Int.J.Curr.Microbiol.App.Sci (2024) *13*(10): 122-132

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 13 Number 10 (2024)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2024.1310.017

Effect of Natural Preservatives and Thermal Processing on the Microbiological and Physicochemical Properties of Chicken Sausage during Cold Storage

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Keywords

Poultry, Sausage, Spices, Physicochemical, Microbiology

Article Info

Received:
20 August 2024
Accepted:
29 September 2024
Available Online:
10 October 2024

A B S T R A C T

Poultry meat is gaining popularity because of its competitive price, short growing period, positive nutritional image. In order to fulfil consumer's desire for healthier meat product, local chickens were used to processed chicken sausage using local spices. Two different samples unpasteurized and pasteurized sausage were successfully processed. Physicochemical and microbiological analysis were carried out during cold storage. The pH of the chicken thigh, chicken breast, pasteurized and unpasteurized sausage ranged from 5.96 to 6.09. Dry matter, ash content, liquid content and protein content was recorded as 32.86±4.6, 1.73±0.4, 23.51±3.3, 16.68±0.11 respectively for pasteurized sample and 33.57±1.71, 1.82±0.21, 29.66±3.35, 17.28±0.46 for the unpasteurized sample. Microbiological evaluation for the pasteurized and unpasteurized sample over 45 days heighted the effect of natural spices and the thermal treatment. Enterotoxigenic *Staphylococcus aureus*, ASR bacteria and *salmonella* were not detected in all samples. These results successfully created a standardized method of sausage formulation using natural spices as preservatives and which can easily be adopted by households.

Introduction

Agricultural sector has an important contribution for the economy of developing countries, by generating a large share of the gross domestic product (GDP) (Soviadan *et al.,* 2022). Poultry area is a sub-sector of agricultural which is still growing around the world and it helps the expansion of that sector (McLeod *et al.,* 2009). Chickens, the most important of the domesticated birds, represents more than 63% of all avian breeds because the poultry products are appreciated for their meat and eggs (Pym, 2013). Poultry meat is gaining popularity because of its competitive price, short growing period, positive nutritional image and very few religious restrictions (McDermott *et al.,* 2010). In fact, in developing countries poultry sector contributes to the livelihoods of poor

households as economically income, food nutrient source, in particular protein, sociocultural for hospitality and exchanges of gifts to strengthen social relationship (Tadelle *et al.,* 2003). However, local production is not enough to cover all the needs in poultry meat and it is more expensive than the imported chicken meat, encouraging the importation of more than 99.4% of chicken poultry products in Togo (Touglo *et al.,* 2023).

To reduce the importation, it is necessary to improve the local production of poultry products. In fact, the Government of Togo, has executed different programmes in manner to boost sustainable rural development (UNDP, 2011; Ouédraogo, 2012). In Togo, the National Program for Agricultural Investment and Food Security (PNIASAN) and Agricultural Sector Support Project (PASA) have subvented the smallholder farmers for the embracing of improved technology in traditional poultry activities, in manner to expand the poultry farming, create more wealth, increase food security and lighten poverty (Gauthier & Langlois, 2010). That experience highlighted that the subvention program significantly increased the annual sale of poultry for the participants to the project (283 annual sale) compared to non-participants (31 annual sale) (Soviadan *et al.,* 2022). The increase of the production in poultry sector have to be sustained by projecting conservation and processing methods in manner to avoid losses in case of strong productivity. Sausage is one of the processed products which can be made using chicken meat. Sausage is from the Latin word "salsus", which means salted meat or preserved by salting (Pearson *et al.,* 1996). Sausage is a combination of ground meat, fatty tissue, curing agent, salt, nitrite, sugar and spices filled into casings. It can be heated, fermented and dried in manner to enhance its stability and increase the shelf-life (Cobos and Diaz, 2015; Venturini *et al.,* 2011). In fact, food industries use synthetic chemical preservatives to delay microbial growth, enzymatic activities and oxidative reactions. Preservatives normally stops microbes from multiplying and thereby preventing the spoilage in the processed food product (Dave and Ghaly, 2011).

