ANTIBACTERIAL ACTIVITY OF MICROENCAPSULATED VIA SPOUTED-BED HYDRO-ALCOHOLIC ROSEMARY EXTRACTS

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ABSTRACT

In this study the antibacterial activity of microencapsulated rosemary hydro-alcoholic extracts against Escherichia coli K12 was observed. The tested microcapsules were previously produced in five batches varying the type of the inert core and shell material as well as the content of fresh or concentrated rosemary extract. Batch 1 and batch 3 contained equal amount of non-concentrated rosemary extract where batch 2 and batch 4 consisted of the same amount of concentrated rosemary extract. Microcapsules from the last batch 5 were impregnated with three times higher quantity of the non-concentrated rosemary extract than the previous four batches. All five types of microcapsules were tested by the agar diffusion method and showed inhibitory effect against the bacterial strain. The highest antibacterial activity was obtained for batch 5 followed by the samples with concentrated extract (batch 2 and batch 4) and the least inhibitory effect was observed by the microcapsules with non-concentrated extract (batch 1 and batch 3). Keywords: antibacterial activity, Rosmarinus officinalis, E. coli K12, hydro-alcoholic extracts, agar diffusion method.

INTRODUCTION

In the last decades the food industry together with the health authorities are focusing on producing safe food products for consumers. Synthetic additives have been widely used and the trend is to decrease their use. Search for natural additives, especially of plant origin has notably increased in recent years. Therefore, the development and application of natural products with both antioxidant and antibacterial activities may be necessary and useful to prolong their storage shelf life and potential for preventing food diseases [1, 2].

Rosmarinus officinalis is an aromatic evergreen plant belonging to the family Lamiaceae and is the only representative of this species that is wild in the Mediterranean regions [3, 4]. Rosemary has a broad range of applications, from culinary to medical, because of its aromatic properties and benefits to human health [5]. It is well known that the biological properties of this species are mainly due to phenolic compounds [6]. The numerous biological activities of rosemary include antioxidant, antimicrobial, antidiabetic and antidepressant, anti-cancer and anti-inflammatory action. Essential oils or extracts of rosemary are used in the food industry, cosmetics, aromatherapy and medicine [7, 8]. Studies show that the inhibitory effect of rosemary is a result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnusic acid, rosmanol and isorosmanol [6]. Moreover, according to the experimental data by Romano et al. 2009, the antibacterial activity of the rosemary extract is significantly higher than the one of additives used in the food industry such as butylated hydroxytoluene (BHT)

The most widely used, easy and fast method to isolate the active ingredients from aromatic plants is the solid-liquid extraction [11]. The solvents used for the extraction processes have a significant effect on the composition of the extract. Absolute ethanol is used mostly to extract diterpenes (carnosol and carnosic acid) and hot water - for extraction of phenolic acids (e.g. rosmarinic acid). Our previous investigations demonstrated that hydro-alcoholic solvents with concentration of ethanol in the range of 30 - 50 wt. % lead to reasonable extract yield of both carnosic and rosmarinic acids [12].

There are some difficulties when using bioactive compounds such as instability, reactivity with other food matrix ingredients or possess strong odour and/or flavours. Microencapsulation emerges as a potential approach to overcome these problems and at the same time to provide controlled or targeted delivery or release [13].

\textit{Escherichia coli} (\textit{E. coli}) is a Gram-negative, facultative anaerobic, rod-shaped bacteria belonging to the \textit{Enterobacteriaceae} family and the genus \textit{Escherichia} [14]. Most \textit{E. coli} strains are harmless and considered an important part of a healthy human gastrointestinal tract. However, some serotypes are pathogenic and can cause a wide range of clinical illnesses, from food poisoning and gastrointestinal diseases to meningitis, urinary tract infections, and septicemia in humans [15].

The antimicrobial activity of rosemary extracts against important foodborne pathogens such as \textit{E. coli} 0157: H7 and \textit{E. coli} ATCC 35218 was reported previously [16 - 18], whereas corresponding reports on microencapsulated forms of the extracts are missing.

The focus of the present work is to study the antibacterial properties of microencapsulated rosemary extracts against the Gram-negative bacteria \textit{E. coli} K12.

**EXPERIMENTAL**

**Materials**

Liquid and solid (agar) medium, \textit{Luria Bertani} (LB) from HiMedia Laboratories were prepared. The strain \textit{E. coli} K12 was obtained from the Bulgarian National Bank of Industrial Microorganisms and Cell Culture and conserved in our laboratory. The culture was incubated in Shaker ES-20/60. Sterile filter paper discs (6 mm diameter) by HiMedia Laboratories were used for the antibacterial experiments.

