## **Original Article**



## Valorization of Chicken and Quail Egg Shells, Snail Shells and Cuttlefish Bone as Naturals Sources of Micronutrients

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#### ABSTRACT

Good nutrition is the foundation of health and well-being, physical and cognitive development, and individual's economic productivity. Unfortunately, global undernutrition and micronutrient deficiencies still remain unresolved problems. The case of developing countries is still very alarming. This work therefore focuses on the micro-nutritional value of chicken and quail egg shells, snail shells and cuttlefish bone, destined for the trash in order to determine the level of ions such as Calcium (Ca<sup>2+</sup>), Magnesium (Mg<sup>2+</sup>), Potassium (k<sup>+</sup>), Sodium (Na<sup>+</sup>) and iron (Fe<sup>3+</sup>). At the beginning of the work, a survey was carried out among the populations of the commune of Natitingou in the Republic of Benin. This step was followed by the preparation of powders of the four substances. The powders were put into solution then assayed for the quantification of the different ions mentioned above. At the end of the survey, it appears that more than 50% of respondents are aware that calcium can be found in the shells mentioned above and are in favor of their processing to make them edible. The titration of the different solutions reveals a higher level of calcium ion Ca<sup>2+</sup> in cuttlefish bone with a value of (90 mg/100g of powder). The highest level of magnesium was found in quail egg shells (24 mg/g of powder for Mg<sup>2+</sup>). The levels of K<sup>+</sup> and Na<sup>+</sup> ions always remain higher in the case of cuttlefish bone and low in the case of chicken and quail eggs, which are very available and often neglected, are a very good source of calcium and magnesium that can serve as a matrix in the design of food supplements rich in micronutrients.

Keywords: Micronutrient deficiencies; neglected shells; ion rate; dosage; food supplements.

#### **INTRODUCTION**

oods are foodstuffs of animal, plant or mixed origin, intended to provide energy to our body.<sup>1-3</sup> They are made up of nutrients, water and sometimes fiber.<sup>4-5</sup> A balanced diet goes well beyond macronutrients and also includes micronutrients which constitute an aspect of the diet that should not be neglected.<sup>6-9</sup> Micronutrient deficiency can cause fatigue with difficulty sleeping or nonrestorative sleep, irritability, significant stress, paleness, difficulty digesting properly, repeated headaches. permanent muscle tension or palpitations. In the absence of treatment, all of these factors can cause very complicated and complex diseases in the long term.<sup>10-11</sup> The case of underdeveloped countries is even more alarming.<sup>12</sup> Nutritional status also remains a fundamental indicator of overall human and economic development according to the FAO and still remains in a critical state on a planetary scale according to the World Bank.<sup>13</sup> Making natural substances intended to be thrown away in the production of micronutrients profitable constitutes a big step in the search for sources of compensation. It is in this context that different shells intended for the trash were carefully collected and used with the aim of carrying out a comparative quantitative study of their chemical composition in micronutrients in order to promote them in nutrition.

Cuttlefish bone remains an excellent source of micronutrients known and exploited by the beninese population for its numerous virtues. The antimicrobial properties of the substance have been described.<sup>14</sup> This substance strengthens the immune system, is used in the prevention of diabetes, in the reconstruction of membrane tissue. <sup>15-17</sup> It is also used in the composition of medicines against gastric ulcers. It powder also showed a water purifying power. <sup>18-19</sup> Unfortunately this substance is becoming increasingly uncommon. But an alternative can be considered with various shells also having interesting biological properties. These are chicken egg shells,<sup>20-23</sup> quail,<sup>24-26</sup> and snail.<sup>27-31</sup> The objective of this work is to carry out a comparative study of the micronutrient content of these four mineral sources with the aim of contributing to the valorization of these shells which can be used to maintain human body. This research is in line with objectives 2 and 3 of the SDGs relating to quality food for all. Starting from an investigation into the state of knowledge of the nutritional value of the different shells,



the dosage phase of the different powders made it possible to make a comparison of their micro-nutritional composition.

#### **MATERIALS AND METHODS**

Before determining the mineral composition of the different matrices, a survey was carried out in the commune of Natitingou to collect the state of knowledge on the role of micronutrients in the body, on the value of the different shells, etc.

#### Description of the town of Natitingou

As stated in the methodology, our survey focused on the populations of the commune of Natitingou (figure 1). Natitingou is a commune and town in the northwest of Benin, prefecture of the Atakora department. Natitingou has 65 villages and town districts spread across 9 districts, including: 3 urban, namely Natitingou 1, Natitingou 2, Natitingou 3 and 6 rural, notably: Perma, Kouandata, Tchoumi-Tchoumi, Kotopounga, Péporiyakou and Kouaba. There Coordinates are 10° 19' north and 1° 23' south. The language(s) speaking is Waama, Otammari, bariba (baatonum), (French), dendi etc.





