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International Journal of
Food Microbiology 29 (1996) 297–309

International Journal of Food Microbiology

Influence of unsliced delicatessen meat freshness upon bacterial growth in subsequently prepared vacuum packed slices

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Received 25 January 1995; revised 24 May 1995; accepted 29 June 1995

Abstract

Unsliced beef pastrami, reformulated ham and bologna held at 6°C were sliced 21, 17, 12 or 7 days before or at the assigned manufacturer's best before date and vacuum packaged. Packages of sliced meats were held at 6°C for another 7, 12, 17 or 21 days, opened and analyses made for total bacteria, lactic acid bacteria, *Enterobacteriaceae* and *Brochothrix thermosphacta*. The maximum storage interval was 42 days; half this period unsliced, the remainder as repacked slices. Numbers of bacteria on pastrami were significantly greater than on ham and bologna (pastrami > ham > bologna) with the lactic acid bacteria dominating in all products. As unsliced meats approached their best before date, insignificant increases were generally noted for numbers of lactic bacteria, *Enterobacteriaceae* and *B. thermosphacta*. During subsequent storage of slices under vacuum, numbers of total and lactic bacteria increased exponentially at the same rate while *B. thermosphacta* growth was significantly slower. Numbers of *Enterobacteriaceae* remained low and were essentially unchanged during sliced meat storage. Within the context of study storage parameters, shelf-life appeared to be determined by length of time after slicing and repackaging rather than by best before date of the unsliced meat. Packages of sliced meat prepared from wholesale unsliced meats with 21 days left until their best before date or from unsliced meats with 12 days left until their best before date showed similar bacterial levels 21 days later. It was probable that the localization of bacterial growth at the meat surface-packaging film interface of the unsliced meats yielded slices with initially lowered bacterial content when repackaged and sampled from the

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SSDI 0168-1605(95)00048-8

uppermost slice. When *Enterobacteriaceae* and *B. thermosphacta* were absent from unsliced meats, extension of sliced meat package shelf-life beyond the best before date of the parent meat was possible. However, these bacterial groups were not always undetected.

Keywords: Delicatessen meats; Vacuum packaging; Lactic bacteria; Ham; Bologna; Pastrami

1. Introduction

Delicatessen meats are a diverse group of products which commonly, but not exclusively, are cured through the action of $NaNO_2$ to form nitrosylmyoglobin and yield the colour typical of these meats (Bard and Townsend, 1978). Products included in this category may be made from whole muscle cuts, or from chopped, ground or emulsified meats and by-products mixed in various combinations with or without binder plus fat, salt, sugar, spice, other non-meat ingredients (acidulants, anti-oxidants) and the cure. They may be fermented with or without starter cultures and slowly dried without cooking, or they may be cooked after formulation in casings or molds of various shapes and sizes. Uncooked products which satisfy regulatory maximum time requirements (above 15°C) and nitrite requirements during manufacture which have a pH < 4.6 or a water activity (a_w) of < 0.85 are regarded as shelf-stable at room temperature and are termed dry sausage (Agriculture and Agri-Food Canada, 1992). Similarly manufactured products having a pH < 5.3 and an a_w of ≤ 0.90 are also stable at room temperature. Fermented products with pH < 5.3 which have not been dried ($a_w > 0.90$) must be kept refrigerated until consumption. These latter semi-dry and the dry sausages can contain > 8 log CFU/g viable lactic bacteria after manufacture (Holley et al., 1988).

Since delicatessen meats are such a physically and chemically different group of products, general statements regarding shelf-life expectation are difficult to make reliably. In addition, post-process handling practices, sanitation, temperature, light, humidity, packaging film gas (O_2, CO_2) permeability and nitrite stability as well as the nature and ability of the resident microorganisms to produce offensive metabolic end products, also significantly affect shelf-life.

