Shelf life of pork from five different quality classes

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Abstract

A total of 117 loins were selected on the cutting line at 24 h post-mortem to study the long term shelf life (35 days, 4 °C) of vacuum packaged pork from five different quality classes (PSE: pale, soft, exudative; PFN: pale, firm, non-exudative; RSE: red, soft, exudative; RFN: red, firm, non-exudative and DFD: dark, firm, dry). The microbial load at 0 d was not significantly different (P > 0.05) among the pork quality classes, indicating that the initial microflora was influenced by the dressing conditions at the plant, not by the meat quality class. But after 35 d of storage, total aerobic mesophilic and presumptive lactic acid bacteria counts were higher (P < 0.05) in DFD pork due to its higher ultimate pH. RSE was the second quality class most susceptible to spoilage, whereas PFN, RFN and PSE pork had similar microbial loads. Further research is needed to elucidate the causes of the shorter shelf life in RSE pork.

Keywords: microbial growth, pork quality, shelf life.
1. Introduction

Fresh pork has been traditionally classified into three quality categories according to measurements of colour, firmness and drip loss: PSE (pale, soft, exudative), RFN (reddish-pink, firm, non-exudative; normal pork) and DFD (dark, firm, dry). Even though these quality characteristics are interrelated, some independent variation has been observed among these quality attributes leading to inaccurate evaluation of pork quality (Warriss & Brown, 1987; van Laack, Kauffman, Sybesma, Smulders, Eikelenboom & Pinheiro, 1994). For a more reliable quality assessment, taking into account the variation in either colour or exudate, additional quality categories have been described including RSE (reddish-pink, firm, exudative) and PFN (pale, firm, non-exudative) pork (Cassens, Kauffman, Scherer & Meeker, 1992; Warner, 1994). In Canada in early 2000, the incidence of PSE and DFD was estimated at 13% and 10%, respectively (Murray, 2001), however, higher proportions of pork were either pale or soft and exudative (PFN and RSE), hence, intermediate in defect, and 5% was firmer and dryer than normal. Similar or higher proportions of RSE pork were also reported in other countries (US: 30%, Kauffmann, Cassens, Scherer & Meeker, 1992; The Netherlands: 13%, Eikelenboom, Faucitano & Hoving Bolink, 1995).

Meat contains sufficient low molecular weight compounds to sustain microbial growth up to $10^9$ cfu/g or cm$^2$, but several intrinsic and extrinsic factors (e.g., pH, anaerobic packaging, etc.) are likely to influence microbial growth rate and species prevalence (Greer, 1989). As reported in a number of studies (Rey, Kraft, Topel, Parrish et al., 1976; Knox, van Laack & Davidson, 2008; Holmer, McKeith, Boler, Dilger, Eggert, Petry et al., 2009), susceptibility to microbial growth is higher in pork with higher pH.
values (DFD) and lower in pork with lower pH values (PSE). The faster spoilage in DFD pork is promoted by a high ultimate pH but also by the lower content of glucose and glycolytic intermediates that force organisms to utilise amino acids. This leads to unpleasant odours and flavours, and, consequently, to early spoilage (Newton & Gill, 1981). However, the susceptibility to microbial spoilage of RSE and PFN pork has not yet been described. Therefore, the objective of this study was to elucidate differences in the long term shelf life of pork stored under vacuum and belonging to the five different quality classes described above.

2. Material and methods

2.1. Pork quality measurements

A total of 500 primal loins were randomly collected on the cutting line of a commercial abattoir during one production day per week for a total of 5 weeks. Primal loins were cut into commercial loins according to the Canadian Pork Buyer’s Manual (Canada Pork International, 1995) in preparation for the 24 h post-mortem pork quality evaluation. The following quality measurements were taken in the longissimus dorsi (LD) muscle at the ¾ last rib level. The ultimate pH (pHu) was measured with a pH meter (Oakton Instruments Model pH 100 Series, Nilis, IL) fitted with a Cole Parmer spear type electrode (Cole Palmer Instrument Company, Vernon Hills, IL) and an automatic temperature compensation probe. Light reflectance was evaluated using a Minolta Chroma Meter CR 300 (Minolta Ltd., Osaka, Japan) with a D65 light source and 0° viewing angle geometry according to the reflectance coordinates (CIE L*, a*, b*) after
exposing the muscle surface to ambient air for 30 min ("blooming time"). Drip loss was evaluated using the filter paper wetness (FPW) test as described by Kauffman, Eikelenboom, van der Wal, Merkus and Zaar (1986). Briefly, filter paper (Whatman PK100, VWR International Co., Mont Royal, Canada) was placed on the LD cut surface after 15 min of air exposure and weighed using an analytical scale (Sartorius model 1419MP8, Fisher Scientific, Ottawa, Canada) after 3 s of fluid accumulation on the paper. Pork quality class was assigned according to parameters defined in Table 1.

