:11 The effects of lactic acid decontamination and frozen storage on the keeping qualities of calf brain

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INTRODUCTION

Calf brain is highly susceptible to bacterial spoilage. This results from the fact that brain is a particularly rich culture medium allowing even fastidious microorganisms to grow (Rosenow 1919). Moreover, the current stunning methods may allow inoculation of the brain with a potentially hazardous microflora, whilst extirpation and subsequent handling may imply yet other sources of contamination (Smulders et al. 1983).

Virtually all brains from Dutch veal calves are being exported to mainly mediterranean countries (Netherlands Commodity Board for Livestock and Meat, 1983). In general this means that the product is being prepared for consumption at 4-7 days post mortem. From a point of view of public health it is extremely important,therefore, to safeguard the bacteriological quality of calf brain at least until the end of this period.

One of the major determinants of shelf-life is the nature and degree of the initial contamination (Haines 1933, Ingram 1972). Conforming to Good Manufacturing Practices may substantially reduce the initial contamination (Snijders et al. 1984). However, a previous study on the keepability of calf brain revealed that strictly hygienic extirpation and handling would only lead to a small improvement of the bacteriological condition at 8 days post mortem.

Relying on studies with beef,veal and pig carcasses as well as pigs liver the results of which are overviewed elsewhere in the Proceedings (Snijders et al. 1984),treatment with a decontaminating agent such as lactic acid may significantly improve the shelf-life of calf brain.

Purpose of the present study was to investigate the effects of decontamination with lactic acid on the bacteriological condition of calf brain initially and at 8 days post mortem which in practice is the latest time of consumption. In view of the unacceptable discolouration of fat, observed at higher concentrations (Woolthuis and Smulders 1984) a solution of 1.25 % (v/v) was used. The decontaminating effects of lactic acid were tested against a well known preservation method i.e. freezing.

MATERIALS AND METHODS

Collection, decontamination and storage

A series of two experiments was conducted involving a total of 120 calves of the Dutch Friesian (FH-) breed. The animals were stunned by means of a captive bolt. Immediately after bleeding the calves were decapitated. In the first experiment at approximately 1 h post mortem, one person skinned and hot boned the heads, splitted the skulls with an axe and removed the brains. The second experiment relied on the same procedures with the exception of the removal of the brain which was carried out by a second person wearing a fresh pair of surgical gloves during each removal to avoid cross contamination.

The first experiment involved a total of 90 brains. Fourty brains were decontaminated for 15 s with 1.25 % (v/v) L-lactic acid spray prepared from a stock solution of 90 % (Chemie Combinatie Amsterdam) and were allowed to drain for 30 s. Fourty brains served as controls. Half of all brains in each of the treatment groups were sampled at day 1, the other half at day 8 after 7 days of storing at 3±1°C. In addition 10 brains were used to assess weight losses during storage.

The second experiment involved a total of 30 brains which were frozen at -40°C for 7 days and subsequently allowed to thaw at day 8. Twenty brains were examined bacteriologically (10 at day 1 and 10 at day 9), whereas 10 brains were used to assess thawing losses.

Sampling

Samples for bacteriological examination were excised from two locations of the brain viz a) the undamaged hemisphere and b) the site of impact of the captive bolt which is usually the cerebellum. Using sterile scalpels and tweezers cone-shaped samples with a base diameter of approximately 2 cm were excised. Subsequently 10 g brain tissue was macerated in 90 ml of Buffered Peptone Water (van Leusden et al. 1982) in a Stomacher (Gerats and Snijders 1978) and allowed to resuscitate for 1½-2 hours at ambient temperatures.

