Original Research Article

Selected Microbiological and Organoleptic Changes in Vacuum Packed Imported Beef

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Abstract

The aim of this work was to determine selected microbiological and organoleptic changes of vacuum packed cooled beef imported from Argentina as one of the few South American countries which export beef to Europe. The changes were studied during its common shelf life (4 months) and 1 month thereafter in case of longer storage. The storage temperature was -1 °C to +2 °C. Four packages of boneless chilled heart of rump were used; all the values of each package were measured five times. Meat and juice weight, vacuum value, numbers of microbes, yeasts and moulds, pH of meat and juice were determined. Some organoleptic characteristics of meat and juice such as taste, aroma, colour, consistency and clearness of both of juice and broth after boiling were evaluated using Czech technical standards (ČSN). The vacuum value of the first package was 90 kPa; the number of the microbes was increasing adequately to time in all the packages. In the course of the entire shelf life period all the samples fulfilled the allowed limits so the meat was suitable for human consumption. During the tested period neither monitored pathogen microflora nor yeasts and moulds were present; pH of all samples was influenced by storage in the vacuum. Meat stored for 5 months showed changes in odour and presence of coliforms. Commencing spoilage of this sample rendered the meat not suitable for human consumption.

Keywords: Vacuum packed beef; microbiological changes; organoleptic changes; pH and beef ripening.

INTRODUCTION

Only five Latin American countries are allowed to export beef to Europe, including the Czech Republic - Argentina, Brazil, Paraguay, Uruguay and Chile (EUROPA, 2009). Delivery period of vacuum packed beef meat to the Czech Republic reaches between 6-8 weeks after packing (XO Foods, 2010). The shelf life of such meat is usually 4 months.

This research was performed to determine the changes of microbiological and organoleptic features occurring during storage. Meat from Argentina was chosen for analysis.

Changes of redox potential (rH) by reducing of oxygen level are often used as a preventive measure in manufacture of sliced meat products. These are placed into plastic bags, the content of oxygen is reduced to 5-10 mm Hg, and the wrapping is finished by welding the free edges. Cellophane laminated polyethylene, polyamide, etc. is most frequently used for packing in vacuum (Steinhauser et al., 1995).

Shelf life of packaged foods of animal origin depends mainly on changes in microbial picture. Even when using high vacuum packaging the shelf life of foods of animal origin does not significantly increase, because along with anaerobes, the majority of microorganisms present in meat are of microaerophilic nature. Only the growth of moulds and yeasts in the vacuum packaging systems is greatly affected because they are mostly strict aerobes. Since the vacuum packaging films are impermeable for gas and water, meat surface does not dry, and under closely adherent foil keeps a fresh look. Therefore it is very important to keep the beginning level of contamination of meat at a low level. Also temperatures 0 to 2 °C are the most suitable for optimal shelf life (Steinhauser et al., 1995).

On the surface of the packaged meat, variety of microflora (various micrococci, *Achromobacter* groups, *Pseudomonas* sp., *Lactobacillus* sp., coliform germs, streptococci, sarcins, aerobic sporobionts, corynebacteria, fungi, yeast, etc.) occurs. Content of water and water activity (a_w) plays an important role in meat preservation. Decrease of water content and a_w cause a decrease in microbial content (Steinhauser et al., 1995).

In the course of meat storage, ripening processes occur and the meat becomes acceptable as food (Matyáš et al., 1965). Lactic acid generated by anaerobic glycolysis plays an important role. The initial pH decreases due to glycolysis, and the speed of this decrease depends on the storage temperature (Steinhauserová et al., 2007). In case of beef, the lowest pH is around 5.5-5.8. Measurement of pH is an important, but only an ancillary method to assess the freshness of meat. Usually, beef ripens at 10 - 15 °C for 2-3 days, at 1 - 2 °C for 8 - 14 days and at about 1 °C for 21 days (Matyáš et al., 1965).

