

Comparative study of the microbiological and physico-chemical quality of cooked local and imported chicken sausages marketed in Benin

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I. Introduction

In order to curb the dietary problems caused by protein deficiency, the FAO estimates that the demand for meat should increase by 200 million tonnes between 2010 and 2050, which is practically a doubling of the volumes currently produced (ANDRIAMIFIDY, 2001). Given the important place that meat and these processed products occupy in human nutrition, it is important to know how to preserve these perishable foods without losing their nutritional value (FAO, 2007). Indeed, the sausage provides the human body with proteins of good biological value, vitamins, minerals, lipids and energy, like all food products, they deteriorate quickly especially when the storage conditions are bad. (Lobo, 2010; Rakansou, 2008).

In recent years, the meat sector in Benin has been restructured and we have seen the emergence of processing industries of the charcuterie type specializing in the production of fine pate sausage and merguez, etc. Broiler chicken has improved. spectacular productivity, thanks to the concomitant progress in breeding methods, nutrition and veterinary medicine, which is why it is increasingly used by processing industries. A development of methods of processing and storing food products then contributes to the production of food of good quality and having a satisfactory nutritional as well as organoleptic property.

Concerning the production of sausages, several varieties are found on the Beninese market. They are made from a wide variety of raw materials. Among them are broiler meat, beef and fish which still dominate this processing sector. The use of poultry meat occupies a large place because of its distinctive characteristics compared to other types of meat.

The present work mainly aims to enhance the consumption of thin chicken sausage in Benin through the production of sausage in Benin and the evaluation of the microbiological and physico-chemical quality of both imported and local sausages.

Keywords : cooked sausage, local chicken, marketed, quality

1. STUDY FRAMEWORK, MATERIAL AND METHODS

1.1. Study framework

1.1.1. Industry overview

VETAGRO SA is located in the Pahou / Ouidah / Benin district. Created in August 2018, the latter aims to reconcile the needs of both categories by supervising breeders who sell their products (broiler / table eggs) at the factory, which are processed to meet the needs of consumers who can access the factory to dispel their doubts. The company has two (2) departments: Sauces and Charcuteries. The sauces department is responsible for the manufacture of mayonnaise, ketchup, and vinaigrette, while the charcuterie department is responsible for the manufacture of sausages, hams, mortadella and meatballs.

1.1.2. Presentation of analytical frameworks

The microbiological and physico-chemical analyzes on our products were carried out at the Food Technology Engineering Laboratory (GTA) of the Polytechnic of Abomey Calavi (EPAC) and at the Central Food Safety Laboratory (LCSSA), in Benin. .

1.2. Study material

As part of our study, several pieces of equipment were used. These are the equipment used for local sausage production and that used in the laboratory for microbiological and physico-chemical analyzes.

1.2.1. Animal material

The animal material used for production and analysis are: Fat and lean from locally raised broilers; local and imported cooked chicken sausage.

1.2.2. Production material:

Knives, bleeding bars, plucker, freezer / cold room, chopper with several fixed perforated plates, cutter with movable bowl, pusher with portioner, electric cooker, vacuum packer, sterilizer.

1.2.3. Laboratory equipment:

These are the materials (glassware and apparatus) generally found in analytical laboratories.

1.3. Methodology

The present study was carried out in two stages: the first stage is the production of cooked chicken sausage with thin paste; the second stage consists of a comparative study on the microbiological and physico-chemical quality of local and imported sausages.

1.3.1. Technical itinerary of the fine cooked chicken sausage

Before any operation, all the ingredients and additives are weighed. The production of cooked thin chicken sausage begins with chopping, then cutting, pushing or embossing, drying, cooking, packing, canning, and finally storage.

Description of unit operations :

- Reception / slaughter

Broilers are received and slaughtered at a weight greater than or equal to 2Kg. For this purpose knives, the bleeder and the plucker are used.



Photo 1: Bleeding tube



Photo 2: Electric plucker

- Boning / trimming

Boning involves separating the meat of the chicken from the bone. Boning is done with a knife on a cutting plane. Trimming refers to the action of preparing the meat (trimming the meat) by removing the inedible parts.

