POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2005, Vol. 14/55, SI 1, pp. 65-68

EFFECT OF XANTHAN PROTECTIVE COATING WITH *LACTOBACILLUS SAKEI* CULTURE ADDITION ON THE MICROBIOLOGICAL SAFETY AND THE QUALITY OF PORK STORED UNDER REFRIGERATION

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Key words: protective coatings, *Lactobacillus sakei*, *Escherichia coli*, raw pork

The purpose of the present study was to evaluate the effect of protective coating with *Lactobacillus sakei* culture addition for shelf-life prolongation and the improvement of the microbiological safety of raw pork stored under refrigeration. The study was performed on dorsal longissimus muscle, cut into samples of *ca*. 100 g. Four experimental variants were prepared (A, B, C and D). The samples A and B were covered with xanthan protective coating (0.5% of xanthan) with 1% *Lactobacillus sakei* BJ-33 culture addition (3.5 x 10^9 cfu/mL). The samples C and D were left as the controls. Subsequently, by means of surface spraying, the samples A and C were inoculated with the suspension of *Escherichia coli* PCM 2057 test strain (9 x 10^3 cfu/g). Total number of 96 (24 per variant) samples prepared were stored at 2ºC for 9 consecutive days with microbiological determinations (the count of *E. coli*, total plate count and the counts of lactic acid bacteria (LAB) and moulds and yeasts) performed on days 0, 3, 6 and 9. The study revealed that the application of *L. sakei*-containing protective coating inhibited the growth of superficially inoculated *E. coli*. On the 9th day of storage the number of test bacteria in the samples protected with the coating (A) was 0.8 log cfu/g lower than in the *E. coli*-inoculated controls (C), in spite of the similar initial contamination. LAB were determined in the coating-protected samples (A, B) only while they were not demonstrated in the controls (C, D), indicating the predominant growth of *L. sakei*. Concluding, the study indicated that the application of protective coatings with LAB seems advisable for industrial raw meat treatment.

INTRODUCTION

Consumer health care is oriented on the prolongation of shelf-life and the appropriate microbiological quality of meat and its products. Accordingly there is a constant need for the introduction of new technologies in the food industry.

The application of edible protective coatings is one of the methods for the prolongation of the shelf-life of raw meat. The coatings protect meat against the objectionable changes, such as the loss of mass, color changes and the growth of microflora. Moreover, they are accepted by consumers and do not pose any health risk [Malicki *et al*., 2003].

There is also an evidence for the positive effect of the technological cultures of lactic acid bacteria (LAB) on the microbiological status and organoleptic properties of meat and its products [Domaszczyńska, 1997; Niessner *et al*., 1998; Korzeniowski & Kubera, 1999; Tyburcy, 2001]. Lactic acid and bacteriocins are of the most importance among the antibacterial substances synthesized by LAB. Of that group, the psychrotrophic *Lactobacillus sakei* and *L. curvatus* seem to be the best adapted to the conditions occurring in meat and its products. *Lactobacillus sakei* is known to generate specific bacteriocins – sakacin A and P. The efficiency of these substances against the microorganisms contaminating meat and its products, including lactic acid-resistant *Listeria monocytogenes*, were proved in the course of *in vitro* studies [Kröckel, 1999].

The purpose of the present research was to evaluate the impact of the protective coating with *Lactobacillus sakei* culture addition on shelf-life prolongation and the improvement of the microbiological safety of raw pork stored under refrigeration.

MATERIAL AND METHODS

The study was performed on dorsal longissimus muscle (*m. longissimus dorsi*). The meat was cut into samples, each of *ca.* 100 g. Four experimental variants were prepared (A, B, C and D, Table 1). The samples A and B were covered with xanthan protective coating with the addition of *L. sakei* BJ-33 culture (3.5 x 10^9 cfu/mL, Baktoferm™ B-2/B-2-U, Chr. Hansen, Częstochowa, Poland) (Table 2). The samples C and D were left as the controls. Subsequently, by means of surface spraying, the samples A and C were inoculated with the suspension of 18-h culture (tryptone soya broth - TSB, Oxoid, Basingstoke, UK, 37ºC) of *E. coli* PCM 2057 test strain (9 x 10^3 cfu/g).

