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EFFECT OF PEPPER AND SALT BLENDS ON MICROBIAL QUALITY OF *QUANTA*: ETHIOPIAN DRIED RED MEAT

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Abstract
This study was conducted to assess the effect of spice blends varying in salt and pepper concentrations on the microbial quality of Quanta: Ethiopian dried red meat. The experiment had seven treatments: 25% spices, 25% salt, and 50% pepper (T1); 25% spices, 20% salt, and 55% pepper (T2); 25% spices, 15% salt, and 60% pepper (T3); 25% spices, 10% salt, and 65% pepper (T4); 25% spices, 5% salt, and 70% pepper (T5); 100% spices (without salt and pepper), a positive control (T6); a negative control without any added ingredient (T7). Microbiological analyses were performed initially on the raw sliced meat and spice blends, and after application of the treatments on the 10th and 20th days of drying. High initial loads of total bacteria (APC) and Enterobacteriaceae (EC) were observed in the raw meat samples and spice blends and increased over the drying periods (10 and 20 days) in all treatments. No significant difference ($p>0.05$) was observed among the treatments (T1-T7) for APC and EC at a given drying period and between the drying periods. Salmonella spp. was not detected in any of the seven treatments either on the 10th and 20th days of drying. However, Escherichia coli was detected in six (T1-T6) of the dry meat samples except in T7 both on the 10th and 20th days of drying suggesting that the spice blends served as a source of contamination of the dried meat samples with E. coli. However, the spice blends used in combination with drying were effective in inhibiting the growth of Salmonella species in the dry meat samples. Spices as well as the raw meat used for Quanta preparation should be produced and handled under hygienic conditions to minimize the microorganisms that they harbor.

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Introduction

The world population is expected to reach 9.7 billion in 2050 [1]. The increased population growth will lead to increased demand for food including meat and meat products [1,2]. Red meat is rich in many nutrients such as minerals, vitamins, and essential fatty acids and it is an excellent protein source, as the bioavailability of nutrients in meat is high compared to plant-based protein sources [2]. Meat and meat products provide primarily vitamin B12, highly digestible protein and bioavailable iron [3].

In recent years, there appears to be a shift in the consumption pattern of meat (mainly red meat) among the world population with consumption increasing in developing countries and decreasing in developed countries [2]. It is predicted that the consumption of animal protein from red meat such as beef, sheep and goat will increase over the next 2–3 decades among the middle-class population of developing countries especially in Africa and Asia [1,2].

There has been a significant increase in consumption of animal-based foods in the last 50 years in the world owing to economic growth [4]. According to FAO statistics, global meat and fish consumption has increased from 23 kg per capita in 1961 to 42 kg per capita in recent years [4]. Moreover, Henchion et al. [1] reported that animal-source protein supply (g/capita/day) is projected to increase in all regions from 2012 to 2050.

It has been reported that most of the future growth in meat and fish consumption is likely to occur in low-income countries, including Sub-Saharan Africa (SSA), where the current consumption levels are still very low [4]. Strong population growth and urbanization in SSA will reinforce growth in total demand for animal-based foods [4]. Meat production in SSA is projected to increase by 2.7% per annum till 2030, which is high compared to the expected increase in global meat production of 1.4% [4]. In SSA, consumption of animal-based foods is expected to increase by 54–69% if GDP of the region doubles [4].

Ethiopia has the largest livestock population in Africa [5]. However, consumption of animal source food has always been low in the country and declining as a result of

the low livestock productivity and continuously growing human population [6,7]. According to the 2020 FAO statistics, the total meat production in Ethiopia was estimated at 918,564 tonnes [8]. The majority of meat production in Ethiopia comes from cattle (beef) which accounts for 47.14% (433,025 tonnes) of the total meat production in the country [8]. According to Shawel and Kawashima [9], the consumption of meat declined from 20 kg/person/year in 1961 to 8 kg/person/year in 2004 in Ethiopia. The average per capita consumption (kg/year/capita) of meat in Ethiopia based on the FAO food balance sheet data was reported to be 7.99 in 2020 [4].

Fresh meat is a highly perishable product due to its biological composition [3,10]. The diverse nutrient composition of meat makes it an ideal environment for the growth and propagation of meat spoilage micro-organisms and common food-borne pathogens [3]. It is therefore essential that adequate preservation technologies are applied to maintain its safety and quality [10].

