

ANTIMICROBIAL ACTIVITY OF CONSTITUTIONAL WATER FROM AROMATIC HERBS AGAINST TECHNOLOGICAL, ALTERATIVE AND PATHOGENIC MICROORGANISMS



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INTRODUCTION

This experimentation is part of «L'innovazione di processo nella filiera delle erbe aromatiche essiccate-ESSICA» project (Interreg V France-Italy, ALCOTRA 2014-2020), focused on process innovation in aromatic herb chain, in order to obtain high quality products and, thus, increase producer competitiveness.

The partnership includes four members: TERRE di SAVOIA Association, DISAFA (Department of Agricultural, Forest and Food Sciences, University of Turin), FRANCEAGRIMER (Etablissement National des Produits de l'Agriculture et de la Mer), CRIEPPAM (Centre Régionalisé Interprofessionnel d'Expérimentation en Plantes à Parfum, Aromatiques et Médicinales).

A new low-temperature drying technique has been tested, in the project, to improve herb quality, and constitutional waters obtained from the drying have been studied, in the present research, for their antimicrobial properties. Constitutional waters were collected from thyme, mint, savory, lemon balm, oregano and mallow and their antimicrobial activity was tested by performing semi-quantitative trials on traditional plates.



AIM OF THE WORK

- ✓ to check if constitutional waters could be used as natural antimicrobial agents against **altering** and **pathogenic microorganisms** contaminating food products
- ✓ to evaluate if constitutional waters inhibits the growth of **technological microorganisms**, the activity of which is fundamental in fermented foods.

MATERIALS AND METHODS



Figure 1. Cold drier provided by Northwest Technology (Boves, Cuneo, Italy)

Six different herbs (savory, mint, lemon balm, mallow, thyme and oregano) produced in Italy (Piedmont region) and in France (Provence region) were submitted to cold drying (20 °C) and constitutional waters were collected. The cold drier was provided by Northwest Technology (Boves, Cuneo, Italy) (Figure 1).

The microorganisms used for evaluating the antimicrobial activity of constitutional waters belonged to *Lactobacillus* spp., *Lactococcus* spp. and *Streptococcus* spp. genera, *Saccharomyces cerevisiae* and *Candida humilis* species related to fermented food transformation, and to *Penicillium* spp. and *Aspergillus* spp., alterative fungi affecting the shelf-life of different food products. Finally, water properties were tested against the pathogens *Listeria monocytogenes*, *Yersinia enterocolitica* and *Salmonella thymophimum*.

To evaluate the antimicrobial activity of constitutional waters against pathogenic and technological microorganisms, the diameters of the inhibition halos were measured around constitutional water-filled wells (Figure 2) and around paper disks soaked in water (Figure 3) on Petri dishes covered with microbial growth of, respectively, pathogenic and technological microorganisms. To evaluate the antimicrobial activity against altering microorganisms, the diameters of the fungal colonies were compared when the fungi were grown in Malt Agar medium with and without the addition of constitutional water (Figure 4).

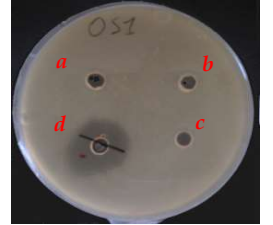


Figure 2. Absence of inhibition halo around lemon balm constitutional water-filled wells on BHI Agar covered with *Listeria monocytogenes* (a,b,c) compared to chloramphenicol-filled wells (d)

RESULTS AND DISCUSSION



Figure 3. Inhibition halos around paper disks soaked in lemon balm constitutional water on M17 medium covered with *Lactococcus lactis* growth.

The constitutional waters did not show inhibition activity against pathogenic species (Figure 2) while a partial inhibition was found against alterative moulds. In particular, the best results were obtained by using constitutional water from thyme and oregano which showed partial inhibition towards *Penicillium citrinum*, *Penicillium aurantiogriseum* and *Aspergillus niger* growth. Moreover, mint constitutional water also showed moderate antifungal activity against *P. citrinum* and *A. niger* (Figure 5).

Constitutional waters obtained from French thyme, French oregano and Italian savory partially limited the growth of some technological microorganisms (Table 1). *Lactobacillus brevis* was the only lactic acid bacteria not inhibited by any of the constitutional water tested in the present study.