Cooked cured delicatessen meats are characterized by low salt (2–4%), high pH (6.0–6.5), low residual nitrite (< 100 $\mu g/g$) and these require constant refrigeration (Boerema et al., 1993). Following heat processing at ≥ 68°C they are commercially sterile but are contaminated to some extent by subsequent handling. Slicing and packaging by the manufacturer has been shown to cause bacterial increases of from 0.5–1.5 log CFU/g (Mol et al., 1971) to 2–3 log CFU/g (Kempton and Bobier, 1970). These workers and others (Steele and Stiles, 1981; Stiebing, 1993) have shown that the contribution of slicing and packaging operations to the total meat bacterial content is determined by the frequency and rigor of sanitation at the slicing location. The oxygen transmission rate (OTR) of the packaging film material used to prepare retail packages of sliced meats significantly influences meat shelf-life. Low OTR films (< 10 $cm^3/m^2/day/atm/23°C$) provide the best protection against light-induced discolouration due to photochemical instability of nitric oxide pigments (Grini et al., 1992) and create a package environment where almost exclusively, bacterial growth is restricted to

psychrotrophic lactic bacteria at refrigerator temperatures (Kempton and Bobier, 1970; Mol et al., 1971; Steele and Stiles, 1981; Bell and Gill, 1982; Grini et al., 1992; Boerema et al., 1993; Yang and Ray, 1994). When appropriate sanitation practices are used to prepare packages, and under desirable storage conditions (< 8°C with O_2 exclusion) numbers of bacteria present can reach 8 log CFU/g without early development of objectionable organoleptic characteristics (Stiles, 1991; Boerema et al., 1993).

Organisms which dominate during refrigerated storage of these cooked cured products are frequently found to be identical to those responsible for controlled fermentation of dry and semi-dry sausages (Schillinger and Lücke, 1987; Korkeala and Makela, 1989; Holzapfel, 1992). When stored in films with low OTR at acceptable chill temperatures, cooked cured meats undergo a post-process fermentation analogous to that seen in the semi-dry sausages; however, meat pH is fairly stable and rarely drops below 6.0 during storage for 3–4 weeks (Kempton and Bobier, 1970; Steele and Stiles, 1981; Holley et al., 1995). It is likely that this difference is due to the almost exclusive fermentation of the cooked meats at the meat surface–packaging film interface. With longer storage, significant reduction in cooked meat pH occurs (Kempton and Bobier, 1970). In the uncooked sausages, starter and adventitious lactic bacteria are blended through the meat batter prior to stuffing which facilitates pH reduction throughout the entire reformed structure.

Higher numbers of bacteria at the cooked, cured meat surface–package interface have been explained by suggestions that: the surface a_w was higher than the interior (Korkeala and Lindroth, 1987); there was a higher level of O_2 at the surface due to its slow diffusion through the packaging film (Bell and Gill, 1982); or physical alteration or stretching caused localized increases in O_2 permeability during package fabrication (Stiebing, 1993). Increasing the area of film contacting the meat surface would therefore be expected to accelerate the rate of bacterial growth on the meats.

Previous work (Holley et al., 1995) has shown that once sliced and packed in low OTR film, ham, bologna and beef pastrami had a shelf-life of about 21 days at 4°C which was unrelated to the 'freshness' of the wholesale meat piece from which the slices were prepared. The wholesale meats used had from 21–46 days remaining before reaching the manufacturers' coded best before date. The current work was undertaken to determine whether unsliced meat freshness, when there was 0 to 21 days left until the best before date, would significantly affect the shelf-life and development of microorganisms on slices repacked in low OTR bags subsequently stored at 6°C for up to 21 days.

2. Materials and methods

2.1. Meats

Three types of cooked, cured delicatessen meats were used in this study. Bologna was manufactured and cooked in 4 kg chubs in its plastic casing. The ham was a reformulated low fat ($< 5\%$) product cooked in a rectangular mold (5.5 kg), removed from the mold and overwrapped in a plastic film before distribution. Beef pastrami was

a whole muscle piece, eye of the round cut weighing 2.5 kg which was cooked, rolled in a non-sterile spice mixture and vacuum packed before distribution. Meats with > 21 days (d) left until the best before date were obtained directly from a local wholesale distributor. For each experiment five pieces of meat with the same coded best before date (BBD) were obtained and placed at 6°C. Meats were held at 6°C until there were 21 d until the BBD, then one package was aseptically opened, sliced, sampled for microbiological content, repacked under vacuum in barrier bags and stored at 6°C for up to 21 d. The other four wrapped, unsliced meat pieces from the same lot were also held at 6°C, opened, sliced, sampled and the slices repacked under vacuum as the BBD approached at days 17, 12, 7 or at the BBD of the meat. Experiments were repeated three times with each of the three meats.