- Weighing and preparation of the scrum

Lean and fat chicken are weighed to a specified amount ready for chopping. Then the various ingredients, additives and dyes are also weighed using an electric balance, taking into account the amount of raw material.



Photo 3: Lean and fat chicken

Photo 4: Ingredients, additives and colorant weighed

- **Hash**

Chopping or grinding is done with an electric chopper. During this operation the lean and the fat are reduced into very small pieces in order to facilitate the work of the cutter.



Photo 5 : Hash

- **Cuttering**

The Cutterage consists of mixing the minced meat with the ingredients, additives and coloring previously prepared while adding water and ice. Temperature controls are carried out in order to avoid temperature increases that are dangerous for the quality of the finished product. Once the emulsion is obtained, the dough is smooth, elastic and shines. It is sent to the pusher using boats for embossing.



Photo 6 : Cutterage

- **Embossing or pushing**

This step consists of placing the scrum in a gut to give it its characteristic shape.

The pusher is equipped with a portioner which allows the face to be pushed in portions in the casings with automatic twisting. The casings used are reconstituted casings, caliber 19mm.



Photo 7: reconstituted casings



Photo 8 : Pusher

- **Drying**

Once pushed, the sausages are placed in the drying cabinet at an air-conditioned temperature (15-18 C) for 2 hours for a uniform set.



Photo 9 : Sausage placed in the drying cabinet

- **The cooking**

Cooking takes place in the electric cooker. It is done by immersion in water, the product is heated so that the internal microorganisms act to stabilize it. The cooking is done in water at 85 ° C for 10 min and 72 ° C at the heart of the product.



Photo 10 : cooker

The recooling

Once the cooking step is complete, the sausages are placed in the cooling piston (water + ice) for 10 minutes to stop cooking (reach around 25 ° C at the core).

Conditioning :

The cooked sausages are peeled (released from the casings), placed in food bags in batches of ten (10) units. Then packaged using the vacuum packaging machine which allows the vacuum to be expelled and the bag to be hermetically sealed.



Photo 11 : Vacuum packaging machine

Appertisation :

The wrapped sausages are placed in the sterilizer at 100 ° C for 3 minutes.

Labeling and storage :

After canning, the products are labeled and put into boxes. The brand of the hospitality company VETAGRO SA is Mori'fresh. The boxes are stored in the cold room at -18 ° C and can be kept under these conditions for 12 months.

