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Desenvolvimento microbiológico em carne suína PSE e normal armazenada sob refrigeração

Microbiological growth in normal and PSE pork stored under refrigeration

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RESUMO

O estudo foi conduzido com o objetivo de avaliar o desenvolvimento microbiológico em carne suína PSE em comparação à carne normal e o efeito da época do ano sobre a velocidade de proliferação dos mesmos. Amostras do músculo *Longissimus dorsi* de carcaças classificadas ao abate como PSE (pH_{45}<5,8) e normais (pH_{45}≥5,8), foram coletadas após 24 horas de refrigeração, no momento da desossa, em um abatedouro comercial, com serviço de inspeção federal, situado na região da Grande Dourados (MS). As amostras (12 normais e 12 PSE) foram coletadas em duas épocas distintas do ano (outono e inverno) para as análises de coliformes totais, coliformes a 45ºC, mesófilos, psicotróficos, pseudômonas, bolores e leveduras aos 0, 5, 10 e 15 dias de armazenamento por refrigeração. O crescimento microbiano comportou-se de maneira semelhante entre os dois tipos de carne ao longo do período de armazenamento, exceto para a contagem de microorganismos aeróbios mesófilos totais, cujo desenvolvimento foi superior em carnes PSE até o 5º dia de armazenamento, igualando-se entre os dois tipos de carne posteriormente. Houve efeito da época do ano sobre a contagem de coliformes totais e a 45ºC, sendo sua velocidade de proliferação superior em carnes provenientes dos animais abatidos na época mais quente (outono).

Palavras-chave: coliformes, microbiologia, suínos, vida de prateleira

SUMMARY

The study was conducted to evaluate the microbiological development in PSE pork compared with normal meat and the effect of season of the year on the rate of proliferation of the same. *Longissimus dorsi* muscle samples from carcasses classified as PSE slaughter (pH_{45} <5.8) and normal (pH_{45} ≥ 5.8) were collected after 24 hours of refrigeration, at the time of deboning, in a commercial slaughterhouse, with service federal inspection, located in the Grande Dourados region (MS). The samples (12 normal and 12 PSE) were collected at two different times of the year (fall and winter) for analysis of total and 45°C coliforms, mesophylls, psychotropic, pseudomonas, yeasts and molds at 0, 5, 10 and 15 days of storage under refrigeration. Microbial growth behaved similarly between the two types of meat throughout the storage period, except for the count of mesophilic aerobic microorganisms, whose development was superior in PSE meat until the 5th day storage, matching between both types of meat later. Was no effect of season on the count of total coliforms and 45oC, and their proliferation rate higher in meat from the slaughtered animals the warmer season (autumn).

Keywords: coliforms, microbiology, shelf life, swine

INTRODUCTION

Pork is a world's a valuable source of animal protein and Brazil is a country that may lead the swine's production for being one of the largest grain producers, condition for sustaining pig's chain. The service expectations of this market require continuous investment to improve productivity and meat quality (BRIDI et al., 2006).

Comply with all the quality specifications are the main challenge for the pork industry. The term meat quality is used and interpreted in different ways according to the point view and interests of producers, industry, trade and consumer. Currently, meat quality may be determined by physical, chemical and technological characteristics, microbial contamination and others (MEINERT et al., 2008).

The PSE meat (pale, soft, exudative) represents a quality problem in the pork industry, due to its flaccid texture, pale color and low water holding capacity, leading to higher water losses during processing than normal meat. The development of PSE meat is a result of the increase in glycolytic rate before and after slaughter, causing a greater concentration of lactic acid and accelerated decline of muscle pH. The combination of carcass low pH and high temperature leads to higher myofibrillar protein denaturation, with consequent reduction in water holding capacity .The rapid detection of PSE meat is particularly important within an industry and, presently, the most practical method is benchmarking carcasses pH at 45 minutes after slaughter and at the end of cooling (ROSENVOLD et al., 2003).

The meat has a chemical composition that makes it an excellent culture media for most organisms. Deficient sanitary conditions during the slaughtering animals, inadequate cooking, improper storage, lack of hygiene utensils, equipment and handlers, in addition to the functional properties of meat, may induce to a risk to consumers (MARCHI et al., 2012).