However, the use of synthetic food additives led to the various food disturbances in human health (Mirza *et al.,* 2017). In fact, the utilisation of Bio-preservation has been shown effective in case of raw and cooked meat that requires the application of different natural extracts obtained from herbs, spices, fruits and vegetables inhibiting lipid oxidation, preserving colour and improving shelf stability. Such benefits were assigned to bioactive compounds like phenolics (flavonoids and non-flavonoids), phenolic terpenes and tannins (Zhang *et al.,* 2010; Hygreeva *et al.,* 2014). In fact, it was reported that garlic implication might inhibit lipid oxidation and increased the shelf-life of chicken sausage (Sallam *et al.,* 2004). Therefore, this study aimed to explore the effect of natural preservatives and thermal treatment on microbiological and physicochemical properties of chicken sausage during cold storage.

Materials and Methods

Local matured Birds were obtained from CERSA (Centre d'ExcellenceRégional sur les Sciences Aviaires) at Université of Lomé, Togo. African spices (ginger, garlic, cloves, black pepper, white pepper, chilli pepper, coriander, cumin, rosemary), sunflower oil, cellulose casing, cow intestine, wheat flour, lemon juice and salt were purchased at the local market of Lomé, Togo.

Sausage Production

Sixty (60) local chickens of 30 weeks old were used in this experiment. The halal (permissible) rules of slaughtering were applied at the slaughterhouse. Ten kilograms of chicken breast meat without bones and skin were ground with the help of a food processor (FUNKOL 800W Electric Meat Grinder) equipped with a 14-cm blade for 5 minutes at the highest speed. 7% of the mixed spice composing of ginger, garlic, chilli pepper, cloves, thyme, black pepper, white pepper, coriander, rosemary and cumin were added in bits to the ground meat while processing. Salt (2%), 5% sunflower oil and 2% of wheat flour were added to the minced meat.

The minced meat was placed in the deep freeze for 1hour 30 minutes. Then it was removed from the fridge and put in the meat grinder for filling into stuffed artificial cellulose casings. Each stuffed sausage weighed approximately 300 g and had a size of approximately 14 cm to 18 cm. The entire processing standardized methodology was carried under in a food processing laboratory.

Pasteurized sample PS was treated with high cooking temperature at 100°C for 10 minutes and the unpasteurized sample (UPS) was kept fresh without heating. Both samples were stored at 4°C for the microbiological analysis which were carried out at day 1, 7, 14, 30 and 45, while the physicochemical analysis were carried out only at the first day.

Physicochemical Analysis

Dry Matter

The dry matter content was determined using AOAC (2002) standard. 20g of each sample was measured into a dried crucible dish of known weight, and transfer to the oven set at 140°C. The samples were dried up to final constant weight, then it was removed and cooled in a desiccator.

Dry matter = $(m_{dm}/m_s) \times 100$

m_{dm}= weight of dry matter and ms = weight of the undried sample.

Ash Content

The Ash content determination, the AOAC (2005), method was used. Five grams of each sample of the initially dried sample (from dry matter) was measured into a previously dried, weighed crucible and transferred into muffle furnace incinerator at 550°C for 6 hours. It was removed and placed to cool in desiccators.

% Ash content = $(m_{ash}/m_s) \times 100$

m_{ash} =mass of ash and m_s = mass of sample

Proteins Content (Semimicro-Kjeldahl Method)

The local chicken sausage sample was weighed as much as 0.5 grams, then put into a 100 ml Kjeldahl flask, then added 0.9 grams of selenium and 2 ml of concentrated H_2SO_4. Then, the solution is heated at a temperature of 410°C for approximately 1 hour until the solution is clear and then cooled. After cooling, 5 ml of distilled water and 20 ml of 40% NaOH were added to the Kjeldahl flask.

