FINAL REPORT

Project Armis No. 4031

The Microbiological Safety and Quality of Foods Processed by the 'Sous Vide' System as a Method of Commercial Catering

The National Food Centre

RESEARCH & TRAINING FOR THE FOOD INDUSTRY

RESEARCH REPORT NO 1

Contents

THE MICROBIOLOGICAL SAFETY AND QUALITY OF FOODS PROCESSED BY THE 'SOUS VIDE' SYSTEM AS A METHOD OF COMMERCIAL CATERING

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SUMMARY

The objective of this project was to improve the quality and safety of sous vide foods by investigating the responses of the food-poisoning micro-organisms to the processing and storage conditions used in this technology. The major food poisoning bacteria of concern in sous vide foods are strains of *Clostridium botulinum*, *Bacillus cereus*, verotoxigenic *Escherichia coli* O157:H7 (VTEC), *Salmonella* spp., *Listeria monocytogenes* and *Yersinia enterocolitica*.

This project had five partners:

Leatherhead Food Research Association, United Kingdom.
Katholieke Universiteit, Leuven, Belgium.
University of Ulster at Jordanstown, Northern Ireland.
University of Thessaloniki, Greece.
The National Food Centre, Ireland.

All the relevant research results are referred to in the summary and conclusions of this report; however, technical details are restricted to the work performed at The National Food Centre.

Initial studies examined bacterial thermal resistance. D-values (the time required to destroy 90% of the bacterial population) were in the range of 11 to 20 minutes at 55°C (D_{55} values) for VTEC in broth, minced meat and potatoes and 0.5 to 1.5 minutes at 60°C. However D_{55} values in beef, pasta and green beans increased to between 29 to 54 minutes while the equivalent D_{60} values were in the range 1.6 to 3 minutes. The corresponding D_{60} values for a cocktail of *Salmonella* strains were 0.95 to 5.5 minutes. Spores of two psychrotrophic strains of *B.cereus* gave D_{90} values of approximately 11 and 13 minutes. The well documented heat resistance of *Cl. botulinum* spores was also confirmed with typical D_{85} values of 50 to 90 minutes. D-values obtained for *L.monocytogenes* and *Y.enterocolitica* in a commercial retort were in the range 1.2 to 96.2 minutes at 48 to 56°C. These results were consistent with those previously generated in our laboratory. A commercially used time-temperature sous vide profile for the processing of rainbow trout and salmon was investigated and found to be insufficient to ensure these products are safe for human consumption.

Exposure to non-lethal temperatures, just above the maximum for growth, increased the thermotolerance of VTEC, *Salmonella* spp., *L.monocytogenes* and *Y.enterocolitica* by between 25 and 500%.

Growth phase also affected thermal resistance. Stationary phase cells showed increased heat stability, even when these cells were subcultured to the exponential phase. Further studies demonstrated that, contrary to current thinking, the induction of heat shock proteins in *L.monocytogenes* and *Y.enterocolitica* was independent of pH and therefore possible in beef products. However, the heat shocked organism was still sufficiently thermolabile to allow currently recommended cooking practices confer a generous safety margin.

In food microbiology the term 'hurdle' refers to preservation factors or techniques. The effects of three additional hurdles (modified atmospheres, lactic acid bacteria and lactate) on the safety and preservation of sous vide foods were examined. Carbon dioxide (CO_2) inhibited the growth of VTEC at 10°C as did a mixture of 20%CO_2/80%O_2. However, it severely limited heat transfer into the product resulting in extended heating times. Lactate (2.4%) inhibited the growth of VTEC, *B.cereus*, *L.monocytogenes* and *Y.enterocolitica*, however at higher concentrations a salty taste was evident. The suitability of several strains of Lactic acid bacteria for use in sous vide products were also examined. However none survived minimal sous vide cooking to subsequently effect the inhibition of foodborne pathogens. Freeze-drying in skimmed milk, a procedure used to enhance the thermal resistance of bacterial cultures, was of little use as carmelisation occurred at temperatures above 60°C.

INTRODUCTION

Changing demographics have increased the market for quality convenience foods. As a result, minimal processing technologies like sous vide have found increasing application (Hollreiser, 1990; Smith *et al.*, 1990). Sous vide is defined as 'a process whereby foods are vacuum packaged and then cooked, chilled and stored refrigerated' (Rhodehamel, 1992). This mild processing procedure and chilled storage provides a product of high sensory quality (Light *et al.*, 1988) that can meet consumer demands for high quality convenience foods (Shellekens and Martens 1992; Armstrong 1996).