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TABLE 1. Characteristics of experimental variants analyzed.

Variant	Xanthan coating	*Escherichia coli*
A	+	+
B	+	-
C	-	+
D	-	-

"+" present or "-" absent in experimental variant

TABLE 2. The composition of the protective coating studied (per 100 mL).

Component	Volume (mL)
Xanthan gum	0.50
Glucose	0.50
NaCl	0.70
1% lactic acid	1.00
L. sakei BJ-33 culture (3.5 x 10^9 cfu/mL)	1.00
Vitamin E	0.55
Water	95.75

The samples were stored at 2ºC for 9 consecutive days. The microbiological determinations: the number of test *E. coli* survivors, total plate count and the counts of LAB and moulds and yeasts, were carried out on 0, 3, 6 and 9 day of storage. Each parameter was tested in 6 samples, giving the total number of 96 samples analyzed.

Escherichia coli cells were restored on chromogenic solid medium (Chromocult Coliform Agar, Merck, Darmstadt, Germany, 24 h, 37ºC). For the purpose of additional identification, all the colonies stained from dark blue to violet were treated with Kovacs reagent for indol. The change of coloration to cherry-red, appearing in a few seconds (positive result of indol test), confirmed the presence of *E. coli*. The remaining microbiological parameters were determined following the Polish Standards. The total plate count was quantified on agar plates (24 h, 30ºC) by means of colony-count technique [PN-EN ISO 4833: 2003]. LAB were enumerated on MRS Agar (Oxoid, Basingstoke, UK, 48 h, 30ºC) [PN-ISO 15214: 2002], whereas moulds and yeasts – on agar with yeast extract, glucose and chloramphenicol (72 h, 25ºC) [PN-ISO 7954: 1999].

Logarithmic transformation of the bacterial counts and their statistical analysis were carried out with the aid of Microsoft® Excel 2000 and Statistica 5, Version 97 software. The significance of the mean value differences was established with the aid of Student's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The total plate count increased in the course of the entire experiment in the uncoated controls (D), with significant changes demonstrated during the initial 3 days of storage and between days 6 and 9 (Figure 1). After 9 days of storage, the aforementioned parameter increased by *ca* 4.0 log cfu/g compared to the initial level. The preliminary value of total plate count in samples A, B and C was, however, higher than in the controls (D). In the course of storage in turn, the parameter discussed increased only by 1.4-1.8 log cfu/g, depending on the experimental variant. That phenomenon evidently reflected the addition and the changes in numbers of *L. sakei* and/or *E. coli* cultures, since as the aerobes or facultative anaerobes, the aforementioned microorganisms are in a greater part enumerated as the components of total plate count.

Up to the 6th day of storage the counts of superficially applied test *E. coli* did not differ significantly between coated (A) and uncoated (C) samples (Figure 2). The decrease of *E. coli* by *ca.* 1.1 log cfu/g was demonstrated after 3 days of the experiment. During the consecutive 3 days, the count of *E. coli* increased by *ca.* 0.3 log cfu/g both in samples A or C. That phenomenon seems to reflect the adaptation of bacteria to a new environment. The significant differences between the experimental variants discussed appeared, however, between the 6th and the 9th day of the study. The number of *E. coli* still increased in uncoated samples (C), while the significant decrease of test microorganisms was demonstrated in A samples. In the samples of B and D variant the bacteria tested were not detected throughout the study.

The decrease of *E. coli* count in the A samples was accompanied by the inconsiderable (by 0.86 cfu/g) growth of LAB (Figure 3). The latter group seemed to consist predominantly of *L. sakei*, since LAB were determined in the coated (A, B) samples only, whereas they were not detected in samples C and D.

Regardless the experimental variant, yeasts and moulds were not detected in any of the samples studied.

