INHIBITION OF MICROBIAL GROWTH OF A SALAD WITH MEAT IN MAYONNAISE BY DIFFERENT PACKAGING TECHNOLOGIES

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Abstract

The objective of the research was the shelf life extension of a salad with meat in mayonnaise. The microbial stability of the salad with meat in mayonnaise packaged under vacuum, in modified atmosphere, and using *'Sous vide'* technology was examined and compared with conventionally packaged (in ambient air) salad. The salads were packaged in polyamide/polyethylene pouches with barrier properties as well as in pouches made from biodegradable plasticized poly-β-hydroxybutyrate or polylactic acid film. Control samples without preservatives were packed in traditional polypropylene containers covered with non-hermetic lids. PURASAL Powder Opti Form (sodium lactate) in the amount 5 g kg^{-1}, 10 g kg^{-1}, and 15 g kg^{-1}, and ethyl alcohol in the amount of 2 ml were used as single preservatives for separate samples. The studies of the samples were carried out after 1, 3, 7, 10, 15, 18, 25, 29, 42, and 52 storage days at the temperature of +4±0.5 °C. Experimentally the quality of the salad with meat in mayonnaise was characterized by measuring the total plate count of microorganisms. The obtained results indicate that different packaging technologies and addition of preservatives show significantly different influence on the microbial growth in different samples. *'Sous vide'* packaging technology was effective for the shelf life extending and guarantee of safety of the salads with meat in mayonnaise.

Key words: shelf life, packaging, total count of microorganisms, meat salad.

Introduction

Food preservation has been a long time desire of human beings. The day when prehistoric humans discovered fire, it started significant developments in food preservation. The humans used indigenous methods of food preservation such us pickling, oiling, and salting of different food types, whether raw or processed. Some of the earlier techniques are still in use and are available in several commercial formats. The major developments and needs in food processing and preservation started during the wars, when extended shelf life of foods became a necessity. As a matter of fact, several food processing techniques such us ready-to-eat food in pouches, aseptic packaging of milk and liquid foods with particiles, and ohmic electric resistance heating of foods were developed to achieve extended shelf life of foods for soldiers in wars. A transfer of technology occurred when consumers started demanding a food product with fresh-like characteristics, along with extended shelf life. The food market increased and consumers became more and more educated about adding food preservatives and their adverse effects on long-term health (Geeraerd et al., 2000; Juneja, 2006).

Nowadays food industries need to become more efficient while simultaneously satisfying the consumer's growing demand for yet higher quality in food. Considering all the small units producing these products, it is essential to ensure that the products are safe for the consumers (Nissen et al., 2002).

The market for salads with meat in mayonnaise has become popular in the recent years in Latvia. In the 1980s such kind of salads was popular only in coffee-bars. In the 1990s it was possible to find only some kinds of no packed salads on the market, but in 1996 the first salads in packaging boxes came appeared at the market showcases. The packaging boxes were polyethylene (PE) or polypropylene (PP) trays with non hermetical lids. The safety of those products was limited (Levkane et al., 2008).

Food safety and security are significant issues in the food industry today. Companies which ones are producing salads with mayonnaise, increasing demand for new technologies to extend the shelf life and to keep/enhance the quality of different kinds of salads in mayonnaise. Nowadays, on the commercial network it is possible to find different modifications of salads in mayonnaise packaged in plastic containers with non-hermeticaly sealed lids, as well as salads packaged under vacuum or modified atmosphere (Levkane et al., 2008). An innovative approach to packaging that can increase shelf life of salads is necessary.

A perspective method to extend the shelf life of food

products is vacuum packaging and packaging in the protective gas mixture or modified atmosphere (MAP) in which content of oxygen has been reduced and carbon dioxide content – increased (Devlieghere et al., 2000; Robertson, 2006; Spenser, 2005). Food packaging in modified atmosphere side by side with vacuum packaging prolongs the shelf life for some days or weeks, maintaining food quality, taste, and aroma. Many research studies have demonstrated the antimicrobial activity of gases at ambient and subambient pressures on microorganisms important in foods (Nyati, 2000; Wang et al., 2004). The major safety consideration in extending shelf life of foods by MAP technology is the loss of sensory cues to spoilage provided by bacterial growth. Without spoilage bacteria indicators, it is conceivable that a food could have acceptable organoleptic quality, but be unsafe. The effect of loss of competitive inhibition by spoilage bacteria is most pronounced on the facultative anaerobic pathogenic bacterial populations in foods under altered atmospheres (Wang et al., 2004).

