis	2	2
S. epidermidis	2	0
S. hominis	0	0.6
S. hyicus	0	0.6
S. lentus	0	2
S. xylosus	4	6.6

season, the TVC in the contents of the eggs were lower than on the shells. This could possibly be due to the increased presence of moisture on the shells that could facilitate the migration of organisms into the egg through the pores (Frazier and Westhoff, 1988; De Reu *et al*., 2004; Soljour *et al.,* 2004).

In conclusion, results from this study have shown that the majority of the chicken keepers and egg consumers surveyed were aware of the nutritional benefits of eggs. Only a small minority however were aware that eggs could be contaminated and could therefore pose a health threat if not properly prepared and stored. This is probably why, in most cases, the chicken-keepers did not wash their hands prior to egg collection, and soiled and clean eggs were collected and stored together. It is further known that washing of soiled eggs does not eliminate the dangers of microbial contamination (Pienaar *et al*., 1994; James *et al.,* 2002) especially if one takes into consideration the fact that the majority of people who participated in this study do not store their eggs in a refrigerator. The growth of the mentioned organisms could be easily controlled and minimised merely by exposing the eggs to a cold shock before storage at room temperature (Theron, 2003b). The analysed eggs were generally of a good microbial quality and since the majority of the consumers prefer their eggs well cooked and consume them immediately after cooking (Table 2.5), the health risks posed by these eggs seem to be minimal. In these households cross-contamination would also play a limited role since 98% (Table 2.5) of the respondents clean the working surface and equipment after contact with raw

eggs. The undesirable presence of *Salmonella* spp. could probably be addressed by educating this community about nest hygiene and its essential part in producing even safer eggs with the backyard chicken system.

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CHAPTER 4

THE INFLUENCE OF PREPARATION METHODS ON THE DECREASE OF *STAPHYLOCOCCUS* SPP. AND FAT-SOLUBLE VITAMINS IN BACKYARD-PRODUCED CHICKEN EGGS

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4.1 Abstract

Eggs were collected, from randomly selected households of individuals keeping backyard chickens, during the cold-dry (May-July), mild-dry (August-September) and warm-wet (October-February) seasonal periods in the Phomolong township, near Hennenman, Free State, South Africa. A total of 150 egg samples were collected and analysed for the presence of *Staphylococcus* spp. The species identified included *Staphylococcus epidermidis, S. hominis, S. capitis* and *S. xylosus.* The vitamins A and E concentrations within the eggs were also determined, and the influence of cooking (boiling, frying and scrambling) on their stability was investigated. The results obtained revealed that *S. epidermidis* in general had a high mortality rate during scrambling (0 $cfu.g^{-1}$) while *S. hominis* proved to be more susceptible to boiling (7.00 $X10^2$ $cfu.g^{-1}$) and frying (1.36 $X10^3$ $cfu.g^{-1}$). *S. xylosus* was more heat labile during frying (0 $cfu.g^{-1}$), with *S. capitis* having the highest mortality rate during boiling (1.37 $X10^3$ $cfu.g^{-1}$). It was further noted that vitamin A decreased faster (1.5µg.min^{-1}, 1.1µg.min^{-1} and 0.1µg.min^{-1} during boiling, frying and boiling respectively) than vitamin E (0.02µg.min^{-1}, 0.02µg.min^{-1}, and 0.001µg.min^{-1}) which indicated that vitamin A oxidized at a rate higher than vitamin E at the selected cooking temperatures. Although the scrambling method exposed the eggs to oxygen and temperature, the time of exposure to the latter resulted in a lower degradation rate than the other preparation methods.

Keywords: Eggs, preparation*, Staphylococcus* spp., fat-soluble vitamins.

4.2 Introduction

Backyard chickens are kept by most rural and peri-urban homesteads in Africa and Asia, where these chickens scavenge for food (Mushi *et al.,* 2000). In a study done in Qwa-Qwa in the northern-eastern Free State Province of South Africa, Thekishoe *et al.* (2003) found that backyard chickens are often provided with night shelters which are made from inexpensive wire mesh and discarded corrugated iron sheeting. This author further stated that these chickens are bred and raised for their meat and eggs, which serve as a rich source of animal protein for the rural poor in most parts of Africa. Surai and Sparks (2001) also reported that eggs are a rich and balanced source of essential amino and fatty acids as well as of minerals and vitamins.

Vitamins are essential for the human metabolism since they have a catalytic function in both the anabolic and catabolic pathways (Turner *et al.,* 2001; Chatzimichalakis *et al.,* 2004; Kledjus *et al.,* 2004; Lukaski, 2004). Based on their solubility, vitamins are classified into two classes: 1) fat-soluble vitamins (A, D, E, and K) which are soluble in non-polar organic solvents, and 2) water-soluble vitamins (vitamin C and eight B-complex vitamins), which can be extracted from foods with aqueous solvents (Luque-García and Luque de Castro, 2001; Chatzimichalakis *et al.,* 2004; Heudi *et al.*, 2004; Lukaski, 2004; Mata-Granados *et al.*, 2004). All four fat-soluble vitamins are present in the egg yolk, with water-soluble vitamins present in either the white or yolk or both (Fox and Cameron, 1995). A medium-sized egg (60g) is known to contain about 260 International

Units (IU) of vitamin A (Bennion, 1990), as well as a lower concentration of vitamin E as this vitamin has a lower transfer efficiency from the hen to the egg (Hossain *et al.,* 1998)

