Original Research Article

Comparative Evaluation of some bioactive compounds in raw and boiled egg varieties: Eggs, potential nutraceuticals?

ABSTRACT

Aims: To comparatively evaluate some bioactive compounds (egg white proteins) of chicken (exotic and local), turkey, quail and guinea fowl eggs in their raw and boiled forms. It also aimed at ascertaining claims on egg being a functional food.
Study design: Experimental.
Place and Duration of Study: Department of Biochemistry, University of Calabar, Calabar and Department of Pharmacology, University of Nigeria, Nsukka, February to July 2017.
Methodology: Freshly-laid poultry-bred eggs were purchased, cleaned and divided into 2: one batch was broken and the egg white separated while the other was boiled by submerging the eggs in boiling water at 100^{o}C for 10min, before taking out the egg whites. The raw and boiled albumen were homogenized before analyses. A combination of methods involving separation of egg white proteins using ion-exchange chromatography, purification using tangential flow filtration and quantification using the colorimetric Bradford assay. Results of the quantitative estimation of avidin, lysozyme, ovalbumin, ovotransferrin and flavoprotein concentrations were statistically compared using analysis of variance (ANOVA).
Results: It was observed that concentrations of the bioactive compounds (except ovalbumin) were significantly ($P < 0.05$) higher among the raw eggs than the boiled ones. Raw turkey egg had the highest avidin content (15.83 ± 0.15 µg/g) and this was significantly different ($P < 0.05$) from the others, while quail had the lowest avidin concentration (8.47 ± 0.20 µg/g) even among the boiled samples. Ovalbumin, a storage protein, was the most abundant of the egg white proteins (50-55%).
Conclusion: Quail eggs are healthier due to their relatively safer content of avidin, higher contents of flavoprotein and ovotransferrin; turkey egg with exceptionally higher avidin concentrations, should be consumed in moderation in order to reduce the risk of biotin deficiency. The presence of these bioactive compounds in significant quantities also show that eggs may serve as functional foods.

Keywords: bioactive compounds, eggs, nutraceuticals, proteins

1. INTRODUCTION

Food is any material of plant or animal origin that consists of essential body nutrients such as carbohydrates, fats, proteins, minerals or vitamins, and is ingested or assimilated by an organism to produce energy, sustain growth and maintain [1]. The nutrient composition of various foods depend on several factors which include species, breeds, cultivars, ecological factors, post-harvest handling, preservation and storage techniques. Some foods are considered healthy depending on their nutrient content while others are considered unhealthy [2].

The importance of good and adequate nutrition for maintenance of health can never be overemphasized. Poverty, diseases, harmful economic systems, food insecurity, inadequate food and agricultural policies, poor nutrition education, adverse climatic changes, conflicts/wars are some of the causes of hunger and malnutrition [3]. With the increase in malnutrition and poverty globally, it has become necessary to develop strategies and remedies to the growing number of nutrition-related diseases that populations are being faced with. There are food based approaches and agriculture based approaches employed in tackling the problem of malnutrition. Food-based approaches, particularly utilizing animal source foods (such as meat and eggs) offer potentially sustainable solutions to multiple deficiencies [4].

Research has shown that food biodiversity can provide some sustainable solution for combating food insecurity and malnutrition [4]. Consumption of different breeds/varieties may have significant impact on nutrition and health outcomes. Some specific data are required to promote the use of biodiversity for food and nutrition. These information which include food composition and consumption from various varieties/breeds and their dietary contribution to nutrition and human health, have been increasing as well as awareness. Different varieties/breeds vary significantly in their nutrient compositions [5]. Macronutrients from different varieties of the same species could vary by 10-fold, and micronutrients by up to 1000-fold [6] by virtue of the genetic resource itself.

Bird eggs which are of different varieties, many of which are consumed by man for food, are made up of the yolk, albumen and shell. The albumen (egg white) consist primarily of water (87%) and protein (13%) with no cholesterol and little, if any fat [7]. According to Abeyrathne [8], ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme (3.5%) and ovomucin (3.5%) are the major egg white proteins while avidin (0.05%), cystatin (0.05%), ovoflavoprotein (0.8%), ovomacroglobulin (0.5%), glycoprotein (1%) and ovoinhibitor (1.5%) are the minor ones. Each of these proteins are recognized for their functional importance and they possess many functional properties such as gelation, emulsification and coagulation [8].

