

Changes in Hygienic Quality of Vacuum-packed Pork During Storage

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Abstract

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Hygienic quality of boned vacuum-packed pork was monitored during storage by sensory, chemical, and microbiological methods. Pork was vacuum-packed into polyamide/polyethylene (PA/PE) foil 72 h after slaughter. Initial temperature did not exceed 9° C. The meat was stored at $2.5 \pm 0.5^\circ\text{C}$ in the dark for 35 days and samples for laboratory examinations were collected at days 14, 21, 28, and 35 to assess appearance, colour, odour, and characteristics of released meat juice, to determine pH and concentrations of ammonia and D- and L-forms of lactic acid, and to enumerate lactacidogenic bacteria. *Lactobacillus curvatus* and *Lactobacillus xylosus* were identified as the causative agents of meat spoilage using the API system. The results were subjected to correlation analyses. The shelf life of boned vacuum-packed pork varied between 21 and 28 days, depending on initial pH. The results of correlation analyses indicated that only ripe pork with the optimum pH = 5.8 to 5.95 is suitable for vacuum-packing. In addition to the lactacidogenic bacteria count (LAC), the shelf life can be estimated also from ammonia concentration (LAC/ammonia $r = 0.9584$, $P < 0.05$), pH (LAC/pH $r = -0.9317$, $P < 0.05$) and the concentration of D-lactate (LAC/D-lactate $r = 0.9867$, $P < 0.01$).

Pork, vacuum packing, lactobacilli, ammonia, pH, lactic acid

Meat spoilage is a complex process in which microorganisms present in the muscular tissue due to secondary contamination during processing are involved, and which depends on ambient temperature. The storage of meat is associated with changes in quality resulting from microbial activity, shift of pH, production of toxic substances, and aberrant odour (Huis 1996). Most prone to spoilage are foods with a high protein content, such as meat, poultry, fish, and milk, which have a high dietetic value, neutral, or lightly acid pH, and a high water content providing favourable conditions for bacterial growth (Huis 1996). Bauer (1995) demonstrated that enzymes of the muscular tissue and bacterial enzymes are involved in chemical changes (production of biogenic amines, changes in colour and fat) occurring during storage. The most important factors influencing the development and growth of spoilage microorganisms on raw meat in cold storage include the initial relative number of psychrotrophic flora and its growth dynamics at low temperatures. Qualitative and quantitative composition of the microbial population responsible for meat spoilage depend on pH, water activity, storage atmosphere, and among-species relations (Gould 1995). Dominant in the microbial population of vacuum-packed meat are lactacidogenic bacteria which participate in the development of an unpleasant odour. Dainty (1996) detected indol and hydrogen sulphide by gas chromatography and identified them as the cause of this sensory defect. The microbial activity results in the production of typical metabolites including invariably the D and L forms of lactic acid. D-lactate is produced by bacteria exclusively and is regarded as an indicator of the growth of specific microbial flora (Sinell and Luke 1979; Schneider et al. 1983). Further metabolites produced by the

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indicator microbial flora include biogenic amines (tyramine, cadaverine, putrescine, and others) and a number of further compounds, such as ethanol, acetic and lactic acids, which are responsible for sensory changes developing in vacuum-packed fresh or cooled meat. Borch (1996) identified *Lactobacillus* spp. as the major agent causing spoilage of vacuum-packed meat manifested by changes in colour, odour, and taste, and by gas production.

Microbiological examination of meat in the Czech Republic is regulated by the Notice No. 294/97 Coll. as amended by the Notice No. 91/99 Coll. These documents list food commodities with indication of tolerated counts of selected microbial groups. Foods in which the counts are not exceeded are regarded as safe and can be marketed.

Microbiological examination of foods is focused not only on causative agents of human diseases, but also on microorganisms causing spoilage and affecting shelf life. Meat is exposed to many environmental factors which are favourable for the survival and propagation of the contaminating microbial flora.

