TWELTH EUROPEAN MEETING OF MEAT RESEARCH WORKERS, Oslo, August, 1966 B

INFLUENCE OF THE COMPOSITION OF THE GASEOUS ENVIRONMENT ON THE CHANGES TAKING PLACE IN SMOKED WILTSHIRE BACON DURING STORAGE

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### SUMMARY

A survey was carried out on retail vacuum-packed bacon to determine its sensory, microbiological and chemical characteristics. The volume and composition of the residual gas in the packs were also determined. Lactobacilli were the most numerous micro-organisms in the bacon and the residual gas in the packs contained up to 0.75 atmosphere partial pressure of carbon dioxide.

Storage of commercial vacuum-packs of bacon at constant temperatures showed that in general lactobacilli increased markedly in numbers whereas other micro-organisms did not. The pH of the bacon fell slightly while the partial pressure of carbon dioxide in the packs progressively increased. On accasions when the partial pressure of carbon dioxide did not increase appreciably the gram-positive cocci present on the bacon multiplied extensively.

Experiments in which bacon was stored in atmospheres of constant composition showed that the micro-organisms present were relatively insensitive to the partial pressure of oxygen; that carbon dioxide in the absence of oxygen could inhibit the growth of gram-positive cocci and yeasts but not lactobacilli; and that in the presence of oxygen, carbon dioxide could inhibit the proteolytic spoilage normally brought about by grampositive cocci.

It is considered that the extended shelf-life of vacuum-packed bacon is probably due in large measure to the effect of the increased partial pressure of carbon dioxide on the growth and metabolism of the grampositive cocci.

RÉSUME

Une expertise fut faite sur le bacon emballé sous vide pour vente au détail afin de déterminer ses caractéristiques sensorielles, microbiologiques et chimiques. Le volume et la composition du gaz résiduel dans les emballages étaient également évalués. Les microorganismes les plus nombreuses dans le bacon étaient les lactobacilles et le gaz résiduel dans les emballages contenait jusqu'à 0,75 atm. pression partielle de gaz carbonique.

Les emballages sous vide de bacon de commerce conservés à des températures constantes montrerent en général que les lactobacilles augmentaient fortement en quantité tandis que les autres microorganismes n'augmentaient pas. Le pH du bacon se baissa légèrement tandis que la pression partielle de gaz carbonique dans les emballages s'augmenta progressivement. Lorsque la pression partielle du gaz carbonique ne s'augmenta pas beaucoup, les coques gram-positifs dans le bacon se multiplièrent beaucoup. Des expériences où le bacon fut maintenu dans des atmosphères de composition constante montrèrent que les microorganismes présents étaient relativement peu sensibles à la pression partielle de l'oxygène; que le gaz carbonique en l'absence d'oxygène pouvait empêcher la croissance de coques gram-positifs et des levures mais non pas celle des lactobacilles; et qu'en présence d'oxygène, le gaz carbonique pouvait empêcher des déchets protéolytiques provenant normalement de coques gram-positifs.

Il est estimé que la durée de vie prolongée du bacon emballé sous vide est due probablement en grande mesure à l'effet de la pression partielle accrue du gaz carbonique sur la croissance et le métabolisme des coques gram-positifs.

#### ZUSAMMENSTELLUNG

Eine Messung an verkäuflichen vakuum-verpackten Speck wurde ausgeführt, um seine sensitiven, mikrobiologischen und chemischen Eigenschaften zu bestimmen. Das Volumen und die Zusammensetzung des zurückbleibenden Gases in den Verpackungen wurden ebenfalls bestimmt. Laktobazillen waren die zahlreichsten Mikroorganismen in dem Speck und die zurückbleibenden Gase in den Verpackungen enthielten bis zu 0.75 atmosphärischen Partialdruck der Kohlensaure.