Then the distillation process was carried out at 100°C. The distillation results are collected in 125 ml Erlenmeyer flask containing a mixture of 15 ml of 4% boric acid (H_3BO_3), two drops of methyl red and methyl blue. After the distillate volume reaches 40 ml and is bluish-green, the distillation process is stopped. The distillate is irritated with 0.1 N HCl until it changes into pink colour. The titrant volume is read and recorded. Then make a blank solution in the same way without the sample. Protein content was analysed by the following formula:

% N Total = (ml HCl – ml white) x N HCl x 14.008gr sample x 1000 x100%

Protein content (%) = N (%) x conversion factor = 6.25

Total Lipids

The total lipids were extracted with a Soxhlet. The test portion (2 g) dried in an oven at 105° C and ground in the porcelain mortar was introduced into the bags of dried and weighed filter paper. The extraction of the oil was carried out using a Soxhlet (Fat-Extractor E-500 by BUCHI Company) with petroleum ether for a period of 6 hours. Oil content was calculated at 0% humidity by the difference in mass of the bag before and after extraction full of lipids.

The oil content, H per 100g of sample expressed in percent (%) relative to the dry mass is given by the following formula:

$$H = \frac{P_1 - P}{P_1 - P_2}$$

- P_1 the mass of the glass cup containing the test portion before extraction

- P the mass of the glass cup containing the test portion after oil extraction

- P_2 the mass of the empty glass cup

pH Measurement

The pH value of the homogenized sample was recorded in triplicate using a pH meter calibrated with acid (pH 4.01) and neutral (pH 7.00) technical buffer solutions (Seven Easy pH, Mettler-Toledo GmbH, Greifensee, Switzerland) at 22°C according to manufacturer's instruction. The pH of the chicken thigh, breast meat was measured and noted after slaughtering, given that these were the parts used for the sausage formulation. pH of the different samples was noted after processing.

Microbiological Analysis

This aspect of analysis was based on the assessment of the possible microbes present in the processed sausage samples (pasteurized and unpasteurized) before the sensorial analysis. It started with the preparation of

disposable Petri dishes, 0.1% sterile peptone water, preparation of media, serial dilution, labelling of Petri dishes, plating/incubation and ended with counting (enumeration) of observed microbes. Two (2) samples were analysed according to the EC Regulation N°1441/2007. The different methods used for the enumeration of the sausage microorganism were shown in Table 1

Statistical Analysis

Microsoft Excel 2013 spreadsheet was used in organising the raw data, calculating the mean and standard deviation. Microbiological data were converted to log10 CFU/g and subjected to statistical analyses. The mean values of microbiological and physicochemical analysis sampling days were compared by using analysis of variance (ANOVA) post-hoc Tukey's test and LSD and t-student test using a SPSS software statistical analysis was (Version 23.0.1.0, IBM SPSS Statistics, Armonk, New York, USA) was used for all analyses.

Results and Discussion

Physicochemical Properties

pH

The pH of the chicken thigh, chicken breast, pasteurized and unpasteurized sausage were noted during this work. The initial pH values were similar in all the variants of sausages and ranged from 5.96 to 6.09 (Table 2). There was no significant different between the pH of the chicken thigh, breast meat, and unpasteurized sausage. They did not differ from the results of an earlier study conducted on the same samples of vacuum-packed sausages, where the average values measured on the first day ranged from 5.82 to 5.95 (Stangierski *et al.,* 2020). In this study, the pH was significantly lower in the pasteurized sausage sample than unpasteurized samples. Other authors observed a similar tendency in their studies (Muguerza *et al.,* 2002; Delgado-Pando *et al.,* 2010; Domingez *et al.,* 2017). The decrease in the pH of chicken sausage might be caused by the denaturation of certain protein chains during the cooking process.

Dry Matter

The water content in a food product can affect the appearance, texture, taste, and shelf life of the food product (Zhang *et al.,* 2018). Based on table 3, the sausage dry matter content was at 33.02±1.30 and 32.92±0.73 for UPS and PS samples respectively. The dry matter content of two samples presented no significant difference ($p< 0.05$), showing that the heat treatment had no major effect on the dry matter content. This research findings gave a higher value of dry matter comparatively to the work of Piotrowicz *et al.,* (2015). Serdaroglu and Degirmencioglu (2004) reported that a decrease in moisture contents is directly proportional to increasing levels of fat. Similarly, Andre´s *et al.,* (2004) reported that sausages with a higher fat composition contained less moisture.

