Preparation of dry egg powder from commercially available chicken eggs and assessment of their nutritive value

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Roll No.: 0119/02

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Biochemistry

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June 2020

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have made

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DEDICATED TO MY
RESPECTED AND BELOVED
FAMILY AND TEACHERS

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The author

June 2020

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List of Abbreviations

%	Percentage
ANOVA	Analysis of variance
CHO	Carbohydrate
CP	Crude protein
CVASU	Chattogram Veterinary and Animal Sciences University
et al	Et alii/ et aliae/ et alia
etc	Et cetera
FAO	Food and Agricultural Organization
HCl	Hydrochloric Acid
HNO_3	Nitric Acid
mg	Mili Gram
SPSS	Statistical Package for Social Science
MS	Master of Science
gm	Gram

Abstract

Egg plays a pivotal role in fulfilling human beings' daily nutrient requirements. Chicken egg is one of the most versatile foods. It is a rich source of protein that is of high biological value. However, due to its fragile nature and perishable characteristic the chicken egg is required to be preserved. Dehydration is a good process of food preservation. The aim of the present study was to measure the physical characteristics and nutritive value of different chicken egg powder such as Brown egg and White egg. Eggs were collected from the local kitchen markets, dried and subjected to nutritional assessment. Descriptive analysis including percentage, mean, standard deviation and independent sample t-test were performed to find out the label of significance at $p<0.05$. Brown egg had both higher weights than white egg. On the other hand, brown eggs contained higher in albumen content. In case of nutritional components of albumen, brown egg contained higher protein (74.2 %) than white egg (73.6%). On the other hand yolk of white egg contained higher protein (31.4%) than brown egg (31.2%). Moreover, the fat content in albumen part ranges from (0.16% - 0.42%) whereas the yolk portion had (47.22% to 49.2%) in both brown and white egg. The albumen of the different poultry eggs was contained higher moisture than yolk. Brown egg albumen had higher calcium (611.8mg/100gm), Phosphorus (290mg/100gm), Zinc (4.65mg/100gm) and Potassium (240mg/100gm) compared to white egg. Conversely, in yolk of white egg also had higher calcium (645 mg/100gm), magnesium (146mg/100gm), Iron (4.49mg/100gm), Potassium (254mg/100gm) and manganese (0.187mg/100gm). The dried eggs of poultry contained considerable amount of Vitamin A, E and B_2 .White egg had higher vitamin E (8.16 IU/100gm) and vitamin B_2 (2.52 mg/100gm) than brown egg. White egg contained higher cholesterol (704.68 mg/100gm) and triglycerides (7148.56 mg/100gm) than brown egg. On the other hand, brown egg contained higher amount of high density lipoprotein (HDL) (21.58 mg/100gm) than white egg.

Keywords: Egg powder, Egg albumen, Egg yolk, Brown egg, White egg, Nutritional composition

Chapter I: Introduction

Egg is an encapsulated source of macro and micronutrients for infants and adults. It contains a wide range of biologically allure components and an ideal source of balanced and diversified nutrients. Chicken eggs are one of the most versatile foods, which contain high quality proteins, carbohydrates, easily digestible fats, vitamins and minerals (Huopalahti, 2007). It represents the least cost animal protein source, essential lipids, fat and water soluble vitamin like A, D, E, vitamin B_{12} (cyanocobalamin), riboflavin, choline, trace elements like zinc, calcium, iron and moderate calorie source (about 140kcal/100gm) (Rehault *et al*., 2018). For growing children and teenagers, egg contributes significantly to the body nutrients demand (Stadelman and Cotterill, 1995).

Egg white is valued for its high nutritional value, particularly the protein content. Liquid egg white and egg white powder have been extensively used in the food industry, for example, in baking, dressing, confectionary and meat products due to their functional properties of gelation, foaming capacity, emulsifying agents, water holding capacity and flavor. Egg white can create a fluffy, light baked product with good volume and texture in cake mixture. Moreover, egg white has the gel capability and is widely used as a binding agent in many different prepared foods. Accordingly, it is a key ingredient in several bakery products (Arzeni *et al*, 2012), and are very good potential sources of raw materials for the pharmaceutical and cosmetic industries. In recent years several different methods and systems for producing eggs have evolved. These systems vary in how the birds are housed, fed and managed. Layer nutrition and husbandry system significantly influences the sensory characteristics and the chemical composition of eggs (Ternes & Leitsch, 1997).

Due to their bulkiness, fragility, and vulnerability to the surrounding environment as their perishable nature, thus fresh raw eggs are difficult to transport (Jay, 2000). During transportation of fresh raw egg considerable loss of 2.5% occurs due to breakage (Jayaraman *et al.,* 1976). Day by day the commercial egg production has been increasing in Bangladesh, however, the price of eggs fluctuates very often, which is an obstacle in the growth of poultry industry. The producers face problems in preserving the raw eggs. As a result, the wastage of eggs causes huge economic loss. Therefore, eggs have to be utilized

to greater extent possible to reduce wastages and to protect price structure. Because of the increased production and the disadvantages in the storage of whole egg, there is a need to preserve the egg for domestic consumption and to promote export (Rao *et al.,* 1995).

Recently, the use of shell egg in food production, as the raw material has reduced with the technological developments around the world food industry, and egg products such as frozen egg, pasteurized liquid egg and dried egg products have gained popularity. Dried egg powder (egg white and egg yolk) is widely used in food preparation because it is microbiologically safe and easy to transport compared to unshelled or liquid eggs. Dried egg powder is also comparatively easier to store, handle, measure and obtain uniformity, extended shelf life of the product (Caboni *et al.,* 2005). Prasad *et al* (2004) suggested that biscuits enriched with spray-dried egg powder can form a good substitute instead of shell eggs for armed personnel stationed in remote areas. Further, the product may find use as protein-rich biscuits for infants and children. Moreover, spray dried egg powder has great importance for food industry with respect to handling and hygienic aspects. Spray drying allows preparation of stable and functional powder products (Koc *et al*., 2010; Liu and Liu, 2009; Sagar and Suresh, 2010) and can be implemented for large scale throughputs (Chávez and Ledeboer, 2007; Youssefi *et al*., 2009). The properties of eggs are very delicate, and the final quality of the dried product can be significantly affected by drying conditions (Koç *et al.,* 2011). For egg powder production, most of the researches in the literature were related with the method of spray drying and process conditions (Caboni *et al*., 2005; Lechevalier *et al*., 2007). Franke and Kiebling (2002) studied the effect of inlet air temperature and nozzle pressure of spray dryer on functional properties of egg powder and found that protein solubility and foaming power decreased as the inlet air temperature increased.

Some recent studies shows that egg plays a beneficial role for humans, including physically active people and some authors demonstrated that egg cholesterol was not well absorbed that's why does not get significantly impact on blood cholesterol concentration (Rehault *et al*., 2018). Therefore, the people are getting to be aware with respect to the wellbeing and they are more interested to consume distinctive parts of eggs independently. For illustration, a few people groups require as it were egg whites and other needs yolk. To

fulfill their necessities, it's essential to create powder shaped of eggs. However, So far we gather knowledge about the preparation of egg powder and their dietary evaluation was not done in the reference of Bangladesh. Another critical point is that, the individuals express their opinion regarding diverse sorts of poultry eggs. But, there are restricted data with respect to the nutrient levels among different types of poultry eggs.

Dried egg powder is very much useful because it possesses different kinds of nutrients abundantly such as calcium, calories, protein and minerals. The calcium of powdered eggs is necessary for the proper development of the teeth and bones. The calories content is low so that it is a healthy alternative for weight loss. The protein is high in powdered egg; the minerals content are potassium and sodium, which are important and essential for muscle and nerve cells. Furthermore, powdered egg has a longer shelf life. And it is not necessary to store the eggs in a refrigerated area. It is required to preserve at cool to moderate temperature and a dark cabinet or cupboard. Apart from these, eggs that have been dried require much less storage space, so substantial supplies are possible in already small space. The eggs can be added to baked recipes in powdered form without the need to mix them with a liquid ahead of time. More to the point, powdered eggs are generally fairly cheap too. Purchasing powdered eggs costs significantly less then purchasing an equivalent number of fresh eggs. Because of this superiority, they are widely used by food producers as well as restaurants, often for making dishes and baking goods. Finally, during the drying process micro-organism is being killed, which is very much beneficial for long time storage and is safe to eat. The consumer will also use powder eggs in dough preparation of making food without any fear of bacteria.

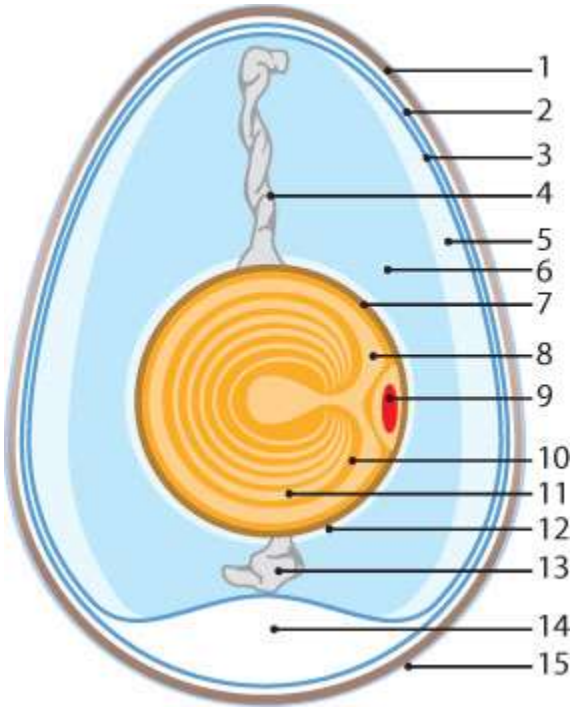
Aims and objectives

- To assess the weight of different parts of eggs at raw and dried condition.
- To produce egg albumen and egg yolk powder of different types of chicken eggs.
- To determine and compare the proximate components of albumen and yolk powder.
- To determine and compare the minerals both albumin and yolk.
- To evaluate the vitamins and lipid profiles of dried yolk powder.