Although the studies on edible coating application for meat preservation are infrequent in the available literature, their results seem promising. The positive effect of the gelatin protective coating on the microbiological status of raw meat was already revealed in our previous study [Malicki *et al*., 2003]. The efficiency of protein-based coating against spoilage and pathogenic microorganisms in the ground beef stored at 4ºC was also proved by Ouattara *et al.* [2002]. It should be noted, however, that the latter authors combined the coating application with the 0.5% ascorbic acid addition and gamma irradiation of meat prior the storage. Recently, milk protein-based edible films containing 1.0% oregano, 1.0% pimento, or 1.0% oregano-pimento (1:1) essential oils mix, applied on beef muscle slices, have been demonstrated to control the growth of pathogenic bacteria and increase the shelf-life during storage at 4ºC. The application of the aforementioned films on meat surfaces containing 10^3 cfu/cm^2 of *E. coli* O157:H7 or *Pseudomonas* spp. showed that the film containing oregano was the most effective against both the bacteria studied [Oussalah *et al.*, 2004].

Protein-based coatings might be, however, the source of nutrients for the microorganisms colonizing the meat surface. Accordingly, xanthan – the polysaccharide of microbiological origin, exhibiting the eligible physicochemical properties and resistant to enzymatic degradation, was used as a base for the coating in the present study. A comparative analysis of the results obtained throughout the present study with our

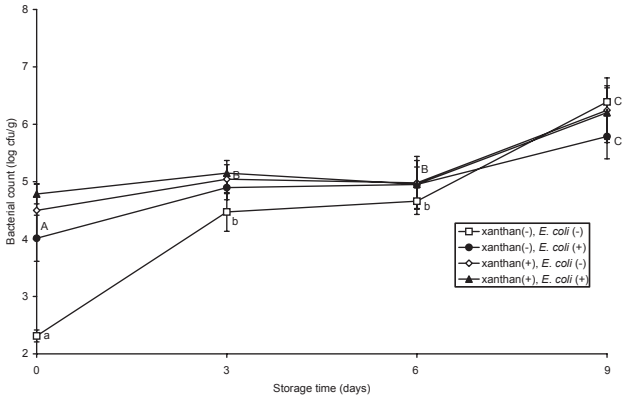

FIGURE 1. Total plate count in coated and uncoated raw pork stored at 2ºC for 9 consecutive days (error bars represent standard deviations, A-C, a-b = statistically significant differences, $p \leq 0.05$).

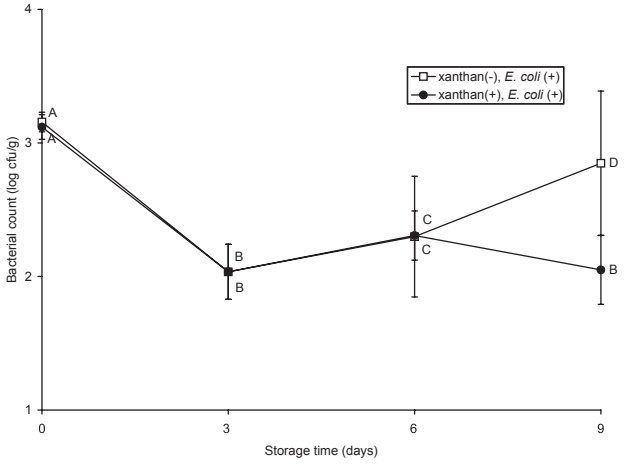

FIGURE 2. The counts of test *E. coli* PCM 2057 in coated and uncoated raw pork stored at 2ºC for 9 consecutive days (error bars represent standard deviations, A-D = statistically significant differences, $p \leq 0.05$). In variants B and D the bacteria tested were not detected in the course of the study.

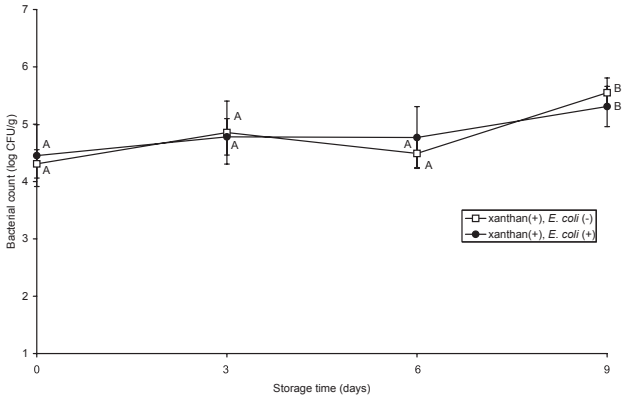

FIGURE 3. The counts of LAB in coated and uncoated raw pork stored at 2ºC for 9 consecutive days (error bars represent standard deviations, A-B = statistically significant differences, $p \leq 0.05$). In variants B and D the bacteria tested were not detected in the course of the study.

