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REDUCTION OF *STAPHYLOCOCCUS* Spp. IN JERKED BEEF SAMPLES AFTER IRRADIATION WITH Co-60

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ABSTRACT

This work aimed to isolate and identify *Staphylococcus* genus microorganisms in jerked beef before and after radiation doses between 2, 4 and 6kGy. Jerked beef samples were obtained on a Recife-PE supermarket network and divided into three lots. Under sterile conditions, the meat was cut and weighed. Sub-samples were assigned to the control group and to the irradiation source of cobalt-60 on doses of 2, 4 and 6kGy. The sub-samples were added to an Erlenmeyer flask with 225 ml of sterile water and stirred for 15 minutes creating wash water, and another part was added to an Erlenmeyer flask with 225 ml of sterile distilled water that was at rest at room temperature for 14 hours there is the formation of a water desalting. 1µL aliquots of this water was removed and sown by depletion in sheep blood agar medium and incubated at 35 °C for 24 hours for analysis of bacterial growth. After Gram staining colonies classified as Gram positive arranged in bunches were subjected to biochemical tests for identification. Were isolated and identified 94 strains of the genus *Staphylococccus* being 72 (76%) of the control group and 22 (24%) after irradiation. Of the 22 isolates, after irradiation, with 2 kGy 7 species were identified as *Staphylococcus succinus*, *Staphylococcus carnosus* sub. *carnosus*, *Staphylococcus fleurettii*, *Staphylococcus saprophyticus* sub. *saprophyticus*, *Staphylococcus simulans*, *Staphylococcus auricularis* all coagulase negative and coagulase positive *Staphylococcus aureus* sub. *anaerobius* (2). At a dose of 4kGy were identified six species: *Staphylococcus epidermidis*, *Staphylococcus xylosus* (2), *Staphylococcus intermedius*, *Staphylococcus warneri*, *Staphylococcus fleurettii* (2), *Staphylococcus aureus* sub. *anaerobius*. *Staphylococcus simulans* (3), *Staphylococcus saprophyticus* sub. *saprophyticus* (2), and *Staphylococcus lugdunensis* were isolated and identified after a dose of 6 kGy. Was observed that irradiation significantly reduced microbial load, and increased dose decreased the number of species found.

Keywords: jerked beef, Co-60, *Staphylococcus* spp.

1. INTRODUCTION

From the standpoint of public health, the population must be within reach not only food of good origin, but also in good sanitary conditions. Since foods are susceptible to contamination by different etiologic agents, which can lead to diseases manifested by the action of pathogenic microorganisms or their toxins. According to the Food and Agriculture Organization (FAO), one fifth of the world eats meat, so there has been increasing concern to give people a healthier meat because this food is characterized by the nature of the protein consists not only in terms of quantity and quality [1, 2].

Considered to be salted meat products, meat and shredding products subjected to the action of common salt and other curing ingredients in solid form or in brine, in order to ensure their preservation for future consumption [3].

The jerked beef is a product similar to jerky. The main difference is in the flowchart of processing, which allows the addition of sodium nitrite, at the beginning of the process during the step of wet curing. This technique gives red color to meat and moisture content of at most 56%. At the end of the process is, necessarily, vacuum-packed [4, 5, 6, 7].

The food processing came from the need to preserve food at harvest to final product and has been developed with the implementation of new technologies that improve the quality and safety and can help minimize risks and maximize benefits [10].
In order to maximize consumer safety, ways to control or reduce contamination are increasingly employed in particular irradiation. In this case the radiation can eliminate pathogenic microorganisms present in the meat and make it safe for consumption and it can increase its shelf life. Studies have demonstrated the effectiveness of the implementation of γ radiation in reducing levels of *Staphylococcus* in salt beef typical of Africa called biltong beef [8, 9, 10].

The objective of this study was to evaluate the effect of radiation doses of 2, 4 and 6kGy in reducing microbial load jerked beef.

2. MATERIALS AND METHODS

2.1 Selection of samples

Were used two batches of jerked beef and each batch containing three samples as determined by the RDC No. 12 January 2001, which instructs that each sample unit must contain at least three samples of each lot. The samples were obtained from a large supermarket network active in the city of Recife –Pernambuco-Brazil, in packages weighing 500g (a pound) each.