Chapter II: Review of Literature

Eggs are a 'complete protein', meaning they contain all the essential amino acids needed for healthy body functions. The content of the hard-shelled produced by a bird, considered as food. Though the main role of the egg is to reproduce the species, most eggs laid by domestic fowl, excluding those specifically set aside for hatching, are not fertilized but are sold mainly for human consumption. Eggs come from chickens, ducks, geese, turkeys, guinea fowl, pigeons, pheasants, and quail. For their weight, eggs provide the highest quality protein of all foods. This protein is highly digestible, which helps weight management

2.1 Egg Composition and Formation - Anatomy & Physiology



1. Eggshell
2. Outer membrane
3. Inner membrane
4. Chalaza
5. Exterior albumen (outer thin albumen)
6. Middle albumen (inner thick albumen)
7. Vitelline membrane
8. Nucleus
9. Germinal disk (blastoderm)
10. Yellow yolk
11. White yolk
12. Internal albumen
13. Chalaza
14. Air cell
15. Cuticle

Figure 2.1: Composition of egg

Size

- Varies significantly between species.

Shell

The shell provides physical protection. It protects the embryo from microorganisms and regulates evaporation so that the embryo does not become desiccated. The shell is also a source of calcium carbonate, which is essential for bone formation in the embryo.

Shell membranes

- They were organized into an inner and outer layer which lie in close apposition.
- Part at one end forms the air cell, where the hatching chick draws its first breath.
- Meshwork of protein fibers (protein and glycoprotein) with minor collagen, hydroxyproline and hydroxylysine.
- Prevent desiccation and infection.

Testa

- The main thickness of the shell
- Matrix fibers and calcium carbonate
- Calcium carbonate layers contain pores to permit gas exchange.
- Pigmented by porphyrin and biliverdin

Cuticle

- Water repellent
- Barrier to infection

Yolk

- Protein and lipids are synthesized in the liver and travel to the oocyte in the ovary, where they are made into yolk (vitellogenesis).
- Principle stored nutrients provides primary nutrient for the embryo.
- Produces water and energy, allowing survival in arid environments.

- Thick and viscous
- Contains maternal antibodies, primarily IgG.

Albumen

- Less dense than yolk
- Composed mainly of water and protein.
- Buffers the embryo from sudden temperature changes.
- It serves as a shock-absorber to protect the embryo.
- A thin layer encloses the yolk membranes and suspends the yolk in the center of the egg by twisted strands called Chalazae.

Germinal Disc

- Small, circular, white spot on the surface of the yolk.
- It contains cytoplasm and the oocyte

Embryonic Membranes

Within the egg, three extra embryonic membranes support the life and growth of the embryo:

Amnion

- Surrounds only the embryo
- An inner layer of cells secretes amniotic fluid in which the embryo floats.
- The fluid keeps the embryo from drying out and provides protection.

Chorion

- It surrounds all embryonic structures and serves as a protective membrane (chorioallantoic membrane).
- Also important for:
    - Transpiration
    - Metabolism
    - Waste collection
    - Calcium transfers from the shell to the embryo.

Allantois (or allantoic sac)

1. Grows larger as an embryo grows
2. It fuses with the chorion and is called the chorio-allantoic membrane.
3. Works together with chorion to permit respiration (exchange of oxygen and carbon dioxide) and excretion.
4. Important in the storage of nitrogenous wastes (uric acid).

2.2 Nutrient composition of eggs:

Egg proteins are distributed similarly between the egg white (a) and egg yolk (b), whereas lipids, vitamins, and minerals are basically concentrated in egg yolk (Figure 2.2). Water constitutes the central part of egg (Figure 2.2) and, notably, the egg is devoid of fiber. The relative substance of egg minerals, vitamins, or specific fatty acids may change from one national reference to another but remains all-inclusive comparable when considering significant constituents such as water, proteins, lipids, and carbohydrates. In a whole, raw, and freshly laid egg, water, protein, fat, carbohydrates, and ash represent almost 76.1%, 12.6%, 9.5%, 0.7%, and 1.1%, respectively. (USDA National Nutrient Database, 2018)

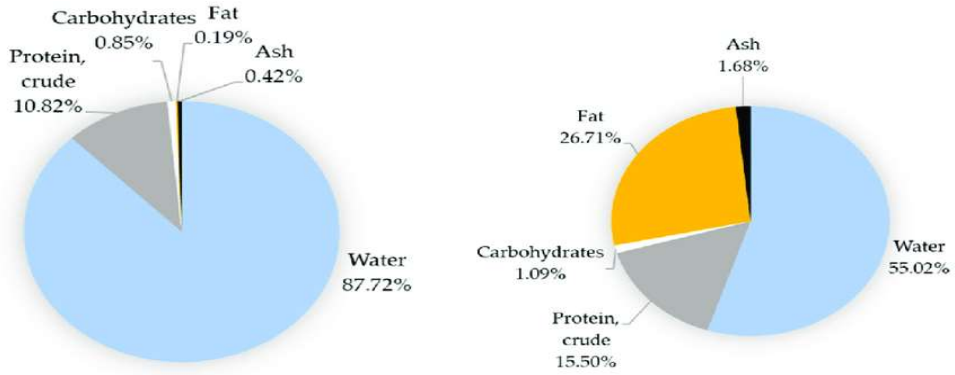


Figure 2.2: **Basic composition of edible parts of the egg. (a) Egg white (b) Egg yolk**

Retrieved on 01/11/2019 from the Ciqual homepage https://ciqual.anses.fr/ (French Agency of food environmental and Occupational Health & Safety. ANSES-CIQUAL).

2.2.1 Macronutrients

2.2.1.1 Proteins

Egg white and egg yolk are highly concentrated in proteins. Egg yolk has a hepatic origin essentially, while the egg white is synthesized and secreted after ovulation of the mature yolk in the hen's oviduct (Nys *et al.,* 2011).

The concentration of proteins is, on average, 12.5 g per 100 g of entirety raw fresh egg, whereas egg yolk with its vitelline layer and egg white contain 15.9 g protein and 10.90 g protein per 100 g, respectively. These values are marginally altered by hen genetics and age. About 1000 different proteins have been identified in the chicken egg, counting the eggshell (Mann, 2007) (Gautron *et al.,* 2007).

The yolk may be a complex milieu containing 68% low-density lipoproteins (LDL), 16% high-density lipoproteins (HDLs), 10% livetins and other soluble proteins, and 4% phosvitins. Apolipoprotein B, apovitellenin-1, vitellogenins, serum albumin, immunoglobulins, ovalbumin, and ovotransferrin are the most abundant proteins of egg yolk, representing more than 80% of add up to egg-yolk proteins (Rehault *et al.,* 2018). The yolk is firmly associated with the vitelline membranes, which comprise two particular layers (Bellairs *et al.,* 1963) that frame an extracellular protein matrix embracing the yolk. The egg white may be a gel-like structure that lacks lipids and is composed of basically water (about 88%) (Bellairs *et al.,* 1963) (Figure 2.2), fibrous structural proteins (ovomucins), glycoproteins (ovalbumin, protease inhibitors), antibacterial proteins (lysozyme), and peptides (Mann, 2008) (Back *et al.,* 1982) . The average volume of egg white is evaluated to be 30 mL (for an egg weighting 60 g, eggshell included), and Nutrients 2019, 11, 684 4 of 26 protein concentration is almost 110 mg/mL of egg white. In total, 150 particular proteins have been identified in egg white (Gautron *et al.,* 2007).

Egg white is concentrated in antibacterial lysozyme that's right now utilized as an anti-infectious operator in many pharmaceuticals and as a food preservative. The viscous aspect of egg white is essentially due to ovomucin (Howthrone, 1950). Remarkably, egg white is additionally characterized by four highly abundant protease inhibitors (Saxena, 1997) that will delay the absorption of egg components, mainly when egg white is utilized as a raw ingredient in a few food preparations.

2.2.1.2 Lipids

The overall lipid substance is generally stable within the egg ranging from 8.7 to 11.2 per 100 g of whole egg when considering different EU countries and USA egg composition tables (Seuss-Baum and Nau, 2011). These lipids are only concentrated within the egg yolk (Figure 2.2) and a small portion may stay firmly associated with vitelline films (Trziska *et al.*, 1982) (Shinn *et al*., 2016). Lipids are a portion of yolk lipoproteins whose structure comprises of a core of triglycerides and cholesteryl esters, encompassed by a monolayer of phospholipids and cholesterol in which apoproteins are inserted (Anton *et al.,* 1997). An increase of fat in eggs basically depends on the increase of yolk-to-egg-white proportion. The relative sum of unsaturated (monounsaturated + polyunsaturated) to immersed fatty acids in yolk (5.31 g versus 2.64 g per 100g of whole egg, Table 2.1) is highly compared to other animal-derived food sources. The amount of cholesterol in eggs is 400mg per 100gm of whole egg.

Name	Egg whole, Raw			Egg yolk, Raw		
	Average Content	Min Value	Max Value	Average Content	Min Value	Max Value
FA saturated	2.64	0.05	3.13	8.47	7.13	9.55
FA 4:0	<0.05	0	-	0	-	-
FA 6:0	<0.05	0	-	0	-	-
FA 8:0	<0.05	0	-	0.009	-	-
FA 10:0	<0.05	0	-	0.009	-	-
FA 12:0	<0.05	0	-	0.009	-	-
FA 14:0	0.024	0	0.038	0.091	0.077	0.1
FA 16:0	1.96	0.05	2.43	6.04	5.03	6.86
FA 18:0	0.65	0.05	0.89	1.73	-	2.42
FA monounsaturated	3.66	0.05	6.73	11.9	10.2	13.8
FA 18:1 n-9 cis	3.51	3.03	3.65	10.4	9.69	11.2
FA polyunsaturated	1.69	0.05	3.39	4.07	3.33	4.66

FA 18:2 9c, 12c (n-6)	1.38	1.18	2.7	3.28	-	3.62
FA 18:3 9c,12c,15c (n-3)	0.061	0.02	0.58	0.15	-	0.27
FA 20:4 5c,8c,11c,14c (n-6)	0.12	-	0.13	0.37	-	0.4
FA 20:5 5c,8c,11c,14c,17c (n-3) EPZ	0	-	0.003	0.01	-	0.011
FA 22:6 4c,7c,10c,13c,16c,19c (n-3) DHA	0.09	0.045	0.18	0.25	0.11	0.46
Cholesterol	0.398	0.344	0.423	0.939	-	1.280

Table 2. 1 Egg lipids

Retrieved on 01/17/2019 from the Ciqual homepage https://ciqual.anses.fr/ (French Agency for Food, Environmental and Occupational Health & Safety.