2.2. Slicing

At slicing, the casing or wrapper was treated with 80% ethanol, partly (25%) peeled back, any brine present was allowed to drain and a 2–3 cm piece was aseptically cut from the end of the meat block. Slices 1–2 mm thick were cut transversely with a slicer (Model 725, Globe Slicing Machine Co., Stamford, CT). Before each use and between meats the machine was washed and treated with 80% ethanol. Care was taken to ensure complete blade disinfection between samples by removal of the blade cover. Slices were allowed to fall on a fresh sheet of aluminum foil to prevent re-contacting the machine.

2.3. Slice storage

Eighteen slices were prepared from each meat piece, collected in groups of three slices, placed in Winpak Deli #1 bags (XPAE-2060, Winpak, Winnipeg, MB) and vacuum packaged (Model A-300, Multi-vac, Sepp Haggenmüller, Wolfertschwenden, Germany). Packages were stored at 6°C, evaluated immediately, and after 7, 12, 17 or 21 d storage. At sampling, one bag was taken, opened, and a complete slice was weighed, added to 90 ml of 0.1% peptone and stomached for 60 s (Colworth Stomacher 400, A.J. Seward, Canlab, Toronto). Decimal dilutions were prepared in 0.1% peptone. Total numbers of bacteria were determined using Plate Count Agar to yield a standard plate count, SPC (PCA, Difco Laboratories, Detroit, MI) at 35°C for 48 h. Lactic acid bacteria (LAB) were analyzed by growth on de Man Rogosa Sharpe agar containing 0.1% thallous acetate (MRST, Mol et al., 1971) with incubation at 25°C for 72 h under CO_2 (Gaspak, BBL, Cockneysville, MD). *Brochothrix thermosphacta* were enumerated using streptomycin-thallous acetate-actidione agar (STAA, Holzapfel, 1992) by incubation at 21°C for 72 h. *Enterobacteriaceae* were enumerated on violet red bile glucose agar (VRBG, Difco) with incubation at 35°C for 48 h. Dilutions were spread-plated onto prepoured media with the exception of VRBG where the conventional overlay method was used. The weight of meat used was factored into the calculation of numbers of organisms/g recorded.

2.4. Statistical analysis

Multi-factor analysis of variance was performed to determine the main effects of Trial (replicate), Product (meat type), Time (time of storage after slicing), and BBD

(best before date of unsliced meats as assigned by the manufacturer) and to determine significant interactions between factors using STATGRAPHICS® PLUS (Manugistics, Rockville, MD). Differences were considered statistically significant at the 5% level.

3. Results

Levels of significance and residual mean square error from results of the ANOVA for SPC, LAB, *Enterobacteriaceae*, and *B. thermosphacta* are summarized in Table 1. Significant effects of Trial and Product were noted for all four bacterial types, with a significant BBD effect found with LAB, *Enterobacteriaceae* and *B. thermosphacta* where their numbers increased in the unsliced meats as they approached the BBD. Time was not a significant factor for *Enterobacteriaceae*, i.e., these organisms generally did not grow in the sliced meat packages during storage.

Significant Trial × Product, Trial × BBD, and Product × BBD interactions were found for all bacterial types. This clearly shows differences in the microbial numbers present in meats manufactured at different times. Numbers of bacteria recovered on SPC, MRST, and STAA media were highest in Trial A and generally lowest in Trial B. A significant Product × Time effect was found with SPC and LAB. Numbers of bacteria on these media reached a maximum within a week of sliced pastrami storage, but on ham slices their growth continued at the same rate until day 21 of slice storage. Increases in numbers of bacteria (SPC and LAB) present on bologna slices were initially the same as in ham slices, but after 2 weeks tended to remain almost constant during continued slice storage.

