Extending the Shelf-Life of Vacuum-Packaged Pork Liver Paté

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ABSTRACT

Processing conditions to achieve a minimum shelf life of 1 month at 4°C for a vacuum packaged pork liver paté were defined. Cooking to a core temperature of 70°C and maintaining less than 25% added water prevented spoilage by lactic acid bacteria. Higher added water levels caused spoilage due to greening resulting from the proliferation of Lactobacillus viridescens. Glycerol at levels up to 1% (w/w) accelerated sour spoilage but glucono-delta-lactone (GDL) at 0.5% (w/w) markedly reduced the growth of lactic acid bacteria. However, with GDL yeasts tended to proliferate. Overall shelf life was best maintained by control of the a₁ of the product through regulation of added water or the use of GDL.

The keeping quality of edible offals depends on the microbial load which they acquire during evisceration and on their subsequent storage conditions (26). The initial level is comparable with that of carcass meat (17) but good temperature control is essential (8) to control bacterial activity. The safety of pork liver has been questioned due to frequent isolations of Salmonella (22). However, other workers have found a low incidence of Salmonella in pork liver (4,15).

Processing of offals can produce a product with a substantially longer shelf-life than the original material and a higher profit margin (26). Paté, which is primarily a perishable comminuted meat product containing nitrite and optionally nitrate and-or ascorbate, is an example of such a processed product (25).

With the establishment of local companies producing pork liver paté guidelines were developed (14), and a cooperative study undertaken with one manufacturer to define the processing conditions required to ensure a minimum shelf life of one month for vacuum-packaged tubs (220 g) of pork liver paté held at 4°C.

MATERIALS AND METHODS

Enterobacteriaceae counts (coliforms) and Salmonella detection were performed as previously described (15). Brochothrix thermosphacta was enumerated on STAA agar (5). Staphylococcus aureus was enumerated on Baird-Parker agar (Oxoid CM275) and yeasts and molds (YAM) on malt extract agar (Oxoid CM59) plus 50 mg/l chloramphenicol (Sigma 000378). Aerobic plate counts (APC) were made on nutrient agar (Oxoid CM3) and total anaerobe counts (TAC) on brain heart infusion agar (Oxoid 375) plus 3 g/l yeast extract (Oxoid L21) (1). YAM, APC, and TAC plates were incubated at 22°C for 3 d, TAC plates in a Forma Scientific Model 1024 (Marietta, Ohio, USA) anaerobic cabinet with an atmosphere of 85% N₂, 10% H₂, 5% CO₂ (all v/v). All other media were incubated according to manufacturers or authors instructions.

Preliminary trials were conducted to determine which microorganisms would be significant during spoilage, and two distinct types of spoilage were noted. Paté stored for an extended period produced sour odors when the pack was opened, while packs opened after shorter periods subsequently developed visible slime and discoloration if stored in air for several days. Sour spoiled samples had high TAC counts and elevated levels of lactic acid. Flora analyses showed that the dominant population was lactic acid bacteria.

Pack opened and left in air, however, had high APC counts and flora analyses showed Bacillus, Micrococcus, and Corynebacterium spp. to be dominant. It was noted that lactic acid bacteria could produce pinpoint colonies on nutrient agar, as had also been found in previous studies (16). To differentiate between the causes of the spoilage conditions found, one being distinctly aerobic and the other anaerobic, it was necessary to ensure the lactic acid bacteria were not enumerated as part of the APC. Hence, nutrient agar plates were checked using hydrogen peroxide (30% v/v) and very small catalase negative colonies discounted. This ensured the APC was a measure of the microorganisms capable of causing aerobic spoilage.

Colonies from TAC plates were also checked with hydrogen peroxide to ensure the anaerobic cabinet was oxygen-free. Catalase positive colonies were inoculated into Hugh and Leifson medium (23) to ensure fermentation was taking place. Growth of obligate aerobes would indicate leakage, and the work would be repeated after repairs.

Samples (10 g) for microbial analysis were blended (2 min), using a stomacher (Colworth 400), in 90 ml peptone saline diluent (Oxoid CM9). All subsequent dilutions used 9 ml portions of this diluent. All sampling for microbial analyses was in duplicate.