2.2.1.3 Carbohydrates

Egg does not contain any fibers, and its content in carbohydrates is low (0.7%). Egg carbohydrates are distributed between egg yolk and egg white (Figure 2). Glucose is the dominant free sugar in the egg (about 0.37 g per 100 g of whole egg) and is essentially present in egg white (0.34 g per 100 g of egg white versus 0.18 g per 100 g of egg yolk) (USDA National Nutrient Database for standard, 2018). Trace amounts of fructose, lactose, maltose and galactose have been detected in raw egg white and raw egg yolk (USDA National Nutrient Database for standard, 2018).

2.2.2 Micronutrients

2.2.2.1 Vitamins and choline

Egg yolk is a vitamin-rich food that contains all vitamins except vitamin C (ascorbic acid). The absence of vitamin C in the egg may result from the fact that birds can satisfy their vitamin C requirements by de novo synthesis from glucose. (Chatterjee *et al*., 1973)

The egg yolk contains a high amount of vitamin A, D, E, K, B_1, B_2, B_5, B_6, B_9, and B_{12}, while egg white possesses high amounts of vitamins B_2, B_3, and B_5 but also significant

amounts of vitamins B_1, B_6, B_8, B_9, and B_{12} (Table 2.2). Eating two eggs per day covers 10% to 30% of the vitamin requirements for humans. Eggs represent a major source of choline, which is essentially concentrated in the yolk (680 mg/100 g in the egg yolk versus 1 mg/100 g in the egg white) (USDA National Nutrient Database for standard, 2018).

It has essential and diverse functions in cellular maintenance and growth across all life stages. It plays some roles in neurotransmission, brain development, and bone integrity (Wiedeman *et al.,* 2018)

Name	**Egg whole, Raw**	**Egg yolk, Raw**	**Egg white, Raw**
Vitamin A or Retinol	160	371	0
Vitamin A precursor or Beta-carotene	0	88	0
Vitamin D or Cholecalciferol	2.0	5.4	0
Vitamin E or Alpha-tocopherol	1050	2580	0
Vitamin K or Phylloquinone	0.3	0.7	0
Vitamin C	0	0	0
Vitamin B_1 or Thiamin	40	176	4
Vitamin B_2 or Riboflavin	457	528	439
Vitamin B_3 or Niacin	75	24	105
Vitamin B_5 or Pantothenic acid	1533	2990	190
Vitamin B_6	170	350	5
Vitamin B_8 or Biotin	16.5–53.8 [2]	27.2–49.4 [2]	5.7–7.9 [2]
Vitamin B_9 or Folate	47	146	4
Vitamin B_{12} or Cobalamin	0.89	1.95	0.09

Table 2. 2 Egg vitamins

Retrieved on 01/17/2019 from Department of Agriculture, Agricultural Research Service (2014). USDA National Nutrient Database for Standard Reference, Release 27. http://www.ars.usda.gov/ba/bhnrc/ndl 2

2.2.2.2 Minerals and Trace Elements

Egg is rich in phosphorus, calcium, potassium and contains moderate sodium (142 mg per 100 g of whole egg) (Table 2.3). It also includes all essential trace elements including copper, iron, magnesium, manganese, selenium, and zinc (Table 2.3), with egg yolk being the major contributor to iron and zinc supply. The presence of such minerals and micronutrients in an egg is quite interesting as a deficiency in some of these (Zn, Mg, and Se) has been associated with depression and fatigue (Wang *et al.,* 2018)

Name	Egg whole, Raw	Egg yolk, Raw	Egg white, Raw
Calcium	56	129	7
Copper	0.072	0.077	0.023
Iodine	0.021	0.18	0.002
Iron	1.75	2.73	0.08
Magnesium	12	5	11
Manganese	0.028	0.055	0.011
Phosphorus	198	390	15
Potassium	138	109	163
Selenium	0.030	0.056	0.02
Sodium	142	48	166
Zinc	1.29	2.30	0.03

Table 2. 3 Egg minerals and trace elements (average content; mg/100g)

Retrieved on 01/17/2019 from Department of Agriculture, Agricultural Research Service (2014). USDA National Nutrient Database for Standard Reference, Release 27 http://www.ars.usda.gov/ba/bhnrc/ndl and from the Ciqual homepage https://ciqual.anses.fr/ (French Agency for Food, Environmental and Occupational Health & Safety. ANSES-CIQUAL) for iodine content.

2.3 Antinutritional Factors

Major proteins of the egg include protease inhibitors that may delay the proper degradation of egg proteins by inhibiting digestive enzymes, including pepsin, trypsin, and chymotrypsin. Indeed, egg white is a significant source of ovostatin, ovomucoid, ovoinhibitor, and cystatin (Back *et al.,* 1982). Moreover, some of these molecules (ovoinhibitor, ovomucoid, cystatin) possess many disulfide bonds that are likely to confer

moderate resistance to denaturation by proteases and gastric juices. Some of these antinutritional factors may be partly denatured by heat (Evenepoel *et al.,* 1998) during the process of cooking, thus facilitating protein access to digestive proteases.

2.4 Egg Nutraceuticals

The egg is not solely a primary food of high nutritional value but it also contains many bioactive compounds (lipids, vitamins, proteins, and derived hydrolytic peptides) (Kovacs-Nolan *et al.,* 2005) of significant interest for human health. Egg proteins display a broad spectrum of antimicrobial activities that could contribute to intestine health. Many efforts have been made in the last decades to characterize further biological activities of egg-derived hydrolytic peptides that may naturally occur during the digestive process (Evenepoel *et al.,* 1998). Interestingly, some of these bioactive peptides are specifically generated after limited proteolysis of denatured egg proteins (Vilcacundo *et al.,* 2018), after boiling.

2.5 Antimicrobials

Egg antimicrobials in edible parts are essentially concentrated in egg white and the vitelline membrane. Depending on the protein considered, these antimicrobials may exhibit antibacterial, antiviral, antifungal, or antiparasitic activities. Their antibacterial effect relies on several bactericidal or bacteriostatic mechanisms.

2.6 Antioxidant Activities

Long-term oxidative stress in the gastrointestinal tract can lead to chronic intestinal disorders. There is increasing interest in investigating the potential of food-derived antioxidants, including egg antioxidants, in intestinal health. Chicken egg contains many antioxidant compounds that encompass vitamins, carotenoids, minerals, and trace elements but also major egg-white proteins (Abeyrathne *et al.,* 2018) such as ovotransferrin, in its native form or as hydrolytic peptides (Abeyrathne *et al.,* 2018), ovomucoid and ovomucoid hydrolysates (Chang *et al.,* 2013), ovomucin hydrolysates and derived peptides (Chang *et al.,* 2013), and egg yolk-proteins including phosvitin (Yousr *et al.,* 2015). The authors

concluded that supplementation of the diet with egg yolk-proteins might be a novel strategy to reduce intestinal oxidative stress (Young *et al.,* 2010*)*.

2.7 Anti-Cancerous Molecules

Food-derived proteins and peptides can also be beneficial to prevent and to cure cancer diseases (Hernandez-Ledesma, 2017). The anticancerous effect of egg tripeptides (Liao *et al.,* 2019) and hydrolytic peptides from ovotransferrin (Ibrahim *et al.,* 2009) have also been published. Information in this field is relatively scarce, but it may be worth investigating such activities. Some interesting data may arise from studies on egg protease inhibitors (Saxena *et al.,* 1997) since similar molecules existing in other food product, including legumes like pea, have been described as potential colorectal chemopreventive agents (Clement *et al*., 2014).

2.8 Immunomodulatory Activities

Several egg proteins have potential immunomodulatory activities. Among these, egg-white lysozyme is a promising agent for the treatment of inflammatory bowel disease. The biological significance of the possible immunomodulatory activity of egg white pleiotrophin in human intestine remains very speculative. In contrast, some valuable immunomodulatory activities might emerge from ovotransferrin and egg yolk vitellogenin hydrolysates (Liu *et al.,* 2017) after partial degradation by digestive proteases.

2.9 Antihypertensive Activities

Considering the prevalence and importance of hypertension worldwide (over 1.2 billion individuals) (Kearney *et al.,* 2004), there is increasing ongoing research to find ways to regulate this multifactorial disease. At the population level, the most essential factors of long-term blood pressure control are sodium and potassium intake and the importance of the renin-angiotensin-aldosterone system. Most egg-derived peptides with anti-hypertensive activities exhibit inhibitory activities against the angiotensin-converting enzyme (ACE). This enzyme triggers the processing and activation of angiotensin I into the active vasoconstrictor angiotensin II. Some of these peptides contain only three amino acids (Liao *et al.,* 2016). Some of these tripeptides were demonstrated to be active in vivo—

the oral administration of these peptides that have been administrated orally in hypertensive rats contributed to significantly reducing blood pressure (Majumder *et al.,* 2015) and, thus, may help in diminishing the occurrence of cardiovascular diseases (Liao *et al.,* 2016).

Variability in egg weight (10.8%) among different sources can affect different parts of eggs and quality of eggs as well as the consumer desire for eggs as consumer typically prefers larger eggs (USDA, 2000).

Differences in egg weight have been linked to hen age and stain (Zita et al., 2009), dietary protein/amino acids, energy, and fat/fatty acids, housing density and condition (cages vs. floor), health status, environmental stress, and feed intake (Attia et al., 1994).

Variation in egg quality and nutritional value has a substantial impact on consumer health; welfare and a variety of other factors can influence egg quality. The breed and strain of layers (Kucukyilmaz *et al*., 2012), dietary composition (Calislar and Kirik, 2009); birds' health, environmental condition and storage, processing and handling of eggs (Ryu *et al*., 2011; Khan *et al*., 2013) are some factors which involves in egg quality and nutritional factors.

Genotype (Kucukyilmaz et al., 2012), the amount and type of dietary fats (Rezaei et al., 2008), and husbandry practice influence on egg lipids (Kucukyilmaz et al., 2012).

Minerals included in eggs, such as calcium, phosphorus, copper, iron, manganese, and zinc, are among the most important and basic microelements required for human and chicken nutrition. As a result, they are frequently added to the poultry diet (Hashish et al., 2012).

The mineral content of eggs has significant health implications, and there is increasing interest in supplementing eggs with minerals such as selenium, iron, chromium, and zinc for human health benefits (Attia et al., 2010). Furthermore, mineral concentrations in eggs and eggshells vary greatly depending on the dose and form of the element, as well as other factors such as physiological reasons, husbandry practice, geographic location (Hashish et al., 2012), and supplemented feed additives (Giannenas et al., 2009)

2.10 Conclusion

Egg quality is affected by a vast extend of variables in expansion to bird strain and nutritional status. It is significant for keeping a high rate of eggs to get how the hens' physiology impacts egg quality in favour of positive characteristics while restricting the more negative aspects. Bird age is the primary factor, and a tremendous effort has already gone into the selection to limit age-related impacts on egg weight, egg whites quality, and shell quality.

