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INFLUENCE OF PLANT EXTRACTS ON THE MICROBIOLOGICAL SHELF LIFE OF MEAT PRODUCTS

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Key words: spices and herbs, phenolic compounds, antimicrobial activities, meat products

The purpose of the study was to examine the possibility of applying different plant extracts as additives to meat products of the "meat-ball" type in order to extend their microbiological shelf life.

Experimental procedures included: the production of model meat products under laboratory conditions with the addition of 0.2% plant extracts (cranberry, rosemary, lovage); the storage of the products at a temperature of 10°C for 16 days; the microbiological evaluation of the meat-balls in terms of the total count of aerobic bacteria (analyses every 4th day) and pH examination (analyses also every 4th day).

The assumption of this study was that natural plant extracts are a group of additives having a considerable influence on growth inhibition of the microflora in meat products. It was concluded that the extract from rosemary was characterised with the strongest activity to microbes, which points to the longest shelf life of the products examined (13.3 days). During the entire storage period the most intense growth of microorganisms was observed in the control sample and the product with lovage addition.

INTRODUCTION

Food poisoning is still a concern for both consumers and the food industry despite the use of various preservation methods. Food processors, food safety researchers and regulatory agencies are continuously concerned with the high and growing number of illness outbreaks caused by some pathogenic and spoilage microorganisms in foods. The increasing antibiotic resistance of some pathogens that are associated with foodborne illness is another concern [Meng *et al*., 1998; Perreten *et al*., 1998; Stermitz *et al*., 2000]. Consumers are also concerned about the safety of foods containing synthetic preservatives. Therefore, there has been increasing interest in the development of new types of effective and nontoxic antimicrobial compounds. There is growing interest in using natural antibacterial compounds, such as extracts of spices and herbs, for food preservation [Smid & Gorris, 1999].

Spices and herbs have been added to foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives [Beuchat, 1994; Nakatani, 1994; Cutler, 1995]. In addition to imparting characteristic flavors, certain spices and herbs prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bacteriostatic or bactericidal activity, also to foodborne pathogenic bacteria [Beuchat & Golden, 1989; Shelef *et al*., 1980]. However, the extent of inhibition depends on the combination of spice, microorganism and further storage factors (temperature, humidity, preservatives, *etc*.) [Zaika, 1998]].

Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies [Smid & Gorris, 1999]. Being natural foodstuffs, spices and herbs appeal to many consumers who question the safety of synthetic food additives. Some spices and herbs used today are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities. The extracts of many plant species have become popular in recent years and attempts to characterise their bioactive principles have gained momentum for varied pharmaceutical and food processing applications. The antimicrobial activities of plant extracts form the basis for many applications, including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies [Lis-Balchin & Deans, 1997].

Many studies have reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant and pharmaceutical properties [Cai *et al*., 2004; Shan *et al*., 2005; Wu *et al*., 2006]. Some studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects [Hara-Kudo *et al*., 2004]. There has been no large scale systematic investigation of the relationship between bacterial inhibition and total phenolic content of spices and herbs. Previous studies [Cai *et al*., 2004; Shan *et al*., 2005] showed that a highly positive linear relationship exists between antioxidant activity and total phenolic content in some spices and herbs. However, there is no

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reported data on the relationship between antibacterial activity and antioxidant capacity of spices and herbs.

The purpose of the study was to examine the possibility of applying different plant extracts as additives to meat products of the "meat-ball" type in order to extend their microbiological shelf life.

MATERIAL AND METHODS

Material

Model meat products in the form of meat balls made under laboratory conditions, each weighing 50 g±1 g, were baked until reaching a temperature of 75°C inside the product. Ready-made products, after chilling to the room temperature, were packed in oxygenic atmosphere – every product separately – into welded pteroglutamic bags (the Stomacher Apparatus' bags, polythene, the thickness of 0.066 mms, penetrable for water vapour – 8.96 g/m^2/24 h±0.28, penetrable for oxygen – 888 cm^2/m^2/24 h) and stored at a temperature of 10°C for 16 days. The product contained beef from the hind (boneless) and pork from the blade (boneless), used in a ratio of 1:1; breadcrumbs (10% of product mass); UHT milk containing 2% of fat (100 mL per 1000 g of meat); onions (100 g per 1000 g of meat); and salt (1.2% of product mass). The meat was bought at one of the Warsaw supermarkets.