Lagerung von handel süblichen vakuum-verpackten Speck bei gleichbleibenden Temperaturen zeigte, das Laktobazillen im allgemeinen sich in auffallender Anzahl vermehrten, im Gegensatz zu anderen Mikroorganismen. Das pH des Specks fiel etwas ab weil der Partialdruck der Kohlensäure sich in den Verpackungen schrittweise vermehrte. Gelegentlich, wenn der Partialdruck der Kohlensäure sich nicht zunehmlich vermehrte, die anwesenden grampositiven Kokken an dem Speck vermehrten sich weitgehendst.

Versuche, in welchen der Speck in Atmosphären von gleichbleibender Zusammenstellung gelagert wurde, zeigten, das die anwesenden Mikroorganismen verhältnissmässsig unempfindlich zu dem Partialdruck des Sauerstoffs waren, das Kohlensäure in der Abwesenheit von Sauerstoff, das Wachstum der grampositiven Kokken und Hefen, aber nicht Laktobazillen, hemmen sollte, und das in der Gegenwart von Sauerstoff, die Kohlensäure den proteolytischen Ausschluss hemmen sollte, welcher normalerweise van den grampositiven Kokken verursacht wird.

Es wird erwogen, das die verlängerte Lagerfähigkeit von vakuum-verpackten Speck wahrscheinlich zu einem grossen Mass von der Wirkung des vermehrten Partialdruckes der Kohlensäure an dem Wachstum und Stoffwechsel der grampositiven Kokken veranlasst wird.

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## Influence of the composition of the gaseous environment on

# the changes taking place in smoked Wiltshire bacon

### during storage

### Introduction

There is an increasing trend towards the vacuum packing of perishable foods, both raw and cooked, for in addition to the many commercial advantages of pre-packing - centralized packing, brand-labelling, and improved hygiene - vacuum-packed foods have a considerably extended 'shelf-life' in comparison with that of unpacked foods or of foods prepacked in films fully permeable to air. The reasons for the extended shelf-life, at least as far as the bacterial spoilage of meats is concerned, are not fully understood (Ingram, 1962; Noskova & Peck, 1963).

The investigations described here were undertaken to provide a better understanding of the spoilage of vacuum-packed bacon, and in particular, to determine the effect on spoilage of the partial pressures of oxygen and carbon dioxide in the gaseous atmosphere surrounding the bacon. The work was planned in three main phases:

- (i) A survey of commercial vacuum-packed bacon at the point of retail sale
- (ii) The storage of commercially vacuum-packed bacon under controlled conditions
- (iii) The storage of bacon in atmospheres of known and maintained composition.

All the bacon used in the experimental work was smoked.

# (i) Retail Survey

During the period January 1964 to March 1965, over 250 vacuumpacks of bacon, mainly of four commercial brands, were purchased from retail shops and subjected to detailed chemical, microbiological and sensory examination, including determinations of the volume and composition of the residual gas in the pack. The results of this survey of retail vacuumpacked bacon are summarised in Tables 1 and 2.

It is clear from the results in Table 1 for the composition of the residual gas that there had been production of carbon dioxide in the packs, with an accompanying reduction in the proportion of oxygen present; partial pressures of carbon dioxide of up to almost 0.75 atmosphere were observed in the packs. There is ample evidence that carbon dioxide at this concentration and, for that matter, at partial pressures of 0.25 atmosphere or above, is inhibitory to many organisms (Tomkins, 1932; Coyne, 1933; Haines, 1933; Ogilvy & Ayres, 1951, 1953). There is also evidence that oxygen at a partial pressure of around 0.01 atmosphere (1 per cent concentration at atmospheric pressure) is sufficient to allow maximum growth of many microorganisms, even so-called aerobes (Mossel & Ingram, 1965). This value is below that found in the majority of the bacon packs.