In addition, freshly laid eggs are known to be generally sterile but contamination can occur shortly after laying due to the washing water, packaging material and extensive handling of eggs by humans (Frazier and Westhoff, 1988). *Pseudomonas, Acinetobacter, Proteus, Aeromonas, Alcaligenes, Escherichia, Micrococcus, Salmonella, Enterobacter* and *Staphylococcus* are among the bacteria known to contaminate eggs (Jay, 2000). Staphylococci are Gram-positive, non-motile and non-sporing facultative anaerobes. They are normally associated with the skin and mucous membranes of warm-blooded vertebrates but are often isolated from food products, dust and water (Wieser and Busse, 2000). Wieser and Busse (2000) further stated that staphylococci are ubiquitous in nature, with *Staphylococcus aureus* used as an indicator of personal hygiene. This organism is also known to be of major importance in food products for human consumption because some strains give rise to foodborne intoxication (Desmarchelier *et al*., 1999; Edwards *et al*., 2001).

Intoxication results from the ingestion of food in which enterotoxigenic strains have grown to sufficient levels to allow a toxic dose of staphylococcal enterotoxin to be produced prior to consumption (Desmarchelier *et al*., 1999; Vanderlinde *et al*., 1999; Borch and Arinder, 2002). The infective dose for *Staphylococcus*

aureus is 10^5 cfu.g^{-1} (Atanassova *et al.,* 2001). Staphylococcal food poisoning is one of the most common foodborne illnesses, and is found in almost all parts of the world (Yang *et al*., 2001). The author further stated that a wide variety of foods, including bakery products containing custards or cream, ham, poultry products, milk and milk products as well as eggs have been reported to be involved in outbreaks of staphylococcal intoxication.

The vitamin content of food is known to be reduced during processing in two ways, namely by oxidation or by dissolving into the cooking water. The latter applies mainly to water-soluble vitamins (Frazier and Westhoff, 1988; Bennion, 1990). Likewise, high temperature is the principal method used to kill organisms (Banwart, 1989; Fox and Cameron, 1995). This study therefore aimed at quantification of vitamins A and E in eggs produced from backyard chickens and assesses the decrease of these vitamins during common cooking methods applied by the population in Phomolong township, near the town of Hennenman. Concomitant evaluation of the decrease in staphylococcal counts during the mentioned cooking procedures were also conducted towards establishing method of cooking that lowers the bacterial counts on the eggs without significantly destroying the vitamins.

4.3 Materials and methods

4.3.1 Sampling/interviews

The majority of the eggs were collected from the areas where eggs were kept after collection from the nest by the keepers. The storage areas were generally in or under cupboards, usually in used egg cartons. In total 150 eggs (two per household) were collected for the quantification of *Staphylococcus* spp. and 71 eggs were collected for the quantification of vitamins A and E. Subsequent quantification of the decrease of the *Staphylococcus* spp. counts and vitamin concentrations within the eggs during cooking was conducted. In order to acquire information on the most common preparation method used in the study area, sixty questionnaires were administered. The questionnaires were compiled in English, but during the interviews the questions were translated into the respondents' preferred language which included Sesotho and Xhosa.

4.3.2 Microbial quantification

Staphylococcus spp. was quantified using Baird-Parker Agar (Biolab-RSA) with 50ml egg-yolk tellurite emulsion added (Merck-RSA, Nikanen and Aalto, 1978). After inoculation the plates were incubated at 36℃ for 48 hours. *Staphylococcus* spp. isolated from the Baird-Parker Agar was further purified on Nutrient Agar with 50ml added sheep blood and identified to species level using the API-Staph system (Omnimed-RSA; Nagase *et al.*, 2001).

4.3.3 Cooking procedure and inoculum

The purified *Staphylococcus* spp. was propagated in Nutrient Broth for 24 hours at 37℃ before being inoculated into test eggs. Sub sequent preparation of the eggs using common preparation methods (boiling, frying and scrambling) as ascertained from the questionnaires was applied. Tests on the mortality of the different *Staphylococcus* spp. using simulated domestic conditions as described by Humphrey *et al*. (1989) were further conducted.

4.3.4 Baseline vitamin quantification

The extraction of vitamins A and E from both raw and cooked eggs was conducted according to the method proposed by Qian and Sheng (1998). Chromatographic separations were performed using a C_{18} silica column. HPLC grade methanol (Merck, SA) was used as a mobile phase at a flow rate of 1.5 $ml.min^{-1}$ and a pressure of 1000 p.s.i. All stock and standard solutions of fat-soluble vitamins were prepared in ethanol. For the preparation of the calibration curves for each vitamin, five different concentrations of each standard were used.

4.3.5 Vitamin stability assessment

For the vitamin stability assessment the collected eggs were boiled for 7 minutes, fried for 8 minutes or scrambled for 5 minutes. The degradation of the vitamins during the mentioned cooking methods was quantified as presented in section 4.3.4.

4.4 Results and discussion

The average *Staphylococcus* spp. count identified on the eggs was higher on the eggshells than in the egg contents (Figure 4.1). On the eggshells the counts ranged from 8.49 X 10^1 cfu per two eggs to 3.88 X 10^1 cfu per two eggs and 5.64 X 10^0 cfu per two eggs for the cold-dry (May-July), mild-dry (August-September) and the warm-wet (October-February) seasonal periods respectively. The increased presence of *Staphylococcus* spp. on the eggshell compared to the egg contents indicates that the eggs were extensively handled by the keepers since the majority of the species identified were associated with humans (Table 4.1). *S. capitis* and *S. epidermidis* are generally isolated from the microbiota populating the human skin (Otto *et al*., 2000; Miragaia *et al*., 2002) while *S. xylosus* have been isolated from human nares (Nagase *et al.*, 2001).

