

THE EFFECT OF ROSEMARY PREPARATIONS ON THE MICROBIAL QUALITY AND TBARS VALUE OF MODEL PORK BATTERS

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Background. Rosemary (*Rosmarinus officinalis* L.) extracts have a potent antioxidant and antibacterial activity and are widely used in the food industry. The effect of added rosemary preparations on the microbiological quality and process of lipid oxidation of model pork batters, immediately after preparation (“0”) and 1, 3 and 7 days of chill-storage (4-6°C) was analysed in the study.

Material and methods. Experiments were conducted with three types of rosemary preparations, i.e.: dried spice, essential oil and a commercial preparation (TasteGuard P). The experimental material consisted of meat batter produced from porcine *musculus longissimus dorsi* and water. Microbiological examinations covered determinations of the total count of mesophilic aerobic microorganisms, psychrophilic bacteria, coliforms and enterococci. In turn, chemical analyses involved determination of the TBARS value.

Results. The rosemary preparations did not exhibit either antibacterial properties against aerobic mesophilic or psychrophilic bacteria. The essential rosemary oil was observed to inhibit the growth of coliform bacteria and enterococci, whereas the dried spice examined was found to increase the counts of aerobic mesophilic bacteria, coliforms and enterococci. None of the rosemary preparations terminated the lipid oxidation process.

Conclusions. The results obtained in this study point to the necessity of continuing investigations to determine the dose of rosemary preparations that would inhibit the growth of microflora being the most frequent cause of raw materials and products spoilage and, simultaneously, restrict oxidation of their lipids.

Key words: rosemary, extracts, antibacterial and antioxidative activity, TBARS value

INTRODUCTION

Prevention of lipid oxidation processed meat products is important for keeping their high quality and safety. It is obvious that natural antioxidants, are preferred. Many works are connected with the use of extracts from different herbs and extracts [Kobus-Cisowska et al. 2010, Zarena and Sankar 2009, Roman et al. 2009].

Rosemary (*Rosmarinus officinalis*) is a small ever-green bush, belonging to the Labiatae family. It grows principally in the basin of the Mediterranean Sea, while in Poland it is usually cultivated in pots. Active substances present in *Rosmarinus* yield it a series of properties, desirable from the point of view of the food industry and medicinal phytochemistry [Rumińska and Ożarowski 1990, Djeddi et al. 2007].

The majority of data found in literature [Madsen and Bertelsen 1995, Beltran et al. 2004, Fernandez-Lopez et al. 2005, Moreno et al. 2006, Georgantelis et al. 2007, Pietrzak and Myron 2008] refers to the anti-oxidative properties of *Rosmarinus officinalis*. A rosemary extract, in the form of an emulsion, powder or oil solution, may be used as a substitute for BHA (butylated hydroxyanisole), added to dehydrated chicken eggs, meat and fish, while rosemary extracts may be added to sausages, macaroni, peanut butter and oil.

Essential oils and extracts, both the aqueous and oil ones, obtained from rosemary are characterised by a high antimicrobial activity. Bacterial strains especially susceptible to the activity of essential rosemary oils include: *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Klebsiella pneumoniae* [Mangena and Muyima 1999, Celikbas et al. 2007, Djeddi et al. 2007].

Rosemary extracts display a relatively poor inhibiting effect on Gram-negative bacteria but, at a level of 0.06-1%, they inhibit the growth of Gram-positive pathogens, such as: *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus*. Moulds of the *Penicillium* and *Botrytis* genus are developing much slower in the environment containing a rosemary extract, while carnosol and carnosic acid (components of rosemary extracts) inhibit the vital activities of drug-resistant bacteria of the *Staphylococcus aureus* strain. Especially susceptible to the activity of rosemary extracts are also bacteria of the *Lactobacillus* and *Brochothrix* genus [Del Campo et al. 2000, Moyosoluwa et al. 2004, Fernandez-Lopez et al. 2005].

In view of the above data, the reported study was aimed at determining the effect of rosemary preparations on the saprophytic microflora and lipid oxidation processes in a model pork batter during its storage in chilling conditions (4-6°C).