Ash Content

Ash content in food is a measure of the mineral content, which includes minerals like calcium, phosphorus, and magnesium. The ash content was 1.82 ± 0.21 and 1.73 ± 0.4 for unpasteurized sausage (UPS) and pasteurized sausage (PS) respectively (Table 3). There was no significant difference in the samples. Thus, temperature had no effect on the mineral composition of the local chicken sausage. The standard ash content value for chicken sausage, like other meat products, can vary depending on the specific recipe and processing methods used by the manufacturer. It's typically expressed as a percentage of the sample's weight. The average sausage ash content has a value range of 1.73% -1.82% which falls in line with the standardized quantity noted in minced poultry meat <3% according to the maximum standard of SNI 3820-2015 (Herlina *et al.,* 2021). Reddy *et al.,* (2020) results differed slightly from ours, they had values of 2.02 to 2.14.

Lipid Content

The lipid content range between 23.51±3.3 and 29.66±3.35 for PS and UPS respectively (Table 3). Among the samples there was a significant different and this confirms with the works of Herlina *et al.,* (2021) who also found the same values in their study on "physical, chemical, and sensory characteristics of chicken sausage with analogue meat substitution'' During cooking, sausage meat liquefy, this is a process called rendering, where connective tissues such as collagens begin to contract and squeeze out pink/brown juice from within muscle intracellular fibers into the spaces between the intercellular fibers and out to the surface (frying pan) and that is how liquid is being lost (Gerber *et al.,* 2009). This explains the reason why we recorded a lower lipid content in PS sample. Lipids are

significant nutrients for humans and help many functional and regulatory activities in the human body, such as signal transduction, myelination, and involved in the structural developments of the human body (Goodman, 2010; Zheng *et al.,* 2019).

Protein Content

The average protein contents (g/100 g) were 16.68 ± 0.11 and 17.28 ± 0.46 for the pasteurized (PS) and unpasteurized sample (UPS) respectively (Table 3). There was a significant difference ($p>0.05$). Samard & Ryu (2019) had a similar report in their study, though they worked with broiler chicken breast meat. This shows that there no major difference in the protein content of broiler birds or local birds. All the measurement revealed that samples, had the total proteins content which was between the limits (13 – 25%) imposed by FAO law for meat and meat product, so both products are indicated as a good source of essential amino-acids for human nutrition. Proteins are long chain molecules that is denatured at high temperatures, coagulate when heated, these chains break apart and lose moisture reasons why there was a slight drop in protein value in the pasteurized sample.

Microbiological Analysis

Enterotoxigenic Staphylococcus aureus and *Salmonella* spp. were not found in pasteurized and unpasteurized samples, but ASR was not noticed in the pasteurized samples and in the unpasteurized samples it was less than <10 CFU/ml. All of the bacteria analysed in this study showed a significant decrease due to the heat treatment. The total germ count was found to be less than 5 $\log_{10}$ CFU/g, Enterobacteria count was less than 2 $\log_{10}$ CFU/g and *E. coli* count was less than 2 $\log_{10}$ CFU/g, which are the acceptance level fixed for a ready-to-eat food (Authority, 2009). These results night ensure that the treatment applied in this study is efficient certifying the microbiological quality of our cooked sausage. High Temperature heat treatment at short time applied to pasteurized meat and poultry products after packaging or before packaging serves as an effective methods of eradicating most pathogenic bacteria (Yuste *et al.,* 1999).

Total Germs Count

Total bacterial, colonies enumerated with similar growth trend were discovered in the both samples and showed in figure 1. At the first day, the total germ count was significantly high in unpasteurized samples (7.40 $\log_{10}$ CFU/g) as compared to pasteurized sausage (4.17 $\log_{10}$ CFU/g). In unpasteurized sausage there was no a significant growth over the storage period, but in the pasteurized sample we observed a significantly increased at the day 45th. These results might suggest that the storage have some effect on the microbial growth.