2.2 Muscle sampling and microbial analysis

A sub-sample of the loins (117) evaluated for pork quality were selected in order to have 25 loins in each pork quality category for the microbiological study, except for the PFN class that contained the only 17 loins available. Loins were deboned and two adjacent LD muscle chops (6 cm long) were sampled at the eye of the loin. Sterile gloves were worn at all times during the microbial sampling. The first chop was taken from the extremity and was swabbed immediately at the newly cut section (day 0), while the second one was vacuum-packed at the plant and stored for 35 days at 4 °C. All samples were kept on ice for transportation to the AAFC pork quality laboratory in Sherbrooke (QC). Each LD muscle chop was swabbed at the loin eye surface using sterile sponges kept in a sterile Whirl-pak™ sampling bag (#B0124E, Nasco, Fort Atkinson, WI) soaked with 10 ml of 0.1% peptone water for the determination of total aerobic mesophiles (TAM), coliforms, *Escherichia coli*, and presumptive lactic acid bacteria (LAB) counts. A volume of 15 ml of peptone water was added to each bag and samples were homogenized at high speed for 2 min using a Stomacher (Model 400, Seward Laboratory...
Systems Inc., Bohemia, NY). Appropriate serial dilutions of the homogenate were made in 0.1% peptone water and each dilution was plated in duplicate. TAM counts were performed on 3 M Petrifilm incubated at 35 °C for 48 h (MFHPB-33; Health Canada, 2001a). *E. coli* and coliform counts were performed using 3 M Petrifilm incubated at 35 °C for 24 h (MFHPB-33; Health Canada, 2001b). Presumptive LAB counts were performed on De Man, Rogosa and Sharp (MRS) agar incubated at 25 °C for 48 h in an anaerobic jar containing a disposable H₂ and CO₂ generator envelope No. 70304 (GasPaK®, BBL®; Saucier, Gendron & Gariépy, 2000).

2.3 Statistical analysis

For meat quality data, classes were compared by analysis of variance using the SAS software MIXED procedure with an all pair-wise test using a Tukey adjustment for multiple comparisons (SAS, 2002). Cell counts were log-transformed prior to analysis. Spearman correlation coefficients were calculated to establish the relationship between the microbial counts and the pork quality parameters.

3. Results and discussion

3.1 Meat quality traits variation among quality classes

Of the 500 loins evaluated, 21% were scored as PSE, 3% as PFN, 47% as RSE, 13% as RFN and 2% as DFD. The remaining loins (14%) could not be classified according to the quality criteria set for this study (Table 1). The proportions of PSE and RSE were higher than those previously reported for Canadian pork (Murray & Johnson,
and demonstrated that pork softness and exudation (PSE and RSE) are major problems for the pork industry.

Table 2 shows the comparisons of meat quality traits between pork quality classes based on measurements from the 117 loins selected for the microbial study only. As already reported (Warner, Kauffman, & Greaser, 1997; Van Laack & Kauffman, 1999; Lee, Norman, Gunasekaran, van Laack, Kim & Kauffman, 2000), the pH_u of PSE pork was lower than that of RSE (P < 0.01), RFN and DFD pork (P < 0.001). The pH_u of PSE pork was similar to that of PFN pork, which was also different from the pH_u of RFN (P < 0.05) and DFD (P < 0.001) pork. The pH_u PFN pork was also similar to RSE pork. These results differ from those reported by van Laack et al. (1994), who only found a difference in pH_u between PFN and DFD pork. As expected, higher (P < 0.001) L* values (paler colour) were found in PSE and PFN pork compared to the other quality classes (Table 2). As in a number of previous studies (van Laack et al., 1994; Warner, 1994; Warner et al., 1997), the L* value of RSE pork was similar to that of RFN pork. In other studies (van Laack & Kauffman, 1999; Lee et al., 2000), the differences in the L* values between these two classes were significant, but small (0.2 units). If the colour difference between PSE and RSE pork can be explained by the rate of pH decrease, which induces protein denaturation (van Lack & Kauffman, 1999), the colour variation between PFN and RSE loins is more difficult to explain since the pH_u values of these two classes is similar. This result confirms that protein denaturation or solubility, which is the basis for meat colour variation, is not different in PFN and RSE pork, as already reported by van Laack et al. (1994). Higher FPWs (higher drip loss) were found in PSE loins followed by RSE loins, whereas lower FPWs were found in DFD pork followed by RFN and PFN pork (Table 2).
This result confirms that RSE pork is a mild form of PSE pork. The difference in exudation between PSE and RSE pork may be explained by the higher post-mortem rate of pH decrease in PSE pork (van Laack & Kauffman, 1999) rather than by colour variation. Note that the correlation between colour and exudation is commonly rather low ($r=0.30-0.50$; van Laack et al., 1994; Huff-Lonergan, Baas, Malek, Dekkers, Prusa & Rothshild, 2002; Correa, Méthot & Faucitano, 2007). According to van Laack et al. (1994), only one-third of the variation in drip loss can be ascribed to variation in the $L^*$ value in pork meat. Even with a difference of almost 10 units, similar FPWs were measured for PFN and RFN pork, and for RFN and DFD pork. These results do not agree with those of Kauffman, Sybesma, Smulders, Eikelenboom, Engel et al. (1993), who reported significant differences in FPW between these pork quality classes.

