Review Article

IMPACT OF PRESERVATIVES USED IN SELECTED DELICATESSEN PRODUCTS ON *LISTERIA MONOCYTOGENES* SURVIVAL. PART II AND III

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Abstract

This article deals with second and third part of the study about impact of benzoic and sorbic acids on survival of L. monocytogenes in connection with possibilities to prolong the shelf life of delicatessen products. For experiments carrots salad and coleslaw salad as representatives of vegetable salads were used. Spreads with high fat contents and low a_w, as different product was choosen. In vegetable salads, number of inoculated L. monocytogenes decreased substantially, despite of the fact, that concentrations of preservants were in substance much lower than in czabay spread. Carrots salad, due to decrease of pH under pH 4.0 may be considered as the product not supporting L. monocytogenes growth. In case of spreads also concentrations of preservants were sufficient enough for considerable decrease of L. monocytogenes counts and shelf life prolongation.

For experiments representatives of spreads czabay spread, hermelín spread, cheese/garlic spread and picant spread were used. Results gained proved again that the use of benzoic and sorbic acids enables not only to prolong a shelf life, but also to concern spreads treated with the above preservatives as products not supporting L. monocytogenes growth too.

Key words: L. monocytogenes, carrots salad, coleslaw salad, czabay spread, hermelín spread, cheese/garlic spread and picant spread, benzoic and sorbic acids.

INTRODUCTION

Vegetables present one of the most complex areas of food production, distribution and marketing. The seasonal nature of production and highly perishable nature of produce caused that until recently the trade and manufacture of vegetable salads has been concentrated trough small specialist shopkeepers. This thinking was changed when problems associated with long distance haulage were overcame together with sophisticated storage methods of high quality standards vegetables. Even if quality of vegetables is based on their perishable nature and freshness is an all-important quality parameter, which may be discerned by untrained eye, wholesomeness depends largely on sanitary meassures implemented in the course of growing, harvesting and storing technologies. A large outbreak of adult and perinatal infection due to L. monocytogenes was positively associated with the cabbage component of coleslaw. The cabbage was grown in fields fertilised with sheep manure from flock with listeriosis. Subsequent cold storage of cabbages had led to multiplication of small initial inoculum of L. monocytogenes to significant population size (Schlech at al., 1983). The ability of L. monocytogenes to grow under temperatures from –1.5°C to 45°C (Petran, 1989; Hudson et al., 1994) makes this germ problematic in food industry.

Some food components protect L. monocytogenes against heat, like fat (Sutherland, 1993) and on the other hand this germ may grow under a_w lower than 0.90 (Juntila et al., 1989). In dry meat products survival of L. monocytogenes was observed under a_w 0.67–0.68 (Trussel, 1999).

Some combinations of ingredients in a perishable base may be ecologically unstable and be prone to microbiological problems. Recipe Hazard Analysis (RHAS) should therefore be applied in development of products of this type (Sutherland et al., 1986). Also pre-prepared fresh salads on trays or in containers as carrots salad have become a future of many supermarkets (Schlech et al., 1983).

Heisick et al. (1989) conducted tests on ten types of fresh produce to detect L. monocytogenes. It was isolated from cabbage, cucumbers, potatoes and radishes. L. innocua was isolated from cucumbers, lettuce, mushrooms, po-

tatoes and radishes. L. seeligeri was isolated from cabbages and radishes and L. welshimeri from cucumbers, potatoes and radishes. No listeria spp. was isolated from broccoli, carrots, cauliflower or tomatoes. However this does not mean that there was no L. monocytogenes contamination in the lots from which these units were drawn. In fact, there is 1 chance in 20 that Listeria contamination exceeds 3% when 92 independent units are observed to be negative. Some study, however suggest that some components of carrots may be toxic to Listeria spp. (Heisick et al., 1989).

Douglas et al. (2002) reported about cohort study of an outbreak of acute fibrile gastroenteritis among attendees of a catered party. Illness was associated with ingestion of pre-cooked sliced turkey and vegetarian sandwiches. Epidemiological studies provide strong evidence that both sporadic and common-source outbreaks of Listeriosis are foodborne with very serious consequences (EFSA 2005; EPIDAT 2009).

In the Czech Republic from year 2005 to year 2007 appeared 227 cases of disease and 31 deaths, 3 abbortuses and 42 cases of neonatal listeriosis were recorded. Studies suggest that up to 10% of human gastrointestinal tracts may be colonized by L. monocytogenes (Ramaswamy et al., 2007). The annual rate of infection is 3 times higher among persons ower the age of 70 years and more than 17 times higher among pregnant women (Broome, 1993). The risk of listeriosis is marketly increased among immunocompromised patiens, particularly those undergoing renal transplantation or receiving high doses of corticosteroids and those with AIDS or cancer. The risk of infection is estimated to be 100 to 300 time higher in AIDS patiens than in the general population (Schuchat et al., 1992). Listeriosis also concerns public health authorities because it is associated with mortality rate of 23%, whereas other foodborne diseases such as salmonellosis are rarely fatal.

Epidemic of listeriosis with high fatality rates (Centers for Disease Control 1985; Fleming et al., 1985; Schlech et al., 1983) have resulted in concern about the incidence and control of L. monocytogenes in food supply and environment.

Recalls, illness and deaths associated with Listeria in foods have been reported over the last years. These incidences have increased the awarness, that additional techniques may be needed for controlling Listeria in food producing plants.

As to organic acids, a low pH environment has adverse effect on the growth of L. monocytogenes (Buchnan et al., 1993). Several researchers have noted that in culture media, acetic acid has more potent antilisterial effects than lactic acid, which in turn, is inhibitorier than hydrochloric acid; (Ahamad, 1990; Jorgensen et al., 1999; Vasseur et al., 1999). Several experiments in culture media demonstrated, that inhibitory effect of an acides are greater at lower temperatures (Gill and Houtsma et al., 1996; Buchnan et al., 1997).

Other factors, such as presence of salt and other compounds used as preservatives, may modify the effect of organic acids on L. monocytogenes (Kamat and Nair, 1996). Organic acids can interact with other preservatives to enhance their effects. Acetic and lactic acids enhance the antilisterial effects on monolaurin (Oh and Marshall, 1996).

However, it should be noted that the effects of organic acids are not always positive in terms of food safety. Listeriae, which are exposed to these acids and survive may repair themselfs during storage at low temperatures and begin to multiply if other barriers are not present (Cheroutre-Vialette et al., 1998; Greer and Dilts, 1995; Palumbo and Williams, 1994).

Since L. monocytogenes can grow on a variety of food products at refrigeration temperatures, a variety of chemicals which destroy or limit the growth of pathogens have been tested. Amongst other preservatives used in control of L. monocytogenes, firstly NaCl should be mentioned. NaCl in foods can be a source of osmotic stress by decreasing water activity. However, L. monocytogenes is remarkably salt-tolerant and withstand higher salt concentrations than Salmonella spp. and Yersinia spp. (Houtsma et al., 1996). Although L. monocytogenes is halotolerant, salt is a stress and does depress growth rates (Vasseur et al., 1999). In combination with other compounds, NaCl is one factor contributing to the destruction or inhibition of L. monocytogenes (Fernandez et al., 1997).

Nitrate also is not very effective antilisterial agent. However, as with salt, in the presence of other agents (Fernandez, 1997) or lactocin 705 (Vignolo, 1998), nitrate can contribute to the suppression of L. monocytogenes at refrigeration temperatures.

Trisodium phosphate has been used for decontamination of poultry carcasses (Salva, 1997) and can reduce bacterial contaminants by 1–2 log.

Smoking also has been shown to inhibit Listerias growth (Niedzela, 1998), as well as a variety of herbs and spices. Plant extracts exhibiting antilisterial activity include for example: pimento leaves (Hao, 1998), rosemary (Lis-Balchin, 1997) leaves and cinnamic acid (Ramos-Nino, 1996) to mention some of them at least.

To the group of other additives lysozyme, sorbate and methyl paraben may be added Bacteriocins, as proteinaceous antimicrobial compounds also for the sake of listeria control are used. Nisin is currently used for the preservation of some foods for well known antilisterial effects. Lysin is more effective in more ac-

id‘s foods, but L. monocytogenes which has adapted to acidic conditions, becomes more tolerant of lysin. This tolerance along with the development of nisin-resistant strains and mutants of L. monocytogenes may limit the effectiveness of nisin in some applications. On the other hand, starter cultures of bacteria producing other antilisterial bacteriocins along with nisin may be used.

Pediocin with its resistence to thermal degradation, reuterin as a broad–spectrum antimicrobial agent (El-Ziney, 1998) and sarcin, which inhibits a growth of L. ivanovii may be also used.

For the sake to complete survey of the various techniques used in listeria intervention, following methods have to be mentioned. Thermal processes, irradiation, modified atmosphere packaging, high pressure, pulsed electric fields and electrolysed oxidising water, ultraviolet light and ultrasound.

Even if various techniques used for Listeria intervention currently cannot be utilized in manufacture of delicatessen products fully, they may be used in treating their components harmless and thus to contribute to wholesomeness of final products.