No significant interactions were noted between Trial and Time, and between Time and BBD. This suggests that regardless of how bacterial numbers varied with Trial and BBD, the ability of the bacteria to grow in both the unsliced and sliced meat packages did not change markedly.

Table 1
Significance levels and residual mean square error from ANOVA conducted with three replicates using bacterial recoveries showing main effects and interactions

Source of variation	Total bacteria	Lactic bacteria	*Brochothrix thermosphacta*	*Enterobacteriaceae*
Main effects				
Trial	< 0.0001	< 0.0001	< 0.0001	0.0212
Product	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Time	< 0.0001	< 0.0001	0.0009	0.6915
BBD	0.2269	0.0001	0.0147	0.0006
Interactions				
Trial × Product	< 0.0001	< 0.0001	< 0.0001	0.0011
Trial × Time	0.3621	0.2887	0.1610	0.8406
Trial × BBD	0.0001	0.0446	< 0.0001	< 0.0001
Product × Time	< 0.0001	< 0.0001	0.3060	0.8700
Product × BBD	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Time × BBD	0.9853	0.8668	0.9900	0.9784
Residual error	1.7401	1.6728	1.6831	0.6369

Since *Enterobacteriaceae* numbers were generally < 1 log CFU/g in all samples, detailed results are not reported here. However, it should be noted that in two different trials with pastrami, *Enterobacteriaceae* were found in slices cut from two different packages. Although their numbers remained constant at about 3 log CFU/g from 12 to