The presence of *Staphylococcus* spp. in the egg contents probably resulted from horizontal transmission (Cox *et al*., 2000). Horizontal transmission is the invasion of bacteria into the egg through the shell after the egg has been laid (Cox *et al*., 2000). There are no guidelines for food contaminated with *Staphylococcus* spp. other than *Staphylococcus aureus* with 10^5 cfu.g^{-1} being indicative for food poisoning (South African Department of Health, 2000). When determining seasonal influence on the presence of *Staphylococcus* spp. on both the eggshells and in the egg contents it was found that during all the seasons sampled, the egg contents were less contaminated than the egg shells (Figure 4.1).

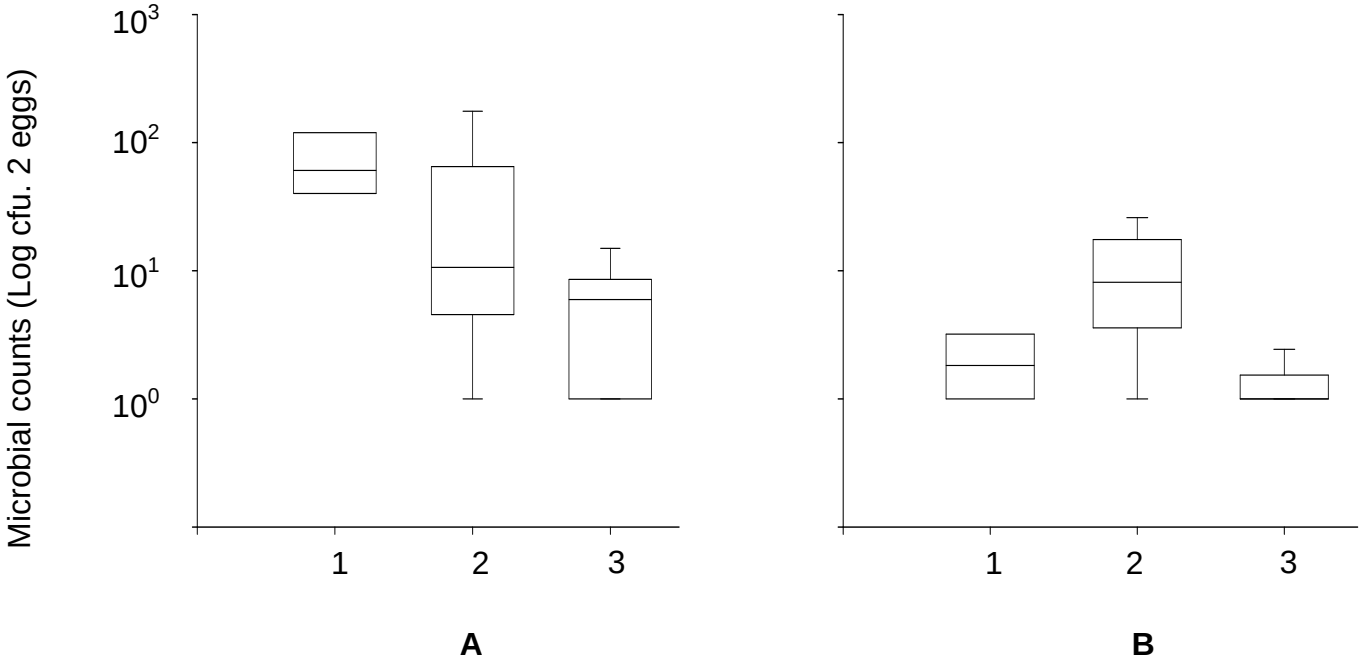

Figure 4.1 *Staphylococcus* spp. identified on backyard chicken eggs on eggshells (A) and in the egg contents (B) during the cold-dry (1), mild-dry (2) and warm-wet (3) seasonal periods

Table 4.1 *Staphylococcus* spp. identified on backyard-produced chicken eggs in the township of Phomolong, near Hennenman

Staphylococcus spp.	Presence (%) on all shells sampled (n=150)	Presence (%) in all egg contents (n=150)
S. capitis	2	2
S. hominis	0	0.6
S. epidermidis	2	0
S. xylosus	4	6.6

The warm-wet season produced the lowest counts of *Staphylococcus* spp. compared to the other two seasons. The presence of this organism during this season could be due to contamination from human-related *Staphylococcus* spp. The majority (68%) of the people did not wash their hands prior to egg collection (Table 2.1, Chapter 2), and a further possibility is that the increased presence of *Staphylococcus* spp. during the cold-dry and the mild-dry season may be from the human nasal passage since people sneeze and cough more often during this time of the year. The majority (87%) of the people indicated that they did not store eggs in the fridge, thus less contamination of the egg contents. Frazier and Westhoff (1988) stated that the temperature of an egg after laying is higher and when stored in the fridge the egg contents shrink, encouraging the penetration of bacteria through the pores into the egg contents.