'Sous vide' is an interrupted catering system in which raw or precooked food is sealed into a vacuumized, laminated plastic pouch or container, thermal treated (pasteurized) by controlled cooking, rapidly cooled, and stored at 0 to +4 °C refrigerated temperatures. The product is sold in the same package in which the product was processed and stored (Church and Parsons, 2000; Geeraerd et al., 2000). Pasteurization time should be kept to a minimum to retain maximum quality in *'Sous vide'* products, while ensuring consumer safety. A time-temperature treatment must be determined for each product in order to render the product free from non-spore forming pathogens effectively. The *'Sous vide'* method produces food with a better flavour, colour, texture and nutrient retention than conventionally cooked foods (Church and Parsons, 2000).

Salads in mayonnaise belong to the group of high risk ready-to-eat foods and relatively few data have been published on the survival and growth of spore-forming bacteria in the products packed by several packaging technologies. Several guidelines give interpretation of microbiological analysis of some ready-to-eat foods (Food Safety authority of Ireland, 2001; Ohosone, 1997), but there is not mentioned that those products are salads in mayonnaise. Some studies (Nissen et al., 2002) declare results obtained from three-year experimental '*Sous vide*' packed ready-to-eat foods testing, and find that in the '*Sous vide*' packed ready-to-eat products the chances of survival and growth of pathogens seem very low since psychrotrophic, toxin-producing strains of bacillus or *Clostridium spp.* are rare or nonexistent. Therefore at low storage temperature the health risk of these products seems small, accordingly for microbial testing of the ready-to-eat end products, traditional plating will suffice.

A novel process for preparing pasteurized meat and vegetable containing salads in mayonnaise having a long shelf-life under refrigerated storage was proprietary (US Patent 4191787, 1980). This process includes essential steps of acid food treatment to pH 4.5 and short time bulk heat treatment before packaging at the temperature of +65 to +75 °C. US Patent 5114733 (1992) relates to a process for preparation of salad mixture with oil emulsion not requiring any preservatives. Prepared salad is placed in container and airtight closed, pasteurized under increased pressure, refrigerated, and storage time achieved in several weeks. Another finding (US Patent 5320856, 1994) informs about separate independent ingredients specific thermal stabilization treatments, cooling and after combination into the desired complex food article which is finally sealed in a package.

Ready-to-eat products need preservatives or mild pasteurization to give them a commercially acceptable shelf life. As natural preservative and antimicrobial substance could be used PURASAL® – produced from natural L(+) lactic acid E325 (sodium lactate), widely used to enhance shelf life and food safety of several food products. Formulating salads and dressings with PURAC FRESH (lactic-acid-based acidulants) results in products with a milder flavour maintaining microbial stability and safety. The slightly salty taste of PURASAL S, sodium lactate, enhances the meaty flavor of meat and poultry products (Jay et al., 2005; Meng and Genigeorgis, 1994; PURAC: The source…, http://www.purac.com/).

The aim of this study was to extend the shelf life of the salad with meat in mayonnaise. To determine the effect of vacuum, '*Sous vide*' and modified atmosphere packaging, and the effect of natural preservative and antimicrobial PURASAL Powder Opti Form (sodium lactate) addition on the shelf life and quality, as well as to obtain information about the microbial growth of the salad with meat in mayonnaise prepared for retail by different packaging technologies, refrigerated, and stored at the temperature of +4±0.5 °C.

Materials and Methods

Experiments were carried out at the Department of Food Technology of the Latvia University of Agriculture in 2008. The object of the research was salads with meat in mayonnaise. Salads with meat in mayonnaise produced for a local market were used for the experiments. The ingredients in the salads were boiled