Researchers report that the main obstacles to the microbiological stability of meat products are water activity, pH and temperature (LEISTNER et al., 2000). The optimum values for bacterial growth are located around 0.990 to 0.995, and the water activity of fresh meat is 0.99 or higher, to the development of many types of bacteria.

The sanitary quality of animal source foods is a concern, due to the possibility of pathogenic microorganisms' transmission. The scope of this study was to evaluate the microbiological development in PSE pork compared with normal meat and the effect of season of the year on the rate of proliferation of the same.

MATERIAL AND METHODS

The experiment was carried out in Federal University of Grande Dourados, in Dourados, MS. The meat samples were obtained from a local commercial slaughterhouse, with a slaughtering capacity of approximately 2,300 animals per day. In order to select and collect the samples, the slaughterhouse was visited twice (in two different season of the year – autumn and winter). In those occasions, the slaughtered pigs presented an average of 115kg of live weight. The animals were all from the same genetic strain, and they were selected for high lean deposition in carcass.

A standard pre-slaughter management was implemented for all animals. The effective fasting time preceding the

transport was about six hours. The heavier animals per pen were selected for shipment, and their transfer was performed using the management of boards, resulting in a mixture of lots from that time. The animals were then loaded to the truck by the use of ramps. The transport density of the pigs to the slaughterhouse was standardized between 250-280kg of live weights per m^2. After arrival, animals were transferred to the resting piggery and housed in density of $0.65m^2$ per animal. The animals were directed to the slaughter using management boards upon completing the resting time. At the syringe, and only then, the electric baton was used in order to stimulate the animals to walk on the treadmill. After the electronarcosis stunning, the animals were slaughtered according to standard procedures, with horizontally bleeding. The carcasses were submitted to scalding, depilation, toilet, evisceration, serrate and inspection.

At 45 minutes after slaughter (pH_{45}), the carcasses pH was assessed in the center of the *Longissimus dorsi*, between the 12th and 13th thoracic vertebra in the left half carcass, using portable pH meter with probe penetration. The carcasses that pH_{45} was <5.8 were classified as PSE, and those whose pH_{45} was > 5.8 were classified as normal (D'ALESSANDO et al., 2011; O'NEILL et al., 2003). At the moment of deboning, 24 hours after slaughter, 12 carcasses in each visit (autumn and winter) were randomly selected (6 normal and 6 PSE), which were sampled and sectioned into steaks of 2.5cm thick, from the *Longissimus dorsi* muscle. The system of sampling was carefully conducted with the aid of sterilized materials. After the assortment, the samples were packed in sterile plastic bags, and packaged in an isothermal box with ice (7 ± 1ºC). Afterwards, they were transported immediately to the Laboratory of Microbiology, at the Faculty of Health Sciences, Federal University of Grande Dourados, where the samples were analyzed.

The excess of surface fat was immediately removed, and each sample was divided into four sub-samples, which were individually packaged in plastic trays. Each sample was wrapped in plastic wrap and kept under refrigeration (7 ± 1ºC), in order to simulate shelf conditions, for bioburden risk at 0, 5, 10, and 15 days of storage.

The total coliforms analyzes and coliforms at 45ºC, mesophylls, psychotropic, yeasts and molds were carried out according to the methods proposed by the APHA (2001). The culture media utilized for microbiological analysis were: peptone water, lauryl broth, EC, EMB, Sabouraud agar, cetrimide agar, PCA and sodium citrate. The culture media were prepared according to the manufacturer's specifications. All materials utilized in the analyses (Petri dishes, test tubes and pipettes) were sterilized by autoclaving at 121°C for 15 minutes. In cetrimide agar was added 1% glycerol after sterilization in an aseptic manner. In order to perform the analysis, 25g of meat were cut and weighed by placing them in a solution of sodium citrate (10^{-1} dilution); 1.0ml of the solution was mixed with 9.0ml tube containing peptone water (dilution 10^{-2}) and diluted to the ratio 10^{-6}. From each dilution 1.0 ml was removed to each tube containing 9.0ml of broth lauryl in triplicate and the solution was incubated for 24h at 37°C for coliforms analysis.