Meat safety can be assessed by testing for indicator (marker) germs. Suitable for this purpose are microbial species or groups which propagate and can be identified readily and which, by manifestation of life processes, yield the required information. One of the typical indicators are coliform bacteria. Currently, tests for the presence and number of coliforms are used to assess the rate of total contamination of foods and the hygienic standard of food manufacture. The assessment must be based on the rate of contamination. Indicator values are specific for individual commodities and depend on input materials and technological procedures. Increased counts of coliform bacteria are indicative of failures in sanitation and very high counts can be dangerous to human health. This does not mean, however, that all foods that are free from coliform bacteria are safe. Relevant for the assessment are almost exclusively increased counts. Coliform bacteria are regarded as negative indicators, the presence of which is undesirable. On the other hand the demonstration of positive indicators can be used as a sign of good quality only exceptionally.

A special group of indicators are fungi. Their demonstration always indicates a poor quality of raw materials or inappropriate storage conditions.

Most papers dealing with effects of packaging are focused on pathogenic bacteria that can induce alimentary diseases (Lát 1991). Also in this case, factors relevant to shelf life and safety include temperature, pH, water activity, redox potential and packaging technology (Roberts 1980).

Materials and Methods

Thirty boned pork loins were selected randomly in the carving shop of a meat processing plant and five slices of each of them were vacuum-packed and stored. Initial a_w values were determined and ammonia and D- and L-lactic acid (lactate) concentrations were determined, pH was measured, and lactacidogenic bacteria were enumerated in samples opened at days 14, 21, 28, and 35. Assessment of sensory characteristics of cold samples was done on these occasions.

Pork

Boned pork loin was cooled to 7 °C within 48 h after slaughter and sliced samples were vacuum-packed 72 h after slaughter. The temperature did not increase above 9 °C during this period.

Packaging material

PA/PE foil, permeability for oxygen 50 mL mL⁻² d⁻¹, for carbon dioxide 150 mL mL⁻² d⁻¹, for nitrogen 10 mL mL⁻² d⁻¹, weight 87.0 g/m², drawing quality longitudinal 45%, transversal 35%.

Storage conditions

The samples were stored in the dark at 2.5 ± 0.5 °C. The temperature was recorded twice a day with the digital thermometer AMA-digid Precision.

Chemical analyses

Ammonia concentration was determined by the Conway method (microdiffusion followed by titration with sulphuric acid after displacement of ammonia into boric acid with potassium carbonate) (Helclová et al. 1990a). pH was measured using the stab glass electrode ORION RESEARCH Ross.

D- and L-lactic acids were determined by an LDH-catalysed reaction in which the acid is oxidised by coenzyme NAD⁺ to pyruvate and NADH. The resulting amount of NADH is equivalent to the content of lactic acid. The concentration of NADH is measured photometrically at 340 nm (Boehringer Mannheim 1995).

The a_w value was measured using the NOVASINA apparatus with an electrolytic sensor at 25 °C. The instructions of the DEFENCOR company were observed.

Microbiological examination

Lactacidogenic bacteria were enumerated according to the ISO Standard 13 721 (56 0125) using MRS agar as the culture medium and identified using the API 50 CH System (BioMérieux). The identity was confirmed by 49 biochemical tests for which five typical colonies (white, glossy, 0.5 to 1 mm in diameter, formed by ovoid immobile rods occurring separately or in pairs) were selected from each dish.

Sensory assessment. The samples were assessed in terms of colour, odour, and appearance of released meat juice using three descriptors for each characteristic with the following verbal definitions:

- Colour: 1 - fresh, light pink
2 - moderately altered
3 - markedly altered, greyish to greenish
- Odour 1 - typical of meat
2 - agreeable, milky
3 - repulsive, typical of beginning spoilage
- Meat juice 1 - no released juice
2 - small amount, lightly opalescent
3 - markedly turbid, dense to sticky

Statistical processing

Means \pm SD were calculated and correlation was tested using the STAT Plus software (Matoušková et al. 1992).

Results

Water activity was determined at the beginning of the storage period only. The results varied between 0.962 and 0.990. This value was no more considered after forming of groups defined by initial pH.