The causes of the spoilage are most often bacteria of the genus *Lactococcus, Enterococcus, Streptococcus, Leuconostoccus, Pediococcus*, but most important is the genus *Lactobacillus*, of the *Lactobacteriaceae* family, when lactic acid bacteria counts reach more than 106 cfu/g (colony forming units per gram). This occurs under hygienic conditions within 28 days (Huis in 't Veld, 1996).

Spoilage of beef by psychrophilic and psychrotropic clostridial species renders meat unacceptable resulting in

financial looses and reduced consumer confidence. A number of clostridial strains, including *Clostridium algidicarnis, Clostridium algidixylanolyticum, Clostridium esterheticum, Clostridium frigidicarnis and Clostridium gasigenes*, have been implicated in red meat spoilage. Unlike other spoilers, these clostridia are able to grow in anaerobic conditions and at low temperatures (some grow at -1.5 °C, the optimal storage temperature for chilled red meat). The spoilage they cause is characterized by softening of the meat, production of large amount of drip (exudates), offensive odour in the case of *C. estertheticum and C. gasigenes* production of gas. Spoilage occurs following the introduction of clostridial spores into vacuum packages during processing (Adam et al., 2010).

Silva et al. (2011) isolated psychrotrophic clostridia from Brazilian vacuum-packed beef cuts (spoiled or not) and identified the isolates by using 16S rRNA gene sequencing. Populations of psychrotrophic anaerobic vegetative microorganisms of up to 10(10) cfu/g, were found in 'blown pack' samples, while in non-spoiled samples populations of 10(5) cfu/g was found. This was the first report on the isolation of psychrotrophic *Clostridium* (*C. gasigenes* and *C. algidicarnis*) in Brazil. This study showed that psychrotrophic *Clostridium* may pose a risk for the stability of vacuum-packed beef produced in tropical countries during shelf life and highlights the need of adopting control measures to reduce their incidence in abattoir and the occurrence of 'blown pack' spoilage.

Degradation processes can either start from outside (aerobic) or from the depth of meat (anaerobic) (Matyáš et al., 1965). Anaerobic changes in meat are slow until cfu (total number of colony forming units) reaches a maximum population of 108 to 109 cfu/g. Acetic acid, lactic acid, citric acid and tartar acid, respectively, increase the shelf life of meat during storage. These acids are sprayed on meat surface after slaughter (Steinhauserová, 2002). The most common germs developed in packaged meat include lactic acid bacteria. These microbes may cause meat spoilage or reduction of product quality.

According to Steinhauserová (2002), *Leuconostoc mesenteroides*, *Lactobacillus paracasei* spp *tolerans* and *Bronchotrix termosphacta* were present as a cause of spoilage of vacuum-packaged meat. Carbohydrates were converted into lactic acid, the active amines, ethanol, acetate and lactate.

Crowley (2010) studied trimmings stored first in vacuum packs for 7, 10, 14 or 22 days at 0 or 5 °C and later minced and stored aerobically at 0 or 5 °C for up to 7 days. They were daily examined to determine total viable, *Pseudomonas*, lactic acid bacteria, *Brochothrix thermosphacta*, and *Enterobacteriaceae* counts, colour and odour. Mincing reduced the counts, particularly of *Pseudomonas*, *B. thermosphacta* and *Enterobacteriaceae*, probably because of the action of free radicals released from muscle and bacterial cells. Storage of vacuum-packed trimmings for 22 days resulted in improved mince colour and inhibition of the growth of *Pseudomonas*. The shelf life of mince from trimmings is directly influenced by the trimmings storage conditions, and longer-term vacuum storage of trimmings produced improvements in mince quality.

Ercolini et al. (2011) monitored the microbial metabolites and bacterial diversity in beef chops stored at 4 °C under different packing conditions: in air, modified-atmosphere, in vacuum and in bacteriocin-activated antimicrobial packaging. After 0 to 45 days of storage, analyses were performed to determine loads of spoilage microorganisms, microbial metabolites and microbial diversity. The microbiological shelf life of meat increased with increasing selectivity of storage conditions. In vacuum packed meat many different bacteria, several of which are usually associated with soil rather than meat were identified. Lactic acid bacteria dominated during the late phases of storage.

