Review articles

IMPACT OF PRESERVATIVES USED IN SELECTED DELICATESSEN PRODUCTS ON *LISTERIA MONOCYTOGENES* SURVIVAL PART I

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Abstract

Epidemics of food borne listeriosis since 2006 became in ČR and EU a serious health problem. The aim of the study was to determine whether selected salads made by technologies using preservatives (benzoic acid and sorbic acid) may be cosidered according to Regulations EC 2073/2005 as sufficiently safe ones, not enhancing L. monocytogenes growth for the whole period of shelf life at least. For this reason correlation between inicial and final preservatives concentrations and numbers of CFU/gm was examined. The use of the above additives in tolerated limits proved substantial decrease in experimentaly inoculated L. monocytogenes amounts.

Key words: *Listeria monocytogenes*, delicatessen products, preservatives, EC Regulation 2073/2005

INTRODUCTION

Listeria monocytogenes as causative agent of food borne disease has been known from eighties when Schlech et al. (1982) reported the first outbreak of invasive L. monocytogenes illness to be epidemiologically linked to contaminated coleslaw, other fooods have also been associated with such outbreaks; this foods include soft cheese (Schuchat et al., 1992), raw milk, (Rorvik et al., 2000), hot dogs, (Schwartz et al., 1989), delicatessen meats, seafood and fresh vegetables (Miettinen et al., 1999).

Genus Listeria, at present time, represents 6 species (Vazquez-Boland et al., 2001) out of which *L. monocytogenes* is pathogenic for humans and animals, *L. ivanovii* is causing disease in sheep and cattle (Alexander et al. 1992), and exceptionaly in humans (Cummins et al., 1994, Chand, Sadana, 1999). All listeria species are widely spread in nature and in gastrointestinal tract of wild animals too. From risk management point of view it is important that alimentary disease is caused by primarily contaminated foods and raw materials of animal origin or by secondary contamination in proces of their manufacture and storage (Easter, 2007; Demnerova et. al., 2008; Pospisilova et al., 2007; Salva et al., 1995).

Listeriosis presents usually as septicemia, meningitis, or abortion, and occurs most commonly in neonates and immunosuppressed patiens. The disease may be either sporadic or epidemic (Fleming, 2009). There are a few casis of professional disease known in case of veterinary surgeons and butchers which were in direct contacts with diseased animals (Bedna at al., 1996; Sramova et al., 2000). EPIDAT data present in 1999 in the Czech Republic 13 cases of listeriosis but in 2008 there is increase to 99 cases and in January and February 2009 already 13 cases were registered (SZU Report, 2009). According to EFSA data, in the EU the highest incidence of listeriosis on 100 000 inhabitants revealed Denmark 0.9, Belgium 0.8, Finland 0.7 and Germany 0.6. Respond to this situation was EU Regulation EC 2073/2005 reviewed by Regulation EC 1441/2007 deviding on the basis of patogen etiology into three Groups: supporting and not supporting *L. monocytogenes* growth and for special medicial purposes and infants. Each category is liable to different microbial criteria as to *L. monocytogenes*. Foods supporting their growth must not exceed more than 100 CFU/gm, but before they are released for market, number of L. monocytogenes must not exceed zero incidence in 25 gms (samples collection aggregate 5×25 gms). Foods not supporting *L. monocytogenes* growth must not exceed limit of 100 CFU/gm. In the third category zero incidence of *L. monocytogenes* in 25 gms is allowed.

Delicatessen products below to risk foods together with non pasteurised products, smoked meat products, ripening cheeses, vegetables and not sufficiently thermicaly processed foods. Performance standards spell out the objective level of food safety performance that establishments must meet as GMP and HACCP system implementation. In delicatessen products as salads especially it is necessary to ensure that ingredients meet their technological requirements with respect to their organoleptic features as well as ensuring that ingredients do not adversely affect safety and stability of the finished products. Therefore an extention of HACCP known as Recipe Hazard Analysis (RHAS) (Zottola and Wolfe, 1981) may be found to be of value in assessing the role of ingredients with respect to salads quality and safety especially. Systems based on RHAS may be beneficial during formulation and it has to be stressed that a final point must be made with respect to formulation. Salad additives as preservatives may at certain circumstances be added unnecessarily and on the other hand avoidance of "redundant" additives is beneficial from an economic and often health viewpoint.