4.4.1 *Staphylococcus* spp. decrease during cooking

This section of the study focused on the mortality rate of different *Staphylococcus* spp. identified on backyard chicken eggs (Table 4.1) during preparation - scrambling, frying and boiling (Table 2.5). High temperature is known as the principal method used to kill organisms in food (Banwart, 1989; Fox and Cameron, 1995). The overall results indicated that the scrambling method, which was applied for five minutes, was the best preparation method, resulting in the elimination (0 $cfu.g^{-1}$) of all *Staphylococcus* spp. inoculated, followed by frying and boiling respectively (Table 4.2). The complete elimination of the *Staphylococcus* spp. during scrambling might be due to the fact that the

scrambling method exposes the egg to more heat as the egg is spread all over the surface of the pan during cooking whereas in the other two cooking methods this is not the case. On the other hand, frying, which was done for four minutes on each side of the egg, exposed the egg to more heat than boiling. It was found that the eggshell which covers the egg contents might limit the amount of heat transferred to the egg, thus the lower mortality rate of *S. hominis* and. *S. capitis.* The various *Staphylococcus* spp. showed a different trend of mortality during the various preparation methods (Table 4.2). *S. capitis* liability was completely eliminated during frying and scrambling and had a higher mortality rate during boiling (1.37×10^3 $cfu.min^{-1}$). When comparing the mortality rates of the various *Staphylococcus* spp. during the three cooking methods, *S. epidermidis* was more heat sensitive than the other *Staphylococcus* spp. followed by *S. xylosus, S. capitis* and *S.hominis* respectively.

Table 4.2 The mortality rates of different *Staphylococcus* spp. in backyard-produced chicken eggs during cooking

Boiling		Frying		Scrambling	
Inoculated	Post-preparation	Inoculated	Post-preparation	Inoculated	Post-preparation
2.53×10^4 cfu. g^{-1}	[a] 0 cfu. g^{-1}	2.97×10^4 cfu. g^{-1}	[a] 0 cfu. g^{-1}	2.5×10^3 cfu. g^{-1}	[a] 0 cfu. g^{-1}
	[b] 7.00×10^2 cfu. g^{-1}		[b] 1.36×10^3 cfu. g^{-1}		[b] 0 cfu. g^{-1}
	[c] 0 cfu. g^{-1}		[c] 2.40×10^3 cfu. g^{-1}		[c] 0 cfu. g^{-1}
	[d] 1.37×10^3 cfu. g^{-1}		[d] 0 cfu. g^{-1}		[d] 0 cfu. g^{-1}

[a] = *S. epidermidis*

[b] = *S. homonis*

[c] = *S.xylosus*

[d] = *S. capitis*

4.4.2 Vitamin concentration in backyard chicken eggs

In all eggs sampled the vitamin A concentration was significantly higher than that of vitamin E. These results correspond with reports of Sungpuag *et al.* (1999) and Flachowsky *et al.* (2002), who reported the same relation in commercial eggs. The levels of both vitamins A and E were, however, much lower than was reported for commercially produced eggs. On average backyard-produced eggs contained 9.63×10^{-4} $mg.ml^{-1}$ ± 9.63×10^{-1} $\mu g.ml^{-1}$ vitamin A and 6.03×10^{-6} $mg.ml^{-1}$ ± 6.03×10^{-3} $\mu g.ml^{-1}$ vitamin E (Figure 4.2). Sungpuag *et al.* (1999) and Flachowsky *et al.* (2002) reported the average vitamin A and E in commercially produced eggs to be 1.62×10^{2} $\mu g.100g^{-1}$ and 1.12 $mg.60g^{-1}$ ± 1.12×10^{3} $\mu g.60g^{-1}$ respectively. This increased concentration (compared to the backyard eggs) might be a direct result of the difference in the fodder fed to commercial chickens and/or the breeds of chickens used in the studies. Jacobs and Miles (2000) mentioned that the vitamin content of chicken eggs varies significantly due to 1) the dietary vitamin intake of the chicken and 2) the transfer efficiency of the vitamin from the hen to the egg. This was also clear in this study as a high standard deviation was evident for both vitamins A and E.