2.2 Microbiological analysis

The experimental procedure consisted of two series of microbiological tests. The first was made with the product samples before irradiation and the second irradiated samples. Microbiological tests were performed at the Applied microbiology and antimicrobials essays laboratoryat Federal University of Pernambuco. Were acquired six samples of 500g and two different batches (three samples each). Under sterile conditions, the meat was cut and placed in Petri dishes and then weighed in order to obtain a more representative number of samples of each batch. Eight sub-samples of each batch weighing 25g was made and then generating 48 subsamples. Of these, 12 sub-samples were assigned to the control group and the remaining (36 sub-samples) were designed to irradiation. The sub-samples were added to an Erlenmeyer flask with 225 ml of sterile water and stirred for 15 minutes creating wash water, and another part was added to an Erlenmeyer flask with 225 ml of sterile distilled water that was at rest at room temperature for 14 hours there is the formation of a water desalting. 1μL aliquots of these samples were sown in the medium by exhaustion in sheep blood agar in Petri dishes and were incubated at 35 ° C for 24 hours for analysis of bacterial growth and microbial population counts. After the incubation period were made counts of colony forming units per gram (CFU / g) [11, 12].

2.3 Gamma irradiation in ^{60}Co source

To carry out irradiation, sub-samples were packaged in Petri dishes, in order to avoid subsequent contamination, the entire procedure was performed in a laminar flow hood and then properly identified as the irradiation dose to be submitted. The material prepared as described was subjected to gamma irradiation (Co-60- irradiator, Gammacell 220 Excel, with a dose rate of 6,619 kGy/h) using doses 2, 4 and 6kGy. The procedure was performed in GammaLab the Department of Nuclear Energy at the Federal University of Pernambuco.

3. RESULTS AND DISCUSSION

In the wash water was observed no growth of microorganism. All strains were obtained from desalting water. Were isolated and identified 94 strains of the genus *Staphylococccus* being 72 (76%) of the control group and 22 (24%) after irradiation. Of the control group 72 microorganisms were isolated, 15 in one batch, lot 32 in 2 and 25 on the lot three. 22 strains were isolated after irradiation**.**

3.1 Isolation and identification of *Staphylococcus* isolated after irradiation of 2 kGy

Were isolated seven species after irradiation with a dose of 2 kGy, six strains in lot 1, one strain in lot 2 and one in the lot 3. Species isolated were *Staphylococcus succinus, Staphylococcus carnosus* sub. *carnosus, Staphylococcus fleurettii, Staphylococcus saprophyticus* sub. *saprophyticus, Staphylococcus simulans, Staphylococcus auricularis* all coagulase negative and coagulase positive *Staphylococcus aureus* sub. *anaerobius* (2).

So in the discussion were only related the samples for risk of human diseases. *Staphylococcus succinus* according Taponen et al. (2008) isolated from cows with mastitis [14].

Staphylococcus saprophyticus is generally found in various locations in the human skin which is observed transiently and at low concentrations, but has a preferential adherence to exfoliated cells from the urogenital epithelium [15]. The fact of having them found in jerked beef can be suggested that the handlers did not use good hygiene practices. A strain of one batch was found that fermented glucose, maltose, sucrose, mannitol and trehalose (Figure 1), and identified as Staphylococcus saprophyticus sub. bovis.

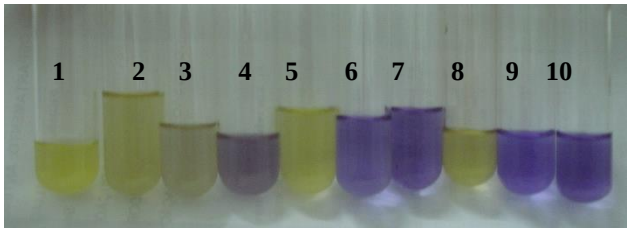

Figure 1. Proof of use of *S. saprophyticus sub. Bovis* carbohydrates. Glucose bovis (1), Maltose (2) Sucrose (3) Lactose (4) Mannitol (5), Mannose (6), arabinose (7) Trehalose (8) Xylose (9) Raffinose (10) . Yellow: positive test (using sugar) Purple: negative test (not using sugar).

