

Effects of Natural Compounds on Food-related Microorganisms

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Abstract.

The aim was to identify natural compounds, alternative to antimicrobial chemical additives used in the fermented meat industry, able to inhibit spoilage microorganisms without affecting the growth of useful ones. Moreover, the effect of natural compounds on protein expression of different food-related bacteria was studied.

The antimicrobial activity produced by natural compounds was detected by the agar well diffusion assay. The effectiveness of natural compounds in different concentrations was detected evaluating the microbial growth in meat broth medium. The effect of natural compounds on the protein expression of food related microorganisms was evaluated by electrophoresis with microfluidic techniques.

*The results evidenced that three natural compounds (*Rosmarinus officinalis*, *Citrus compositum* and *Malpighia punicipholia*) were the most effective in order to inhibit spoilage microorganisms such as *Clostridium sporogenes*, *Brochothrix thermosphacta* and *Pseudomonas fluorescens*, without affect the growth of *Lactobacillus sakei*, *Staphylococcus xylosus* and *Kocuria varians*, used as starter in fermented meat products. Electrophoresis evidenced that natural compounds are also able to affect the expression of the microbial protein pattern.*

The research produced promising results on the possibility to obtain innovative fermented meat products without chemical additives or with their reduction. Moreover, the results highlighted a relationship between the sensitivity/resistance of microorganisms to natural compounds and their whole-cell protein expression.

Keywords. Bio-control, antimicrobial natural extracts, chemical additives reduction

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Introduction

The use of natural compounds may represent an important innovation in the food preservation, also considering the consumer demand, that requires safe foods in terms of reduction in the use of chemical additives (Holley & Patel 2005). Several spices and essential oils may be utilized as natural additives alternative to chemicals, thanks to their strong antimicrobial activity (Nychas 1995; Tassau *et al.* 2000). There are many scientific reports that highlight antimicrobial effects of several natural compounds on pathogenic or spoilage bacteria (Delaquis *et al.* 2001; Holley & Patel 2005). However the literature is very poor in studies focusing on the activity against both undesirable and useful microorganisms. This aspect has to be contemplated, considering that the presence of many food-related microorganisms assumes a key role in the production of fermented foods. Microorganisms are able to react in different ways to the presence of natural compounds, but the mechanisms of response, in terms of susceptibility or resistance, are still not completely identified. Since several Authors (Burt, 2004; Bakkali *et al.* 2008) demonstrated that the microbial response to stress conditions involves the induction of specific proteins, we can suppose that a similar mechanism could be adopted by microorganisms to protect themselves against natural compounds, whose presence could be considered as a stress condition for the microbial cell. In this study different natural compounds were studied in order to assess their ability to inhibit spoilage bacteria without affecting the growth of useful ones. Moreover, the effect of natural compounds on the protein expression of different food-related bacteria was studied.

Materials and Methods

Bacterial strains and natural compounds

Five undesirable microorganisms (*Clostridium sporogenes* DSM 795T, *Pseudomonas fluorescens* DSM 50090T, *Enterococcus faecium* DSM 20477T, *Brochothrix thermosphacta* DSM 20171T, *Staphylococcus aureus* DSM 20714T) and three useful ones (*Lactobacillus sakei* DSM 20017T, *Staphylococcus xylosus* DSM 20266T and *Kocuria varians* DSM 20033T) were used in this study. Natural compounds used in this study were extracted by *Carica papaya*, *Citrus compositum*, *Malpighia punicifolia*, *Medicago composita*, *Propolis*, *Raphanus niger*, *Rosmarinus officinalis*, *Spirulina pacifica*. Substances were supplied from Bioma (®Swiss).

Detection of antimicrobial activity

The inhibitory action of each compound was assessed by the agar well diffusion assay (Tremonte *et al.* 2007). Clear zones around the wells were described as a low (L < 5 mm), moderate (M between 5 and 8 mm) or strong (S > 8 mm) inhibitory effect. The presence of growth around the well was considered as absence of inhibition (N, neutral).

The antimicrobial effectiveness was detected by the evaluation of the growth of each microorganism inoculated in meat broth added of different concentrations of natural compounds (0.1%; 0.5%; 1%). The initial inoculum size was 10^4 cfu/ml of each bacterium. After incubation for 24 h at right temperatures, the bacterial growth was quantified to evaluate the antimicrobial activity as $\Delta\text{LOG UFC/ml}$ (difference of growth between 24 h of incubation and time zero).

Detection of the effect of natural compounds on whole-cell protein

Bacterial strains were inoculated in proper growth media in the presence of each natural compound at 1% concentration. Broths without natural compounds were used as control. After incubation for 72h, whole cell proteins were extracted and protein patterns were separated and

analysed by electrophoresis with innovative microfluidic techniques. Data were analysed and compared with the bio-rad experion system software tools (BIO-RAD, USA).

Results

Antimicrobial activity

The results of the agar well diffusion assay are summarized in Table 1. *Carica papaya*, *Propolis* and *Raphanus niger* showed a low (L) antimicrobial activity. On the other hand, *Rosmarinus officinalis*, *Citrus compositum* and *Medicago composita* were characterised by the largest spectrum of activity against both undesirable bacteria and useful ones. *Spirulina pacifica* and *Malpighia punicifolia* showed an intermediate antimicrobial activity, but interestingly *Malpighia punicifolia* produced inhibition only against undesirable microorganisms, without affect useful ones.