Food handling, preparation, and preservation practices in Ethiopia are based on indigenous knowledge that is handed over to the present generation, which is an invaluable and intangible asset as they are the outcome of repeated research and practical experiment by many generations [11]. Moreover, research on meat and meat products in Ethiopia has been given the lowest attention [12]. The traditional dried meat product of Ethiopia and East African countries [13], called "*Quanta*" (in Amharic), is similar to the dried meat Biltong. People in Ethiopia prepare *Quanta* with the application of salt (salting) and different spices [14] on the surface of red meat. Blends of spices used in the pre-treatment of the raw material used for the preparation of *Quanta*, that is, the sliced fresh meat include *Mitmita (Capisicum frutescene)* and *Berbere (Capisicum anmum)* and they are prepared as cooking aid or condiment [15]. The composition of the ingredients for the above-mentioned pre-treatment of raw meat is reported to be 50% pepper, 25% salt, and 25% spices [13].

However, the ingredients used in the preparation of the pre-treatment materials vary in their type and amount due to different factors and their inhibitory effect against pathogens and spoilage microorganisms has not been studied to date. Besides, the proportion of the ingredients (pepper, salt, and spices) in the blend which results in better inhibitory effects without affecting the sensory quality of the meat has also not been studied so far. Improvement and proper use of traditional meat preservation techniques like preparation of *Quanta* can play a significant role to ensure sustainable food supply through reduction of post-harvest (post-slaughter) losses of meat. Moreover, improving the quality of *Quanta* through effective pre-drying treatments may alleviate the problem of seasonal availability of meat in the country, may help to develop a uniform type of dried meat, and create an opportunity for exporting meat in a dried form. Therefore, this experiment was conducted to evaluate the treatment effect of blends varying in concentrations of salt and pepper on the microbial quality of dried red meat (beef), *Quanta.*

Methodology and methods

Preparation of blends used for pre-drying treatment

Mareqo type red pepper *(Capisicum anmum)* which is African chilies indigenous to Ethiopia [16], and table salt (NaCl) were purchased from Assela market on the relative quality basis (cleanness, color, and size) with the help of experienced women. The items purchased for the blend preparation were taken to a blend processing place (a private compound in Assela town which was rented for this experimental season) with independent plastic bags and they were subjected to wet cleaning (washing) and/or dry cleaning (picking, trimming, etc.) based on the requirement of each ingredient. Each type of spices, pepper and salt were sun-dried for 10 days in independent plastic trays. The sole spice blend was made to consist of a combination of 4% Basil (*Ocimum basilicum*), 4% Rue (*Ruta graveolens*), 4% Rosemary (*Rosmarinus officialis*), 7% Fenugreek (*Trigonella foenum-graecum*), 7% Bishop's weed (*Carum copticum* L.), 7% Black cumin (*Nigella sativa* L.), 15% Garlic (*Allium sativum*), 15% Shallot (*Allium cepa*), 15% Ginger (*Zingiber officinale*) and 22% Ethiopian Cardamom (*Aframomum corrorima*). The spices were mixed by pounding them with a mortar and pestle. The red pepper was also pounded separately with a mortar and pestle to a size of about 5 mm with the traditional size reduction process called "*shikesheka*" in Amharic.

The preparation of blends, which were used to treat meat slices, was based on FAO [13] ingredients estimation for traditional meat drying of *Quanta* viz., 50% pepper, 25% salt, and 25% spices. Thus, this blend level was prepared and used as one pre-drying treatment in the experiment. Besides, the other four blends varying in the proportion of pepper and salt but having the same amount of the other blend of spices were prepared. The blending of the mixtures was done by pounding with a mortar and pestle. This blending step is called '*Deleza*' in Amharic. A blend formulated with spices only was prepared to be used as a positive control. The blends were placed in the sun again for 3 days for drying. All six lots of blends were subjected to a careful traditional art of low heat treatment, further drying, on a metal sheet one after the other independently. This traditional art of heat drying is called '*Emesa*' in Amharic. Finally, milling of the above-stated six blends was done independently, one after the other, in one of the commercial mills in the town of Assela.