In fact, raw chicken legs stored at 4°C presented a significant increase of total germ count between the day 1 (5 $\log_{10}$ CFU/g) and day 14 (9.13 $\log_{10}$ CFU/g) and its level increased over the storage period (Katiyo *et al.,* 2020). The absence of difference in our study might be associated to the presence of spices which inhibited the microbial growth. In fact, the plant-based products play an important antimicrobial effect in different meat products. Essential Oil of Thymus showed a decrease of level of different microorganism (Juliano *et al.,* 2000). The extracts and essential oils of herbs and spices are generally recognised for their strong antioxidant, antimicrobial and antifungal activities in foods. In fact, in beef, pork, mutton, turkey, and chicken meat products the addition of these herbs and spices and their essential oils extracts showed some effect on the growth inhibition of several food borne pathogens (Vaquero *et al.,* 2010; Hasapidou & Savvaidis, 2011; Hygreeva *et al.,* 2014; Xiong *et al.,* 2022).

Enterobacteria

The level of *Enterobacteria* explored in both samples was shown in figure 2. At the first day Enterobacteria level was significantly high in unpasteurized sausage (4.28 $\log_{10}$ (CFU/g)) compared to pasteurized sausage (1.88 $\log_{10}$ (CFU/g)). In the unpasteurized sausage there was no significant changes in *Enterobacteria* count, while in the pasteurized sausage a significant decrease was observed after 7 days of storage, then it remained constant for over the storage period.

However, the Enterobacteria count was found to significantly increase during the first 10 days of storage in chicken meat during a cold storage, before remaining constant during the rest of storage (Katiyo *et al.,* 2020). The absence of changes in the unpasteurized sausage and the decrease of Enterobacteria count in pasteurized samples might be related to the effect of the natural spices used during the production of the sausage. In fact, the combination of the extract of cloves and rosemary showed an important inhibition of Enterobacteria growth in samples stored at 4°C for 15 days (Zhang *et al.,* 2016).

Table.1 Different food microbes enumerated in the chicken sausage samples

Germ	Reference of the method used	Reactive culture media	Level of dilution	Temperature/duration of incubation
Total aerobic mesophilic germs	NF EN ISO 4833-1	Plate Count Agar	-1, -3, -5	30°C/24 -72hours
Enterobacteria	NF EN ISO 21528-2	VRBL	0, -1	30°C/24hours
E.coli	NF EN ISO 16649-2	Brillance E-Coli	0, -1	44°C/ 24hours
Anaerobic Sulphite Reductors Germs	NF EN ISO 15213	Typotone sulfite Neomycine		37°C/24-48hours
Staphylococcus aureus	NF EN ISO 6888-1	Baird Parker	-1, -2, -3	37°C/24-48hours
***Salmonella*sp.**	NF EN ISO 6579-1	EPT, Rappaport, Hektoen, Galery API 20^{E}		37°C/24hours
Yeast and Molds	NF EN ISO 21517-152	Sabourand + Chloramphenicol	0, -2	30°C/48-72hours

Table.2 pH values of the different parts of chicken and different sausage samples

Samples	Chicken Thigh	Chicken Breast	Pasteurized Sausage	Unpasteurized Sausage
pH content	6.06 ± 0.07^{a}	6.09 ± 0.06^{a}	5.92 ± 0.13^{b}	6.06 ± 0.04^{a}

Alphabets with same letter means No statistically significant differences between means in the same rows ($p < 0.05$; mean ± standard deviation; and different letter means statistically significant differences

Table.3 Nutritional analysis of the pasteurized and unpasteurized sausage

Sample/ Parameter	Dry Matter Content	Ash Content	Lipid Content	Crude Protein Content.
Pasteurized sausage	32.92 ± 0.73^{a}	1.73 ± 0.4^{a}	23.51 ± 3.3^{a}	16.68 ± 0.11^{a}
Unpasteurized Sausage	33.02 ± 1.30^{b}	1.82 ± 0.21^{a}	29.66 ± 3.35^{b}	17.28 ± 0.46^{b}

Alphabets with same letter means No statistically significant differences between means in the same rows ($p < 0.05$; mean ± standard deviation; and different letter means statistically significant differences

Figure.1 Total bacteria Growth for the unpasteurized and pasteurized sausage over the storage period. Different letters indicate significant difference among samples ($p \leq 0.05$), where the small letter referred the difference due to the storage time and the big one to the effect of thermal treatment. The "a" and "A" letters were assigned to the highest value.

