PE8.15 Gas formation in vacuum-packed beef - a new-old phenomenon? 86.00

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***Abstract* — In chilled vacuum-packed beef, gas formation, mainly caused by *Clostr. estertheticum*, occurs occasionally. This effect is followed by slow inflation of the packages and the development of an unpleasant odour. In the present study however, no differences could be detected neither in routine microbiological analyses nor in pH value between blown and normal packages. The formed gas was characterized by an important increase in CO_2- and especially in H_2-concentration, whereas O_2- and N_2-concentration were markedly reduced in comparison to ambient air. The best marker was found with butyric acid in the beef, which was 135 times more concentrated than in packages without gas formation. It was concluded that either determination of butyric acid in the meat itself or the detection of H_2 in the formed gas can be a good and rapid indicator for gas formation caused by Clostridia ssp. in beef, although more complex analytical methods are necessary for a clear identification of the causes.**

***Key words* — Clostridium estertheticum, beef, vacuum package, gas formation, blown pack**

I. INTRODUCTION

In chilled beef vacuum packages, the occurrence of gas formation is reported occasionally from the Swiss meat sector during the last years. Up to now, one similar observation was also made in imported vacuum-packed venison.

The phenomenon starts usually with the formation of small bubbles which accumulate in the package and then highly inflate it. The final balloon-like appearance is often described as “blown packs”. The phenomenon comes along with an unpleasant odour. According to the literature [1-4], it is caused by various kinds of bacteria of the Clostridium family.

A group of English researchers already described the phenomenon in the late 1980s [1]. The gas produced on this occasion was qualified initially as “sulphurous“, after 5 minutes as “fruity“ and “similar to a solvent“ and then after 10 minutes as “strongly cheesy” and “resembling butyric acid“. The authors were able to identify the cause: it was a species of Clostridia but they did not give any further information. In a study from New Zealand, also psychrotrophic Clostridia spp. were associated with “blown-pack” spoilage of chilled vacuum-packed red meats [2].

In a recent study of the Max Rubner Institute in Kulmbach (MRI), new research results were presented in which the bacterium *Clostridium estertheticum* - among other Clostridia - was identified as being the main cause of the “blown-pack” phenomenon [3]. According to the authors, the determination of *Clostr. estertheticum* with current methods is not possible without a large investment in time and work (duration: up to 8 weeks), because a specific culture medium for this bacterium, which grows very slowly, is not available. For this reason, a molecular biology method (PCR, with two pairs of primers) was developed at the MRI which now makes it possible to reliably determine *Clostr. estertheticum* [3].

One of the risk factors is probably contamination by the skin/coat (→ stained with excrements from the oxygen free digestive tract or soil particles?) with very resistant Clostridia spores and their propagation to the meat during slaughter, as has been shown by a New Zealand research team [4]. Other risk factors are the long lengths of storage of cooled beef (sometimes several months). This may not only be due to the long transport periods (i.e. from Brazil to Europe), but also to the longer maturation time for beef, as it is often allowed especially to high-quality beef. Identification of *Clostr. estertheticum* in beef originating from warm countries raises various questions as regards their means of propagation, especially since this species of bacteria was, it seems, first detected in the Arctic. In addition to the contamination of the meat by spores of Clostridia, it is necessary to take into account other promoting factors such as low temperature and the absence of oxygen being ideal growth conditions for psychrotrophic Clostridia. It is well known that under these conditions spores of Clostridia attain a vegetative state (grows down to -1.5 and 2°C, optimum: 12-15°C, growth inhibited at temperatures above 20°C), which is accompanied in the present case by gas formation. This may also happen in particular when beef is vacuum-packed (→ absence of oxygen) too rapidly after slaughter even if the temperature is certainly still too high, but ideal for Clostridia.

Earlier experiences with Emmentaler cheese have shown that blown cheeses are caused by *Clostridium*

tyrobutyricum, which could be mainly related to silage feeding of the corresponding cows [5]. Therefore, specific parameters, which permit rapid initial detection of inflated packaging, were also examined for vacuum-packed beef samples at Agroscope Liebefeld-Posieux Research Station (ALP).

II. MATERIALS AND METHODS

After being contacted by a butcher, 5 blown and 3 normal beef vacuum packages were collected for further analyses. The samples were stored at 2°C until analyses occurred.

Routine microbial analyses for total viable counts, lactic acid producers, enterobacteria and *Clostr. perfringens* were performed by using standard methods according to the Swiss Food manual [6]. Gasforming anaerobic sporeformers were counted with dextrose potato agar after 8 days of anaerobic incubation at 37°C by using a MPN technique. pH was determined by potentiometry [7].

Gas chromatography was used for gas-composition characterization [8] as well as for the determination of the profile of volatile carboxylic acids [9].

Statistical analyses were performed with a t-test on a significance level of $P \leq 0.05$.

III. RESULTS AND DISCUSSION

According to table 1, no differences could be seen from routine microbiological analyses of vacuum-packed beef with gas formation. This could be due to the fact that at temperatures above 20°C, growth of *Clostr. estertheticum* is inhibited, thus explaining why it is not detected during routine microbiological analyses. Although significant in one case, these results indicate that certain groups of germs do not survive well in the gas which was formed. The same was also true for the pH value of the beef, where no differences between normal and blown packs were observed.

However, during analysis of the gas composition which formed in the head space of packages, marked differences were determined compared to the composition of ambient air (table 2). The increase in carbon dioxide (CO_2), which in general, correlates with microbial metabolic activity, was very obvious. The hydrogen (H_2) levels, which were very high, may lead to explosion which in practice should not be underestimated. A low oxygen (O_2) content was also noted, which was to be expected since it is an indispensable condition for the survival of Clostridia.

