

# Fermentation Enhancement by Spices: Identification of Active Component

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## ABSTRACT

Manganese was identified as a factor in spices responsible for the enhancement of acid production by meat starter bacteria. Stimulatory activity of 0.1N HCl extracts of spices increased with increasing Mn concentration. Extracts of clove, cardamom, ginger, celery seed, cinnamon, and turmeric, all with high Mn contents, were strongly stimulatory. Clove had the highest Mn content and exerted the greatest stimulatory effect. Spice extracts or standard  $Mn^{++}$  solutions enhanced acid production by *Lactobacillus plantarum* more than by *Pediococcus acidilactici* in a beef extract-sugar medium. Fermented sausages without spices but with added  $1 \times 10^{-5}M Mn^{++}$  and those with spices developed a similar level of acidity.

## INTRODUCTION

DURING STUDIES on sausage fermentation, it was observed that a mixture of spices accelerated lactic acid production by the lactic acid starter culture or by natural microflora present in ground meat (Zaika et al., 1978). In subsequent experiments the effect of individual spices on Lactacel MC starter culture, composed of *Lactobacillus plantarum* and *Pediococcus cerevisiae* (now designated *P. acidilactici*), was tested in liquid medium (Kissinger and Zaika, 1978; Zaika and Kissinger, 1979a, b). The starter bacteria grew in the presence of up to 12g/liter of the majority of the spices tested, but were severely inhibited by clove and oregano. When growth occurred in the presence of spices, it was accompanied by enhanced acid production with the degree of stimulation dependent on the spice used.

A few reports on stimulatory properties of spices have appeared in the literature. Studying the effect of spices on fermentation of Belgian dry sausage, Vandenriessche et al. (1980) confirmed that spices stimulated the fermentation of carbohydrate to lactate. Fermentation was effected by natural microflora in meat. Skjelkvåle and Nes (1981) studied the effect of clove, pepper, cinnamon, ginger, and garlic on commercial *L. plantarum* and *P. cerevisiae* starter cultures and found that addition of up to 10g/liter of the spices, alone or in combination, led to increased acid production except in the case of clove. Sreenivasamurthy and Krishnamurthy (1959) observed (no data presented) that an aqueous extract of ginger stimulated growth and acid production by *Lactobacillus casei*, while Park et al. (1980) reported that this organism was inhibited by a homogenate of fresh ginger added to TGY medium.

It has also been reported that spices can stimulate other microbial species such as micrococci (Salzer et al., 1977) and yeast (Corran and Edgar, 1933; Wright et al., 1954). These observations suggested that there is a common component in a variety of spices, present at various concentrations, that stimulates lactic acid production by meat starter culture bacteria. Therefore, the objective of the present study was to isolate, identify, and characterize this component as a potential means of accelerating lactic acid fermentation of meats.

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## MATERIALS & METHODS

### Microorganisms

*Lactobacillus plantarum* (Lactacel 804, Microlife Technics, Sarasota, FL), *Pediococcus acidilactici* (Lactacel 110, Microlife Technics) were used for studies in liquid medium. A mixed culture of *L. plantarum* and *P. acidilactici* (Lactacel MC, Microlife Technics) was used for preparation of fermented sausages.

### Liquid medium

The fermentation medium was prepared by dissolving 3g beef extract (Difco), 5g tryptone (Difco), 20g sucrose, and 20g glucose in 1 liter of distilled water. The pH of the medium was adjusted to 6.5 with 6N  $H_2SO_4$  (giving a post-sterilization pH of 5.7-6.3). Aliquots (250 ml) of the medium were dispensed into 500-ml Erlenmeyer flasks and sterilized for 15 min at 15 psi.

### Spices

Commercially dried and ground spices were obtained as "Purified" spices from Griffith Laboratories, Inc., Union, NJ. Spices used in the fermentation experiments contained less than 100 organisms/g.