potatoes and eggs, cooked beef, pickled cucumbers, and mayonnaise Provansa purchased on the local market. The characteristics of 18 investigated salad samples using different packaging and product treatment technologies are summarized in Fig. 1. Vacuum packed and '*Sous vide*' technology treated salads with meat in mayonnaise were placed in PA/PE film pouches with barrier properties thickness of 20/45 µm and size of 200 x 300 mm, as well as in pouches made from biodegradable, commercially produced in Brazil, plasticized poly–β-hydroxybutyrate (PHB) films with thickness of 65±2 µm, and polylactate (PLA) film thickness 40 µm, size 200 x 300 mm. Pouches were sealed by chamber type machine MULTIVAC A 300/16. For modified atmosphere, MAP (40% CO_2 and 60% N_2) packaging thermoformed ready-made polypropylene (PP) containers (size 210 x 148 x 35 mm) were placed in polyamide/polyethylene (PA/PE) film pouches with barrier properties of thickness of 20/45 µm. Pouches were sealed by chamber-type machine MULTIVAC A 300/16. As preservatives, PURASAL Powder Opti Form E325 (sodium lactate) in the amount of 5 g kg^{-1}, 10 g kg^{-1}, and 15 g kg^{-1}, and ethyl alcohol C_2H_5OH 96±1% in the amount of 2 ml were used for each packing. For '*Sous vide*' studies, the samples were pasteurized in a water bath – Clifton Food Range at the temperature of +65±0.5 ºC. Generally the treatment time was within 50 min, including warming up (15 min), holding time (20 min) while the core temperature of the sample +63±0.5 ºC was reached. The cooling occurred in two steps: with water from an artesian well at +10±1 ºC temperature for 5 min, which was follow by ice water cooling at +1 to +2 ˚C for 5 min (Muizniece-Brasava et al., 2007). As a control the salad with meat in mayonnaise without preservatives was packed in traditionally used polyethylene (PE) containers covered with non-hermetical lids. Mass of each sample was 200±1 g. All samples were stored in Commercial Freezer/Colder ELCOLD at +4±0.5 ˚C temperature (recorded by MINILog, Gresinger electronic) within 52 days under fluorescent light (OSRAM Lumilux De Luxe) with radiant fix at 100–800 lux (measured by Light meter LX-107). Throughout the storage period, the samples were randomly interchanged to minimize temperature fluctuations and light conditions. At each time of measurement, two identical packages for each treatment were randomly selected on sampling days 1, 3, 7, 10, 15, 18, 25, 29, 42, and 52, for analysis.

To define pH values, all samples of 200±1 g were homogenised with mixer BOSCH Easy Mixx 260. The pH values of the salad with meat in mayonnaise samples were determined by JENWAY 3510 pH-meter using an electrode JENWAY (3 mol/KCl). Two identical packages were analyzed on three randomly selected locations on each sample. To control the shelf life of salads with meat in mayonnaise, the total plate count test was performed by the methods of colony count technique at +30 ºC in accordance with International Standard ISO 4833:1991.

