5:4 Hot-boning of pork - a microbiological evaluation of different packaging principles

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Introduction

Little is known about the types and numbers of microorganisms present on pork as compared to red meat, but shelf-life of pork has been found to be inferior to that of red meat. Pork is probably also more easily contaminated with potential pathogenic bacteria than red meat. Hot-boning of meat is a new technique which has many potential advantages including reducing cooling space and refrigerating energy, increasing cut yield and facilitating centralized processing.

On the other hand potential microbiological advantages (shelf-life) and disadvantages (hygiene), particularly in connection with the packaging of hot-boned meat are relatively unknown.

To develop a packaging system which offers optimal microbiological safety and optimal shelf-life to hot-boned pork is therefore of great importance.

The aim of the present investigation has been to find out whether hot-boning of pork involves increased hygienic risks and to examine the effect of different gas packaging systems on the microbiological shelf-life of packaged hot-boned pork.

Materials and Methods

Boneless top loin roasts of pork, hot-boned 4 hrs after slaughter (meat temperature 20°C) and boneless top loin roasts of pork, cold-boned 24 hrs after slaughter (meat temperature 5.5°C) were packaged as follows:

1. Conventional vacuum packaging.
2. Gas packaging in a mixture of 90% CO_2 and 10% N_2 and with a headspace of 0.2 litres.
3. Gas packaging in 100% CO_2 and with a headspace of 3 litres.

The packaging material was Saran-laquered Mylothen. All the packages were rapidly chilled at -1°C and an air velocity of 0.5 m/s, single-layered on trays for 5 hrs followed by storage at 4°C. After 9 and 20 days of storage twin samples from each packaging system were subjected to the following examinations: Gas analysis, microbiological analysis, drip loss and sensory evaluation (colour and odour).

Gas analysis of CO_2 and O_2

Before the plastic bags were opened for microbiological sampling 0.5 ml gas was withdrawn and analysed for CO_2 and O_2 using a gas chromatograph (Varian 920) fitted with Porapac Q and a molecular sieve 5A column. The vacuum package were first filled with 100 ml helium to create sufficient gaseous volume to facilitate sampling of the content.

Microbiological analysis

Samples for microbiological analysis were taken from pork roasts before packaging and from all packaging systems after 9 and 20 days of storage at 4°C.

At the end of each storage period all pork roasts were repackaged in oxygen permeable film and stored for 3 days at 4°C after which time they were again sampled microbiologically.

The microbiological sampling was carried out according to Enfors et al. (1979). The samples were examined for Total aerobic count, Enterobacteriaceae, Lactic acid bacteria, Clostridia, Staphylococcus aureus and Enterococci.

All the packaged meat samples and an additional 10 samples of fresh hot-boned pork roasts were also examined for the presence of: Yersinia enterocolitica, Aeromonas hydrophila, Campylobacter jejuni and Erysopelothrix rhusiopathiae.

Isolation and identification

From countable plates from total aerobic count agar (Tryptone-glucose-extract agar, TGE) and from plates used for identification of Enterobacteriaceae (Violett-red-bile-dextrose-agar, VRBD), 20 colonies/plate were picked for identification from samples of fresh meat before packaging and from stored samples in each packaging system.

400 bacteria strains isolated from TGE were identified of which 80 represented the initial bacteria flora from hot-boned and cold-boned pork.

From VRBD 200 bacteria strains were isolated and identified. From samples which had been stored in air for 3 days a total of 160 strains were isolated from TGE plates. No isolations were made from VRBD plates.

Erysipelothrix-like organisms were tested further for H_2S formation on TSI-agar.

Drip loss

Amount of drip loss i.e. free meat juice in the packages was weighed and calculated as percentage of the sample weight.

Sensory evaluation

Colour and odour were tested directly after breaking the packages and after storage of the samples in air for 15 min.

Results and Discussion

Gas atmospheres

Analysis of CO_2 and O_2 concentrations in the packages after storage for 20 days at 4°C showed that the CO_2 concentration in the vacuum packages had increased to 80% both in hot and cold-boned samples. The oxygen concentration was 1-7% in the vacuum packages but only 0.1-0.6% in the gas packaging systems (Table 1).