### Fermentation in liquid medium

Spices, spice residues, or extracts were added aseptically to the flasks of sterile liquid medium. An inoculum was prepared from a 24-hr culture of the lactic acid bacteria in the liquid medium. All flasks were inoculated with 1 ml of the culture diluted with 0.1% peptone water, such that the initial bacterial population in the flasks ranged from  $10^3$ - $10^5$  cells/ml. Flasks were incubated statically at 35°C for up to 7 days. Samples for bacterial counts and titratable acidity were taken at 24-hr intervals.

### Titratable acidity

A 25-ml portion of each sample was centrifuged at 20,200  $\times$  g for 15 min to remove the cells. 10 ml of supernatant, diluted with 50 ml of distilled water, was titrated with 0.1N NaOH to pH 7.0 using a Fisher Accumet Model 325 pH meter equipped with a Corning combination pH electrode. The titratable acidity was expressed in terms of ml of 0.1N NaOH/10 ml medium. The titratable acidity of uninoculated medium was 0.33-0.65 ml.

### Enumeration of bacteria

Bacterial counts were made by conventional pour plate techniques using APT agar (Difco). Plates were incubated for 48 hr at 35°C.

### Extraction of spices with solvent

Spices, 2g, were weighed into 33  $\times$  94 mm cellulose extraction thimbles (Whatman) and extracted for 16 hr in a Soxhlet extractor with 130 ml of petroleum ether, b.p. 35-60°C, or other solvents. After extraction, the thimbles containing the insoluble spice residues were placed into sterile wide-mouth bottles, capped with cotton, and the solvent was allowed to evaporate.

For fermentation experiments, the contents of the extraction thimble were added to a flask containing 250 ml sterile medium. The mixture, representing 8g/liter whole spice, was inoculated and incubated as described above.

### 0.1N HCl extracts of spices

Spices, 5g, were extracted with chloroform as described above to remove oleoresin, and the residual solvent was allowed to evaporate. Each spice residue was transferred from the cellulose thimble into a 125-ml Erlenmeyer flask and mixed with 100 ml 0.1N HCl. The

mixtures were shaken several times during a period of 3 hr and were allowed to stand for 2 days. The insoluble residues were removed by centrifugation, and the acid extracts were filtered through Whatman #44 ashless filter paper. The extracts were stored at 4°C.

For fermentation studies, 5 ml aliquots of the 0.1N HCl extracts were added to 250-ml portions of sterile medium, giving a concentration equivalent to 1g of spice/liter. For the control, 5 ml of 0.1N HCl was added to 250 ml medium.

#### Manganese analysis

The manganese content of the 0.1N HCl extracts of spices was determined with the aid of a Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer. An oxidizing air-acetylene (50:40) flame was used, and measurements were made at 279.5 nm. Standard solutions containing 2, 6, and 20 ppm Mn were prepared by dilution with 0.1N HCl of a 1000 ppm manganese reference solution (Atomic Absorption Standard, Fisher Scientific Co.).

#### Standard manganese solutions

Solutions of  $MnSO_4 \cdot H_2O$  ranging from 1 to  $1 \times 10^{-6}M$  were prepared using sterile glassware and sterile glass-distilled water. For fermentation studies, 2.5 ml aliquots of the standard solutions were added to 250 ml of sterile media to give concentrations in the media of  $1 \times 10^{-2}$  to  $1 \times 10^{-8}M Mn^{++}$ .

#### Preparation of fermented sausages

Fermented sausages were prepared and processed as previously described (Zaika et al., 1978). All sausages were formulated with 30g NaCl, 20g glucose, 20g sucrose and 100 mg  $NaNO_2$  per kg of beef. Lactacel MC starter culture was added at a level of 0.1%. For experiment 1, three types of sausages were prepared in 1-kg batches: (1) control, no additive; (2) 1 ml of  $1 \times 10^{-2}M MnSO_4 \cdot H_2O$ ; (3) 8g of a 9-component spice mixture (Zaika et al., 1978). For experiment 2, four types of sausages were prepared in 2-kg batches: (1) control, no additive; (2) 2 ml of  $1 \times 10^{-3}M MnSO_4 \cdot H_2O$ ; (3) 2 ml of  $1 \times 10^{-2}M MnSO_4 \cdot H_2O$ ; (4) 2 ml of  $1 \times 10^{-1}M MnSO_4 \cdot H_2O$ . Thirteen sausages, each weighing approximately 150g, were prepared from each batch. Three sausages from each of the four batches were taken daily for analysis.