From dilutions of 10^{-2} and 10^{-3}, 0.1ml were removed and spread with Drigalski Strap on Sabouraud agar and cetrimide agar, and then the samples were incubated for seven days at room

temperature (Sabouraud) and refrigerator (cetrimide) for analysis of yeasts and molds. From the dilutions of 10^{-2}, 10^{-3} and 10^{-4}, and amount of 1.0ml were removed and poured on PCA agar, with smooth movements to homogenize 4 plates to each sample. In the next step, two of them are incubated in 37°C for 24 hours to proceed mesophylls count while the other two were incubated in refrigerator unit (7° ± 1°C) for seven days to count psychotropic microorganisms. During the reading of total coliform in broth lauryl, positive samples were divided to seed, with the aid of a platinum loop in tubes containing 9.0ml of EC broth and incubated in a water bath for 24 hours. The positive samples (with gas evolution) were streaked with the assistance of a platinum loop on EMB agar plates and then taken to a stove at 37° C for analysis of coliforms at 45°C.
The contaminated materials disposal were sterilized by autoclaving at 121°C, during 30 minutes, and properly discarded as medical waste.
The experiment was conducted in a completely randomized design in factorial scheme 2 x 4 (two meat types and four storage times) The values of microbial counts at 0, 5, 10 and 15 days of storage of the two meat types (normal and PSE) were transformed into the log CFU/g, and variance analysis was applied to the data, using the GLM procedure SAS (STATISTICAL ANALYSIS SYSTEM, 2002). Means were compared by Tukey test, at 5% significance.

RESULTS AND DISCUSSION

There was no interaction between the type of meat and the storage time, so the main effects were discussed separately. No presence of mesophylls microorganisms was found on the 1st day of storage in both treatments. However, they proliferated faster in PSE meat until the 5th day (1.76 x 0.53log CFU/g) ($p < 0.05$), and after this time, the count was similar in both types of meat (Figure 1).
The mesophilic microorganisms have an optimum temperature multiplication between 25°C and 40°C, and minimum multiplication around 5°C and 25°C, and a maximum between 40°C and 50°C. Theses temperatures are particularly valuable for food safety, including most pathogens of interest. Most of these microorganisms found in live animals are aerobic mesophilic, and only a few achieve to develop at temperatures below 7°C. Microbial counts of these organisms is used as an indicator of food quality and, when present in large numbers indicates failures during production (CARDOSO et al., 2005), which was not observed in this study.
All samples, both of PSE meat as normal, were negative for total coliforms and coliforms at 45°C on day zero (1st day of storage), and to fecal coliforms there was no microbial growth until the 5th day of storage.
The presence of coliform bacteria group, which most of them are found in the intestinal tract of humans and other warm-blooded animals, indicates environmental and fecal contamination in the product (ISHII & SADOWSKY, 2008). The enumeration of coliforms is utilized to assess the product hygienic conditions, as high numbers indicates contamination resulting from failure during processing, improper cleaning or inadequate heat treatment. The detection of a high number of coliforms bacteria in food is inferred as the presence of intestinal pathogens since the population of this group consists in

a high proportion of *Escherichia coli* (SOUZA et al., 2006). Thus, this low initial counting observed in the present study reflects the careful handling of the samples, and this condition is better than those commonly found in slaughterhouses or deboning rooms.