Table 2 shows that among the predominant microorganisms in the vacuum packaged bacon, including bacon cured by the traditional Wiltshire process, were lactobacilli; this group is not usually considered to form an important part of the microflora of unpacked bacon (Garrard & Lockhead, 1939; Brooks <u>et al</u>, 1940; Patterson, 1960; Hansen & Riemann, 1962), although on one occasion Ingram (1960) found a high proportion of lactobacilli to be present. It is, however, known to be resistant to the inhibitory effects of high partial pressures of carbon dioxide (Ingram, 1962).

#### (ii) In-pack storage under controlled conditions

In order to follow the changes occurring in the composition of the residual gas in the pack, in the number and composition of the microbial flora present on the bacon, and in its sensory quality, samples of vacuumpacked bacon were obtained directly from the manufacturers and duplicate sets of samples were stored at 21°C and at 5°C and examined at intervals. Six experiments were carried out, each with a different brand of bacon; the packs of bacon used in each experiment came from a single side of bacon (except for one experiment where they came from two sides).

From these experiments, a 'typical picture' of changes emerged. The oxygen concentration fell to between 2 and 5 per cent and the carbon dioxide concentration rose to 40 to 50 per cent. The lactobacilli increased rapidly in numbers over the first five days and became the predominant group even when present initially to the extent of less than 1 per cent of the microbial population, while the micrococci and staphylococci generally showed little tendency to increase. The pH of the bacon fell slowly by about 0.3 of a pH unit, except for the bacon known to have been cured with sugar, where the pH fell by about one unit. This 'typical picture' is shown in Figures 1, 2 and 3.

Occasionally this 'typical picture' of changes did not occur at one temperature of storage although the duplicate samples stored at the other temperature behaved normally. In particular, the carbon dioxide concentration did not rise nor the oxygen concentration fall, and the micrococci and staphylococci multiplied extensively, as did the lactobacilli despite the high partial pressure of oxygen. One such atypical example is shown in Figure 4.

#### (iii) Storage in atmospheres of constant composition

The retail survey and in-pack storage experiments suggested that the preservative effect of vacuum packaging on bacon depends less on the absence of oxygen than on the presence of carbon dioxide, the overall effect being to inhibit micrococci, staphylococci and yeasts, while allowing lactobacilli, which are known not to be appreciably proteolytic, to develop. It was not considered feasible to investigate the precise effects of the partial pressures of oxygen and carbon dioxide unless these partial pressures could be held constant during the duration of an experiment. To allow this to be done, the following experimental arrangement was devised.

The eye muscles of a series of bacon slices were removed and suspended in perspex cells as illustrated in Figure 5. The cells were linked together in the arrangement shown also in Figure 5, so that a slow continuous stream of gas (10 litres per hour), containing the desired concentrations of carbon dioxide and oxygen, with nitrogen as an inert diluent, and humidified to 98 per cent relative humidity, could be passed through them. Four of these arrangements were used in each experiment, thus allowing four gaseous atmospheres to be directly compared: an atmosphere of nitrogen was included in each experiment to The actual compositions of the gases were monitored serve as a control. at intervals using a gas chromatographic apparatus. Cells were detached successively from the experimental arrangement at suitable intervals and the bacon was removed and subjected to chemical, microbiological and sensory examination.

The effect of oxygen concentration on microbial growth in bacon is shown in Figure 6. It is clear that a reduction in the partial pressure of oxygen to 0.01 atmosphere and below restrained the growth of micrococci and yeasts but had little effect on staphylococci and lactobacilli. The rate of spoilage of the bacon increased with higher concentrations of oxygen (in the range of 0.01 to 0.5 atmosphere partial pressure) and in addition, the presence of oxygen completely altered the spoilage pattern. In nitrogen (and in vacuum packs) the noxious volatile products of proteolysis did not occur, the observed spoilage being of a 'sour-sweet' character. With as little as 1 per cent of oxygen in the storage atmosphere production of ammonia and sulphides occurred and the pH rose, reaching values of above 7.0 in 20 per cent oxygen.