Type of package	Storage (days)	% CO_2 in the packages		% O_2 in the packages		Total count TGE, 28°C (Log N/cm²)		Lactic acid bacteria ACA 30°C (Log N/cm2)		Entero bacteriaceae VRBD, 37°C (Log N/cm2)		Drip loss (W/W %)	
		cold-boned	hot-boned	cold-boned	hot-boned	cold-boned	hot-boned	cold-boned	hot-boned	cold-boned	hot-boned	cold-boned	hot-boned
Vacuum packaging	0	-	-	-	-	4.1	2.4	0.8	0	1.6	0	-	-
	9	82.2	83.0	1.8	1.1	4.2	3.3	3.8	1.3	0.6	0	3.2	2.5
	20	60.6	82.2	2.0	7.3	7.7	6.1	6.4	5.7	5.5	3.2	1.3	3.5
Gas mixture (90% CO_2+10% N_2)	0	-	-	-	-	4.1	2.4	0.8	0	1.6	0	-	-
	9	59.0	60.5	0.2	0.3	4.0	2.6	3.6	2.3	1.5	0	2.5	2.5
	20	50.7	55.9	0.4	0.3	7.4	4.8	6.8	2.2	3.8	0	3.5	2.8
100% carbon dioxide	0	-	-	-	-	4.1	2.4	0.8	0	1.6	0	-	-
	9	99.5	95.7	0	0.6	3.5	2.5	2.8	0.1	1.1	0	1.2	0.8
	20	99.5	98.5	0	0.1	6.0	3.2	5.9	1.9	1.3	0	0.9	1.9

Table 1. The microflora of gas packaged hot-boned and cold-boned pork loin roasts after 20 days of storage at 4°C.

Gas composition of the packages at the time of opening is also given.

This is in agreement with results obtained by Erichsen & Molin (1981) on packaged beef and by Blickstad et al. (1981) on packaged pork.

In the gas packages originally containing 90% CO_2 and 10% N_2 the CO_2 concentration had decreased to 50-60% during storage while the oxygen concentration was 0.2-0.4% (Table 1). The reason for the reduction of the CO_2 concentration in these packages could be explained by the low headspace in the packages (2 dl) and by absorption of CO_2 into the meat. No difference was found in the CO_2 concentration of hot-boned pork compared to cold-boned pork in spite of assumed higher biochemical activities of hot-boned meat which could possibly affect the gas atmosphere. Cuthbertson (1977) found that the higher respiration rate of hot-boned beef did not result in any noticable higher concentration of CO_2 in gas packages.

In packages containing 100% CO_2 the CO_2 concentration had hardly changed during the storage period (Table 1).

Odour and appearance

After 20 days of storage all samples irrespective of gas packaging system were fully acceptable as far as colour and general appearance are concerned. This also applies to the samples subsequently stored in air for 3 days. However, on cold-boned samples, vacuum packaged or packaged in the gas mixture, the microbial load was so high after 3 days in air that these samples probably would develop objectionable off-odours within 1-2 days of further storage in air (Table 3).

Drip loss

No difference was found in the drip loss between hot-boned and cold-boned pork samples in any of the packaging systems.

Initial bacterial load

Great differences existed in the initial bacterial numbers on samples from hot-boned and cold-boned pork. Total aerobic count was almost 2 log units lower on hot-boned pork (Table 1). Also the composition of the microflora differed markedly. On hot-boned pork mesophilic micrococci dominated the microflora while meat spoilers like Enterobacteriaceae occurred only in small numbers. On cold-boned pork, gram negative meatspoilers were more abundant (Table 2). Numbers as well as types of microorganisms originally present on meat is of great importance for the shelf-life of packaged meat during refrigeration storage (Ingram, 1962).

Gas atmosphere / Microorganisms	Before packaging		Vacuum packages		Gas mixture (90% CO2 + 10% N2)		100% CO_2	
	cold-boned	hot-boned	cold-boned	hot-boned	cold-boned	hot-boned	cold-boned	hot-boned
Micrococcus spp.	30	82	-	-	-	-	-	-
Lactobacillus spp.	-	-	45	57	98	90	100	100
Streptococcus spp.	-	-	-	3	2	-	-	-
Corynebacteria	20	5	5	-	-	-	-	-
Enterobacter spp.	-	-	-	10	-	10	-	-
Serratia spp.	-	-	37	23	-	-	-	-
Acinetobacter spp.	20	-	-	-	-	-	-	-
Flavobacterium spp.	5	-	3	-	-	-	-	-
Pseudomonas spp.	2	-	-	-	-	-	-	-
Proteus spp.	10	-	10	-	-	-	-	-
Moraxella-like	13	13	-	-	-	-	-	-
Erysipelothrix-like	-	-	-	7	-	-	-	-
Total aerobic count (Log N/cm²)	4.1	2.4	7.7	6.1	7.4	4.8	6.0	3.2

Table 2. The microflora of hot-boned and cold-boned pork loin roasts packaged in different gas atmospheres after 20 days of storage at 4°C. Percent of the total microflora.