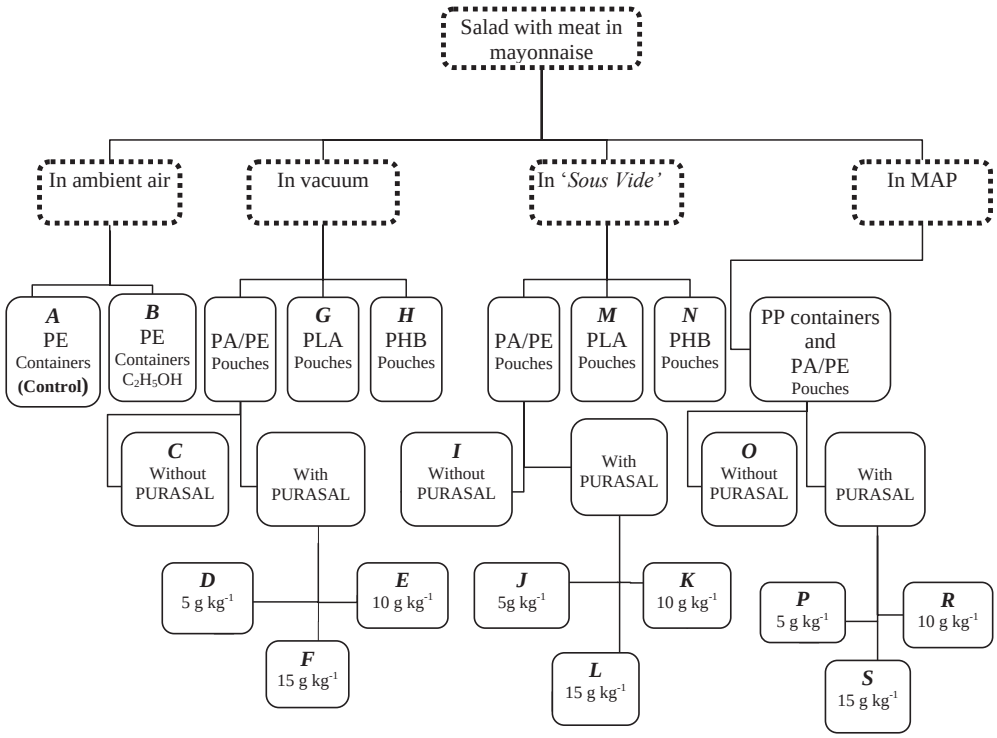

Figure 1. Structure of performed experiments.

A salad sample with meat in mayonnaise (amount of each 10 g) was removed from each package, placed in a stomacher bag, 90 ml of 0.1% peptone water were added and then homogenized with a stomacher (Bag Mixer 400) for three seconds. After preparing serial decimal dilutions of the homogenate with 0.1% peptone water, duplicate plates were prepared using pour plate method for enumeration. Total viable counts were determined on Plate Count Agar with incubation at +30±1 ºC for 72±3 h. After the specified period of incubation, each dish containing 15–300 colonies, the total count of microorganisms (CFU) was counted and multiplied by the dilution factor to determine CFU g^{-1} of the salad with meat in mayonnaise. The results were evaluated by Guidance Note No. 3, 2001 Guidelines for the Interpretation of Results of Microbiological Analysis of Some ready-to-eat foods Sampled at Point of Sale (Food Safety Authority of Ireland, 2001). According to the guidelines, salads are adapted to vegetable food group (prepared mixed salads and crudités) and meat food group (meat, sliced (cooked ham, tongue)) which belong to category D, accordingly could be acceptable up to 10^6 - 10^7 cfu g^{-1}. The results are defined like < 10^6 cfu g^{-1} satisfactory (means that the test results indicate good microbiological quality), 10^6 - <10^7 cfu g^{-1} acceptable (is suggested to take account of the limitations in microbiological sampling, handling, testing and wide confidence limits in enumeration - due to these factors, some samples will fall between what is considered satisfactory and unsatisfactory), and ≥ 10^7 cfu g^{-1} unsatisfactory (indicates that the acceptability threshold has been exceeded) (Food Safety Authority of Ireland, 2001).

The results were processed by mathematical and statistical methods. Statistics on completely randomized design were determined using the General Linear Model (GLM) procedure SPSS 16.00. Two-way analyses of variance ($p \leq 0.05$) were used to determine significance of differences.

Results and Discussion

The goal of this experiment was to substantiate the extended shelf life of salad with meat in mayonnaise for each of 18 investigated variants and find out the optimum shelf life as well as to get the information about microorganism growth in the examined packaging and treatment modes of the salads.