The results of sensory assessment are presented in Tables 1 and 2. Initial samples examined immediately after vacuum packing showed light pink colour, typical odour of meat, and only negligible amount of released juice. Sensory changes observed between days 21 and 28 included an increased amount of turbid meat juice, marked milky odour of meat, and change in colour to light grey. Signs of beginning spoilage were apparent at day 35 from unpleasant odour, greyish-green colour and milky appearance of released meat juice. At this stage, the meat was no more consumable. Significant differences in the descriptors used in the sensory analyses were found only between days 28 and 35 ($P < 0.05$ for all the descriptors).

Table 1
Mean values of sensory descriptors during the storage period

Initial pH	Descriptor	Storage period (days)				
		1	14	21	28	35
5.20–5.30	Colour	1.0	1.0	2.0	2.5	3.0
	Odour	1.0	1.0	2.0	3.0	3.0
	Meat juice	1.5	2.0	2.5	3.0	3.0
5.45–5.66	Colour	1.0	1.0	1.5	1.5	3.0
	Odour	1.0	1.0	2.0	2.0	3.0
	Meat juice	1.5	1.5	1.5	2.0	3.0
5.71–5.80	Colour	1.0	1.0	1.5	2.0	3.0
	Odour	1.0	1.0	1.0	1.5	3.0
	Meat juice	1.5	1.5	1.5	2.0	3.0
5.92–6.16	Colour	1.0	1.0	2.0	2.0	3.0
	Odour	1.0	1.0	1.5	2.0	3.0
	Meat juice	1.5	1.5	2.0	2.0	3.0

Table 2
Sum of mean values of sensory descriptors during the storage period

Initial pH	Storage period (days)					Sum
	0	14	21	28	35	
5.20–5.30	3.5	4.0	6.5	8.5	9.0	31.5
5.45–5.66	3.5	3.5	5.0	5.5	8.0	25.5
5.71–5.80	3.5	3.5	4.0	5.5	9.0	25.5
5.90–6.16	3.5	3.5	5.5	6.0	9.0	27.5

Initial pH measured before vacuum packing was considered in the assessment of results of chemical and microbiological examinations. All the samples were divided into four groups in terms of this variable. Table 3 shows ammonia concentrations and pH (means \pm S.D.) as dependent on the length of the storage period. The corresponding data on dynamics of lactate concentrations and lactacidogenic bacteria counts (LBC) are represented in Figs 1 through 4.

Table 3
Changes of ammonia concentration and pH during the storage period (mean \pm SD)

Initial pH	Parameter	Storage period (days)				
		1	14	21	28	35
5.20–5.30	pH	5.25 \pm 0.05	5.57 \pm 0.51	5.59 \pm 0.51	5.66 \pm 0.1	5.62 \pm 0.11
	Ammonia*	16.09 \pm 0.0	20.17 \pm 0.0	22.31 \pm 0.02	29.12 \pm 6.0	30.94 \pm 20.00
5.45–5.66	pH	5.55 \pm 0.05	5.59 \pm 0.09	5.59 \pm 0.09	5.55 \pm 0.12	5.46 \pm 0.23
	Ammonia*	14.4 \pm 0.0	16.23 \pm 0.01	22.85 \pm 0.05	23.38 \pm 0.30	23.94 \pm 2.40
5.71–5.80	pH	5.75 \pm 0.02	5.81 \pm 0.07	5.75 \pm 0.02	5.67 \pm 0.03	5.60 \pm 0.11
	Ammonia*	13.45 \pm 0.0	16.25 \pm 0.02	18.42 \pm 0.04	20.16 \pm 0.52	27.33 \pm 1.80
5.92–6.16	pH	6.11 \pm 0.09	6.02 \pm 0.03	6.01 \pm 0.03	6.07 \pm 0.05	5.95 \pm 0.01
	Ammonia*	17.00 \pm 0.0	20.85 \pm 0.0	25.38 \pm 0.03	29.8 \pm 0.20	32.88 \pm 0.05

* (mg/kg)