Several steps are involved in sous vide processing including, pre-cook preparation, packaging under vacuum, pasteurisation, rapid chilling, storage at 0 to 3°C and reheating before consumption. Preservation is achieved by the combination of vacuum packaging, mild cooking, rapid chilling and chilled storage.

There are several codes of practice for sous vide processors throughout the world. French regulations require a heat treatment equivalent to 100 or 1,000 minutes at 70°C for products with a 24 or 48 day shelf-life, respectively (Betts, 1992). In the United States the National Advisory Committee on Microbiological Criteria for Foods (USNACMCF) require a thermal process sufficient to achieve a minimum 4 log reduction in *L.monocytogenes* (USNACMCF, 1991). United Kingdom regulations, drawn up by the Advisory Committee on the Microbiological Safety of Food (ACMSF), are even more severe because these deal with the threat posed by the extremely dangerous obligate anaerobe *Clostridium botulinum*. Hence they recommend a heat treatment sufficient to achieve a 6 log reduction in *Cl.botulinum* spores. In contrast the UK Department of Health (DOH) suggest a 70°C (core temperature) cook for 2 minutes is sufficient for products with a 5 day shelf.

However, the Food and Drug Administration (FDA) has expressed concern about the risks associated with sous vide processing. Pathogenic organisms can survive minimal heat processing and grow under anaerobic conditions in the event of temperature abuse (Simpson *et al.*,1994). Indeed, both *L.monocytogenes* and *Y.enterocolitica* are capable of growing at refrigerated

▲ *Sous vide chicken and mushroom in a cream sauce, one of many such recipes satisfying customer demand for quality convenience foods.*

temperatures under anaerobic conditions (Gill and Reichel, 1989; Walker *et al.*, 1990; Hudson *et al.*, 1994) and pose an ever increasing health risk in sous vide products (Schofield, 1992; Smith *et al.*, 1990). This is especially true of meats, which may be contaminated with these pathogens (Wendlandt and Bergann, 1994; Logue *et al.*, 1996) but receive minimal sous vide processing (Schellekens, 1996). Indeed, in the absence of other hurdles, mild cooking may result in the sous vide process becoming selective for these facultative anaerobic psychrotrophs.

Thus it is important that sous vide products receive sufficient heat treatment to reduce the number of pathogens, such as *L.monocytogenes* and *Y.enterocolitica*, while at the same time maintaining the sensory attributes of the product. The microbiological objectives of this project were two-fold:

I. To increase the safety and quality of sous vide foods through an understanding of the response of micro-organisms to the processing and storage conditions specific to sous vide foods

II. To design and validate additional hurdles to the survival and growth of micro-organisms.

HEAT RESISTANCE OF *L.MONOCYTOGENES* AND *Y.ENTEROCOLITICA* IN SOUS VIDE COOKED BEEF PREPARED IN A COMMERCIAL RETORT

All of the regulations for sous vide products are based on laboratory generated data. However the heating pattern in the laboratory waterbath is dissimilar to that of a commercial retort. The first experiment, therefore, was

designed to investigate the thermal properties of *L.monocytogenes* and *Y.enterocolitica* in a commercial system, thereby allowing comparison with the wealth of similar laboratory generated data. D-values (the time required to destroy 90% of a particular bacterial population) were obtained for the two organisms in both solid and minced beef using a Barriquand Steriflow commercial retort. At 48°, 52° and 56°C the D-values obtained were in the range of 1.2 to 96.2 minutes (Table 1) and therefore consistent with the corresponding values reported in the literature. On the basis of these results, laboratory generated data is applicable in the commercial setting and the mildest sous vide cook currently recommended is more than adequate to ensure the total destruction of any *L.monocytogenes* and *Y.enterocolitica* in sous vide beef products.

▲ *The Barriquand Steriflow retort used in commercial sous vide preparation.*

Table 1. D-values for *L.monocytogenes* and *Y.enterocolitica* in solid and minced beef when heated in a Barriquand Steriflow commercial retort