Mauriello et al. [16] mentions that *S. saprophyticus* is often isolated from salami in southern Europe. Samelis [17] said that *S. saprophyticus* should be something to be evaluated with potential for use as starter culture, but another author disagrees saying this be recognized as an opportunistic pathogen [18].

Several species of coagulase-negative staphylococci are reported as common microflora in milk and fur in domestic ruminants and more common in bovine milk is *S. simulans*. The authors identified this microorganism in goat teats and mastitis in milk from these [19]. It may be suggested that the presence of this microorganism in the jerked beef may be because the product was made with a dubious origin of meat.

Staphylococcus auricularis was found in meat and slaughterhouses of South Africa at levels above the National Guidelines (10^2 UFC.g^{-1}), the authors suggest that this contamination rolled off the production line (handlers, surface equipment and slaughtering environment). So evidencing bad hygiene practices on behalf of refrigerators [20]. The same can be said of the material analyzed. Three isolated strains of *S. auricularis* derived Lot 2, the fermentation characteristics are shown in Figure 2.

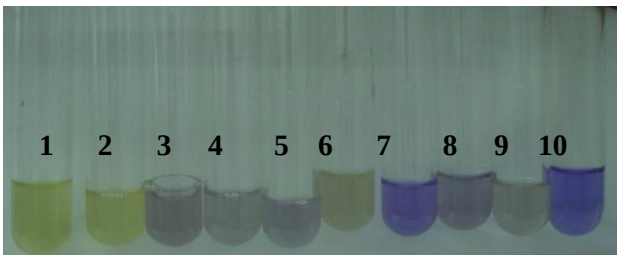

Figure 2. Proof of use of *S. S. auricularis* carbohydrates. Glucose bovis (1), Maltose (2) Sucrose (3) Lactose (4) Mannitol (5), Mannose (6), arabinose (7) Trehalose (8) Xylose (9) Raffinose (10) . Yellow: positive test (using sugar) Purple: negative test (not using sugar).

Staphylococcus aureus can produce more than one type of toxin which can cause symptoms of poisoning, accompanied mainly by vomiting and diarrhea. Other virulence factor is represented by toxin-1 of the toxic shock syndrome being recognized as a cause of fever, hypotension, congestion of various organs and lethal shock [21]. This species is the most prevalent in staphylococcal food poisoning outbreaks; however, the *S. intermedius* and *S. hyicus* can also produce enterotoxins [22] [23] and have been implicated in several outbreaks of food, especially in products of animal origin [23].

3.1.2 Identification of *Staphylococcus* isolated after irradiation of 4 kGy

Eight strains of *Staphylococcus* were isolated in six species after irradiation with a dose of 4 kGy, seven in lot 1, none in lot 2 and one in the lot 3. Were identified *Staphylococcus epidermidis*, *Staphylococcus xylosus* (2), *Staphylococcus intermedius*, *Staphylococcus warneri*, *Staphylococcus fleurettii* (2), *Staphylococcus aureus* sub. *Anaerobius*, as shown in Figure 3.