According to Li-Chan *et al.* [9], many egg white proteins have also been found to possess various antimicrobial and antioxidant properties. Technologies have now been developed for separating egg white proteins commercially, hence there are possibilities of using these egg white proteins for their antimicrobial and antioxidant properties [10]. Ovalbumin is the major egg white protein synthesized in the hen's oviduct and accounts for 54% of the total egg white proteins [11]. Conalbumin was renamed as ovotransferrin after findings that it can bind iron. One molecule of ovotransferrin can bind 2 molecules of iron, and it transports iron in the body [8]. Lysozyme is another important egg white protein found in nature as a monomer, but is occasionally present as a dimer with more thermal stability. It is considered as a strong basic protein present [12]. Ovomucin, one of the major egg white proteins, has more coiled regions at its extremities, like the structure of human mucin; there are the soluble and insoluble forms. Previous studies have shown that at least 3 types of carbohydrate chains are found in ovomucin, which are composed of galalctose, galactosamine, sialic acid, and sulfate with a molecular ratio of 1:1:1:1. On average, 33% of ovomucin is carbohydrates [13]. Ovomucoid is one of the most highly glycosylated proteins found in egg white [14]. A research [15] reported that ovomucoid can be used to control *Streptomyces erythraeus*. Ovomucin is also considered as a trypsin inhibitor, which is a negative property of the protein; it has the capability to control microorganisms and so can be used as an antimicrobial agent in foods.

Separations of egg white proteins have been done for many years but new, simple, economical, and sequential methods with better yield and purity are emerging. Among the egg white proteins, lysozyme is currently used as antimicrobial agent in the food industry, and others proteins such as ovalbumin has a strong potential as a drug carrier, ovotransferrin as an antimicrobial agent or iron carrier, ovomucin and ovomucoid as antimicrobial and immunomodulating agents. Peptides derived from ovotransferrin, ovalbumin, ovomucoid, and ovomucin showed cytotoxic, anticancer, immunomodulating, ACE-inhibitory, antimicrobial, and antioxidant activities, and have high potentials to be used in the pharmaceutical, nutraceutical, and food industries [8].

Numerous species of bird eggs exist which are nutritious but there seems to be scant information on the nutritional and non-nutritional content of different egg varieties, except the popular chicken egg. According to Kiple [16], the chicken egg is the most consumed by humans but other eggs including those of quail, guinea fowl, goose, turkey and duck are also important in human nutrition; however, information on egg quality characteristics have been quite limited to chicken eggs and comparative evaluation of egg varieties are not many.

This study therefore seeks to evaluate and compare the concentrations of five bioactive compounds in some popularly consumed bird egg varieties namely exotic chicken, local chicken, turkey, quail and guinea fowl eggs, in their raw and boiled forms.

2. MATERIALS AND METHODS

2.1 Egg sample collection and preparation

Eggs were sourced from poultry houses in Nsukka, Nigeria; only freshly laid eggs were purchased for the purpose of this research. The different egg varieties (5 samples each) were cleaned and prepared separately for analyses.

2.1.1 **Preparation of raw egg samples**: Shells of fresh eggs were cleaned, broken and the egg whites carefully emptied into clean glass beakers. The raw egg white content was then homogenized and placed in clean, labelled beakers and sealed with parafilm.

2.1.2 **Preparation of hard-boiled egg samples**: The fresh eggs were boiled by adding them to tap water already boiling at 100^{o}C. The tap water was put to cover the eggs in the pot. The eggs were left to boil for 10 min, immediately after which they were removed and allowed to cool in tap water at room temperature. Each type of eggs was boiled separately (5 samples per egg variety) and then shelled and carefully incised using a scalpel blade to remove the egg whites before homogenization, after they had cooled. The homogenised boiled eggs were placed in clean, labelled beakers and sealed with parafilm.

After homogenising, 20g of each of the samples (raw and boiled samples) were stored in properly labelled, air-tight sample glass bottles until ready for analyses. All the reagents used in the laboratory analyses were of standard analytical grade (AR).

2.2 Determination of bioactive compounds

This was carried out according to the method of He [17] and Bradford [18]. The bioactive compounds (egg white proteins) were analysed using a combination of methods involving egg white preparation, separation of egg white proteins using ion-exchange chromatography, purification using tangential flow filtration and quantification using the colorimetric Bradford assay.

2.2.1 Principle and Procedure: Ion-exchange chromatography separates ions based on their charged groups. A change in pH changes the charge on the particular molecules and therefore alters binding. The molecules then start eluting based on the changes in their charges from adjustments. In the Bradford assay, the separated proteins form a protein-dye complex with the Bradford reagent and the concentration of the protein is colorimetrically determined using a spectrophotometer.

2.2.1.1 Egg White Preparation: Fresh eggs (raw) were broken, and egg white and yolk were separated using a kitchen egg separator by removing the whole yolk. The boiled egg whites were separated from the yolk using a scalpel blade. Both raw and boiled egg whites were homogenised and then diluted 10-fold with 10mM Tris-HCl and 10mM disodium orthophosphate. This was stirred for 30 min and stored overnight at 4℃. The diluted samples were then centrifuged at 10000g for 30 min to remove all insoluble debris, and the supernatants were collected. Supernatants was prepared for ion exchange chromatography.