Therefore, the compositions of spice (S) blends prepared for the pre-drying treatments and their respective treatments were as follows: S1 with 25% spices, 25% salt, and 50% pepper (T1); S2 with 25% spices, 20% salt, and 55% pepper (T2); S3 with 25% spices, 15% salt, and 60% pepper (T3); S4 with 25% spices, 10% salt, and 65% pepper (T4); S5 with 25% spices, 5% salt, and 70% pepper (T5);

a positive control, S6 with 100% spices (without salt and pepper) (T6); and, a negative control, without any added ingredient (T7). Therefore, the experiment had a total of seven treatments.

Preparation of meat slices

Meat from two pairs of hind legs of two male beef cattle (Arsi breed) was purchased from private butchers in Assela town. The two hind legs of both oxen were cut into whole cut meat (deboned meat) and sliced into strips 1 cm thick and 40 cm long according to FAO [13]. Slicing was performed by the researchers with the assistance of two experienced women.

Pre-drying treatment of meat slices

The amount of blend used for the pre-drying treatment of sliced meats was determined according to Jay et al. [17]. The minimum inhibitory concentration (MIC) of most spices required to inhibit growth of sensitive organisms ranges from 1 to 5%. Thus, by taking the average concentration level, which is about 3%, dilutions were prepared by mixing the pre-drying treatment blends with distilled water. About 4 L of pre-drying treatments were prepared and used for every six treatments including a blend of sole spices. Two kilos of sliced meat lots were used for each treatment and uniformly treated by dipping in their respective dilutions for 10 minutes by turning them up and down. Similarly, the negative control, without any spice treatment, was treated with distilled water to control the deviation that could occur because of the water used in the dilution of other treatment blends. Seven clean plastic pans (bowls) were used to treat the seven experimental treatments individually in the first block (sliced meat lots of the first ox) and the bowls were reused for the second block (sliced meat lots of the second ox) of similar treatments after thorough cleaning and disinfection with 70% alcohol.

Drying of meat samples

Drying of the meat slices was done in a room with a 4 m × 4 m area having windows and a door for adequate ventilation, and the room openings were covered with mesh wire to prevent the entrance of flies according to FAO [13]. The ambient temperature of the experimental site, Assela town, was between 9.17 °C and 22.63 °C during experimentation. A drying bed (string) 2 m high, 2 m wide and 3 m long was constructed in the drying room. The drying bed had two blocks with a 50 cm gap between them. Both of the drying blocks were made to have 14 rows (7 pairs) of stretched ropes (5 mm diameter) with a 20 cm gap between the rows. Drying was done by suspending sliced meat lots on ropes (Figure 1). Each treatment had two independent hanging rows across the blocks. A uniform arrangement of meat slices was made with no surface contact between the neighboring meat slices. Drying was done for twenty days and identification cards were suspended together with drying meat slices.

Figure 1. Treated meat samples hanging on ropes during drying

Experimental design

The design used to conduct this experiment was Randomized Complete Block Design (RCBD). Two sources of meat, meat samples obtained from two different oxen, were used as a block after having been sliced in order to avoid the variation in meat quality obtained from the two animals. About 14 kg of slices were made from the first ox meat and divided into seven lots each containing two kilos. The seven treatments were randomly allocated to the seven meat slices in the first block. The same was done to the slices made from the second ox meat in the second block. In this experiment, two varying factors, namely salt and pepper, were used. Five different blends were prepared from the spices, salt, and pepper by varying only the ratio of pepper to salt and keeping the level of spices constant in all five blends. In addition, two types of controls were used in the experiment; a positive control that was a blend made from spices only (without pepper and salt), and the other was a negative control without any pre-drying treatment with spices or salt. A total of seven treatments, six spice blends, and the negative control (without any pre-drying spice treatment), were applied to each of the two blocks.

Microbiological analysis

In the current study, microbiological analysis was done at four different times to assess: the aerobic plate count (APC), *Enterobacteriaceae* count, and presence of the pathogens, *Escherichia coli* and *Salmonella* spp. The first microbiological analysis was done on the sliced fresh meat samples of both meat sources (from two types of sliced meats sourced from different oxen) before they were treated and dried, and on the six different types of spice blends used (Table 1) to assess their initial microbial load. The second, third and fourth microbiological analyses were conducted after the application of pre-drying treatments and on the 10^{th} and 20^{th} days of the drying experiment, respectively to determine the change in microbial population over the drying period.