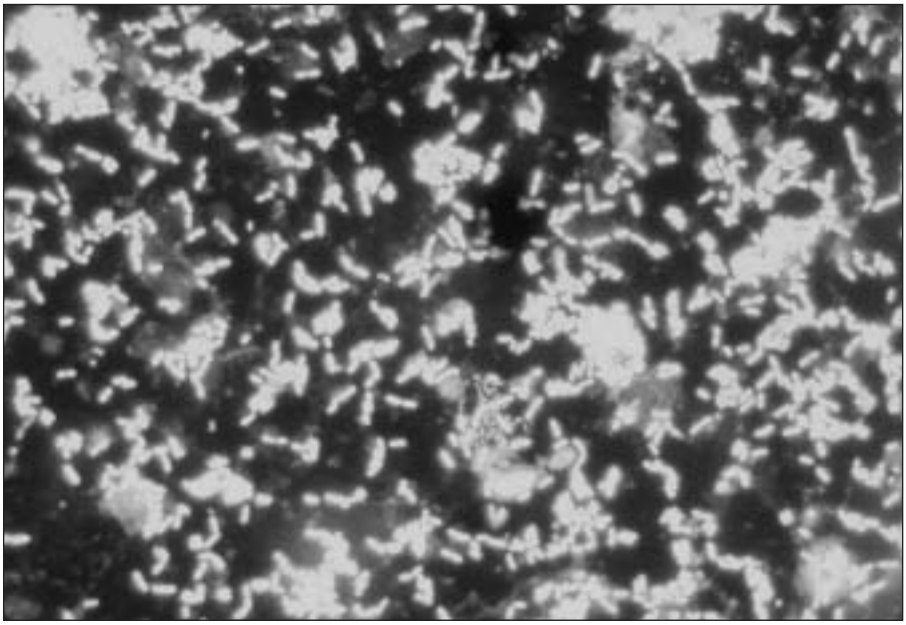
Temperature (°C)	Beef Sample	D-value (min)	
		L.monocytogenes	*Y.enterocolitica*
48	Solid	96.2	78.4
48	Mince	71.2	43.7
52	Solid	29.3	9.8
52	Mince	21.3	7.1
56	Solid	3.3	1.4
56	Mince	3.0	1.2

THE EFFECT OF CELL GROWTH PHASE ON INDUCTION OF THE HEAT SHOCK RESPONSE IN *L.MONOCYTOGENES* AND *Y.ENTEROCOLITICA*

When bacterial cells are heated at sub-lethal temperatures or heated slowly (as is the case with sous vide cooking) proteins, called 'heat shock' proteins, are formed. These increase the thermal resistance of the organism. Growth phase greatly affects this process. The induction of heat shock proteins in *L.monocytogenes* and *Y.enterocolitica* and the obvious implications for current cooking recommendations, was investigated by heating these cells at 48°C for 10 minutes and 45°C for 30 minutes, respectively, to induce the formation of heat shock proteins. This procedure was performed on exponential:exponential cells (cells grown to the mid-exponential phase of growth, subcultured and tested when the second exponential phase was reached), stationary:exponential cells and stationary:stationary cells of each bacterium. Although the D-values increased significantly (Table 2), the mildest sous vide time-temperature profile currently recommended would achieve at least a 30 $\log_{10}$ cfu/g reduction in *L.monocytogenes* numbers and a 37 $\log_{10}$ cfu/g in *Y.enterocolitica* levels. Since this amount of contamination is unrealistic, current regulations are more than sufficient to ensure the product is safe, regardless of whether or not the sous vide process induces the formation of heat shock proteins.

Table 2. The effect of heat shock formation on the thermal resistance (D_{55}, D-values at 55°C) of *L.monocytogenes* and *Y.enterocolitica*.

Culture	*L.monocytogenes*		*Y.enterocolitica*	
	D-value (min)			
	Non heat shock	Heat shock	Non heat shock	Heat shock
Exponential:exponential	2.19	5.07	0.50	2.08
Stationary:exponential	7.21	6.42	1.53	7.46



▲ *Bacterial cells like* L.monocytogenes *form 'heat shock' proteins when exposed to sub-lethal temperatures.*

THE EFFECT OF pH ON THE PRODUCTION OF THE HEAT SHOCK RESPONSE IN *L.MONOCYTOGENES* AND *Y.ENTEROCOLITICA*

Regardless of heating rate, the relatively low pH of beef may inhibit the formation of heat shock proteins (Hansen and Knochel, 1996). This was investigated by heating *L.monocytogenes* and *Y.enterocolitica* cells at 48°C for 10 minutes and 45°C for 30 minutes respectively in BHI broth at pH 5.5, 6.0 and 6.5 as well as in meat broths at pH 5.6 and 6.6. Increased thermal resistance was detected in each case suggesting, contrary to current thinking, that heat shock protein formation is independent of pH and may occur in meat products (Table 3).