3.2 Microbial analysis among quality classes

Microbial analysis of the refrigerated (4 °C) pork stored under vacuum was performed at days 0 and 35 for the total aerobic mesophilic, presumptive LAB, coliforms and $E.\ coli$ counts. Throughout the experiment, all $E.\ coli$ counts remained below detection level (1.1 log cfu/per loin eye of 47 cm$^2$). At day 0, coliforms were only detected in low number on no more than eight samples out of 25 per class. Counts varied from 1.10 to 2.18 log cfu/per loin eye of 47 cm$^2$. The TAM and presumptive LAB ranged from 0.48 to 0.66 and 0.54 to 0.56 log cfu/cm$^2$, respectively. Initial cell counts among the different pork qualities were not significantly different ($P > 0.05$), indicating that all classes started their storage life with a similar microbial profile (Table 3). Hence, the initial contamination is not related to the meat quality class but rather to the carcass.
dressing conditions at the plant. The same observation was also obtained by Knox et al. (2008) for aerobic, psychrotrophic, *Enterobacteriacea* and LAB plate counts over a pH range of 5.5-6.5. These authors, however, assigned pork groups to ranges of pH not pork quality characteristics.

At day 35, coliforms were still detected in relatively low numbers on no more than six samples out of 25 per class. Counts varied from 1.10 to 2.18 log cfu/per loin eye of 47 cm² and were not significantly different among pork quality classes (P > 0.05). At 35 d, TAM counts increased, from below 1 log unit (0.48-0.66 cfu/cm²) at day 0, to 5.46, 3.89, 3.08, 2.96 and 2.64 log cfu/cm² (SEM = 0.38) for the DFD, RSE, RFN, PFN and PSE pork, respectively (Figure 1). Similarly, the presumptive LAB counts increased, again, from below 1 log unit (0.54-0.56 log cfu/cm²) at day 0, to 5.69, 4.65, 3.94, 3.69 and 3.92 log cfu/cm² (SEM = 0.45) for the DFD, RSE, RFN, PFN and PSE pork, respectively, at day 35 (Figure 1). Lactic acid bacteria are known to exert a competitive exclusion effect on less desirable organisms such as coliforms (Dainty & Mackey, 1992). The maintenance of low coliform counts and the increase in presumptive LAB during storage indicated that the anaerobic conditions created by packaging under vacuum induced the proper microbial ecology shift in favour of the LAB (Dainty & Mackey, 1992). A significant interaction (P = 0.0002) between the meat quality classes and the type of microorganisms tested (TAM and presumptive LAB) was observed. The presumptive LAB counts were less dependent on the pork quality class than TAM (Figure 1). When TAM and presumptive LAB counts were compared per meat quality class, TAM counts were significantly lower than presumptive LAB counts for PSE pork (P < 0.0001) but not for DFD and PFN pork (P > 0.05; Figure 1). These results suggested
that PSE pork was more favourable for establishing a desirable LAB microflora. For RFN and RSE pork, TAM counts tended to be lower than presumptive LAB counts at 35 days of storage ($P = 0.07$ and $0.09$, respectively; Figure 1). These differences might have been greater if the pork had been stored for a longer period of time.