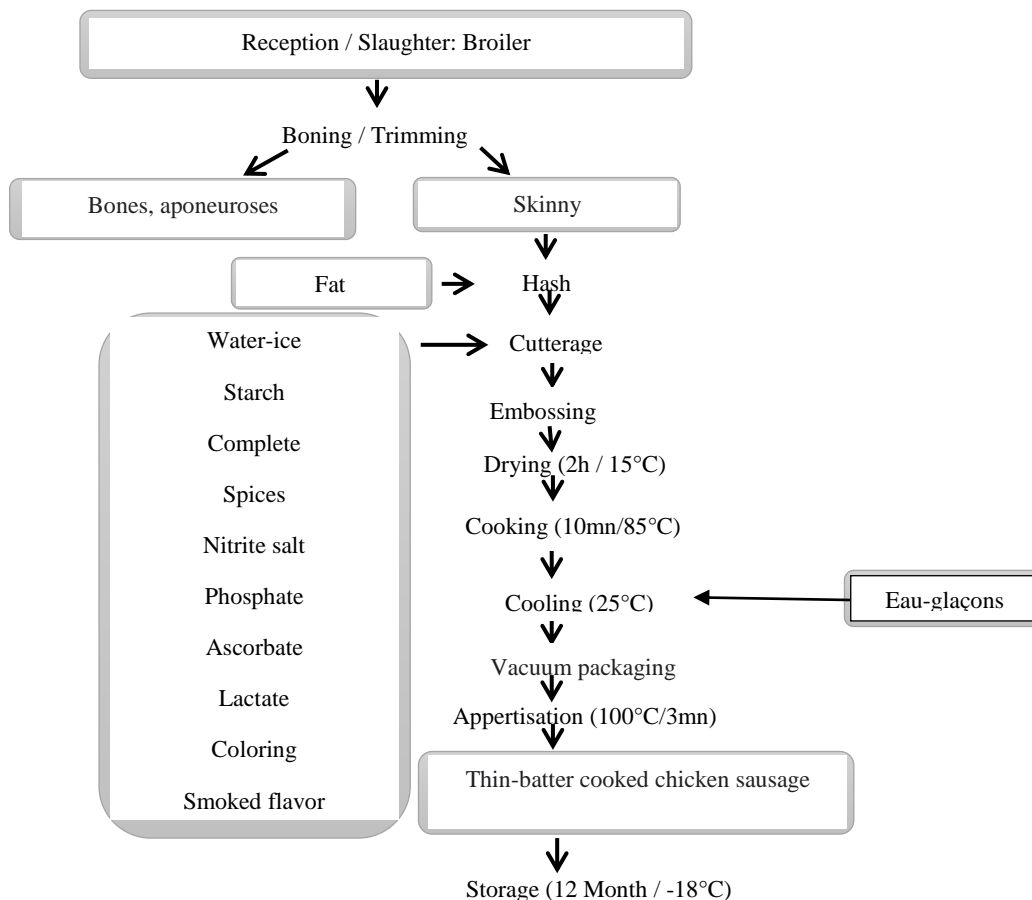


Figure 1: Diagram of how cooked chicken thin-crust sausage is made.

1.3.2. Analysis methods

1.3.2.1. Sampling

Sampling is an often delicate fundamental step in the analysis of products. According to our survey of sausage production and consumption in Benin, two (2) brands of sausages: Mori'fresh (local sausage) and Minuano (imported sausage) are the most consumed in Benin (Gbaguidi et al., 2021). The quality review covers these two (2) brands of cooked chicken thin-crust sausage.

The samples to be analyzed were collected randomly in fishmongers and markets in the municipalities of Abomey-Calavi, Cotonou and Porto-Novo according to the following criteria :

- Availability of both brands,
- Hygienic conditions,
- Installation and equipment

The transport of the samples is done in a clean and sterilized cooler containing ice cubes and sent directly to the laboratory.

Once in the laboratory, samples from the same brand were mixed to obtain the representative sample of the brand to be analyzed.



Photo 12 : Local sausage



Photo 13: Imported sausage

1.3.2.2. Microbiological analysis

In order to assess the sanitary quality of the two (2) types of sausages selected, five steps were necessary:

▪ Preparation of culture media

The instructions to follow are written on the label of the box containing it and depend on each culture medium.

▪ Preparation of stock solution and decimal dilutions

Samples of local sausages (SL) obtained from fishmongers and markets in Cotonou, Abomey-Calavi and Porto Novo were ground using a laboratory mortar (disinfected with alcohol). This same operation is carried out on samples of imported sausages (SI).

Then 5 g of the ground material from each SL and SI sample are each transferred to 10 ml of peptone water. The decimal dilutions are shown in photo 14.

❖ Seeding and incubation

The inoculation is made from dilutions on selective media and specific to each type of microorganism sought and then incubated.

❖ Enumeration

This assessment was carried out using standard microbiological analysis techniques as supports. The germs counted are:

- Aerobic Mesophilic Germs: according to the NF EN ISO 4833-1, 2013 method on PCA culture medium at 30 ° C for 72 hours, from the stock solution to 10⁻⁵.
- Enumeration of Escherichia coli: according to the NF EN ISO 16649-2, 2001 method on TBX culture medium at 44 ° C for 24 hours, from the stock solution to 10⁻².
- Enumeration of coagulase positive staphylococcus: according to the NF EN ISO 6888-1, 2004 method on Baird Parker agar at 37 ° C for 48 hours, from the stock solution up to 10⁻².
- Enumeration of sulfite-reducing anaerobic bacteria (ASR): according to the NF ISO 15213 method on TSC culture medium at 37 ° C for 24-48 hours in a tube, from the stock solution to 10⁻².

▪ **Search for pathogenic germs**

- **monocytogenic listeria** : The search was made according to the standard NF EN ISO 11290-1.
- **Salmonella research** : The search was made according to the standard NF NE ISO 6579; 2017.