Sampling

About 200 g of samples were taken from each sliced fresh meats, spice blends, and sliced and dried meat samples in the respective microbiological analysis seasons. Sampling was done randomly and samples were transported to the Microbiology Laboratory of Quality and Standards Authority of Ethiopia by putting them in an icebox after they were packed into polyethylene bags and labeled properly. Sampling of spice blends and meat lots for microbiological analysis was done by aseptically weighting 25 g from each sample type. Sample dilution (1:10) was performed with 225 ml of buffered peptone water [18] and homogenized for two minutes using a stomacher (Seward Medical, London). Serial (10-fold) dilutions (10^{-1} to 10^{-7}) were prepared by transferring 1 ml of the previous dilution (1:10) into test tubes containing 9 ml of 0.1% peptone water [18]. Separate sterile pipettes were used for transferring samples during serial dilutions and all dilutions were thoroughly mixed before they were plated. The presence of the pathogens *Escherichia coli* and *Salmonella* spp. was detected using samples from the initial dilution level (1:10). However, the aerobic plate count (APC) and *Enterobacteriaceae* count were made using the appropriate dilutions that yielded countable colonies (30–300 colonies/dish). Every analysis was performed in triplicate.

Escherichia coli detection

Detection of *Escherichia coli* was done according to the method described by Roberts and Greenwood [19] following four sequential incubation steps. The first incubation step was done at 37 °C for 48 h [19] by transferring 1 ml representative sample from 1:10 (10^{-1}) dilution into test tubes containing Lauryl Tryptose (LT) broth (Lab M Limited, UK). The second incubation step was done by transferring 1 ml representative sample from the Lauryl Tryptose (LT) broth into test tubes containing Brilliant Green Bile (BGB) broth (Lab M Limited, UK) and incubated at 35 °C for 24 h, and gas production was considered as an indicative test for the presence of *Escherichia coli* according to ISO [19]. The third incubation step was done in the selective media for pathogenic *Escherichia coli*, MacConkey Sorbitol medium (Lab M Limited, UK), for 24 h at 45.5 °C [19] after transferring 1 ml representative sample from Brilliant Green Bile (BGB) broth. Colonies grown on this medium were subjected to the fourth and the final confirmatory test. The fourth and the confirmatory test was done by transferring about 10% of typical colonies grown on MacConkey Sorbitol medium into test tubes containing peptone water and incubated at 44 °C for 24 h. Then, the peptone water in the test tubes was tested with Kovac's reagent (5 ml) for the presence of indole. The production of bluish color was considered as an indicator for the presence of indole in the samples, that is, a positive test for the existence of *Escherichia coli* [19].

Detection of Salmonella spp.

Detection of *Salmonella* spp. was done according to ISO [20] following five consecutive incubation steps. The first incubation step, pre-enrichment, was performed at 37 °C for 48 h by taking about 150 ml of representative samples from the 1:10 dilution levels. The second incubation step was done by transferring an aliquot from the completed pre-enrichment step into a selective enrichment medium, Rappaport Vassiliadis Soya (RVS) broth (Lab M Limited, UK), and incubating at 41 °C for 24 h. The third incubation step was performed by plating the sample enriched in the second incubation step into solidified Hektoen Enteric (HE) selective medium (Lab M Limited, UK) and incubating at 37 °C for 24 h. Then, the fourth incubation step was done by transferring about 10% of typical colonies selectively grown on Hektoen Enteric (HE) medium into solidified Nutrient agar medium (Lab M Limited, UK) and incubating at 37 °C for 24 h. The fifth and final incubation step was done by transferring about 10% of typical colonies grown on Nutrient agar, plating into Urea agar medium (Lab M Limited, UK) and incubating at 37 °C for 24 h. Finally, smooth colonies (colorless, translucent, or pale colonies) that were 2–4 mm in diameter were considered as a positive test for the presence of *Salmonella* spp.

Aerobic plate count
and Enterobacteriaceae count

Aerobic plate count and *Enterobacteriaceae* count were made using samples from appropriate dilution levels. One milliliter of a sample was pipetted into appropriately marked Petri dishes. Enumeration of the APC was performed after incubating samples using plate count agar (Lab M Limited, UK) at 30 °C for 72 h according to ISO [18] method 4833. Incubation of *Enterobacteriaceae* was done using Violet Red Bile (VRB) agar (Park Scientific Limited, UK) at 30 °C for 48 h and all red-pink colonies with a diameter of greater than 1 mm were counted [21]. The colonies were counted using a colony counter (Wissenschaftlich Technische, Werkstatten, Germany) and the estimated average numbers per gram of sample were calculated according to Maurin and James [21] for APC and *Enterobacteriaceae* count, and then data were presented in $\log_{10}$ cfu/g.