Chapter III: Materials and Methods

The experiment was conducted at the Biochemistry Laboratory of Physiology, Pharmacology and Biochemistry Department, Food Processing and Engineering Department and Poultry Research & Training Centre of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh. It has been completed during December 2020 to August 2021 to manufacture quality egg powder and also to determine nutrient contents of it. The egg was collected from local markets of Chattogram.

3.1 Egg collection and sample preparation

3.1.1 Collection of eggs

100 eggs were randomly collected of two types (white and brown) of chicken egg from Zaotola kitchen market, Chattogram City, in December 2020. An equal number of egg samples of brown egg (50) and white egg (50) were purchased. Then eggs were carefully carried from the local market to the Biochemistry lab of CVASU.

3.1.2 Samples preparation for nutritional composition analysis

The collected eggs were cleaned with clothes for further processing. Then whole weight of egg was measured. The eggs were washed by water spray and dried at room temperature to remove surface moisture. The eggs were carefully broken and separated egg yolk and egg albumen and egg liquid were inspected visually for any spoilage. To optimize the drying time, the egg liquid foam was dried using a cabinet drier at 60˚C. After drying, the samples were blended and sieved to get fine egg yolk and egg albumen powder. The process of egg powder preparation is summarized in flow chart as follow:

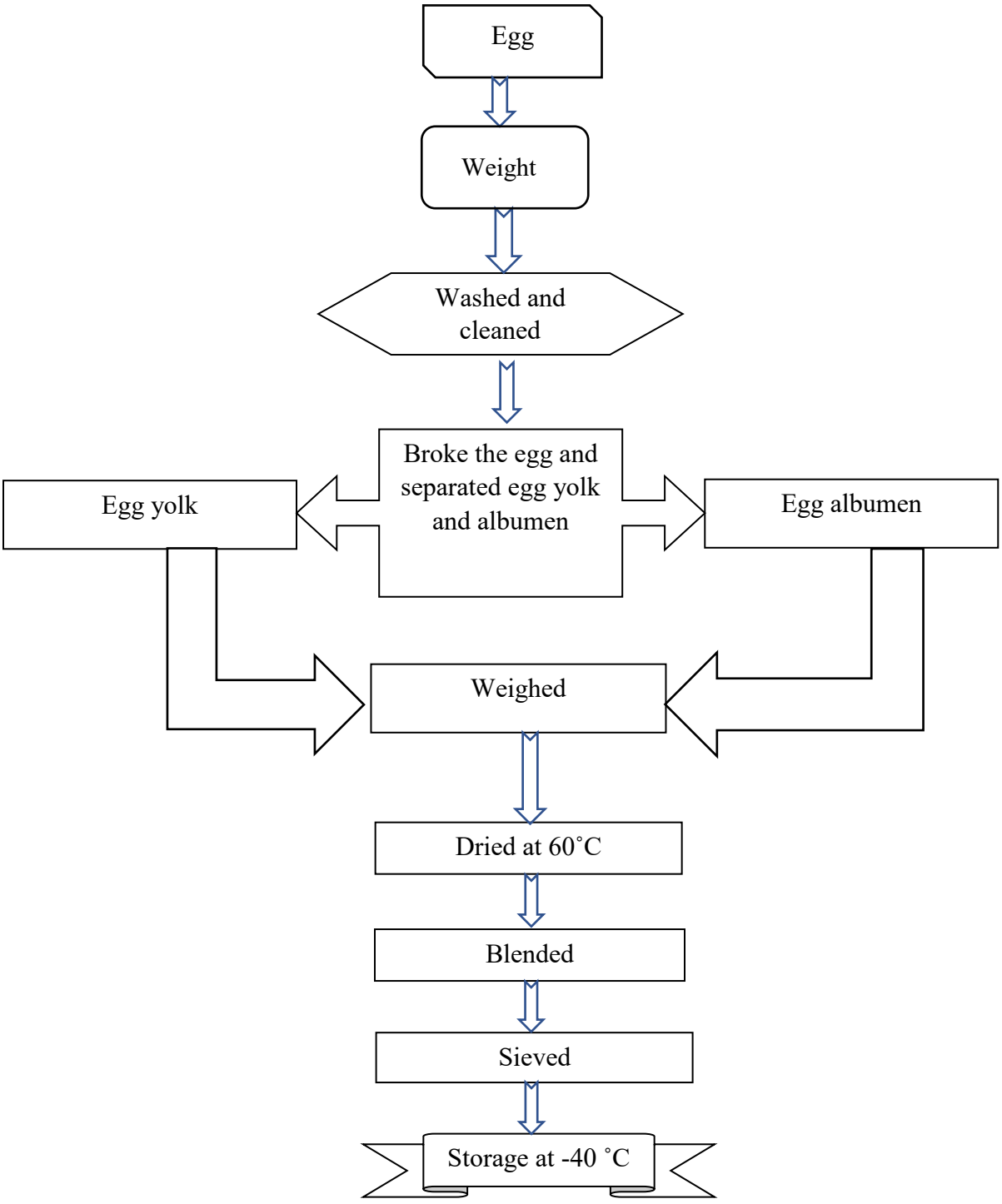


Figure 3. 1: Schematic diagram of egg powder production

3.2 Chemical analysis of egg powder

Egg samples were analyzed for Moisture content (MC), Ash content (AC), Proteins, Fats, Crude fibre and total Carbohydrates. All determinations were done in triplicate and the result was expressed as the average value.

3.2.1 Determination of moisture content

Apparatus: Crucible**,** hot air oven**,** desiccator**,** weighing balance

Procedure: Moisture is always present in food staffs. Estimation of moisture is done simply by heating at 104-105°C for 3-4 hours in the oven and is cooled in a desiccator to absorb moisture. The process is repeated for several times until the constant weight shows by the sample.

Calculation: The percent of moisture was calculated as follows:

$$\text{Moisture \%} = \frac{\text{Initial weight} - \text{Final weight}}{\text{sample weight}} \times 100$$

3.2.2 Determination of ash content

Apparatus: Porcelain, gas burner, muffle furnace

Procedure: The ash fraction contains all the mineral elements. This method performs oxidization of all organic matter by incineration and determines the weight of remaining ash.

At first weighed the empty crucible. About 5 gm of dried samples was ignited in the crucible with the help of a suitable burner for about an hour. Completed the ignition by keeping in a muffle furnace at 550-600ºC for about 3 hours until grey color ash was obtained. Then the crucible was taken from muffle furnace and cooled in desiccators. Later on the crucible was weighted.

Calculation: The ash content was calculated by the following expression:

$$\text{Ash \% of Sample} = \frac{\text{Amount of ash of supplied sample}}{\text{Sample wt}} \times 100$$

3.2.3 Protein determination

Apparatus: Kjeldahl flask**,** Condenser**,** Kjeldahl digestion unit

Reagent required

1. Concentrated Sulphuric acid (0.2%)
2. Digestion mixture (K_2SO_4& $CuSO_4$)
3. Boric acid solution
4. Alkali solution.
5. Mixed indicator solution
6. Standard HCl (0.2 N)

Procedure: The estimation of nitrogen is done by Kjeldhal method**.** The protein content of food stuff was obtained by estimating the nitrogen content of the material and multiplying the nitrogen factor by 6.25.

Digestion

The digestion step was done to break down the intricate structure and chemical bonds of feed substance to simple ionic structure. In digestion procedure, proteins and other forms of nitrogen were broken down and converted to ammonia.

0.3g sample was weighed accurately. Then 4g digestion mixture was added. Further 5ml of conc. H_2SO_4 was added to the mixture. After that the digestion flask was placed on Kjeldahl digestion set. After digestion removed the flask from the chamber and cooled at room temperature.

Distillation

In Distillation steps, ammonia-nitrogen was separated from the digested end product. It involved the conversion of ammonium (NH_4^+) ion to ammonia (NH_3). Distilling the ammonia, nitrogen was separated, and collected the distillate in a suitable trapping medium. Collection of ammonia is usually done by absorption into a solution of 2% boric acid. The ammonia is bound to the boric acid in the form of ammonia borate.

At first 25 ml distilled water was added. Then content was transferred to distillation flask. After that 10 ml 40% NaOH solution was added and set the condenser10 ml 2% Boric acid solution and mixed indicator were added in conical flask. Heat the distillation flask and continue up to collection of app. 100ml of distillate.

Titration

Determination of the amount of nitrogen on the condensate flask can be accomplished by several methods. The most common method is titration of the ammonia with a standard solution of N/10 HCl in the presence of mixed indicator.

The receiving solution was titrated with 0.2N HCl solution until turn into grey color. The percentage of crude protein calculated by following formula:

Calculation: Percentage of nitrogen and protein calculated by the following equation:

$$\text{Protein \%} = \frac{\text{Titration value} \times \text{Normality of HCl} \times 0.014}{\text{Sample wt}} \times 6.25 \times 100$$

3.2.4 Crude fat determination

Apparatus: Soxhlet apparatus, Thimble

Principle: Fat was determined by dissolving the sample into organic solvents (chloroform, methanol) separating the filtrate by filtration. The filtrate was placed in separating funnels and then separated mixture was dried to measure the extract and the percentage of fat was estimated.

Procedure: The dried sample was taken in a thimble and plugged the top of the thimble with a wood of fat free cotton. The thimble was dropped into the fat extraction tube attached to a Soxhlet apparatus. The anhydrous petroleum ether was poured through the sample in the tube into the flask. Top of the fat extraction tube was attached to the condenser. The sample was extracted for 16 hours or longer on a water bath at 70-80°C.

At the end of the extraction period, the thimble from the apparatus was removed and distilled of the petroleum ether by allowing it or collected in Soxhlet tube. The ether was poured off when the tube was nearly full. When the ether was reached a small volume, it was poured into a small, dry (previously weighed) beaker through a small funnel containing plug cotton. The flask was rinsed and filtered thoroughly using ether. The ether was evaporated on a steam bath at low heat, it was then dried at 100°C for 1 hour, cooled and weighed. The difference in the weights was the ether-soluble material present in the sample.

Calculation: The percent of crude fat was expressed as follows:

$$\text{Fat \% of sample} = \frac{\text{Wt of extract}}{\text{Sample wt}} \times 100$$

3.2.5 Crude Fiber Determination

Apparatus: Liebig condenser**,** Reflux condenser**,** Gooch crucible

Reagent required:

1. 0.255N sulphuric acid solution
2. 10.0% Potassium sulphate solution;
3. Asbestos- Gooch grade.