Ethanol and water extracts made from rosemary, lovage and cranberry representing 0.2% of the product mass were added to pulps obtained from the minced meat mixed with all the pulp raw materials. The amount of extracts added was determined in preliminary tests on the basis of a sensory evaluation. Plant extracts came from the Department of Physiological Sciences of the Faculty of Veterinary Medicine, Warsaw University of Life Sciences. Prior to being incorporated to the pulp, weighed amounts of plant extracts were crushed in a mortar with a small amount of milk (part of the milk to be added to the pulp).

Four study series were conducted. The product without any extract added constituted a control group.

Extract preparation

Plant extracts (rosemary, cranberry and lovage) used in the study were prepared in compliance with the following methodology: the herbs were ground and a mixture of 95% ethanol (7 volumetric parts) and water (3 volumetric parts) was added – the ratio between herbs and the mixture was 1:6; the tincture was kept in the darkness for 24 h and then filtered; the ethanol was evaporated in a vacuum evaporator in the atmosphere of nitrogen; the remaining water fraction was lyophilized; extract lyophilizates were stored at the temperature of – 20°C.

Microbiological study

A microbiological study was conducted with the use of a traditional deep plate method in conformity with the Polish Standard PN-85/A-8205. Determination was performed with respect to an overall number of aerobic microorganisms. Biocar Diagnostics-made nutrient agar was used as a medium for the study. At four-day intervals, 5 g of the weighed amount was sampled and put in a Stomacher bag and 45 mL of sterile fluid for dilution (Biocar Diagnostics peptone water) was added. Subsequently homogenization was conducted for two minutes at a standard speed in a Stomacher 400 apparatus. Following homogenization, a number of tenfold-diluted samples were prepared. Inoculation was carried out from three subsequent dilution of samples at a time, 1 mL for each group of three dishes. Next, the dishes were incubated at the temperature of 37°C for 48 h.

Testing pH levels

pH was measured on a model CP-501 Elmetron pH--meter, taking into account the ambient temperature. Product samples (10 g ± 0.1 g each) were taken and homogenized with 10 mL of distilled water. The homogenate obtained was used for the testing.

Statistical analysis

The statistical analysis of results was carried out using Microsoft Excel and GraphPad 4.0 statistical programs. The arithmetical mean and the standard deviation were calculated in Microsoft Excel. The other statistical (the linear regression analysis, the analysis of variance) calculations were executed on GraphPad.

RESULTS AND DISCUSSION

Analysis of microbiological results

Findings received in the testing conducted prove that the addition of plant extracts rich in polyphenolic compounds affects microbiological changes taking place in the course of meat product storage. Growth curves of the total count of aerobic bacteria in the meat-ball type products, depending on various additives, are presented in Figure 1.

The total bacteria count in stored products increased with the storage time. The number of microorganisms in initial samples ranged from 3.23 to 3.24 log cfu/g. On the last storage day, the total bacteria count in the ascending order,

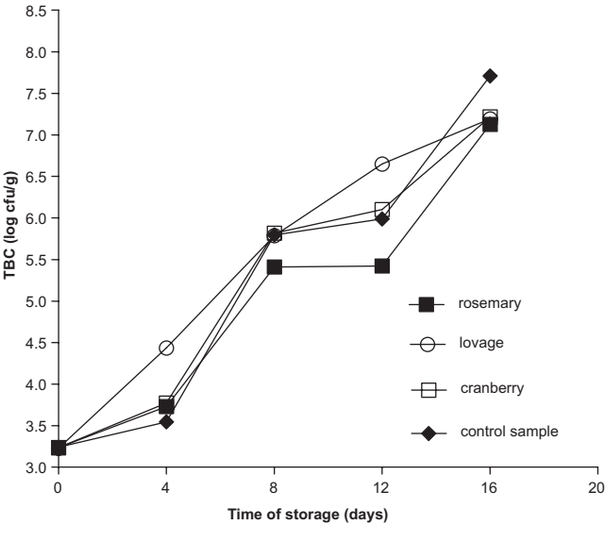

FIGURE 1. Growth curves of the total bacteria count in meat-ball products stored at a temperature of 10°C, depending on different plant extracts added.

amounted to: 7.13 log cfu/g for the sample with rosemary addition, 7.19 log cfu/g for the sample with lovage addition, 7.22 log cfu/g for the sample with cranberry addition and 7.71 log cfu/g for the control sample.