Statistical analysis

Analysis of variance (ANOVA) for Randomized Complete Block Design (RCBD) was carried out using PROC General Linear Model (GLM) of the Statistical Analysis System [22] Version 9.1. Microbial counts were first transformed to logarithmic values ($\log_{10}$) before statistical analysis. Differences between treatment means were determined using the least significant difference (LSD) technique. All comparisons were made at a 5% level of significance.

Results and discussion

Red meat is a highly perishable product and soon becomes unfit to eat and possibly dangerous to health through microbial growth, chemical change, and breakdown by endogenous enzymes [23]. Drying is amongst the effective and simple methods for the preservation of red meat [24]. Although commercialization and production of *Quanta* can help in alleviating the variation in meat availability and price in the country, to date there is no documented information about its processing steps, and no research has been conducted to enhance its quality attributes and storage stability. The current study investigated the effect of spice blends varying in concentrations of salt and pepper on microbial quality of the Ethiopian dried red meat (beef), *Quanta.*

Detection of pathogens in the raw material

The results for the detection of the pathogens *Escherichia coli* and *Salmonella* spp. in the raw materials, the sliced raw meat and the spice blends used in this experiment are presented in Table 1. *Escherichia coli* is the most frequently identified pathogen associated with beef products [25]. The incidence of *E. coli* is not very variable in domestic or export beef meat [26]. Since its discovery by Theodor Escherich in 1885, it has been receiving much greater importance due to the pathogenicity of certain strains to both humans and animals [27]. In the current study, *Escherichia coli* was not detected in the two sliced raw meat samples. The finding disagrees with that of Gwida et al. [28] who reported a high percentage of *E. coli* isolated from raw meat and unprocessed ready-to-eat products. However, *E. coli* was detected in all the six spice blends analyzed. Possible sources of contamination of spices by pathogenic microorganisms were reported to include storage equipment, handling, unhygienic surroundings, vehicular transmission, atmospheric particles and air [29]. On the other hand, *Salmonella* spp. was not detected in any of the spice blends; however, its presence was exhibited in the sliced raw meat of both animal sources. In the process of converting live animals into meat, microbial contamination of carcass surfaces is unavoidable [25]. While most of the microfloras transferred to the carcasses during the slaughtering process are nonpathogenic, there is a possibility that pathogens like *Salmonella* spp. may be present and it represents one of the most critical safety challenges for the meat industry [25]. *Salmonella* spp. is most commonly associated with animal products and is only present in vegetables through cross-contamination [30,31]. This may explain the absence of detection of the pathogen *Salmonella* spp. in the spice blends analyzed.

Table 1. Occurrence of the pathogens *Escherichia coli* and *Salmonella* spp. in raw sliced meat samples and different spice blends used in the experiment

Type of sample	Occurrence of the pathogens	
	Escherichia coli	*Salmonella* spp.
Sliced meat (B1)	-ve	+ve
Sliced meat (B2)	-ve	+ve
Spice blend (S1)	+ve	-ve
Spice blend (S2)	+ve	-ve
Spice blend (S3)	+ve	-ve
Spice blend (S4)	+ve	-ve
Spice blend (S5)	+ve	-ve
Spice blend (S6)	+ve	-ve

n = 3, number of samples; B1 and B2 are sliced meat samples from two different sources used in block 1 and block 2, respectively; S1 up to S6 are spice blends used to treat the meat samples in this study; -ve shows the absence of the pathogen and +ve shows the presence of the pathogen.