Microbial quality of packaged pork

After 20 days of refrigeration storage a certain difference was observed in microbial numbers between samples in different gas packaging systems and between hot and cold-boned pork (Table 1). The microbial load was consistently lower on hot-boned pork all through the storage period and in all packaging systems. The quality promoting effect of the different gas packaging systems seems to increase in the following order: vacuum $< 90\% \ CO_2 + 10\% \ N_2 < 100\% \ CO_2$. This effect was reflected not only in lower total aerobic count in samples packaged in 100% CO_2 but also in the composition of the microflora after storage for 20 days att 4°C (Table 2). In vacuum packaged samples the number of lactic acid bacteria constituted 45% of the total flora while the gram negative meat spoilage bacteria constituted 50%. In samples packaged in the 90/10 gas mixture the microflora consisted mainly of lactic acid bacteria while most of the gram negative types had been suppressed (Table 2). Finally, in pure CO_2 atmosphere, the microflora was completely dominated by lactic acid bacteria.

It could be argued that the differences in the microbial numbers between the 90/10 gas packaging alternative and the pure CO_2 alternative was caused by differences in the headspace, i.e. total amount of CO_2, rather than differences in CO_2 concentration. This argument may be valid to the extent that microbial numbers do not reflect the difference between 90% and 100% CO_2 but between 60% and 100% CO_2 (Table 1). Because of the smaller gas volume in the 90/10 alternative the composition of the gas phase changes when CO_2 is absorbed in the water phase of the meat and part of the CO_2 molecules are transformed into HCO_3^-. As a consequence the difference in microbial numbers would have been somewhat smaller if comparison had been made using a greater gas volume in the 90/10 gas alternative.

With reference to a recent work by Molin (1983) it seems more appropriate to assume that a smaller but significant difference in microbial numbers also exists between 90% and 100% CO_2 under otherwise comparable conditions.

The fact that the CO_2 concentration found in the vacuum packages was higher (ca 80%) than in the 90/10 gas alternative (60%) may seem contradictory to the observation above that vacuum packaging is inferior from a bacteriological point of view - several explanations are possible. (1) The different method used when analysing the gas in the vacuum packages gave results which are difficult to compare. (2) The amount of CO_2 as such is of importance for an extended shelf-life. (3) The oxygen concentration was considerably higher in the vacuum packages. (4) In the 90/10 gas alternative the CO_2 concentration was high right from the start of the storage period while in vacuum packages it was successively built up. The last explanation may seem the most likely one.

The composition of the microflora after storage for 20 days was unaffected by the boning procedure. A favourable governing effect on the microflora could be noticed on hot-boned pork stored in pure CO_2. Lactic acid bacteria dominated the microflora completely in these packages even though the total aerobic count was still low (3.2 log units/cm^2).

The only difference found between hot-boned and cold-boned pork was the lower initial number of microorganisms on hot-boned pork, and that part of this difference was maintained all through the storage period. After 20 days of storage the difference in number of organisms was somewhat smaller in vacuum packages and greater in gas packaged alternatives (Table 1). To gain a better advantage of the favourable initial microbial condition of hot-boned pork, therefore, gas packaging in high concentrations of CO_2 should be used.

Storage in air

Based on total aerobic count obtained after storage for 20 days hot-boned pork tolerated a further 3 days storage period in air at 4°C, irrespective of previous packaging system. With cold-boned pork this was only achieved in samples previously stored in pure CO_2 atmosphere. Composition of the microflora was not affected in a crucial way by subsequent storage of the pork samples in air (Table 3).

Type of package	Storage (days)	Total count TGE 20°C (log N/cm^2) cold-boned	hot-boned	Lactic acid bacteria ACA 30°C (log N/cm2) cold-boned	hot-boned	Enterobacteriaceae VRBD, 37°C (log N/cm^2) cold-boned	hot-boned
Vacuum	9	4.2	3.3	3.8	1.3	0.6	0
Vacuum + air	9 + 3	4.9	3.4	4.6	3.2	1.3	0.3
Vacuum	20	7.7	6.1	6.4	5.7	5.5	3.2
Vacuum + air	20 + 3	9.8	6.8	6.8	5.9	8.1	4.1
Gas-mix	9	4.0	2.6	3.6	2.3	1.5	0
Gas-mix + air	9 + 3	5.2	3.9	4.9	3.8	1.4	0
Gas-mix	20	7.4	4.8	6.8	2.2	3.8	0
Gas-mix + air	20 + 3	7.5	6.3	7.2	4.9	4.4	1.9
100% CO_2	9	3.5	2.5	2.8	0.1	1.1	0
100% CO_2 + air	9 + 3	4.5	2.7	4.4	1.9	0.9	0.1
100% CO_2	20	6.0	3.2	5.9	1.9	1.3	0
100% CO_2 + air	20 + 3	6.5	4.3	6.4	3.4	2.4	0.6