Procedure: Crude fiber is the water insoluble fraction of carbohydrate consists mainly of cellulose, hemicellulose and lignin. It was estimated through digestion of fat free known amount of food sample by boiling it in a weak solution of acid (1.25% H_2SO_4) for 30 minutes followed by boiling in weak solution of alkali (1.25% NaOH) for 30 minutes at constant volume and then deducting ash from the residue obtained.

About 2-5ml of moisture and fat free sample were weighed into 500 ml beaker and 200 ml of boiling 0.255 N (1.25%w/v) sulfuric acid is added .the mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. At the end of this period, the mixture was filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker and 200ml of boiling 0.313 N (1.25%) NAOH added. After boiling for 30 min, the mixture was filtered through muslin cloth. The residue was washed with hot water till free from alkaline followed by washing with some alcohol and other. It was then transferred to a crucible, dried overnight at 300-1000ºC and weighed. The crucible is heated in a muffle furnace at 6000ºC for 2-3 hrs. Cooled and weighed again. The difference in the weight represents the weight of crude fiber.

Calculation: The loss in weight represents crude fiber

$$\text{Crude fiber \%} = \frac{\text{Weight of residue with crucible} - \text{Weight of ash with crucible}}{\text{Sample weight}} \times 100$$

3.2.6 Determination of total carbohydrates

The carbohydrate content was determined by calculating the difference of Nitrogen Free Extractive (NFE). It was given as the difference between 100 and a sum total of the other proximate components. The formula bellow:

% CHO = 100% - % (Protein + Fat + Fibre + Ash + Moisture content).

3.3 Determination of mineral contents

This method involves the extraction of minerals from the organic food matrix by digestion through wet digestion. The mineral contents in the digested compounds was determined by spectrophotometer (Humalyzer 3000®).

Apparatus: Beaker, Measuring pipets, Volumetric flask, Analytical balance, Heating mantle or hot plate, Filter paper, Whatman® No. 541

Required reagent: Nitric acid and Perchloric acid

Procedure: One (01) g of dry sample was weighted in a conical flask. For dried samples, 7.5 ml conc. HNO_3, and 2.5 ml conc. $HClO_4$ in the ratio of 2:1 was prepared. For wet sample, 5 ml HNO_3 and 1 ml $HClO_4$ was added (HNO_3: $HClO_4$ = 5:1). Then the flask was placed in a hot plate at 200W for 1-2 hours until full digestion. After digestion, it was cooled at room temperature. Then transferred the digested samples into 100 ml volumetric flask and diluted up to 100 mark with Deionized water and mixed well. Later, the solution was filtered through Whatman® filter paper No. 1 and transfer to Eppendorf Tube for mineral quantification.

Figure 3. 2: Mineral determination

3.3.1 Determination of Sodium (Na^+)

Sodium is precipitated as a triple salt with magnesium and Uranyl acetate. The excess of uranyl ions are reacted with Ferro cyanide in an acidic medium to develop a brownish color. The intensity of the color produced is inversely proportional to sodium concentration in the sample.

Procedure:

Table 3.1: Sodium (Na^+) determination

Step 1: Precipitation

	Pipette into cuvette	
	Blank	**Standard**
Precipitating Reagent(L1)	1.0 ml	1.0 ml
Sodium Standard	20 µl	-
Sample	-	20 µl

Mix well and let stand at R.T. for 5 mins. With shaking well intermittently. Centrifuge at 2500 to 3000 RPM to obtain a clear supernatant.

Step 2: Color Development

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Acid Reagent(L2)	1.0 ml	1.0 ml	1.0 ml
Supernatant from step 1.	-	20 µl	20 µl
Precipitating Reagent(L1)	20 µl	-	-
Color Reagent(L3)	100 µl	100 µl	100 µl

Calculations:

$$\text{Sodium in mmol / L} = \frac{(A)\text{blank} - (A)\text{sample}}{(A)\text{blank} - (A)\text{standard}} \times \text{Standard conc. (mmol / L)}$$

3.3.2 Determination of Chloride Ion (Cl^-)

Principle: Chloride ions combine with free mercuric ions and release thiocyanate from mercuric thiocyanate. The thiocyanate released combines with the ferric ions to form a red

brown ferric thiocyanate complex. Intensity of the color formed is directly proportional to the amount of chloride present in the sample.

Procedure:

Table 3.2: Chloride ion (Cl^-) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	0.01ml
Deionized water	0.01ml	-	
Standard	-	0.01ml	-
Reagent	1.0ml	1.0ml	1.0ml

Calculation:

$$\text{Chloride in mmol / L} = \frac{(A)\text{sample}}{(A)\text{standard}} \times \text{Standard conc. (mmol / L)}$$

3.3.3 Determination of Calcium (Ca^{++})

Principle: In an alkaline medium, calcium ions form a violet complex with O-Cresolphthalein complexone.

Procedure:

Table 3.3: Calcium (Ca^{++}) determination

	Pipette into cuvette		
	Reagent blank SO	**Standard SI**	**Sample**
Sample	-	-	25µl
Distilled water	25 µl	-	
Standard	-	25 µl	-
Working Reagent	1.0ml	1.0ml	1.0ml

Calculation:

$$\text{Concentration in mg / dl} = \frac{(A)sample}{(A)standard} \times \text{Standard conc. (mg/dl)}$$

3.3.4 Determination of magnesium ($Mg^{2+)}$

Principle: The method is based on the specific binding of calmagite, a metallochromic indicator and magnesium at alkaline pH with the resulting shift in the absorption

wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of magnesium in the sample.

Procedure:

Table 3.4: Magnesium (Mg^{2+}) determination

	Pipette into cuvette		
	Blank	**CAL. Standard**	**Sample**
Sample	-	-	10µl
CAL. Standard	-	10µL	-
R1. Reagent	1.0ml	1.0ml	1.0ml

Calculation:

$$\text{Magnesium (mg /dl)} = \frac{(A)\text{sample}}{(A)\text{standard}} \times \text{Standard conc. (mg/dl)}$$

3.3.5 Determination of phosphorus (P):

Principle: Inorganic phosphate reacts with ammonium molybdate in the presence of sulfuric acid to form a phosphomolybdic complex which is measured at 340 nm.

Procedure:

Table 3.5: Phosphorus (P) determination

	Pipette into cuvette		
	Blank	**CAL. Standard**	**Sample**
Sample	-	-	10µl
CAL. Standard	-	10µL	-
R1. Reagent	1.0ml	1.0ml	1.0ml

Calculation:

$$\text{Phosphorus concentration (mg / dl)} = \frac{(A)\text{sample}}{(A)\ \text{Standard}} \times \text{Standard conc. (mg/dl)}$$

3.3.6 Determination of Potassium (K^{+})

Principle: Sodium tetraphenyl boron reacts with potassium to produce a fine turbidity of potassium tetraphenyl boron. The intensity of turbidity is directly proportional to the concentration of potassium in the sample.

Procedure:

Table 3.6: Potassium (K^+) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	0.02ml
Deionized water	0.02ml	-	-
Standard	-	0.02ml	-
K^+ Reagent	1.0ml	1.0ml	1.0ml

Calculation:

$$\text{Potassium in (mmol / L)} = \frac{(A)\text{sample}}{(A)\text{standard}} \times \text{Standard conc. (mmol / L)}$$

3.3.7 Determination of Iron (Fe)

Principle: The iron is dissociated from transferring-iron complex in weakly acid medium. Liberated iron is reduced into the bivalent form by means of ascorbic acid. Ferrous ions give with FerroZine a colored complex. The intensity of the color formed is proportional to the iron concentration in the sample.

Procedure:

Table 3.7: Iron (Fe) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	200µl
Standard	-	200µl	-
Reagent	1.0ml	1.0ml	1.0ml

Calculations:

$$\text{Iron in µg / dl} = \frac{(A)\text{sample} - (A)\text{sample blank}}{(A)\text{standard}} \times \text{Standard conc. (mg/dl)}$$

3.3.8 Determination of Zinc (Zn)

Principle: Zinc in an alkaline medium reacts with Nitro-PAPS to form a purple coloured complex. Intensity of the complex formed is directly proportional to the amount of Zinc present in the sample.

Procedure:

Table 3.8: Zinc (Zn) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Distilled Water	50 µl	-	-
Zinc Standard	-	50 µl	-
Sample	-	-	50 µl

Calculations:

$$\text{Zinc in µg/dl} = \frac{\text{Abs of Sample}}{\text{Abs of standard}} \times \text{Standard conc. (µg/dl)}$$

3.3.9 Determination of Copper (Cu)

Principle: Copper, reacts with Di-Br-PAESA to from a colored complex. Intensity of the complex formed is directly proportional to the amount of Copper present in the sample.

Procedure:

Table 3.9: Copper (Cu) determination

	Pipette into cuvette (Step- 1)		
	Blank	**Standard**	**Sample**
Sample	-	-	2.0 ml
Methanol	10 ml	-	-
Standard	-	2.0 ml	-
S1	-	2.0 ml	2.0 ml
S2	-	2.0 ml	2.0 ml
S3	-	4.0 ml	4.0 ml
Final volume(S4)	10 ml	10 ml	10 ml

Calculations:

$$\text{Copper in µg/dl} = \frac{\text{Abs of Sample}}{\text{Abs of standard}} \times \text{Standard conc. (µg/dl)}$$

3.3.10 Determination of Manganese (Mn^{2+})

Principle: Manganese solution in sodium hydroxide when mixed with brucine followed by HCl produces pink color and the absorbance is measured at 480 nm. This color reaction has been developed for the colorimetric determination of manganese (Hashmi *et al*., 1969).

Procedure:

Table 3.10: Manganese (Mn^{2+}) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Buffer Reagent (L1)	500 µl	500 µl	500 µl
Colour Reagent(L2)	500 µl	500 µl	500 µl
Distilled Water	50 µl	-	-
Copper Standard	-	50 µl	-
Sample	-	-	50 µ

Trade name: Manganese standard solution. Catalogue number: 119789. Manufacturer name: Merck KGaA ,Germany.

Shake, heat at 60-65°C for 2 mins in water bath until formation of orange yellow color and cool under tap water and take 5 ml standard and sample from S4 and blank will be untouch.