On the last day of storage, the number of microorganisms in all the samples exceeded the level admissible for meat products compliant with the Polish Standard PN-85/A-8205, *i.e.* 6 log cfu/g, and therefore they were not fit for human consumption.

The day on which the total bacteria count reaches the value of 6 log cfu/g, is deemed the end of a product shelf-life. The storage day on which that level has been reached and thereby the expiry date for meatball-type products containing various additives is presented in Table 1.

The longest shelf-life which was determined experimentally pursuant to the total bacteria count, *i.e.* 13.3 days, had the sample with rosemary extract added. The subsequent samples in terms of their shelf-life length were the control sample (12 days), the sample with cranberry extract (10.3 days) and the sample with lovage added (9 days).

The total content of polyphenols in extracts added is presented in Table 2.

The highest total content of polyphenols from among the extracts used was found in the preparation with rosemary (14.5 mg/g extract), which was also probably reflected in the test results. The samples with the addition of the rosemary extract exhibited stronger antibacterial properties than those with the addition of the cranberry extract (5.1 mg of polyphenols per gram of extract), and lovage extract (2.7 mg/g extract). The sample with the addition of the lovage extract had the poorest test results from among samples with the addition of natural plant extracts; it was additionally characterised by the lowest content of polyphenols. It can be concluded that the more polyphenols in an extract, the stronger its antimicrobial action.

Differences observed in the growth of microorganisms both in products with an addition of plant extracts and in the control sample without any additives, were not significant. Taking into account statistical data of the linear regression analysis and correlation between storage time and bacteria count, the most intense growth of microorganisms was observed in the control sample and the product with the addition of lovage (in both cases the correlation coefficient accounted for 0.93, while the determination coefficient R^2 – for 0.87) (Figure 2). In all the cases, the p value was statistically significant (it reached the value <0.0001). The storage time affected to a lesser degree the growth of microorganisms in products with the addition of rosemary and cranberry (R^2 amounted to 0.69 and 0.66 respectively, while R – to 0.83 and 0.81, respectively). Solely at the conclusion of the storage cycle, *i.e.* on the 16th day of storage, all the samples with extract addition were of slightly higher microbiological quality than the control sample.

Studies conducted in Germany [Grohs *et al*., 2000; Grohs & Kunz, 2000] with regard to possibilities of using spice mixtures with an objective of extending meat shelf-life have given positive effects. The studies were based on the fact that the effectiveness of antibacterial action of various spices had been known for a long time, and their application in microbiological stabilization of ground meat has been repeatedly acknowledged. Literature data report that spice mixtures may delay bacteria growth in fresh pork and beef [Grohs *et al.,* 2000]. In order to prove the antibacterial action of specific spices mixtures, their influence on bacteria growth rate in fresh pork was studied. The study results proved that due to the use of bacteria reproduction process in pork was not completely impaired but it was considerably delayed as compared with meat without condiments [Grohs & Kunz, 2000]. Besides, the antibacterial action of spice mixtures increased the longer the meat storage time was [Grohs *et al.,* 2000].

Research on pharmacological properties of rosemary (*Rosmarinus officinalis* L.) was conducted by Klemens [2004]. In the study he used oil of Rosemary (*Oleum Rosmarini*) and galenic preparations obtained from rosemary leaves: Tinctura Rosmarini and Extractum Rosmarini 50. The raw materials and preparations came from the plant Phyto Pharm Klęka

TABLE 1. Approximate shelf-life of meatball-type products, depending on the type of extract.

Extracts	Approximate shelf-life (days)
Rosemary extract	13.3
Cranberry extract	10.3
Lovage extract	9.0
Control (with no additive)	12.0

TABLE 2. The total content of polyphenols in the extracts added.

Ethanol and water extracts (0.2%)	The total content of polyphenols (mg/g extract)
Rosemary	14.5
Cranberry	5.1
Lovage	2.7

Source: Wilczak *et al.* [2001]

