SENSITIVITY OF COMMON MEAT SPOILAGE BACTERIA TO SELECTED ORGANIC ACIDS, FATTY ACIDS, AND ESSENTIAL OILS

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Keywords: meat, spoilage, bacteria, inhibition.

INTRODUCTION With the ultimate goal of developing active packages, with antimicrobial properties, for the preservation of meat products, a preliminary study was undertaken to select the organic substances most efficient to inhibit the growth of common meat spoilage organisms. To keep in line with previously published work, the short chain (<C4) or cyclic carboxylic acids will be referred to as organic acids, while the medium to long chain acids ($\geq$C12) will be called fatty acids, although the distinction is quite arbitrary.

MATERIALS AND METHODS *Carnobacterium piscicola (Cp)*, *Lactobacillus curvatus (Lc)*, and *Lactobacillus sake (Ls)* were obtained from the ATTC culture collection and grown in lactobacilli MRS broth. *Pseudomonas fluorescens (Pf)*, *Brochothrix thermosphacta (Bt)*, and *Serratia liquefaciens (Sl)* were isolated from various meat products and grown in brain heart infusion broth (BHIB).

To evaluate the effects of organic acids (acetic, citric, lactic, propionic, benzoic, and sorbic) on bacterial growth, the acids were first added to the growth media (MRS or BHIB) to reach final concentrations of 0.1, 0.2, 0.3, 0.5, 0.75, or 1% (w/v). The media were subsequently inoculated with the appropriate organisms (about 10^5 cells.ml^{-1}, final concentration), introduced in a 96 well microplate, and incubated aerobically (*P. fluorescens, B. thermosphacta, S. liquefaciens*) or in a CO_2-enriched atmosphere (the others organisms), at 20°C, without agitation. At regular intervals, absorbance (540 nm) was read with an automatic plate reader, after suspension of the sedimented cells on a rotary shaker. The experiment was repeated with hydrochloric acid to enable one to dissociate the effects of organic acids from the effects of pH alone.

Growth inhibition by fatty acids (lauric, linoleic, linolenic, myristic, oleic, palmitic, palmitoleic, stearic) and essential oils (black pepper, cinnamon, clove, cumin, garlic, oregano, pimento, rosemary, thyme) was evaluated after mixing these substances in molten MRS or BHI agar, cooled to 45°C, to reach final concentrations of 50-2500 μg.ml^{-1} (acids) and 1/10, 1/100, or 1/1000 v/v (oils). The agars were subsequently poured into Petri dishes, surface-inoculated with the appropriate organisms (0.1 ml of a 10^5 cells.ml^{-1} culture), incubated at 20°C as above, and examined over time for the presence of bacterial colonies. In addition, the essential oils were analyzed by GLC to determine their contents in 17 aromatic substances commonly found in spice extracts.

RESULTS AND DISCUSSION In general, the presence of organic acids in the growth media resulted in growth inhibition, which took the form of longer lag periods, lower growth rates, and/or lower bacterial numbers in stationary phase. Large variations were observed, however, in the extent of inhibition, depending on the bacterium and on the acid evaluated. The strongest inhibition was observed with *C. piscicola* which did not grow in the presence of acetic or propionic acid, even in low concentrations (0.1% in the growth medium). On the other hand, the highest concentration of citric acid used (1%) did not delay the growth of *L. curvatus* or *P. fluorescens* for more than 48 h and 72 h, respectively. Intermediate degrees of inhibition were observed for all other bacterium-acid combinations. Growth inhibition by benzoic and sorbic acids was only tested on *B. thermosphacta*, *P. fluorescens*, and *S. liquefaciens* (results not shown). Both acids partially inhibited the growth of the three organisms at a concentration of 0.15%. Higher concentrations could not be used due to the limited solubility of these acids in water.

The minimum concentrations which resulted in complete growth inhibition (no detectable increase in absorbance during the whole experiment, 0-120 h) were referred to as minimum inhibitory concentrations. These enable one to easily compare the relative effectiveness of the various acids to inhibit bacterial growth. Accordingly, acetic acid was found to be the most inhibitory organic acid for all the bacteria tested (Table 1), followed by propionic and lactic acids, while citric acid was the least effective to prevent bacterial growth. It must be stressed, however, that the abilities of the various organic acids to hinder bacterial growth were only slightly different.

Under comparable pH conditions, growth inhibition was generally more pronounced in the presence of organic acids than in the presence of hydrochloric acid (not shown). This indicated that the molecules of organic acids had an inhibitory effect of their own. In addition, the organic acids most detrimental to bacterial growth, i.e. acetic and propionic, were also the least dissociated at a given concentration, due to their higher pKa (4.75 and 4.90, respectively, compared to 3.1 for the other acids). Both findings are consistent with the well established fact that undissociated organic acid molecules can penetrate bacterial cells and inhibit growth through disruption of pH homeostasis.