Results were statistically analysed by analysis of variance (10). Presumptive Lactobacillus spp. were initially characterized by standard methods (23) then identified using the API 50CHL system (API System, S.A., La Balme les Grottes, France).
Lactic acid was determined enzymatically (Sigma kit 726-UV, Saint Louis, Missouri, USA) and water activity (a_w) was determined using a Protimeter DP 680 (Marlow, Buchi, UK) dew-point meter. In both cases triplicate samples were analysed.

Paté was prepared in one commercial premises using ingredients routinely available: pork liver 2.3 kg, bacon trim 3.2 kg, rusk (white, superfine) 0.9 kg, water 1.4 kg, dried mixed herbs 170 g, mixed spices 110 g. Herbs, spices, and rusk (T. Lucas Ltd, Kingswood, Bristol, UK) were standard butchers wholesale supplies. Sodium nitrite was added as a solution made with part of the water to give a final level of 150 mg kg^-1. The liver and bacon were blanched in boiling water for approximately 5 min before blending with the other ingredients (2 min) in a Robot Coupe R15 blender.

A Franke U.L. 21E fan assisted oven (120°C) was used for all cooking. Temperatures were measured using Thermocouple Instruments model 55 electronic thermometer (Penewyn, Wales). The probe was marked to allow the sensor to be accurately placed at the center of a tub. The temperature measured is referred subsequently as the core temperature. Six samples were measured to provide mean temperatures.

Plastic tubs (approximately 12 x 9 cm oval, 3 cm deep) were filled with 220 g of paté and after cooking placed in vacuum pouches (Intervac, Hemel Hempstead, GB) with transmission rates (20°C, 100% relative humidity) of 1.69 g/m²/d (H_2O), 40 ml/m²/d (O_2), and 120 ml/m²/d (CO_2). After cooling overnight at 2°C, the pouches were evacuated and sealed.

Whenever possible, a single large batch of paté was prepared, subdivided, then individual treatments carried out. Five treatments, including a control, were usually studied at one time, i.e. 120 tubs which were loaded in a randomized fashion to allow for temperature variations in the oven. Treatments were studied in duplicate.

Raw ingredients were sampled at the premises used for cooking, eight times over a period of four weeks. Sampling of paté during storage trials, in duplicate, took place twice a week for up to 34 d.

To determine the lowest core cooking temperature, a thermocouple was fixed at the center of a tub in the center of the oven and 22 tubs were removed at 45, 55, 65, 75, and 80°C. This study used 220 g packs as models for wholesale 2.2 kg tubs, but the smaller packs were adopted by the manufacturer as retail packs, hence subsequent experiments used 4°C storage to simulate good display chills rather than ideal wholesale storage at 1°C.

RESULTS

The initial work aimed to define the lowest core cooking temperature allowing a storage life of one month at 1°C. Water activity fell linearly with increasing temperature -

\[ a_w = 0.969 - 1.06 \times 10^{-1} T, \quad p < 0.05 \]  

(T = temperature in °C)

From the microbial analyses of the raw ingredients (Table 1), the raw paté would have had a mean APC of 2.37 x 10^7 g^-1. Cooking decreased the APC by 2 to 4 log cycles. Little increase in numbers was detected in any samples during the first 22 d of storage.

While the APC was controlled by even the shortest cooking process followed by vacuum packaging and storage at 1°C, the TAC showed no lag phase after cooking to 46.3°C (Fig. 1). Longer cooking gave a marked reduction in numbers and slower subsequent multiplication. Analysis of the TAC at 34 d showed a linear relationship between the core temperature after cooking and log_{10} TAC. Since the growth of anaerobes leading to souring was the main problem it was decided to keep numbers below 1% of the potential spoilage level of about 10^7 g^-1. Using the regression equation the log_{10} TAC for 65 and 70°C were 4.86 and 4.45 respectively. To allow for the variation in temperatures seen in the samples, a core temperature of 70°C was recommended and adopted in the studies described below.