Table 3. The effect of pH on the formation of heat shock proteins and hence thermal resistance (D_{55}, D-values at 55°C) of *L.monocytogenes* and *Y.enterocolitica*.

pH	*L.monocytogenes*		*Y.enterocolitica*	
	Non heat shock	Heat shock	Non heat shock	Heat shock
BHI broth:				
pH 5.5	1.82	4.42	2.35	6.28
pH 6.0	2.90	4.94	3.07	7.71
pH 6.5	3.90	4.79	3.91	7.54
Meat broth:				
pH 5.6	4.16	5.20	2.90	3.64
pH 6.6	4.35	6.81	3.11	5.68

THE EFFECT OF A COMMERCIAL SOUS VIDE THERMAL PROCESS ON THE SURVIVAL OF *L.MONOCYTOGENES* AND *Y.ENTEROCOLITICA* IN RAINBOW TROUT AND SALMON FILLETS

In co-operation with a sous vide producer, current sous vide fish processing was investigated. At present rainbow trout (*Oncorhynchus mykiss*) and salmon (*Salmo salar*) fillets are cooked at 65°C for 5 minutes and 9 minutes, respectively (personal communication). Current DOH guidelines recommend heating to a core temperature of 70°C for 2 minutes or the equivalent. In this case the equivalent cook at 65°C is for 10.4 minutes. The commercial sous vide fish process may therefore be insufficient and warranted investigation. Our research shows, that with an initial inoculum level of $10^{6\text{-}7}$ bacteria/g, the commercial treatment achieved approximately a 1 $\log_{10}$ cfu/g to 5 $\log_{10}$ cfu/g reduction in the numbers of both heat shocked

and non-heat shocked *L.monocytogenes* and *Y.enterocolitica* (Table 4). While the latter is sufficient to ensure the product is safe for human consumption (according to USNACMCF guidelines), a 1 $\log_{10}$ cfu/g reduction in bacterial numbers is not.

Table 4. The effect of sous vide cooking on the levels of *L.monocytogenes* and *Y.enterocolitica* in fish

Fish	*L.monocytogenes* present after the sous vide cook ($\log_{10}$ cfu/g)		*Y.enterocolitica* present after the sous vide cook ($\log_{10}$ cfu/g)	
	Non heat shock	Heat shock	Non heat shock	Heat shock
Trout (surface)	3.96	4.87	3.32	4.58
Salmon (surface)	NA*	3.64	NA	1.81
Trout (core)	3.13	5.55	3.32	4.74
Salmon (core)	NA	3.64	NA	1.81

* NA = not analysed

THE EFFECT OF SODIUM LACTATE ON THE GROWTH OF *L.MONOCYTOGENES* AND Y.ENTEROCOLITICA IN SOUS VIDE COOKED MINCED BEEF

At present, sous vide processing is dependent on two hurdles to ensure the microbial safety of the product. These are the application of heat, when pasteurising the product, followed by rapid cooling to below 3°C and chilled storage. However, these hurdles could be complimented by the addition of substances which inhibit the growth of foodborne pathogens. Sodium lactate is one such substance and has been used successfully in meat and poultry products. The effect of this salt on the growth and survival of *L.monocytogenes* and *Y.enterocolitica* was therefore examined in minced beef

processed by sous vide. At 0°C neither organism grew, regardless of the presence or absence of sodium lactate (Figures 1 & 2). At the storage abuse temperature of 10°C, however, considerable growth was detected in the absence of sodium lactate but not when the salt was added (Figures 3 & 4). Sodium lactate might therefore be advantageously applied in sous vide meat products.

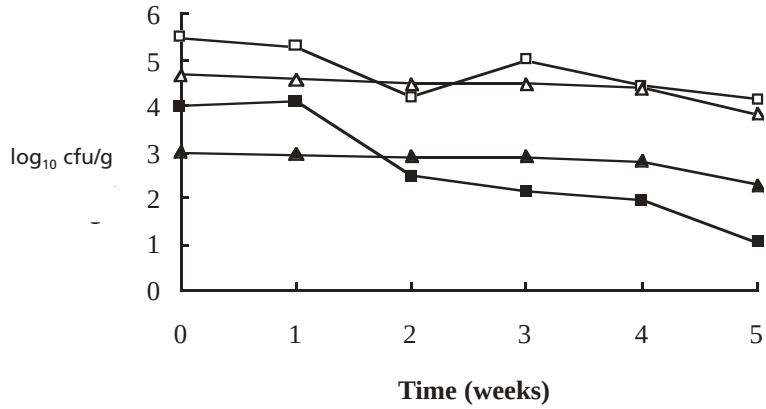


Figure 1. Survival of *L.monocytogenes* at 0°C in minced beef containing 0% sodium lactate (selective agar(■), non-selective agar (□)) or 2.4% sodium lactate (selective agar (▲), non-selective agar (△)).