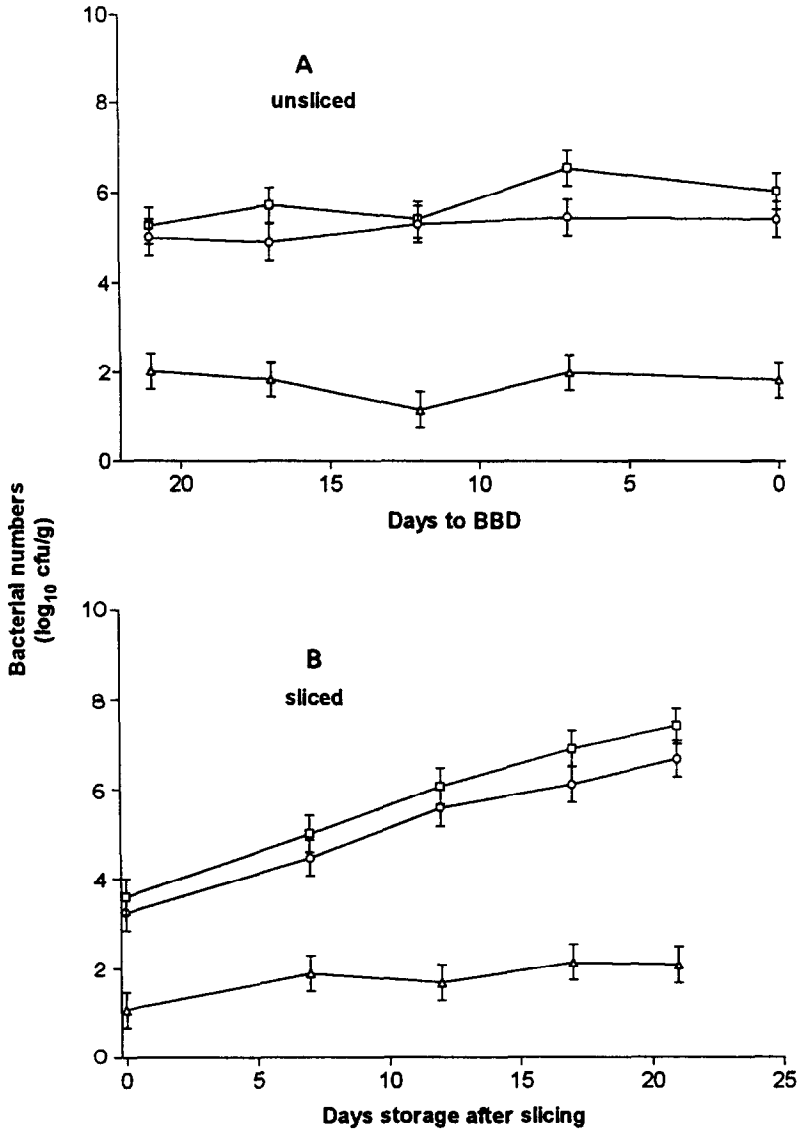

Fig. 1. (A) Main effects of best before date (BBD) of unsliced meats (i.e. days prior to BBD that sample is repackaged) on bacterial growth. SPC, total bacteria (○); MRST, lactic bacteria (□); STAA, *Brochothrix thermosphacta* (△). Meats at entry into the study had 21 days to BBD (Fresh = 21 d; aged = 0 d BBD). (B) Main effects of storage time on bacterial growth in sliced meat packages. SPC, total bacteria (○); MRST, lactic bacteria (□); STAA, *Brochothrix thermosphacta* (△). Values are means for the three meats.

21 d storage in one trial, numbers increased in the other from 5.9 log CFU/g at day 7 of sliced storage to 7.3 log CFU/g at 21 d. After their first detection in unsliced products, all subsequently sampled sliced meat packages fabricated from the same unsliced meat piece were positive. It is suspected an organism like *Serratia liquefaciens* which has been isolated from vacuum packed pastrami slices was responsible for this result (Holley, unpublished results).

Numbers of bacteria recovered on SPC, MRST and STAA were significantly higher in the first than in the other two trials and numbers of *B. thermosphacta* on STAA were significantly lower than those recovered on SPC and MRST. Almost without exception

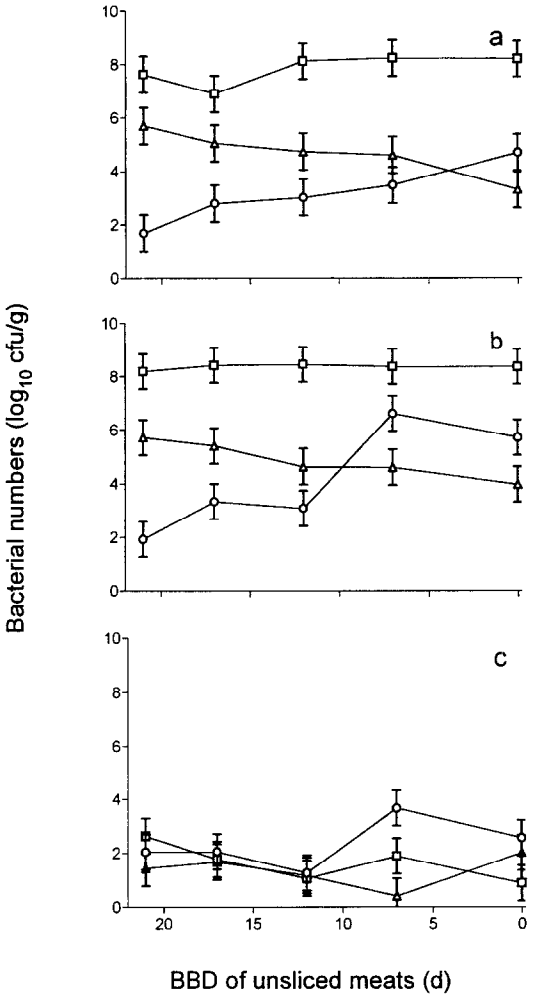

Fig. 2. Influence of interactions between bologna (○), pastrami (□) and ham (△), and best before date (BBD) of unsliced meats upon total bacteria (a), lactic bacteria (b) and *Brochothrix thermosphacta* (c) present in sliced meats. Fresh = 21 d BBD; aged = 0 d BBD.

in packages containing unsliced (Fig. 1a) or sliced (Fig. 1b) meats, the dominant bacteria present were LAB. These figures also illustrate the effects of BBD and Time, respectively, upon bacterial numbers. Pastrami had the highest overall SPC and LAB numbers but bologna supported growth of *B. thermosphacta* the best of the three meats in both unsliced and sliced packages. Differences in BBD, as the unsliced meat aged, did not appear to have a substantial influence upon bacterial numbers, since there was only a minor trend toward higher LAB numbers as the BBD was approached (Fig. 1a). However, there was a Product × BBD interaction which is hidden in Fig. 1a by the relatively constant and high numbers of LAB from pastrami during storage of the unsliced meat (Fig. 2). Numbers of LAB and *B. thermosphacta* increased on bologna but decreased on ham as the unsliced meat aged.