Ripened raw beef is cherry red, with characteristic odour, vacuum-packed beef has a small amount of pure dark red juice, the consistency of meat is firm, flexible, crispy, after compression the surface returns back to its original position. Boiled beef is fine, juicy, with a special taste and pleasant aroma. Broth is clear with specific pleasant taste and aroma. Aroma and taste of meat vary with changing aromatic components. During *rigor mortis* their content decreases, in the next stage of ripening the content of aromatic components significantly increases (Matyáš et al., 1965).

For our study purposes, meat from Argentina was used as a representative sample of South American imported vacuum packed beef presently available for purchase at the Czech market.

MATERIALS AND METHODS

Materials: Four packages of boneless chilled heart of rump ("corazon de quadril" in Spanish, "cheio de alcatra" in Portuguese and "hovězí zadní" in Czech) were used. Fifteen different samples were taken of each package: > 5 from the meat surface, 5 from the depth of meat, 5 from the meat juice. All the packages were of Argentinean origin, slaughtered and cut in Argentina and imported to the Czech Republic 6 weeks after manufacturing under following conditions: vacuum packaged, in temperature between -1 °C and +2 °C, labeled with a note: "Once the packaging is opened, consume within the next 4 days." The best before date was 4 months after the manufacturing date. Packaging was made 2 days after slaughter.

Methods: All of measured characteristics were monitored once in a month since the meat entered the Czech Republic (first sampling 2 months after packaging) while the meat was still in period of shelf life (up to 4 months after packaging) and also 1 month after its shelf life (5 months after packaging).

Before opening all the 4 packages of meat were kept in recommended conditions (from -1 °C up to + 2 °C, vacuum packed) in "CRYOVAC" vacuum shrink bags. All the values of each package were measured 5 times.

Vacuum value

For orientation vacuum value of the first package before opening was measured by method usually used in food processing industry. The package was submerged into a water bath in a glass hermetic closed aquarium. When the plastic bag of the package started to inflate, the vacuum air pump started. It means the equation of the pressures in the package and inside a water bath. The pressure was scale read on the barometer.

Weight determination

All the packages were unwrapped just before examination. Weight of meat, meat juice samples and the packing materials were measured using a laboratory scale. First the total weight of each package was measured (Total weight [g]). Subsequently the juice weight which was poured out into the prepared glass beaker after the opening of a bag was recorded (Juice and beaker [g]). Later the weight of the wrapping with the rest of the juice (Wrapping and rest juice [g]) and the wrapping weight after drying (Wrapping [g]) were recorded. Total weight of the juice was determined as juice flown into the beaker together with juice remained inside the bag. Total weight of the meat was calculated as a difference between the total weight of vacuum packaged sample and total weight of the juice plus weight of the wrapping. All these data are shown in Tables 1 and 2.

Microbes, yeasts and moulds

Five samples from different places of each package were taken and the content of microbes, yeasts and moulds on the surface of the meat, in the meat juice and in the inner part of the meat was counted using Czech standards (ČSN). The microbiological tests were done in accordance with the time schedule of the whole survey. In Table 3 all kinds of tested microorganisms as well as numbers of used ČSN standards are stated.

Growth of microbes, yeasts and moulds on the meat surface

The swabs of 10 cm^2 of the meat surface were taken. The swab was submerged into 10 ml of saline and left for 10-15 minutes. The first dilution was done mixing with 90 ml of saline solution. Then second, third and fourth dilutions were prepared in the same way. Diluted solutions were poured on Petri dishes with culture or subsequently covered by diagnostic media depending on the tested microorganism. The contents of microorganisms were counted in accordance with ČSN standards.