MATERIALS AND METHODS

For experiments about impact of benzoic and sorbic acids on survival of L. monocytogenes and possibilities, to prolong shelf life of delicatessen products, in the second and third part of study two kinds of salads (colleslaw and carrots salads) and four kinds of spreads (czabay spread, hermelin spread, cheese-garlic spread and picant spread) were used.

Samples were taken six times for six consecutive days. Randomly selected original wrappings (containers a 250 gms) intended for retail with declared shelf life 11 days were used. Samples were transported to laboratory in cool boxes and in laboratory stored in refrigerator less than 8°C. Stock of L. monocytogenes originating from the Czech Microbes Collection Institute in Brno was used for inoculation. Examinations were performed in accordance with ČSN EN ISO 11290-1 for diagnosis of L. monocytogenes and ČSN ISO 11290-2 for total count of L. monocytogenes. As culture media commercial products of BioRad (France) - liquid medium Fraser ½ and solid diagnostic medium AL Agar-listeria were used. Confirmation was not performed as samples before inoculation were tested on absence of Listeria according to ČSN EN ISO 11290 (zero in 25 gms). Incubation was done in incubator BT120M (ČR) equiped with calibrated thermometer. Samples homogenisation was done by the use of STOMACHER homogenizer (UK) for 30 seconds. Scales Balance Metter (Switzerland) for weighing up were used. Meassuring of pH was performed by accredited method by the use of pH meter WTW, type pH 90 (BRD) calibrated at given temperature with buffers WTW pH 4.0 and pH 7.0. Water activity (a_w) by accredited method with the use of Thermoconstanter TH-2 (Switzerland) was performed.

Contents of preservative matters by HPLC method using HPP 5001 apparatus with detector LCD 2082 ČR was performed. Salt contents by the use of accredited method ČSN ISO 1841 was performed. For experiments, samples were inoculated by final concentration of L. monocytogenes stated in graphs chpt. results. Ten gram of tested samples into microtene bags were weighed, than inoculated by L. monocytogenes collection strain CCM 4469. Inoculum was diluted by way enabling before set up number of colonies to be inserted, than samples were homogenized mechanically and stored in refrigerator at 8°C. At the same time, 25 gm of samples were stored for the sake to measure pH ranges in products tested.

Appropriate samples dilutions on AL agar were inoculated on 4 plates, in paralel two plates from each dilution, which in accordance to ČSN EN ISO 7218 (2008) were performed. From results gained, average number of typical blue-green colonies were counted after 48 hrs incubation and recorded in tables under chapter Results. At beginning of experiments and after their finish, in paralel control samples, no presence of L. monocytogenes was found. At the same time, a_w, pH, NaCl and preservatives contents in samples were meassured. Further studies on pH ranges and microbiological picture were concentrated, as these two parameters were in study most important. Both examinations were tested at fixed intervals: 1 hour, 1 day, 2 days, 3 days, and 5 days after manufacture of salads.

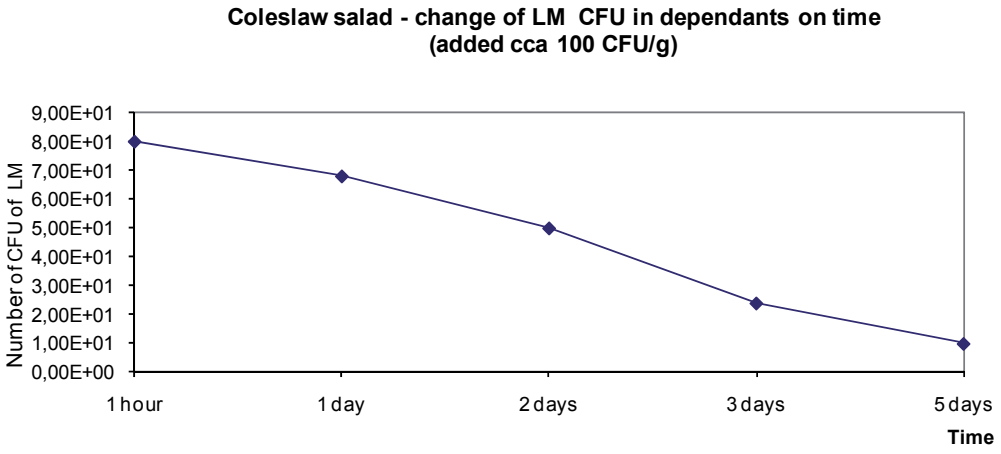
RESULTS

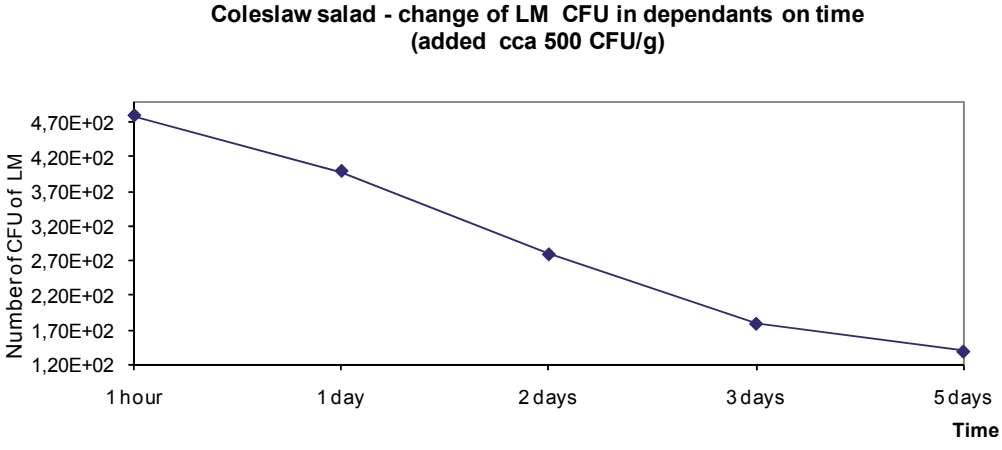
Results presented in graphs are selfexplanatory. From values of a_w and salt it is obvious, that products checked provide suitable environment for L. monocytogenes growth. Contents of preservatives, which was in limit of tolerated figures, during storage decreased. Concentrations of preservants were substantially different according to products compositions. Fact that quantities of preservatives were considerably lower, in colleslaw and carrots salad L. monocytogenes amounts decreased substantionaly.

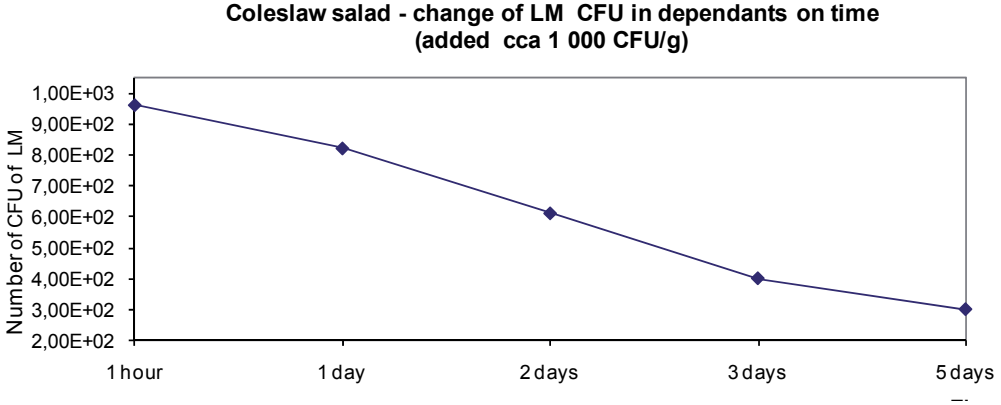
Sample No. 1: Coleslaw salad

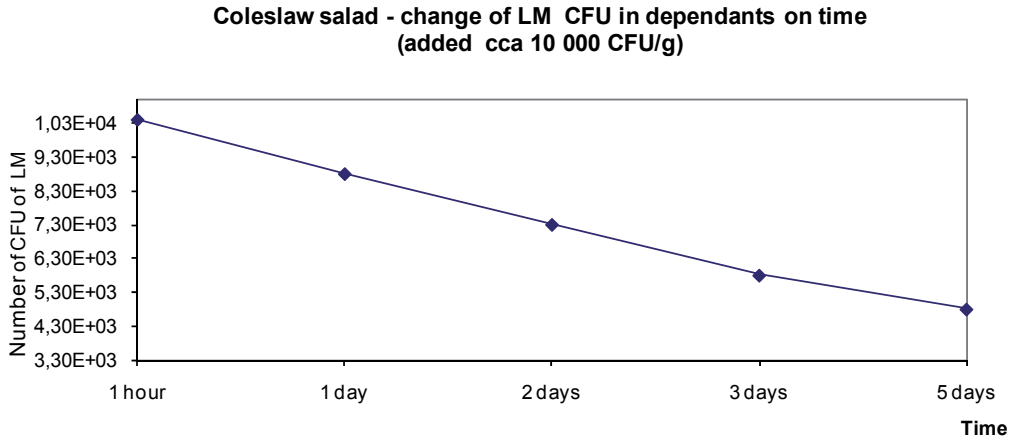
Shelf life: 4 days		
Water activity a_w: 0.969		
Content of NaCl: 1.56%		
Preservatives content (mg/kg):		
	start of experiment	end of experiment
Benzoic acid	23.85	14.6
Sorbic acid	4.6*	2.26*
Total	28.55	16.86