MATERIAL AND METHODS

The experimental material consisted of meat batter obtained from porcine *musculus longissimus dorsi* (90%) and water (10%). Rosemary (*Rosmarinus officinalis* L.) was added to the batter in the form of dried spice (P.P.H.U. "Ben"), a commercial preparation TasteGuard P (Christian Hansen, Poland) or an essential oil (Pollena-Aroma). All three forms are further referred to as rosemary preparations.

In each case, about 400 g of a muscle tissue was minced in a laboratory grinder, using a net with mesh diameter of 3 mm, divided into four equal portions, and mixed with water and the rosemary preparation. Four batter variants were produced, differing

in the type of rosemary preparation used, the quantity of which was determined on the basis of producer recommendations and data found in the available literature:

- C – control variant, containing no rosemary preparation
- D – variant containing dried rosemary (5% in relation to batter weight)
- P – variant containing preparation TasteGuard P (0.08% in relation to batter weight)
- O – variant containing rosemary oil (1.0% in relation to batter weight).

Samples of meat batter, prepared as described, were vacuum packed, using multi-layer foil bags and chill-stored (4-6°C).

A 20 g portion of meat batter was taken with a sterile spoon after the opening of the bag, and mixed with 180 ml of 0.1% peptone water. Each sample was homogenized in a Stomacher Lab Blender (model 400 Circulator, Seward Laboratory) for 30 seconds. Serial 10-fold dilutions were prepared by diluting 1 ml of the sample in 9 ml of 0.1% sterile peptone water. Two plates were prepared from each dilution. Microbiological examinations covered the determination of the total count of mesophilic aerobic microorganisms (PCA fluid agar, bio-Mérieux), psychrophilic bacteria (PCA fluid agar, bio-Mérieux), coliforms (VRBL, BTL, Poland) and enterococci (Slanetz and Bartley, Oxoid LTD). Before the actual experiment the preparations were analysed for microbial quality. Duplicate plates were counted after incubation at: 28°C for 72 hours for mesophilic, at 4°C for 10 days for psychrophilic and at 37°C for 48 hours for coliforms and enterococci. The counts were expressed as the colony forming units (cfu/g).

Chemical analyses involved determination of the TBARS value [Pikul et al. 1989]. To this end, a 10 g portion of batter was mixed with 34.25 ml of 4% HClO₄ (Sigma Aldrich) and 0.75 ml of 0.01% BHT (Sigma Aldrich) in a laboratory homogenizer (MPW-120, Mechanika Precyzyjna, Poland). Next, the sample was filtrated to a 50-ml measuring flask and filled up to the volume of 50 ml. For analyses, 5 ml of the filtrate were transferred to a test tube containing 5 ml of 2-thiobarbituric acid (TBA, Sigma Aldrich). The tubes were tightly closed and placed in a boiling water bath for 1 hour and cooled in cold water. Absorbance was measured spectrophotometrically (Spectronic 20 GENESYS) at 532 nm against a blank that contained all the reagents except the meat. The samples were analysed twice and results were expressed as mg malonaldehyde/kg sample.

Both the microbial and chemical determinations were performed on batters containing rosemary preparations after 1, 3 and 7 days of storage in chilling conditions (4-6°C) and in control samples (containing no rosemary) directly after preparation of the batter ("0") and after 1, 3 and 7 days of storage.

The results obtained were subjected to a statistical analysis, using the Statgraphics Plus 4.1 software. Analysis of variance (ANOVA) was conducted for each storage period and addition of rosemary preparations to determine significance of differences between the mean values determined for the particular samples. Differences were considered significant at $p < 0.05$. The statistical significance of differences between means values was analysed with the LSD test.

RESULTS

Out of the preparations used in the experiment the highest contamination with mesophilic aerobes, yeasts and moulds, as well as the coli group bacteria, was observed

for that containing dried rosemary: 5.0×10^4 , 1.5×10^1 and 9.1×10^1 cfu·g⁻¹, respectively. The preparations with Taste Guard P or oil showed only low contamination with mesophilic aerobes, yeasts and moulds, i.e. < 10 cfu·g⁻¹, while coliforms were not identified in 0.1 g of any of the samples containing the commercial preparation or the oil.