Calculation:

$$\text{Manganese (µg/2ml)} = \frac{Y}{0.0002}$$

Where, 0.0002 = slope, Y=Absorbance

3.3.11 Determination of Selenium (Se^{2-})

Principle: The reaction of selenium with potassium iodide in acidic medium liberate iodine. The liberated iodine bleaches the violet colour of azure B to colourless leucoform and the absorbance is measured at 620 nm (Narayana and Mathew, 2006).

Procedure:

Table 3.11: Selenium (Se^{2-}) determination

Pipette into cuvette			
	Blank	**Standard**	**Sample**
Sample	-	-	1.0 ml
Distilled water	1.0 ml	-	-
Standard	-	1.0 ml	-
S1	1.0 ml	1.0 ml	1.0 ml
S2	1.0 ml	1.0 ml	1.0 ml
S3	500 µl	500 µl	500 µl

Gently shaken each cuvette after adding S2 solution to yield yellow color. Again shaking well 2 mins after adding S3 solution. Centrifuge at 3000 RPM for 15 min to obtain a clear supernatant.

Pipette into cuvette (Step- 2)			
	Blank	**Standard**	**Sample**
S4	10 ml	5.0 ml	5.0 ml
Add S3	-	5.0 ml	5.0 ml

Trade name: Sodium Biselenite. Batch number: M0M091209. Manufacturer name: Qualikems fine chem. Pvt Ltd.

Calculation:

$$\text{Selenium } (\mu g/ml) = 8.654x + 1.830$$

Where, 8.654= slope, 1.830= intercept, x= absorbance

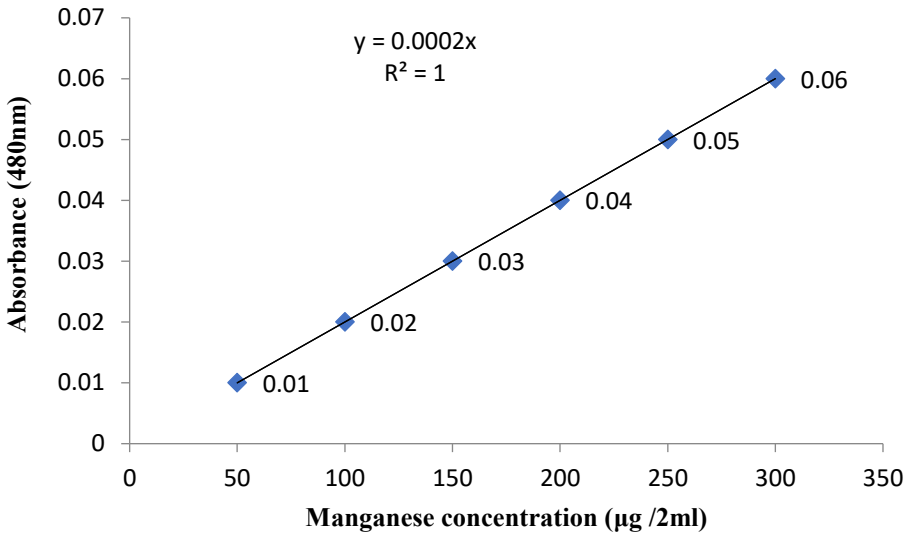


Figure 5: Calibration curve for the determination of Manganese

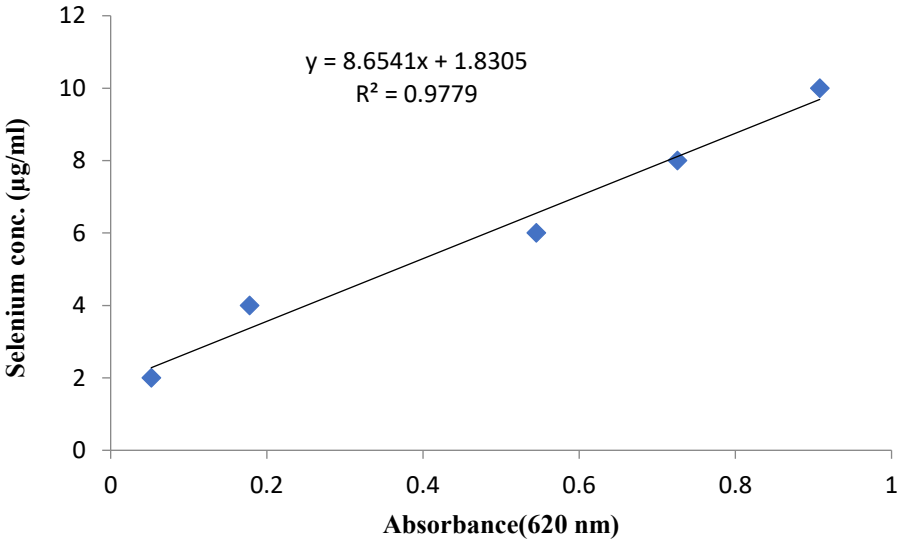


Figure 6: Calibration curve for the determination of selenium

3.4 Determination of Vitamin content

3.4.1 Determination of Riboflavin (Vit B2)

Principle: After oxidizing interfering substances permanganate, riboflavin is extracted into acetic acid-pyridine-butanol mixture and measured calorimetrically (Morell and Slater, 1946).

Procedure:

Table 3.12: Riboflavin (Vit B2) determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	500 µl
Distilled water	500 µl	-	-
Standard	-	500 µl	-
S1	500 µl	500 µl	500 µl
S2	1 drop	1 drop	1 drop
S3	2 drop	2 drop	2 drop
Shake gently for 1 min. If the purple color is not destroyed within 10 sec, add a further drop of S3 and warm to 21˚C.			
S4	1.5 ml	1.5 ml	1.5 ml
Shake vigorously up and down 25 times over 1 min and Stand to allow the layers to separate. Add a small amount of S5, rotate between the hands until the alcohol layer clears and Stand for a min or two for the layers to separate fully**.**			
Alcohol extracts	1.0 ml	1.0 ml	1.0 ml

Absorbance read at 540 nm in colorimeter using excitation at 480 nm, zeroing against the blank. The alcohol extracts are stable for at least 2 hr. To get an improved riboflavin reading, expose the alcohol extract to strong ultraviolet light for 60 min after taking the initial reading. Then read the final absorbance at 540nm and the difference is due to riboflavin.

Trade name: Riboflavin standard. Manufacturer name: Koch-Light Laboratories Ltd.

Calculation:

$$\text{Riboflavin (mg/l)} = \frac{\text{Difference of sample Abs}}{\text{Difference of standard Abs}} \times \text{Standard conc. (mg/l)}$$

3.4.2 Determination of retinol and carotene (Vit-A) using TFA

Principle: Proteins are precipitated with alcohol and retinol and carotenes extracted into light petroleum. After reading the intensity of the yellow colour due to carotenes, the light petroleum is evaporated and the residue dissolved in chloroform before carrying out the colour reaction. Allowance is made for the carotene contribution to the reaction (Bradley and Hornback, 1973). Retinol present in sample reacts with trifluoroacetic acid (TFA). During the reaction of sample and TFA, a blue color is observed indicating the presence of retinol in the sample. The blue color is transient, so if the color develops, it must be observed within 2 seconds after adding the reagent (Makhumula *et al*., 2007).

Procedure:

Table 3.12: Carotene determination

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	1.0 ml
S1	-	-	2.0 ml
S2	6.0 ml	-	3.0 ml
Standard	-	6.0 ml	-
Total volume (S3)	6.0 ml	6.0 ml	6.0 ml

Trade name: Beta carotene. Batch number: 9899207. Manufacturer name: Sisco research laboratories.

Mix well with a vortex mixer, mechanical shaking for 10 min, centrifuge the tubes for 10 min at 3000 RPM. Finally take 2 ml blank and standard and collect 2 ml supernatant from sample. Read the absorbance at 420 nm against the blank. Do this without delay to prevent solvent evaporation and destruction of carotenoids by light.

Table 3.13: **Retinol determination**

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample (from S3)	-	-	2.0 ml
Evaporate the contents of the sample cuvette to dryness in a 50 ℃ water bath.			
S4	100 µl	-	100 µl
Standard	-	100 µl	-
S5	1.0 ml	1.0 ml	1.0 ml

Trade name: Retinol acetate. Batch number: G05Y/1305/1006/62. Manufacturer name: S.D. fine- chem. Limited, Mumbai 400025.

Mix well with a vortex mixer. Recording the absorbance (A_{620}) at exactly 2s after adding the reagent. Because S5 reagent is a strong acid with an irritant vapour.

Calculation:

$$\text{Carotene (mg/l)} = \frac{Y-0.0041}{0.0133}$$

Where, 0.0133= slope, 0.0041= intercept, Y= absorbance

$$\text{Retinol (mg/l)} = \frac{Y-0.01}{0.00267}$$

Where, 0.00267= slope, 0.01= intercept, Y= absorbance

3.4.3 Determination of tocopherol (Vit -E)

Principle: Tocopherols can be measured by their reduction of ferric to ferrous ions which then form a red complex with α,α′- dipyridyl. Tocopherols is first extracted into xylene and the absorbance is read at 480 nm to measure the tocopherols. A correction for the tocopherols is made after adding ferric chloride and reading at 520 nm (Baker and Frank, 1968).

Procedure:

Table 3.14: **Tocopherol (Vit -E) determination**

	Pipette into cuvette		
	Blank	**Standard**	**Sample**
Sample	-	-	1.5 ml
S2	1.5 ml	-	-
Standard	-	1.5 ml	-
S1	1.5 ml	-	1.5 ml
S2	-	1.5 ml	-
S3	1.5 ml	1.5 ml	1.5 ml
Supernatant	1.0 ml	1.0 ml	1.0 ml
S4	1.0 ml	1.0 ml	1.0 ml

Trade name: DL-α-Tocopherol. Batch number: L225501707. Manufacturer name: Loba Chemical Pvt.Ltd.