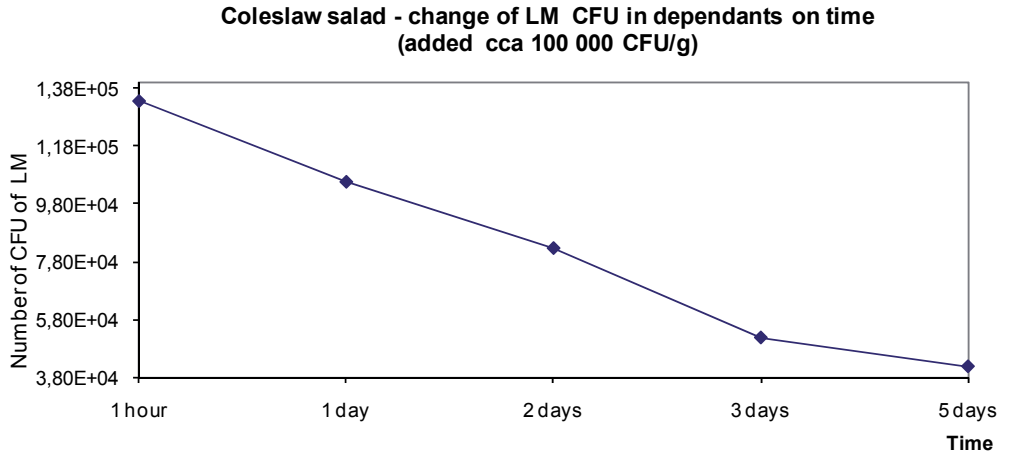
*This figures are quoted just for interest. Due to inaccuracy of low figures there are in protocol stated as < 10

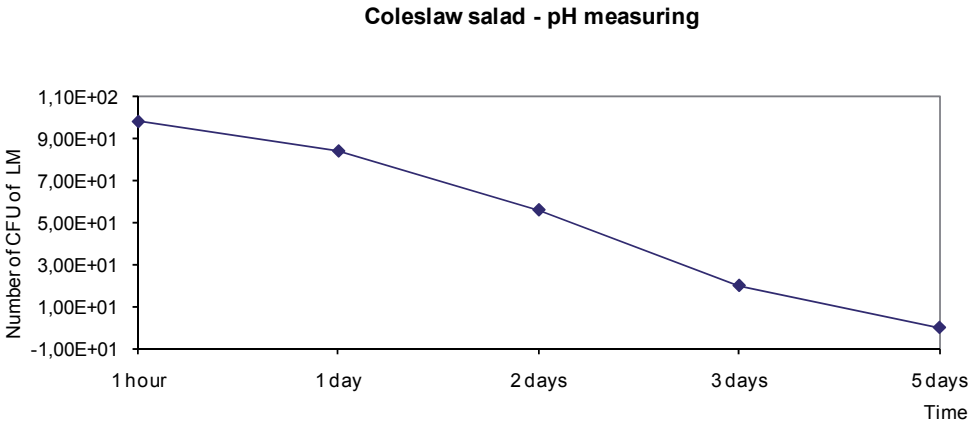










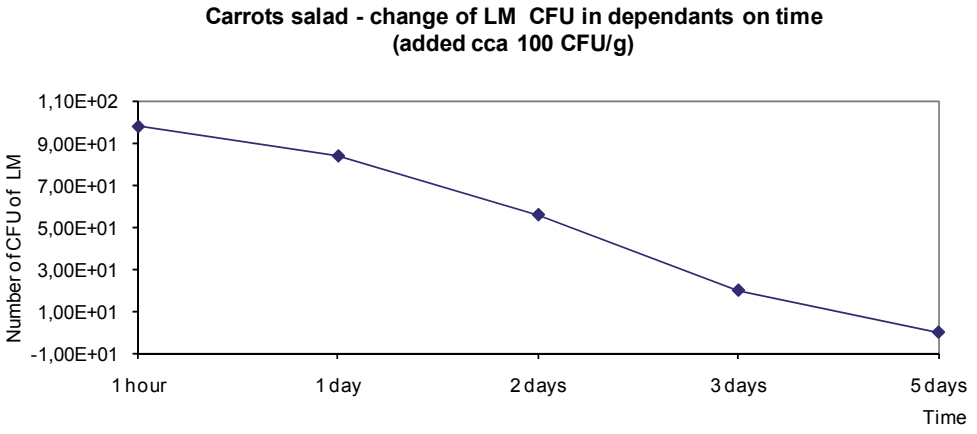


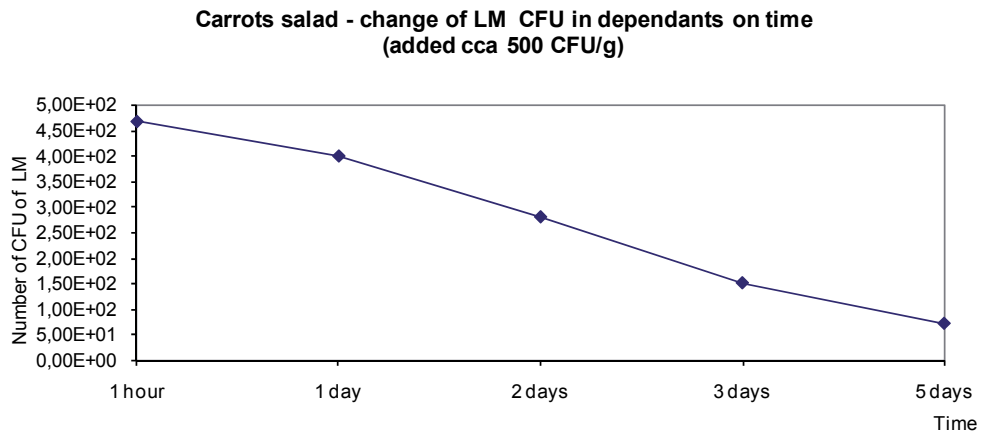
Sample of No. 2: Carrots salad

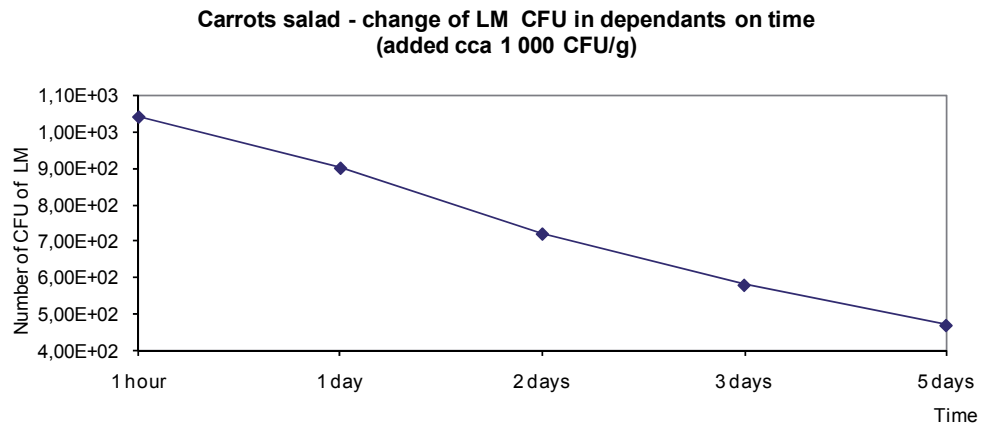
Shelf life: 4 days
Water activity a_w: 0. 959
Content of NaCl: < 0.29%
Preservatives content (mg/kg)

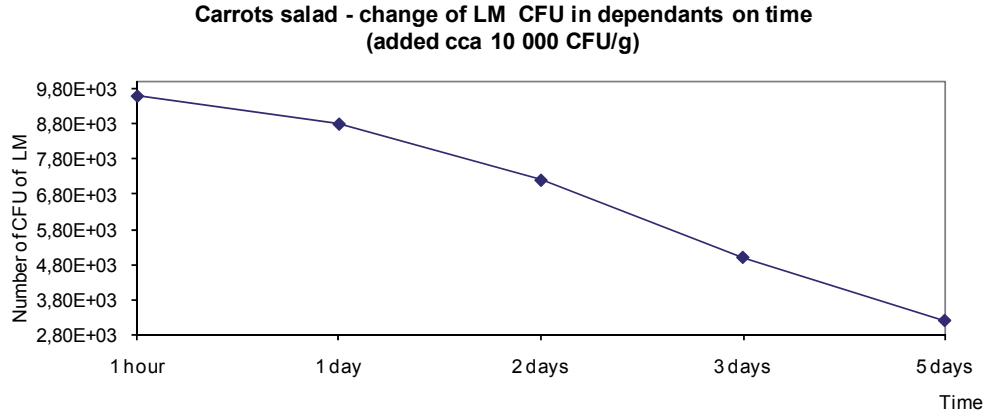
	start of experiment	end of experiment
Benzoic acid	22.21	11.36
Sorbic acid	6.17*	3.58*
Total	28.38	14.94