Irrespectively of the batter variant, in each of the samples examined, an increase was recorded in the count of mesophilic aerobic microorganisms (Table 1). The highest contamination with aerobic microorganisms over the whole storage period was observed for the batter samples produced with the addition of dried rosemary (D). The lowest number of mesophilic aerobes was identified in the control batter analysed directly after preparation ("0"). With the increasing time of cold storage, irrespectively of the rosemary preparation added, the number of mesophilic aerobes was increasing significantly ($\alpha = 0.05$).

Table 1. Total number of aerobic mesophilic and psychrophilic bacteria in pork batters with addition of rosemary preparation stored in chilling conditions (4-6°C; cfu·g⁻¹; means)

Storage time days	Variable of batter			
	control (C)	dried (D)	preparation (P)	oil (O)
Total number of aerobic mesophilic bacteria, cfu·g ⁻¹				
0	$2.9 \cdot 10^{2\text{ a b A}}$			
1	$5.0 \cdot 10^{3\text{ a A}}$	$6.1 \cdot 10^{3\text{ a B}}$	$3.6 \cdot 10^{3\text{ a AB}}$	$4.9 \cdot 10^{3\text{ a AB}}$
3	$8.0 \cdot 10^{4\text{ ab A}}$	$3.1 \cdot 10^{4\text{ ab B}}$	$3.2 \cdot 10^{4\text{ ab AB}}$	$5.0 \cdot 10^{4\text{ ab AB}}$
7	$8.4 \cdot 10^{4\text{ b A}}$	$4.0 \cdot 10^{5\text{ b B}}$	$9.1 \cdot 10^{4\text{ b AB}}$	$1.7 \cdot 10^{5\text{ b AB}}$
Number of psychrophilic bacteria, cfu·g ⁻¹				
0	$1.5 \cdot 10^{4\text{ a A}}$			
1	$6.1 \cdot 10^{4\text{ a A}}$	$1.8 \cdot 10^{4\text{ a A}}$	$1.5 \cdot 10^{4\text{ a B}}$	$5.1 \cdot 10^{4\text{ a AB}}$
3	$3.5 \cdot 10^{5\text{ a A}}$	$2.6 \cdot 10^{4\text{ a A}}$	$1.1 \cdot 10^{4\text{ a B}}$	$2.6 \cdot 10^{4\text{ a AB}}$
7	$1.5 \cdot 10^{5\text{ a A}}$	$2.8 \cdot 10^{4\text{ a A}}$	$8.4 \cdot 10^{4\text{ a B}}$	$5.8 \cdot 10^{4\text{ a AB}}$

a, b – values from the same column, with different superscripts, are significantly different at $\alpha = 0.05$.
A, B – values from the same row, with different superscripts, are significantly different at $\alpha = 0.05$.

In each of the examined batters containing rosemary preparations an increase was observed in the count of the psychrophilic microflora (Table 1), which is often the cause of meat products spoilage. Irrespectively of the batter variant, in majority of the samples examined the number of psychrophilic microorganisms tended to increase over the whole cold storage period. In control (C) batter samples, as well as in those containing dried rosemary (D), the number of psychrophilic microorganisms was significantly lower ($\alpha = 0.05$) when compared to the samples produced with the addition of TasteGuard P preparation (P).

In all the samples of pork batter, irrespectively of the type of rosemary preparation, the number of coliforms, determined after 1 day of storage, was significantly ($\alpha = 0.05$) the highest and decreased after 3 and 7 days of cold storage (4-6°C; Table 2). The lowest number of coliforms was observed in most of the samples containing essential oil (O),

Table 2. Number of coliforms and enterococci in pork batters with addition of rosemary preparation stored in chilling conditions (4-6°C; cfu·g⁻¹; means)

