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ANTIBACTERIAL ACTIVITY OF OREGANO AND SAGE PLANT EXTRACTS AGAINST DECARBOXYLASE-POSITIVE ENTEROCOCCI ISOLATED FROM RABBIT MEAT

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ABSTRACT

The effect of plant extracts (sage, oregano) against decarboxylase-positive enterococci from rabbit back limb meat was reported in this study. Oregano plant extract inhibited the growth of all 34 tested enterococci (the inhibitory zones: 12 to 45 mm). The growth of the majority of strains (n=23) was inhibited by oregano plant extract (the high size inhibitory zones (higher than 25 mm). The growth of 11 strains was inhibited by oregano extract reaching medium size inhibitory zones (10 to 25mm). The most sensitive strain to oregano extract was *E. faecium* M7bA (45 mm). Sage extract was less active against tested enterococci (n=16) reaching lower inhibitory zones (up to 10 mm).

Keywords: *Labiatae*; effect; inhibition; meat; rabbit

INTRODUCTION

Enterococci belong to the Phylum Firmicutes, to the Family Enterococcacae and to the genus Enterococcus. They represent Gram-positive, catalase-negative, coccus-shaped bacteria producing lactic acid as the major product of glucose fermentation. Currently, 37 species of enterococci are validly described **(De Vos et al., 2009)** which fall into 7 species groups on the basis of 16S rRNA gene similarity **(Franz et al., 2007)**. Although some enterococci have possessed probiotic properties **(Franz et al., 2007; Szabóová 2011)** on the other hand, some strains can also cause food intoxication through production of biogenic amines and for virulence traits **(Kučerová et al., 2009)**. Biogenic amines (BA) are natural antinutrition factors and are important from a hygienic point of view **(Shalaby 1996)**. In spite of amines being considered as endogenous to food originating from plant matter, such as fruits and vegetables, BA are formed in other foods such as fish and meat products, eggs, cheeses, etc. as a result of microbial action during aging and storage. The most important BA occurring in foods are histamine, putrescine, cadaverine, tyramine, tryptamine, p-phenylethylamine, spermine, and spermidine. The factors which influence the formation of BA in foods include the availability of free amino acids, the presence of microorganisms that can decarboxylate amino acids, the favourable conditions of such microorganisms for the growth and production of their enzymes **(Shalaby 1999)**. Concerning the meats, enterococci can be associated with processed meat products, but they can be already found in raw meats **(Lauková et al., 2011; Talon et al., 2011)**, rabbit meat including **(Szabóová 2011)**. Rabbit meat is a lean meat rich in proteins of a high biological value and it is characterized by high levels of essential aminoacids. Furthermore, rabbit meat is also an important source of highly available micronutrients, such as vitamins and minerals. Rabbit meat is characterized by its lower energetic value compared with red meats **(Dalle Zotte 2004)** due to its low fat content. Fat content varies widely depending of the carcass portion from 0.6 to 14.4% with an average value of 6.8% **(Hernández 2008)**, proteins level is 20-21% **(Dalle Zote 2004)**. The amount of cholesterol in rabbit meat is about 59 mg/100 g of muscle **(Combes 2004)**, lower values than those presented in meat from other species (61 mg in pork, 70 mg in beef, 81 mg in chicken). The mineral fraction of rabbit meat is characterized by its low contents in sodium (49 and 37 mg.100 g^{-1}; **Dalle Zote 2004)**. Rabbit meat is also an important source of B vitamins. Consumption of 100 g of rabbit meat contributes to 8% of daily vitamin B2, 12% of vitamin B5, 21% of vitamin B6, and 77% of vitamin B3 requirements, and provides a fulfillment of the daily vitamin B12 requirement **(Combes 2004)**.

Rabbit breeds selection by producers tends to improve the quantitative aspects of the production, such as growth rate and development of muscularity, but pays less attention to meat quality aspects. Meat quality can be evaluated objectively by measuring some biophysical or biochemical traits such as pH, water holding capacity, colour, texture, flavour. These attributes are determined by both, biological and productive factors as well as by *ante*-mortem, and *post*-mortem treatments **(Dalle Zote 2004)**. *Post*-mortem biochemical changes in the muscle determine the transition from muscle into meat and can influence the final meat quality. Safety and shelf life of meat are limited by microbial growth and contamination.