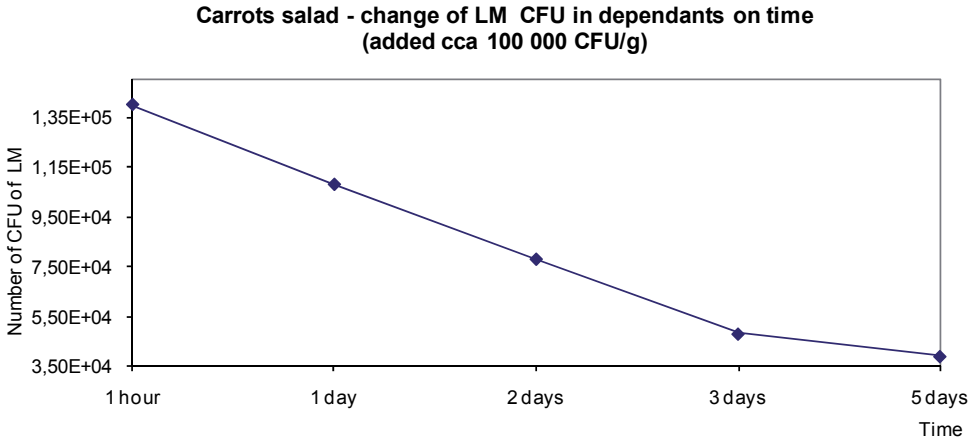
*Primarily total count examination revealed no growth of LM, after re-cultivation using growth supporting medium LM were found

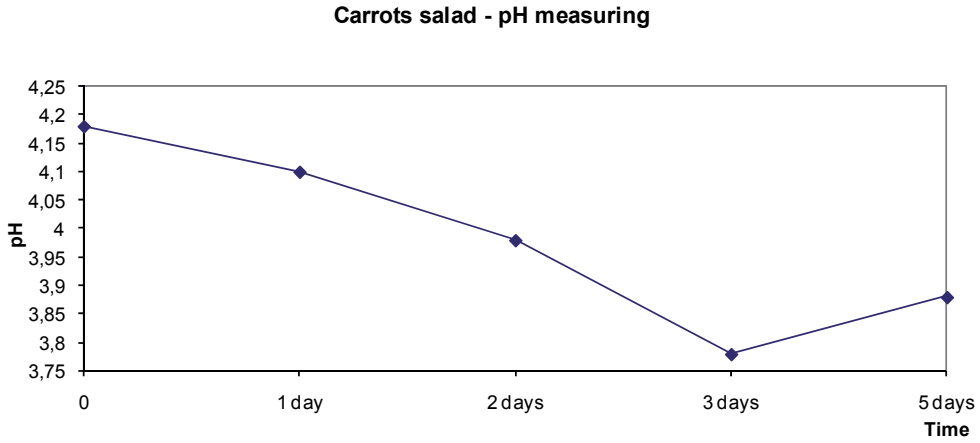

Carrots salad - change of LM CFU in dependants on time
(added cca 100 CFU/g)
Number of CFU of LM
1,10E+02
9,00E+01
7,00E+01
5,00E+01
3,00E+01
1,00E+01
-1,00E+01
1 hour
1 day
2 days
3 days
5 days
Time


Carrots salad - change of LM CFU in dependants on time
(added cca 500 CFU/g)
Number of CFU of LM
5,00E+02
4,50E+02
4,00E+02
3,50E+02
3,00E+02
2,50E+02
2,00E+02
1,50E+02
1,00E+02
5,00E+01
0,00E+00
1 hour
1 day
2 days
3 days
5 days
Time


Carrots salad - change of LM CFU in dependants on time
(added cca 1 000 CFU/g)
Number of CFU of LM
1,10E+03
1,00E+03
9,00E+02
8,00E+02
7,00E+02
6,00E+02
5,00E+02
4,00E+02
1 hour
1 day
2 days
3 days
5 days
Time


Carrots salad - change of LM CFU in dependants on time
(added cca 10 000 CFU/g)
Number of CFU of LM
9,80E+03
8,80E+03
7,80E+03
6,80E+03
5,80E+03
4,80E+03
3,80E+03
2,80E+03
1 hour
1 day
2 days
3 days
5 days
Time

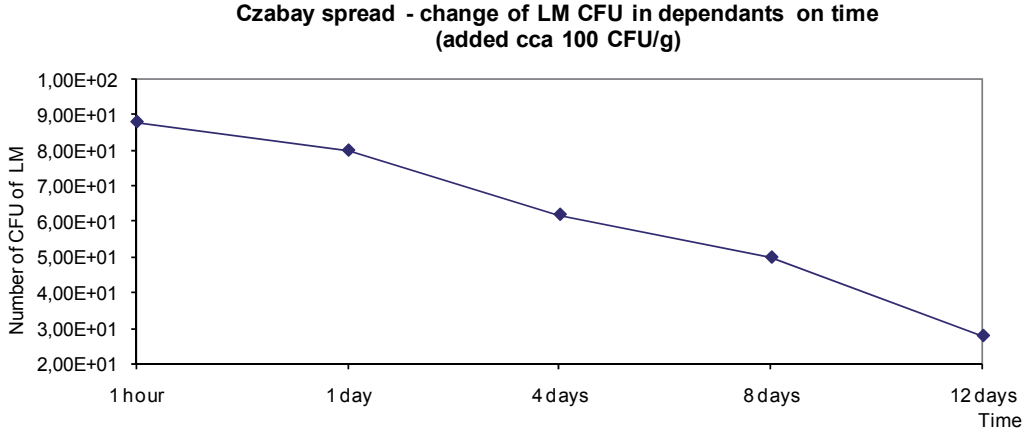


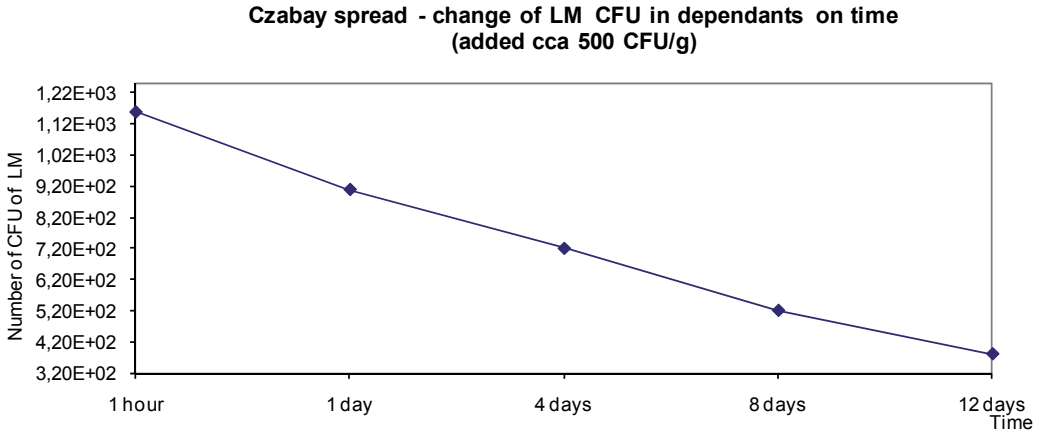


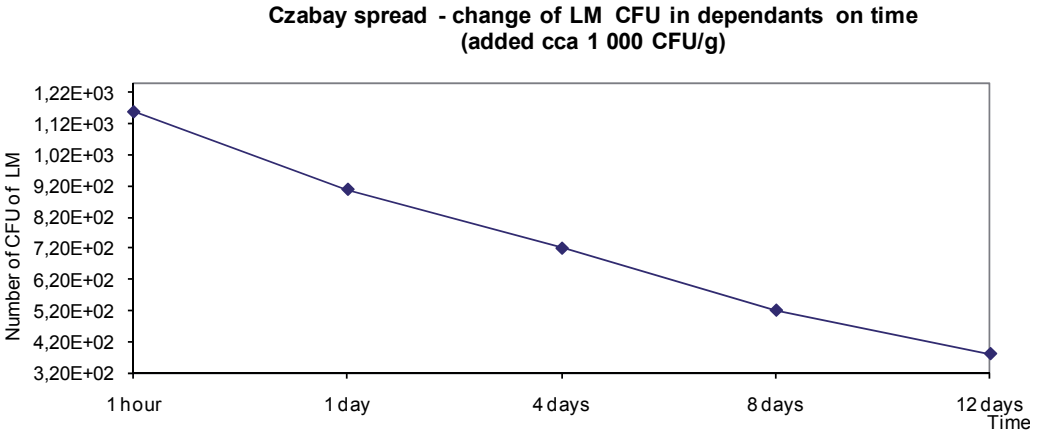
Sample of No. 3: Czabay spread

Shelf life: 11 days
Water activity a_w: 0.962
Content of NaCl: 1.75%
Preservatives content (mg/kg)

	start of experiment	end of experiment
Benzoic acid	380.8	368.97
Sorbic acid	360.8	341.1
Total	741.6	710.04




Czabay spread - change of LM CFU in dependants on time
(added cca 500 CFU/g)
Number of CFU of LM
1,22E+03
1,12E+03
1,02E+03
9,20E+02
8,20E+02
7,20E+02
6,20E+02
5,20E+02
4,20E+02
3,20E+02
1 hour
1 day
4 days
8 days
12 days
Time