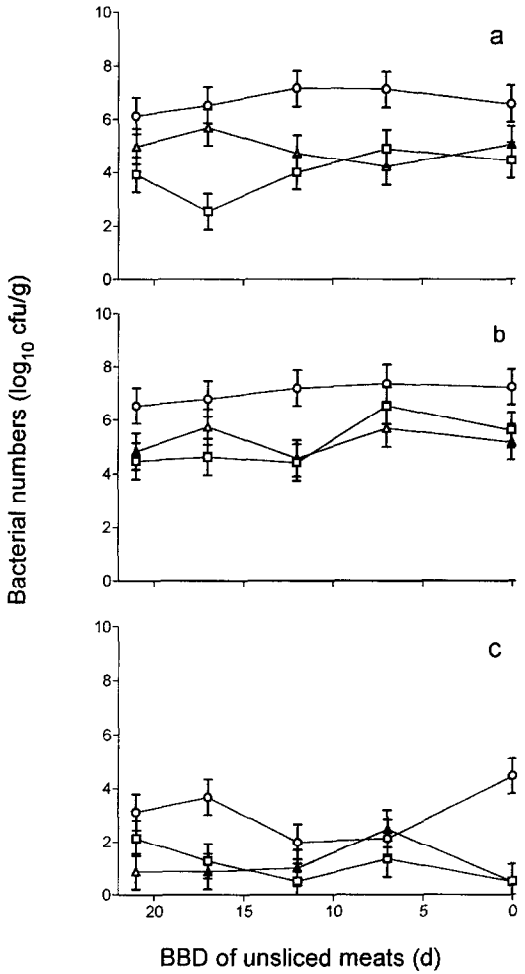

Fig. 3. Influence of interactions between Trials A (○), B (□) and C (△) and best before date (BBD) of unsliced meats upon total bacteria (a), lactic bacteria (b) and *Brochothrix thermosphacta* (c). Fresh = 21 d BBD; aged = 0 d BBD.

The BBD did not always have a significant effect upon bacterial numbers. This effect was influenced by Trial (Fig. 3). Generally, numbers of total and lactic bacteria were higher in sliced packages when they were fabricated closer to the BBD of the unsliced meat and this was most noticeable with bologna. It was of interest that during the storage of sliced meats both total and LAB increased exponentially at essentially the same rate, while the growth of *B. thermosphacta* was significantly slower (Fig. 1b).

Product type had a significant influence upon the rate of bacterial growth in sliced meat packages (Fig. 4). Little growth was noted in pastrami since numbers of total and LAB were initially high. In contrast, considerable growth was noted on ham, with significantly less growth on bologna (Fig. 4a and b). *B. thermosphacta* grew periodically on bologna but did not grow well on ham and pastrami (Fig. 4c).