The effect of altering the quantity of water used was then studied (Table 2). The upper limit was determined experimentally as that which gave a product judged by the paté manufacturers to be slightly too soft for the consumer. The effects of the change in a_w are shown in Fig. 2. Lowering the a_w reduces the rate of exponential growth (p<0.01) and the TAC after 28 d was directly proportional to the a_w (P<0.05). Again the APC was well controlled and the maximum numbers seen were 3.72 x 10^7 g^-1. Sour spoilage odors were not detected at any time.

Analysis of variance showed that acid production did not significantly affect the pH at any a_w (P<0.05). How­

ever, at the highest a_w the concentration of lactic acid rose from 4 to 510 µg/g during 28 d storage. In contrast in the lowest a_w sample it rose to 25 µg/g. Source spoilage odors

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Log_{10} (APC g^-1)</th>
<th>Log_{10} (yeasts and molds g^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>liver</td>
<td>6.91</td>
<td>&lt;1</td>
</tr>
<tr>
<td>cured pork</td>
<td>5.03</td>
<td>0.52</td>
</tr>
<tr>
<td>rusk</td>
<td>3.34</td>
<td>0.32</td>
</tr>
<tr>
<td>spices</td>
<td>4.52</td>
<td>0.22</td>
</tr>
<tr>
<td>herbs</td>
<td>5.79</td>
<td>0.26</td>
</tr>
</tbody>
</table>

![Figure 1](http://example.com/fig1.png)
TABLE 2. Additions of water to pate mix and consequent effects on $a_w$ drip and microbial counts.

<table>
<thead>
<tr>
<th>Water added (g/kg)</th>
<th>Values after 28 d at 4°C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$a_w$ (Log$_{10}$/g)</td>
<td>drip (g/pack)</td>
</tr>
<tr>
<td>0</td>
<td>0.938</td>
<td>0.30</td>
</tr>
<tr>
<td>215</td>
<td>0.958</td>
<td>1.56</td>
</tr>
<tr>
<td>430</td>
<td>0.960</td>
<td>2.14</td>
</tr>
<tr>
<td>645</td>
<td>0.964</td>
<td>3.49</td>
</tr>
<tr>
<td>860</td>
<td>0.982</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2. Growth of TAC at different values of $a_w$ during storage at 4°C. Anaerobic bacteria had highest survival at an $a_w$ of 0.96.

were detected after 28 d in samples with an $a_w$ of 0.982. Samples at the two highest $a_w$ values turned green on exposure to air. Of 20 anaerobes selected at random from the TAC plates of these samples 16 were identified as Lactobacillus viridescens. No coliforms, S. aureus, or yeasts and molds were detected.

To give the required shelf life, 215 ml/kg of added water was chosen. This value also showed low drip loss on storage (Table 2) and drip in packs was reported as unsightly by retailers test marketing the pate.

The effect of humectants on extending the shelf life were then studied. Glycerol (0.1 and 1% w/w) and delta-gluconolactone (DGL) (0.1 and 0.5% w/w) were selected and the upper concentrations determined as the maximum acceptable to the sensory assessment panel. DGL also acts as an acidulant and caused a sour taint at 1% (w/w).

Only DGL at 0.5% (w/w) increased the shelf life (P<0.05) as assessed by TAC (Fig. 3). It also caused a reduction in pH of 0.6 units, to a value of $5.77 \pm 0.04$, meaned over the 30 d storage period.

The $a_w$ 0.96 in the control, fell to 0.94 and 0.93 with 0.1 and 1.0% glycerol respectively, while DGL (0.1% and 0.5%) caused respective reductions to 0.95 and 0.93.

Pates prepared with glycerol gave sour odors on opening the packs after 28 d and rapidly turned green. Again Lactobacillus viridescens was isolated. No yeasts and molds, S. aureus, coliforms, or B. thermosphacta were detected in the control or glycerol containing pates.

Pates containing DGL did not exhibit greening or souring. With the lower level of DGL no yeasts and molds, S. aureus, coliforms, or B. thermosphacta were detected. With the higher level of DGL none of the organisms noted above were found but after 16 d, $10^2$ yeasts/g were found, increasing to $2.51 \times 10^4$ yeasts/g at 28 d. The mean generation time of the yeasts was 36 h and growth was in the exponential phase at 28 d. APC were below $10^6$ in all cases.