Czabay spread - change of LM CFU in dependants on time
(added cca 1 000 CFU/g)
Number of CFU of LM
1,22E+03
1,12E+03
1,02E+03
9,20E+02
8,20E+02
7,20E+02
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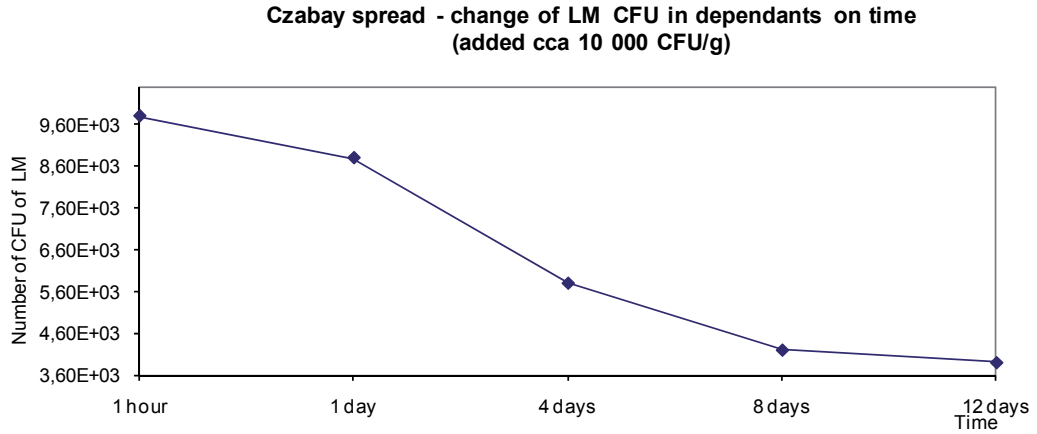
1 hour
1 day
4 days
8 days
12 days
Time


Czabay spread - change of LM CFU in dependants on time
(added cca 10 000 CFU/g)
Number of CFU of LM
9,60E+03
8,60E+03
7,60E+03
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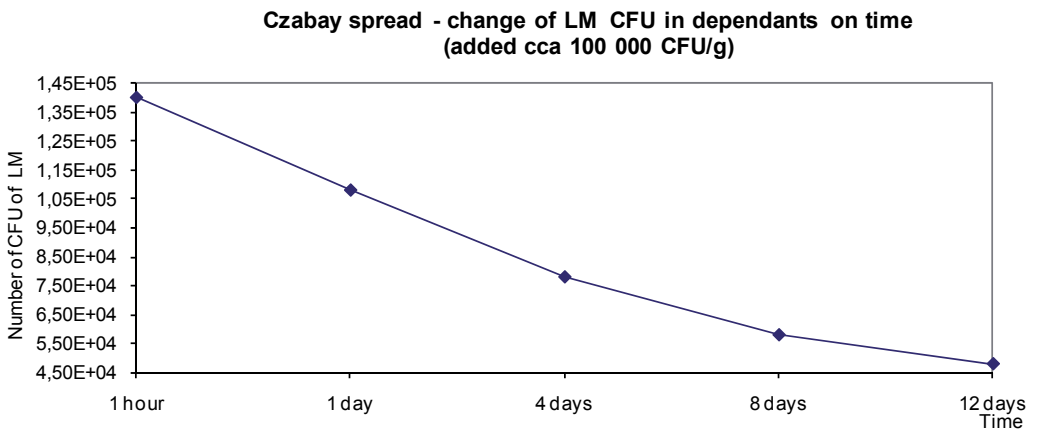
5,60E+03
4,60E+03
3,60E+03
1 hour
1 day
4 days
8 days
12 days
Time


Czabay spread - change of LM CFU in dependants on time
(added cca 100 000 CFU/g)
Number of CFU of LM
1,45E+05
1,35E+05
1,25E+05
1,15E+05
1,05E+05
9,50E+04
8,50E+04
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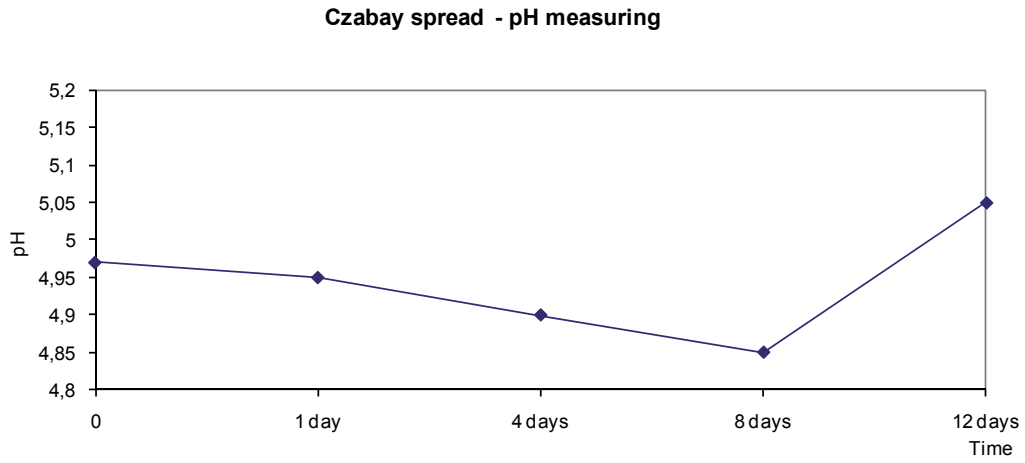
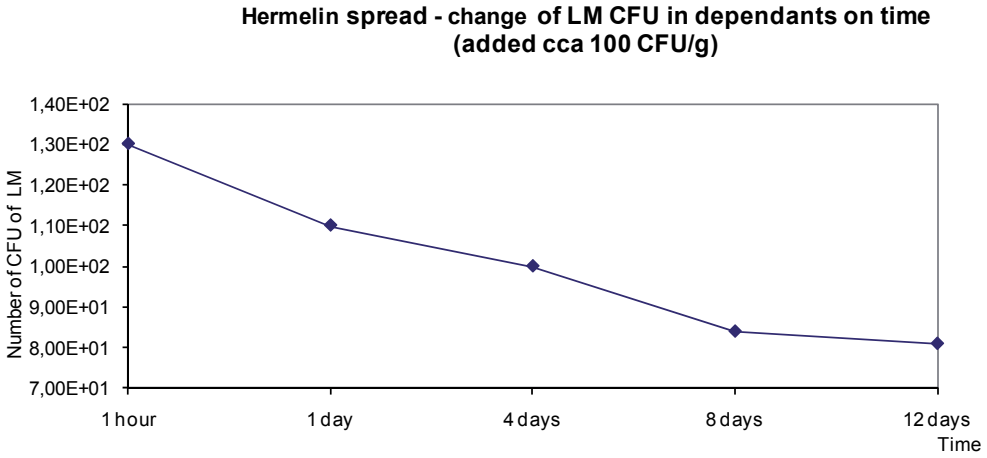
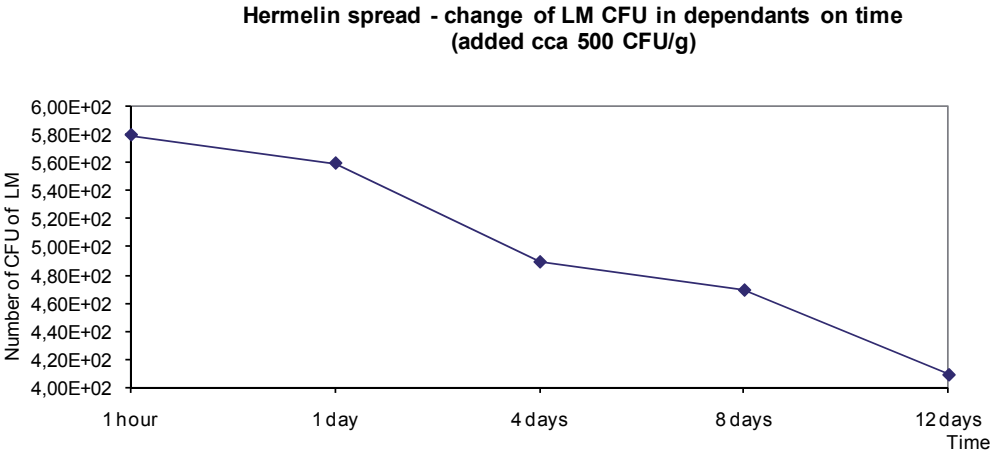
4,50E+04
1 hour
1 day
4 days
8 days
12 days
Time



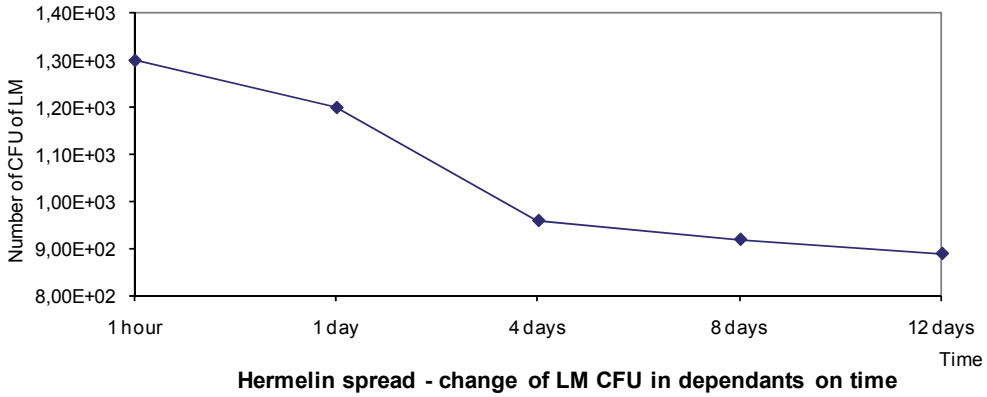
Sample of No. 4: Hermelin spread

Shelf life: 11 days	
Water activity a_w: 0. 967	
content of NaCl: 1. 64 %	
Preservatives content (mg/kg)	
Benzoic acid:	744. 4
Sorbic acid:	724. 2
Total	1468. 6