As former mentioned, enterococci can be detected in the meat as a result of environmental influence i.e. during processing or slaughtering **(Simonová et al., 2009; Lauková et al., 2011).** The herbal extracts and/or essential oils obtained from plants are considered one of the most important natural substances with antimicrobial activity.

They are also known for their antioxidant and anti-inflammatory properties (borneol and cineol obtained from *Salvia* sp.; **Milos et al., 2000**). In general, the efficiency of herbal extracts and/or plant essential oils has been found common for many years before and just at present time it is becoming increasingly topical because to return to all natural. **Delamare et al. (2007)** presented *in vitro* antimicrobial effect of sage extract; the growth of *Bacillus cereus, B. subtilis* and *B. megatherium* as well as of *E. coli* and *S. aureus* was inhibited. There are many studies demonstrating the potential antibacterial effects of essential oils and components obtained from *Origanum vulgare* L. (such as carvacrol, thymol) in food and feed **(Burt 2004).**

MATERIAL AND METHODOLOGY

Table 1 The inhibitory activity of sage and oregano plant extracts against indicator strains – enterococci from rabbit meat.

Indicator strain	Oregano PE	Sage PE
	Inhibitory zone in mm and -/+/++	
EF M1C	25 / ++	5 / -
EF M2C	24 / +	8 / -
EF M7C	31 / ++	12 / +
EF M7b	17 / +	10 / +
EF M4C	15 / +	9 / -
EF M6C	28 / ++	9 / -
EF M5a	28 / ++	8 / -
EF M3b	30 / ++	7 / -
EF M1b	21 / +	9 / -
EF M2a	30 / ++	10 / +
EF M2cA	12 / +	11 / +
EF M2cB	35 / ++	10 / +
EF M3a	16 / +	11 / +
EF M4aA	40 / ++	9 / -
EF M4aB	16 / +	11 / +
E.sp.M5aA	32 / ++	8 / -
EF M5aB	12 / +	9 / -
E.sp.M6b	31 / ++	9 / -
EF M7bA	45 / ++	0 / -
EF M7bB	26 / ++	11 / +
EF 1BM	34 / ++	8 / -
E.sp.3AM	40 / ++	10 / +
EF 4BM1	25 / ++	10 / +
EF 4BM2	30 / ++	10 / +
EF 5BM1	18 / +	9 / -
E.sp.5BM2	26 / ++	12 / +
EF M1B	20 / +	8 / -
EF M2c	25 / ++	8 / -
EF M1c	25 / ++	14 / +
E.sp.M5a	25 / ++	11 / +
EF M6c	32 / ++	8 / -
E.sp.M3A	28 / ++	10 / +
EF M4B	30 / ++	7 / -
EF M2A	34 / ++	10 / +

PE – plant extract; EF – *Enterococcus faecium*; E.sp. – *Enterococcus* species; the size of inhibitory zone < 10 mm (-; no inhibitory zone); the size of inhibitory zone in the range from 10 to 25 mm (+; inhibitory zone- medium size); the size of inhibitory zone higher than 25 mm (++; inhibitory zone- high size)