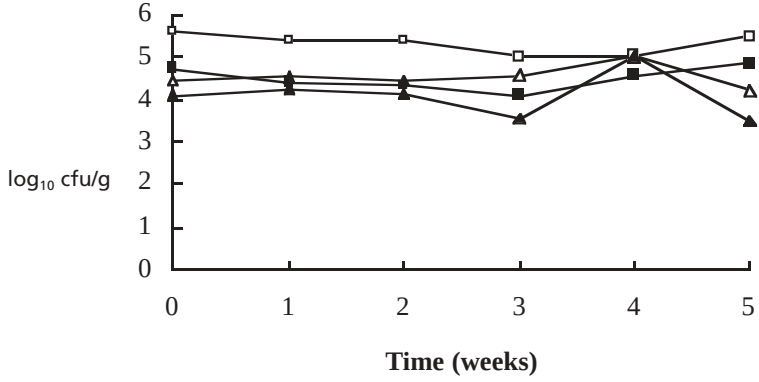


Figure 2. Survival of *Y.enterocolitica* at 0°C in minced beef containing 0% sodium lactate (selective agar(■), non-selective agar (□)) or 2.4% sodium lactate (selective agar (▲), non-selective agar (△)).

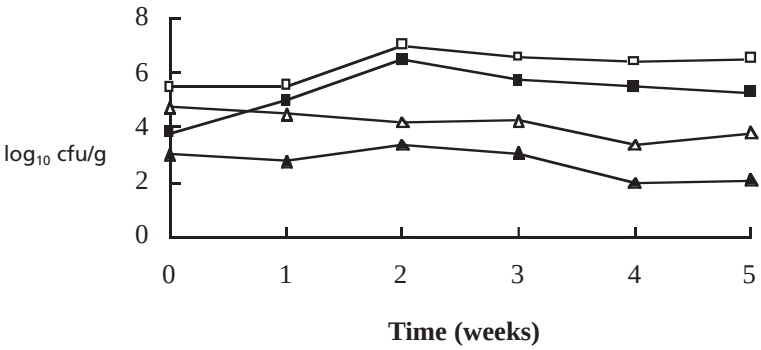


Figure 3. Survival of *L.monocytogenes* at 10°C in minced beef containing 0% sodium lactate (selective agar(■), non-selective agar (□)) or 2.4% sodium lactate (selective agar (▲), non-selective agar (△)).

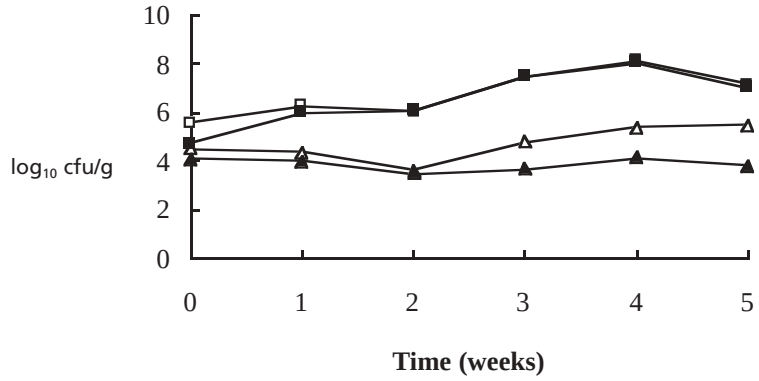


Figure 4. Survival of *Y.enterocolitica* at 10°C in minced beef containing 0% sodium lactate (selective agar(■), non-selective agar (□)) or 2.4% sodium lactate (selective agar (▲), non-selective agar (△)).

CONCLUSIONS

- The extensively available laboratory generated thermoinactivation data on pathogenic bacteria may be validly applied to scaled up commercial sous vide processes.
- From all the evidence gathered there would not appear to be a problem with the survival of non-spore-forming pathogens in sous vide products, with the exception of the survival of vegetative cells in trout and salmon. However, the spores of *B.cereus* and *Cl.botulinum* may survive. Limiting the risk associated with these organisms is dependent on the use of raw materials of good microbiological quality and strict low temperature storage (< 3°C) after processing.
- Carbon dioxide is not suitable for use in modified atmospherically packed sous vide products as it limits heat transfer resulting in considerably increased processing times or severe under-processing.
- Sodium lactate (2.4%) is a suitable preserve for sous vide prepared meat products and should be used to protect meat products against the undesirable microbiological consequences of temperature abuse. However, concentrations above 2.4% result in a salty taste from the product and may enhance the thermostability of any pathogenic contaminants.

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