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Microbial and Qualitative Analyses of Stingless Bee Bread using Dry Preservation Methods

Rofela Combey*

Department of Conservation Biology and Entomology, School of Biological Science, University of Cape Coast

ABSTRACT

Bee bread is an extremely nourishing tonic made of honey and pollen by bees. However, most bee keepers do not harvest this hive product due to lack of knowledge on the proper preservation method. The main objective for the research was to access the best method of preserving stingless bee bread using dry preservatives. The research involved collecting bee bread pots from a stingless bee hive and subjecting them to various dry preservative methods such as refrigeration, freezing and drying under base parameters such as pH, taste, color, moisture content and microbial load which were carried out in the Department of Molecular Biology and Biotechnology laboratory. Plate Count Agar was used for the detection of bacteria while Sabouraud Agar for the detection of fungi. Yeast and Penicillium were found to be the main fungal group associated with the bee bread. Results obtained also showed that drying preservation method had the least amount of bacteria counts of 1.83×106cfu/g which implies that dry preservative method is the best method of preserving bee bread as product development strategy in Beekeeping industry.

Keywords: *beebread, bacteria, yeast, stingless bees, shelf life*

INTRODUCTION

Bee bread is an extremely nourishing tonic made of pollen and honey [1,2]. This is the main source of protein food for most honey bees and their larvae which is gathered by the worker bees [3-5]. Bees collect pollen as a high protein product to feed young bees and brood in the hive. However, it is usually fed to young brood as 'bee bread', that is pollen which has been stored in the cells of the comb and undergone a natural fermentation process. Stingless bee bread has a distinctive bush taste, a mix of sweet and sour with a hint of fruit. The taste comes from plant resins which the bees use to build their hives and honey pots and varies at different times of year depending on the flowers and trees visited [6].

Most bees store pollen in the comb or pots, by further addition of sugars and enzymes which creates bee bread through lactic acid fermentation [7]. According to Roubik [8] the term bee bread refers to the pollen stored by the bees in their combs where the bee bread is processed by the bees for storage with the addition of various enzymes and honey, which subsequently ferment.

This hive product consists of 55% carbohydrates, 35% protein, 3% minerals and vitamins, 2% fatty acids and 5% other substances considered to be antioxidants, including beta-carotene, lycopene, selenium, flavonoids, vitamins C, E and K [9,10]. However, Green [11] and Wong [3] reported that, bee bread contains more than 40% plant carbohydrates, 5% plant fats and 40% protein which is far more amino acids than beef, eggs and cheese of equal weight.

The composition of most bee bread changes from species to species, thus species of the genus Trigonia are known to have bee bread containing 25% vegetable protein, including at least 18 amino acids and also more than a dozen vitamins, variation in absolute amounts of the different compounds can be very high. Protein contents of above 40% have been reported in stingless bee bread but the typical range is 7.5% to 35% (typical sugar content ranges from 15% to 50% and starch content is very high [12]. Partially fermented pollen mixture stored in the stingless bee combs, also

referred to as "bee bread" has a different composition and nutritional value than the field-collected pollen pellets. It is the food given to stingless bee larvae and eaten by young worker bees to produce royal jelly [13]. Bee bread contains enzymes which are essential biological catalysts during the digestive process. The enzymes found in bee bread include amylase, catalase, cozymase, cytochrome, dehydrogenase, diaphorase, diastase, lactic acids, pectase and phosphatase [12,14].

Economic Importance of Bee Bread

Although not a 'perfect food' as so often claimed, its nutritional value could be useful to those with unbalanced or deficient diets. In developing countries, the use of bee bread may be more preferable to fresh pollen because the nutritional value is higher, the product has a longer shelf life and it can more easily be transported to market. It is also attributed with antibiotic properties and honey can be added to make it less sour and more attractive to taste [12].

It is known that bee bread allows more oxygen to reach the body and brain cells by acting almost as an atherosclerotic flushing agent thus stamina is increased for the individual. It also causes rapid weight gain and energy increase for convalescents and demonstrates a regulatory action on intestinal functions; both on constipation as well as in cases of diarrhoea. Bee bread produces a calming, tranquilizing, and sedating effect without any side effects; it also provides a rapid increase in haemoglobin, especially in cases of anemia and helps to flush out the impurities and toxins that constantly pile up in capillaries from stress, the taking of drugs, and exposure to the various pollutants of modern societies [15]. Bee bread also provides those chemical substances from which are formed glands, muscles, hair and vital organs such as the heart. In addition, it also furnishes those essential materials that are necessary for the repair of any worn-out cells or tissues. Furthermore, bee bread regulates the intestines by destroying or weakening any harmful bacteria while simultaneously promoting the growth of health-giving species [12].