#### Acid production in sausages

Sausage, 50.0g, and 200 ml of distilled water were homogenized in a Waring Blender for 2 min. The mixture was centrifuged in the cold at  $8800 \times g$  for 20 min and the supernatant was filtered through glass wool to remove fat. A 50-ml aliquot of the filtrate was diluted with 50 ml of distilled water and titrated to pH 7 with 0.1N NaOH. This titratable acidity was expressed as percent lactic acid.

## RESULTS & DISCUSSION

SPICES were extracted with various solvents, and the effect of these fractions on the lactic acid bacteria was determined,

Table 1—Effect of spices<sup>a</sup> before and after extraction with petroleum ether on Lactacel MC<sup>b</sup> starter culture

	24 hr		96 hr	
	TA <sup>c</sup>	Count/ml	TA	Count/ml
Control medium	0.63	$1.0 \times 10^8$	1.88	$7.3 \times 10^7$
Cardamom	0.29	$6.0 \times 10^7$	2.53	$5.5 \times 10^4$
Cardamom—PE <sup>d</sup>	2.28	$4.5 \times 10^8$	7.50	$2.5 \times 10^8$
Rosemary	0.05	$2.4 \times 10^7$	4.86	$3.1 \times 10^8$
Rosemary—PE	1.66	$4.4 \times 10^8$	6.29	$7.2 \times 10^7$
White pepper	0.18	$7.1 \times 10^7$	3.28	$5.3 \times 10^6$
White pepper—PE	1.98	$4.1 \times 10^8$	5.88	$2.8 \times 10^8$
Control medium	0.60	$1.3 \times 10^8$	1.56	$2.6 \times 10^7$
Oregano	0.00	$1.3 \times 10^1$	0.05	<1
Oregano—PE	1.72	$1.5 \times 10^8$	6.80	$5.3 \times 10^6$
Control medium	0.63	$1.8 \times 10^8$	1.73	$5.5 \times 10^7$
Clove	0.00	$3.1 \times 10^4$	0.06	<1
Clove—PE	2.13	$2.4 \times 10^8$	7.17	$3.9 \times 10^7$

<sup>a</sup> 8g/liter of medium.

<sup>b</sup> Inoculum of  $10^4$  cells/ml was used in each experiment.

<sup>c</sup> TA = titratable acidity produced, ml 0.1N NaOH/10 ml medium.

<sup>d</sup> PE = spice residue, insoluble in petroleum ether.

in order to isolate the component that stimulates lactic acid bacteria. Extraction with various organic solvents, such as petroleum ether, removed the inhibitory oleoresin fraction, thereby enhancing the stimulatory activity (Table 1). For example, oregano and clove, which completely inhibited the starter culture bacteria at a level of 8g/liter, were strongly stimulatory after extraction. Tests with commercially available oleoresins of black pepper, nutmeg, capsicum, and clove indicated that these substances had little or no effect on Lactacel MC at levels of 400 ppm. Essential oils had more pronounced inhibitory effects. Oil of cassia and oregano were bactericidal at levels of 500 and 200 ppm, respectively. Oil of nutmeg, 500 ppm, delayed bacterial growth for 4 days, while oil of black pepper, 400 ppm, had no effect. These results are in agreement with the observations of Nes and Skjelkvåle (1982), who found that while spices stimulated acid production by *L. plantarum*, their oleoresins had no effect.

The stimulatory factor of spices was only partially extractable with water, even after extensive washing, but was soluble in dilute acids. As an example, the effect of sequential extraction of oregano on acid production by lactic acid bacteria is depicted in Fig. 1. Addition of the insoluble residues from oregano (equivalent to 0.3% added to medium) after organic solvent extraction ( $O_1$ ) followed by water extraction ( $O_2$ ) increased acid production five- and fourfold, respectively, with some accompanying stimulation of growth. The insoluble fraction after acid extraction ( $O_3$ ) was inactive.