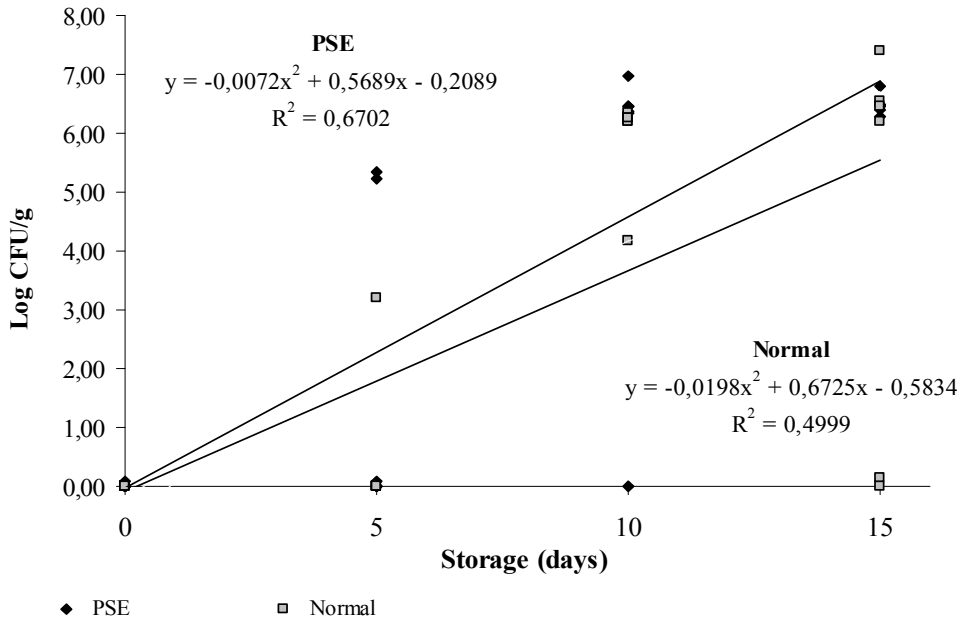


Figure 1. Growth curve of mesophilic microorganisms in normal and PSE pork throughout 15 days of storage

The mean values of total coliforms at 10th day of storage there were 3.09 and 3.97log CFU/g, and at 15th day there were 4.03 and 4.31log CFU/g in normal and PSE meat, respectively. The results did not differ among meat types ($p>0.05$). Values of Coliforms at 45°C were 2.52 and 2.26log CFU/g at the 10th day of storage for PSE meat and normal, respectively, and 3.19log CFU/g on day 15 for both types of meat ($p>0.05$).

Regarding the yeasts and molds, the initial microbial count was 1.27log CFU/g in normal meat and 1.36log CFU/g in PSE meat ($p>0.05$). During the storage period, these microorganisms developed similarly in both types of meat, reaching the maximum growth rate at 10 days of cooling (6.00 and 6.62log CFU/g in normal PSE meat, respectively) and with a trend to decrease after this period.

Both types of meat were positive at the beginning of the experiment to psychrotrophic microorganisms, with mean values of 1.80 and 2.35log CFU/g for normal and PSE meat, respectively no difference was found in the growth rate of these microorganisms between the types of meat ($p>0.05$).

The initial values for Pseudomonas count were 0.51 and 0.68log CFU/g for normal and PSE meat, respectively, reaching the maximum growth after 10 days of storage (4.35 and 4.97log CFU/g), and declining thereafter until the 15th day, with no differences among the types of meat ($p>0.05$).

Pseudomonas fragi species growth showed similar behavior of that observed for analyses of total psychrotrophic organism. Psychrotrophic microorganisms that predominate in the carcasses may multiply, even slowly at 0°C or below temperatures. These microorganisms are responsible for most of the changes and products deterioration, which makes the conservation of commercial life of meat, fish, eggs and other products (ALCANTARA et al., 2012), depends on the number of microorganisms after their production.

In natural environments and experimental conditions, when nutrients and space are limited, some factor could become negative. Any of these circumstances, isolate or in association, inhibit growth, causing a decline in the number of viable cells in the population, which could explain the reduction observed in this search of the total mesophilic microorganisms, psychrotrophic, yeasts or reduction in growth after 10 days of storage. As the nutrients viability decreases, cells become less able to generate ATP, and the growth rate reduces. The duration of the exponential growth phase is highly variable, depending on both bacteria genetic characteristics and environmental conditions; however no significant difference for PSE meat compared to normal meat. Observing the medium microbial growth from 1st to 15th day of storage, normal meat and PSE pork did not differ ($p>0.05$) for any of the microorganisms evaluated, with the highest growth rate regardless of the meat type, observed for psychrotrophic organisms, yeasts and molds (Figure 2).