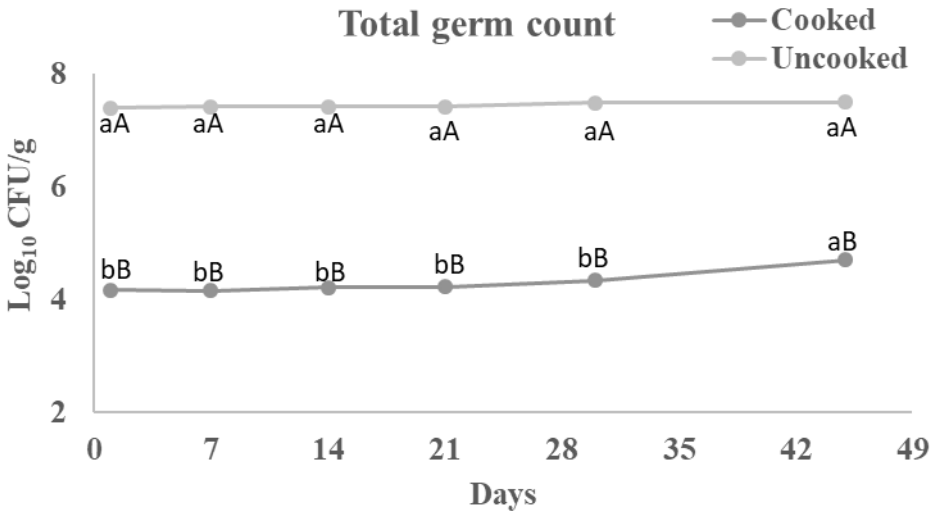


Figure.2 Enterobacteria Growth for the unpasteurized and pasteurized sausage over the storage period. Different letters indicate significant difference among samples ($p \leq 0.05$), where the small letter referred the difference due to the storage time and the big one to the effect of thermal treatment. The "a" and "A" letters were assigned to the highest value.

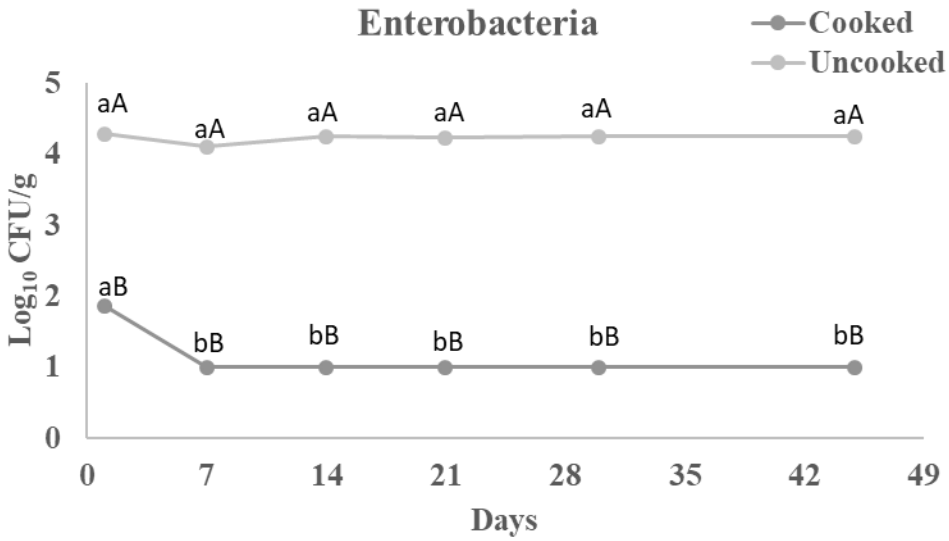


Figure.3 *E. coli* growth rate for the unpasteurized and pasteurized sausage over the storage period. Different letters indicate significant difference among samples ($p \leq 0.05$), where the small letter referred the difference due to the storage time and the big one to the effect of thermal treatment. The "a" and "A" letters were assigned to the highest value.