Compared to organic acids, the fatty acids had only limited antibacterial properties against meat spoilage bacteria. Four of the fatty acids used (myristic, oleic, palmitic, and stearic acid) did not inhibit the growth of any organism, even at the highest concentration tested (not shown) and the four remaining fatty acids were also ineffective against *B. thermosphacta*, *P. fluorescens*, and *S. liquefaciens* (Table 1). However, growth inhibition by fatty acids, when it occurred, was achieved with much lower (about 10 fold) acid concentrations than inhibition by organic acids. Interestingly, the specific nature of the fatty acid used (lauric, palmitoleic, linoleic, or linolenic) had little influence on the resulting antibacterial effect, since only marginal differences were found between the acid concentrations required to inhibit growth.

Table 1: Minimum inhibitory concentration of various organic or fatty acids relative to the growth of meat spoilage bacteria[1]

	Lc	*Ls*	*Cp*	*Bt*	*Pf*	*Sl*
Acetic	0.75	0.75	0.1	0.3	0.5	0.50
Propionic	0.75	0.75	0.1	0.3	0.5	0.50
Citric	>1	1	0.2	0.5	1	1
Lactic	0.75	0.75	0.2	0.5	0.5	0.75
Lauric	0.050	0.050	0.025	*ni*[2]	*ni*	*ni*
Palmitoleic	0.045	0.045	0.035	*ni*	*ni*	*ni*
Linoleic	0.065	0.065	0.060	*ni*	*ni*	*ni*
Linolenic	0.050	0.065	0.050	*ni*	*ni*	*ni*

[1] minimum concentration (%) which prevented growth of the organism for at least 48 h.
[2] no inhibition detected at a concentration of 0.25%.

The failure of the fatty acids used in this study to hinder the growth of *P. fluorescens,* and *S. liquefaciens* was not surprising since most Gram-negative bacteria are known to be resistant to the inhibitory effects of medium and long chain fatty acids. In contrast, the uninhibited growth of *B. thermosphacta* in the presence of fatty acids, not previously reported, was not expected since Gram-positive bacteria are generally sensitive to fatty acids. Indeed, the other Gram-positive bacteria tested, i.e. the lactobacilli and *C. piscicola*, could not grow in the presence of lauric, palmitoleic, linoleic, and linolenic acids, in concentrations above the 0.025-0.065% range (250-650 $\mu g.ml^{-1}$; Table 1).

All essential oils selected for the study displayed some degree of antibacterial activity against meat spoilage bacteria (Table 2). The least efficient oil was thyme which failed to inhibit the growth of *P. fluorescens* and *S. liquefaciens*, even at the highest concentration tested (1/10, v/v). In contrast, clove, cinnamon, and rosemary oils, in that order, were found to be the most efficient oils, inhibiting the growth of some organisms at the 1/100 (v/v) concentration.

Depending on the oil, the 17 aromatic substances commonly found in spices represented from 20% to 80% of the total concentration of volatile compounds detected by GLC (not shown). All of the oils contained at least one of the 17 substances, and most oils contained several. These substances can be used, if wanted, to compare the actual concentration of essential oils in this study and in other studies. Clove oil, found to be the most inhibitory towards meat spoilage bacteria (Table 2), was particularly rich in eugenol, a known antibacterial agent, while cinnamon oil contained eugenol and cinnamaldehyde, another antibacterial agent, in large amounts. No known antibacterial agent was found in rosemary oil but most of the oil contents (80%) was not identified (not shown).

Table 2: Inhibitory effect of various essential oils on the growth of meat spoilage bacteria.[1]

	Lc	*Ls*	*Cp*	*Bt*	*Pf*	*Sl*
Cinnamon	++	++	++	+	+	+
Clove	++	++	++	++	+	++
Cumin	+	+	+	+	+	+
Garlic	+	+	+	+	+	+
Oregano	+	+	+	+	+	+
Black pepper	+	+	+	+	+	+
Pimento	+	+	+	+	+	+
Rosemary	++	++	++	+	+	+
Thyme	+	+	+	+	*ni*	*ni*

[1] ++: no growth after 48 h of incubation on agar containing 1/100 (v/v) of essential oils; +: no growth after 48 h of incubation on agar containing 1/10 (v/v) of essential oils: *ni*: growth in the presence of essential oils at the 1/10 (v/v) concentration.

Among the 6 organic acids, the 8 fatty acids, and the 9 essentials oils evaluated in this study, 3 organic acids (acetic, propionic, lactic), 4 fatty acids (lauric, palmitoleic, linoleic, linolenic), and 3 essential oils (clove, cinnamon, rosemary) were found to be the most effective substances to inhibit the growth of common meat spoilage bacteria, under favourable conditions of incubation (rich growth medium, optimum temperature). Obviously, additional experiments are now required to test the ability of these substances to delay or suppress the growth of meat spoilage organisms on the surface of packaged meat products, under storage conditions prevailing at the retail level. However, since bacteria are generally less resistant to stresses in more adverse environments, it can be expected that the substances selected in this study will also be efficient in controlling the growth of bacteria indigenous to meat when these will have to face lower temperature, nutrient limitations, and competition from neighbouring organisms.