previous report [Malicki *et al*., 2003] indicates that the xanthan coating with the addition of the technological culture of *L. sakei* improves the microbiological quality of raw pork stored under refrigeration and prolongs its durability even more evidently than the protein-based film alone.

The antibacterial effect of protective coating against *E. coli* seems to be related either to its barrier properties or to the antagonistic activity of LAB. The latter microorganisms inhibit the other microflora, generating lactic acid and numerous bacteriocins [Kröckel, 1999; Vermeiren *et al.*, 2004]. The polythene film coated with a bacteriocin produced by *Lactobacillus curvatus* 32Y was proved to reduce the count of *Listeria monocytogenes* in pork steak and ground beef stored at 4ºC [Mauriello *et al.*, 2004].

The additional advantage related to the application of the technological cultures of LAB is their positive effect on the ripening of meat and its sensory properties.

CONCLUSION

The application of the edible protective coating with the addition of *L. sakei* technological culture is a profitable alternative for the improvement of microbiological quality and the prolongation of stability of raw meat.

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WPŁYW OCHRONNEJ POWŁOKI KSANTANOWEJ Z DODATKIEM KULTURY *LACTOBACILLUS SAKEI* NA BEZPIECZEŃSTWO MIKROBIOLOGICZNE I JAKOŚĆ MIĘSA WIEPRZOWEGO PRZECHOWYWANEGO W WARUNKACH CHŁODNICZYCH

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Celem pracy była ocena zastosowania powłoki ochronnej z dodatkiem kultury technologicznej bakterii fermentacji mlekowej w celu przedłużenia trwałości i poprawy bezpieczeństwa mikrobiologicznego surowego mięsa wieprzowego przechowywanego w warunkach chłodniczych. Materiałem do badań był mięsień najdłuższy grzbietu, który podzielono na próbki o masie około 100 g. Doświadczenie wykonano w 4 wariantach (A, B, C i D). Na próbki A i B nanoszono ksantanową powłokę ochronną z dodatkiem kultury *Lactobacillus sakei* BJ-33 (tab. 1). Próbki C i D pozostawiono jako kontrolne. Następnie na próbki A i C nanoszono metodą natryskową zawiesinę szczepu testowego *Escherichia coli* PCM 2057. Ogółem przygotowano 96 próbek, które przechowywano przez 9 dni w temperaturze 2ºC, w 0, 3, 6 i 9 dniu wykonując oznaczenia mikrobiologiczne (liczbę przeżywających pałeczek szczepu testowego *E. coli*, ogólną liczbę bakterii tlenowych oraz liczby: bakterii fermentacji mlekowej i pleśni i drożdży). Przeprowadzone badania wykazały, że zastosowanie powłoki ochronnej zawierającej szczep *L. sakei* wpływa hamująco na rozwój naniesionych powierzchniowo testowych pałeczek *E. coli*. Po 9 dniach przechowywania przy podobnym poziomie zanieczyszczenia początkowego, liczba bakterii testowych w próbkach zabezpieczonych powłoką z dodatkiem *L. sakei* (wariant A) była o 0.8 log jtk/g niższa niż w próbkach kontrolnych zakażonych *E. coli* (C) (rys. 2). Obecność bakterii fermentacji mlekowej stwierdzono jedynie w próbkach zabezpieczonych powłoką (A, B), podczas gdy ich wzrostu nie wykazano w materiale jej pozbawionym (C, D), co wskazuje na dominujący wzrost drobnoustrojów *L. sakei* (rys. 3). Uzyskane wyniki wskazują na celowość zastosowania na skalę przemysłową powłok ochronnych mięsa surowego wzbogaconych w kultury bakterii fermentacji mlekowej.