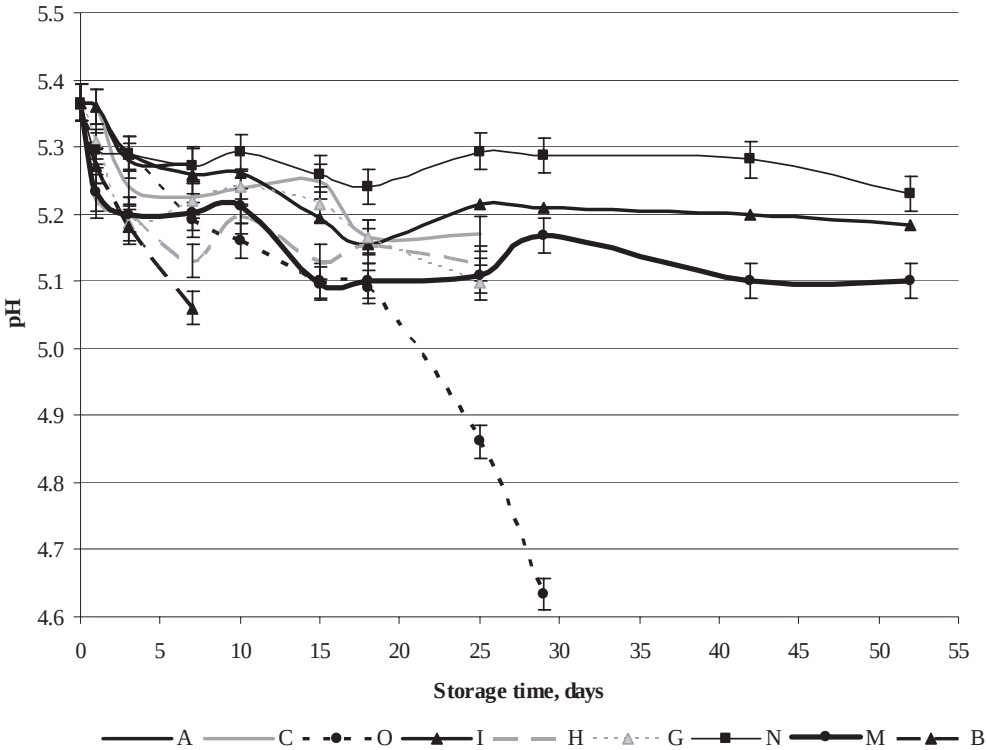

Figure 2. Dynamics of pH values at the storage time for the samples without PURASAL:

A – in ambient air (control); **B** – in ambient air, C_2H_5OH; **C** – in vacuum without PURASAL; **G** – in vacuum, PLA; **H** – in vacuum, PHB; **I** – in *'Sous vide'* without PURASAL; **M** –*'Sous vide'*, PLA; **N** –*'Sous vide'*, PHB; **O** –in MAP without PURASAL.

The pH value of a freshly prepared control sample was 5.3. Significant differences in pH values during the storage time of 52 days were observed among all investigated groups of the salad with meat in mayonnaise samples packed by different packaging technologies, using various packaging materials, pasteurization temperatures, and natural antioxidant additives ($p<0.05$). Whereas pH of the samples packed in MAP significantly decreased (from 5.3 to 4.6) already after 25 days of refrigerated storage at +4 °C. Significant differences in the pH values of the salads with meat in mayonnaise packed in pouches made from PHB and PLA composite materials, during the examined storage time from 25 till 52 days, were not found. The pH value of salads packaged under vacuum in pouches made from PHB and PLA films, decreased from 5.3 to 5.1, and using '*Sous vide*' technology – from 5.3 to 5.2 ($p<0.05$).

