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 (pH_u) 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 0º 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 H_2 and CO_2 generator envelope No. 70304 (Gas PaK®, 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,

136 1998; Murray, 2001) and demonstrated that pork softness and exudation (PSE and RSE)
137 are major problems for the pork industry.

138 Table 2 shows the comparisons of meat quality traits between pork quality classes
139 based on measurements from the 117 loins selected for the microbial study only. As
140 already reported (Warner, Kauffman, & Greaser, 1997; Van Laack & Kauffman, 1999;
141 Lee, Norman, Gunasekaran, van Laack, Kim & Kauffman, 2000), the pH_u of PSE pork
142 was lower than that of RSE ($P < 0.01$), RFN and DFD pork ($P < 0.001$). The pH_u of PSE
143 pork was similar to that of PFN pork, which was also different from the pH_u of RFN ($P <$
144 0.05) and DFD ($P < 0.001$) pork. The pH_u PFN pork was also similar to RSE pork. These
145 results differ from those reported by van Laack et al. (1994), who only found a difference
146 in pH_u between PFN and DFD pork. As expected, higher ($P < 0.001$) L^* values (paler
147 colour) were found in PSE and PFN pork compared to the other quality classes (Table 2).
148 As in a number of previous studies (van Laack et al., 1994; Warner, 1994; Warner et al.,
149 1997), the L^* value of RSE pork was similar to that of RFN pork. In other studies (van
150 Laack & Kauffman, 1999; Lee et al., 2000), the differences in the L^* values between
151 these two classes were significant, but small (0.2 units). If the colour difference between
152 PSE and RSE pork can be explained by the rate of pH decrease, which induces protein
153 denaturation (van Lack & Kauffman, 1999), the colour variation between PFN and RSE
154 loins is more difficult to explain since the pH_u values of these two classes is similar. This
155 result confirms that protein denaturation or solubility, which is the basis for meat colour
156 variation, is not different in PFN and RSE pork, as already reported by van Laack et al.
157 (1994). Higher FPWs (higher drip loss) were found in PSE loins followed by RSE loins,
158 whereas lower FPWs were found in DFD pork followed by RFN and PFN pork (Table 2).

159 This result confirms that RSE pork is a mild form of PSE pork. The difference in
160 exudation between PSE and RSE pork may be explained by the higher post-mortem rate
161 of pH decrease in PSE pork (van Laack & Kauffman, 1999) rather than by colour
162 variation. Note that the correlation between colour and exudation is commonly rather low
163 (r=0.30-0.50; van Laack et al., 1994; Huff-Lonergan, Baas, Malek, Dekkers, Prusa &
164 Rothshild, 2002; Correa, Méthot & Faucitano, 2007). According to van Laack et al.
165 (1994), only one-third of the variation in drip loss can be ascribed to variation in the L^*
166 value in pork meat. Even with a difference of almost 10 units, similar FPWs were
167 measured for PFN and RFN pork, and for RFN and DFD pork. These results do not agree
168 with those of Kauffman, Sybesma, Smulders, Eikelenboom, Engel et al. (1993), who
169 reported significant differences in FPW between these pork quality classes.

170

171 *3.2 Microbial analysis among quality classes*

172 Microbial analysis of the refrigerated (4 °C) pork stored under vacuum was
173 performed at days 0 and 35 for the total aerobic mesophilic, presumptive LAB, coliforms
174 and *E. coli* counts. Throughout the experiment, all *E. coli* counts remained below
175 detection level (1.1 log cfu/per loin eye of 47 cm^2). At day 0, coliforms were only
176 detected in low number on no more than eight samples out of 25 per class. Counts varied
177 from 1.10 to 2.18 log cfu/per loin eye of 47 cm^2. The TAM and presumptive LAB ranged
178 from 0.48 to 0.66 and 0.54 to 0.56 log cfu/cm^2, respectively. Initial cell counts among the
179 different pork qualities were not significantly different ($P > 0.05$), indicating that all
180 classes started their storage life with a similar microbial profile (Table 3). Hence, the
181 initial contamination is not related to the meat quality class but rather to the carcass

182 dressing conditions at the plant. The same observation was also obtained by Knox et al.
183 (2008) for aerobic, psychrotrophic, *Enterobacteriacea* and LAB plate counts over a pH_u
184 range of 5.5-6.5. These authors, however, assigned pork groups to ranges of pH_u, not
185 pork quality characteristics.