Group 1 - initial pH 5.20 to 5.30

This group consisted of three samples. pH increased continuously from the initial 5.25 \pm 0.05 to reach 5.62 \pm 3.23 at the end of the storage period. As can be seen in Fig. 1, the changes in pH during the storage period were inconsiderable. Ammonia concentrations increased from the initial 16.06 \pm 0.15 mg/kg to the final 30.94 \pm 3.23 mg/kg. LBC varied considerably during the storage period. The initial zero count increased to values in the order of 10⁶ CFU/g. A marked increase in LBC was observed after day 28. Standard deviation was very low, as can be seen in Fig. 1. The concentration of D-lactate increased up to day 28 and remained unchanged up to the end of the storage period. On the other hand, the concentration of L-lactate decreased from the initial 10.12 \pm 0.32 g/kg up to day 28 and remained unchanged thereafter.

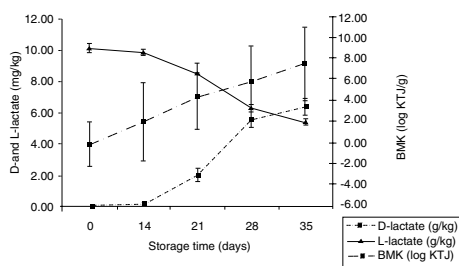


Fig. 1. Dependence of indices under study on storage time (musculature samples with initial pH 5.20-5.30)

Group 2 - initial pH 5.45 to 5.66

Laboratory tests were carried out in 19 samples of this group. Mean initial pH 5.55 \pm 0.05 remained unchanged during the first 28 days of storage and decreased to 5.46 \pm 0.23 by its

end at day 35. Ammonia concentration increased from the initial 14.40 ± 0.15 mg/kg to 22.85 ± 1.89 mg/kg at day 21 and to 23.94 ± 4.36 mg/kg at day 35. The range of standard deviation was rather wide. LBC increased from the initial zero value moderately up to day 21 and then more markedly up to day 35 when values in the order 10^6 CFU/g were found. The standard deviation range was narrow. The concentrations of the D- and L-lactic acid forms were similar to those found in Group 1 and standard deviation was negligible.

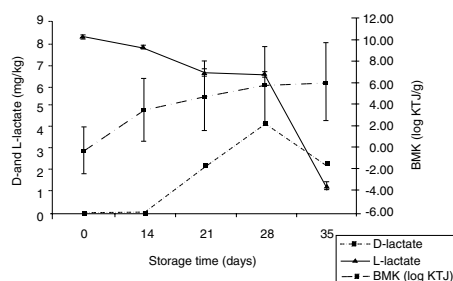


Fig. 2. Dependence of indices under study on storage time (musculature samples with initial pH 5.45-5.66)

Group 3 - initial pH 5.71 to 5.80

Laboratory tests were carried out in 5 samples of this group. Only negligible changes in pH were observed during the storage period. The initial value 5.75 ± 0.02 decreased to 5.60 ± 0.11 at its end. Ammonia concentrations increased from the initial 13.45 ± 0.47 mg/kg to 20.16 ± 0.71 mg/kg at day 28 and 27.33 ± 4.09 mg/kg at day 35.

LBC increased from the initial zero value to reach the order 10^6 CFU/g at day 35. The standard deviation range was rather narrow. Fig. 3 shows that a marked increase occurred after day 28. The concentration of L-lactate decreased from the initial 8.145 ± 0.355 g/kg to 7.53 ± 0.260 at day 35 and that of D-lactate increased during the same period from 0.02 ± 0.0075 g/kg to 2.48 ± 0.260 g/kg.

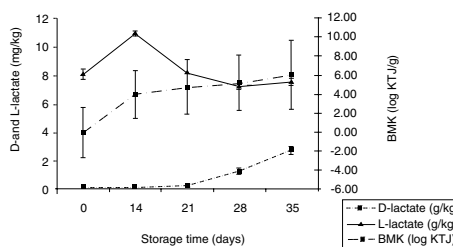


Fig. 3. Dependence of indices under study on storage time (musculature samples with initial pH 5.71-5.80)