Enumeration of microorganisms in the raw materials

Evaluation of the microbiological quality and safety of food products is commonly carried out by determination of total viable counts and the indicator organisms *Enterobacteriaceae* and *E. coli* [32,33]. Aerobic plate count is used to estimate the bacterial population in a food sample. It is not an evaluation of the entire bacterial population nor does it indicate differences among bacterial types in a food product. It provides an estimate of the number of microorganisms that can grow aerobically at ambient temperatures. The APC may be used to judge sanitary quality, sensory acceptability, and conformance with good manufacturing practices (GMPs) [34]. The results for the aerobic plate count and *Enterobacteriaceae* count ($\log_{10}$ cfu/g) of the raw sliced meat samples and different spice blends used for the treatment of the meat samples are presented in Table 2. A very low APC of <1 $\log_{10}$ cfu/g was found in the meat sample obtained from the first block (B1), while 5.91 $\log_{10}$ cfu/g was found in the raw meat samples obtained from the second block (B2). The variation in the APC between the two meat sources could be from the hygienic practice followed during slaughtering and post slaughtering of the animals. The high count exhibited in one of the beef sources (B2) was also in the range (4.0 to 7.05 $\log_{10}$ cfu/g) of earlier research reports for the microbiological status of fresh beef cuts at different countries' retail markets [35,36,37]. A major problem in food hygiene is the fecal contamination of beef and chicken meat with the family *Enterobacteriaceae* [28]. *Enterobacteriaceae* are a large family of facultatively anaerobic, gram-negative bacilli that inhabit the intestines of many animal species. This family includes pathogenic *Escherichia*, *Salmonella* serovars, and *Klebsiella* species [28,38]. The high prevalence of *Enterobacteriaceae* could be attributed to inadequate sanitary conditions and poor general hygiene. In the present study, a very low *Enterobacteriaceae* count (<1 $\log_{10}$ cfu/g) were found in the raw sliced meat samples of the two beef sources used in the preparation of *Quanta*. Crowley et al. [39] reported *Enterobacteriaceae* levels ranging from 6.54 to 6.98 $\log_{10}$ cfu/g in fresh, unpackaged, and minced beef. Abdelrahman et al. [40] reported $6.3 \times 10^4 \pm 2.8 \times 10^4$ cfu/g counts for fresh ground beef. Zulfakar et al. [37] identified 5.05 ± 0.87 $\log_{10}$ cfu/g in a bacterial contamination study on beef sold at selected wet markets in Selangor and Kuala

Lumpur. As compared to these earlier studies, a substantially lower APC and *Enterobacteriaceae* count reflects the hygienic status of the sliced meats from two different sources used in the current study.

Table 2. Aerobic plate count and *Enterobacteriaceae* count (log_{10} cfu/g) in raw sliced meat samples and different spice blends used for treatment of the meat samples

Type of sample	Bacterial count (log_{10} cfu/g)	
	APC	Enterobacteriaceae
Sliced meats (B1)	<1	<1
Sliced meats (B2)	5.91 ± 0.11	<1
Spice blend (S1)	5.89 ± 0.04	5.28 ± 0.01
Spice blend (S2)	6.15 ± 0.04	5.28 ± 0.15
Spice blend (S3)	6.24 ± 0.01	<1
Spice blend (S4)	5.89 ± 0.16	5.79 ± 0.13
Spice blend (S5)	5.83 ± 0.08	5.79 ± 0.04
Spice blend (S6)	6.44 ± 0.03	6.07 ± 0.01

n = 3, number of observations; B1 and B2, and S1 up to S6 are as indicated in Table 1; APC is aerobic plate count; Values in the table are means ± SD of three observations.

On the other hand, the APC and *Enterobacteriaceae* count of most of the spice blends analyzed were above 5.0 log_{10} cfu/g except for the spice blend S3 where a low detectable count of *Enterobacteriaceae* was found (Table 2). Bakobie et al. [29] reported that spices and herbs can serve as sources of microbial contamination of foods, in which they are used as condiments or cooking aids. In the present study, high initial loads of both total bacteria and *Enterobacteriaceae* were observed in the spice blends that ranged from 5.89 to 6.44 log_{10} cfu/g and from <1 log_{10} cfu/g to 6.07 log_{10} cfu/g for the aerobic plate count and *Enterobacteriaceae* count, respectively. The high bacterial load in the spices is an indication of unhygienic practices during their preparation. In the microbiological quality study of the spice used in the production of Kilishi which is a product similar to that in our study, *quanta*, a comparable high aerobic plate count of 8 log_{10} cfu/g was reported by Shamsuddeen [41]. According to Shamsuddeen [41], spices like other food substances may carry some bacteria, yeasts, molds spores, and even some insects. The predominant flora is generally composed of aerobic spore-forming bacteria; non-spore-forming bacteria, indicator organisms, and some pathogens can also be found according to the International Commission on Microbiological Specifications for Foods [42].