Storage time days	Variable of batter			
	C	D	P	O
Number of coliforms, cfu·g ⁻¹				
0	2.2 · 10 ² a A			
1	8.7 · 10 ⁴ b A	9.1 · 10 ⁴ b B	4.4 · 10 ⁴ b A	3.1 · 10 ⁴ b A
3	1.5 · 10 ⁴ a A	3.1 · 10 ⁴ a B	3.9 · 10 ⁴ a A	4.4 · 10 ³ a A
7	2.6 · 10 ³ a A	2.7 · 10 ³ a B	1.6 · 10 ³ a A	1.5 · 10 ³ a A
Number of enterococci, cfu·g ⁻¹				
0	5.2 · 10 ⁵ b			
1	4.5 · 10 ⁵ b AB	5.9 · 10 ⁵ b B	9.7 · 10 ⁴ b AB	2.3 · 10 ⁵ b A
3	2.3 · 10 ⁴ a AB	2.4 · 10 ⁴ a B	8.3 · 10 ³ a AB	1.8 · 10 ⁴ a A
7	1.8 · 10 ³ a AB	1.4 · 10 ³ a B	2.5 · 10 ³ a AB	2.1 · 10 ³ a A

Description as in Table 1.

while significantly ($\alpha = 0.05$) the highest contamination with coliforms during the whole storage period was observed for the batter samples produced with the addition of dried rosemary (D).

In all the batter samples examined, irrespectively of the type of rosemary preparation added, the number of enterococci decreased significantly ($\alpha = 0.05$) over the whole cold storage period (4-6°C; Table 2). In the case of all the samples examined the number of

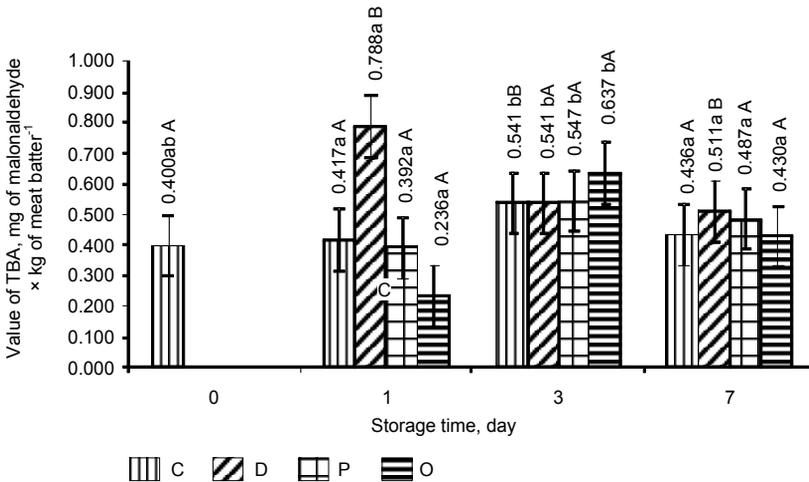


Fig. 1. Value of TBARS measured in pork batters with addition of rosemary preparation stored in chilling conditions (4-6°C; mg of malonaldehyde × kg of meat batter⁻¹; mean and standard deviation). Description as in Table 1

enterococci determined directly after the batters were produced and after 1 day of storage was significantly ($\alpha = 0.05$) higher than that recorded after 3 and 7 days of storage.

The lowest enterococci contamination was observed for the batter sample containing oil (O), while the highest – for the sample containing dried rosemary (D).

The addition of dried rosemary (D) resulted in a significant increase of TBARS value, measured in cold stored batter samples (Fig. 1). In majority of batter samples (except for the variant containing dried rosemary – D) the values of TBARS increased from the lowest, recorded after 1 day of storage, to the highest after 3 days, to finally decrease once more after 7 days of storage. Significantly the lowest TBARS value after 1 day of storage was reported for the batter containing rosemary oil (O), with this tendency observed to sustain also after 7 days of meat samples storage.

DISCUSSION

Numerous authors [Wieczorkiewicz-Górnik 2003, López et al. 2005, Hać-Szymańczuk et al. 2009] have suggested that the differentiated effect of rosemary preparations on selected microorganisms depends on their composition, the characteristics of the plant material and concentration used. The plant material contains among much else: essential oils (1.5-2.5%), tannins, flavonoids, terpenes, saponins, rasins, phytosteroles, rosemary acid and many others. Essential oil is responsible for the strong, camphor aroma of rosemary. Its basic components are: camphor (14.5%), cineol (12%), borneol (10.5%), pinene (8.5%) and camphene (7%) [Rumińska and Ożarowski 1990, Djeddi et al. 2007]. Moreno et al. [2006], analysing rosemary extracts containing 30% of carnosic acid, 16% of carnosol and 5% of rosemary acid, observed that they were more effective against Gram-positive than Gram-negative bacteria or yeasts.