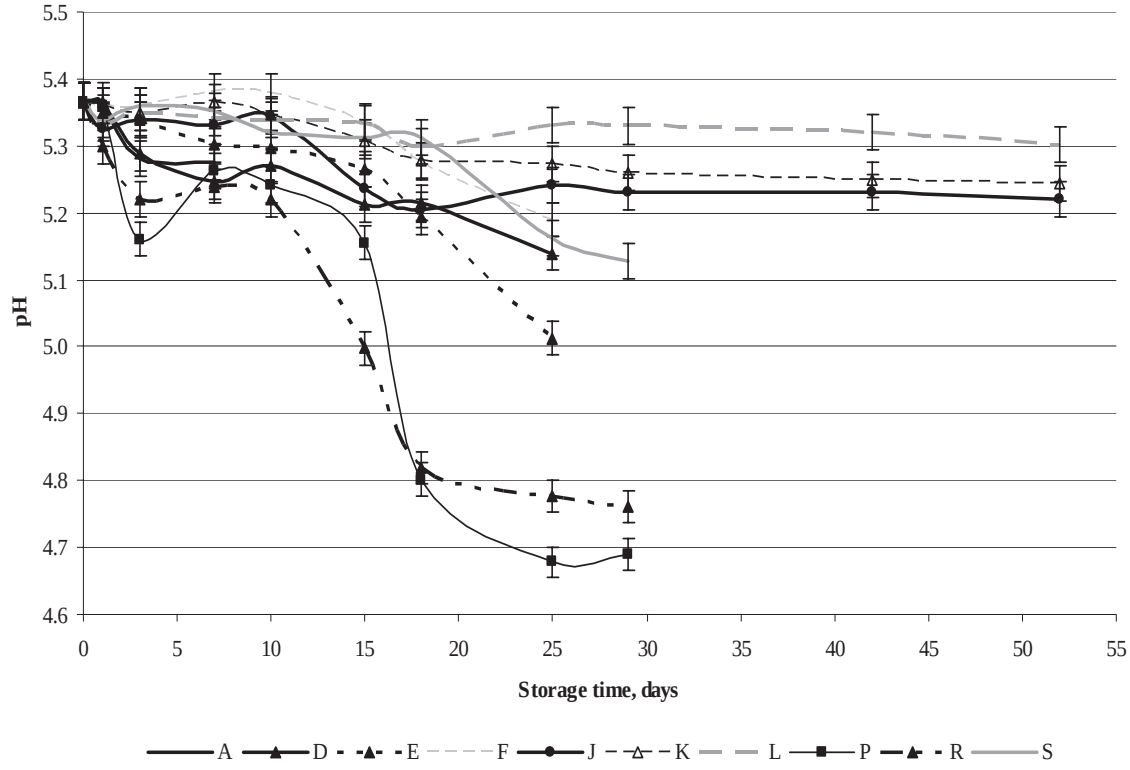

Figure 3. Dynamics of pH values at the storage time for the samples with PURASAL:

A – in ambient air (control); **D** – in vacuum with PURASAL 5 g kg^{-1}; **E** – invacuum with PURASAL 10 g kg^{-1}; **F** – in vacuum with PURASAL 15 g kg^{-1}; **J** – in '*Sous vide*' with PURASAL 5 g kg^{-1}; **K** – in *'Sous vide'* with PURASAL 10 g kg^{-1}; **L** – in *'Sous vide'* with PURASAL 15 g kg^{-1}; **P** – in MAP with PURASAL 5 g kg^{-1}; **R** – in MAP with PURASAL 10g kg^{-1}; **S** – in MAP with PURASAL 15g kg^{-1}.

Figure 3 shows the pH values after addition of PURASAL. The MAP technology in the amount of 5 g kg^{-1} and 10 g kg^{-1} decreased the pH values during the 29 day storage time of approximately from 5.3 to 4.7 ($p<0.05$), which has been recommended for successful shelf life extension to several weeks (PURAC: The source…, http://www.purac.com). Whereas in the pH of the samples packed in '*Sous vide*' with PURASAL in the amount of 5g kg^{-1}, 10g kg^{-1}, and 15g kg^{-1}, significant differences during the examined storage time till 52 days were not found (from 5.3 to 5.2). The pH of the salads with meat in mayonnaise in the vacuum technology with PURASAL in the amount of 5g kg^{-1}, 10g kg^{-1}, 15g kg^{-1} decreased only slightly - from 5.3 to 5.0.

The microorganism growth in salads with meat in mayonnaise was affected by the packaging methods (vacuum, '*Sous vide*' ,and MAP), as well as by addition of PURASAL (E325) and ethyl alcohol (C_2H_5OH) (Figs. 4 and 5). In order to evaluate the microbiological quality and acceptability of salads with meat in mayonnaise, in accordance with Guidance Note No. 3 (2001) they conform with prepared mixed vegetable salads as well as cooked meat which belongs to category D, accordingly could be acceptable till lg CFU 10^6–$<10^7$ cfu g^{-1}. The

principal spoilage mechanisms that limit the shelf life of cooked and processed meat products as ingredients of the salad in mayonnaise are microbial growth, colour change, and oxidative rancidity. Consequently, spoilage of cooked meat products appears due to post-process contamination by microorganisms as a result of neglecting manufacturing hygiene and handling practices.