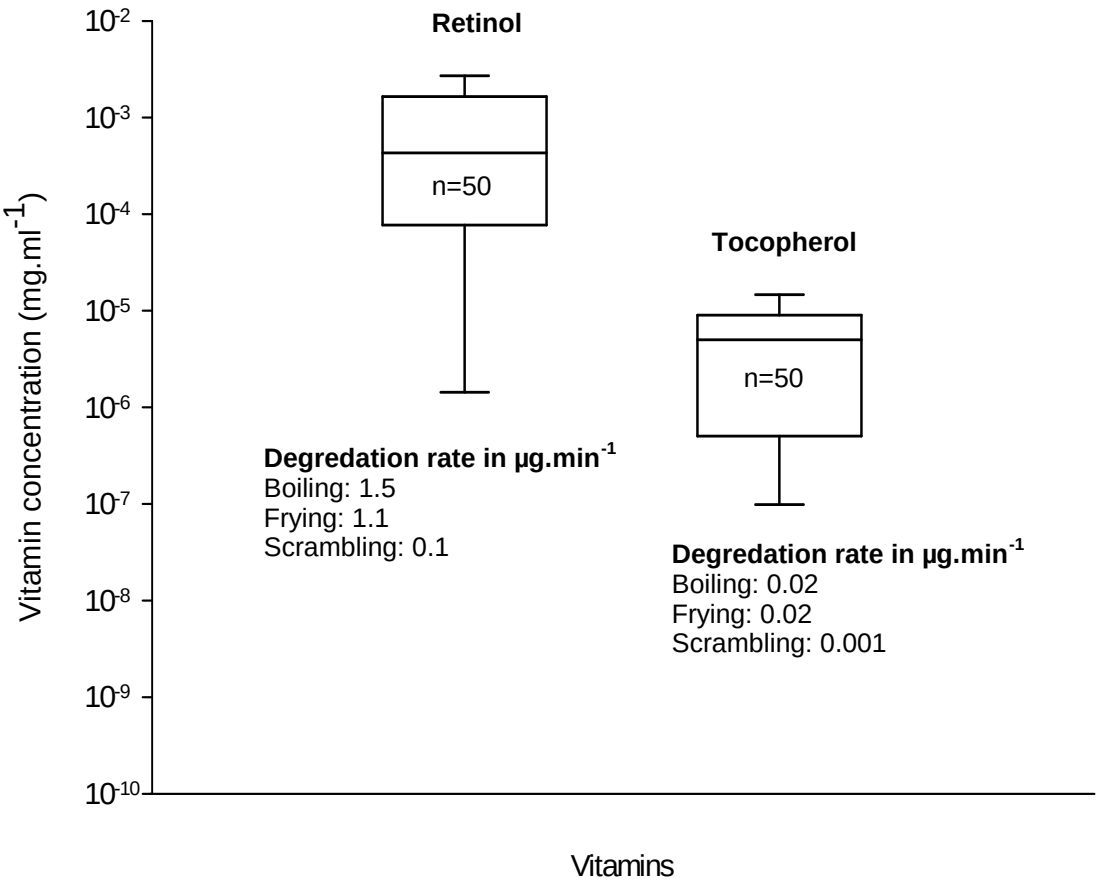

Figure 4.2 The concentration of vitamins A (retinol) and E (tocopherol) in backyard-produced chicken eggs and the degradation of these vitamins during various cooking methods

4.4.3 Vitamin degradation during cooking

73% of the respondents preferred scrambling, while 17% chose frying as the preferred method to prepare chicken eggs. In this section of the study, the degradation of vitamins was determined in terms of the mentioned preparation methods and preparation times. The scrambling method produced the least degradation of both vitamins A and E followed by frying and boiling respectively (Figure 4.2). This scenario was rather unexpected although the explanation might be related to the difference in time taken to prepare the eggs. Scrambling resulted in less degradation because the eggs were exposed for a lesser period to oxygen and temperature. Ipek *et al.* (2005) mentioned that vitamins are easily broken down in the presence of oxygen and high temperature. Vitamin A proved to be less heat stable than vitamin E during all three preparation methods. The former degraded at a rate of 1.5µg.min^{-1} during boiling, 1.1µg.min^{-1} during frying and 0.1µg.min^{-1} during scrambling. In all cases vitamin A degraded faster than vitamin E. Wirakartakusumah (1998) and Miquel *et al*. (2004) mentioned that vitamin E can be affected by light, oxygen and temperature and that vitamin A is heat labile in the processing environment.

In conclusion, it was observed that *S. epidermidis* had the highest mortality rate throughout the experiment compared to the other organisms which had different rates of mortality depending on the various cooking methods. Although heat may kill the cells, most of the species used in this experiment are toxin producers and if enterotoxins are produced, the toxin may persist since it is more heat stable

than the organism. On the other hand, backyard-produced eggs had a higher concentration of vitamin A than vitamin E. The low concentration of vitamin E found in backyard-produced eggs indicates that vitamin E has a medium or low transfer efficiency from the hen to the egg, as was reported by Hossain *et al*. (1998). In most cases vitamin A was the more sensitive vitamin during cooking when compared with vitamin E. Therefore it is recommended that a study should be conducted to determine the survival of the toxins of various *Staphylococcus* spp. after using various common cooking methods as toxins are more harmful than the organism. It is further recommended that proper hand washing should be practised by the chicken-keepers as the majority of the *Staphylococcus* spp. identified were human-related: as these species are toxin producers they might cause a health threat to the more susceptible groups in the community. Finally, the best preparation method that resulted in a high mortality rate of the majority of the *Staphylococcus* spp. while preserving both vitamins, is the scrambling method, with a recommended scrambling time of five minutes. It is also suggested that the people should be encouraged to feed the chickens maize, which provides most of the essential nutrients as stated by the South African National Department of Agriculture (NDA). In this way, the costs of chicken keeping will be reduced since the chickens will be fed a nutritious fodder that will improve chicken health and egg production.

4.5 References

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CHAPTER 5

GENERAL CONCLUSION

5.1 Introduction

Limited information is available on the bacterial quality of backyard-produced chicken eggs on national and international level. Since backyard chickens are mostly kept by the disadvantaged people in the community who need to consume high-quality food, the aims of this study were 1) to assess the people's practices associated with the production of backyard chicken eggs; 2) to quantify and identify microbiota associated with backyard chicken eggs; 3) to determine the influence of cooking on the decrease of *Staphylococcus* spp. and the stability of fat-soluble vitamins in backyard-produced chicken eggs during various preparation methods. The nest-keeping, egg collection and storage practices of these people will have an influence on the type and number of microorganisms identified on the chicken eggs. At the same time, the common preparation methods that the people use will determine whether they consume eggs that are safe and nutritious.