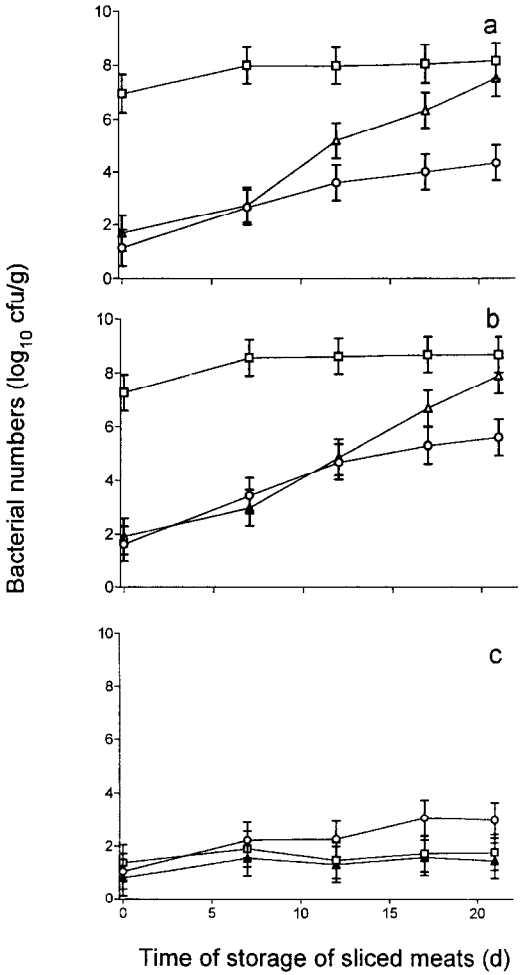

Fig. 4. Influence of interactions between bologna (○), pastrami (□) and ham (△), and days storage of sliced meats upon total bacteria (a), lactic bacteria (b) and *Brochothrix thermosphacta* (c).

4. Discussion

Initial numbers of lactic acid bacteria found in this study were higher on pastrami (by 2–3 log CFU/g) but lower on bologna (0.5 log CFU/g) and ham (0.5–2.7 log CFU/g) than found in an earlier study using similar products (Holley et al., 1995). Mean values for the latter study were 4, 3 and 3 log CFU/g for freshly sliced pastrami, bologna and ham, respectively, when prepared from meats 21 d prior to manufacturers' BBD. Differences noted could have been due to the greater nitrite stability in bologna with the least stability seen in stored pastrami. Just as in the earlier study, and as expected, dominating organisms during storage were LAB. There were two instances in sliced pastrami where bacteria were present in VRBG. These were probably *Serratia liquefaciens* (Nielsen, 1983) but were not identified here, and were introduced during the slicing procedure since they were not recovered from unsliced meats. Early spoilage of these types of meats by *Enterobacteriaceae* has been noted, their growth being more noticeable in meats packed in film of higher O_2 permeability, stored at higher temperature (Stiebing, 1993).

Levels of *B. thermosphacta* in unsliced meats during storage until the BBD did not reach levels where they would have influenced meat quality (Figs. 1–3). However, slicing accelerated the rate of *B. thermosphacta* growth in Trial A. With bologna held 9 d before being sliced and subsequently held 12 d after slicing, numbers were high enough to have caused off odours (sweaty, cheesy). In packages held longer (> 9 d) before as well as after slicing (> 12 d) numbers of these organisms were significant (5–8 log CFU/g) and could have caused problems (Stiles, 1991). With ham from the same trial and pastrami from a different trial a similar result was obtained in some sliced meat packages. Unsliced meats were an important source of the organisms and in these cases it would be important not to exceed the BBD coded by the manufacturers when code dating the sliced meat packages, i.e., placing 21 d shelf life expectation on packages of meat sliced from these blocks when there was only 12 d left before the BBD would probably have yielded poor quality sliced products. With other sliced packs fabricated from different unsliced blocks during this trial and other replicates, microbiology results indicated that an additional 21 d storage was possible after the BBD was reached, particularly by ham in Trial C.

Better growth of *B. thermosphacta* than LAB was reported by Nielsen (1983) in vacuum packed bologna but there were also significant numbers of *Enterobacteriaceae* in the samples studied which suggested that sanitation, higher than required storage temperature and greater than desirable O_2 concentrations were contributory factors. In the present study high numbers of *B. thermosphacta* were not coincident with large numbers of *Enterobacteriaceae*. After study completion, the manufacturer checked the OTR of the bags used to pack slices and found it to be 10.48 $cm^3/m^2/day/atm/23°C$ and this would have been sufficient to prevent *B. thermosphacta* growth if sliced meats were satisfactorily packaged under vacuum. Perhaps the brief exposure to O_2 during repacking slices was enough to allow their growth on slices made from blocks where they already were present at low (3–4 log CFU/g) levels. Boerema et al. (1993) reached a similar conclusion in a study of modified atmosphere packaged ham, but packaging films with 3-times greater OTR than required to prevent *B. thermosphacta* growth were

used. Normally (and in most samples studied here, Fig. 3), LAB successfully compete with and inhibit *B. thermosphacta* in these types of products (Stiles, 1991), however LAB growth in bologna was not extensive (Figs. 2 and 4) and this was probably due to a combination of nitrite stability, which was 2- and 10-times greater in 21-day-old sliced bologna than in ham and pastrami, respectively, stored at 8°C, as well as lower a_w (bologna a_w was 0.960, ham and pastrami were 0.967; Holley et al., 1995).

