CARBON DIOXIDE PACKAGING AS A MEANS OF CONTROLLING THE SPOILAGE FLORA OF DFD MEAT

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INTRODUCTION

The packaging of DFD meat (Dark, Firm, Dry meat) under vacuum has been shown to reduce shelf life and cause discolouration of the meat due to the development of high numbers of Pseudomonas putrefaciens and/or Enterobacteriaceae (Nicol et al., 1976, Taylor & Shaw, 1977, Petterson & Gibbs, 1977, Bem et al., 1976, Newton & Gill, 1980).

Considering the fact that the frequency of DFD meat in Swedish beef carcasses on an average is about 6-8%, the technical and practical implications of the short shelf life of DFD beef is considerable.

Gas packaging of DFD beef in pure CO_2 has been shown to control the microflora of the meat, i.e., lactic acid bacteria increase and microorganisms more deteriorative for the meat quality decrease (Erichsen & Molin, 1981). This effect may be utilized to prolong the shelf life of refrigerated DFD beef.

The aim of the present study was to get further proof that packaging in pure CO_2 is a suitable method to increase the microbiological shelf life of refrigerated DFD beef. This was done by studying the microbial flora of normal pH beef and of DFD beef after storage in CO_2 and in vacuum packages.

MATERIAL AND METHODS

Experimental design

Four muscles of beef (Longissimus dorsi) with a pH of 5.5 and an equal number of the same type of muscle with a pH of 6.6 were selected for the experiments. Half of the material had been electrically stimulated by the low voltage method (Rudèrus & Fabiansson, 1981).

The beef muscles were cut into halves and each half wrapped in laminated plastics of saran-laquered poly with low gas permeability (O_2=10 cm^3/cm^2 - 24 hrs, CO_2=10 cm^3/cm^2 - 24 hrs). Four packages of DFD beef and four packages of normal pH beef were vacuum drawn (1 kPa) and the corresponding numbers of packages were filled with 100% CO_2. The headspace in the gas-filled packages was about 10 litres. All samples were stored at 4°C for 34 days.

Microbiological sampling

The microbiological sampling was carried out according to Enfors et al. (1979). Samples for bacteriological examination were taken from all the muscles before packaging and from all the packages after storage. Using conventional dilution procedures viable counts were obtained from the suspensions on Plate Count Agar (PCA, Oxoid), Vilett-Red-Bile-Dextrose-Agar (VRBD, Oxoid) MRS-agar (deMan et al., 1960) and on Triple-Sugar-Iron-Agar (TSI, Difco).

The PCA plates were incubated at 28°C for 3 days, the VRBD plates at 37°C for 24 hrs., the MRS plates at 30°C for 5 days and the TSI plates at ambient temperatures for 5 days in N_2 atmosphere.

Classification of isolates

Identification tests were carried out on microorganisms isolated from the fresh beef samples and from samples stored under vacuum and in CO_2 atmosphere. The isolates were picked from countable PCA plates used for the aerobic count. Twenty colonies were randomly selected from each plate and a total of 480 colonies were isolated of which 160 represented the initial flora from the two types of beef.

The classification of the PCA isolates was performed according to Enfors et al. (1979) and Blickstad et al. (1981) except for Enterobacteriaceae which were classified by the system Micro-ID (General Diagnostics).

Gas analysis

Before the plastic bags were opened for microbiological sampling a layer of silicon rubber was glued to each one of them. Through this layer a gas-tight syringe was inserted and 0.5 ml gas was withdrawn and analysed for CO_2 and oxygen. The vacuum-packaged

samples were first filled with 100 ml of helium to create sufficient gaseous volume to facilitate sampling of the contents. A gas chromatograph (Varian 920) fitted with Porpak Q and a molecular Sieve 5A column were used for CO_2 and oxygen.

Two samples were taken from each bag and the mean values of the analyses were determined.

RESULTS

Table 1 shows the increase of the bacterial counts on the different types of beef during storage.

No differences were found in bacterial numbers or bacterial types present on electrically stimulated and non-stimulated samples of beef during storage under vacuum and in pure CO_2 atmosphere. The values presented are therefore the mean values from bacterial examinations of both electrically stimulated and non-stimulated beef samples.

It can be seen that the total flora was higher on DFD beef than on normal beef in both packaging systems after storage for 34 days at 4°C. Even the count of Enterobacteriaceae on VRBD plates was higher on DFD beef than on normal beef. There was, however, a striking difference in the growth of Enterobacteriaceae in the two packaging systems. In 100% CO_2 atmosphere Enterobacteriaceae were clearly retarded as compared to vacuum-packaged samples both on DFD and of normal pH beef.

The lactic acid count was higher on beef packaged in CO_2 atmosphere than on beef packaged in vacuum and somewhat higher in normal pH beef than on DFD beef.

In normal beef the lactic acid count seemed equal to the total count while the count on DFD beef was lower in both packaging systems. Vacuum-packaged samples were all spoilt with strong off-odours after storage for 34 days at 4°C. Normal beef showed no discolouration while a greenish discolouration was observed in connection with DFD beef. In these samples high concentrations of H_2S-producing microorganisms were observed (Table 1). Samples of both normal beef and DFD beef in CO_2 atmosphere had retained their normal red colour and did not produce any off-odours during the storage period. In CO_2-packaged beef samples H_2S-producing microorganisms had also developed but not to the same extent and no discolouration was evident after storage.

On normal beef samples H_2S-producing bacteria did not develop in any of the packaging systems.