DISCUSSION

Prior to cooking, pork liver carried the most contamination (Table 1), representing about 95% of the APC. Since the APC of liver rapidly increases unless properly cooled (8,15) good manufacturing practice could reduce this load significantly. Local livers were reported with an APC (Log$_{10}$ CFU/g) of 4.21 (15) and maintaining these levels would have a marked effect on the microbial quality of the pate.

However, since the microbial contamination of liver
is largely confined to its surface (6) the blanching procedure should significantly reduce numbers.

The combination of low temperatures and low oxygen tension in the product kept the APC low during all of the studies. Since the APC was always below 10^9/g no problems with aerobic bacteria occurred, and hence, aerobic spoilage can be discounted during storage.

Spoilage always occurred due to souring or greening and both of these activities are attributable to lactic acid bacteria, enumerated in the TAC.

The paté studied was similar in nature to luncheon meat, being a cured, comminuted meat product given a mild heat treatment resulting in a perishable product. Studies with raw luncheon meat (2) showed a mean APC of 1.11 x 10^9/g compared with an estimated 2.37 x 10^9/g for the paté studied. Counts of Lactobacillus (Log_{10}/g) after cooking the luncheon meat (2) were 2.78 while a TAC of 2.59 + 0.72 (n=16) was found in our study. Hence the numbers after cooking are similar, despite the luncheon meat spending 89 min in a water bath at 68.5°C, and the core temperature chosen appears appropriate.

Lowering a_w values in such products can reduce the rate of microbial growth and the maximum number of bacteria (11). Although 3-fold changes in added water caused a change of only 0.006 in the a_w (Table 4) reducing the a_w from 0.967 to 0.961 prevented the spoilage of liver sausage (12).

The greening seen in the high a_w patés was due to hydrogen peroxide production by L. viridescens when the paté was exposed to air (18). It is therefore essential that a_w values below 0.964 are maintained.

Glycerol has been used to increase the microbiological stability of foods (3,9,11) but generally at higher levels than in this study — about 12-30%. Since 1% was used, only minor improvements were expected. However, patés with glycerol soured more quickly than the controls. B. thermosphacta can utilize glycerol as a carbon source (13) and can cause sour spoilage of meat products (7,8). But it was not detected in any patés during this study.

Studies on the taxonomy of lactic acid bacteria from vacuum-packed meats showed that 94% of cluster I (non-aciduric streptobacteria) isolates could ferment glycerol (24). This property is rare in strains of lactobacilli (20). It was also noted (21) that the group 1 organisms were more resistant to high temperatures than the other lactobacilli studied. Therefore, it is probably that the use of glycerol as a humectant simply provided a fermentation substrate for the more thermotolerant lactobacilli, leading to rapid souring.

GDL was much more suitable as a preservative. By combining the effects of an acidulant and a humectant, effective microbial control was seen (Fig. 3). The reduction in a_w to 0.93 moved the paté from the "highly perishable" (a_w>0.95 and pH>5.2) category to "perishable" (a_w>0.95 or pH>5.2) (19). In Australia, fresh beef to be vacuum packed must be below pH 5.8 to prevent rapid microbial spoilage (27).

However in the paté with 0.5% GDL the growth of yeasts was seen for the first time during this study. The reduced a_w and pH, coupled with the reduction in growth of the competing bacteria, may have allowed the yeasts to proliferate. Therefore, care in deciding the final a_w would be required to ensure bacterial spoilage was not simply replaced by spoilage by yeasts.

Overall, the major factors affecting the shelf life of vacuum-packaged pork liver paté were studied. The product usually spoiled due to souring or greening caused by lactic acid bacteria. Microbial control was best exercised by manipulation of the a_w, either by regulation of the water included in the mix or by the addition of a humectant and it also reduced the pH with consequent benefits in shelf-life extension.

GDL had no adverse effects on flavor or texture at the level used (0.5%), as assessed by a sensory analysis panel.

REFERENCES