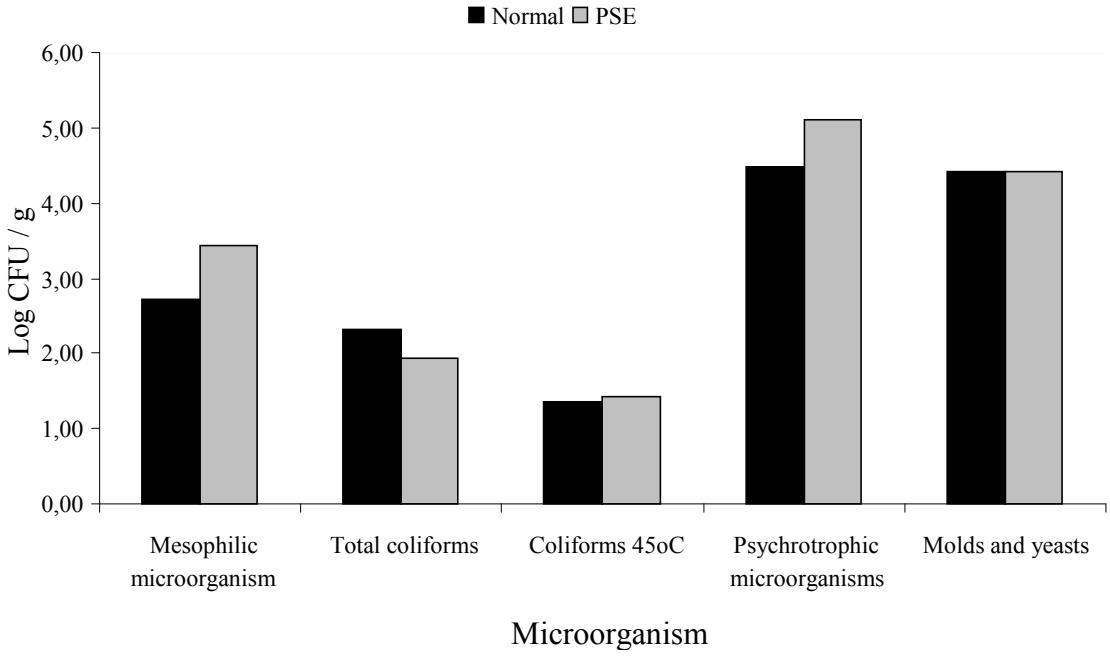


Figure 2. Average microbial growth of normal and PSE pork between 1 º and 15th day of storage

Most of the bacteria present an optimal growth in an environment with almost neutrality pH. Bacteria grow in pH ranges between 5.4 to 8.5 not being this way the pH of PSE meat the determining factor for the higher or lower bacterial growth.

However, PSE meat present greater water activity in relation to the normal meat because has a higher content of free water due to its lower water binding capacity with protein (KUO & CHU, 2003), making it more vulnerable to further bacteria development (YU et al., 2009). Thus, it was expected found greater rate of bacterial growth in these meats.

The microorganisms may be categorized regarding capacity for growth and metabolites due to aw condition (PELIZER et al., 2003), and most microorganisms, including pathogenic bacteria develop faster when water activity features are between 0.90 - 0.99 (ARAÚJO et al., 2005). Water activity of fresh meat is around 0.98-0-99, which may become an appropriate environment for the development of many types of bacteria.

According Gock et al. (2003) molds and yeasts develop sound under water activity values lower (>0.7), which may explain that there was no difference in those microorganisms' growth in meat with different values of water activity.

The sample collections were performed in two distinct periods, in which the environmental parameters showed reasonable variation (Table 1).

This may have influenced the microbial development, and their effects were further analyzed. The average temperature and relative humidity of the week in which the sampling was collected were taken into account. Regardless of the standard meat quality (normal or PSE), an effect of the environmental variables was found ($p<0.05$) on the development of some microorganisms.