Group 4 - initial pH 5.92 to 6.16

Laboratory tests were carried out in 3 samples of this group. Only this groups showed a gradual decrease of pH from 6.11 ± 0.09 at the beginning to 5.95 ± 0.01 at the end of the storage period. Ammonia concentration increased almost twofold during this period from 17.00 ± 6.87 mg/kg to 32.88 ± 4.79 mg/kg. Standard deviation for this concentration was the largest (Fig. 4). LBC increased from the initial zero to the order of 10^7 CFU/g. The concentration of L-lactate increased from the initial 7.37 ± 0.03 g/kg to 8.37 ± 0.19 g/kg at day 21 to decrease during the rest of the storage period to 4.65 ± 0.06 g/kg. The concentration of D-lactate increased from the initial zero to 2.75 ± 0.24 g/kg at the end of the storage period.

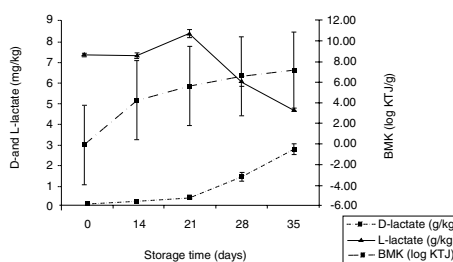


Fig. 4. Dependence of indices under study on storage time (musculature samples with initial pH 5.92-6.160)

Discussion

Values of water activity found in our experiments were comparable with those regarded as common for raw pork, which are optimal for the propagation of microbes of the genus *Lactobacillus* (Lát 1991; Topinková 1996).

Typical microbial flora responsible for anaerobic spoilage of meat is lactic acid producing bacteria (LAPB) of the genus *Lactobacillus* (Huise 1996). LAPB, including the genera *Lactobacillus* and *Leukonostoc*, predominate in meat stored under anaerobic conditions. Anaerobic decomposition of meat is a slow process setting in as soon as the bacterial population reaches 10^8 to 10^9 CFU/g. Shelf life can be shorter if the hydrogen sulphide-producing species *Lactobacillus sakei* prevails (Pipek 1995).

The lactobacillar population on meat usually includes the species *L. sakei*, *L. curvatus*, and *L. plantarum* (Hugas 1998). Gill (1983) identified *L. curvatus* and *L. sakei* as representatives of psychrotropic microbial flora on vacuum-packed meat.

Samelis (2000) identified *L. sakei* as the dominant species present on vacuum-packed pork and found a good correlation between the propagation of LAPB and relevant intrinsic factors including pH and water content in meat.

Fermentation of saccharides by lactobacilli results in the production of lactic acid associated with a decrease of pH and an odour change. LAPB are a component of the original microbial flora of meat that readily propagates on cooled and vacuum-packed meat. Their growth in red (beef and pork) vacuum-packed meat stored in cooling rooms inhibits the propagation of G- bacteria. The propagation of LAPB is accompanied by very moderate fermentation processes which, in the initial phase, do not induce alterations of sensory characteristics due to the low concentration of saccharides in and a high buffering capacity of meat. LAPB can, via various mechanisms, inhibit the propagation of pathogenic bacteria (Hugas 1998). Metabolites produced by LAPB that inhibit the growth of other bacteria include lactic and acetic acids, carbon dioxide, hydrogen peroxide, diacetyl, and bacteriocines.

Yost (2000) used PCR to identify LAPB strains isolated from vacuum-packed pork stored at 2 °C. Of the 70 isolates, 52 were identified as *L. sakei* and the remaining 18 as *Leukonostoc* spp.

Lactobacilli isolated within our experiments were identified as *L. xylosum* and *L. curvatus*. The latter ranks with bacteria producing the racemic form of lactic acid, while the former produces its L-form only. The amount of lactic acid produced in vacuum-packed meat depends primarily on the content of residual saccharides. Lactic acid can be further metabolised by *L. curvatus* which shows L-lactate oxidase activity. This enzyme uses oxygen as an electron acceptor giving rise to hydrogen peroxide as one of the factors inhibiting the growth of undesirable microbial flora (Kameník 1994).

Coefficients of correlation between LBC on the one side and the other variables under study on the other side were calculated for the four groups of samples differing in initial pH.