Source : <u>https://fr.wikipedia.org/wiki/Fichier:Atakora\_communes.png</u>

With a view to promoting micronutrients contained in quail and chicken egg, snails shells and cuttlefish bone, the investigation sheet was designed to find out if the population is aware of the role of micronutrients in body. The work mainly took place in Kaba Research Laboratory in Chemistry and Applications (LaKReCA) for the University of Technical Sciences, Engineering and Mathematics (UNSTIM), of Republic of Benin.

#### Preparation of the survey sheet and conduct of the survey

This is a cross-sectional descriptive study by direct interview with the populations of the commune of Natitingou (figure 1). It was initiated in our laboratory and the methods used are inspired by those of Yolidje and other authors.<sup>32-35</sup> In total, 120 people were asked to answer our questions developed on the survey sheets based on the objectives to be achieved. It was carried out over a period of 30 days from June 1, 2023 to June 30, 2023. The survey took into account men and women in the age group from 15 to 65 years and over. It affected both educated and non-educated people. It took place both in the town of Natitingou and in surrounding villages. Several languages were used during the survey depending on the subjects to be investigated (French, Wama and Ditamari).

The data recorded on the survey sheets were then entered and analyzed with Microsoft Excel 2013 software to allow us to obtain the various tables and graphs.

#### Preparation of powders to analyze

The dry bone purchased in a sales center from traditional practitioners, egg and quail shells, acquired in a local cafeteria and snail shells collected from a seller of snail meat were material used as a source of micronutrients.

The method used for the preparation of the powder was inspired by that used by Ben Nasr et al. and by Fred et al.<sup>36,37</sup> Each material is brought to a boil for approximately 15 minutes to remove odors and sterilize it. It is then dried in an oven at 40 degrees for 24 hours. Each material, pounded in a wooden mortar, was then ground into fine powder using an electric grinder, then sieved with a 10 mm sieve.<sup>38</sup> At the end of the operation, four powders were obtained (that of dry bone, chicken, quail and snail shells).

Each prepared powder was then instantly put into solution for the determination of the levels of Calcium (Ca<sup>2+</sup>), Magnesium (Mg<sup>2+</sup>), Potassium (k<sup>+</sup>), Sodium (Na<sup>+</sup>) and iron (Fe<sup>3+</sup>).<sup>39-40</sup>

#### Description

The determination of water hardness is based on the addition of eriochrome black T (NET) to the water to be analyzed to form Mg-NET and Ca-NET complexes (1). The solution takes on a red-violet color characteristic of these complexes. We reach pH 10 by adding a buffer solution. The solution obtained is then titrated with ethylene diamine tetra-acetic acid (EDTA). There is initially formation of Mg-EDTA and Ca-EDTA complexes with the free Mg<sup>2+</sup> and Ca<sup>2+</sup> ions (i.e. not complexed with NET) (2) then the ions complexed with NET (3). This releases the NET into solution, and the solution takes on a blue color characteristic of this molecule at pH 10.

#### **RESULTS AND DISCUSSION**

#### Analysis of the Surveys

This study, as stated above, was initiated with the aim of promoting different shells rich in micronutrients generally intended for the trash in the design of food supplements. The work began with a survey of the population to assess their level of knowledge on the importance of micronutrients and more particularly calcium in the body.



Some aspects were taken into account during the investigation. This concerns the sex of the respondents, their ages, their levels of study, their locations, their state of knowledge of the role of micronutrients, the probable sources of calcium that they know, the source of origin of their recipes, etc.

#### Gender of respondents

Of the 120 people surveyed, 68% were male and 32% were female. The percentage of men is the double that of women. The men therefore made themselves more available to answer our questions when women being busier. Furthermore, as the investigators are predominantly men, they would have had an easier time collaborating with men.

#### Level of study of respondents

Concerning the level of study of the respondents, 50% have university level (figure 2). This rate remains the highest. This percentage is followed by that of respondents with a second cycle level with a rate of 24%. The rate of uneducated people is as follows with a percentage of 15%. Primary and undergraduate level respondents are the least numerous with percentages of 5 and 6%.





The high rate noted among the respondents is explained by the fact that the investigators are more in contact with the university environment. This rate is made up of both learners and university teachers.