5.2 Concluding remarks on Chapters 2, 3, and 4

In Chapter 3, microbiota associated with backyard chicken eggs were compared during three different seasons, namely, the cold-dry (May-July), mild-dry (August-September) and the warm-wet (October-February) seasonal periods. Eggs from backyard-produced chickens contained relatively low levels of contamination compared to commercially-produced eggs from the same region, as shown by Theron (2003). In most cases, the eggshells were found to be more contaminated than the egg contents and the majority of the organisms

enumerated on the eggshells and in the egg contents were not affected by seasonal changes, except for the Total Coliforms and the Total Viable Counts. *Staphylococcus* spp., which was further identified to species level, revealed that most of the species identified during the study were of human origin, with the exception of only two species, *S. hyicus* and *S. lentus* which have been previously associated with chickens (Takeuchi *et al*., 2000; Nagase *et al*., 2001; Le Loir *et al*, 2003).

When determining the best cooking method resulting in the highest mortality rate for the different *Staphylococcus* spp. identified on backyard chicken eggs, it was found that scrambling (5 minutes) was the best method, followed by frying (8 minutes) and boiling (7 minutes) respectively (Figure 5.1). The various *Staphylococcus* spp. had different mortality trends throughout the experiment with *S. epidermidis* being completely eliminated with the scrambling method. The majority of the species used in the study were toxin producers and it is therefore recommended that a study be conducted on the stability of toxins produced by the *Staphylococcus* spp. identified in this study after exposure to various common cooking methods.

Furthermore, the stability of vitamins generally present in eggs was determined using commonly used preparation methods (boiling, frying and scrambling) as obtained from the questionnaire results (Chapter 2). In terms of vitamin concentration, it was found that the backyard-produced eggs in this study had

lower concentrations of vitamins A and E compared to those produced commercially as evidenced in the studies done by Sungpuag *et al*. (1999) and Flachowsky *et al.* (2002). This is probably due to the differences in the fodder that is given to commercially-produced chickens and backyard-produced chickens. Commercial chickens are fed a 'complete' feed which contains sufficient proteins, vitamins and other nutrients necessary for proper growth and egg production. This is not the case with backyard chickens; therefore it is recommended that backyard chickens be fed maize which provides the majority of the essential nutrients thus limiting costs of chicken keeping while improving chicken health and egg production.

It was finally concluded that the preparation method which caused the least decrease of vitamins was the scrambling method, followed by the frying and boiling methods. Similar to the vitamin analysis, it was found that the best preparation method to reduce the number of Staphylococci organisms was the scrambling method, followed by the frying and boiling methods. Therefore, when selecting the best preparation method which would result in limited vitamin degradation while still destroying most of the Staphylococci, it was found that the scrambling method proved the most efficient.

5.3 Recommendations

In conclusion it is recommended that the chicken nests be cleaned regularly to decrease contamination on the eggshells. This would also reduce the prevalence of contamination of faecally-borne pathogenic organisms that include *Salmonella* spp. on and in the egg. The washing of eggs prior to storage should be discouraged since this practice removes the cuticle, which acts as a covering that inhibits bacterial penetration by closing the pores within the shell (Wang and Slavik, 1998). On the other hand the washing of hands prior to egg collection should be encouraged since the majority of the *Staphylococcus* spp. identified on and in the egg contents were of human origin.

The uncovered storage of eggs exposes them to various external contamination factors and this practice should be discouraged. The respondents indicated that they collect both soiled and clean eggs at once and this encourages cross-contamination from soiled to clean eggs. The use of cracked or broken eggs should be discouraged, as broken or cracked eggs favour penetration of organisms into the egg contents.

Vitamin A was further found to degrade faster than vitamin E during cooking (Figure 5.2). The best cooking method found to preserve vitamins A and E was the scrambling method which was done for 5 minutes. The cooking method which was identified to decrease the *Staphylococcus* spp. counts while still preserving vitamins was also the scrambling method, done for 5 minutes.