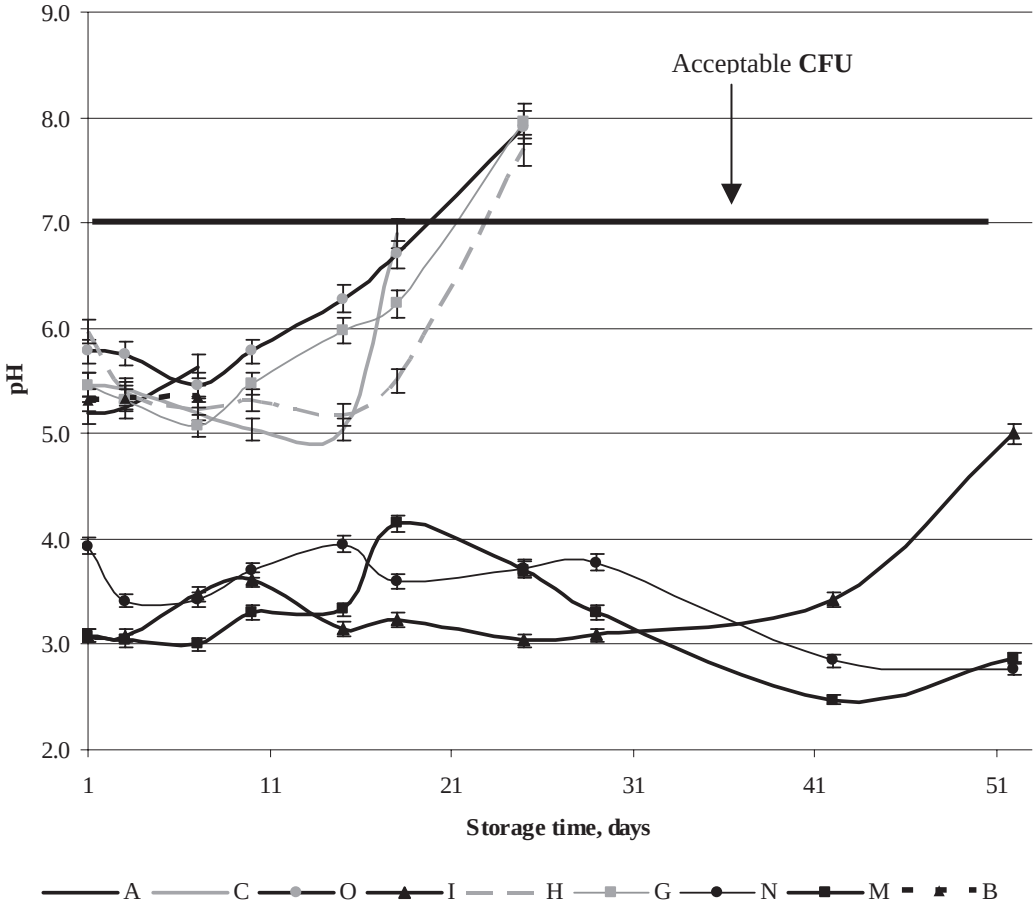

Figure 4. Microbial growth in the salads with meat in mayonnaise without PURASAL:

A – in ambient air (control); **B** – in ambient air, C_2H_5OH; **C** – in vacuum without PURASAL; **G** – in vacuum, PLA; **H** – in vacuum, PHB; **I** – in *'Sous vide'* without PURASAL; **M** – in *'Sous vide'*, PLA; **N** – in *'Sous vide'*, PHB; **O** – in MAP without PURASAL.

Packing foods in a modified atmosphere offers extended shelf life and improved product presentation in a convenient container, making the product more attractive to the retail customer. All investigated salad with meat in mayonnaise samples packed by different packaging technologies, using various packaging materials, pasteurization temperatures and PURASAL after one day storage show different results. Our experiments demonstrated that the storage time of vacuum and modified atmosphere (CO_2 - 40%; N_2 - 60%) packaged salads extends till 18 days, maintaining the quality of the salads by slowing chemical and biochemical deteriorative reactions and by slowing. or in some instances preventing, the growth of spoilage organisms. While the shelf life of control sample as well as of sample with ethyl alcohol addition packed in air ambiance is determined only seven days, experiment still need to continue. The thermal treatment process ('*Sous vide*') kills the vegetative bacteria cells and inactivates enzymes. For '*Sous vide*' it is important that cooking achieves at least a three-decimal reduction in CFU already after the treatment.