2.2.1.2 Ion Exchange Chromatography (Liu *et al*., 2012) [19]: The separation of egg white proteins was carried out using tandem ion-exchange chromatography. This method incorporates both HiLoad 26/10 High Performance Q and SP Sepharose Fast Flow columns for the separation of both anionic and cationic species, on an ÄKTA explorer FPLC System (GE Healthcare). Columns were washed with elution buffer (10mM Tris-HCl, 10mM DSOP, 1M NaCl, pH 7). Further equilibration with 4 column volumes of equilibration buffer (10mM Tris-HCl, 10mM DSOP, pH 7). 100mL of diluted egg white (1:10 equilibration buffer) was loaded at a flow rate of 8.0mL/min initially onto the Q Sepharose column to bind anionic compounds. The unbound cationic components were then trapped onto the SP Sepharose column. Compounds that did not bind either Q or SP Sepharose were collected as the flow through. Egg white proteins were first eluted from the Q Sepharose column with elution buffer with isocratic steps at 5% for 30 minutes, 10% for 30 minutes, 20% for 20 minutes, and 100% for 20 minutes. Afterwards, the in-line valve was switched to isolate the SP Sepharose column. Elution was then achieved with elution buffer on the HiLoad SP Sepharose column using isocratic steps at 5% for 20 minutes and 100% for 20 minutes.

2.2.1.3 Purification of fractions: Tangential Flow Filtration (TFF) and Stirred Cell Filtration were used. Tandem ion-exchange chromatography separated diluted egg white into nine fractions. Fractions were concentrated on two 1 kDa nominal molecular weight limit (NMWL) via TFF using a ProFlux M12

(Millipore, Bedford, MA, USA) at a trans membrane pressure of 40 psi. Ultrafiltration and buffer exchange (diafiltration) were used to ensure conductivity of the sample was approximately ≤300 µS.

Owing to the large hold-up volumes associated with TFF, an Amicon stirred cell with a 1 kDa NMWL regenerated cellulose ultracel membrane (millipore) was used to further concentrate protein fractions and filter the samples in PBS. Nitrogen gas was applied at a maximum pressure of 75 psi while the sample was stirred at 25 rpm to avoid "gelling" and accumulation of egg white proteins on the filter membrane. Concentration of each protein fraction was quantified using the colorimetric Bradford assay. The purified sample fractions were stored at −40°C.

Laboratory results (in triplicates) were analysed and compared using analysis of variance (ANOVA) on the Statistical Package for Social Sciences (SPSS) software version 20.0

3. RESULTS AND DISCUSSION

3.1 Bioactive compounds

Table 1 shows the results of the quantitative estimation of bioactive compounds (egg white proteins). Comparing the raw and boiled samples, it was generally observed that the bioactive compounds concentrations were significantly higher ($P < 0.05$) in the raw egg samples than the boiled samples; for instance, the concentration of avidin in raw quail egg was 8.47 ± 0.20 µg/g but it was 4.60 ± 0.10 µg/g in the boiled sample. This was the trend except for ovalbumin which had slightly higher concentrations in the boiled egg samples. Among the raw samples, turkey egg had the highest concentration of avidin (15.83 ± 0.15 µg/g) and this was significantly ($P < 0.05$) different from the others, while quail had significantly ($P < 0.05$) lower concentration (8.47 ± 0.20 µg/g) even in the boiled samples. Ovalbumin was of greater percentage composition with values ranging between 47 and 53 g/100g egg white in both raw and boiled samples. The avidin content of quail egg reduced by half after boiling. It was also observed that for lysozyme, all the raw and boiled concentrations varied significantly ($P < 0.05$) except for guinea fowl egg where boiling did not significantly affect the lysozyme content. Ovotransferrin concentrations of raw exotic chicken, quail and guinea fowl eggs, were statistically similar ($P > 0.05$) and higher than that of local chicken and turkey egg which were also statistically similar ($P > 0.05$).