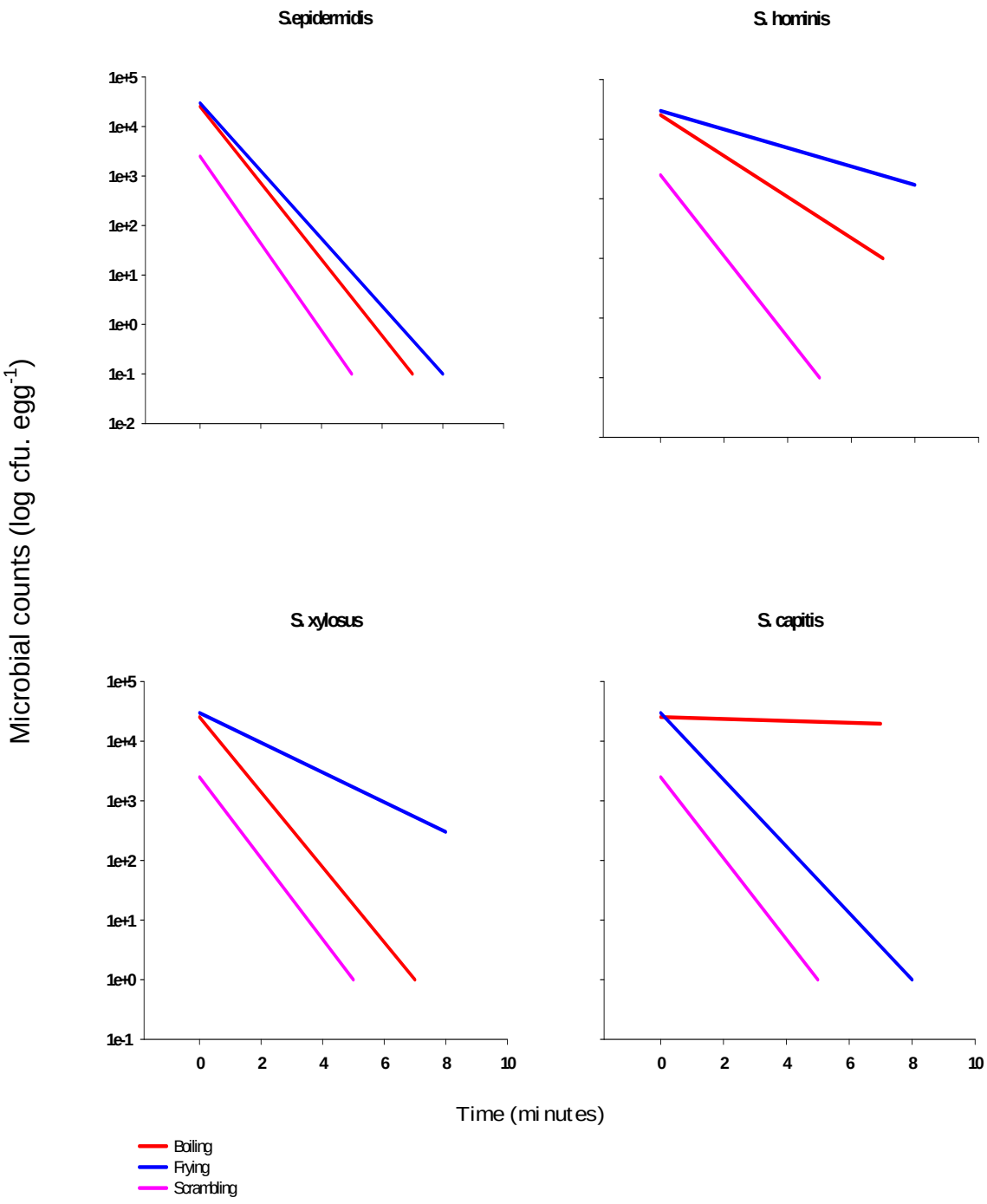

Figure 5.1 The decrease of various *Staphylococcus* spp. subjected to different cooking methods

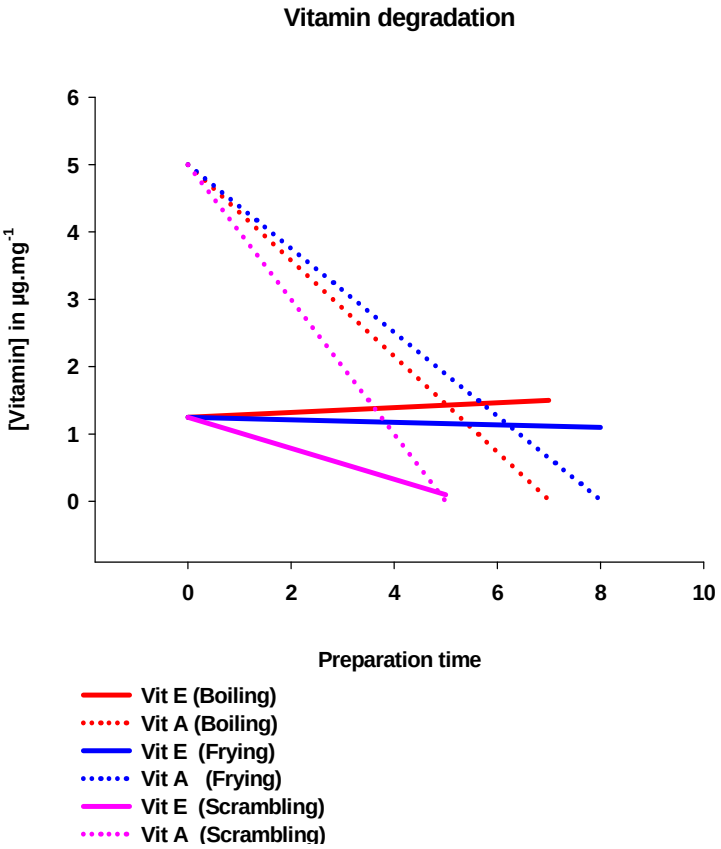

Figure 5.2 The degradation of vitamins A and E subjected to commonly used preparation methods

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APPENDIX A

THE KEEPING OF CHICKEN NESTS, COLLECTION AND PREPARATION OF EGGS

THE KEEPING OF CHICKEN NESTS, COLLECTION AND PREPARATION OF EGGS

INTRODUCTION

A. Please read the questions carefully before answering.

B. Your answers to the questions in this questionnaire will be regarded as strictly confidential.

C. Answer all questions by ticking the appropriate block.

PERSONAL INFORMATION OF THE INTERVIEWEE **For Office Use**

Household number ☐☐ 1-2

Position in the household

Mother	1	☐ 3
Grandmother	2	
Other	3	

Marital status

Single	1	☐ 4
Married	2	
Divorced	3	
Widow/Widower	4	

Gender

Female	1	☐ 5
Male	2	

Age

20-30	1	☐ 6
31-40	2	
41-50	3	
50 and above	4	

Educational level

None	1	☐ 7
Primary	2	
Secondary	3	
Tertiary	4	

CHICKEN KEEPING AND NEST HYGIENE

How many hens [1] and how many roosters [2] do you have? [ ][ ] 8-9 [ ][ ] 10-11

Where do you keep your chickens?