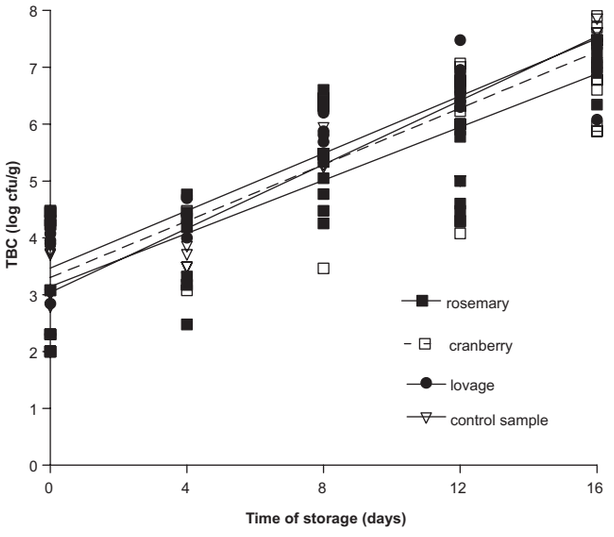

FIGURE 2. Curves of linear regression of total count of bacteria in meatball products stored at a temperature of 10°C, depending on different plant extracts added (n=5).

S.A. Klemens [2004], after 3-4 days of incubation of dishes infected with a bacteria suspension containing adequate concentrations of the preparation, determined the lowest concentrations of substances under study which inhibit the growth of microorganisms. The results obtained prove that the highest antibacterial action was exerted by essential oil (Oleum), followed by the extract (*extractum* 50) and the tincture which appeared to be the most active against the *Staphylococcus aureus* FDA 209 P strain.

In another experiment conducted by Erdoğrul [2002], 0.01 mL of test strain cultures prepared (concentration: 10^5 cfu/mL), were inoculated in sterile Petri dishes. Next, 15 mL of agar medium (Mueller-Hinton) were added and thereon were placed rings saturated with rosemary extracts prepared in advance: ethyl acetate, methanol extract, chloroform extract and acetone extract. Bacteria under study were also subjected to the action of selected antibiotics: Ampicillin, Cefodizime, Cefuroxim, Cefalotin, Oxacyclin, Tobramycin, Ofloxacin and Vancomycin. The rosemary extracts used inhibited the growth of all the bacteria subjected to testing. Solely the acetone extract turned out to be ineffective against the *Yersinia enterocolitica* strain. Besides it was found that part of antibiotics under study (*i.a.* Ampicillin, Cefodizime and Oxacyclin) were less effective than the rosemary extracts against the *Yersinia enterocolitica* strain – no growth inhibition zones) were reported [Erdoğrul, 2002].

The studies referred to the above were conducted on isolated bacteria strains and artificial media and not in food products; however, the results obtained show that rosemary preparations possess a highly antibacterial power. Therefore it can be expected that rosemary preparations should also inhibit the growth of microorganisms in food products. This hypothesis is further confirmed by tests conducted for the needs of this study.

Rosemary (*Rosmarinus offinalis*) constitutes a very promising dietary supplement because it contains a variety of compounds including: carnosol, carnosic acid, rosmanol, 7-methyl-epirosmanol, isorosmanol, rosmadial, caffeic acid, 1,8 cineol, camphor and a-pinene, with substantial antimicrobial and antioxidant activity *in vitro* [Baratta *et al*., 1998a; Flamini *et al*., 2002; Iban˘ez *et al*., 2003; Saenz-Lopez *et al*., 2002]. Carnosic acid is the most active antioxidant component [Cuvelier *et al*., 1996; Saenz-Lopez *et al*., 2002], while 1,8 cineol, a-pinene [Baratta *et al.,* 1998b] and camphor [Pandit & Shelef, 1994] are the most active antimicrobial components present in rosemary.

In a research work by Klemens [2004], rosemary essential oil distinguished itself by the highest antimicrobial activity, being the richest of all the preparations in active compounds. Therefore in further research it would be necessary to compare the action of both the extract and essential oil made of rosemary. It is most probable that in food products essential oil would show a stronger action than the extract. It is, however, difficult to forecast whether an addition of essential oil would be sensorically tolerated by consumers and at what amount; therefore such an assessment should be carried out as well.

Not only ethanol-and-water rosemary extracts but also those made from cranberry and lovage may exert bactericidal and bacteriostatic action due to various biologically-active compounds. These compounds include, in the case of lovage, mainly essential oils, flavonoids, terpenes, phytosterols and organic acids. Whereas juice and infusion made from dried bilberries (blueberries) has been used for a long time to treat diarrhoea and destroy digestive tract parasites. Dyes comprised in bilberries (representing *ca*. 7% of their content) neutralize food toxins and block absorption thereof *via* mucous membranes. The blue anthocyanin dye inhibits bacteria growth as well [Beuchat, 1994].

