

The effect of reheating on shelf life of Unrefrigerated cooked prerigor beef dipped in Gut content

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SUMMARY

This study was conducted to evaluate the microbial quality and shelf stability at 22°C of prerigor beef samples deliberately contaminated by dipping in gut content, followed by aerobic packaging in oriented polypropylene bags and subjected to different cooking and reheating procedures. Reheating the cooked packaged samples after initial storage periods of 4, 10 or 24 hrs did not improve the shelf life of the products. The samples cooked at 75°C internal temperature for 30 min reached high levels of mean aerobic plate count APCs (Log 10/g) by day 7 of storage irrespective of the reheating time. The cooking procedure, 100°C for 40 min, followed by reheating after 4 to 24 hrs of initial storage gave inconsistent results especially by day 7 of storage. By day 14 of storage at 3°C or at 22°C, all the samples reached high levels of mean APCs (Logio/g) regardless of the cooking and reheating procedures.

INTRODUCTION

During the slaughter of meat animals the carcass surface becomes contaminated with large numbers of microorganisms (Embey & Scott, 1939). Studies of meat spoilage have been concerned largely with the processes occurring at shill temperatures. At higher temperatures mesophilic species which form the major part of the initial bacterial contamination (Newton *et al.*, 1978) should be able to grow and possibly displace the psychrotrophic species.

The importance of extending the shelf life of cooked meat at ambient temperature is well recognized in the face of the inadequate chilling facilities in many developing countries (In some instances, no such facilities exist).

Previous studies on the extended shelf life of unrefrigerated prerigor cooked meat indicated that regardless of the heat treatment applied to prerigor beef samples packaged in highly oxygen permeable bags (Oriented polypropylene, OPP), spoilage occurred during storage at 22°C. It was claimed that some thermoduric organisms could have survived the heat treatment and caused the spoilage. The results also revealed the importance of determining the generation time needed for heat injured cells or spores to germinate so that they could be dealt with whenever is practical (Abugroun *et al.*, 1993). The objective of this study was to evaluate the microbial quality and shelf stability at 22°C of prerigor beef samples deliberately contaminated by dipping in gut content, followed by packaging in OPP bags and subjected to different cooking and reheating procedures.

MATERIALS AND METHODS

In this experiment six heads of beef were used. Triceps brachii muscle was dissected from one side 45 min of exanguination of the animals, and samples of 2 x 3 x 5 cm³ were prepared.

Dipping & Packaging

Gut content was collected immediately after evisceration of the carcasses 30 gm of gut content was diluted in 300 ml distilled water to make 1:10 dilution. The dilution was sieved through a cheese cloth. Each sample was dipped in the diluted gut content with stirring before being placed in the oriented polypropylene bags.

The bags were then vacuum sealed using a vacuum sealing machine (Multivac Agw, Koch Supplies Inc., Kansas City).

Heat treatment

Two cooking procedures were applied. In one, the packaged samples were heated in 100°C water for 40 min. In the second, the packaged samples were heated in 100°C water to an internal temperature of 75°C (7 min) and then held in a 75°C water bath for 30 min. Previous work showed that heating the 2x3x5 cm³ samples in 100°C water for 7 min was equivalent to an internal samples temperature of 75°C (Abugroun *et al.*, 1993).

Forty-nine samples were prepared as previously described, and were assigned to seven treatment groups and then treated as follows: Four dipped uncooked packaged samples were stored at 3°C as a control.

One dipped fresh sample was placed in a sterile stomacher bag and kept overnight at 3°C for microbiological analysis. Three groups, two of seven and one of eight dipped packaged samples, were heated at 75°C internal temperature for 30 min as described above. Another three groups similar to the above were heated in 100°C water for 40 min. After the heat treatments they were cooled, sampled for microbiological analysis and stored at 22°C. The three groups of seven samples each, of the two cooking procedures were divided equally into three initial storage groups of 4, 10 and 24 hrs. At the end of each initial storage period at 22°C the group was sampled for microbiological analysis and reheated in 100°C for 10 min. The reheated samples were then cooled as previously described, sampled for microbiological analysis and reheated at 22°C.