186 At day 35, coliforms were still detected in relatively low numbers on no more
187 than six samples out of 25 per class. Counts varied from 1.10 to 2.18 log cfu/per loin eye
188 of 47 cm^2 and were not significantly different among pork quality classes ($P > 0.05$). At
189 35 d, TAM counts increased, from below 1 log unit (0.48-0.66 cfu/cm^2) at day 0, to 5.46,
190 3.89, 3.08, 2.96 and 2.64 log cfu/cm^2 (SEM = 0.38) for the DFD, RSE, RFN, PFN and
191 PSE pork, respectively (Figure 1). Similarly, the presumptive LAB counts increased,
192 again, from below 1 log unit (0.54-0.56 log cfu/cm^2) at day 0, to 5.69, 4.65, 3.94, 3.69
193 and 3.92 log cfu/cm^2 (SEM = 0.45) for the DFD, RSE, RFN, PFN and PSE pork,
194 respectively, at day 35 (Figure 1). Lactic acid bacteria are known to exert a competitive
195 exclusion effect on less desirable organisms such as coliforms (Dainty & Mackey, 1992).
196 The maintenance of low coliform counts and the increase in presumptive LAB during
197 storage indicated that the anaerobic conditions created by packaging under vacuum
198 induced the proper microbial ecology shift in favour of the LAB (Dainty & Mackey,
199 1992). A significant interaction ($P = 0.0002$) between the meat quality classes and the
200 type of microorganisms tested (TAM and presumptive LAB) was observed. The
201 presumptive LAB counts were less dependent on the pork quality class than TAM (Figure
202 1). When TAM and presumptive LAB counts were compared per meat quality class,
203 TAM counts were significantly lower than presumptive LAB counts for PSE pork
204 ($P < 0.0001$) but not for DFD and PFN pork ($P > 0.05$; Figure 1). These results suggested

205 that PSE pork was more favourable for establishing a desirable LAB microflora. For RFN
206 and RSE pork, TAM counts tended to be lower than presumptive LAB counts at 35 days
207 of storage ($P = 0.07$ and 0.09, respectively; Figure 1). These differences might have been
208 greater if the pork had been stored for a longer period of time.

209 The analysis of variance revealed a significant interaction between the day of
210 sampling and the pork quality class for the TAM counts ($P < 0.001$). TAM and
211 presumptive LAB counts increased significantly from day 0 to day 35 ($P < 0.001$). The
212 DFD pork had the highest TAM counts and was significantly different from the four
213 other pork quality classes ($P < 0.01$; Table 3), as was to be expected because of its higher
214 pH_u (Newton & Gill, 1981; Table 2). The PSE and RSE pork TAM counts were also
215 significantly different at 35 days of storage ($P < 0.05$; Figure 1). The higher susceptibility
216 to spoilage of RSE pork compared to RFN, PFN and PSE pork is further expressed by the
217 number of samples that reached the threshold limit of log 6 cfu/g or cm^2 for TAM. At a
218 microbial load of log 7 cfu/g or cm^2, spoilage is evident and meat is rejected without
219 further analysis (Knox et al., 2008). After 35 days of storage under vacuum packaging,
220 five DFD pork samples reached the log 6 cfu/cm^2 threshold limit for TAM compared to
221 three for RSE meat samples. No sample reached that limit for the other remaining pork
222 quality classes. For the presumptive LAB enumerated on MRS agar under anaerobic
223 conditions, DFD pork counts were higher than PFN ($P < 0.01$), PSE ($P < 0.01$) and RFN
224 ($P < 0.05$) pork counts but they were similar to RSE pork counts ($P > 0.05$). No other
225 differences were observed when each of the pork quality classes was compared to one
226 another (Table 3).

227 The TAM and presumptive LAB counts were significantly correlated with the pH_u
228 and L^* values ($P < 0.001$), and TAM counts were significantly correlated with the FPW
229 ($P < 0.01$; Table 4). Even when the high pH of DFD pork were not included in the
230 analysis, the pH_u correlations for TAM and presumptive LAB remained significant ($P =$
231 0.02 and 0.03, respectively). It is known that the growth of meat microflora is influenced
232 by post-mortem pH_u variation (Knox et al., 2008). The higher pH_u value of DFD pork is
233 less growth restrictive, whereas the low pH_u value of PSE pork represses microbial
234 growth (Newton & Gill, 1981). Holmer et al. (2009) indicated that 87% of the variation
235 in aerobic plate counts could be explained by pH_u variation. In this study, the pH_u value
236 of DFD pork differed by 0.5 unit from that of RFN pork and there was only a 0.19 unit
237 difference in pH_u value among the four other classes, suggesting that pH_u alone cannot
238 explain the microbial count variation between RFN, RSE, PFN and PSE pork.