Enterococci were isolated from healthy farming rabbits-breed Hyplus, age 56 days; they were identified and characterized as was described previously by **Szabóová et al. (2012).** Among 34 isolates, 28 were allotted to the species *Enterococcus faecium* **(Szabóová et al., 2012a)** and 6 isolates were not specified. But all strains were found dacarboxylase-positive **(Pleva et al., 2012)**. These strains were tested to their sensitivity to oregano and sage plant extracts respectively effect of both extracts was tested against enterococci. For the test, the amount 10 µl of both extracts was used and the agar spot test **(De Vuyst et al., 1996).** Testing was provided on Brian Heart Infusion Agar (0.7 and 1.5 %; Becton and Dickinson, USA). Oregano plant extract obtained from the tops of *Origanum vulgare* L. (*Lamiaceae* family; density: 0.959 ± 0.002 g/cm^3; refractive index: 1.515 ± 0.001; gas chromatography analysis: carvacrol: 55.000 ± 3.000 %; Calendula a.s., Nová Ľubovňa, Slovak Republic) as well as sage plant extract obtained from the leaves of *Salvia officinalis* L. (*Lamiaceae* family; density: 0.915 ± 0.001 g/cm^3; refractive index: 1.469 ± 0.001; gas chromatography analysis: cineol: 15.000 ± 1.000 %, thujone: 24.000 ± 1.000 %, borneol: 18.000 ± 1.000 %; Calendula a.s., Nová Ľubovňa, Slovak Republic) were kindly provided by Dr. Šalamon and Dr. Poráčová (University of Prešov, Slovak Republic). Effect of both extracts against tested strains was expressed as the size of the inhibitory zones in mm (Table 1).

RESULTS AND DISCUSSION

Oregano plant extract inhibited the growth of all tested enterococci (the inhibitory zones in the range from 12 to 45 mm, Table 1). The growth of the majority of strains (n=23) was inhibited by oregano plant extract reaching the medium size inhibitory zones (higher than 25 mm, ++, Table 1). The growth of 11 strains was inhibited by oregano reaching medium size inhibitory zones (10-25 mm, +, Table 1). The most sensitive strain to oregano plant extract was *Enterococcus faecium* M7bA (45 mm). Sage plant extract was less active against tested enterococci; the growth of 16 strains was inhibited (inhibitory zones up to 10 mm, +). **Di Pasqua et al. (2005)** confirmed the inhibitory effect of oils (extracts) isolated from *Origanum vulgare* L. and *Salvia officinalis* L.; in their *in vitro* experiment were the most sensitive strains *Staphylococcus* sp. including *S. aureus*, *E. coli* O157: H7, *L. monocytogenes* ATCC 7644 and *Lactococcus* sp. **Smith-Palmer et al. (1998)** reported bacteriostatic and bactericide activity of sage extract on the growth of *E. coli* and *S. aureus*. The most important components from chemical composition view of *Origanum vulgare* are thymol and carvacrol; in *Origanum* sp. were detected also acyclic monoterpenoids geraniol, linalool, kamfor and borneol. The concentration of plant essential oils components fluctuates during the seasons **(Skoula & Harborne, 2002).** There is a relationship between chemical structure of essential oil components and its antibacterial potential, it is described as the big correlation with the concentration of essential oil and its active compounds (phenols, alcohols, ketones, esters) and pH of test media under *in vitro* conditions **(Baricevic & Bartol, 2002)**. **Bozin et al. (2006)** demonstrated an antibacterial activity of oregano essential oils/extracts against multiresistant strains of *Pseudomonas aeruginosa* and *E. coli* in *in vitro* experiments. The mechanism of inhibition is probably given by damage of permeability and the integrity of cell membrane, pH homeostasis (pH gradient - ΔpH, **Helander et al., 1998**), the balance of inorganic ions (membrane potential - Δψ; **Sikkema et al., 1995**). Carvacrol, the active component of many essential oils, destabilizes the cytoplasmic membrane of cell, reduces the pH gradient, causes ATP depletion by leakage of ions, amino acids and nucleic acids followed by cell death **(Ultee et al., 2002)**. The antimicrobial effect of plant extracts obtained from *Origanum vulgare* L. and *Salvia officinalis* L. was reported under *in vitro* as well as *in vivo* conditions e. g in rabbits; reduction of coagulase-positive staphylococci and *Clostridium*-like sp. was demonstrated after administration oregano and sage plant extracts **(Szabóová et al., 2011; 2012b)**.

CONCLUSION

Oregano and sage plant extracts are effective bioactive substances. The results obtained are useble to prevent contamination during rabbit meat processing. Consumers prefer dietetic healthy, nutritive and non-contaminated food. In addition, natural alternatives are requested to replace the additives used up to now but recently banned.

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