The concentrations of bioactive compounds found to be higher in the raw eggs than in the boiled ones, may help to explain why raw eggs are prescribed for use as functional foods for 'treating' conditions such as hypertension, infections and hyperlipidemia because these bioactive compounds have been reported to have antihypertensive, hypolipidemic, anticancer and antibacterial properties [20]. In another research, Verrinder-Gibbins [21] indicates that there are potential approaches for increasing egg white proteins through genetic engineering. This will afford possibilities for enhancing functional properties and increasing their use for industrial and pharmaceutical applications. The high content of avidin in raw turkey egg causes it not to be recommended for individuals who need increased levels of biotin (vitamin B_7). This is because avidin binds biotin and reduces its bioavailability to the body. In an article [22], it was reported that biotin improves glycemic control in diabetic patients and hence, biotin supplementation is one of the recommended treatments for diabetes since the vitamin helps in lowering blood sugar and also functions in energy production. Quail egg had the least avidin concentrations (both in the raw and boiled samples) and may be a healthier choice for diabetic patients who choose drink raw eggs for perceived health benefits. Ovalbumin had the highest concentration and this agreed with the findings of Stevens [23] and Zabik [24] who reported ovalbumin as the major egg white protein. During storage, ovalbumin is changed to s-ovalbumin, an extra heat-stable form [25]. This may be the reason for the observed non-decrease of its concentration in the boiled sample. Ovotransferrin makes up about 13% of the egg white proteins and its ability to bind to iron is related to its antimicrobial activity [24]. According to Akkouche *et al*. [26], when egg white is heated, its globular proteins are prone to changes in structure and conformation. Depending on the temperature and duration of heating, these changes can range from denaturation, to gelation or coagulation. This may help explain the decrease in concentration of some of the bioactive compounds in the boiled samples. Flavoprotein is reported to have the highest selenium content (a potent antioxidant) of 1800 ng/g, among the egg white proteins [27]. This may be responsible for some of the speculated anticancer activities of raw eggs. Of all the species, raw turkey eggs were found to be richest in flavoprotein.

Table 1. Bioactive compounds (egg white proteins) in raw and boiled egg varieties.

EGG SPECIE	Avidin (µg/g EW)	Lysozyme (g/100g EW)	Ovalbumin (g/100g EW)	Ovotransferrin (g/100g EW)	Flavoprotein (g/100g EW)
Raw samples					
Exotic chicken	9.37 ± 0.18^{b}	4.47 ± 0.07^{b}	49.90 ± 0.64^{b}	1.50 ± 0.12^{a}	0.25 ± 0.00^{c}
Local chicken	9.77 ± 0.07^{b}	4.23 ± 0.09^{b}	52.37 ± 0.18^{c}	1.70 ± 0.06^{b}	0.26 ± 0.00^{c}
Turkey	15.83 ± 0.15^{c}	5.30 ± 0.12^{c}	52.17 ± 0.74^{c}	1.17 ± 0.03^{b}	0.31 ± 0.01^{d}
Quail	8.47 ± 0.20^{a}	3.63 ± 0.09^{a}	47.63 ± 0.32^{a}	1.73 ± 0.03^{b}	0.16 ± 0.01^{a}
Guinea fowl	9.40 ± 0.17^{b}	4.50 ± 0.12^{b}	51.20 ± 0.35^{c}	1.40 ± 0.10^{a}	0.22 ± 0.00^{b}
Boiled samples					
Exotic chicken	7.17 ± 0.07^{c}	2.37 ± 0.15^{a}	53.37 ±1.05^{b}	1.27 ± 0.07^{c}	0.21 ± 0.01^{b}
Local chicken	6.30 ± 0.12^{b}	3.30 ± 0.12^{b}	52.67 ± 0.81^{b}	1.20 ± 0.06^{b}	0.21 ± 0.01^{b}
Turkey	11.40 ± 0.17^{d}	4.17 ± 0.03^{c}	53.47 ± 0.19^{b}	1.17 ± 0.03^{b}	0.25 ± 0.01^{b}
Quail	4.60 ± 0.10^{a}	3.23 ± 0.07^{b}	42.53 ±1.22^{a}	1.27 ± 0.03^{c}	0.83 ± 0.03^{c}
Guinea fowl	6.77 ± 0.29^{b}	4.17 ± 0.07^{c}	53.60 ± 0.21^{b}	1.07 ± 0.03^{a}	0.13 ± 0.03^{a}

Values are expressed as mean ± SEM, n = 3. Values with different superscripts in the same column are significantly ($P < 0.05$) different from each other. EW means egg white.

4. CONCLUSION

Comparative evaluation of the bioactive compounds of some bird eggs has shown that most egg white proteins are present in higher amount in the raw eggs than boiled. Some egg varieties have relatively higher content of certain egg white proteins than others. These bioactive compounds have also been reported to have antimicrobial and antioxidant activities, thus causing the eggs to have the ability to serve as functional foods. In order to reduce the risk of biotin deficiency, consumption of quail egg (with lower avidin levels) should be more recommended, especially for diabetics who need more biotin due to its hypoglycaemic activity; while turkey eggs with exceptionally higher avidin levels should be consumed in moderation.

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