Taking into consideration the above facts, the use of preservatives in delicatessen salads is wellfounded. A low pH environment has an adverse effect on the growth of *L. monocytogenes* but it is not only the specific pH of the medium which is important but also the type of acid, temperature and other antimicrobial features which are present (Buchanan, Golden, Whiting, 1989).

Other factors, such as presence of salt used as preservatives may modify the effects of organic acids on *L. monocytogenes* (Houtsma et al., 1996; Kamat, Nair, 1996). However, it should be noted that the effects of organic acids are not always positive in term of food safety. Listeriae which are exposed to these acids and survive may repair themselves during storage at low temperatures and begin to multiply if other bariers are not present (Greer, Dilts, 1995; Palubo, Williams, 1994). In order to increase antimicrobial aktivity and to prevent changes in sensoric properties of preserved foods, combination of organic acids and their salts are used eg. PURAC, PURASAL. Among commonly used organic acids belong milk acid, ascorbic acid, sorbic acid, citric acid, acetic acid, propionic acid to name some of them. Their use must always be considered in relatin to kind of food, expected results and last but not least whether an additive is for certain food permitted and amount used does not exceed limits stated ni Decree MZ4/2008 Col.

Therefore cooperation of researchers with commercial enterprises is streamed to find out the most suitable technologies garanting production of harmless foods. Both theoretical and practical studies for this reason are performed (eg. AFSSA France, 2009) with the aim to seek optimal solution for particular food product. In the Czech Republic, there studies concerning preservatives by Brychta et al. (2007), Pařilová (2008) and Čeřovský (2009) were performed, to mention at least some of them.

Also target of this study was to assess technologies using benzoic acid and sorbic acid in manufacture of salads for prevention of *L. monocytogenes* growth. Examined and designed technology has to garantee that in products, providing that GMP is observed, no growth of *L. monocytogenes* will appear. In this case demands of Regulation EC 2073/2005 concerning performance of studies proving that food may be considered as not risky and safe will be realizable.

MATERIALS AND METHODS

In the first part of study three kinds of salads "vlašský" salad, potatoes salad and "hermelín" salad were used. Samples were taken six times for six consecutive days. Randomly selected original wrappings (containers a 250 gms) intended for retail with declard shelf life 11 days were used. Samples were transported to laboratory in cool boxes and in laboratory stored in refrigerator under 8°C. Stock of *L. monocytogenes* originating from the Czech Microbes Collection Institut in Brno was used for inoculation. Examinations were performed in accordance with ČSN EN ISO 290-1 for diagnosis of *L. monocytogenes* and ČSN ISO 11290-2 for total count of Listeria 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 was used. Meassuring of 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 aktivity (aw) by accredited method with the use of Thermoconstanter TH-2 (Switzerland) was performed.

Contents of preservative matters by HPLC method using HPP5001 apparatus with detector LCD 2082 ČR were 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 gms 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, aw, 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, 4 days, 8 days, and 11 days after manufacture of salads.

RESULTS

In order to prevent unnecessary lengh of article with giving details, crucial results are presented in Figures 1–6.