Seal integrity test

Leakage tests were performed after samples had been randomly selected for microbial analysis. The integrity of the seal area was tested by visual inspection, then by submerging the packaged samples in a water-filled vacuum desicator. As vacuum was drawn on the vessel, entrapped gases expanded to exert a pressure on the package. Microbiological analysis was performed on samples that passed the seal integrity test.

Microbiological analysis

The samples per treatment were analyzed for aerobic plate count (APC) at both storage temperatures. The APC was determined on the samples randomly picked from each treatment, immediately after each heat treatment and after each initial storage and at weekly intervals during storage.

Each storage bag was opened with a sterile scalpel and its content was transferred into a preweighed sterile stomacher bag. This step and the rest of the microbiological analysis were conducted under a Laminar flow hood (Environmental Air Control, Inc., Hagerstown, Maryland) equipped with a HEPA filter to reduce airborne contamination. The exact weight of the sample was determined and a sterile 0.1% (w/v) peptone water (Diffco), nine times the weight of the sample, was added to the stomacher bag containing the sample to make 1:10 dilution. A stomacher lab-Blender 400 (Seward & Co., London, England) was used to homogenize the dilution for 2 min. Homogenates were serially diluted using sterile dilution bottles containing 99 ml of 0.1% (w/v) peptone water (Diffco), and duplicate plate count agar (Diffco) pour plates were prepared from appropriate

dilutions: Plates were inverted and incubated aerobically at 30°C for 48 hrs.±2 hrs; colonies were counted and the APC reported as Log CFU/g. Because transformed microbiological data approximate a normal distribution (Jarvis, 1989), Log CFU/g values were used to calculate the mean Log CFU/g values that were used in statistical analysis.

Statistical analysis

Analysis of variance using least square differences (LSD) and Newman-Keuls mean separation were determined using the Statistical Analysis System (SAS, 1989).

RESULTS

The results indicate that the mean APCs (Log_{10}/g) of the samples after the three initial storage periods, in both cooking procedures, were not significantly different. They did not differ from their levels at 0 hr following the heat treatments ($P > 0.05$). Nevertheless, the mean APCs (Log_{10}/g) after 24 hr of initial storage reached $> 3 \text{ Log}_{10}/\text{g}$ that were not different from the mean APCs (Log_{10}/g) of the fresh samples or of the uncooked samples stored at 3°C for 7 days ($P > 0.05$).

The mean APCs (Log_{10}/g) of the samples at day 0 of storage at 22°C, after the reheating following the three initial storage periods in both cooking procedures, were not different ($P > 0.05$). Irrespective of the decontaminating effects of the reheating, all the mean APCs (Log_{10}/g) of the samples cooked at 75°C for 30 Min reached similar high levels by day 7 of storage at 22°C and remained high through out the storage period ($P > 0.05$).

The cooking procedure, 100°C water for 40 min followed by reheating after the three initial storage periods, gave inconsistent results especially the two treatments with the reheating applied after 4 and 24 hr of initial storage (Table). By day 7 of storage at 22°C the mean APCs (Log_{10}/g) of the samples in these two treatments were significantly lower than the mean APCs (Log_{10}/g)

of the samples cooked at 75°C for 30 min (**P**> 0.05). By day 14 of storage, both cooking procedures resulted in high mean APCs (Log_{10}/g) that were not different (**P**> 0.05).

Least squares means and standard errors of aerobic plate counts (Log_{10}/g) for oriented polypropylene packaged and inoculated pre-rigor beef samples as influenced by cooking and storage procedures (**N** = 6 for each treatment).