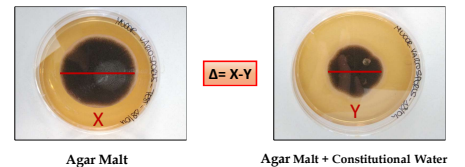


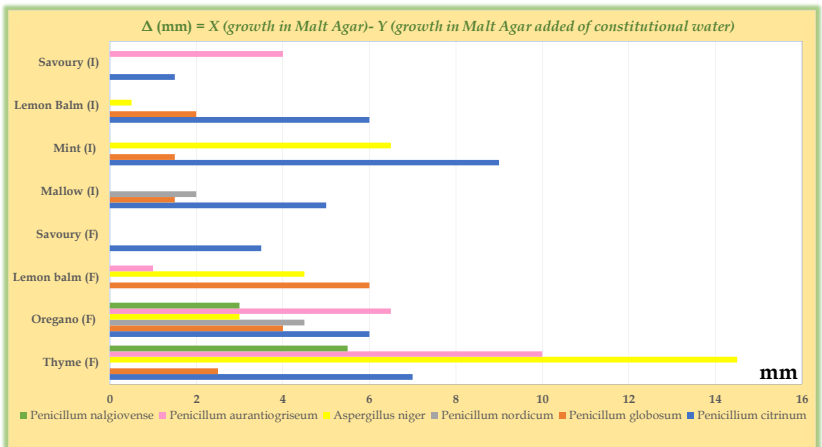
Figure 4. Different growth expansion (Δ) of *Aspergillus niger* mycelium on Malt Agar plates with/without the addition of French thyme constitutional water

Table 1. Inhibition halos (mean and standard deviation, mm) around paper disks soaked in constitutional waters on media covered with technological microorganisms.

SPECIES	STRAIN CODE	Thyme (F)	Oregano (F)	Lemon balm (F)	Savory (F)	Mallow (F)	Mint (F)	Lemon Balm (F)	Savory (F)
<i>Lb. brevis</i>	CNRZ 734	a*	a*	a*	a*	a*	a*	a*	a*
<i>Lc. lactis lactis</i>	DSM 20481	a*	a*	a*	a*	a*	a*	a*	1,13 ± 1,36
<i>Lb. casei shirota</i>	shirota	3,83 ± 1,17	a*	a*	a*	a*	a*	a*	a*
<i>Lb. paracasei</i>	DSM 5622	a*	1,13 ± 1,21	a*	a*	2,33 ± 1,15	a*	a*	a*
<i>Lb. plantarum</i>	ATCC 4908	1,43 ± 1,40	1,50 ± 1,31	a*	a*	a*	a*	a*	1,25 ± 1,39
<i>Lc. lactis cremoris</i>	DMS 20069	1,00 ± 0,53	a*	2,33 ± 1,53	a*	a*	a*	a*	2,25 ± 0,89
<i>St. thermophilus</i>	DSM 20617	a*	a*	a*	a*	1,67 ± 0,58	a*	a*	a*
<i>Lb. fermentum</i>	LA S 02	2,00 ± 1,63	3,88 ± 3,44	a*	a*	1,67 ± 0,58	a*	a*	a*
<i>Lb. plantarum</i>	A 16	a*	3,13 ± 1,55	a*	a*	a*	a*	a*	a*
<i>S. cerevisiae</i>	L11 B01	1,00 ± 0,00	1,63 ± 0,53	a*	a*	a*	a*	a*	1,00 ± 0,00
<i>S. cerevisiae</i>	A 37	1,00 ± 0,00	1,00 ± 0,00	a*	a*	a*	a*	a*	a*
<i>C. humilis</i>	A 11	1,88 ± 0,35	a*	a*	a*	a*	a*	a*	a*

a*: absence

Figure 5. Different growth (Δ) of mycelium of alterative fungal species on Malt Agar plates with/without the addition of constitutional waters



CONCLUSIONS

On the basis of the data obtained, the constitutional waters tested in the present study are not active against some of the most common microorganism in food products. On the contrary, constitutional waters especially from thyme and oregano showed moderate antifungal activity which could be considered for some anti-moulds applications in foods. Finally, the low activity on technological microorganisms should not give rise to concern for possible applications on fermented foods. Those results will be further investigated, in fact, constitutional water could be considered a by-product of interest, in food manufacturing, as natural preservative.