It is clear that the type of organisms which develop in these products during refrigerated storage is more important in determining shelf-life than is the total number of bacteria which develop. Early spoilage by *B. thermosphacta* and *Enterobacteriaceae* can normally be controlled by good post-process sanitation and use of high O_2 barrier packaging film materials for vacuum packs (Stiles, 1991; Grini et al., 1992; Boerema et al., 1993). Numbers of LAB on these products are frequently extremely high (> 8 log CFU/g) before signs of spoilage become visible (Steele and Stiles, 1981; Stiles, 1991; Boerema et al., 1993). Sensory evaluation was not a part of the current study.

Perhaps one of the most unexpected trends seen in the present work was the apparent shelf-life extension which occurred when slices were repackaged. This was particularly noticeable with ham where total and LAB numbers appeared to decrease as the BBD of the unsliced meat approached (Fig. 2). Our earlier work indicates this observation is to some extent a sampling artifact. Although results are implied to mean that numbers of bacteria recovered are from the unsliced meat, in reality the results presented in Fig. 2 are from analysis of a transverse slice from the unwrapped meat block. Since bacterial growth was localized at the unsliced meat surface–package film interface and was usually ≥ 3 log CFU/g higher than levels inside the meat (Holley et al., 1995) the type of sampling used here probably diluted bacterial numbers at the peripheral edge of the slice yielding what appeared to be a decrease in numbers as the unsliced meats aged. Nonetheless, it is clear that the process of slicing adds between 0.5–2 log CFU/g to the meat slices (Kempton and Bobier, 1970; Mol et al., 1971; Holley et al., 1995) and this probably happened here but methods used were not designed to detect this difference.

When all three types of unsliced meats with 21 d left to their BBD were sliced and repackaged there were instances where levels of *B. thermosphacta* or *Enterobacteriaceae* could have prevented attainment of 21 d sliced storage life. However, there were many more observations where the combined unsliced plus sliced packed shelf-life was clearly longer (sometimes 21 d sliced shelf-life alone) than the unsliced BBD. To the extreme, in all three trials with pastrami held to the BBD and sliced, the repackaged slices were microbiologically acceptable 21 d later. With ham and bologna in two of three trials, the same observation was made. On average, slicing extended the manufacturer's BBD by 10–12 d. Slicing and repackaging had a significant impact upon potential shelf-life and early slicing may compromise the shelf-life of unsliced meats with greater than 21 d to their BBD. That is, for maximum shelf-life meats should be kept unsliced as long as possible before slicing. Refrigerated shelf-life of sliced delicatessen meats seems to be determined more by the number of days the slices are in the final package and not by the 'freshness' of the unsliced meat piece.

Provided unsliced meats do not contain detectable levels of *B. thermosphacta* or *Enterobacteriaceae* during storage until their BBD, there is a very strong possibility that they may have up to an additional 21 d shelf-life after slicing and repackaging. This is

only possible if excellent hygienic practices are followed, low OTR packaging film is used, adequate vacuum and acceptable refrigeration conditions ($\leq 6°C$) are met. It is believed that this extension of shelf-life is made possible by the almost exclusive growth of bacteria at the meat surface–package interface in both the unsliced and sliced meat packages. Although not investigated here, it is possible that storage of packed sliced meats past the original BBD may provide opportunity for growth of psychrotrophic pathogens which might be present.

Acknowledgements

Joanne Elsaesser and Ted Millard are recognized for their interest in this work and their excellent technical expertise during conduct of the experiments. Meats used in this study were obtained through the cooperation of Keith McEvoy, Meat Procurement Manager, Loeb Inc., Ottawa. This is Contribution No. 2302 from the Centre for Food and Animal Research, Agriculture and Agri-Food Canada.

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