Bee bread protects against any insufficiencies in vitamins, minerals and amino acids especially during pregnancy, lactation, and intensive physical or mental work and also permits achievement of optimal physical and intellectual output [7]. Bee bread works wonders in a weight-control or weight-stabilization regimen by correcting a possible chemical imbalance in body metabolism that may be involved in either abnormal weight gain or loss. The normalizing and stabilizing effects of this perfect food from the bees are phenomenal [1]. Stingless bee bread is very rich in nutrients and can be used as food supplement for man but not much has been done on its preservation method. Neither has its potential as value added product in bee hives been explored for additional income. In view of this, it has become necessary to carry out this study: microbial and qualitative analyses of stingless bee bread using dry preservation methods for bee bread in stingless bees.

MATERIALS AND METHODS

Study Area

The research involved both field work and laboratory work. The field work was carried out at the International Stingless Bee Keeping Centre at Abrafo in the Central Region of Ghana. Abrafo is about 5km from the University of Cape Coast campus and 1.5km from Kakum National Park. The International Stingless Bee Keeping Centre is a research Centre of the University where training, research and stingless bee culturing are carried out while laboratory work was carried out at the Microbiology Laboratory at the Department of Molecular Biology and Biotechnology, University of Cape Coast.

Field Work

Three different stingless bee hives were opened in turns using a hive tool that has been sterilized to prevent the introduction of any bacteria and was critically observed to identify pots containing bee bread. A knife was used to cut pillars that have been created by the bees to secure the pots in the bee hive. Though bee bread pots are separate from honey pots, the two were joined together and so both were collected and placed in a tray, the bee hives were then closed. The pots were then observed critically for isolation of the bee bread pots from the honey pots. The bee bread pots were then placed in plastic envelopes and brought to the lab.

Laboratory Work

Determination of various base parameters

The following base parameters: pH, moisture content, microbial load, taste and colour were tested for and recorded before the various preservative methods were administered to the bee bread. To test for the microbial load, plate count agar (PCA) and sabouraud agar were used. The plate count agar was used to detect the presence of bacteria, whilst the sabouraud agar was used to test for the presence of fungi. For the plate count agar, 8.75g of powdered PCA was weighed into 1000ml beaker and 500ml distilled water was poured into the beaker, a rod was placed in it and the mixture was placed on a magnetic stirrer for five (5) minutes for uniform stirring of the mixture. It was then transferred into a volumetric flask and corked. The mixture was autoclaved at 121^0C for 45 minutes after which it was left in the hood for a day for it to solidify.

In preparing 500ml sabouraud agar, 20g maltose, 5g peptone and 7.5g agar was weighed into a 1000ml beaker. 500ml distilled water was poured into the beaker, a rod was placed in it and placed on a magnetic stirrer for five (5) minutes to ensure uniform stirring of the mixture. The mixture was then transferred into volumetric flask and autoclaved at 121^0C for 45 minutes after which it was left in the hood for a day to solidify.

To determine the microbial load of the bee bread, 2g of the bee bread was weighed and dissolved in 18ml of sterile distilled water and serial dilutions of the beebread were prepared to 106.1ml of the 106 diluent was inoculated onto the plate count agar and sabouraud agar using the pour plate method. The PCA cultures were incubated for 18 hours at 30^0C. After the incubation, the cultures were observed and identified using morphological features. The number of variable colonies grown on these cultures was counted using free hand. Cultures for the isolation of fungi were incubated at room temperature for 5days, the fungi isolate was identified based on morphological features such as color and shape. The identification was done by the help of Dr Sekyi Agyirifo. All microbial work was done under aseptic conditions.

In determining the moisture content of bee bread, 80g of bee bread was weighed and air dried until the attainment of a constant weight which was recorded. The moisture content was calculated as follows: $\%MC = \frac{A-B}{B} \times 100$

Where:

% MC=Percentage moisture content

A=Weight of wet sample (grams)

B=Weight of dry sample (grams)

To assess the pH of the bee bread, the serial dilutions of the bee bread made for the detection of the microbial load was used and this was done by using a pH meter to measure the pH of each diluent of the bee bread. The color was determined by personal observation whilst it was tasted to determine the taste.