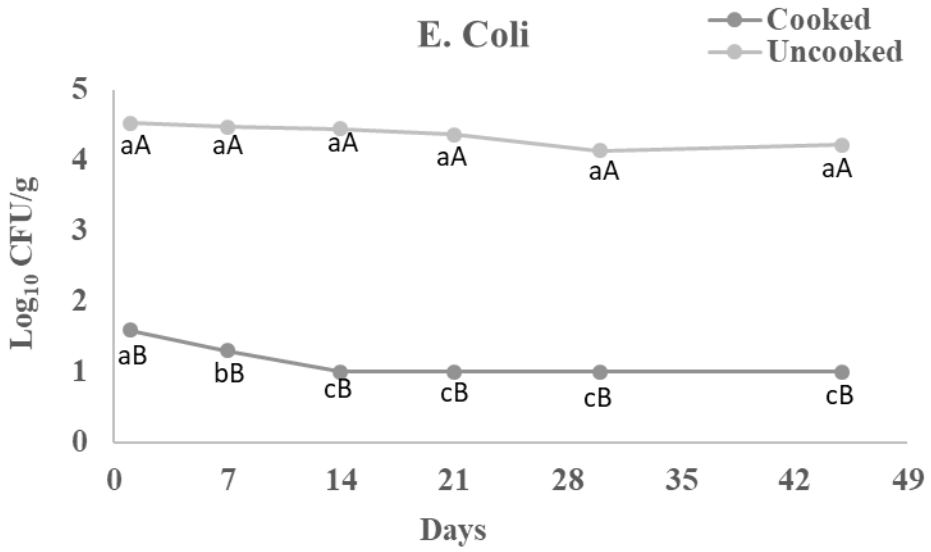
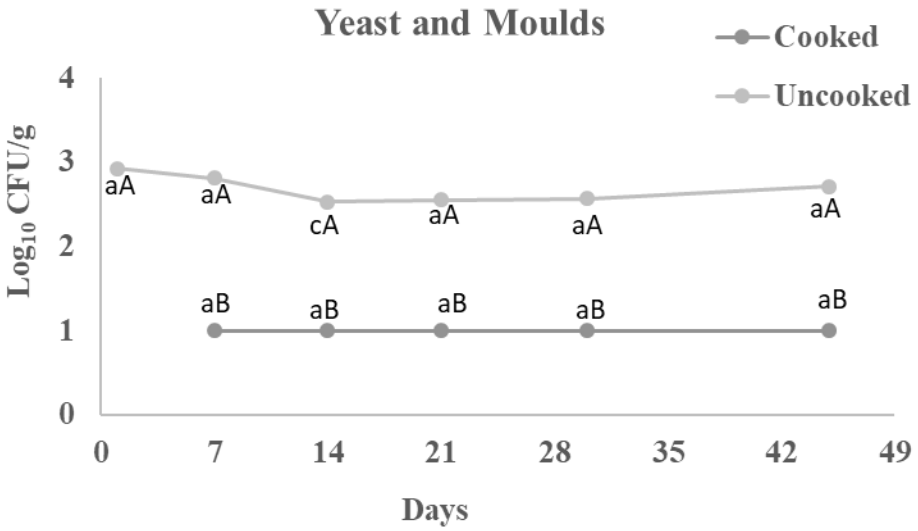


Figure.4 Yeast and moulds growth in the unpasteurized and pasteurized sausage over the storage period. Different letters indicate significant difference among samples ($p \leq 0.05$), where the small letter referred the difference due to the storage time and the big one to the effect of thermal treatment. The "a" and "A" letters were assigned to the highest value.