1.3.2.3. Physico-chemical analysis

This analysis is based on:

- **pH determination**

The pH measurement of the sausage samples was carried out using a TOOGOO (R) (PH-009 (I)) brand electronic pH meter equipped with an electrode and a digital display screen. . On a mixture obtained from 10 g of sample ground in 90 ml of distilled water. The measurements were taken by immersing the electrode of the pH meter in 10 ml of the filtrate obtained from the mixture. The pH values were read directly from the display screen.

- **Determination of water content (humidity)**

The water content was determined according to the AOAC method (2008). 5g of each sausage sample were weighed in crucibles and then placed in an oven at 105 ° C for 48 hours. After drying, the crucibles containing the samples were cooled in a desiccator and then weighed again. The dry matter content (TMS) of each sample was obtained by the formula :

- P1 (g) = weight of the sample $TMS(\%) = \frac{P1 - P0}{P} \times 100$ and of the crucible after passing through the oven,
- P0 (g) = weight of the empty crucible,
- P (g) = weight of the sample before passing through the oven;
- The water content (TE) itself is determined by the formula :

$$TE (\%) = 100 - TMS (\%)$$

- **Determination of protein content**

The method used is that of Kjeldahl which makes it possible to determine the level of crude proteins from the nitrogen content and using 6.25 as a conversion factor. The method has three (3) steps: mineralization, distillation and titration. 1g of sausage sample weighed in a flask is mineralized by means of concentrated sulfuric acid. Distillation separates and traps ammonia in a solution of boric acid. The titration then makes it possible to determine the ammonium ions contained in the distillate with hydrogen chloride (HCl) (0.1N). The expression used to calculate the protein content (PT) is as follows :

$$TP(\text{en \% de MS}) = \frac{1,401 \times 6,25 (V_e - V_b)}{P_e \times TMS}$$

- Ve = Volume of HCl for sample titration;
Vb = Volume of HCl used for the titration of the control;
6.25 = Nitrogen to protein conversion factor;
Pe = sample weight;
TMS = Dry matter rate (%)

- **Determination of lipid content**

The method used is that of Soxhlet (AACC, 1983) which consists in extracting the free lipids from the sausage sample with petroleum benzine, which is then evaporated. Balloons containing 3 granules of pumice stone are dried, cooled and weighed. 200mL of petroleum ether is poured into it. Then 5g of the sample is introduced into a cartridge closed with degreased cotton. The cartridge and balloon assembly is mounted in a Soxhlet type extractor. Extraction ends after 6 hours and the flasks are removed and evaporated. These are then weighed after drying for 1 hour in an oven (105 ° C) and then cooled in a desiccator.

The lipid content (TL) of the sample is finally determined using the formula :

$$TL (\%) = \frac{P_f - P_i}{P_0 \times TMS}$$

- Pi = Weight of the balloon with the pumice stones before extraction;
Pf = Weight of the balloon assembly, pumice stones and lipids after extraction;
Po = Weight of the sample;

TMS = dry matter content of the sample.

- Determination of the Total Volatile Basic Nitrogen (ABVT) content

The ABVT content of the different sausage samples was determined according to the EC 2074/2005 method. It was carried out in three stages: deproteinization with perchloric acid, steam distillation and then neutralization with hydrochloric acid. In fact, to 10g of crushed sausage were added 90mL of 0.6N perchloric acid and then homogenized. 50 mL of the resulting filtrate was mixed with 6.5 mL of 2N NaOH solution with a few drops of phenolphthalein and silicone antifoaming agent. The mixture was distilled for 10min. The vapor obtained is condensed and collected in 100 ml of boric acid, the mixture is titrated with 0.01 N hydrochloric acid.