The effect of carbon dioxide, at partial pressures of 0.5 to 1.0 atmosphere, was to inhibit the growth of yeasts, micrococci and staphylococci, as shown in Figure 7, while leaving the growth of lactobacilli largely unaffected. Spoilage in atmospheres of 50 to 100 per cent carbon dioxide was slower than in nitrogen and was of the same non-proteolytic nature, with pH values falling slightly.

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Finally, the effect of mixtures of oxygen and carbon dioxide Growth of the micrococci, yeasts and staphylococci was investigated. was inhibited by carbon dioxide in concentrations of 50 per cent and above, even in the presence of oxygen, although to a much smaller extent than in its absence. This is shown for micrococci in Figure 8. The important effect of carbon dioxide, however, was not on growth but on the spoilage In a concentration of, for example, 50 per cent, with 10 per pattern. cent oxygen, the proteolytic spoilage which occurred in 10 per cent oxygen in the absence of carbon dioxide was replaced by the 'sour-sweet' type of spoilage with a slight fall rather than a marked rise in pH. The 'shelf lift' of the bacon was thus similar to that in nitrogen and in vacuum-packs.

#### Discussion

It is clear from the investigation of the spoilage of bacon in various constant gaseous atmospheres that the partial pressure of carbon dioxide is more important in influencing spoilage than is the partial pressure of oxygen. Even at as low as 0.01 atmosphere partial pressure of oxygen, in the absence of carbon dioxide, spoilage as a result of proteolysis was relatively rapid (14 days or less) whereas in the presence of oxygen up to 0.1 atmosphere partial pressure, a high partial pressure of carbon dioxide delayed spoilage for considerably longer than 14 days by interfering, by some means not yet known, with the metabolic processes leading to the proteolytic breakdown of the bacon. When the partial pressure of oxygen was below 0.01 atmosphere, high partial pressures of carbon dioxide delayed spoilage for periods in excess of four weeks.

It is not yet known to what extent the findings from the experiments in constant gaseous atmospheres are directly relevant to the spoilage of bacon commercially vacuum-packed in plastic film. In such packs the ratio of residual gas to bacon is small, several slices of bacon are in close contact and the extent to which the gases dissolved in the tissues are in equilibrium with the free gases is not known. Also, there will be diffusion of both oxygen and carbon dioxide through the film and the composition of the atmosphere within the pack will be influenced by the metabolic activity both of the bacon and of the resident microflora. There are, however, four factual observations which appear to be relevant:

- (i) Vacuum-packed bacon develops a high partial pressure . of carbon dioxide
- Lactobacilli, rather than micrococci, staphylococci and yeasts, predominate on bacon in vacuum-packs. This cannot be explained solely on the basis of the small absolute amounts or low partial pressures of oxygen present.

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(iii) Spoilage of bacon in vacuum packs is non-proteolytic even when micrococci and staphylococci are present in large numbers and oxygen is present at partial pressures of 0.01 to 0.1 atmosphere, and

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(iv) The 'shelf life' of vacuum-packed Wiltshire bacon is about 14 to 21 days. This is somewhat less than that of bacon in a constant atmosphere of 50 per cent carbon dioxide and 10 per cent oxygen, but the difference may be attributed to the high partial pressure of oxygen and low partial pressure of carbon dioxide initially in the packs.

These observations all appear to support the view that the spoilage changes taking place in vacuum-packed bacon can be validly interpreted on the basis of the results obtained in the experiments described above.

#### Acknowledgments

These investigations were carried out with the financial support of the Pig Industries Development Authority. Our thanks are due to Mr. J.W. Selby for undertaking the gas analyses and to Mr. G.A.Lea for manufacturing the perspex cells. Dr. Susan Sarsfield and Mr. A.R.A. Court were largely responsible for the chemical and microbiological analyses.