Detection of pathogens in the treated meat samples

With an increase in global trade and consumer awareness of the hygienic quality of meat in recent years, international attention is being focused on ways to improve the microbial quality and safety of foods [25]. Rapid, accurate, and reliable detection and identification of bacterial foodborne pathogens are critical for food safety. The occurrence of *Escherichia coli* in the treated meat samples over a drying period of 10 and 20 days and that of *Salmonella* spp. in the raw meat sample is indicated in Table 3. *Escherichia coli* is a member of the family *Enterobacteriaceae*. *E. coli* is known to microbiologists as «enteric bacteria», because it lives in the intestinal tract of humans and animals. *E. coli* colonizes the gastrointestinal tracts of a wide range of wild and domestic animals, especially animals raised for human consumption [43]. *Escherichia coli* was not detected in the raw sliced meat samples as indicated in Table 3; however, it was detected in six (T1-T6) of the dry meat samples treated with spice blends on the 10th and 20th days of the drying experiments. *Escherichia coli* was not detected in T7 (the negative control, which did not contain the spice blend) throughout the experimental period. As *E. coli* was not detected in the raw meat samples, the detection of *E. coli* on the 10th and 20th days of the drying experiments was attributed to the presence of *E. coli* in the spice blends used in the experiment (Table 1). Thus, it seems that the spice blends used served as a source of contamination of the meat by *E. coli*. This calls for careful and scrupulous hygienic measures during handling and preparation of spices used for treatment of the dried meat *Quanta*. Similarly, occurrence of microorganisms that are potentially pathogenic in spices used in Suya (dried smoked meat) and Kilishi (sun dried spiced and grilled meat snack) preparation was reported as a major cause of gastrointestinal disturbances resulting from the consumption of these meat products in Nigeria [41]. Contaminated spices were reported to be

Table 3. Occurrence of *Escherichia coli* and *Salmonella* spp. in raw sliced meat (day 1) and meat samples treated with different spice blends (day 10 and 20 of drying)

Treatments	Occurrence of pathogens					
	Escherichia coli			*Salmonella* spp.		
	Day 1	Day 10	Day 20	Day 1	Day 10	Day 20
T1	-ve	+ve	+ve	+ve	-ve	-ve
T2	-ve	+ve	+ve	+ve	-ve	-ve
T3	-ve	+ve	+ve	+ve	-ve	-ve
T4	-ve	+ve	+ve	+ve	-ve	-ve
T5	-ve	+ve	+ve	+ve	-ve	-ve
T6 (positive control)	-ve	+ve	+ve	+ve	-ve	-ve
T7 (negative control)	-ve	-ve	-ve	+ve	-ve	-ve

n= 3, number of observations; T1 up to T7 are experimental treatments (sliced meat samples treated with different spice blends as indicated in Table 1; spice blend number corresponds to the treatment number except for T7, which did not receive pre-drying spice treatment); +ve refers to presence and –ve refers to absence.

causes of food-borne illness and spoilage of food and were associated with food-borne pathogenic microorganisms [44,45]. According to Toldra [46], unless spices are treated to reduce their microbial content, they may add high numbers and undesirable kinds of organisms to food, in which they are used. In the current study, the spice blends used for the pre-drying treatment of the sliced meats were treated with low heat treatment during the preparation step. However, the heat treatment applied was mild as it was intended only for drying and did not help in reducing the bacterial contamination of the spices. Therefore, spices should be subjected to treatment that would reduce their microbial load to avoid the introduction of undesirable kinds of spoilage and pathogenic organisms.

Salmonella spp. was detected on day one in all the raw meat samples (treatments) (Tables 1 and 3); however, it was not detected in the treated meat samples on the 10th and 20th days of drying (Table 3). The absence of *Salmonella* spp. in the meat samples on the 10th and 20th days of the drying period suggests that the different concentrations of the spice blends used in combination with drying are effective in inhibiting the growth of *Salmonella* species in the meat samples. Drying inhibits microbial growth in foods by reducing its water activity. According to Murano [47], the removal of biologically active water through drying helps stop the growth of microbes. In general, bacteria other than halophiles will not grow at 0.83 a_w or below, and most are inhibited markedly at 0.90 a_w or less [48]. Ghaly et al. [49] documented that the growth of pathogens is prevented by a_w at 0.85 and USDA [50] reported that the minimum water activity for growth of *Salmonellae* associated with dried meat products is 0.94. In the present study the disappearance of *Salmonella* spp. can be related to the combined inhibitory effect of the spice blends and drying that led to a reduction of water activity of all treatments.