No significant correlation was found in Group 1 (pH 5.20 to 5.30). The respective coefficients were as follows: LBC/pH $r = 0.3917$; LBC/ammonia $r = 0.7816$; LBC/total lactate $r = -0.7177$; LBC/L-lactate $r = 0.8033$; LBC/D-lactate $r = -0.8248$.

Only one significant correlation between LBC/L-lactate ($r = -0.9754$, $P < 0.05$) was found in Group 2 (pH 5.45 to 5.65). The remaining coefficients were as follows: LBC/pH $r = -0.1084$; LBC/ammonia $r = 0.7257$; LBC/total lactate $r = -0.6960$; LBC/D-lactate $r = 0.5234$. Only the correlation coefficient of LBC/L-lactate was $r = -0.9754$ ($P < 0.05$).

Rather interesting were the correlations between LBC and the remaining variables in Group 3 (pH 5.71 to 5.80), which were expressed by the following coefficients: LBC/pH $r = -0.9317$, $P < 0.05$; LBC/ammonia $r = 0.9584$, $P < 0.05$; LBC/total lactate $r = 0.2395$; LBC/D-lactate $r = 0.9867$, $P < 0.01$; LBC/L-lactate $r = -0.5160$.

Only one significant correlation LBC/D-lactate ($r = 0.9724$, $P < 0.05$) was found in Group 4 (pH 5.92 to 6.16). The remaining coefficients were as follows:

LBC/pH $r = -0.6903$

LBC/ammonia $r = 0.8141$

LBC/total lactate $r = -0.3415$

LBC/L-lactate $r = -0.9112$

It is evident from the results of the correlation analyses that ammonia and D-lactate concentrations and pH could be used for fixing the shelf life of vacuum-packed pork in samples of Group 3 only. It should be noticed however, that initial pH corresponded to values found in well ripened meat only in samples of this group and that the above characteristics cannot be used for vacuum-packed meat showing an atypical ripening course.

A comparison of results obtained by the different methods indicates that sensory aberrations follow changes detectable by biochemical and microbiological methods with a delay of 5 to 7 days. Even so, objective assessment of hygienic quality of vacuum-packed meat cannot be based on separate determination of a single characteristic.

Změny v hygienické jakosti vakuově baleného vepřového masa

Cílem práce bylo sledování hygienické jakosti vakuově baleného vepřového masa bez kosti na základě senzoričského, chemického a mikrobiologického vyšetření. K pokusu bylo použito vepřové maso 72 h post mortem, jehož teplota nepřesahovala 9 °C. Jako obalový materiál byla použita PA fólie koextrudovaná PE. Skladování probíhalo při teplotě prostředí 2.5 ± 0.5 °C ve tmě po dobu 35 dnů. Během této doby byly odebrány vzorky (14., 21., 28. a 35. den) k laboratornímu vyšetření. Senzoricky byl hodnocen celkový vzhled, barva, vůně a charakter uvolněné masové šťávy. Chemicky byla stanovena hodnota pH, obsah amoniaku, D- a L- formy kyseliny mléčné. Mikrobiologicky byl stanoven počet bakterií mléčného kvašení. API systémem byly identifikovány jako původci kažení *Lactobacillus curvatus* a *Lactobacillus xylosus*. Výsledky byly zpracovány s použitím korelační analýzy. Doba použitelnosti vakuově baleného vepřového masa bez kosti se pohybovala v rozmezí 21–26 dnů. Její délka je odvislá na počáteční hodnotě pH masa, určeného k balení. Na základě výsledků korelační analýzy jsme zjistili, že pro vakuové balení vepřového masa je vhodné použít pouze maso vyzrálé, jehož optimální hodnota pH = 5.80 – 5.95. Jako kritéria pro hodnocení doby použitelnosti je možno použít kromě stanovení počtu bakterií mléčného kvašení (BMK) i stanovení obsahu amoniaku (BMK/amoniak $r = 0.9584$, $P < 0.05$), hodnoty pH (BMK/pH $r = -0.9317$, $P < 0.05$) a obsahu D-laktátu (BMK/D-laktát $r = 0.9867$, $P < 0.01$).

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