#### References

- 1. Brooks, J., Haines, R.B., Moran, T. & Pace, J, (1940). The function of nitrate, nitrite and bacteria in the curing of bacon and hams. <u>Food Invest</u>. <u>Spec.Rept</u>, No.49. H.M.S.O. London.
- 2. Coyne, F.P. (1933). The effect of CO<sub>2</sub> on bacterial growth. <u>Proc.Roy.Soc.</u>, <u>B</u>, 113, 196.
- 3. Garrard, E.H. & Lockhead, A.G. (1939). A study of bacteria contaminating sides of Wiltshire bacon with special consideration of their behaviour in concentrated salt solutions. <u>Canad. J. Res.</u>, <u>17</u>, 45.
- 4. Haines, R.B. (1933). The influence of carbon dioxide on the rate of multiplication of certain bacteria, as judged by viable counts. J.Soc.Chem.Ind., Lond., 52, 13T.

5.	Hansen, N-H. & Riemann, H. (1962). Mikrobiologische Beschaffenheit von vorverpacktem Fleisch und vorverpackten Fleischwaren. <u>Fleischwirtschaft</u> , <u>14</u> , 861.
6.	Ingram, M. (1960). Bacterial multiplication in packed Wiltshire bacon. <u>J.appl.Bact.</u> , <u>23</u> , 206.
7.	Ingram, M. (1962). Microbiological principles in prepacked meats. <u>J.appl.Bact.</u> , <u>25</u> , 259.
8.	Mossel, D.A.A. & Ingram, M. (1955). The physiology of the microbial spoilage of foods. <u>J.appl.Bact.</u> , <u>18</u> , 232.
9.	Noskova, G.L. & Peck, G.Y. (1963). The influence of vacuum on the microflora of refrigerated food products (In Russian). <u>All-Union Sci. Res. Inst. Refrig. Industr.</u> , pp.38.
10.	Ogilvy, W.S. & Ayres, J.C. (1951). Post-mortem changes in stored meats.ll. The effect of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. <u>Food Technol.</u> , Champaign, 5, 97.
11.	Ogilvy, W.S. & Ayres. J.C. (1953). Post-mortem changes in stored meats. V. Effect of carbon dioxide on microbial growth on stored frankfurters and characteristics of some microorganisms isolated from them. Food Res., 18, 121.
12.	Patterson, J.T. (1960). Studies on the keeping quality of sliced, vacuum packed, bacon. <u>Res.Exp.Record Min.Agric</u> . Northern Ireland, 10, 139.
13.	Tomkins, R.G. (1932). The inhibition of the growth of meat attacking fungi by carbon dioxide. J.Soc. Chem.Ind., Lond., 31, 261T.
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		Chemical composition of the bacon					Volume & composition of the residual gas		
	Number of	NaC 1	KN03	NaNC2	phenols	pH	Volume	CO2	02
Brand	Samples	g./100g.water	g./100g.meat	p.p.m.meat	p.p.m.meat		ml	%	%
A.Non-Wiltshire cure	65	6.92 (4.09 <b>-</b> 9.74)	0.032 (0.0-0.157)	122 (19-326)	0.7 (0.1-1.6)	6.13 (5.50- 6.92)	0.52 (0.11-1.42)	41.4 (6.0-63.0)	10.4 (3.4–15.7)
B,Wiltshire cure	63	7.60 (4.54–12.70)	0.093 (0.003–0.278)	206 (0-532)	1.0 (0.2 <b>-</b> 3.9)	6.30 (5.65- 7.16)	2.81 (0.15-12.83)	29.8 (5.4–60.7)	6.40 (0.7 <b>-</b> 18.9)
C.Non-Wiltshire cure	63	4.66 (2.66-7.92)	0.036 (0.0-0.101)	88 (11-259)	2.5 (0.8-5.5)	6.21 (5.56- 7.32)	3.87 (0.19-22.4)	9.4 (1.2-64.1)	12.5 (0.9-18.7)
D.∏iltshire cure	20	7.94 (5.5-10.25)	0.050 (0.003-0.131)	131 (14-395)	2.0 (0.6-3.1)	6.06 (5.84- 6.40)	0.22 (0.15-0.27)	38.8 (20.0–73.2)	12.5 (2.8-19.4)
Miscellaneous	40	7.63 (4.02-11.68)	0.035 (0.0-0.127)	164 (6-629)	0.8 (0.1-1.8)	6.18 (5.41- 7.07)	4.1 (0.3-22.0)	19.0 (0.0-49.6)	8.5 (0.4-22.0)