Pipette 1.5ml of the mixture into colorimeter cuvettes and read the absorbance (A_{480}) of the sample and standard against the blank at 480 nm. Then, in turn, beginning with the blank add 0.33 ml ferric chloride solution, mix, set the wavelength to 520 nm and 1.5 min after mixing read the absorbance (A_{520}) of the sample and standard against the blank

Calculation:

$$\text{Alpha tocopherols (mg/l)} = \frac{A' \text{ of unknown}}{A' \text{of standard}} \times 10$$

Where $A' = A_{520} - 0.29 \times A_{480}$

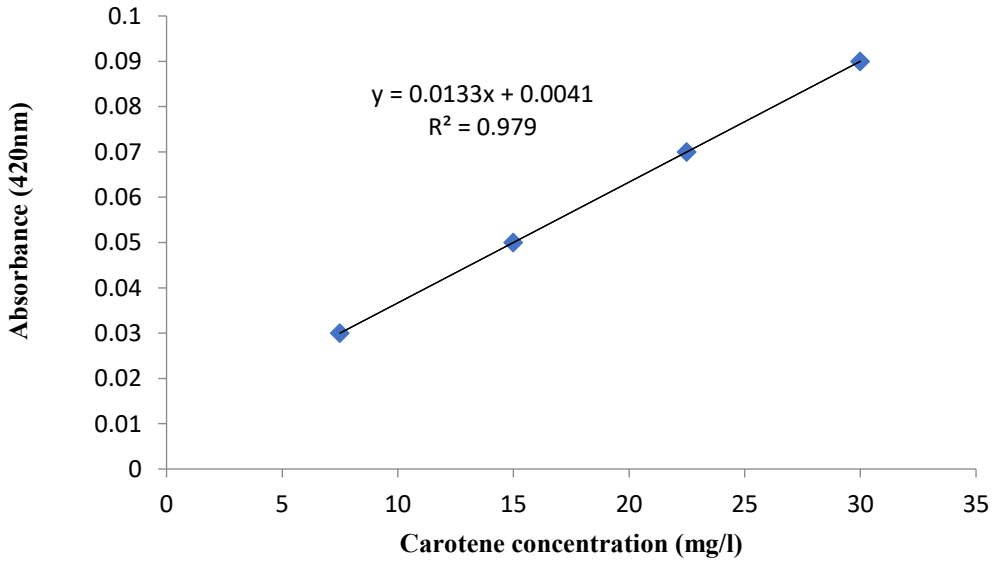


Figure 7: Calibration curve for the determination of carotene

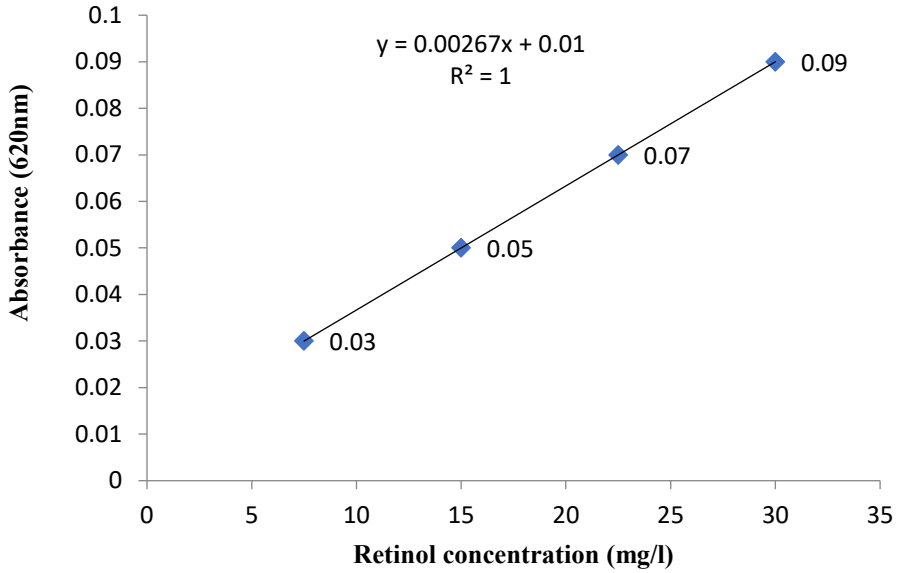


Figure 8: Calibration curve for the determination of retinol

3.5 Determination of lipid profile

3.5.1 Determination of Cholesterol

Principle: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen per oxide 4 aminophenazone in the presence of phenol and peroxidase.

Procedure:

Table 3.15: **Cholesterol determination**

	Pipette into cuvette		
	Reagent Blank	**Standard**	**Sample**
ddH_2O	5µl	-	-
Standard	-	5µl	-
Sample	-	-	5µl
Reagent	500µl	500µl	500µl

Calculation

$$\text{Cholesterol in mg/dl} = \frac{\text{Abs of Sample}}{\text{Abs of standard}} \times \text{Standard conc. (mg/dl)}$$

3.5.2 Determination of Triglycerides

Principle: The triglycerides are determine after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen per oxide 4 aminophenazone and 4 chlorophelol under the catalytic influence of peroxidase.

Procedure:

Table 3.16: Triglycerides determination

	Pipette into cuvette		
	Reagent Blank	**Standard**	**Sample**
ddH_2O	-	-	-
Standard	-	5µl	-
Sample	-	-	5µl
Reagent	500µl	500µl	500µl

Calculation

$$\text{Triglycerides in mg/dl} = \frac{\text{Abs of Sample}}{\text{Abs of standard}} \times \text{Standard conc. (mg/dl)}$$

3.5.3 Determination of High Density Lipoprotein (HDL)

Principle: Low density lipoproteins and chylomicron fractions are precipitated quantitatively phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined.

Procedure:

Table 3.17: **High Density Lipoprotein (HDL) determination**

	Pipette into cuvette		
	Reagent Blank	**Standard**	**Sample**
ddH_2O	50µl	-	-
Standard	-	50µl	-
Sample Supernatant	-	-	50µl
CHOL Reagent	500µl	500µl	500µl

Calculation

$$\text{High Density Lipoprotein (HDL) in mg/dl} = \frac{\text{Abs of Sample}}{\text{Abs of standard}} \times \text{Standard conc. (mg/dl)}$$

Chapter IV: Results

4.1 Overall weight distribution of different types of chicken egg

Table 4.1 showed (Mean±SD) the overall weight distribution of different poultry such as commercial brown egg and white egg from chicken at Chattogram division. The whole egg (Raw) weight of brown egg found high weight (60.17±5.90) g than white egg (56.72±4.776) g. In raw egg, the albumen content was found the highest in brown egg (37.53±6.041) g whereas this was found the lowest in white egg (30.87±4.776) g. On the other hand, the yolk and shell weight of white egg had the highest weight (20.5.5±.596; 5.37±1.639) g among other types of eggs. In case of dried condition, the albumen content was found the highest in white egg (5.17±1.312) g while this was found lowest in brown egg (5.13±1.272) g. The highest weight of dried yolk was found in white egg (9.25±1.656) g whereas in the brown egg was found the lowest (8.59±1.420) g. The ratio of raw albumen to raw yolk and dry albumen to dry yolk was found the highest in white egg.

Table 4.1: Comparative egg quality character of commercially available white and brown chicken egg

Parameters		White egg (N=46)	Brown egg (N=50)	
	Min-Max	**Mean ± SD**	**Mean ± SD**	**p-value**
Raw (gm)	46-68	56.72±3.810	60.17±5.90	0.003
Raw albumen (gm)	18-43	30.87±4.776	37.53±6.041	0.000
Raw Yolk (gm)	17-32	20.5±2.890	17.53±.596	0.000
Raw albumen : Raw yolk	-	1.54±0.344	2.20±0.488	0.069
Dry albumen (gm)	3-9	5.17±1.312	5.13±1.272	0.993
Dry Yolk (gm)	5-15	9.25±1.656	8.59±1.420	0.035
Dry albumen : Dry yolk	-	0.57±0.163	0.61±0.157	0.024
Shell (gm)	3-10	5.37±1.639	5.28±1.598	0.865
Raw Weight : Shell Weight	-	11.29±2.665	12.52±4.247	0.393

The mean difference is significant at 0.05 level

Legends: All values showed Mean±SD of data and expressed in gm. SD= Standard Deviation.

4.2 Nutritional composition of white and brown chicken egg

Table 4.2: Proximate component of albumen between white and brown egg of commercial chicken per 100 gm

Parameters	Albumen		
	White egg (N=5)	Brown egg (N=5)	
	Mean ± SD	Mean ± SD	p-value
Crude protein	73.99±2.422	74.83±2.957	0.636
Fat	0.22±.048	.45±.162	0.030
Ash	4.23±.120	4.27±.177	0.717
Moisture	10.51±.224	11.32±.553	0.016
Carbohydrates	11.05±2.620	9.12±2.445	0.264
Energy(Kcal/100gm)	339.91±1.84	342.15±.98	0.044

The table 4.2 shows the proximate component of Albumen between White and Brown Egg of commercial chicken. Fat percentage between white and brown egg differ significantly ($P<0.5$). Moisture percentage found higher significantly in white egg as compared to brown egg ($P<0.5$). Energy value found higher significantly in brown egg than white egg ($P<0.5$).

Table 4.3 Proximate component of yolk between white Egg and brown egg of commercial chicken per 100 gm

Parameters	Yolk		
	White egg (N=5)	Brown egg (N=5)	
	Mean ± SD	Mean ± SD	p-value
Crude protein	31.40±1.34	31.20±.837	0.784
Fat	49.2±2.49	47.2±1.92	0.193
Ash	3.44±.152	3.68±.217	0.077
Moisture	6.60±3.053	7.45±1.207	0.583
Carbohydrates	10.41±.029	10.41±.123	0.383
Energy(Kcal/100gm)	592.59±13.58	607.43±21.95	0.235

The Table 4.3 shows the proximate component of yolk between white egg and brown egg. There were no significant difference between white egg and brown egg in terms of crude protein, fat, ash, moisture, carbohydrate and energy value.

4.3 Mineral composition of different types of chicken egg

Table 4.4: Mineral composition of different type of brown and white egg (mg/100g)

Parameters	Types	Na	K	Cl	Ca	P	Mg	Zn	Fe	Mn	Se
Albumen	Brown	43±.032	240±.406	1013.87±1.23	611.8±2.09	290±.98	96±.523	4.506±11.22	4.654±14.50	.058±1.310	.145±.121
	White	47±1.032	204±.70	1510.17±1.96	477.8±2.65	228±.52	144±.33	4.39±3.902	3.706±8.51	.0455±.51	.142±.32
	P-value	.84	.40	.000	.552	.40	.11	.942	.634	.407	1.0
Yolk	Brown	161±.23	206±.75	1212.39±1.03	631±2.72	256±.73	92±.26	4.56±.097	4.23±1.97	0.182±0.03	.131±1.54
	White	156±.94	254±1.49	1212.39±1.20	645±8.12	228±1.11	146±.32	4.02±4.45	4.49±.97	0.187±.32	.129±1.2
	P-value	.343	.539	.000	.003	.913	.019	0.03	.666	.003	.035

The mean difference is significant at the 0.05 level

Legends: All values showed ME±SD of data and expressed in percentage. ME= Mean, SD= Standard Deviation.