The ABVT content of each sample is determined by the formula :

$$T_{ABVT} \text{ (en mg/100g)} = \frac{(V1 - V0) \times 0,14 \times 2 \times 100}{M}$$

V1 = volume of 0.01 mol / L hydrochloric acid in mL for the sample

V0 = volume of 0.01 mol / L hydrochloric acid in mL for the control

M = mass of the sample in g

- Determination of energy

It is obtained by summing proteins, carbohydrates and lipids with their coefficients. Our samples are alcohol free.

$$\text{Energy} = 4P + 4C + 9L + 7A$$

P: Proteins

C: Carbohydrates

L: Lipids

A: Alcohol

II. Results And Discussions

2.1 Results

2.1.1 Analysis of the local sausage manufacturing process

Good manufacturing practices (GMP) and good hygiene (BPH) are more or less respected throughout production. The effort of the staff in terms of hygiene is remarkable. Regarding the raw material used for the sausage, the company works directly with broiler breeders. The director of VETAGRO being a veterinarian, he ensures a permanent follow-up of the farms in order to have raw materials in accordance with the requirements of the factory. The chopping of fat and lean chicken allows us to break them up (come out in the form of granulate) and not only to facilitate the cuttage but also to reduce the cuttage time.

2.1.2 Results of microbiological analyzes

The results of the microbiological analyzes of the various samples are presented in Table 1 below:

Table 1 : Comparative Table of Microbiological Results

Parameters	Method references	Number of colonies (CFU) in the SI sample	Results SI	Number of colonies (CFU) in the SL sample	Results SL	Criteria acceptabilities (CE and AFSSA)	Microbiological quality
Aerobic germs mesophiles (FAMT)	ISO 4833-1, 2013	1,6.10 ⁴ CFU / g	< C.A	2,8.10 ³ ufc / g	< C.A	3. 10 ⁵ ufc/g	Satisfactory
<i>Escherichia coli</i>	ISO 16649-2, 2001	< 10 CFU / g	< C.A	Absence	< C.A	10 ² CFU / g	Satisfactory
<i>Staphylococcus aureus</i>	ISO 6888-2, 2003	17 CFU / g	< C.A	10 ² ufc/g	< C.A	10 ³ CFU / g	Satisfactory
Total Coliforms	JORA : N° 5 du 27-05-1998	< 10 CFU / g	< C.A	Absence	absence	10 CFU / g	Satisfactory
ASR	ISO 15213	30 CFU / g	< C.A	< 10 CFU / g	< C.A	10 ³ CFU / g	Satisfactory

Listéria monocytogènes	ISO 11290-1	absence	Absence	Absence	Absence	abs/25g	Satisfactory
Salmonella spp	ISO 6579-1, 2017	absence	Absence	Absence	Absence	abs/25g	Satisfactory
Yeasts and molds	ISO 21527-1 2008	Absence	Absence	1	< C.A	Présence	Satisfactory

SI = imported sausage; SL = local sausage

If only one of the germs (GAM, E. coli, Coagulase + Staphylococcus, ASR, Salmonella and L. monocytogens) sought is present in a number greater than the reference criterion in a sample, the product concerned is of unsatisfactory microbiological quality.

2.1.3 Results of physico-chemical analyzes

In Table 2 are presented the physicochemical results of imported and local sausages. Analysis showed that the different samples consist of macronutrients (carbohydrates, proteins and fats) and other minor constituents.

Table 2 : Physicochemical results of imported and local sausages

For 100g of sausage	Imported Sausage	Local Sausage	Regulation UE N° 1169 / 2011
Energy	206,42 Kcal	191,68 Kcal	271 Kcal
Carbohydrates	1,5g	1,35g	1,24g
Lipids	16,5g	14,68g	23,4g
Protein	12,98g	13,54g	13,7g
Dietary fiber	1g	1g	> 0,5g
Sodium chloride salt (Nacl)	2,2g	1,76g	2g
Water content	56,7%	58,4%	60%
Ph	5,9	6,1	5,8-6,5
ABVT	17,97mg / 100g	18,01mg / 100g	- Good freshness: <15mg / 100g - Normal freshness: 15 to 40mg / 100g - Doubtful case: from 40 to 600 mg / 100g - Unsuitable:> 600mg / 100g

The diagrams below show the mass summaries of each type of sausage.