The gas composition was similar to that found in the above mentioned study [1] where 59-70 ml of CO_2, 27-38 ml of hydrogen, 1.6-3 ml of nitrogen and 0.1-0.3 ml of oxygen were found (values per 100 ml). In this study, defined quantities of argon, an inert gas, were introduced into the normal packages to allow determination of the gas composition (which otherwise would not be possible for lack of gas). The results showed concentrations of 72-73 ml of CO_2, 1 ml of hydrogen, 24-27 ml of nitrogen and 0.1-3 ml of oxygen (values per 100 ml). The similar concentrations of CO_2 found in both packages (with/without gas formation), are again an indication of a general microbial processes, whereas the differences in the levels of hydrogen and nitrogen probably relate more to the makeup of the microbial flora present.

Determination by gas chromatography of the contents of the volatile formic and acetic carboxylic acids showed some differences, even if they were not significant (table 3). However, a very clear and highly significant difference ($P \leq 0.001$) in the butyric acid content was found in the packages with gas formation and was 135 times higher than in the normal packages. As is well known, it is especially the Clostridia which are responsible for the formation of butyric acid. One can thus conclude that the determination of the concentration of butyric acid should be a good indicator of the presence of Clostridia and could provide the first signs of the presence of the agent responsible for gas formation in beef vacuum packages. These results confirm those of Dainty et al. [1], in which the formic acid content in packages with gas formation was, compared to the standard, higher by a factor of 3 and the butyric acid content by a factor of 84 to 250. It is especially the latter which could explain, at least partially, the sometimes intense change in odour, mentioned above, even if in the study of Dainty et al. [1] high percentages of biogenic amines, hydrogen sulphide and various other volatile aromatic components were also found.

IV. CONCLUSION

The present study shows that the analysis of butyric acid content, in addition to the molecular biology method developed at the MRI for the determination of *Clostridium estertheticum*, is a further and quick possibility to obtain indices of the agent for gas formation in vacuum packed chilled beef. However, when it is a question of identifying various critical

points in the production process (carryover from skin to meat) in an establishment with vacuum-packed beef, it is not possible to carry out controls with the tested alternative methods. But for this purpose the molecular biology method of the MRI [2] is a new very valuable instrument to aid in solving these kinds of problems. Henceforth it will be possible to directly determine one of the most important vectors of the blown-pack problem within a more than reasonable time.

Meat transformation companies must at the same time limit the risk of germs multiplication in their establishments, particularly hygiene during slaughter. There it is necessary to avoid any possible contact of meat with the skin, dirt or particles of excrement (transmission of spores when removing the skin?). During other stages of transformation, such as cutting or storage of the meat, contamination by spores of Clostridia by the means of the above-mentioned vectors cannot be excluded and it is thus necessary to take essential hygienic measures. In addition, it is recommended that early vacuum packaging of the meat should be avoided due to prevent too high core temperatures which would be favourable for the development of *Clostr. estertheticum*.

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Table 1: pH values and number of germs in vacuum packed cooled beef

		Without gas formation (n = 3)	With gas formation (n = 5)	Sign.
pH value		5.53 ± 0.02	5.51 ± 0.05	n.s.
Total viable counts	*CFU/g*	$6.1 \times 10^6 \pm 6.8 \times 10^6$	$1.3 \times 10^6 \pm 2.5 \times 10^6$	n.s.
Lactic acid producers	*CFU/g*	$3.7 \times 10^5 \pm 2.6 \times 10^5$	$5.5 \times 10^4 \pm 4.4 \times 10^4$	n.s.
Enterobacteria	*CFU/g*	$5.4 \times 10^2 \pm 1.1 \times 10^2$	n.d. ($< 10^2$)	*
Clost. perfringens	*CFU/g*	n.d. ($< 10^2$)	n.d. ($< 10^2$)	n.s.
Butyric bacilli	*Spores/g*	n.d. (0)	n.d. (0)	-

CFU = colony forming units; n.d. = non detectable (in brackets, detection limit)
*n.s. = not significant, * = significant ($P \leq 0.05$), - = no statistical evaluation possible*

Table 2: Gas composition of the vacuum packed beef, compared to ambient air [*ml / 100 ml*]

	Ambient air	With gas formation (n = 5)
Oxygen (O_2)	20.94	0.57 ± 1.19
Carbon dioxide (CO_2)	0.04	68.08 ± 6.19
Nitrogen (N_2)	78.08	4.18 ± 3.64
Hydrogen (H_2)	traces	26.17 ± 3.19
Ammonia (NH_3)	n.d.	n.d.

n.d. = non detectable

Table 3: Concentrations of volatile carboxylic acids in vacuum packed cooled beef [*mmol/kg*]

	Without gas formation (n = 3)	With gas formation (n = 5)	Sign.
Formic acid	0.71 ± 0.44	1.13 ± 0.23	n.s.
Acetic acid	1.64 ± 0.74	1.38 ± 0.18	n.s.
Propionic acid	n.d.	n.d.	-
Butyric acid	0.07 ± 0.08	9.40 ± 2.21	*
Isobutyric acid	n.d.	n.d.	-
Isocaproic acid	n.d.	n.d.	-
Isovaleric acid	n.d.	n.d.	-

*n.d. = non detectable; n.s. = not significant, * = significant ($P \leq 0.05$), - = no statistical evaluation possible*