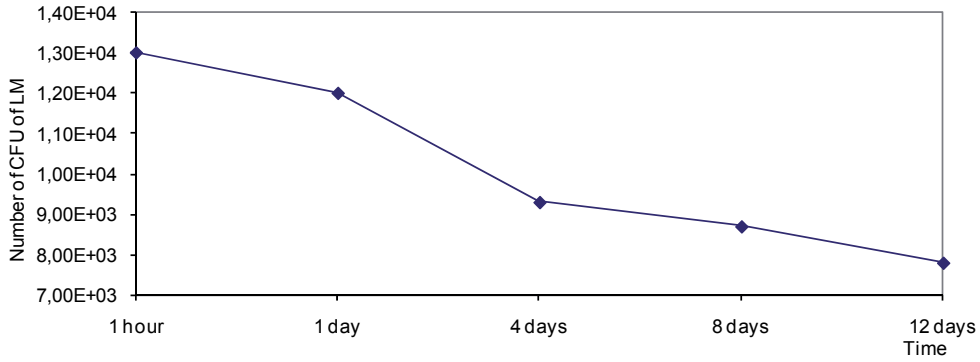




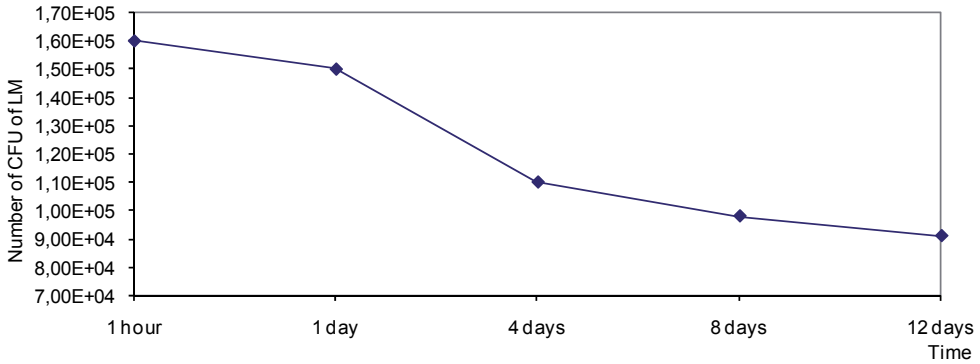
Hermelin spread - change of LM CFU in dependants on time (added cca 1 000 CFU/g)



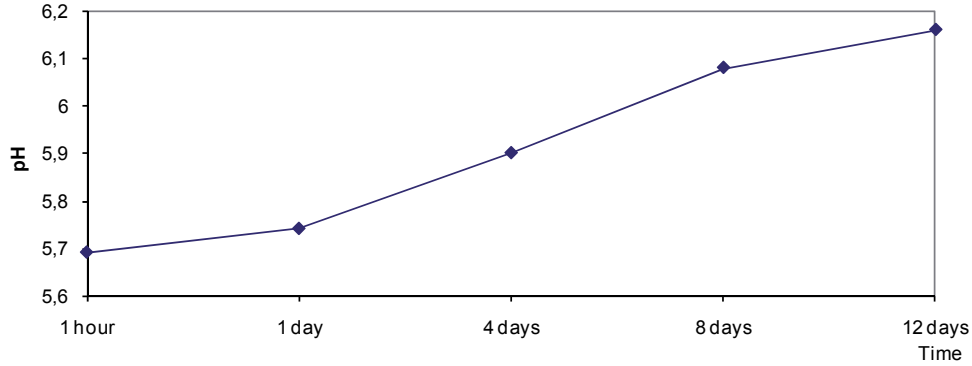
Hermelin spread - change of LM CFU in dependants on time (added cca 10 000 CFU/g)



Hermelin spread - change of LM CFU in dependants on time (added cca 100 000 CFU/g)