Storage time		Cooked at	Cooked at	Uncooked
75°C ^h for		100°C for		
30 min		1.00 (0.52) ^h		40 min 1.66 (0.52) ^{g^h}
Storage at 3°C, d		0		4.16(0.52)
7				3.85(0.52)
14				6.36(0.52)
22°C initial storage (4hr)		storage after reheating,		1.43(0.52) ^h
d		1.26(0.52) ^h		
0		1.03(0.52) ^h		1.06(0.57) ^h
7		8.34(0.57) ^d		5.14(0.52) ^d
14		8.20(0.52) ^d		7.17(0.52) ^{de}
22°C initial storage (10hr)		storage after reheating,		1.79(0.57) ^{g^h}
d		1.27(0.57) ^h		
0		1.3(0.57) ^h		1.24(0.52) ^h
7		8.31(0.52) ^d		7.09(0.52) ^{de}
14		8.49(0.57) ^d		7.94(0.52) ^{de}
22°C initial storage (10hr)		storage after reheating,		3
d		3.25(0.57) ^{fgh}		1.00(0.52) ^h 1.03(0.52) ^h
7		8.11(0.57) ^d		5.26(0.52) ^d
14		8.51(0.52) ^d		6.92(0.52) ^{de}

* Sample were dipped in 10⁻¹ dilution of gut content before packaging.

^b Heated to 75°C in 100°C water. Reheating at 100°C water for 10 min after initial storage.

defgh Means bearing different superscripts are significantly different (**P**< 0.05).

The uncooked samples reached a high mean APC (Logio/g) by day 14 of storage that was not different from the mean APC (Logio/g) of all the cooked samples stored at 22°C.

DISCUSSION

The results of this experiment show that more than 10 hr is needed for heat injured cells or spores to germinate during storage at 22°C, since by 24 hr of storage more than 3 (Logio/g) were reached in both cooking procedures. The results also indicate that reheating the samples at different initial storage periods does not guarantee shelf stability of the products when stored at an ambient temperature.

The gut contains different species of bacteria including Clostridia and other faecal bacteria (Haines, 1937). In addition to the contamination deliberately introduced with large numbers of microorganisms during slaughter (Embey, & Scott, 1939), the majority of microflora is composed of saprophytic species and various enterobacteriaceae (Stringer *et al.*, 1969). Both lactic acid bacteria and Bacillus species are initially present in low numbers (Ingram & Simonsen, 1980).

The OPP film used prevents post cooking bacterial contamination (Ronsivalli *et al.*, 1966). The heat sensitive psychrotrophic organisms are not expected to survive the cooking treatment used in this study. Therefore, spoilage microflora can only develop from those thermotolerant microorganisms that survive the cooking process (Steinke & Foster, 1951; Bell & Gill, 1982). The OPP, being highly permeable to oxygen, is expected to encourage the growth of aerobic spore formers and to prevent the growth of strict anaerobes.

The relatively low mean APCs (Logio/g) by day 7 of storage at 22°C, of the samples cooked in 100°C water for 40 min and reheated after 4 or 24 hr of initial storage reflect the inconsistency observed with these two treatments. This inconsistency could be explained by the fact that the samples might differ in their initial microbial load and composition before the dipping treatment depending on their location within the Triceps brachii muscle. Accordingly, the microbial load and composition after the dipping treatment would also be different.

Although the mean APC (Logio/g) of the control samples, by day 14 of storage, was not different from the mean APC (Logio/g) of the cooked samples stored at 22°C, the microflora would be different. At chill temperatures the spoilage flora of meat are composed of psychrotrophic species with pseudomonads usually predominating under aerobic conditions (Gill & Newton, 1978). At higher temperatures mesophilic species, which form the majority of the initial bacterial contamination (Newton *et al.*, 1978) should be able to grow and multiply.

It is clear from this study that reheating samples dipped in gut content at 4, 10 or 24 hr of initial storage at 22°C does not guarantee their shelf stability at such storage condition. The heat treatments applied should be supported by one or more protective mechanisms if the cooked product is intended for storage at ambient temperatures.

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