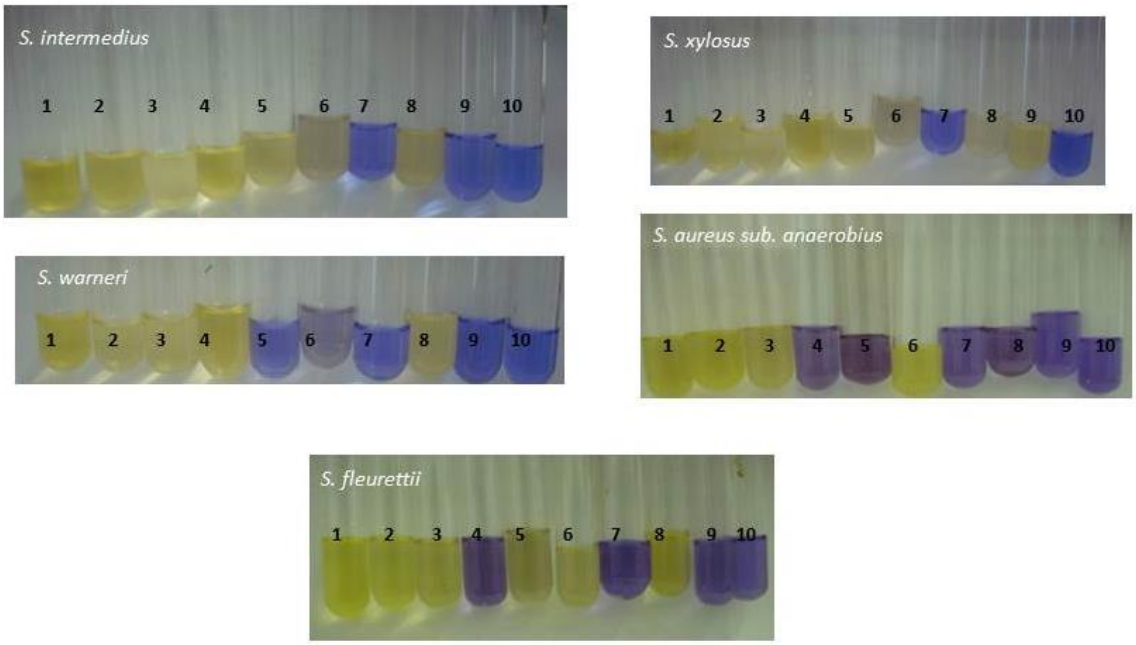

Figure 3. bovis (1), Maltose (2) Sucrose (3) Lactose (4) Mannitol (5), Mannose (6), arabinose (7) Trehalose (8) Xylose (9) Raffinose (10) . Yellow: positive test (using sugar) Purple: negative test (not using sugar).

In a study of raw and refrigerated milk, 40% of strains were coagulase-positive staphylococci among these was found *S. intermedius* and the authors suggest that the production of enterotoxin by *Staphylococcus* is strongly associated with the production of coagulase. Highlighting the risk of food consumption that has such microorgnisms. According to the Systematic Bacteriology and Veterinary Society *Staphylococcus intermedius* can be isolated from wounds caused by animal bites in humans, the causal agent of pyoderma [24].

A study to verify the production of enterotoxin by Staphylococcus coagulase negative noticed that *S. warneri* was able to produce in cooked ham and milk type long life [25].

Staphylococcus epidermidis despite being normally present in human skin and mucosa, presents medical interest, since according to some studies this species can cause food poisoning through the production of enterotoxins [26].

Staphylococcus epidermidis and *Staphylococcus intermedius* were found in the proportion of 57 % and 36 % in a study conducted in Jordan with fresh meat and frozen meat, the authors of this study report the danger of having enterotoxigenic staphylococci in the flesh, because those who consume them will contract an infection staphylococcal [27].The isolation of this bacterium in the material analyzed reveals the potential danger of consumption due to its reported enterotoxigenic power.

3.1.3 Identification of *Staphylococcus* isolated after irradiation of 6 kGy

Six microorganisms in three species were isolated after irradiation with dose of 6 kGy , all of the lot 3. Three were identified: *Staphylococcus saprophyticus* sub. *saprophyticus* (2*), Staphylococcus simulans* (3)and *Staphylococcus lugdnensis.*

A study says that the participation of coagulase negative staphylococci in human disease is increasing , and these a stake of 30% in bloodstream infections and that among the coagulase negative *S. lugdunensis* is the most pathogenic to present several virulence factors.

4. CONCLUSION

The results allowed to evaluate the effectiveness of gamma radiation at doses of 4 and 6kGy in control of microorganisms in samples of jerked beef. Thus ensuring greater safety for consumers, by the fact that by reducing the microbial load overall, certainly the level of pathogenic microorganisms present in this product will also be reduced. Greater demand is being made about food safety and the consumer market becomes more demanding about the product that consumes, so the radiation takes on a very important role as an assurance alternative for safe food production.

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