239 Besides being attributed to higher pH_u values, early spoilage in DFD pork has also
240 been associated with low glycogen and glucose muscle reserves, leading to microbial
241 utilisation of amino acids as a carbon source (Newton & Gill, 1981). Glucose and
242 glucose-6-phosphate are the preferred substrates for microbial growth but, once these
243 substrates are exhausted, growth of bacteria on amino acids produces spoilage odours
244 (Newton and Gill, 1981; Greer, 1988). It has been established that low molecular weight
245 compounds used for growth are present in sufficient quantity in meat exudates to support
246 growth up to log 9 cfu/g or cm^2 without the contribution of proteolysis and lipolysis
247 (Greer, 1988 and 1989). A possible explanation for the higher predisposition of RSE pork
248 to spoilage is the presence of readily metabolised compounds such as glucose and
249 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. 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_u 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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Table 1.
Pork quality classification including pH_u, color brightness (L* value) and filter paper wetness (FPW)[a]

Quality class[b]	pH_u	L* value	FPW[c]
			mg
PSE	< 6.0	> 50	≥ 80
PFN	< 6.0	> 50	< 80
RSE	< 6.0	43-48	≥ 80
RFN	< 6.0	43 -48	< 80
DFD	≥ 6.0	< 42	< 40

[a]Modified from Warner (1994).
[b]PSE (pale, soft, exudative); PFN (pale, firm, non-exudative); RSE (red, soft, exudative); RFN (red, firm, non-exudative); DFD (dark, firm, dry).
[c]FPW = 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[w]

Quality class[x]	pH_u	L*	FPW[y] mg
PSE	5.52[d]	53.41[a]	125.10[a]
PFN	5.58[cd]	52.54[ab]	45.12[c]
RSE	5.67[bc]	46.43[b]	99.84[b]
RFN	5.71[b]	45.92[b]	33.16[cd]
DFD	6.21[a]	40.54[c]	24.60[d]
SEM[z]	0.03	0.47	3.92

[w]Means within a row followed by different letter are significantly different ($P < 0.05$).

[x]PSE (pale, soft, exudative); PFN (pale, firm, non-exudative); RSE (red, soft, exudative); RFN (red, firm, non-exudative); DFD (dark, firm, dry).

[w]FPW = Filter paper wetness according to Kauffman et al. (1986) and the guidelines of the National Pork Board (NPB, 2000).

[z]**SEM** = standard error of the mean

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Table 3.

Different P values between pork quality classes at day 0 and 35 on TAM and MRS counts (log cfu/cm^2)

DAY	DFD vs PFN	DFD vs PSE	DFD vs RFN	DFD vs RSE	PFN vs PSE	PFN vs RFN	PFN vs RSE	PSE vs RFN	PSE vs RSE	RFN vs RSE
TAM[a]										
0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
35	***	***	***	**	NS	NS	NS	NS	*	NS
LAB[a]										
0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
35	**	**	*	NS	NS	NS	NS	NS	NS	NS

*, **, *** $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively; NS = not significant.

[a] TAM = Total Aerobic Mesophilic; LAB = Lactic Acid Bacteria.

Table 4.
Correlation coefficients (*r*) between pork quality traits and microbial counts at 35 d

Counts	pH_u	L*	FPW
Coliforms	0.13	0.05	-0.05
TAM[a]	0.48***	-0.49***	-0.30**
LAB[a]	0.37***	-0.39***	-0.12

*, ** , *** $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively; NS = not significant.
[a] TAM = Total Aerobic Mesophilic; LAB = Lactic Acid Bacteria.

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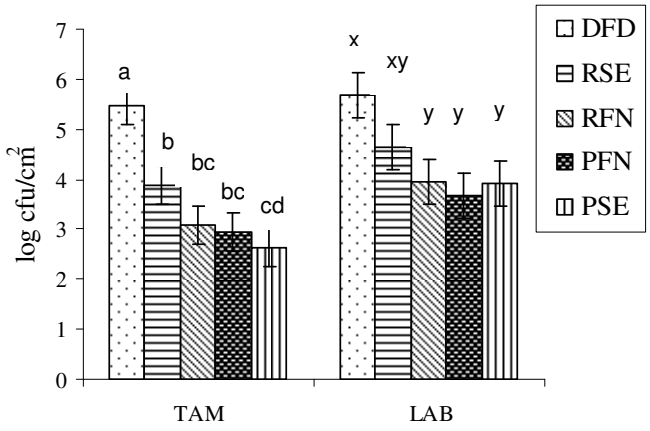

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395 Figure 1.
396 Total aerobic mesophilic (TAM) and presumptive lactic
397 acid bacteria (LAB) counts on DFD (dark, firm, dry), RSE
398 (red, soft, exudative), RFN (red, firm, non-exudative), PFN
399 (pale, firm, non-exudative) PSE (pale, soft, exudative) pork
400 after 35 days of storage at 4°C under vacuum. Bar
401 represents standard error of the mean. Within the same
402 microbial type, pork classes with different subscripts are
403 significantly different ($P < 0.05$).
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