Enumeration of microorganisms in the treated meat samples

The APC and *Enterobacteriaceae* count (log_{10} cfu/g) of meat samples on the 10th and 20th days of drying after application of the treatments are indicated in Table 4. As compared to the total plate count in the raw materials (meat samples and spice blends) reported in Table 2, an increase in the APC was observed in the treated meat samples over the drying period (10th and 20th days). All the meat samples had APC > 7.0 log_{10} cfu/g (Table 4). No significant difference ($p > 0.05$) in the APC was observed among the treatments (T_1-T_7) at a given drying period (10th and 20th days) and also between the two drying times for a given treatment (Table 4). Some researchers stated that the *Enterobacteriaceae* as a whole, and not just *E. coli*, should be taken into account when considering the sanitary standards and hygiene of dry and low-moisture foods [51,52]. High *Enterobacteriaceae* count in food samples is an indication of possible contamination from enteric sources [53].

Similar to the aerobic plate count, all the meat samples (T1-T7) had the *Enterobacteriaceae* count of > 7.0 log_{10} cfu/g (Table 4) during the drying experiment. No significant difference ($p > 0.05$) in the *Enterobacteriaceae* count was observed among the different treatments (T_1-T_7) at a given drying time and also between the 10th and 20th days of the drying period for a given treatment (Table 4). The high microbial counts observed in the spice blends used for pre-treatment of the meat samples (Table 2) in the present study may be responsible for the very high (>7.0 log_{10} cfu/g) APC and *Enterobacteriaceae* count observed in the dried meat samples after 10 and 20 days of treatment. Thus, this calls for scrupulous hygienic measures during the handling and preparation of spice blends used for the treatment of meat samples. According to Frazier and Westhoff [54], spices do not have a marked bacteriostatic effect in the concentrations used in meat products and they may even serve as a source of contamination of the processed product. According to Jay et al. [17], components used as seasoning and other formulation ingredients/additives such as spices can be sources of additional microorganisms. This may explain the increases in the *Enterobacteriaceae* count and total bacteria count over the drying periods (10 and 20 days) of the present study.

Table 4. Aerobic plate count (APC) and *Enterobacteriaceae* count (log_{10} cfu/g) of meat samples on the 10th and 20th days of drying after application of the treatments

Treatments	Bacterial counts (log_{10} cfu/g)			
	Aerobic plate count (APC)		*Enterobacteriaceae* (EC)	
	10th day	20th day	10th day	20th day
T1	7.69 ± 0.18	7.78 ± 0.06	7.70 ± 0.16	7.70 ± 0.20
T2	7.83 ± 0.01	7.73 ± 0.04	7.60 ± 0.16	7.49 ± 0.01
T3	7.75 ± 0.03	7.82 ± 0.15	7.44 ± 0.25	7.55 ± 0.33
T4	7.72 ± 0.01	7.83 ± 0.06	7.56 ± 0.31	7.55 ± 0.29
T5	7.71 ± 0.04	7.74 ± 0.13	7.70 ± 0.23	7.44 ± 0.30
T6	7.42 ± 0.04	7.86 ± 0.04	7.41 ± 0.14	7.65 ± 0.16
T7	7.89 ± 0.11	7.87 ± 0.03	7.72 ± 0.28	7.75 ± 0.05

n = 3, number of observations; T1 up to T7 are as indicated in the Table 3; Values in the table are means ± SD of three observations; No significant difference ($p > 0.05$) in the APC and EC was observed among the treatments (T_1-T_7) at a given drying period (10th and 20th days) and also between the two drying days for a given treatment.

Conclusion

The results of the present study showed that treatment of meat samples with the spice blends served as a source of contamination of the dried meat samples with *E. coli*. However, the spice blends used in combination with drying were effective in inhibiting the growth of *Salmonella* species and resulted in absence of *Salmonella* spp. in the dry meat samples on the 10th and 20th days of the drying period. Based on the findings of this study, the following recommendations are made to improve the quality of dried meat, *Quanta*:

- Spices and herbs applied on meat used for preparation of *Quanta* should be produced and handled under hygienic conditions and should be subjected to treatments that would reduce their microbial load during the blend preparation.
- Extraction of essential oils and active agents of spices may increase the antimicrobial and preservative effects of spices on dried meat. Thus, this needs further investigation.

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