Fig. 1: Overall graph – quantity of added colony of LM cca 100

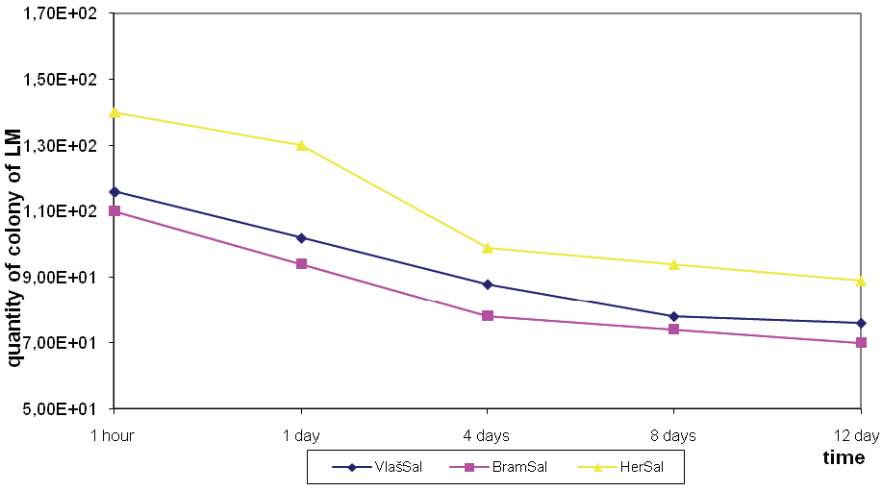


Fig. 2: Overall graph – quantity of added colony of LM cca 500

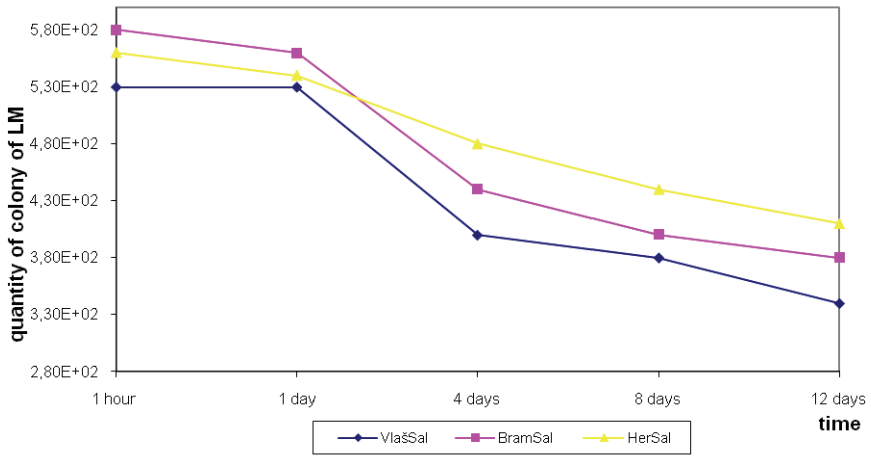


Fig. 3: Overall graph – quantity of added colony of LM cca 1 000