The CO_2 and the oxygen content of each package was determined after the termination of the storage period. Table 1 shows that the CO_2 content in the vacuum-packaged samples of normal beef had increased during storage and reached almost the same concentration as in the CO_2-packaged samples. The final oxygen concentration was higher in the vacuum-packaged samples than in the CO_2-packaged samples.

The composition of the microflora of normal and DFD beef after storage in vacuum and in CO_2 atmosphere is shown in Table 2. On vacuum-packaged beef the microflora was composed mainly of Hafnia alvei and lactic acid bacteria. The major differences between DFD beef and normal pH beef were: the higher level of Hafnia alvei, the lower level of Lactobacillus spp and the presence Erysiphelothrix-like organisms, Yersinia enterocolitica and Brochothrix thermosphacta.

On the CO_2-packaged beef the predominance of Lactobacillus spp on both types of beef was stronger than on the vacuum packaged beef.

DISCUSSION

The initial microbiological load on beef used in the present study was relatively high (Table 1). Consequently, the test conditions for the CO_2 packaging technique were unfavourable and the total viable count at the termination of the storage period was of the same magnitude in both the vacuum packages and in the CO_2 packages. In spite of this fact both normal and DFD beef packaged in CO_2 had a normal visual appearance and produced no off-odours while all the vacuum-packaged beef samples were spoilt.

The good quality status of the CO_2-packaged beef, therefore, seems to be due to the fact that the CO_2-packaged beef developed a less deleterious microflora than the vacuum-packaged beef. From Table 2 it can be seen that the lactic acid bacteria strongly dominates in samples stored in CO_2 while in vacuum-stored samples also Enterobacteriaceae had developed high numbers, especially in DFD beef.

The short shelf life of vacuum-packaged DFD beef has been attributed to the growth of Enterobacteriaceae and to Pseudomonas (Alteromonas) putrefaciens (Patterson & Gibbs, 1977, Newton & Gill, 1980). However, P. putrefaciens was not isolated in the present study, only Enterobacteriaceae, with H. alvei as the major species (Table 2). H. alvei is known to be able to produce H_2S and cause discolouration and off-odours in meat (Hanna et al., 1979). In the present study therefore, H. alvei seems to be the organism mainly responsible for the spoilage of the vacuum-packaged DFD beef.

It could be argued whether the lack of growth of H. alvei in CO_2 packaged beef is due to an inhibition of this organism by CO_2 or whether it is due to a stimulatory effect on the growth of Lactobacillus resulting in turn in an inhibition of H. alvei due to antagonistic effects of Lactobacillus. Possibly both effects are at work.

The present study strongly supports the earlier findings by Erichsen & Molin, (1981) that packaging of DFD beef in a gas atmosphere of pure CO_2 will give the beef a good microbiological shelf life. The method can therefore be recommended for wholesale packaging of DFD beef.

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Table 1.

Microbial counts on normal beef and DFD beef stored at 4°C in vacuum and in CO_2 atmosphere. Gas composition in the packages at the time of opening is also given.

	Initial		Vacuum		CO_2	
	Normal	DFD	Normal	DFD	Normal	DFD
Bacterial count: (log N/cm2)						
Total count	5.6	5.7	7.5	8.4	7.4	8.3
Lactic acid bact.	3.5	2.6	6.9	6.4	7.5	7.0
Enterobacteriaceae	2.6	1.8	6.5	7.6	4.0	4.3
H_2S-producing bact.	0.9	2.3	1.2	6.4	1.1	4.2
Visual appearance:						
colour	red	red	red	green	red	red
off-odours	no	no	yes	yes	no	no
gas atmosphere:						
CO_2	-	-	93	-	94	98
O_2	-	-	1.3	-	0.6	0.3
pH	5.6	6.6	5.4	6.4	5.4	6.1

Table 2

Organisms	Percent Organisms in vacuum-packad and CO_2-packed beef samples					
	Initially		Vacuum		CO_2	
	Normal	DFD	Normal	DFD	Normal	DFD
Lactobacillus spp. (homofermentative)	-	-	61	10	93	84
Brochothrix thermosphacta [1]	6	-	-	6	-	6
Erysipelothrix - like	-	-	1	14	-	6
Staphylococcus spp.	14	15	-	-	1	-
Streptococcus spp.	-	-	-	1	-	-
Enterobacter agglomerans	1	-	-	-	-	-
-"- sakazakii	1	1	-	-	-	-
Hafnia alvei	-	-	36	49	-	-
Serratia rubidea [2]	-	-	-	4	-	-
-"- liquefaciens	-	-	-	1	-	-
Yersinia enterocolitica	-	-	-	9	-	1
Proteus morganii	-	-	-	-	1	-
Acinetobacter calcoaceticus	13	6	-	-	-	-
coryneforms	38	64	-	1	3	-
Flavobacterium spp.	6	4	1	1	2	-
Micrococcus spp.	-	-	-	-	-	3
Moraxella spp.	1	1	-	-	-	-
Pseudomonas "bovis" [3]	18	9	-	-	-	-
-"- fluorescens	1	-	-	-	-	-
-"- fragi	1	-	-	-	-	-
Vibrionaceae	-	-	-	3	-	-
Total aerobic count (log number/cm^2)	5.6	5.7	7.5	8.4	7.4	8.3

1 Lactic acid bacteria according to the defination of Orla-Jensen (1919)

2 Enterobacteriaceae

3 New species suggested by Molin & Ternström (1981)