Determination of Various Methods of Preservation on the Bee Bread

The bee bread pots were divided into three parts. One part of the closed pot of bee bread was placed in a plastic envelope and placed in a refrigerator at a temperature of -5 to -7°C for three days to monitor the qualitative changes that would occur in the beebread. The second part was placed in a plastic envelope and put in a freezer at a temperature of -10°C for three days to assess the changes that would occur in the fresh bee bread. The third portion was placed in a petri dish that was washed with distilled water to ensure that there is no existence of any microbe that may attack the bee bread. The bee bread was then placed in an oven and dried at a temperature of 25 to 30°C for one day since prolonged heating of the bee bread can lead to it burning and also losing most of its nutrients. A closed pot of bee bread was used as a control which was compared with the other bee bread that was administered to the various dry preservative methods and this was kept on a shelf.

Data Analyses

The data was coded and analyzed using the one way ANOVA software to determine the significant difference in the various dry preservative methods. Results are presented in tabular form for each assessment.

RESULTS

In this study that assessed microbial and qualitative analyses of stingless bee bread using different dry methods, oven drying is the best method of preserving bee bread outside the bee hive with least bacterial count of 1.83×10^6 cfu/g, moisture content of 6.5% and very acidic with a pH of 3.5 (Table 1).

Table 1: Qualitative analyses results on bee bread.

Methods of Preservation				
Base parameters	**Control**	**Frozen**	**Refrigeration**	**Oven dried**
pH	4.8	5.4	4.9	3.5
Taste	Sour to bitter	Bitter	Bitter	Bitter
Moisture content	21%	25%	15%	6.50%
Colour	Yellow with whitish substance in it	Yellow with whitish substance in it	Yellow with whitish substance in it	Brown

Table 1 shows the qualitative changes that occurred when the bee bread was administered to the various preservative methods. The frozen and refrigeration method had been bread color as the freshly collected one but the oven dried method changed color to brown after heating.

Assessments that analyzed microbial loads for each preservative method indicated that oven dried bee bread habour least microbes, mainly bacteria and fungi (Table 2).

Table 2: Mean bacterial load on stingless bee bread (×106cfu/g) after culturing**.**

Methods of Preservation				
Replicates	**Control**	**Frozen**	**Refrigeration**	**Oven dried**
1	247	54.7	62	24
2	273	23	34	15
3	289	38	43	16
Mean	269.67	38.57	46.33	18.33

For the three sampling times, the dried bee bread had the least mean bacterial load, 18.3×10^6 cfu/g. The control had the highest bacterial count in the bee bread, 269.7×10^6 cfu/g. Bee bread from the refrigerator had more bacterial load than bee bread that was frozen (Table 3).

Table 3: Fungal load on stingless bee bread (×103cfu/g).

Fungi		Methods of Preservation		
	Control	**Frozen**	**Refrigeration**	**Oven dried**
Penicillium	+	-	-	-
Yeast	++	++	++	++
Legend: - Absent; + Present and ++ Highly Present				

Penicillium sp. was present in the control but absent in the other three preservative methods whilst Yeast was highly present in all the three preservative methods (Figure 1).

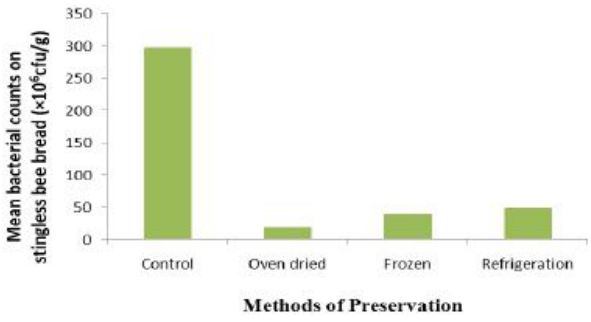

Figure 1: Mean bacterial counts of stingless bee bread cultures (×10⁶cfu/g).

The oven dried bee bread had the least mean bacterial load and control had the highest bacterial count in the bee bread. Bee bread from the refrigerator had more bacterial load than bee bread that was frozen (Appendix).

DISCUSSION

The shelf life is the recommendation of time that products can be stored, during which the defined quality of a specified proportion of the goods remains acceptable under expected (or specified) conditions of distribution, storage and display [12]. For expansion of the shelf life and preservation of useful properties of bee bread, its biological activity does not affect the disruption of the useful components [7].