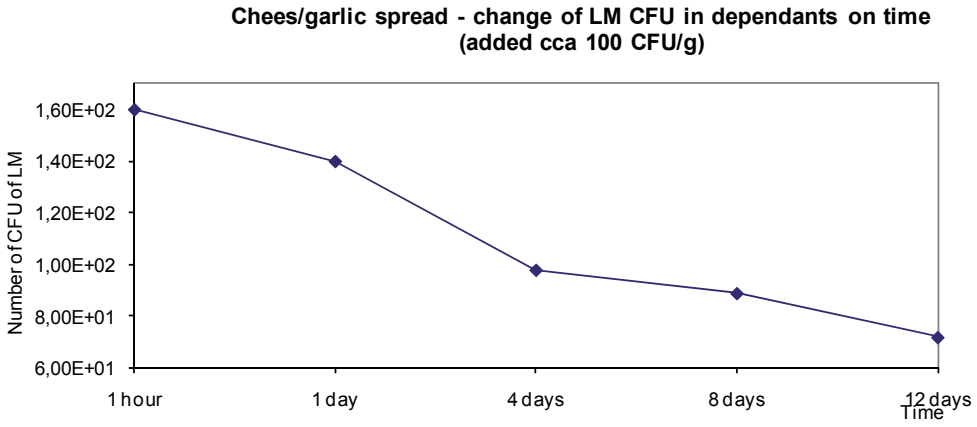


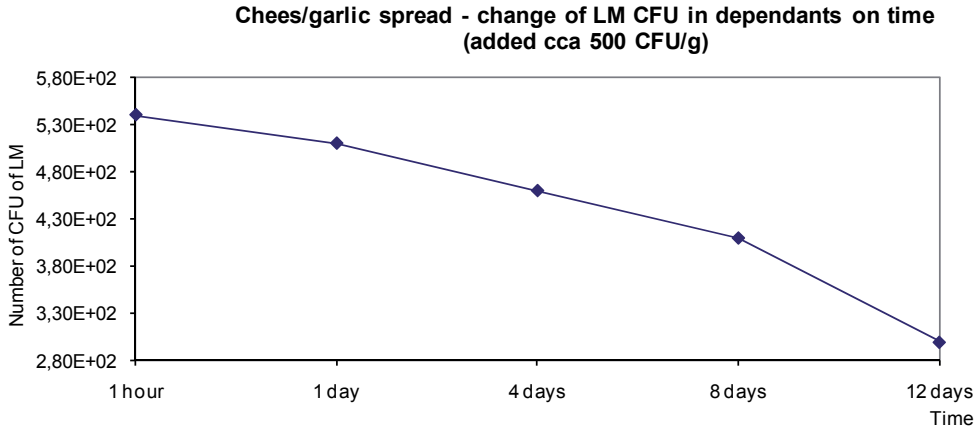
Hermelin spread - pH measuring

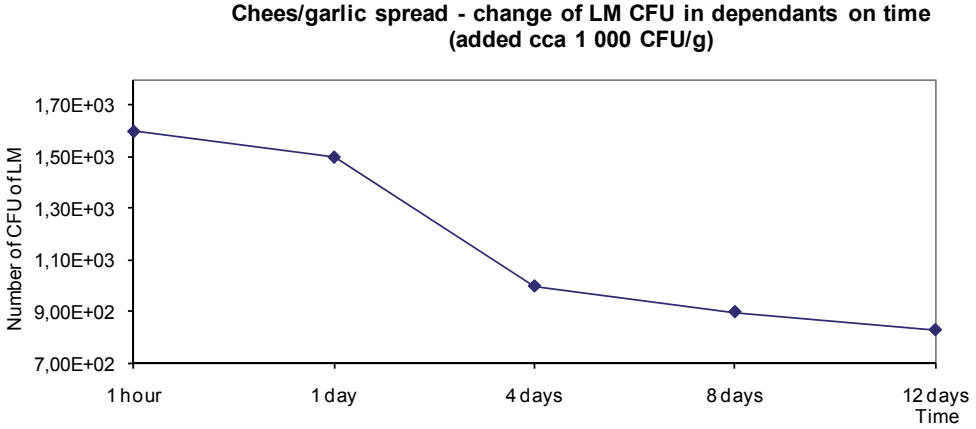


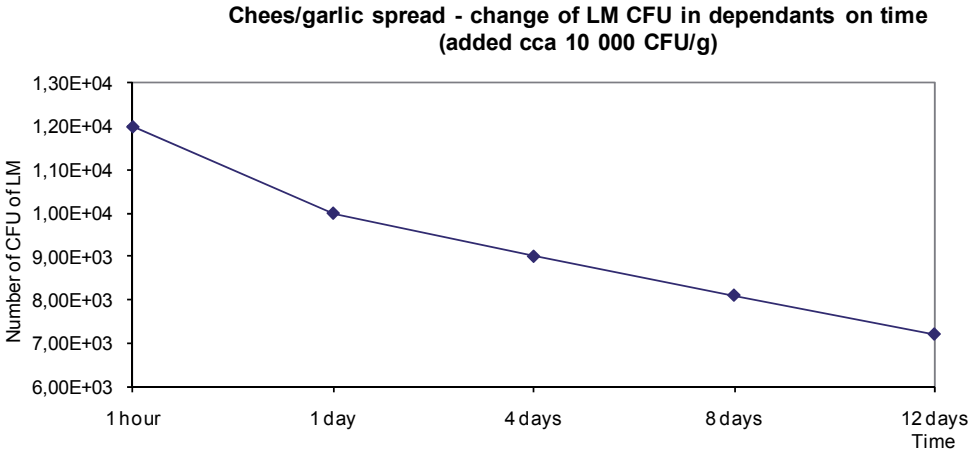
Sample of No. 5: Chees/garlic spread

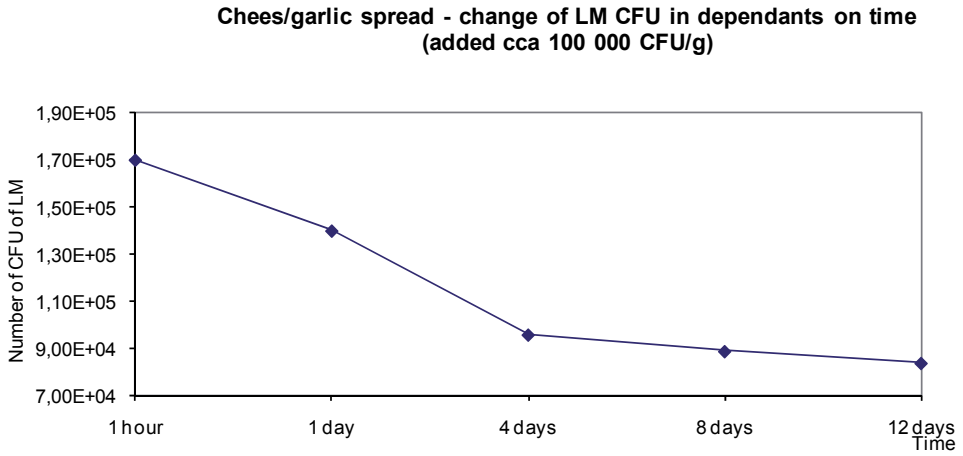
Shelf life: 11 days	
Water activity a_w: 0. 971	
content of NaCl: 1. 56 %	
Preservatives content (mg/kg)	
Benzoic acid	770. 6
Sorbic acid	658. 1
Total	1428. 7