In a cage	1	[ ] 12
They walk around in a confined area	2	[ ] 13
They walk around everywhere	3	[ ] 14

If kept in a cage, how long are they kept in for?

24 hours a day	1	
During night time only	2	[ ] 15
24 hours, but not every day	3	

What do you feed your chickens?

Mealies	1	[ ] 16
Wheat	2	[ ] 17
Other**,** specify……	3	[ ] 18

If kept in a cage, how often do you clean the cage?

Once a day	1	
Once in three days	2	[ ] 19
Once a week	3	
Once a month	4	

How often do you change water and feed for the chickens?

Once a day	1	
Twice a day	2	[ ] 20
Once in two days	3	
Other, specify……	4	

Do you think the water and feed containers should be constructed in a specific way?

Yes	1	[ ] 21
No	2	

If yes, the construction should be in a way that

It minimises dirt	1		
Just to allow the chickens to drink with ease	2		22
Both	3		

Do you ever find dead chickens?

Yes	1		23
No	2		

If yes, how often

Once in a month	1		
Twice in a month	2		24
Once in months	3		
Once in a year	4		

COLLECTION OF EGGS

Do you wash your hands before and after collecting eggs?

Yes	1		25
No	2		

If yes, how do you wash them?

With cold water and disinfectant soap	1		
With hot water and disinfectant soap	2		26
With running tap water only	3		
Other, specify…….....	4		

How often do you collect eggs from the nests

Once a day	1		
Twice a day	2		27
Once a week	3		
Other, specify……	4		

What is the colour of the eggs that you usually collect?

White	1		
Brown	2		28
Other, specify……	3		

What is the size of the eggs that you usually collect?

Small	1	
Medium	2	29
Large	3	

Do you think the eggs are dirty?

Yes	1	
No	2	30

If yes, due to what?

Dust	1	31
Faeces in the nest	2	32
Chicken feathers	3	33

How do you collect the eggs from the nests?

Collect clean eggs first	1	34
Collect both clean and soiled eggs at once	2	

Do you use separate buckets for clean and soiled eggs during collection?

Always	1	
Sometimes	2	35
Never	3	

Do you wash dirty or soiled eggs?

Yes	1	36
No	2	

If yes, how do you usually wash them?

With cold water without disinfectant soap	1	
With cold water with disinfectant soap	2	37
With luke-warm water without disinfectant soap	3	
With luke-warm water with disinfectant soap	4	
Other (specify)……	5	

……

Do you find broken or cracked eggs in the nest?

Yes	1	38
No	2	

If yes, how often?

Every day	1	
Once in three days	2	39
Twice a week	3	

What do you do with broken or cracked eggs?

Use	1	40
Discard	2	

Do you get rotten eggs from the nests?

Yes	1	41
No	2	

If yes, how often

Once a week	1	
Once a month	2	42
Once every six months	3	

STORAGE OF EGGS

Do you store eggs after collection?

Yes	1	43
No	2	
Sometimes	3	

If yes, where do you mainly store them?

In the refrigerator	1	
In a cupboard	2	44
On the table next to the window	3	
Under the table or cupboard	4	
In an egg carton in the cupboard	5	
Other, specify	6	

If stored in the refrigerator, in which part?

Coldest part of the refrigerator	1	45
Built-in egg racks on the refrigerator door	2	

How do you store your eggs?

In an uncovered egg carton	1	46
Covered in a bucket	2	
Uncovered in a bucket	3	

Are eggs moist or wet when removed from the refrigerator?

Yes	1	47
No	2	

How long do you usually store eggs before consuming them?

One day	1	
Two days	2	48
More than three days	3	

EGG PREPARATION

Do you know that eggs are nutritious?

Yes	1	49
No	2	

Do you drink or eat raw egg yolk?

Yes		
No	1	
If yes, why?……..	2	50
……..		

How many eggs do you consume per day?

One	1	
Two	2	51
Three	3	
More, specify……	4	

Who eats most eggs in the house?

The children	1	52
The elderly	2	

How do you prepare the eggs?

Scrambled	1	53
Fried	2	54
Boiled	3	55
In baking	4	56

What do you use to fry the eggs?

Cooking oil	1	57
Butter	2	58
Both	3	59
If other, specify…….	4	60

What type of stove do you use to cook the eggs on?

Gas stove	1	
Electric stove	2	61
Paraffin stove	3	

How do you prefer to consume them?

With the yolk solid and cooked	1	62
With the yolk not solid and still flowing	2	

Have you found any blood spots in the eggs during preparation?

Yes	1	63
No	2	

Do you eat the eggs immediately after cooking?

Yes	1	64
No	2	

If no, where do you store them?

In the refrigerator	1	65
In a closed container at room temperature	2	
In a closed container in the refrigerator	3	
If other, specify……	4	

If you refrigerate them, for how long do you refrigerate?

A few hours	1	66
One day	2	
Two days	3	
Three to four days	4	

What do you do with cooked leftover egg or egg dishes?

Refrigerate and eat them later	1	67
Discard them	2	
Finish all the cooked eggs	3	

Have you ever felt ill after consumption of eggs?

Yes	1	68
No	2	

Do you wash the working surfaces and equipment after contact with raw egg?

Yes	1	69
No	2	

Do you sell eggs?

Yes	1	70
No	2	

Do you buy additional eggs for household purposes?

Yes	1	71
No	2	

Do you think backyard eggs and eggs from the shop taste the same?

Yes	1	72
No	2	73
If no, specify……		

……

THANK YOU FOR YOUR CO-OPERATION