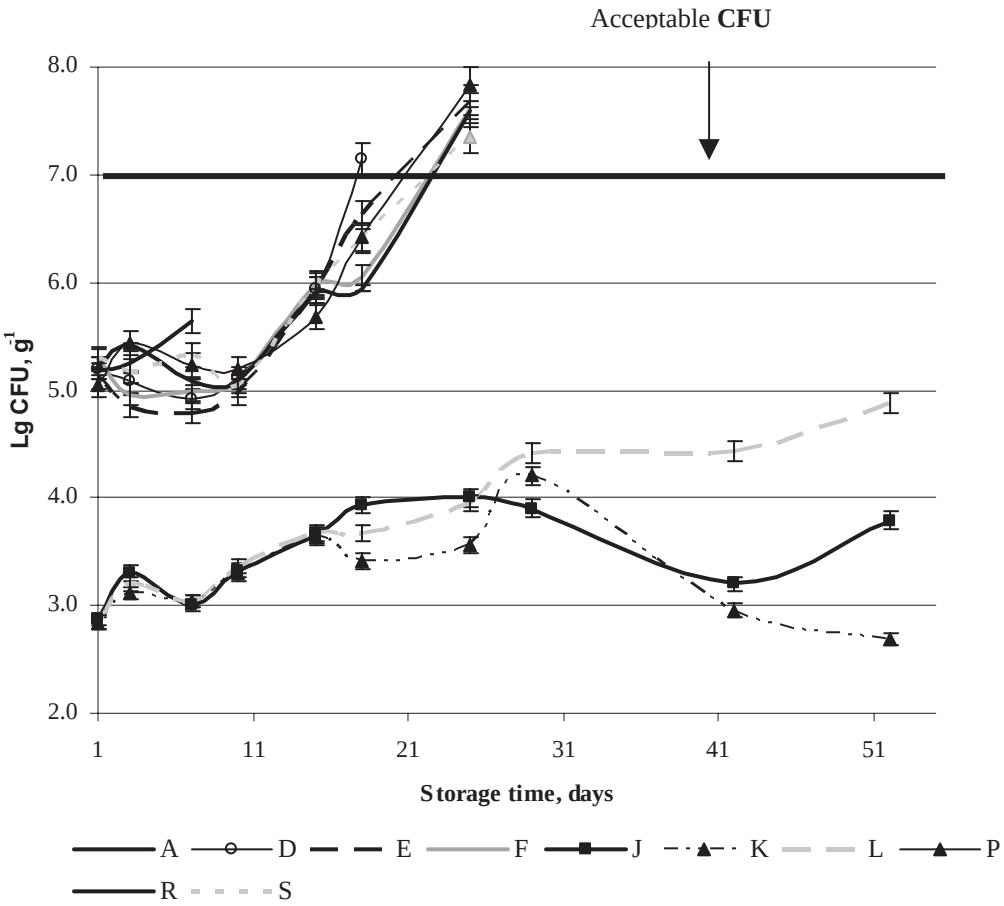

Figure 5. Microbial growth in salads with meat in mayonnaise with PURASAL:

A – in ambient air (control); **D** – in vacuum with PURASAL 5 g kg^{-1}; **E** – vacuum with PURASAL 10 g kg^{-1}; **F** – in vacuum with PURASAL 15 g kg^{-1}; **J** – in '*Sous vide*' with PURASAL 5 g kg^{-1}; **K** – in *'Sous vide'* with PURASAL 10 g kg^{-1}; **L** – in *'Sous vide'* with PURASAL 15 g kg^{-1}; **P** – in MAP with PURASAL 5 g kg^{-1}; **R** – in MAP with PURASAL 10 g kg^{-1}; **S** – in MAP with PURASAL 15 g kg^{-1}.

The results demonstrate that mild '*Sous vide*' treatment is an effective method to prevent microbial growth during all investigated periods of storage and enhances the shelf life of salads with meat in mayonnaise till 52 days. Packaging materials – PA/PE and environmentally friendly packaging plasticized PLA as well as PHB films - do not influence the microbial growth in meat salads with mayonnaise treated by '*Sous vide*' technology. Plasticized PLA and PHB films could be successfully used for '*Sous vide*' thermal treatment at the temperature not higher than +65±0.5 ºC, and the pasteurization effect is similar to the other obtained using conventional packaging films.

Conclusions

Significant differences in pH values were found among all the salad with meat in mayonnaise samples packed in different kinds of material and technologies during 52-day storage. The addition of PURASAL to MAP technology in the amount of 5 g kg^{-1} and 10 g kg^{-1}, decreased the pH values during the storage time of 29 days approximately from 5.3 to 4.7.

The shelf life of salad with meat in mayonnaise (cooked beef, boiled potatoes and eggs, pickled cucumbers, salt, and mayonnaise) was affected by packaging methods (vacuum, *'Sous vide'*, and MAP), as well as by addition of PURASAL (E325). Mild *'Sous vide'* treatment of salads retarded the total plate count.

Environmentally friendly plasticized PHB and PLA packaging films could be suggested for '*Sous vide*' thermal food treatment at the temperature not higher than +65±0.5 ºC.

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