Analysis of pH level study results

The study of pH level change occurring during food product storage showed that an addition of plant extracts at the amount of 0.2% resulted in a small change in the product's acidity (Figure 3).

Changes in pH level in products over the entire food storage cycle were very small. At the beginning, pH amounted to 5.96 for the product with rosemary, 5.99 for the product with cranberry, 5.97 for the product with lovage and 5.99 for the control sample. A slight increase in pH level was observed in the control sample until day 12 of storage and in the samples with an addition of rosemary and lovage extract – until half the storage cycle. On the 8th day of storage it reached the maximum value of 6.05 for the sample with rosemary, 6.08 for the sample with lovage and 6.08 for the control sample on the 12th day of storage. In the second half of the storage period the pH value decreased slightly reaching on the 16th day of storage following values: 5.95 for the sample with rosemary, 5.97 for the sample with lovage and 5.96 for the control sample. In the sample with an addition of cranberry extract, pH level was the most stable for 12 days of storage to fall to the value of 5.91 at the end thereof.

Small pH changes in all the products over the whole storage period are confirmed by results of linear regression analysis and correlation between storage time and pH changes. The determination coefficient R^2 for the sample with lovage and control sample accounted for 0.001, for the sample with rosemary for 0.005 and for the sample with cranberry for 0.019, whereas correlation ratios R amounted to -0.03, -0.04, -0.07 and -0.14, respectively. The time factor did not affect product pH, which was acknowledged by the p value which in all the cases amounted to much in excess of 0.50.

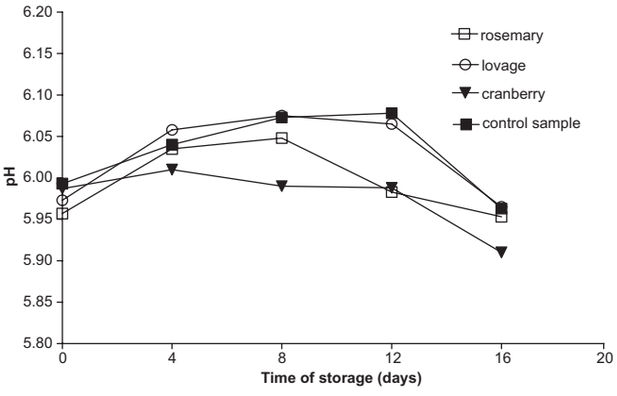

FIGURE 3. Curves of changes in pH of meat-ball products stored at a temperature of 10°C, depending on different plant extracts added.

The final pH value of the stored products was similar to their initial pH value despite considerable differences in the total number of microorganisms between storage days from 0 to 16. The differences amount to: 3.89 log cycles for the sample with rosemary, 3.98 log cycles for the sample with cranberry, 3.96 log cycles for the sample with lovage, and 4.47 log cycles for the control sample.

An indication of meat spoilage is usually an increased pH level which results from putrefactive bacteria growth and release of ammonia into the environment. In the case of saccharolytic microflora growth and carbohydrate decomposition, acids are liberated and pH level decreases. For storage purposes it is important that meat reaches as low pH level as possible in order to ensure meat shelf-life stability. That pH level amounts to *ca*. 5.4. The pH value of 6.4 is considered to be critical for the meat being fit for storage [Prost, 1985]. When pH value exceeds that level, meat shows little resistance to microorganisms and is thereby susceptible to decomposition processes. This is also connected with the activity of enzymes produced by microorganisms especially the proteolytic ones, whose activity optimum is reached at pH value equal to 7.0–8.0. Their activity, however, is inhibited at pH value lower than 6.0 [Prost, 1985].

In the course of this study, during the entire storage cycle, major changes in pH values were not observed, and at the end thereof even a decrease was recorded despite a significant microflora growth. It might have resulted from the influence of the addition of plant extracts on directions of metabolic transformations of microorganisms and enzymes in the product.

CONCLUSIONS

1. Out of all the plant extracts applied, the rosemary extract was characterised by the strongest action exerted against microorganisms, manifesting itself by the longest shelf-life (13.3 days) of products under study stored at the temperature of 10°C.

2. During the entire storage period the most intense growth of microorganisms was observed in the control sample and the product with lovage addition.

3. The addition of plant extracts caused small changes in product acidity which reached a level comparable with that of the control product despite large differences in the initial and final number of microorganisms recorded during the storage period.

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