The acid extractable nature of the stimulatory factor of spices suggested that the increased acid production could be due to the presence of a trace mineral that is limiting for the lactic acid bacteria. It has been well established that lactic acid bacteria, including *Lactobacillus*, *Leuconostoc*, and *Pediococcus*, have a relatively high requirement for manganese (MacLeod and Snell, 1947; Möller, 1939; Efthymiou and Joseph, 1972; Stamer et al., 1964). Data on the manganese content of spices are limited, but indicated that it can range from trace amounts to more than 600 ppm (Christensen et al., 1968; Orlandi et al., 1964), depending on the specific spice. This suggested that the acid-extractable stimulatory factor in spices could be manganese.

To evaluate the possible role of manganese as the stimulatory factor, the manganese content of 0.1N HCl extracts of spice samples (after an initial extraction with chloroform to remove oleoresin) was determined by atomic absorption spectrophotometry (Table 2). The manganese concentrations of the various dry spices ranged from 10–600 ppm. The values obtained were in general agreement with those

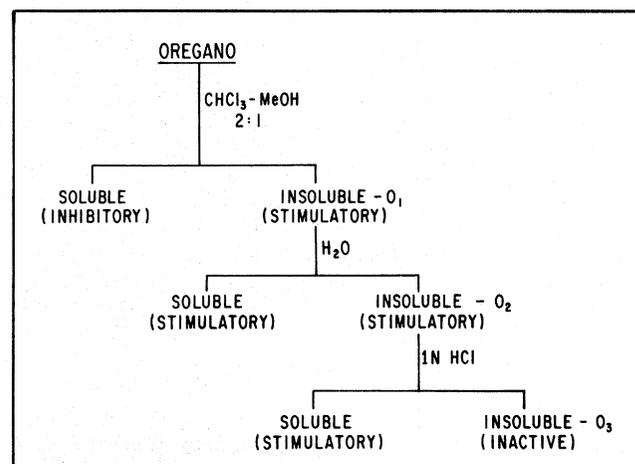


Fig. 1—Sequential extraction of oregano.

reported by Christensen et al. (1968) and Orlandi et al. (1964).

The acid extracts were then evaluated for relative stimulatory activity by adding them to microbiological media to produce a concentration equivalent to 1g spice/liter. The extractor produced a two- to fivefold increase in titratable acidity after 4 days of fermentation with *L. plantarum*. The degree of stimulation was directly related to the manganese content of the extracts (Fig. 2). The titratable acidity of the control culture was 1.24 ml, and the manganese concentration of the medium was below the limits of detection by atomic absorption spectrophotometry. A similar relationship was observed with *P. acidilactici* (Fig. 3), although the degree of stimulation was smaller than that observed with *L. plantarum*. This is in agreement with Nes and Skjelkvåle (1982) who found that the stimulatory effect of spices on acid production by *P. cerevisiae* was only 30% of that observed with *L. plantarum*.

The effect of supplementing medium with  $10^{-8}$  to  $10^{-2}$ M  $MnSO_4$  on *L. plantarum* and *P. acidilactici* was

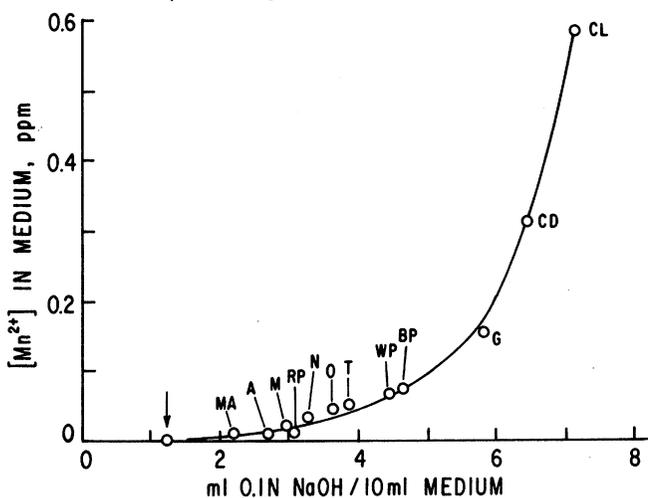


Fig. 2—Effect of 0.1N HCl extracts of spices on acid production by *L. plantarum* after 4 days at 35°C. The arrow indicates the acidity produced in a control medium. A = allspice, BP = black pepper, CD = cardamom, CL = clove, G = ginger, M = mustard, MA = mace, N = nutmeg, O = oregano, RP = red pepper, T = thyme, WP = white pepper.