Bacteriological examination

Numbers of colony forming units (cfu) of the following microorganisms were assessed in the macerate finally obtained: a) Aerobic colony count at 30°C:in poured plates of Tryptone Glucose Beef Extract agar (Difco 0002-01); incubation 3 d at 30°C. b) Aerobic colony count at 4°C: in poured plates of Tryptone Glucose Beef Extract agar (Difco 0002-01); incubation 12-14 d at 4°C. c) *Enterobacteriaceae*: in poured plates of Violet Red Bile Glucose agar (Oxoid CM 485) with overlayer; incubation 1 d at 37°C (Mossel et al. 1962). In addition all samples showing plates containing 7 or more *Enterobacteriaceae* colonies ($\log_{10}$cfu/g≥ 1.8) were tested for salmonellae. For this purpose the remaining macerate was incubated at 37°C during 24 hours whereupon 1.0 ml was inoculated in 10 ml Muller-Kauffmann broth (Oxoid CM 343) and incubated at 43°C for another 24 hours. After plating on Brilliant Green anar (Oxoid CM 329) and incubation during 24 hours at 37 °C, typical colonies were tested by agglutination and type of growth in Kligler Iron agar tubes (Difco 0086-01). c) Lancefield group D streptococci: on spread plates of Kanamycin Aesculin Azide agar (Oxoid CM 481) ; incubation 18 to 20 hours at 37°C; all typical colonies i.e. porcelain-like with black halos were regarded to be Lancefield D streptococci (Mossel et al. 1978).

Mathemathical analysis of data

Colony counts were expressed in colony forming units (cfu) per gram tissue and then converted to logarithms base 10. To determine significance in differences between counts these were analysed using Student-t-tests. Samples with less than 7 colonies on the first decimal dilution plate and therefore inappropriate for colony assessment (Mossel and Drion 1954) were assigned counts corresponding with the limits of detection (for aerobic colony counts at 30 and 4°C, *Enterobacteriaceae* on one side and Lancefield D streptococci on the other 1.8 and 2.8 log cfu/g respectively).

RESULTS AND DISCUSSION

Tables 1 and 2 present the effects of lactic acid decontamination (LAD) on the bacteriological condition of calf brain as assessed at both undamaged (hemispheres) and damaged locations (site of impact of the captive bolt).

Not surprisingly the level of initial contamination at the site of impact of the captive bolt was significantly higher for all parameters examined. Some faecal contamination may occur during the stunning process as may be seen from the positive Lancefield D streptococci count. However in none of the samples at any sampling moment salmonellae were found. This supports earlier findings (Smulders and Woolthuis 1984) indicating that the introduction of salmonellae may be restricted substantially provided adequate cleaning and disinfection of lorries and Good Manufacturing Practices during slaughtering are observed.

Table 1 The effect of 1.25% v/v lactic acid (LA) on the bacteriological condition of calf brain as assessed at day 1 (n=20) or after 7 days of storage at 3±1°C (day 8;n=20); *undamaged hemispheres*, means and standard deviation ($\bar{x}\pm s$ as $\log_{10}$cfu/g)

	day 1				day 8			
	LA		Control		LA		Control	
	%▽	$\bar{x}\pm s$	%	$\bar{x}\pm s$	%	$\bar{x}\pm s$	%	$\bar{x}\pm s$
Aerobic colony count 30°C	100	3.3 ± 0.7^{aΔ}	100	3.9 ± 0.5^{b}	100	7.1 ± 0.8^{c}	100	6.9 ± 0.9^{c}
Aerobic colony count 4°C	85	2.7 ± 0.5^{a}	95	3.1 ± 0.5^{b}	100	7.2 ± 0.8^{c}	100	7.1 ± 0.7^{c}
Entero-bacteriaceae	25	2.3 ± 0.3^{a}	50	2.5 ± 0.4^{b}	100	5.4 ± 0.7^{c}	100	5.3 ± 0.7^{c}
Lancefield D streptococci	0	< 2.8	0	< 2.8	0	< 2.8	0	< 2.8

▽ Percentage of plates appropriate for enumeration from which means have been calculated.

Δ In horizontal rows figures with different superscripts differ significantly ($p<.025$).