#### Age of respondents

In relation to the age of the respondents, the age group from 25 to 35 is the most abundant with a rate of 40% (figure 3). This age group is followed by the age group ranging from 15 to 25 years old with a rate of 31%. The next category is that of 35 to 45 years old with a rate of 20%. The age group from 45 to 65 and over covers a low rate of 9%.



Figure 3: Age of respondents

The high rate noted in the age group from 25 to 35 is due to the fact that the survey mainly took place in the workplace of the investigators where the population is quite young. They would also have had an easier time approaching young people than older people.

#### Participation of calcium in the maintenance of the body

To the question of whether, the respondents knew that calcium is a micronutrient which participates in the maintenance of the body, 87% answered in the affirmative and 13% answered no.

All the educated people answered affirmatively to the question. In addition, most uneducated respondents are also aware of the importance of the nutrient. Calcium, the most abundant mineral in the body 76% of respondents who know the role of the mineral have the information that it is the most abundant mineral in the body. 24% did not have the information. Those who did not have the information found themselves in the ranks of the uneducated.

#### **Calcium intake situations**

When asked in what cases respondents feel the need to take calcium, 48% of respondents take it on medical prescription, 33% take it when they feel tired. 17% of respondents naturally use calcium during children's teething period to help relieve the discomfort they encounter. 2% of respondents use the mineral for skin care.

This paragraph indicates that most of the respondents do not need medical advice before formulating recipes supposed to contain calcium. Biological or synthetic source.

When compensating for the lack of calcium, 86% of respondents would prefer calcium from natural sources.



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The reason for this choice is based on the fact that they avoid the side effects of calcium of synthetic origin. This was mentioned by 91% of respondents.

Type of calcium used by respondents 87% of those who use calcium admitted that there are natural sources that contain it. 13% say that it is a mineral always of synthetic origin.

When asked in which substances calcium can be found naturally, 50% of respondents think that calcium is found in different shells (eggs, guinea fowl, and quails), 34% think that the micronutrient can be found in fruits, vegetables and leaves. Some think that it can be found in dairy products, their rate is estimated at 13%. 3% of respondents believe that calcium can be found in other substances such as meat, fish, etc. (figure 4). It is important to emphasize that some have given several opinions on this question, they think that calcium can be found in the leaves as well as in the shells, etc.



Figure 4: Substances which contain calcium

## Satisfaction after using a natural source of calcium

Among those surveyed, 84% use substances that can serve as sources of natural calcium. The rest get their supplies from pharmacies. Those who use natural substances as sources of calcium are divided into two categories: 88% take it orally and 12% use it by mixing it with their bath soap, especially in the case of teething children. Among those who use the mineral orally, 67% take the calcium source substance directly while 33% accompany it with a meal such as porridge, soups, etc.

Almost all of those who use calcium of natural origin revealed satisfaction after its use as evidenced by the rate which is 88%.

## Opinion on the galenic formulation

They are all in favor of formulations of food supplements rich in calcium based on natural shells or dry bone as

natural sources of the micronutrient. Those who have opted for food supplements rich in micronutrients would consider that the doses can be taken into account during conception. Among those who gave their favorable opinion, 68% preferred dosage forms in capsule form and 32% in tablet form. Those who have opted for capsules think that by wanting to formulate tablets that one may have to resort to chemical compounds, hence their reluctance for this form. In addition, since the capsules are coated, they will not be exposed to an organoleptic problem.

## Source of the traditional practitioners' recipe

The study also looked at some sellers of substances supposed to contain calcium to find out from whom they received the different recipes. It appears that 61% of sellers inherited the different recipes from their parents, 33% got the recipes through research and 6% got the recipe from other sellers. The majority of sellers inherit their parents' income. It therefore remains a family heirloom.

#### Satisfaction rate after using a natural recipe

According to our surveys, sellers reveal that 98% of customers are satisfied with their products.

This sequence relating to the survey on the use of calcium reveals that the population is aware of the importance of calcium in the body. According to the comments collected, generally certain signs indicate the absence of these minerals in the body. Also in certain cases of fracture (reconstruction of bones) and teething of children, they are aware of the provision of minerals to accelerate healing. Populations generally rely on the compositions of traditional practitioners, but an excess of this micronutrient called hypercalcemia is a risk for human health that can lead to serious consequences on the body that can affect organs. But this intake must be reframed because excessive intake can lead to serious complications ranging from cardiovascular problems to kidney stone problems as demonstrated by certain authors.41,42 The quantitative study is therefore very important so as not to exceed the normal dose.