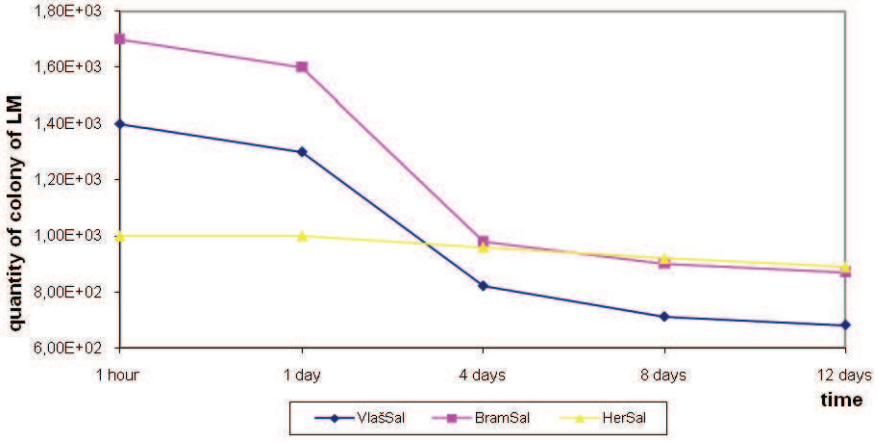


Fig. 4: Overall graph – quantity of added colony of LM cca 10 000

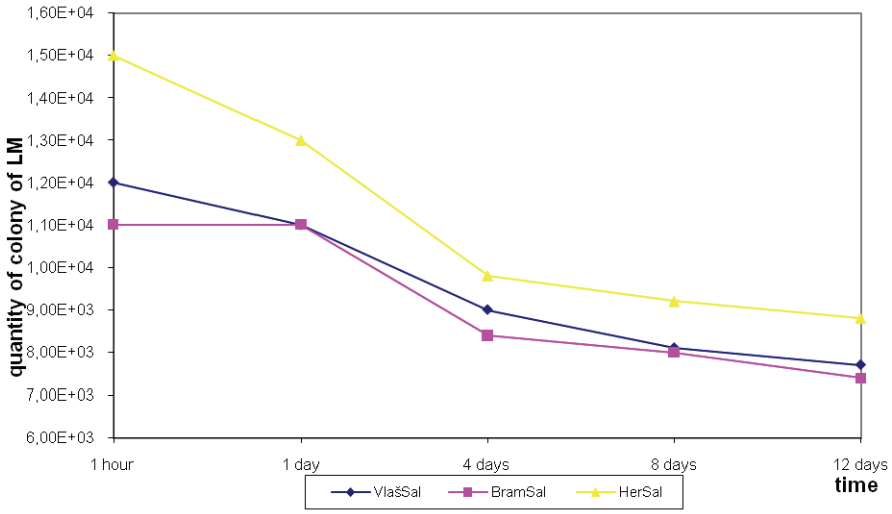


Fig. 5: Overall graph – quantity of added colony of LM cca 100 000

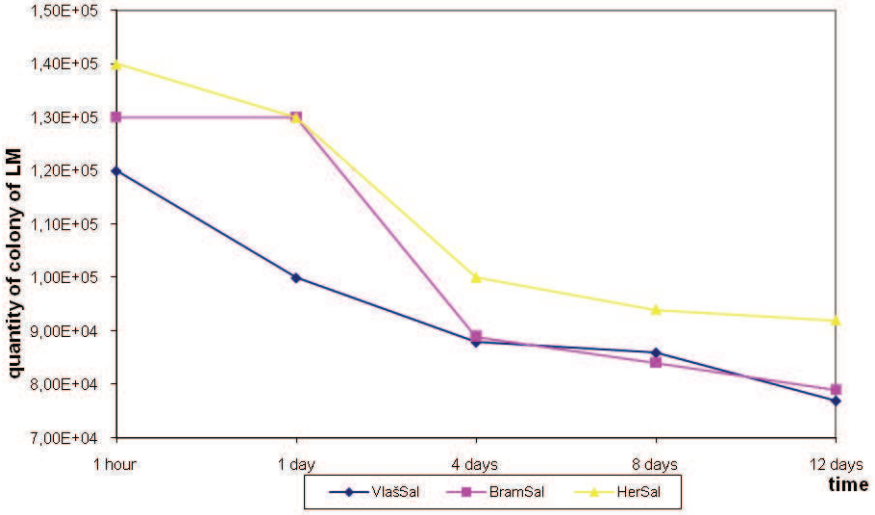
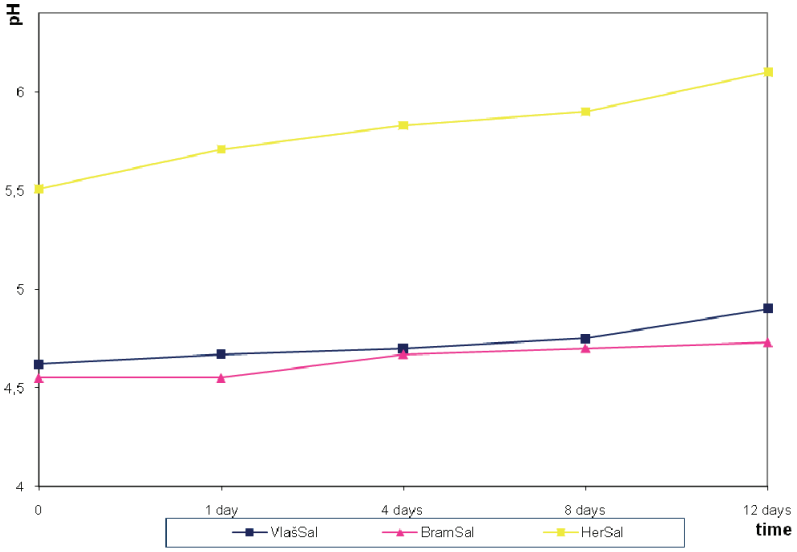