investigated (Table 3). Acid production by *L. plantarum* was strongly stimulated by increasing  $MnSO_4$  levels up to  $10^{-5}$ M which produced a sevenfold increase in titratable acidity after 4 days of fermentation. Further increases in  $MnSO_4$  concentration produced relatively small increases in acid production. Manganese also stimulated bacterial growth to some extent; however, concentrations  $\geq 10^{-4}$ M appeared to delay growth during the initial (day 1) stages of the fermentation. Acid production by *P. acidilactici* increased with increasing  $MnSO_4$  concentration, though again the degree of stimulation was not as great as that observed with *L. plantarum*. A threefold increase in acid production was observed with  $MnSO_4$  levels  $\geq 10^{-5}$ M. Manganese did not affect bacterial growth during the initial phases of the fermentation, but higher concentra-

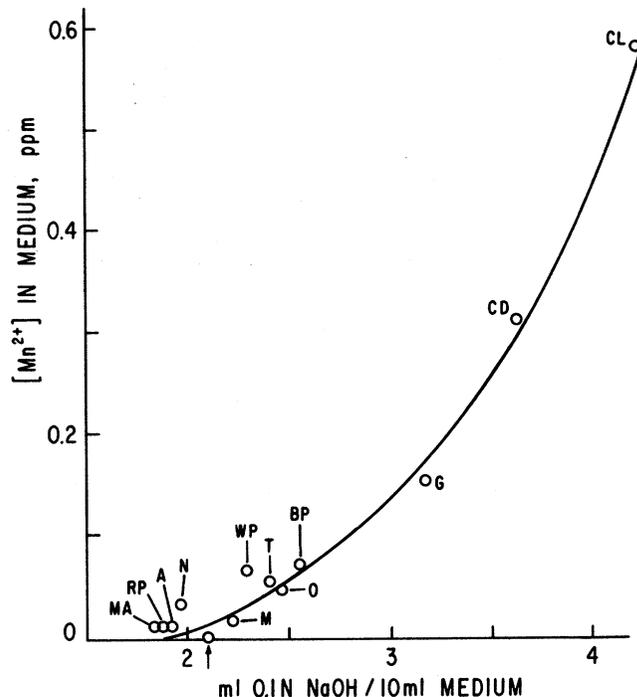


Fig. 3—Effect of 0.1N HCl extracts of spices on acid production by *P. acidilactici* after 4 days at 35°C, symbols as for Fig. 2.

Table 2—Manganese content of spices

	ppm	
	In 0.1N HCl extract	In ground dry spice
Allspice	0.6	12
Anise	1.8	36
Cardamom	16.0	320
Celery seed	5.3	106
Cinnamon	4.8	96
Clove	30.0	600
Coriander	1.2	24
Ginger	8.0	160
Mace	0.5	10
Marjoram	3.1	62
Mustard	0.8	16
Nutmeg	1.7	34
Oregano	2.4	48
Pepper, black	3.8	76
Pepper, white	3.4	68
Pepper, red	0.6	12
Rosemary	1.1	22
Sage	1.8	36
Thyme	2.7	54
Turmeric	6.0	120