Table 1 Results of the “Agar Well diffusion Assay”. Clear zones around the wells indicate different levels of inhibition. (Low, L<5 mm), (Moderate, M between 5 and 8 mm) , (Strong, S > 8 mm). The presence of growth around the well indicates absence of inhibition (Neutral, N).

| Natural extract | Useful microorganisms | | | Undesirable microorganisms | | | | |
|-------------------------------|-----------------------|-------------------|------------------|----------------------------|-------------------------|-------------------|-----------------------|------------------|
| | <i>L. sakei</i> | <i>S. xylosum</i> | <i>K.varians</i> | <i>C. sporogenes</i> | <i>B. thermosphacta</i> | <i>E. faecium</i> | <i>P. fluorescens</i> | <i>S. aureus</i> |
| <i>Carica papaya</i> | N | N | N | L | L | N | N | N |
| <i>Citrus compositum</i> | M | M | M | H | H | H | H | M |
| <i>Malpighia punicifolia</i> | N | N | N | M | H | N | H | L |
| <i>Medicago composita</i> | L | L | H | M | L | L | L | L |
| Propolis | N | N | N | L | L | N | N | N |
| <i>Raphanus niger</i> | N | N | N | L | L | N | N | N |
| <i>Rosmarinus officinalis</i> | H | H | H | H | H | L | H | H |
| <i>Spirulina pacifica</i> | M | M | M | M | H | N | N | L |

This effect was highlighted (data not shown) also in the presence of the lowest concentration of this compound (0.1%). Among the other assayed extracts, *Citrus compositum* and *Rosmarinus officinalis* showed a promising application, exerting their inhibitory activity at the lowest concentration (0.1%) against *C. sporogenes* and *B. thermosphacta* without affecting useful bacteria (data not shown).

Effect of natural compounds on whole-cell proteins

The results, in accordance with other studies (Bakkali *et al.* 2008), evidenced that microorganisms could modify their protein expression in presence of stress conditions such as natural compounds with antimicrobial activity. Particular attention was given to the behaviour of *C. sporogenes*, that was chosen as non-pathogenic microorganism to simulate the behaviour of pathogenic *C. botulinum* (Girardin *et al.*, 2005). Moreover the attention was focused on *Staph. aureus*, which was weakly inhibited by natural compounds. When the strain of *C. sporogenes* resulted strongly inhibited by natural compounds, such as *Malhighia punicifolia*, the degradation/non-expression of high molecular weight proteins was observed. These differences appeared particularly significant comparing (Figure 1A) the two electropherograms (region between 35 and 5 second of migration) related to the growth of *C. sporogenes* in absence of the natural compound (characterized by the presence of five peaks), or in its presence (no evident peak). Different results were appreciated when the growth of assayed strains was only restrained but not completely inhibited by natural compounds. This case is well explicated by the electropherograms (Figure 1B) of whole-cell proteins of *Staph. aureus* cultivated in absence or in presence of *Malhighia punicifolia*. In detail, new peaks (between 45 and 50 seconds of migration) were observed for *Staph. aureus* cultivated in presence of *Malhighia punicifolia*. On this bases, we can assert that some strains, when are weakly inhibited by the presence of

natural compounds, are able to react to the stress through the synthesis of new undefined proteins. Finally, the study highlighted that strains not inhibited by the presence of natural compounds produced similar electropherograms in presence or in absence of the assayed natural compound (data not shown). On the basis of these results we can assert that natural compounds are able to affect not only the microbial growth but also the expression of microbial protein pattern.

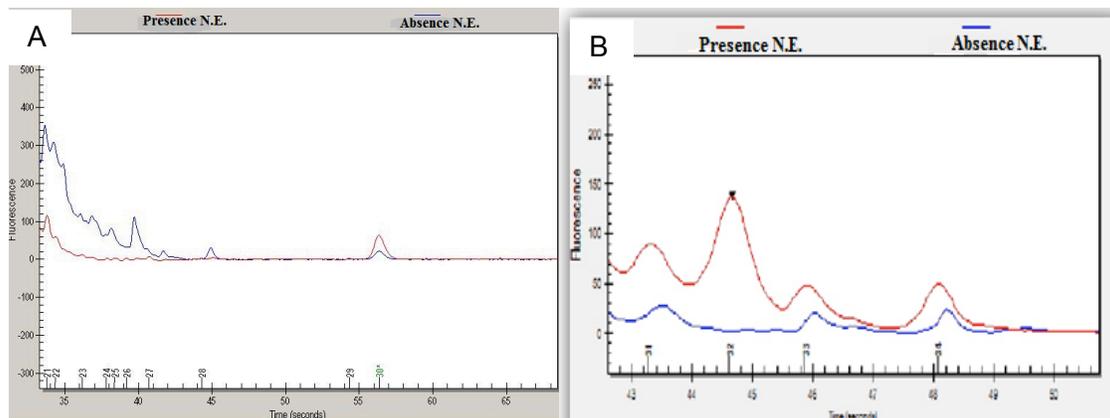


Figure 1AB Electropherograms of whole-cell proteins of (A) *C. sporogenes* or (B) *Staph. aureus* in absence (----) and in presence (----) of *Malpighia puniceifolia*.

Conclusions

This research produced promising results which open new horizons on the possibility to produce innovative products without chemical additives. However, the most important scientific enrichment produced by this study is due to data that describe the relationship between the sensitivity/resistance of microorganisms to natural compounds and their whole-cell protein expression.

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