Fig. 6: Overall graph of PH



Legend: "Vlašský" salad = VlašSal, 2. Potatoes salad = BramSal, 3. Hermelínový salad = HerSal

DISCUSSION

Prepared salads typically consist of chopped vegetables in either a mayonnaise or oil and vinegar base. Such product is retailed in pre-packed containers or from bulk. Care is needed when formulating salads because interaction between ingredients may create conditions suitable for growth of potentially pathogenic microbes. Coleslaw eg. has been implicated as the origin of an outbreak of adult and perinatal disease caused by *L. monocytogenes* (Schlech et al., 1983). In addition to ascertaining that the product formulating inherently meets the required conditions it must be ensured that in practice the formulation is correctly produced. This is likely to involve laboratory testing which should be decided upon at the design stage of the formulation. Results ensued from the study revealed following facts. Levels of water activities in tested products ranged from 0.965 to 0.974. Growth of *L. monocytogenes* was found to be optimal between aw 0.93–0.95 (Bartl, Erben, Herink, Neužilová, 2004). From results obtained in the study it is obvious that salads create suitable conditions for growth of all microbes, *L. monocytogenes* included.

Due to the fact that aw value by the technology used in tested salads manufacture, is not affected and therefore it will not have influence on microbes number, no attention was paid any more to its value.

Salt contents rating from 1.3 to 1.6% has not negative effect on *L. monocytogenes* and therefore in nocsequent examinations attention to NaCl was also not payed.

Value of pH in the course of examinations showed minute differencies, but it was not changed substantially. In case of hermelín salad higher increase of pH value was noted, compared to other two products. Increase of pH value from 5.5 to 6.1 of the above product was noticed at the end of shelf life and it might be caused either by the action of microflora inherent to hermelín cheese (made in principle by the same method as camembert), or it might be pertinent to decomposing processes resulting from hermelín composition. As L. *monocytogenes* is substantially tolerant to pH, it is obvious that pH values only, in examined products, did not inhibit listeria growth significantly. Achieved results showed that technology using preservatives potassium sorbate and natrium benzoate in amounts stated in technical norms of products, together with refrigerator temperature are causing substantial decrease in *L. monocytogenes* growth. It accords with some literature data obtained in different observations (Vařejka et col., 1989; Marth, 1988).

Testing for preservatives used confirmed that their sum has not exceeded limit stated in Decree MZ 4/2008 Col.

Experiments revealed that growth of *L. monocytogenes* for the whole period of shelf life was decreasing in the course of first 24 hrs. Their decrease was observed, whereas the highest growth's decrease 4th day and at the end of shelf life was observed. Taking into cinsideration the aim of study, crucial conclusion is that decrease of *L. monocytogenes* was observed in all products tested, starting from first day up to one day after expiration of shelf life. Examination beyond the shelf life was done purposly due to possible delay in consumption by costumers. Examined technology guarantees no growth of *L. monocytogenes* in selected products and choise of technology is correct.

CONCLUSION

The aim of study was to prove that designed technology of delicatessen salads manufacture prevents, under stated conditions, *L. monocytogenes* growth in selected products, which may be considered as not supporting growth of *L. monocytogenes.* Results are a part of complex study of delicatessen salads and spreads and vegetable salads, aiming to find out appropriate technologies enabling safe, expeditive and economic approach to their manufacture.

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