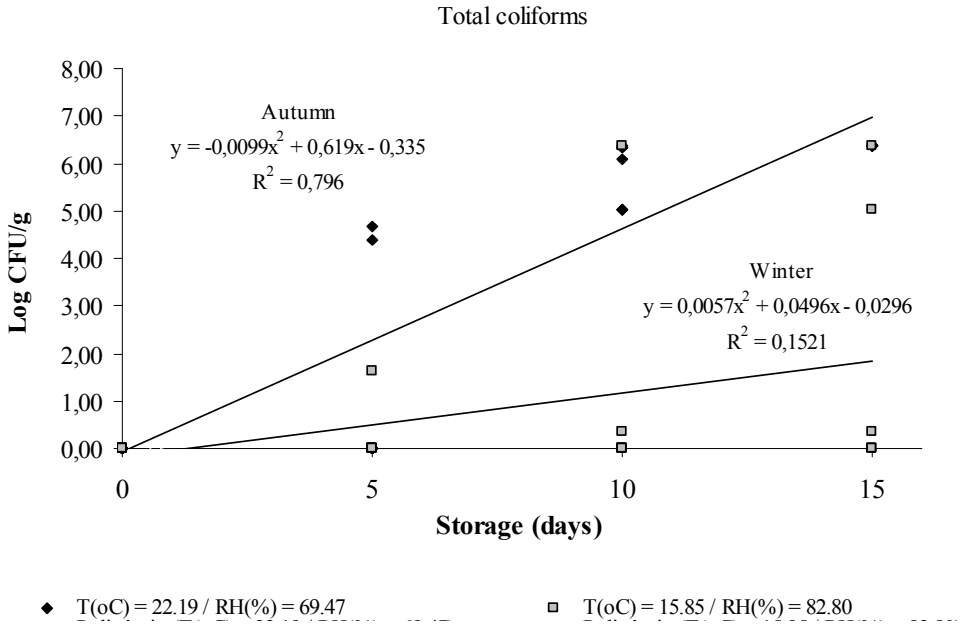
Table 1. Minimum temperature (T*min*), maximum temperature (T*max*), mean temperature (T*m*), minimum relative humidity (RH*min*), maximum relative humidity (RH*max*) and relative humidity (RH*m*) for the week of sampling normal and PSE pork at slaughterhouse

Sampling	T*min* (°C)	T*max* (°C)	T*m* (°C)	RH*min* (%)	RH*max* (%)	RH*m* (%)
1 (autumn)	15,17	29,60	22,19	40,30	96,6	69,47
2 (winter)	11,53	21,44	14,85	63,50	91,8	82,8

Total coliforms and coliforms at 45° C multiplied faster in meat samples from animals slaughtered at high air temperature (fall season). No growth over the 15 days of storage in samples collected during the winter (Figure 3) was found for the fecal coliform.

During animals' growth and development, the skin acquires a large population of microorganisms, including normal flora of the skin and the stemming from soil, water, food and manure. This skin microbial population at slaughter depends on a number of factors such as the production location, the method of transportation, the stable conditions in the slaughterhouse fridge and the season. Considering that the optimum temperature for the development of mesophilic bacteria,

among them those of the coliform group, is between 30-35°C, the season with hot weather becomes more favorable for growth, and consequently contaminating the animals' skin and carcasses.