Karpińska-Tymoszczyk [2008] studied the effect of adding 1% of rosemary oil to turkey forcemeat balls that were subjected to thermal processing and stored at a temperature between -1°C and -3°C , and observed that it inhibited the growth of psychrophilic bacteria. In turn Wieczorkiewicz-Górnik [2003], when examining the effect of rosemary oil on the *Pseudomonas fluorescens* bacteria, reported that the growth of this strain was inhibited only upon oil addition in the concentration exceeding 1% (5%). Antonio et al. [2009], in studies on the stability of a ready salad, using a combination of 2% rosemary oil with enterocyne AS-48, analysed its activity against *Listeria monocytogenes*, which is capable of surviving storage at low temperatures.

The results obtained in the present study, referring to the high activity of rosemary oil and extract against *Escherichia coli* are consistent with findings of other authors [Wolski et al. 2001, Fecka et al. 2002]. This effect is attributed to the considerable concentration of 1.8-cyneole and verbenone in the preparations examined. According to Kazimierczuk and Kozłowska [2006], rosemary oil added to sausages, pies and fish inhibits the growth of *Escherichia coli*. Georgantelis et al. [2007] also examined the antimicrobial effect of rosemary extract while adding it to fresh pork sausage ($260 \text{ mg}\cdot\text{kg}^{-1}$). They observed that the number of microorganisms of the *Enterobacteriaceae* family increased in a sausage prepared without the rosemary extract after 20 days of storage at 4°C , while in the product containing components originating from rosemary it decreased by 1 logarithmic cycle when compared with the initial value.

In studies conducted by Hammer et al. [1999], a determination was made for the minimum bacteriostatic concentration (MIC) of oils from sweet basil, marjoram, oregano, salvia and thyme in relation to bacteria of the *Enterococcus faecalis* species. The highest efficiency in growth inhibition of those bacteria was recorded for oregano (MIC = 0.25%) and thyme (MIC = 0.5%) oils, while the remaining oils showed the bacteriostatic activity only after reaching a concentration of about 2.0%. López et al. [2005], examining the effect of plants used for seasoning on the growth of many bacterial species, including *Enterococcus faecalis*, demonstrated a stronger effect of cinnamon and clove oil than of rosemary oil. Moreover, Erkmén and Özcan [2008] observed that *Enterococcus faecalis* bacteria were more susceptible to laurel than to rosemary essential oil.

Lipid oxidation may be reduced by incorporating natural antioxidants. Some authors have shown that one of the strongest natural antioxidants can be found in the leaves of rosemary (*Rosmarinus officinalis*). It is now well known that the antioxidative activity of this spice is due to phenolics. Carnosol (an odourless and tasteless phenolic diterpenic lactone) and carnosic acid have both been found in rosemary. Rosmanol, epirosmanol, isorosmanol and rosmarinic acid have been isolated from rosemary as well [Madsen and Bertelsen 1995].

The results obtained in the present work did not confirm the data found in literature and indicating a considerable effect of rosemary preparations on the inhibition of lipid oxidation. Beltrán et al. [2004] reported that a 0.04% addition of rosemary extract to minced poultry meat resulted in strong oxidation-stabilising properties. According to Madsen and Bertelsen [1995], rosemary dried or in the form of oleoresins, added to various meat products, to a considerable degree inhibited the oxidation during cold storage or freezing. These authors demonstrated also that rosemary, added in sensorily acceptable amount (0.05%), reduced lipid oxidation. It is worthy of notice that in some investigations a considerable reduction in TBARS value was observed in the early storage period, whereas at the end of the storage period it increased to the level found in the samples without spices addition.