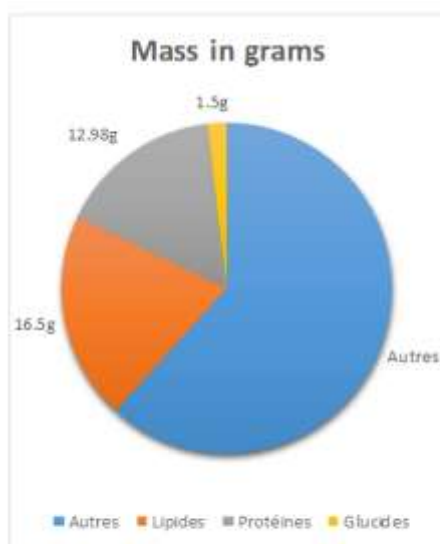


Figure 2: Imported sausage macronutrient bulk summary

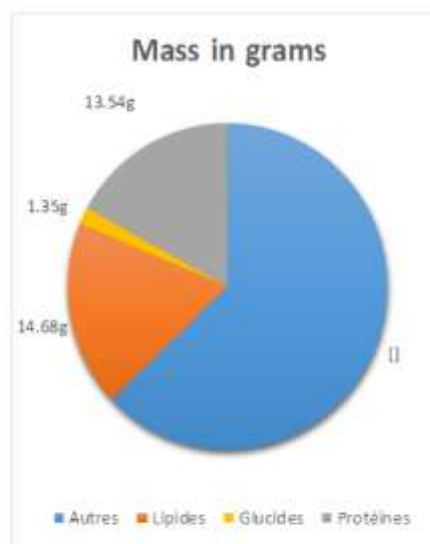


Figure 3: Local sausage macronutrient bulk summary.

2.2 Discussion

The use of ice cubes instead of water allows us to keep the temperature of the face as low as possible to avoid dangerous temperature increases for the finished product (favorable environment for the rapid multiplication of germs, influences the emulsion). Hence the need for permanent temperature control during

cuttering. This rise in temperature is due to the friction of the tears of the cutter. For this the speed and cutterage time go hand in hand. The weight 34g and 12 Cm inscribed in the poursoi for each portion of sausage is in accordance with the characteristics of the frankfurt type sausages available on the Beninese market. Cooking at 72 ° C at the heart allows the solidification of the proteins without their destruction and the gelation of the carbohydrates which gives the final product solidification. Rapid cooling with ice water allows cooking to stop immediately. The vacuum packaging frees the area of the bag and the product and then hermetically seals the bag so the product is stored for longer. Sterilization (canning) eliminates the germs of recontamination of the finished product during handling after cooking. Storage at a temperature of -18 ° C complies with standard EN 12830, limiting the proliferation of microorganisms.

These microbiological results show that neither the SI sample nor the SL sample was contaminated with the *pathogens Salmonella* and *Listeria monocytogenes*. Their absence in 25g of sample is indicative of quality products because the risk of poisoning by these pathogenic germs is eliminated. The absence of these germs testifies to good hygiene and production practice. However, the samples have a contamination rate by Mesophilic Aerobic Germs of $2.8.10^3$ cfu / g for SI and 16.10^3 cfu / g for SL. These contamination rates are lower than that prescribed by the standard (3.10^5 cfu / g). Mesophilic aerobic germs are the primary sources of food contamination, they are also referred to as spoilage germs capable of altering the marketable quality of the product. Their presence beyond the standard can mean a lack of hygiene in handling, during processing of the raw material and poor storage conditions (Poor management of the time-temperature pair; break in the cold chain, etc.). Since this rate is below the standard, the product would be processed in good hygienic and storage condition. For *staphylococcus aureus* with possible coagulase, the quantity of germ enumerated for SI and SL respectively 10^2 cfu / g and <10 cfu / g is lower than the regulatory standard (10^3 cfu / g). *Staphylococcus aureus* are potentially pathogenic germs causing food poisoning when the counted quality is higher than the normative value. Their presence in charcuterie products indicates post-cooking contamination, especially whenever there is direct contact between humans and the product (saliva, sweat, etc.). As for the hygiene indicator germs: *E.coli* and ASR; they are present with a contamination rate lower than the normative criteria. Thus, there is (<10 cfu / g) of *E. Coli* in both SI and SL samples while the standard requires a rate $<10^2$ cfu / g. In general, their presence in products is frequently related to contamination of faecal origin. The ASR contamination rate enumerated is 30 cfu / g for the SI sample and <10 cfu / g for the SL sample. These rates are satisfactory compared to the standard 10^2 cfu / g. This observation may be due to the beneficial effects of cooking; as JAOFARA (2016) says: "cooking is often considered as a means of correcting errors during the preparation phases (poor handling or poorly mastered hygiene)". Contamination in ASR is often before cooking. Their presence in the samples could be the object of non-observance of the cooking time or of improper sterilization. According to the selected microbiological acceptability criteria, for both types of sausage the microbiological quality is satisfied. However, there are some differences in the rate of contamination between the two sausages at the level of GAM germs, *Staphylococcus aureus* and ASR. The presence of *Staphylococcus aureus* and ASR in local sausages is low compared to the imported one. Only the GAM has a high value for the local sausage.