Growth of microbes, yeasts and moulds in the meat juice

Similar method was used to count the microorganisms in the meat juice. The first dilution was done by mixing of 90 ml of saline with 10 ml of meat juice, later second, third and fourth dilution was performed. The rest of the method was analogous with microorganisms counting on the meat surface.

Growth of microbes, yeasts and moulds in the inner part of meat

Microorganisms in the inner part of the meat were obtained by the following method: 10 g of meat from deep part of the meat (approximately 1 cm under meat surface) was cut off. Obtained samples were handled according to specific procedures described in particular ČSN standards.

Table 1: Measured weights

Number of package	Total weight [g]	Juice and beaker [g]	Wrapping and rest juice [g]	Wrapping [g]	Beaker [g]
1	2278	294.3	25.9	13	180.9
2	2335	322.1	16.8	13.9	174
3	1711	259.8	20.4	9.1	173.9
4	1871	216.1	18.3	14.1	174

Table 2: Calculations of the meat and juice weights

Number of package	Weight of juice inside the wrapping [g]	Total juice content weight [g]	Total weight of meat [g]
1	25.9 –13 = 12.9	(294.3 – 180.9) + 12.9 = 126.3	2278 – (126.3 + 13) = 2138.7
2	16.8 – 13.9 = 2.9	(322.1 – 174) + 2.9 = 151	2335 – (151 + 13.9) = 2170.1
3	20.4 – 9.1 = 11.3	(259.8 – 173.9) + 11.3 = 97.2	1711 – (9.1 + 97.2) = 1604.7
4	18.3 – 14.1 = 4.2	(216.1 – 174) + 4.2 = 46.3	1871 – (46.3 + 14.1) = 1810.6

Table 3: Tested microorganisms, ČSN (Czech technical standards)

Tested microorganism	Abbreviation	ČSN number	ČSN
total number of colony forming units	CFU	ČSN EN ISO 4833	Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony-count technique at 30 °C
coliforms	C	ČSN ISO 4832	Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique
coagulase positive staphylococci	CPS	ČSN EN ISO 6888-1	Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium
Escherichia coli	EC	ČSN ISO 16649-2	Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of ß glucuronidase-positive *Escherichia coli* - Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl-ß-D-glucoronide
lactobacilli	L	ČSN 560094	Food products. Enumeration of bacteria of the genus *Lactobacillus*
mesophilic sporogenic anaerobic microbes	MSAM	VLM HP No.6, chapter 4.4.9	Determination of presence and number of anaerobic sporogenic microbes. Technique of colonies counting
Pseudomonas aeruginosa	PA	ČSN ISO 13720	Meat and meat products – Enumeration of *Pseudomonas* spp.
Listeria monocytogenes	LM	ČSN EN ISO 11290-1	Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* – Part 1 – Detection method
Yersinia enterocolitica	YE	ČSN ISO 10273	Microbiology of food and animal feeding stuffs – Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica*
Bacillus cereus	BC	ČSN EN ISO 7932	Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of presumptive *Bacillus cereus* – Colony-count technique at 30 °C
Salmonella	S	ČSN EN ISO 6579	Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.
residues of inhibitory substances	RIS	by VLM HP No. 6 and also by guideline SVS ČR 1999	Determination of inhibitory substances residues, method with phylum *Geobacillus stearothermophillus* by five and four plate method
yeasts	Y	ČSN ISO 21527-1	Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0.95
moulds	M	ČSN ISO 21527-1	Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of yeasts and moulds – Part 1: Colony count technique in products with water activity greater than 0.95

Note: SVS ČR = State Veterinary Administration of the Czech Republic
Source: Standards and investigative methodologies ČSN EN ISO (listed in the References)
VLM HP Veterinary laboratoy methods for food hygiene

Organoleptic characteristics

The following organoleptic characteristics of meat and meat juice and their changes in time of storage were observed: aroma, taste and colour. The meat was examined before and after frying on a frying pan and after boiling in water. The sensory characteristics were evaluated in accordance to ČSN.