The analysis of variance revealed a significant interaction between the day of sampling and the pork quality class for the TAM counts ($P < 0.001$). TAM and presumptive LAB counts increased significantly from day 0 to day 35 ($P < 0.001$). The DFD pork had the highest TAM counts and was significantly different from the four other pork quality classes ($P < 0.01$; Table 3), as was to be expected because of its higher pH$_u$ (Newton & Gill, 1981; Table 2). The PSE and RSE pork TAM counts were also significantly different at 35 days of storage ($P < 0.05$; Figure 1). The higher susceptibility to spoilage of RSE pork compared to RFN, PFN and PSE pork is further expressed by the number of samples that reached the threshold limit of log 6 cfu/g or cm$^2$ for TAM. At a microbial load of log 7 cfu/g or cm$^2$, spoilage is evident and meat is rejected without further analysis (Knox et al., 2008). After 35 days of storage under vacuum packaging, five DFD pork samples reached the log 6 cfu/cm$^2$ threshold limit for TAM compared to three for RSE meat samples. No sample reached that limit for the other remaining pork quality classes. For the presumptive LAB enumerated on MRS agar under anaerobic conditions, DFD pork counts were higher than PFN ($P < 0.01$), PSE ($P < 0.01$) and RFN ($P < 0.05$) pork counts but they were similar to RSE pork counts ($P > 0.05$). No other differences were observed when each of the pork quality classes was compared to one another (Table 3).
The TAM and presumptive LAB counts were significantly correlated with the pH\textsubscript{u} and \textit{L}* values ($P < 0.001$), and TAM counts were significantly correlated with the FPW ($P < 0.01$; Table 4). Even when the high pH of DFD pork were not included in the analysis, the pH\textsubscript{u} correlations for TAM and presumptive LAB remained significant ($P = 0.02$ and 0.03, respectively). It is known that the growth of meat microflora is influenced by post-mortem pH\textsubscript{u} variation (Knox et al., 2008). The higher pH\textsubscript{u} value of DFD pork is less growth restrictive, whereas the low pH\textsubscript{u} value of PSE pork represses microbial growth (Newton & Gill, 1981). Holmer et al. (2009) indicated that 87% of the variation in aerobic plate counts could be explained by pH\textsubscript{u} variation. In this study, the pH\textsubscript{u} value of DFD pork differed by 0.5 unit from that of RFN pork and there was only a 0.19 unit difference in pH\textsubscript{u} value among the four other classes, suggesting that pH\textsubscript{u} alone cannot explain the microbial count variation between RFN, RSE, PFN and PSE pork.

Besides being attributed to higher pH\textsubscript{u} values, early spoilage in DFD pork has also been associated with low glycogen and glucose muscle reserves, leading to microbial utilisation of amino acids as a carbon source (Newton & Gill, 1981). Glucose and glucose-6-phosphate are the preferred substrates for microbial growth but, once these substrates are exhausted, growth of bacteria on amino acids produces spoilage odours (Newton and Gill, 1981; Greer, 1988). It has been established that low molecular weight compounds used for growth are present in sufficient quantity in meat exudates to support growth up to log 9 cfu/g or cm\textsuperscript{2} without the contribution of proteolysis and lipolysis (Greer, 1988 and 1989). A possible explanation for the higher predisposition of RSE pork to spoilage is the presence of readily metabolised compounds such as glucose and glucose-6-phosphate as expressed in the glycolytic potential (GP) of the muscle. Van
Laack and Kaufman (1999) reported significantly higher ($P < 0.01$) GP in PSE pork (163 ± 5 µmol lactate/g) compared to RSE (137 ± 4 µmol lactate/g) and RFN (110 ± 6 µmol lactate/g) pork, with the GP of RSE pork being higher ($P < 0.01$) than that of RFN pork. These results may indicate that microbial growth is promoted in RSE pork due to a greater availability of nutrients, such as glycogen, glucose, and glucose-6-phosphate, which are components of the muscle GP.

These results suggest that further research is needed on the variations in exudate composition, along with the rate of glycogen breakdown (glycogenolysis) in relation to the other physico-chemical factors (pH, colour, drip loss, etc.) for PSE, RSE, RFN and PFN pork. More studies will be needed to clearly establish the influence, contribution and relationship of each of these factors on the microbial shelf life and spoilage of pork.