The physico-chemical analysis reveals that the products are compliant for the determinations made, in accordance with EU Regulation No. 1169/2011 for the various analysis values achieved. But the two sausage samples show variability in the content of its chemical components. For the carbohydrates which constitute the quantity of energy material contained in the sausage, the imported sausage has a higher rate (1.5g) than the local sausage (1.35g). This high carbohydrate value is thought to be due to the starch added during the preparation of the sausage or to the feed of the chickens during breeding (VIERLING, 2003). Regarding the lipid content of sausages, it is between 14.68g and 16.5g for respectively 100g of local sausage and imported sausage. These rates, even when high, are lower than the normative value which is 23.4g. The high level of lipids in the imported sausage could be justified by the fact that the climatic conditions of the countries of origin of these sausages tolerate the consumption of a high level of fat to manage the temperature differences. On the other hand, a high lipid content of local sausages could constitute a health problem for Beninese consumers. For the protein content, the local sausage has a rate of 13.54g while the imported sausage has a rate of 12.98g per 100g of sausage. These different rates meet the standard which is 13.7g per 100g of sausage. This is due to the quality of the meat and the monitoring of the chickens from the farm to the factory. These results corroborate those of Gandemer (1992) who showed that chicken is an excellent source of protein, 40 of the amino acids are essential amino acids. For the water content, it is between 56.7% for imported sausage and 58.4% for local sausage. These values come from the lean and water added in the preparation and due to the properties of the lean to have a high water retention capacity. This can be considered as a quality criterion of the meat used. The total moisture content is not limited, because a meat product is all the more moist the richer in leanness. So the SL is perhaps richer in lean. So the total humidity (HT) cannot be retained as a quality criterion (IBERRAKEN *and al.*, 2006). Total Volatile Basic Nitrogen (ABVT) expresses normal freshness in both sausages (17.97mg / 100g and 18.01mg / 100g). This is because the meats used for making sausages are of adequate quality. From all of the

above, we can retain that the physicochemical characteristics of the sausage samples are satisfactory compared to the normative values even if there is some variability in the value of these different parameters.

III. Conclusion

Of all the above, the VETAGRO company located in Pahou, BENIN, is the only one that produces thin-paste chicken sausage raised locally. This same type of imported sausage most consumed in BENIN is MINUANO brand and comes from BRAZIL (according to the consumption and production survey). The increasing consumption of sausages nowadays means that they are imported in quantity, but we do not know their quality. As a result, the local sausage was compared with the imported and more consumed one. The results of the analyzes of the two sausages enabled us to make a comparison of their microbiological as well as physicochemical qualities. The types of germs detected on these sausages, kept under the same conditions, exhibited contamination rates (cfu) lower than those prescribed by the various reference standards indicated in Table 1. The results of the physicochemical analyzes show a First of all, that our two sausages comply with the standard even if there is some variability in the value of these different components. Then, these analyzes reveal to us that the imported sausage is much more full of fat and salt which are not pleasant to our health here in Benin because of our climatic conditions. Which brings us closer to the fact that it is good to eat local.

Our results demonstrated the hygienic and nutritional quality of the local sausage. A remarkable effort is observed at the level of the VETAGRO company. Good manufacturing practices (GMP) and good hygiene (BPH) are more or less respected throughout production. Regarding the raw material used for the sausage, the company works directly with chicken breeders. The director of VETAGRO being a veterinarian, he ensures a permanent follow-up of the farms in order to have raw materials in accordance with the requirements of the factory.

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