Table I. Retail vacuum-packed bacon - chemical results

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The figures in parentheses are minimum and maximum values.

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		Mean log <sub>10</sub> count per g. meat							
Brand	Number of Samples	lactobacilli	staphylococci	micrococci	yeasts	enterococci			
A.Non-Wiltshire cure	65	4.34 (<2.00-7.13)	4.25 (<2.00-5.60)	4.06 (<2.64-6.60)	3.02 (<1.00-5.91)	2,80 (<1.94–5.68)			
B.Wiltshire cure	63	7.25 (<4.34-8.55)	6.30 (2.82-7.95)	5.98 (3.52 <b>-</b> 8.20)	3.14 (<1.00-8.12)	2.20 (<1.94-3.87)			
C.Non-Wiltshire oure	63	5.08 (<2.00-8.00)	4.07 (2.23-7.74)	3.73 (<2.04-6.89)	2.97 (<1.00-5.95)	2.67 (<1.94-6.26)			
D.Wiltshire cure	20	5.87 (3.50-8.12)	6,61 (<2.00 <b>-</b> 8.02)	5.97 (3.64-7.04)	4.52 (<1.00-6.59)	3.17 (<1.94-7.72)			
Miscellaneous	40	4.59 (<2.32-3.04)	5.08 (2.64-8.11)	4.60 (<2.60 <b>-</b> 7.39)	3.38 (<1.00-6.26)	2.71 (<1.94-5.84)			

# Table 2. Retail vacuum-packed bacon - microbiological results

The figures in parentheses are minimum and maximum values.

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FIG.1. Vacuum packed bacon stored at 21°C - typical changes in composition of residual gas.







FIG.4. Vacuum packed bacon - atypical changes in gas composition and microflore in one experiment at  $5^{\circ}C$ .

# FIG. 5. Experimental arrangement for storage in atmospheres of constant composition

- A. Gas cylinders containing nitrogen, oxygen and carbon dioxide with reducing valves to 10 lb. per sq.in.
- B. Gas supply from appropriate cylinders at 10 lb. per sq.in.
- C. Drawn-out capillary tubing to control flow rate of individual gases and thus composition of mixtures.
- D. Gases of controlled flow rates combined to give required mixtures.
- E. Humidifier containing saturated solution of potassium sulphate (ERH = 98 per cent) and short lengths of glass tubing to reduce flow rate of gas.
- F. Series of perspex sample cells.
- G. Gas sample tube to allow composition of gas to be monitored by gas chromatography.
- H. Flask of water for observation of continuity of gas flow.
- I. Perspex cell in plan, outer plates approximately 11.5 cm. square, inner cell approximately 9 cm. in diameter and 1.5 cm. deep.
- J. Perspex cell, side view.
  - 1. Outer perspex plates
  - 2. Rubber seal
  - 3. Inner perspex cell
  - 4. Tightening screws
  - 5. Gas inlet and outlet.
  - 6. Pegs to hold bacon

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10.5. Experimental arrangement for storage in atmospheres of constant composition.



FIG.6. Effect of oxygen concentration on microbial growth in bacon.

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FIG.7. Effect of carbon discide concentration on microbial growth in bacon.



FIG.8. Effect of a gas mixture containing 50 per cent carbon dioxide and 10 per cent oxygen on the growth of micrococci in bacon.