Appearance of packed samples

Appearance of packed samples before opening was

observed. Colour of meat and amount of juice inside the package were noted.

Characteristics of unpacked samples

Colour, consistency, aroma and clearness of juice were observed. Colour, consistency, aroma and appearance of meat on the surface and cut were noted.

Characteristics of samples after boiling and frying

Quality of broth, colour, aroma and clearness during boiling were observed as well as meat consistency, colour, aroma, texture and taste of meat after boiling. Meat consistency, colour, aroma, taste of meat after frying was also recorded.

pH value

pH values of meat and meat juice were measured by standardized reference method ČSN ISO 2917- Measuring of pH – Meat and meat products. The procedure was repeated five times (each one was done in different part of the meat).

RESULTS

Vacuum value

The vacuum value was measured for orientation only in the first sample and reached 90 kPa.

Weight values and counts

In Tables 1 and 2 the individual values of measured weights are shown.

Microbes, yeasts and moulds contents

The results of counts of the microorganisms on the meat surface [cfu/g] are in Table 4. Table 5 shows counts of the microorganisms in the meat juice [cfu/g] and Table 6 describes counts of the microorganisms inside the meat - deep cut [cfu/g]. All abbreviations used can be found in footnotes to Table 4. There were 5 different measurements made; only the average values are stated.

Organoleptic characteristics

Appearance of packed samples

All pieces of chilled vacuum packed beef in vacuum shrank bags with inserted paper label were dark red colour. Meat juice was of dimly red colour of already mentioned amounts (see Table 2) – the amount of juice in the first two packages was bigger than in last two ones.

Characteristics of unpacked samples

The juice after rewrapping had dark red colour in all packages. The colour on the cut as well as in the inner part was dark red, the appearance and the aroma was proper for beef without any distinctive deviations. Only the last package had a stronger acidulous smell after unwrapping;

Table 4: Mean counts of the microorganisms on the meat surface [cfu/g]

Package	1	2	3	4
CFU	128 000	216 000	184 000	1 800 000
C	0	0	0	1 600
CPS	20	20	30	30
EC	0	0	0	0
L	168 000	146 000	128 000	460 000
MSFAM	36 000	120 000	126 000	320 000
PA	0	0	0	0
LM	negative	negative	negative	negative
YE	negative	negative	negative	negative
BC	0	0	0	0
S	negative	negative	negative	negative
RIS	negative	negative	negative	negative
QRM	streptococci, micrococi	streptococci, micrococi, sporulates	streptococci, micrococi, sporulates	streptococci, micrococi, sporulates, coliforms
Y	0	0	0	0
M	0	0	0	0

Abbreviations: CFU – total number of colony forming units, C – coliforms, CPS – coagulase positive staphylococci, EC – *Escherichia coli*, L – lactobacilli, MSFAM – mesophilic sporogenic anaerobic microbes, PA – *Pseudomonas aeruginosa*, LM – *Listeria monocytogenes*, YE – *Yersinia enterocolitica*, BC – *Bacillus cereus*, S – *Salmonella*, RIS – residues of inhibitory substances, QRM – qualitative representation of microbes, Y – yeasts, M - moulds

Table 5: Mean counts of the microorganisms in the meat juice [cfu/g]

Package	1	2	3	4
CFU	2 400 000	475 000	880 000	1 400 000
C	0	0	80	40 000
CPS	0	20	20	40
EC	0	0	40	80
L	1 600 000	3 800	400 000	480 000
MSFAM	160 000	28 000	3 000	48 000
PA	0	0	0	0
LM	0/25	0/25	0/25	0/25
YE	0/25	0/25	0/25	0/25
BC	0	0	0	0
S	0/25	0/25	0/25	0/25
RIS	negative	negative	negative	negative
QRM	Alfa streptococci, micrococi, sporulates	Alfa streptococci, micrococi, sporulates	streptococci, micrococi, sporulates, coliforms	streptococci, micrococi, sporulates, coliforms
Y	0	0	0	0
M	0	0	0	0