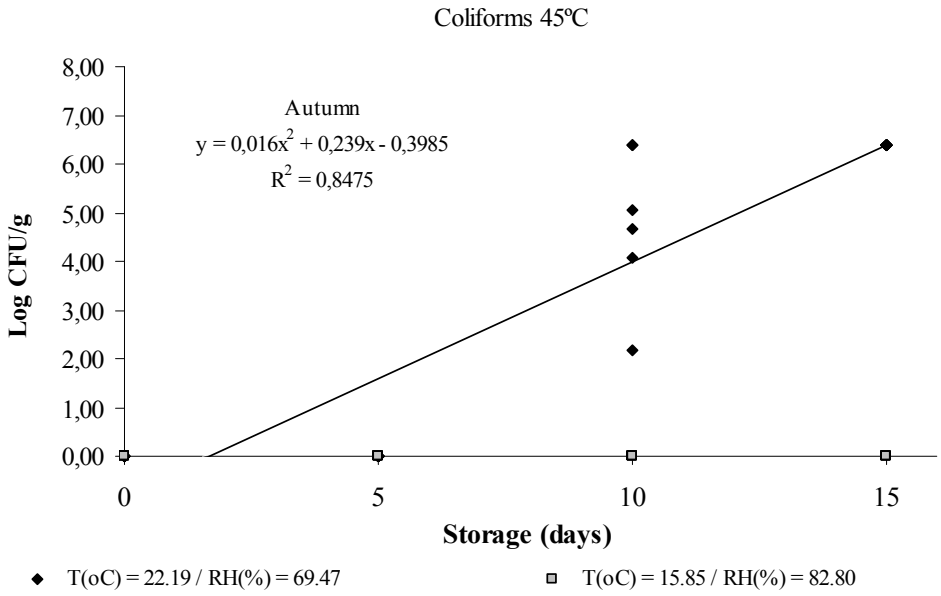


Figure 3. Growth curve of total coliforms and coliforms at 45 ° C, in samples of normal and PSE pork in periods of different environmental variables throughout 15 days of storage

Psychrotrophic microorganisms also developed faster in meat samples collected during warmer period (Figure 4). Although these bacteria are able to multiply at low temperatures, 7°C or lower. The optimum temperature for

growth is around 20-30ºC, which would explain slower proliferation when average ambient temperature was in about 15°C. There was no effect of environmental variables on the growth rate to the total count of mesophilic, psychrotrophic and Pseudomonas.

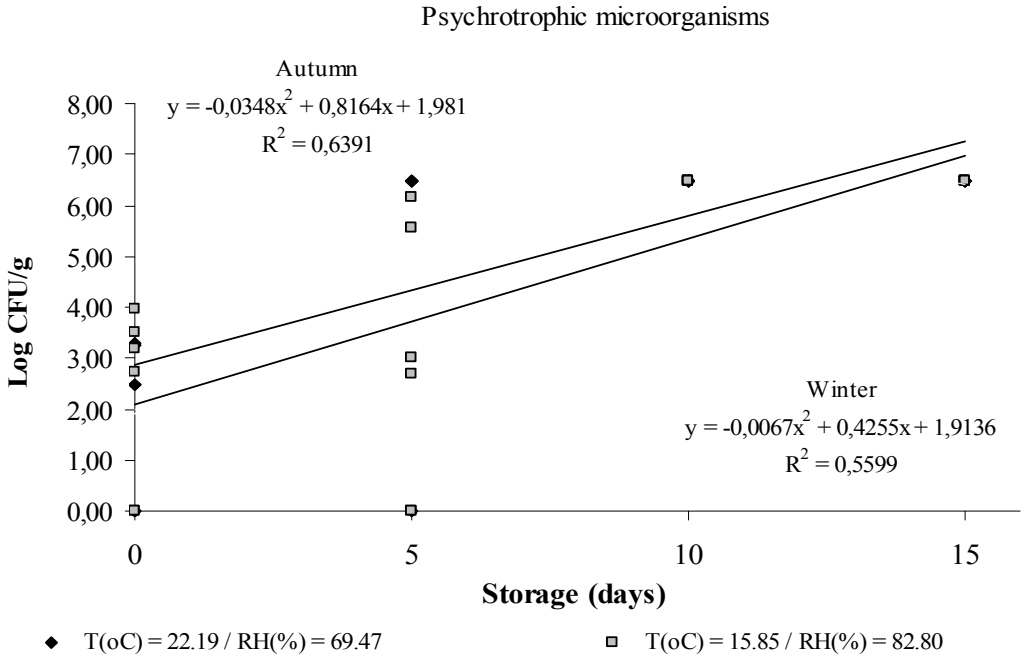


Figure 4. Growth curve of psychrotrophic microorganisms in samples of normal and PSE pork in different periods of environmental variables over 15 days of storage

The development of mesophilic microorganisms, total coliform, fecal coliform, psychrotrophic, molds and yeasts is similar in normal and PSE meat packed under refrigeration for 15 days, showing exponential growth phase until the 10th day of storage.
Environmental conditions, determined by the time of year, may influence the development of some microorganisms, the latter being favored by higher temperatures.

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