Table 3. The microflora of hot-boned and cold-boned pork loin roasts after 20 days of storage at 4°C followed by 3 days storage in air at 4°C.

Safety

Pathogenic bacteria like Yersinia enterocolitica, Campylobacter jejuni, Aeromonas hydrophila, clostridia, enterococci, Staphylococcus aureus and Erysipelothrix rhusiopathiae were not found in any of the samples of pork.

Erysipelothrix-like organisms were, however, identified among isolates picked from the total aerobic count plates from hot-boned pork (Table 2 & 4). The biochemical pattern of these organisms corresponded partly with that of Erysipelothrix rhusiopathiae, but the organisms obviously grew on refrigerated meat and on TGE-agar which would imply that these organisms are not Erysipelothrix rhusiopathiae, i.e. pathogenic organisms. The erysipelothrix-like organisms found should instead be regarded as belonging to a type of spoilage "lactic acid bacteria". Microorganisms like Enterobacteriaceae are commonly present on fresh meat. In this study the number of Enterobacteriaceae was particularly great in vacuum packaged pork after storage for 20 days at 4°C (Table 1). Enterobacteriaceae are present in soil and water and many strains grow well under refrigeration temperatures.

Microorganisms	Pork previously packaged under vacuum: cold-boned	hot-boned	Pork previously packaged in gas-mixture: cold-boned	hot-boned	Pork previously packaged in 100% CO2: cold-boned	hot-boned
Micrococcus spp.	-	-	-	-	-	-
Lactobacillus spp.	25	53	90	48	99	92
Streptococcus spp.	-	-	-	-	-	-
Corynebacteria	3	-	8	-	-	-
Enterobacter spp.	10	12	-	32	-	-
Serratia spp.	60	18	2	5	-	-
Acinetobacter spp.	-	-	-	-	-	-
Flavobacterium spp.	-	-	-	-	1	-
Pseudomonas spp.	-	-	-	-	-	-
Proteus spp.	2	-	-	-	-	-
Moraxella-like	-	-	-	-	-	-
Klebsiella spp.	-	17	-	-	-	-
Hafnia alvei	-	-	-	-	-	2
Erysipelothrix-like	-	-	-	15	-	6
Total aerobic count after air storage (20 + 3 days) (log N/cm^2)	9.8	6.8	7.5	6.3	6.5	4.3

Table 4. The composition of the microflora of hot-boned and cold-boned loin roasts packaged in different gas atmospheres and stored for 20 days at 4°C followed by storage for 3 days in air at 4°C. Percent of the total microflora after storage in air.

In this study a relatiely large number of Enterobacteriaceae were isolated from TGE-plates and from VRBD-plates both from vacuum packaged pork and from pork packaged in the 90/10 gas mixture (Table 5). However, no hygienic hazardous types or higher concentrations of potential pathogenic microorganisms were found.

Organisms	Percent distribution
Serratia rubidaea	26
Serratia liquefaciens	21
Serratia marcescens	9
Enterobacter agglomerans	21
Hafnia alvei	17
Enterobacter amnigenus	5
Providencia rettgeri	1
Enterobacter cloacae	0.5
Klebsiella oxytoca	0.5

Table 5. Enterobacteriaceae isolated from VRBD. 200 strains were identified.

Conclusion

The conclusions of the present study can be summarized as follows:

1. Hot-boning of pork does not imply any special microbiological health risks.
2. At the time of packaging, hot-boned pork had a lower microbial load than cold-boned pork. This implies both an increased shelf-life and a better biological protection against pathogenic bacteria due to development of lactic acid bacteria.
3. From a microbiological point of view the best packaging system for hot-boned as well as cold-boned pork is gas packaging in pure CO_2 atmosphere.
4. To gain full advantage of the good hygienic and microbiological conditions obtained in the hot-boning process, hot-boned pork should be packaged in high concentrations of CO_2 (preferably 100%).

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