4. Conclusion

The high incidence of PSE and RSE pork found in this study means that the production of soft and exudative pork is still an unresolved problem for the pork industry. This study also confirms that RSE and PFN pork are as exudative and as pale, respectively, as PSE pork, which confirms their definition as milder forms of PSE pork. At 24 h post-mortem, microbial loads for $E. \text{coli}$, coliforms, TAM and presumptive LAB on freshly cut loin surfaces was not significantly different among the pork quality classes, indicating that the initial microflora is influenced by the dressing conditions at the plant rather than the meat quality. During storage, however, the characteristics of the meat greatly influence its shelf life. The poor keeping quality of DFD meat is well established and is confirmed in this study. RSE pork is the second quality class most susceptible to
spoilage, whereas PFN, RFN and PSE pork had similar microbial loads. Microbial
growth is multifactorial and the shelf life of meat varies according to the combined effect
of initial microflora, temperature, type (glucose vs. amino acid) and concentration of
nutrients, meat pH and the gas composition of the head space in the packaging material,
to name only a few. Further research is needed to better understand the variation of shelf
life among pork quality classes to allow better control of commercial pork quality.
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References


Table 1.
Pork quality classification including pH_u, color brightness (L* value) and filter paper wetness (FPW)^a

<table>
<thead>
<tr>
<th>Quality class^b</th>
<th>pH_u</th>
<th>L* value</th>
<th>FPW^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSE</td>
<td>&lt; 6.0</td>
<td>&gt; 50</td>
<td>≥ 80</td>
</tr>
<tr>
<td>PFN</td>
<td>&lt; 6.0</td>
<td>&gt; 50</td>
<td>&lt; 80</td>
</tr>
<tr>
<td>RSE</td>
<td>&lt; 6.0</td>
<td>43-48</td>
<td>≥ 80</td>
</tr>
<tr>
<td>RFN</td>
<td>&lt; 6.0</td>
<td>43-48</td>
<td>&lt; 80</td>
</tr>
<tr>
<td>DFD</td>
<td>≥ 6.0</td>
<td>&lt; 42</td>
<td>&lt; 40</td>
</tr>
</tbody>
</table>

^aModified from Warner (1994).
^bPSE (pale, soft, exudative); PFN (pale, firm, non-exudative); RSE (red, soft, exudative); RFN (red, firm, non-exudative); DFD (dark, firm, dry).
^cFPW = Filter paper wetness according to Kauffman et al. (1986) and the guidelines of the National Pork Board (NPB, 2000).
Table 2.
Meat quality measurements on the 117 loins selected for each quality class

<table>
<thead>
<tr>
<th>Quality class</th>
<th>pH</th>
<th>L*</th>
<th>FPW mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSE</td>
<td>5.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>53.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PFN</td>
<td>5.58&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>52.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>45.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>RSE</td>
<td>5.67&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>46.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>99.84&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>RFN</td>
<td>5.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.16&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>DFD</td>
<td>6.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>24.60&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>SEM</sup> = standard error of the mean

<sup>Means within a row followed by different letter are significantly different (<i>P</i> < 0.05).</sup>

<sup>FPW</sup> = Filter paper wetness according to Kauffman et al. (1986) and the guidelines of the National Pork Board (NPB, 2000).
Table 3.
Different P values between pork quality classes at day 0 and 35 on TAM and MRS counts (log cfu/cm²)

<table>
<thead>
<tr>
<th>DAY</th>
<th>TAM</th>
<th>LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DFD</td>
<td>DFD</td>
</tr>
<tr>
<td></td>
<td>DFD</td>
<td>PFN</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>35</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
* *, **, *** $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; NS = not significant.

TAM = Total Aerobic Mesophilic; LAB = Lactic Acid Bacteria.
Table 4.
Correlation coefficients \((r)\) between pork quality traits and microbial counts at 35 d

<table>
<thead>
<tr>
<th>Counts</th>
<th>pH_a</th>
<th>L*</th>
<th>FPW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliforms</td>
<td>0.13</td>
<td>0.05</td>
<td>-0.05</td>
</tr>
<tr>
<td>TAM(^a)</td>
<td>0.48***</td>
<td>0.49***</td>
<td>-0.30**</td>
</tr>
<tr>
<td>LAB(^a)</td>
<td>0.37***</td>
<td>0.39***</td>
<td>-0.12</td>
</tr>
</tbody>
</table>

*, **, *** \(P < 0.05\), \(P < 0.01\) and \(P < 0.001\), respectively; NS = not significant.

\(^a\) TAM = Total Aerobic Mesophilic; LAB = Lactic Acid Bacteria.
Figure 1.
Total aerobic mesophilic (TAM) and presumptive lactic acid bacteria (LAB) counts on DFD (dark, firm, dry), RSE (red, soft, exudative), RFN (red, firm, non-exudative), PFN (pale, firm, non-exudative) PSE (pale, soft, exudative) pork after 35 days of storage at 4°C under vacuum. Bar represents standard error of the mean. Within the same microbial type, pork classes with different subscripts are significantly different ($P < 0.05$).