The frozen and refrigerated bee bread had the same color as the one in the pot which served as the control. This could be due to the fact that the bee bread was not made to come into contact with the external environment. The taste for the bee bread in the pot was sour to bitter compared to the oven dried, freezed and refrigerated bee bread which was bitter due to the qualitative changes that occurred in administering the preservative methods. According to Coe [1] fresh bee bread has a moisture content of 10% to 20%, while dried bee bread is 4% to 6%. The findings of the research indicated that the moisture content of the control experiment was 21% which could be attributed to environmental factors such as humidity, temperature, etc. The moisture content for the various preservative methods administered were as follows; the oven dried method had a moisture content of 6.5% as a result of the fact that much of the water was made to evaporate which is similar to Coe's [1]. The moisture content for the frozen and refrigerated methods were 25% and 15% respectively. This could be due to humid environment of the freezer and refrigerator which subsequently increased the moisture content of the bee bread.

Fermentation produces a pleasant degree of acidity (ideally pH 3.6-3.8). Some bee bread species may promote excessive yeast growth but this does not spoil the bee bread [12]. The results of the experiment show that the pH for the control, frozen and refrigerated bee breads was 4.8, 5.4 and 4.9 respectively. This may be due to the higher moisture content that might have influenced the acidity in spite of the increased activity of bacteria. The oven dried method had a pH of 3.5, which may be attributed to the release of toxins by bacteria when the bee bread was heated, thereby increasing the acidity.

Further, it was observed that there was no color change between the control experiment, the frozen and refrigerated method of preservation due to the high moisture content of the bee bread whilst the oven dried bee bread had a brown color that may be due to the reduced moisture content during the process of drying. The taste of the bee bread for the various methods was very bitter as compared to the control which was sour to bitter.

The results obtained after culturing the stingless bee bread showed that the control method had the highest bacterial count whilst the dried method had the least bacteria count and this could be due to some environmental factors that acted on the bee bread when it was been brought from the bee hive. The least bacterial count for the dried method could be due to the fact that the heat from the oven killed most of the bacteria that might have been present in the bee bread. The frozen and refrigerated methods had moderate amounts of bacterial growth, and this could be due to the fact that some bacteria survived the temperature that the bee bread was frozen and refrigerated though it suppressed the activity of others.

Fungal load for the stingless bee bread recorded *Penicillium sp.* for the control method but was absent for the other preservative method and it can be due to the fact that the fresh bee bread had qualities that favoured the growth of *Penicillium sp.* but when it was administered to the various preservative methods, the conditions in these methods suppressed the growth of the *Penicillium sp.* Yeast was highly present in all the preservative methods which signify that all conditions administered to the various preservative methods favoured its growth which is similar to the findings of food and agricultural organization [12].

The ANOVA analysis showed significant differences among the bacterial load in the various preservative methods. The oven-dried method had the least bacterial load whilst the control had the highest bacterial load which was due to the fact that the temperature at which the bee bread was dried, suppressed the growth of most bacteria whilst in the control the conditions favoured the growth of most bacteria (Appendix). According Anderson et al. [16], in spite of bee breads' microbial diversity its preservation environment is similar to honey, and contains consistently low microbial biomass. Thus, this important hive product must be explored as addition generating venture by stakeholders in beekeeping.

CONCLUSION AND RECOMMENDATION

The research proved that oven dried method had least bacterial count of 1.83×106cfu/g, moisture content of 6.5% and very acidic with a pH of 3.5. Comparing this to the other methods, it can be said that oven drying is the best method of preserving bee bread outside the bee hive.

- Closed pots of stingless bee bread were successfully collected from the bee hive.
- The physical parameters of the freshly collected bee bread had a pH of 4.8, moisture content of 21%, the taste was sour to bitter and the color was yellow with whitish substance in it.
- The taste of the bee bread was bitter for the three preservative methods. The pH was 3.5 for the oven dried method but 4.9 and 5.4 for the refrigerated and frozen method with moisture content being 6.5%, 15% and 25% for oven dried refrigerated and frozen method. The frozen and refrigerated bee bread had the same color of yellow with whitish substance in it but the oven dried bee bread had a brown color.
- The fungi that were associated with the bee bread in the research were *Penicillium sp.* and Yeast with yeast being present in all the preservative methods.
- It is recommended that further research should be carried out on stingless bee bread to find out the specific bacteria associated with it.

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