Abbreviations: see Table 4

Table 6: Mean counts of the microorganisms inside the meat - deep cut [cfu/g]

Package	1	2	3	4
CFU	100 000	36 000	168 000	188 000
C	0	0	0	1 600
CPS	0	0	0	0
EC	0	0	0	0
L	160 000	18 000	24 000	64 000
MSFAM	240 000	76 000	94 000	80 000
PA	0	0	0	0
LM	0/25	0/25	0/25	0/25
YE	0/25	0/25	0/25	0/25
BC	0	0	0	0
S	0/25	0/25	0/25	0/25
RIS	negative	negative	negative	negative
QRM	streptococci, micrococi, sporulates	micrococi, sporulates	streptococci, micrococi, sporulates	streptococci, micrococi, sporulates, coliforms
Y	0	0	0	0
M	0	0	0	0

Abbreviations: see Table 4

however, it disappeared after about 20 minutes. The consistence of meat was firm, elastic. The juice was clear in all packages.

Characteristics of samples after boiling and frying

During boiling a small amount of brown spume was notable on the surface of the broth. The aroma was typical of beef broth in all four packages, the colour was brown and of normal clearness while boiling. After boiling and frying, all meat samples (one of each package) had usual colour, aroma and taste typical of beef meat without any changes. Meat was elastic and firm in all four samples.

pH value

The average values of measured pH are recorded in Table 7.

Table 7: Mean pH values

Package	Meat juice	Inside the meat
1	5.60	5.70
2	5.53	5.70
3	5.59	5.70
4	5.36	5.80

DISCUSSION

Since there was only one exemplary sample of vacuum value measured, we suppose that there were similar vacuum values in all the packages under study. The wrapping on every package was of the same nature and such a minimum possible difference of the pressure should not have any prominent influence on the keeping or the spoilage of the meat. Our measured vacuum value was not different from values reported earlier (Matyáš et al., 1965).

Meat is practically sterile after slaughtering. During the handling some contamination may occur especially on the surface of the meat, inside the meat it occurs only if good manufacture practices are violated. These factors, together with intravital influences (notably overheating, fatigue) may lead to initial differences in the microbial counts in meat intended for vacuum packaging. In accordance to Steinhauser et al. (1995), some of the microaerophilic microbes and some streptococci appeared in our stored beef samples. Yeasts and moulds were not present because they are mostly strict aerobes. Lactobacilli were present as shown in Tables 4, 5 and 6; *Pseudomonas* sp. on the other hand, in our study did appear neither in juice nor in meat. Our findings may suggest the possibility of meat treatment by lactic acid prior to vacuum packing. Under normal conditions according to Steinhauser et al. (1995) and Brychta et al. (2005) pH_1 is lower than 6 during *rigor mortis* and at the beginning of meat ripening. Then, depending on time, temperature, contamination, DFD and PSE status etc., pH_{24} is less than 6.20. Increase of pH is caused by gradual decrease of lactic acid. The highest number of microbes was present in meat juice where the most suitable conditions for their growth exist. In agreement with Matyáš et al. (1965) the lowest pH is around 5.5 -5.8; we found the lowest pH in meat 5.7. These authors thus described the ripened raw beef exactly as we experienced.

As shown in Table 2, there was a larger amount of loosened juice in the first two packages than in last two recorded ones. All four packages were in good condition after opening. Only the last (No. 4) package showed a stronger acidulous smell, the meat itself did not show any acid smell. Variation in loosened juice is depending on age of animal, time of meat hanging in cold room, weight of packed meat etc.

Silva et al. (2011) and Adam et al. (2010) published a similar study on vacuum packed beef reporting clostridial spoilage of the product. This should be the next step in microbiological survey of beef imported to the Czech Republic, because as they alert clostridial species may pose a risk for the stability of vacuum-packed beef produced in tropical countries.

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