Table 3—Effect of manganese on starter cultures in liquid medium

[Mn <sup>2+</sup> ] in medium	Day 1		Day 2		Day 4	
	TA <sup>a</sup>	Count/ml	TA	Count/ml	TA	Count/ml
<i>L. plantarum</i> <sup>b</sup>						
Control	0.10	$1.3 \times 10^7$	0.49	$4.2 \times 10^6$	0.95	$5.6 \times 10^6$
$1 \times 10^{-8}$ M	0.20	$1.0 \times 10^7$	0.51	$7.9 \times 10^6$	1.00	$5.3 \times 10^6$
$1 \times 10^{-7}$ M	0.23	$2.0 \times 10^7$	0.75	$1.1 \times 10^7$	1.25	$1.1 \times 10^7$
$1 \times 10^{-6}$ M	0.51	$9.4 \times 10^7$	2.25	$5.3 \times 10^7$	3.35	$1.7 \times 10^7$
$1 \times 10^{-5}$ M	0.70	$1.3 \times 10^8$	4.10	$1.1 \times 10^8$	6.83	$6.1 \times 10^7$
$1 \times 10^{-4}$ M	0.13	$1.8 \times 10^7$	4.44	$3.2 \times 10^8$	7.25	$1.9 \times 10^7$
$1 \times 10^{-3}$ M	0.00	$8.3 \times 10^6$	4.30	$3.0 \times 10^8$	7.35	$1.1 \times 10^7$
$1 \times 10^{-2}$ M	0.00	$3.2 \times 10^6$	4.45	$4.7 \times 10^8$	8.02	$2.5 \times 10^7$
<i>P. acidilactici</i> <sup>c</sup>						
Control	0.60	$7.8 \times 10^7$	0.97	$7.2 \times 10^7$	1.38	$3.3 \times 10^7$
$1 \times 10^{-8}$ M	0.57	$7.4 \times 10^7$	0.96	$8.1 \times 10^7$	1.37	$2.7 \times 10^7$
$1 \times 10^{-7}$ M	0.77	$1.3 \times 10^8$	1.27	$1.2 \times 10^8$	1.67	$2.9 \times 10^7$
$1 \times 10^{-6}$ M	1.42	$3.2 \times 10^8$	2.32	$3.1 \times 10^8$	3.07	$1.3 \times 10^7$
$1 \times 10^{-5}$ M	1.92	$2.4 \times 10^8$	3.07	$2.7 \times 10^8$	3.81	$5.4 \times 10^6$
$1 \times 10^{-4}$ M	2.13	$3.4 \times 10^8$	3.37	$4.0 \times 10^8$	4.01	$1.6 \times 10^6$
$1 \times 10^{-3}$ M	2.57	$3.5 \times 10^8$	3.92	$4.0 \times 10^8$	4.37	$2.0 \times 10^5$
$1 \times 10^{-2}$ M	2.75	$2.6 \times 10^8$	3.67	$7.6 \times 10^7$	3.72	$3.3 \times 10^5$

<sup>a</sup> TA = Titratable acidity produced, ml 0.1N NaOH/10 ml medium.

<sup>b</sup> Initial count =  $2.8 \times 10^3$  cells/ml.

<sup>c</sup> Initial count =  $8.3 \times 10^4$  cells/ml.

tions appeared to be cytotoxic for *P. acidilactici* during the later stages (day 4). Manganese produced a similar stimulation of acid production (data not shown) when *Pediococcus pentosaceus* was employed.

To test the validity of these results triplicate samples of control medium and media supplemented with  $10^{-6}$ ,  $10^{-5}$ , and  $10^{-4}$  M  $MnSO_4$  were subjected to fermentation by *L. plantarum*, *P. acidilactici*, and *P. pentosaceus*. Reproducibility of titratable acidity values for 1 and 2 days of fermentation were very good, as indicated by low standard deviation values (range 0.02–0.11 ml, avg 0.04 ml).

The identification of manganese as the active component of spices that stimulates lactic acid production was further evaluated by comparing the response of *L. plantarum* to standard  $MnSO_4$  solutions and the 0.1N HCl extracts of various spices selected for their respective  $Mn^{++}$  concentrations (Fig. 4). Plots of titratable acidity after 3 days vs manganese concentrations indicated similar responses for standard solutions and spice extracts. This suggests that manganese is the major component of spices that stimulates acid production. However, at similar manganese content, the spice extracts consistently produced slightly higher acidity values, suggesting that spices may contain additional trace minerals or other components that affect acid production by *L. plantarum*.