**Rosemary extracts preparation**

Microencapsulated rosemary extracts used in this work were produced via spouted bed processing of fresh rosemary extracts obtained with 37 wt. % ethanol in water as a solvent for extraction. Both fresh extracts and their concentrates resulting from vacuum evaporation were employed in the coating solution for spouted bed microencapsulation. Detailed description of the solvent extraction and microencapsulation methodologies can be found elsewhere [12]. The composition of the coating solution for each of the studied five microencapsulated products is shown in Table 1. In addition, each batch contains 220 g of maltodextrin as a core material.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Batch 4</th>
<th>Batch 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composition of the coating solutions, wt. %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Modified starch</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fresh extract</td>
<td>80</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td>80</td>
</tr>
<tr>
<td>Concentrated extract</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>Amount of coating solution, g</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>239</td>
<td>755</td>
</tr>
</tbody>
</table>
Antibacterial experiments

The antibacterial effect of microencapsulated rosemary extracts was tested against the Gram-negative strain - *Escherichia coli* K12. The *E. coli* K12 strain was chosen not only because it is a model strain for pathogenic bacteria with the ability to cause clinical illnesses but also because of the higher resistance that Gram-negative bacteria are showing against antibacterial agents in comparison to Gram-positive bacteria. This higher resistance is due to the more complex cell wall and additional outer membrane of Gram-negative bacteria [19].

The culture was grown, sub-cultured and maintained in LB agar medium and stored at 4°C. For the experiment a single colony of the organism was inoculated into 50 ml LB broth and incubated overnight (24 h) at 37°C with shaking at 220 rpm. 100 μl of bacterial suspension with 1×10^7 cfu/ml were seeded in agar plates with solid LB medium by the pour plate technique. For each plate 3 filter disks were used and all of the batches were performed in triplicate as described previously [20]. The disks were impregnated with 6 μl of 10 wt. % aquatic solution of the microencapsulated rosemary extracts. Inhibition zones around the disks were measured after incubation overnight at 37°C. The formation of a clear zone (restricted bacterial growth) is an indication of antibacterial activity for the obtained materials.

RESULTS AND DISCUSSION

The agar diffusion test was performed for each batch and the results are summarized in Fig. 1.

The smallest inhibition zones are observed for batch 1 (0.83 mm) and batch 3 - 1.00 mm. Batch 2 and batch 4 are showing stronger inhibition and the measured values are 1.20 mm and 1.33 mm, respectively. Batch 5 has the highest antibacterial activity with a resulting zone of 1.85 mm.

As described previously [12], batch 1 and batch 3 contain equal amount of non-concentrated rosemary extract while batch 2 and batch 4 contain the same amount of concentrated extract. Another major difference in the ratio of the used components is that for batch 1 and batch 2 an equal amount of maltodextrin and modified starch is used when for batch 3 and batch 4 only maltodextrin is used. The extract for batch 5 is also non-concentrated but the quantity of the impregnated material is three times higher, which leads to higher quantity of rosmarinic acid in the microcapsules thus stronger antibacterial activity of the sample.

The antibacterial test experiment for the five batches is presented in Fig. 2. As it could be seen, batch 1 and batch 3 show smaller inhibition zones in comparison to those of batch 2 and batch 4. For batch 5 a noticeable difference could be observed due to the above mentioned reasons.

Many authors reported the antimicrobial effect of rosemary extracts against Gram-negative bacteria [21, 22]. At the same time there is lack of information about the antibacterial effect of their microencapsulated forms [23]. To the best of the authors’ knowledge, there are no reports of the antibacterial activity of microencapsulated via spouted-bed hydro-alcoholic rosemary extracts.

![Fig. 1. Inhibition zones of all hydro-alcoholic microencapsulated extracts (batch 1, batch 2, batch 3, batch 4 and batch 5) tested against *E. coli* K12.](image-url)
CONCLUSIONS

The diffusion test used for analysing the antibacterial activity showed inhibitory effect of the microcapsules impregnated with rosemary extracts against the Gram-negative bacteria *E. coli* K12. The results in the present work indicate that the analyses of the microencapsulated rosemary extracts are potentially applicable in the food and pharmaceutical industries.

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REFERENCES