Escherichia coli

The results concerning *Escherichia coli* count in both samples were shown in figure 3. At the first day the level of *E.coli* was significantly high in unpasteurized sample as compared to pasteurized sausage. In fact, the level of *E. coli* was 1.6 $\log_{10}$ (CFU/g) in pasteurized sausage where it was 4.53 $\log_{10}$ (CFU/g). A significant ($p < 0.05$)

decrease in *E. coli* level was observed in pasteurized samples during the first 14 days of storage, then it remained constant over the storage period. While in the unpasteurized sausage there was no significant changes in the *E. coli* count. Oleoresin rosemary, seasoning oil, showed a decrease in *E. coli* population after 9 days of storage (Ahn *et al.,* 2007). Therefore, the spices used in the preparation of the sausage had specific antimicrobial properties against the bacteria, inhibiting its growth and gradually leading to its death phase.

Garlic contains allicin, a compound known for its antimicrobial properties. It has been shown to inhibit the growth of various bacteria, including *E. coli* (Avato *et al.,* 2000). Thyme contains thymol, which has been found disrupt the cell membranes of bacteria, including *E. coli.* Piperine, the active compound in black pepper, has shown same antimicrobial effects (Gholami-Ahangaran *et al.,* 2022).

Our results are consistent with other studies indicating that slaughtering at the slaughterhouse is a critical control point for contamination and transfer of pathogens to chicken meat (Little *et al.,* 1998). Therefore, prevention of cross contamination and careful handling of the products and effective cleaning and sanitation program are essential to produce safer products.

Yeast and Moulds

Yeast and moulds had a different trend on both samples as showed in figure 4. At the first day there were no yeast and moulds in pasteurized sausage while in the unpasteurized sausage, there were 2.93 $\log_{10}$ (CFU/g). An increase in Yeast and Moulds was observed in pasteurized samples up to obtain 1 $\log_{10}$ (CFU/g) on day 7^{th}, then it remained over the storage period.

In the unpasteurized sausage a significant decrease was observed at day 14^{th}, then it remained constant up to day 30^{th}, before creasing at the end of storage. However not all strains of yeast are able to grow at low temperature, there are same strains which can grow at the 4°C (Nielsen *et al.,* 2008).

In fact, it was reported that *Candida Zeylamoides* and *Candida sake* grew at 5°C reaching 6 $\log_{10}$ (CFU/g) at the day 14^{th} and continued to grow up to 28 days of storage (Nielsen *et al.,* 2008). This limited increase of Yeast and Moulds in our samples might be associated to the presence of added spices. In fact, many of the spices we used in the sausage formulation, including garlic, thyme, rosemary, cloves, and ginger, contain natural compounds like allicin, thymol, eugenol, rosmarinic and gingerol, have demonstrated antifungal properties (Shahidi *et al.,* 2018). These compounds can inhibit the growth of bacteria, yeasts and moulds, that might spoil the sausage. By reducing the microbial load, the sausages can remain safe for consumption for a longer period and this was also aligned with the works of Aziz *et al.,* (2018) who used similar spices in their work.

Using the natural preservatives in chicken sausage was successfully processed in the laboratory. A standardized method of sausage formulation was achieved and this method can easily be adapted by firms and households. The physicochemical properties exhibited the good nutritional profile as expected.

The African spices inhibited the growth of microbes on the processed product. The fresh chicken sausages with natural preservatives might be refrigerated for more than a month without strong changes in microorganism growth.

Author Contributions

Agoura Diantom: Investigation, formal analysis, writing—original draft. Nerine Akwa: Validation, methodology, writing—reviewing. Yao Hoekou:—Formal analysis, writing—review and editing. Akpéné Akakpo: Investigation, writing—reviewing. Bouraïma Djeri: Resources, investigation writing—reviewing. Damintoti Karou: Validation, formal analysis, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Agoura Diantom, Nerine Akwa, Yao Hoekou, Akpéné Akakpo, Bouraïma Djeri and Damintoti Karou. 2024. Effect of Natural Preservatives and Thermal Processing on the Microbiological and Physicochemical Properties of Chicken Sausage During Cold Storage. *Int.J.Curr.Microbiol.App.Sci.* 13(10): 122-132.
doi: https://doi.org/10.20546/ijcmas.2024.1310.017