Experiments in liquid medium established that the major stimulatory component of spices was manganese. To verify that this effect occurs in fermented sausages, a Lebanon bologna-type product (Zaika et al., 1978) was

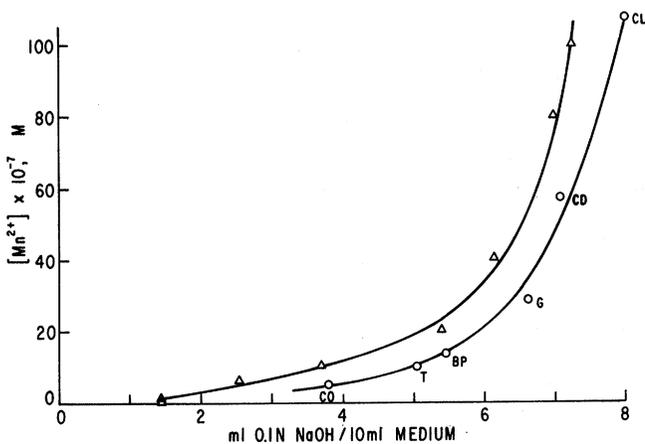


Fig. 4—Effect of  $Mn^{++}$  and 0.1N HCl extracts of spices on acid production by *L. plantarum* after 3 days at 35°C.  $\Delta$  = standard  $MnSO_4$  solutions,  $O$  = 0.1N HCl extracts of clove (CL), cardamom (CD), ginger (G), black pepper (BP), thyme (T), and coriander (CO).

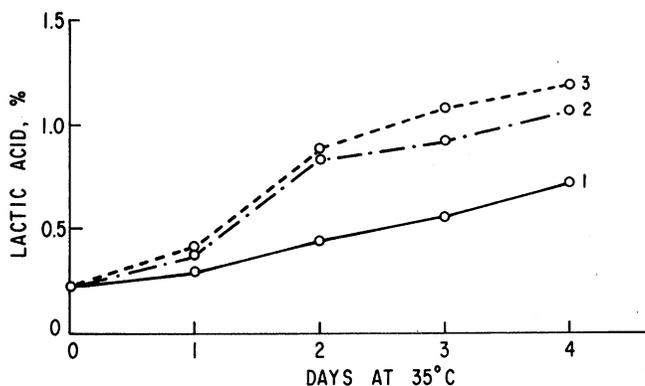


Fig. 5—Effect of  $Mn^{++}$  and spices on fermentation of Lebanon bologna-type sausage. 1 = control, no additive; 2 =  $1 \times 10^{-5}$  M  $MnSO_4$ ; 3 = spice mixture, 8g/kg.

formulated: (1) without additives; (2) with  $10^{-5}$  M  $MnSO_4$ ; and (3) with a spice mixture. A 9-component spice mixture was employed at a level of 8g/kg. The sausages were fermented using Lactacel MC, a mixed starter culture consisting of *L. plantarum* and *P. acidilactici*. The lactic acid production in the three sausage types fermented for 4 days at 35°C is shown in Fig. 5. Acid production was greater in the sausages containing spices or the manganese supplement, as compared to the controls. The amount of added manganese in the  $MnSO_4$ -supplemented and spice-containing sausages was 0.55 and 0.72 ppm, respectively.

The effect of varying the level of  $MnSO_4$  supplementation ( $10^{-6}$ ,  $10^{-5}$ ,  $10^{-4}$  M) on lactic acid formation in sausages is summarized in Fig. 6. Addition of  $10^{-6}$  M  $MnSO_4$  produced a small, but significant, increase in acid formation. A concentration of  $10^{-5}$  M appears sufficient to insure a high level of acid production, particularly during the early stages of fermentation. Rapid acid production early in a fermentation is desirable for controlling the growth of pathogenic microorganisms that may be present in the product.

The stimulatory effect of added manganese, either as  $MnSO_4$  or as a component of spices, can be observed in fermented sausages because the concentration of manganese in muscle meats appears too low to support maximal growth and acid production by starter cultures. Manganese concentrations for beef muscle have been reported ranging from 0.02 ppm (Mitteldorf and Landon, 1952) to 1.7 ppm (Assaf and Bratzler, 1966). Nuurtamo et al. (1980), studying the mineral composition of a large variety of meats and meat products, reported the content of manganese in beef muscle as 0.04–0.17 ppm (mean, 0.1 ppm). The values for pork muscle were similar. Kirkpatrick and Coffin (1975) reported that the concentration of manganese in cured meats ranged from 0.01–1.15 ppm with an average value of 0.26 ppm.