The mineral content of different chicken eggs is shown in the Table 4.4. In dried albumen part, potassium content showed significant difference between two types of eggs. Magnesium content found higher in white eggs, than brown eggs. However, the remaining mineral contents including sodium, calcium, zinc, manganese, selenium, phosphorus, potassium and iron were not differed significantly between them.

Contents of calcium, magnesium, chlorine, zinc, manganese, selenium was found significantly different between different types of dried egg yolk.

4.4 Vitamin Contents in different white and brown egg yolk

Table 4.5: Vitamin contents per 100 grams

Types of egg	Vitamin A (IU)		Vitamin E (IU)	Riboflavin (mg)
	Carotene	Retinol		
Brown Egg (n=5)	971.09±0.73	606.06±1.92	8.11±.27	2.43±1.12
White Egg(n=5)	962.36±2.74	639.54±1.63	8.16±1.72	2.52±1.29
P-Value	.200	.213	.110	.354

The mean difference is significant at the 0.05 level

Legends: All values showed Mean±SD of data and expressed in percentage. SD= Standard Deviation.

Above Table 4.5 showed the vitamin contents in brown and white egg yolk. Carotene is higher in brown egg than white egg while retinol is found higher in white egg. Vitamin E and riboflavin is higher in brown egg than white egg.

Table 4.6 Lipid profiles of Yolk between White Egg (n=5) and Brown Egg (n=5) of commercial chicken egg powder

Parameters	Yolk (mg/100gm)		
	White egg (N=5)	Brown egg (N=5)	
	Mean ± SD	Mean ± SD	p-value
Cholesterol, mg	704.68±180.48	618.11±171.23	0.436
Triglycerides, mg	7148.56±790.52	5813.25±449.15	0.006
High density lipoprotein, mg	10.79±1.80	21.58±16.52	0.184

The Table 4.6 shows lipid profiles of yolk between white egg and brown egg. There were no significant difference between white egg and brown egg in terms of cholesterol, and high density lipoprotein except triglycerides ($P<0.05$).

Chapter V: Discussion

The chicken eggs have been traditionally considered and important source of nutrients for human. The variability in the quality and nutritional values of eggs has a significant impact on consumer's health; simultaneously welfare and many other factors can affect the egg quality. There are some evidences for a genetic influence on the following chemical components of eggs or of factors that might directly affect their chemical composition: 1) relative proportion of yolk and albumen and percentage solids, 2) albumen quality, 3) qualitative protein polymorphism, 4) total protein content, 5) cholesterol, 6) vitamin A, thiamine, riboflavin, 7) fatty acids, 8) enzymes, and 9) deposition of metabolic products in the eggs In the current study, the average weights of eggs were found between 40-66 gm (Washburn, 1978). Considering two poultry species brown egg weight found higher compared to white egg.

In this study, in raw state of brown egg, the albumen content found 2.1 times higher than yolk whereas in dried condition the content of yolk nearly 1.6 times higher as compared to albumen and in raw state of white egg, the albumen content found 1.5 times higher than yolk whereas in dried condition the content of yolk nearly 1.7 times higher as compared to albumen. Differences in egg weight among various sources can affect the quality of eggs, and consumers desire as they usually prefer larger eggs (USDA, 2000). Moreover, egg weight could be varied due to age, strain of hens (Zita et al., 2009; Alsaffar, et al., 2013), diet variations, housing density, housing condition, health status, environmental stress and feed intake (Attia et al., 1994). A number of factors are associated with increasing yolk index with increasing egg weight, age of hens, storage time and poor storage condition. Increasing yolk index results from undesirable changes in interior egg quality due to increase permeability of vitelline membrane for water from albumen to yolk. Furthermore, the high food energy value (FEV) recorded particularly in egg yolk powder, which makes it particularly attractive for infant food formulae (Krause and Mahan, 1984; Vaclevik and Christian, 2008). On analysis of proximate components shows that the albumen portion contains higher moisture content in brown egg compared to white egg. White egg albumen

had higher protein, carbohydrates and ash than Brown egg whereas fat content found higher in yolk than Brown egg. In the present study, the protein, fat, ash percentage found slightly lower in both albumen and yolk than the earlier study. Conversely, the moisture content found higher in this study in both parts of dried egg than the earlier study. These discrepancies are due to different types of chicken eggs, method and time duration of cabinet drying. Cabinet drying is suitable to keep the moisture contents are low enough to extended the shelf life of the egg powders in an environment of low humidity (Jay, 2000). Both egg white and egg yolk are highly concentrated in proteins. Hundreds of different proteins have been identified and are associated with specific physiological function to fulfill time-specific requirements during embryonic development. Egg yolk has essentially a hepatic origin, while white egg is synthesized and secreted after ovulation of mature yolk in the hen's oviduct (Mann, 2008).

In the current study, the overall mineral contents were higher in the egg yolk than the egg white. Both egg yolk and egg white contain iron at daily recommended level. The recommended daily requirement of iron for man is 6-40 mg/kg (Bolt and Bruggenwert, 1978). Moreover, the egg yolk contains more iron than the white, which is dissimilar with the previous study. The reason for this is that iron is present in ferric form and interacts with the phosvitin found in egg yolk. Additionally, the presence of eggs in the diet reduces the bioavailability of iron from other food sources. Combining eggs in the diet with enhancing factors such as vitamin C, citric acid, cysteine-containing peptides or ethanol results in significantly increased bioavailability of iron (Hallberg and Hulthen, 2000). The mineral contents are varied among different egg sources in mineral contents of eggs reflected the nutrition and health status of laying hens. For example, Ca, P, Zn, Mn, Mg, Fe, Cu and Na contents were affected by source of eggs (Rehault, 2019). The mineral of the whole edible parts of eggs was found to fulfill 3.2, 14.3, 4.3, 0.11, 1-1, 8.0, 3.0, 1.8, and 21% of the RDA for Ca, P, Zn, Mn, Mg, Fe, Ca, K, and Na, respectively (Attia, 2014). Differences in mineral contents of eggs could be attributed to dietary mineral contents and sources, husbandry systems (floor vs Cage) (Gianneas et. al., 2009), feed additives (Backenmann and Terners, 2008), organic and conventional rearing system and different geographical area.

The recommended dietary allowance value of calcium is 600-1400mg/kg (Bolt and Bruggenwert, 1978). The present study shows that both Calcium and Potassium high Brown egg powder albumen and white egg yolk powder. Moreover, magnesium content is higher in brown egg albumen and yolk than white egg albumen and yolk powder. Phosphorus content is high in yolk powder than albumen in white egg.

The Recommended Dietary Allowance (RDA) of selenium is 55 micrograms daily for adult men and women. Women who are pregnant and lactating need about 60 and 70 micrograms daily, respectively. In the present study selenium content is higher in brown egg albumen powder and yolk powder than white egg.

Manganese is involved in the formation of bone and in amino acid, lipid, and carbohydrate metabolism. The Adequate Intake (AI) for adult men and women is 2.3 and 1.8 mg/day, respectively. In this study, manganese content is higher in white egg yolk powder than brown egg.

We need little amount of vitamin in our everyday life. Egg is a good source of vitamins compared to other food products. Although the vitamin content between brown and white egg did not differ significantly, retinol content found higher in white egg while carotene in found higher in brown egg. Both vitamin E and B_2 or Riboflavin were found higher in white egg than brown egg. These variations could be due to differences in diet, genotype, housing pattern and age of chicken. The egg yolk contains high amount of vitamin A, D, E, K, B_1, B_2, B_5, B_6, B_9, and B_{12} while egg white possesses high amounts of vitamin B_2, B_3, B_5 but also significant amounts of vitamins B_1, B_6, B_8, and B_{12}.

Some authors have demonstrated that egg cholesterol was not well absorbed, that's why egg doesn't significantly impact blood cholesterol concentration (Kim et. al., 2018). In the study, both Cholesterol and triglycerides were found higher in white egg than brown egg and conversely high density lipoprotein (HDL) were found higher in brown egg.

We may suggest that egg albumen powder might be good choice for people who are suffered from protein deficiency whereas egg yolk powder could be regarded as good fat source. Eating two eggs per day covers 10% to 30% of the vitamin requirements for human. It is noteworthy that the content of liposoluble vitamins (Vitamin A, D, E, K) in egg yolk is highly dependent on the hen's diet (Rehault, 2019).

Chapter VI: Conclusion

Egg is considered as complete food, which are excellent source of high-quality protein, lipids, vitamins and minerals. Egg powder is easy to handle, transport and showed excellent functional properties like foaming, firmness. From the investigations of the present work, it can be concluded that the brown egg weight was higher than white egg. There was significant difference in overall weight. Further, all egg powders differed in nutritional composition where brown egg albumen powder had the highest nutrient content compared to white egg albumen powder. Brown egg yolk had the highest protein compared to white egg. In case of ash (minerals) brown egg yolk powder showed higher content compared to white egg yolk powder.

Chapter VII: Strength & Weakness

7.1 Strength of the study

- There is limited research on egg powder. In this study we produce egg powder separately (egg white and egg yolk powder) and evaluate their nutritional component.
- We used common chicken eggs which were available in the local market.
- Compared the nutritional comparison among three different types of chicken egg.

7.2 Weakness of the study

- Less fund
- The eggs could not collect from different location and different season
- Other parameters like amino acid, vitamin D and K were not done .
- The other important properties of egg powder like foaming properties, gel properties, pH were not analyzed.
- The physical characteristic tests and the self-life of the samples were not analyzed.

Chapter VIII: Recommendations & future projection

- The egg should be collected from different area, different species and in different seasons.
- The other parameter such as amino acid, D and K should be analyzed.
- The physical characteristics test and the shelf life of the egg powder should be analyzed.
- The other important properties of egg powder like foaming properties, gel properties, pH etc. should be analyzed.
- Effect of different drying methods on powdered egg of different poultry should be considered.
- Drying losses should be measured and appropriate dryer should be developed for drying of egg keeping the original quality

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Appendices: Photo Gallery

Egg collection

Cleaning

Breaking

Egg separation

Weighing

Drying

Blending

Sieving

Yolk and albumen powder

Brief Biography

Lipa Chowdhury passed the Secondary School Certificate Examination in 2010 and then Higher Secondary Certificate Examination in 2012. She obtained her B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Biochemistry under the Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, (CVASU). She has immense interest to work in improving health status of poor people through proper guidance and suggestions and create awareness among people about Food Science and Nutrition.