Table 2 The effect of 1.25 % v/v lactic acid (LA) on the bacteriological condition of calf brain as assessed at day 1 (n=20) or after 7 days of storage at 3±1°C (day 8;n=20); *site of impact of the captive bolt*, means and standard deviations ($\bar{x} \pm s$ as $\log_{10}$cfu/g)

	day 1				day 8			
	LA		Control		LA		Control	
	%▽	$\bar{x}\pm s$	%	$\bar{x}\pm s$	%	$\bar{x}\pm s$	%	$\bar{x}\pm s$
Aerobic colony count 30°C	100	4.3 ± 0.4^{aΔ}	100	4.6 ± 0.3^{b}	100	6.9 ± 0.8^{c}	100	7.3 ± 0.7^{c}
Aerobic colony count 4°C	100	3.3 ± 0.6^{a}	100	3.6 ± 0.5^{a}	100	7.0 ± 0.8^{b}	100	7.5 ± 0.7^{c}
Entero-bacteriaceae	60	2.5 ± 0.6^{a}	90	2.6 ± 0.3^{b}	100	5.2 ± 0.6^{c}	100	5.6 ± 0.6^{d}
Lancefield D streptococci	20	3.6 ± 0.7^{a}	0	$< 2.8^{a}$	10	3.0 ± 0.1^{a}	10	4.6 ± 0.1^{a}

▽ Percentage of plates appropriate for enumeration from which means have been calculated.

Δ In horizontal rows figures with different superscripts differ significantly ($p<.05$).

With the exception of the latter group of microorganisms, all other bacterial parameters were significantly lower after LAD. Yet, the reduction was considerably less pronounced as compared with previous experiments in beef,veal and pig slaughterline where decreases of more than 1 log unit were found with similar concentrations as that used in the present study (Snijders et al. 1984).

Although some delayed effect of LAD as observed in previous experiments (Smulders and Woolthuis 1984, Snijders et al. 1984) may have been present earlier, significant differences were only present in the damaged tissue at day 8 post mortem for psychrophilic and Enterobacteriaceae counts. Moreover LAD samples exhibited an unacceptable brown discolouration. Several favctors may have effected this situation. As substantiated by the high bacterial numbers in a previous (Smulders et al. 1983) and the present experiment, brain is extremely prone to bacterial spoilage. Moreover, LA may not have penetrated sufficiently into the numerous gyri and sulci. Ingram et al (1956), discussing various acids, stated that the preservative action of acids might among other factors depend on the demands of microorganisms for nutrients and the extent in which these nutrients are available. Ingram particularly mentioned the B vitamins and allied substances. The easy availability of B vitamins, abundantly available in calf brain (Souci et al 1969) may thus have interfered with the decontaminating potential of LA.

Table 3 presents the effects of freesing on the bacteriological quality of brain assessed at both damaged and undamaged brain locations.

Table 3 The effect of freezing on the bacteriological condition of calf brain as assessed at two locations at day 1 (n=10) and after 7 days of storage at -40°C (day 9;n=8); mean bacterial counts and standard deviations ($\bar{x}\pm s$ as $\log_{10}$cfu/g)

	day 1				day 9			
	damaged tissue		undamaged t.		damaged tissue		undamaged t.	
	%▽	$\bar{x}\pm s$	%	$\bar{x}\pm s$	%	$\bar{x}\pm s$	%	$\bar{x}\pm s$
Aerobic colony count 30°C	100	3.4 ± 1.0	50	2.4 ± 0.4	100	3.3 ± 0.6	80	2.3 ± 0.2
Aerobic colony count 4°C	30	2.2 ± 0.2	0	<1.8	30	2.1 ± 0.2	0	<1.8
Entero-bacteriaceae	20	2.1 ± 0.1	0	<1.8	20	2.0 ± 0.1	0	<1.8
Lancefield D streptococci	10	2.8	0	<2.8	10	2.8	0	<2.8

▽ Percentage of plates appropriate for enumeration from which means have been calculated

Firstly, when comparing the data of Table 3 with those from Tables 1 and 2 the beneficial effect of improved hygiene (extirpation with gloves) as observed in an earlier study (Smulders et al. 1983) is evident.

As also reported for other variety meats (Hanna et al. 1982) freezing results in an extended storage-life: at both locations and with regard to all bacteriological parameters examined,no significant differences were present between day 1 and day 9. However thawing loss (expressed as percentage of the initial weight) was on average 5.1 % which is somewhat higher than the 1.2 % weight loss (expressed as percentage of the initial weight) found after chilled storage. Possibly by applying adequate freezing systems thawing loss may be reduced. In view of public health the meat industry is advised to make efforts to obviate these economical impediments.

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