Govaris et al. [2007] reported that feeding young turkeys over a period of 4 weeks on a feed containing dried rosemary (10 g kg^{-1}) had a favourable effect on the quality of the meat obtained, as the turkey fillet remained fresh for a longer period during cold storage. This is due to the activity of compounds inhibiting lipid oxidation, which in this case reach the tissues still during the birds' life, through diet supplementation. Pietrzak and Myron [2008] examined the effect of a commercial preparation FlavourGuard, added at a concentration of 0.03%, on lipid oxidation processes taking place during storage in chilling conditions of vacuum packed, baked poultry hamburgers. The rosemary extract proved to be a very effective antioxidant. The TBARS value proved to be almost three times lower than that determined for the control sample.

CONCLUSIONS

1. Over the entire period of storage under chilling conditions all the applied rosemary preparations were not inhibiting the growth of mesophilic anaerobic bacteria in vacuum-packed batter samples, which may indicate their resistance to the effects of the preparations at experimental doses.

2. The addition of essential oil of rosemary was observed to inhibit the growth of coliforms and enterococci, which indicates that this oil is a source of substances displaying antimicrobial activity. This is likely to be due to the method of oil preparation (distillation), which greatly affects changes in its chemical profile.

3. The addition of dried rosemary to batter caused an increase in the counts of both mesophilic bacteria, *Coli* group bacteria and enterococci, which may result from its initial contamination.

4. The use of the rosemary preparations in the applied experimental doses had no significant effect on the inhibition of lipid oxidation proceeding in samples of pork batter, packed under vacuum and stored in chilling conditions (4-6°C). This is likely to result from a low content of compounds responsible for the restriction of oxidation processes in the rosemary preparations examined.

The results obtained in this study point to the necessity of continuing investigations to determine the dose of rosemary preparations that would inhibit the growth of microflora being the most frequent cause of raw materials and products spoilage and, simultaneously, restrict oxidation of their lipids.

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WPLYW PREPARATÓW Z ROZMARYNU NA JAKOŚĆ MIKROBIOLOGICZNĄ I WARTOŚĆ WSKAŹNIKA TBA MODELOWYCH FARSZÓW Z MIĘSA WIEPRZOWEGO

Wstęp. Ekstrakty z rozmarynu lekarskiego (*Rosmarinus officinalis* L.) wykazują aktywność przeciwutleniającą i przeciwdrobnoustrojową oraz często są stosowane w przemyśle spożywczym. W pracy określano wpływ preparatów z rozmarynu lekarskiego na jakość mikrobiologiczną oraz przebieg procesów utleniania tłuszczów w modelowym farszu mięsny, bezpośrednio po przygotowaniu farszu („0”) oraz po 1, 3 i 7 dniach przechowywania w warunkach chłodniczych (4-6°C).

Materiał i metody. Zastosowano trzy rodzaje preparatów z rozmarynu: suszoną przyprawę, olejek eteryczny oraz handlowy preparat TasteGuard P. Materiałem badawczym był farsz mięsny wyprodukowany z wieprzowego mięśnia najdłuższego grzbietu oraz wody. Badania mikrobiologiczne obejmowały określenie ogólnej liczby drobnoustrojów mezofilnych, psychrofilnych, bakterii z grupy coli oraz enterokoków. W badaniach chemicznych oznaczano wartość wskaźnika TBA.

Wyniki. Badane preparaty rozmarynu nie wykazywały aktywności przeciwbakteryjnej w stosunku do drobnoustrojów tlenowych mezofilnych i drobnoustrojów psychrofilnych. Olejek eteryczny zahamował wzrost bakterii z grupy coli i enterokoków, natomiast suszony rozmaryn wpłynął na wzrost ogólnej liczby bakterii, bakterii z grupy coli oraz enterokoków. Żaden z badanych dodatków rozmarynu nie zahamował procesu oksydacji lipidów.

Wnioski. Na podstawie wyników uzyskanych w pracy koniecznością wydaje się prowadzenie dalszych badań w celu określenia wielkości dodatku preparatów rozmarynowych, która zahamowałaby wzrost mikroflory najczęściej powodującej zepsucie surowców i produktów oraz ograniczyłaby jednocześnie utlenianie lipidów w nich zawartych.

Słowa kluczowe: rozmaryn lekarski, preparaty, aktywność przeciwdrobnoustrojowa i przeciwutleniająca, jakość mikrobiologiczna, wskaźnik TBA

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