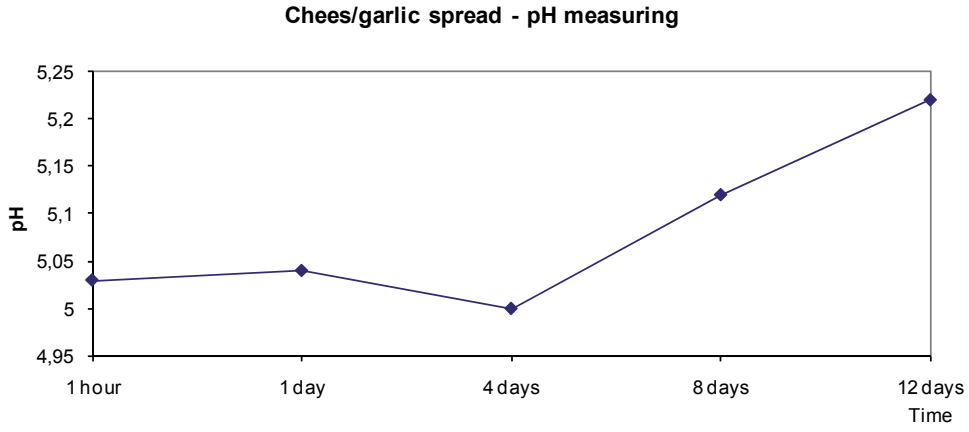






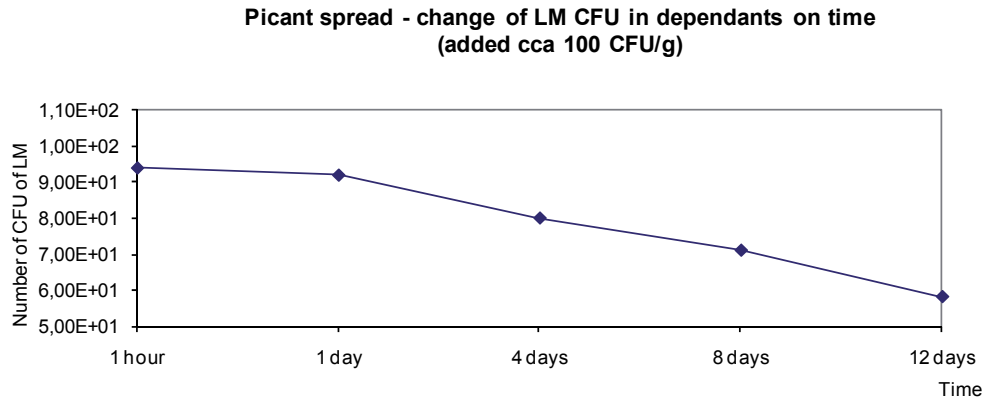


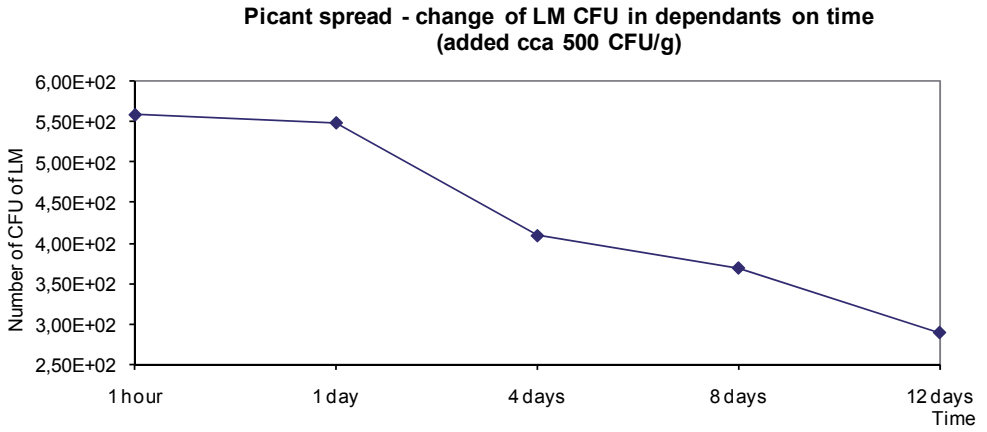


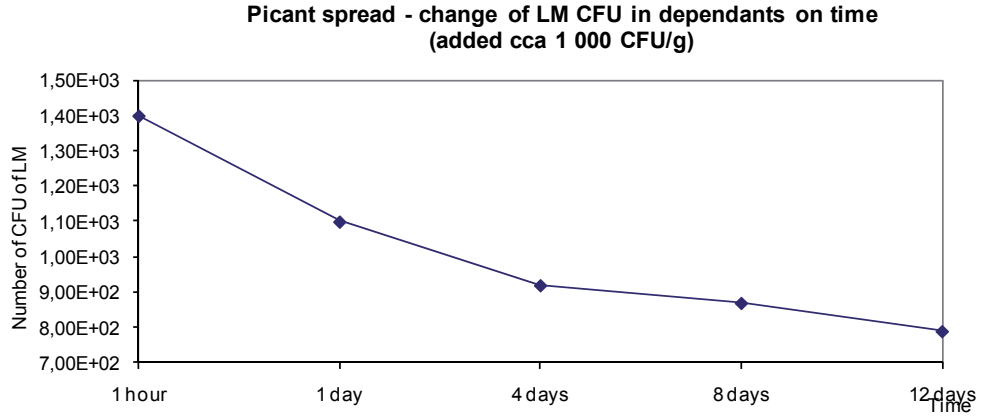


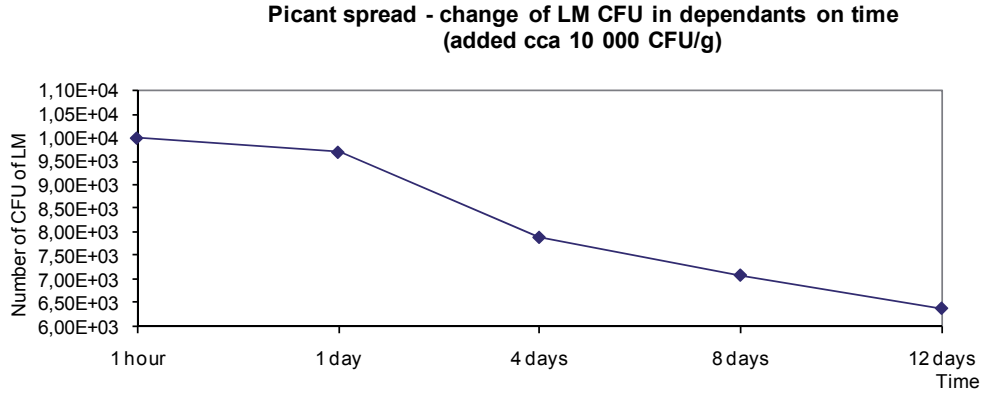
Sample of No. 6: Picant spread

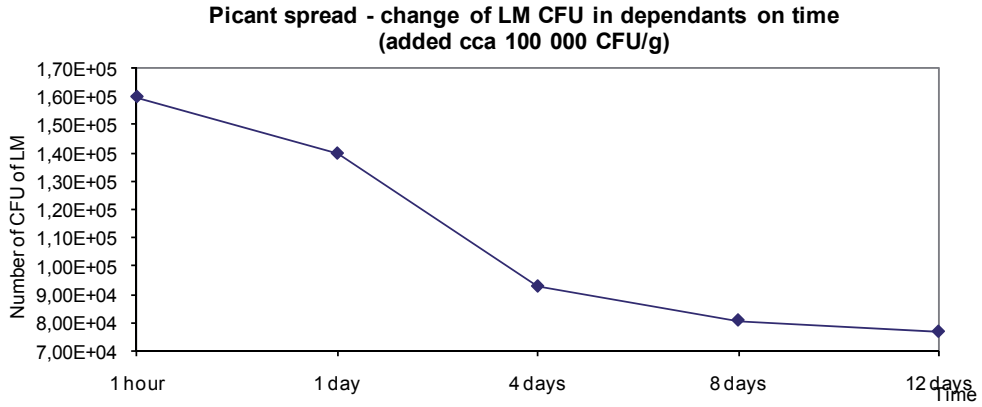
Shelf life: 11 days	
Water activity a_w: 0. 970	
content of NaCl: 1. 45 %	
Preservatives content (mg/kg)	
Benzoic acid:	590. 1
Sorbic acid:	886. 2
Total	1476. 3

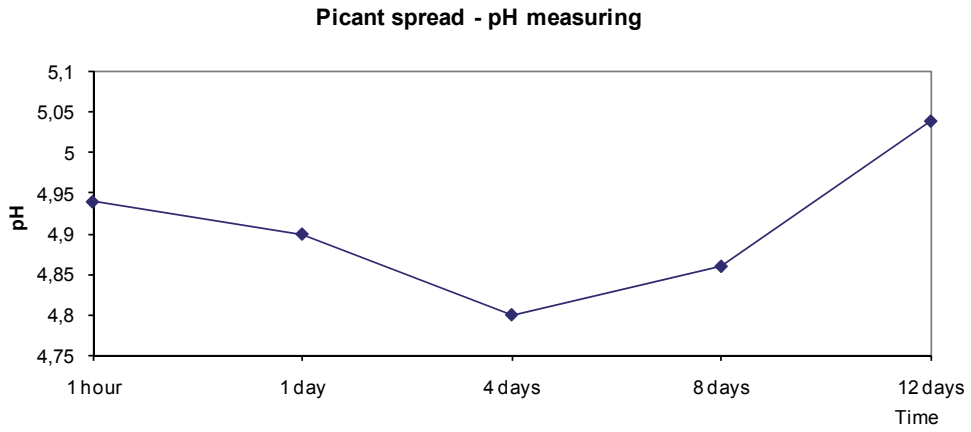

Picant spread - change of LM CFU in dependants on time
(added cca 100 CFU/g)
Number of CFU of LM
1,10E+02
1,00E+02
9,00E+01
8,00E+01
7,00E+01
6,00E+01
5,00E+01
1 hour
1 day
4 days
8 days
12 days
Time


Picant spread - change of LM CFU in dependants on time
(added cca 500 CFU/g)
Number of CFU of LM
6,00E+02
5,50E+02
5,00E+02
4,50E+02
4,00E+02
3,50E+02
3,00E+02
2,50E+02
1 hour
1 day
4 days
8 days
12 days
Time


Picant spread - change of LM CFU in dependants on time
(added cca 1 000 CFU/g)
Number of CFU of LM
1,50E+03
1,40E+03
1,30E+03
1,20E+03
1,10E+03
1,00E+03
9,00E+02
8,00E+02
7,00E+02
1 hour
1 day
4 days
8 days
12 days
Time


Picant spread - change of LM CFU in dependants on time
(added cca 10 000 CFU/g)
Number of CFU of LM
1,10E+04
1,05E+04
1,00E+04
9,50E+03
9,00E+03
8,50E+03
8,00E+03
7,50E+03
7,00E+03
6,50E+03
6,00E+03
1 hour
1 day
4 days
8 days
12 days
Time





DISCUSSION

As it has already been stated in results, quantities of sorbic acid in case of carrots and coleslaw salads, for the sake of information are reported only. The reason is, that such a low amounts are not ussually presented due to insufficient accuracy of detection method used. Results are reported as "lower than 10" i.e. under detection limit of apparatus used.

In case of carrots and coleslaw salads, number of inoculated L. monocytogenes substantially decreased, despite the fact, that concentrations of preservatives were considerably lower than in czabay spread. It may be attached to better penetration of preservatives into products containing high amount of water and chiefly, due to low pH of product. Decrease of pH under pH 4.0 as in case of carrots salad, in principle classifies the above product, in accordance with Regulation EC2073/2002, into products not supporting L. monocytogenes growth. This fact was also proved.

On the other hand, one of factors contributing to decrease and growth preclusion of L. monocytogenes may also be natural antimicrobial feature of used raw materials (Heisick, 1989).

In case of czabay spread, despite of expectation, higher pH increase, due to split components appearence, was not observed. Value of pH even plaucibly has been decreasing. Observed situation might be also caused by the use of starter microbial cultures preventing microbial spoilage.

Organic acids mentioned also in literature survey are used in salads and spreads manufactured due to their antimicrobial activities mainly. For increase of antimicrobial activities and at the same time for diminishing their influence on sensoric characteristics, combination of these matters is utilized. Some of them are manufactured as commercial products like PURAC, PURASAL and others. Bacteriocines are also a part of commercially prepared ingredients as Alta 2341, Fargan 763, Bionex ETA.

In order to assess their use, it is always necessary to take into consideration kind of foods and expected effects of preservatives.

From risk prevention point of view, rather problematic are non-homogenizable products (as open sadwiches) consisting of variously components, both animal and plant origin usually. These factors together with structure of products, their pH, a_w, use of additives and guar-

anted shelf life of products are as to Listeria growth, the most important factors. Therefore, the last part of survey to spreads was devoted. Spreads are more homonegized than salads and are expected to show higher pH due to ripening of certain components and also they show lower a_w than salads. They are therefore causing less suitable environment for microbes growth (Horniková, 2007).

Content of preservatives and persistence of their levels for the whole period of shelf life (11 days) as well as observation of their limits, are playing an important role in products wholesomeness (Annual Report ČZPI, 2008).

As to the samples tested, a_w levels between 0.965 to 0.974 were found, which means that they create suitable environment for growth of all germs, Listerias included (Bartl, 2004). As technology in spreads manufacture does not have an influence on a_w, this parameter was not followed any more. Salt content rating from 1.3 to 1.6 does not have negative influence on L. monocytogenes, therefore it also was not followed any more.

As it can be seen from tables, pH values were changing slightly, but they have not changed substantially, except of hermelín spread, where the increase from pH 5.5 to pH 6.1 was revealed. Even this pH is not for Listerias growth limited and its influence has to be enhanced by combination with other factors (Erban et al., 2004).

Results gained revealed, that technology using in spreads manufacture potassium sorbate and sodium benzoate, in stated limits, together with cold store temperature are causing substantial decrease of L. monocytogenes in tested products. The levels of preservation matters did not exceeded limits stated in Decree Ministry of Health 4/2008 Col.

In case of tested spreads, the above technology seems to be more effective than in salads. The decrease of L. monocytogenes was more rapid and at the end of span life, lower numbers of Listerias was found, when originally inoculated amounts decreased to half nearly.

CONCLUSION

It has been proven, that technology used for the manufacture of delicatessen products, with declared shelf life of 11 days, low a_w and high fat content and high a_w and low fat content, benzoic and sorbic acids may be used. The above preservatives not only prevented L. monocytogenes growth in selected delicatessen products but, were causing a substantial decrease of experimentaly inoculated L. monocytogenes amounts. Technologies of selected delicatessen products, in case if GMP is observed, will prevent L. monocytogenes growth.

Results also support industry demand asking for consideration of selected salads and spreads as to be, in compliance with Regulation EC 2073/2005, declared to be fit for human consumption in case of findings a less than 100 CFU of L. monocytogenes in products in producers premisses.

Valid standards of EU spell out the objective level of food safety performance that establishments must meet. However, they allow establishments to develop and implement processing procedures customized to the nature and volume of their production.

It is important to stress in this connection that in the course of experiments decrease of L. monocytogenes appeared in all delicatessen tested, from the time of their manufacture up to the end of their shelf life and one day more. Prolongation of shelf life was deliberatedly done, with the aim to prove, that a product is from Listerias appearence point of view safe after date of expire, which can happen in the house of consumer.

TeEted technology may serve as model for production of tested delicatessen products.

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