The stimulatory effect of spices is strongly dependent on the growth medium employed. In our studies using a beef extract-sugar medium low in manganese concentration, the stimulatory effect is readily observed. However, in media, such as APT or MRS, which give optimum growth of lactic acid bacteria, the content of manganese is too high ( $7.1 \times 10^{-4}$  M  $Mn^{++}$  in APT and  $2.2 \times 10^{-4}$  M in MRS) to allow the stimulatory effect of spices to be significant.

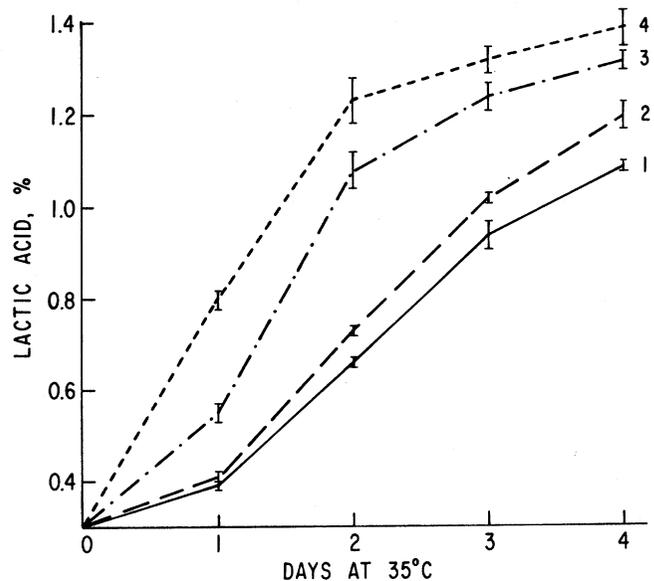


Fig. 6—Effect of  $[Mn^{++}]$  on acid production in Lebanon bologna-type sausage. 1 = control, no additive; 2 =  $1 \times 10^{-6}$  M  $MnSO_4$ ; 3 =  $1 \times 10^{-5}$  M  $MnSO_4$ ; 4 =  $1 \times 10^{-4}$  M  $MnSO_4$ .

Thus, when an 0.1N HCl extract of clove was added to APT medium ( $4.3 \times 10^{-6}$ M added  $Mn^{++}$ ), no significant enhancement of acid production was observed, since acid production by *L. plantarum* was already at a maximum in the control medium. Nes and Skjelkvåle (1982) reported that acid production by *L. plantarum* was enhanced by spices in sausages or in a meat extract-glucose medium, but that no effect on bacterial growth or acid production was observed in MRS medium. On the other hand, Yoo et al. (1978) reported that aqueous extracts of ginger and red pepper added to MRS medium stimulated growth and acid production by *L. plantarum* and *L. fermenti*. The stimulatory effect appears minimal, although their statistical data indicate significant differences.

The stimulatory effect of manganese in fermented meat products was recognized previously. Chalet (1960) reported that addition of a manganese salt markedly increased acid production in sausages fermented with *P. cerevisiae* (or *P. acidilactici*). Raccach (1981) found that addition of  $Mn^{++}$  to the sausage formulation accelerated fermentation by *P. pentosaceus* at low temperatures (15.6–26.7°C) and was effective in reducing or eliminating inhibition of the starter bacteria by antioxidants, such as BHA and BHT, commonly added to fermented meat products.

Manganese supplementation may have a potential as a means of accelerating production of fermented meat products particularly when stimulatory spices are not used or when spices are replaced by oleoresins or other flavorings. Also, increased acidity in the product would have the advantage of increasing protection against *Staphylococcus aureus* and other pathogenic bacteria. The present study indicates that trace metals present in food ingredients may profoundly affect the activity of microorganisms in foods.

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