# Determination of the mineral composition of the different shells

The mineral composition was determined on the different powders obtained as described above. From the description, it appears that the dosage was done instantly on the direct powder without any transformation.

### Determination of Calcium (Ca<sup>2+</sup>) level

Figure 5 shows the calcium level in the different powders prepared instantly. This percentage varies from one powder to another and significantly by location.



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**SS** = Snail Shells; **CES** = Chicken Egg Shells; **CB** = Cuttlefish Bone; **QES** = Quail Egg Shells.

The histogram shows that cuttlefish bone has the highest level of calcium (90 mg/g of powder) followed by chicken egg shell powder (60 mg/g of powder). Quail egg shell powder is in third position with a rate of (40 mg/g of powder). Snail shell powder showed a low level of 15 mg/g. the values obtained may vary from one treatment to another. These are conditions related to temperature and stirring time. It has been shown that when the temperature is varied, the mineral content also varies. Likewise, the longer the agitation lasts, the more the level of minerals also increases.<sup>43,44</sup>

In the context of this work, two explanations are possible; either the calcium is in abundance in the cuttlebone or the calcium contained in the cuttlebone dissolves more quickly. The shells of chicken and quail eggs being less hard, it is normal for their levels to be higher than the level contained in the snail shell. In some works, to maximize the mineral content, the authors use the formation of ash in an oven between 500 and 600 °C. But beyond these temperatures, it has been noticed that micronutrients undergo greater degradation.<sup>45,46</sup> Egg shells contain other elements in addition to minerals such as protein and vitamins with numerous biological properties that it would be interesting to protect during the valorization of these substances.47 The powder made and used at low temperature can be beneficial in terms of valorization and profitability of other micronutrients. But calcined shells can make the ores more available without the other constituents.

#### Determination of the Magnesium (Mg<sup>+</sup>) level

Figure 6 shows the level of Magnesium in the different powders prepared instantly.



Figure 6: Level of Magnesium in the four powders

Through reading this diagram, it appears that quail egg shells have the highest rate (24 mg/g of powder). This rate is followed by that of eggshells (20 mg/g of powder). The levels observed in cuttlefish bone and in snail shells are identical and low. This rate amounts to 3 mg/g of powder. This result shows that the magnesium in quail egg powder solubilizes more quickly or is in large quantities than in other cases. The low rate observed in cuttlefish bone would be due to the fact that magnesium was solubilized in large quantities in seawater before their release on the shores. This rate always remains low with snail shell powder. This shell being harder, the magnesium, even if it was in significant quantity, can struggle to solubilize. The determination of calcium and magnesium levels is generally found optimally in chicken egg shells.

#### Determination of the potassium (K<sup>+</sup>) level

Figure 7 shows the potassium rate in the different powders prepared instantly.



Figure 7: Potassium level in the four powders

The potassium rate remains higher in the case of cuttlefish bone (0.95 mg/g of powder) and snail shell (0.59 mg/g of powder) (figure 7). The level remains low with the shells of quail eggs (0.42 mg/g of powder) and in the chicken



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eggshells (0.38 mg/g of powder). The high level of potassium observed in cuttlefish bone and the snail shell can be explained by their environments of origin. Indeed, the sea is loaded with minerals, this also explains its very salty taste. The snail also lives permanently on the ground also loaded with minerals and sometimes containing fertilizers.

#### Determination of sodium (Na<sup>+</sup>) level

Figure 8 shows the sodium level in the different powders prepared instantly.



Figure 8: Sodium rates in the four powders

The sodium level (2.23 mg/g of powder) remains significantly high in the case of cuttlefish bone compared to other levels. As in the previous case, it is followed by the rate of snail shells (1.45 mg/g of powder). The rate of shells is as follows with a content of 1.18 mg/g of powder. Quail eggshells have the latest sodium level as in the previous cases with a content of 0.9 mg/g of powder. The observation with these two minerals is the same as in the previous case of determine of potassium rates.

#### CONCLUSION

The population at various levels is well aware of the importance of micronutrients for the maintenance of the body. Studies carried out indicate that the calcium level is higher in cuttlefish bone. High levels of magnesium have been noted in quail egg shells. Calcium and magnesium levels remain relatively high in chicken eggshells. These rates are significantly low at the level of the snail shell. When considering the levels of sodium and potassium ions, they are higher in cuttlefish bone and snail shell. Due to the fact that calcium and magnesium are generally the minerals most required in large quantities in the body, compared to a low demand for Na<sup>+</sup> and K<sup>+</sup> ions, it appears that Eggs and quails shells are very good sources of calcium that can be used in the formulation of food supplements rich in micronutrients.

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