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COMPARATIVE STUDY CONCERNING THE EFFECT OF NITRITE AND SORBATE ON *LISTERIA MONOCYTOGENES* IN SAUSAGE (With Two Tables)

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(Received at 25/3/1989)

دراسة مقارنة بخصوص تأثير النيتريت والسوربات على
الليستيريا مونوسيتوجين في السجق

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تم دراسة تأثير نيتريت الصوديوم وسوربات البوتاسيوم على عترتين من الليستيريا مونوسيتوجين هما Scott A, V₇ . تم تجهيز خمس فئات من السجق الأولى منها لا تحتوي على أية إضافات ، الثانية تحتوي على 26.0٪ سوربات البوتاسيوم ، الثالثة تحتوي على 156 جزء بالمليون نيتريت الصوديوم ، الرابعة تحتوي على 78 جزء بالمليون نيتريت الصوديوم بالإضافة إلى 26.0٪ سوربات البوتاسيوم والخامسة تحتوي على 78 جزء بالمليون نيتريت الصوديوم بالإضافة إلى 156 جزء بالمليون نترات الصوديوم . وقد تم حفظ السجق على درجة 4°م وقياس الرقم الهيدروجيني للعينات يومياً وجد أنه لم يتغير كثيراً بالحفظ حيث وجد أنه يتراوح ما بين 6.3 و 6.1 . وثبت من الدراسة أن النيتريت أو السوربات بمفردهما أو كليهما ليس لهما أى تأثير على عترتين الليستيريا اللتين تم إختبارهما فى السجق . وتم مناقشة تأثير سوربات البوتاسيوم ونيتريت الصوديوم على البكتريا تحت الظروف المختلفة .

SUMMARY

The influence of sodium nitrite and potassium sorbate on growth of two strains of *Listeria monocytogenes* was studied. Five batches of fresh sausage were prepared with no additives, 0.26% potassium sorbate, 156 ppm of sodium nitrite, 78 ppm of sodium nitrite plus 0.26% potassium sorbate, 78 ppm of sodium nitrite plus 156 ppm of sodium nitrate. The prepared products were kept refrigerated at 4°C and *L.monocytogenes* growth was monitored. The pH of the sausage samples ranged from 6.1 to 6.3. The presence of sodium nitrite and potassium sorbate, separately or together were without appreciable effect on *L.monocytogenes* strains V₇ and Scott A. The effect of potassium sorbate alone or in combination with nitrite on the growth of different microorganisms was discussed.

INTRODUCTION

Nitrite use in cured meat products has been under federal and public security since the 1950, and the economic, health related and political areas of controversy have been detailed in several recent reviews (GRAY and RANDALL, 1979; SEBRANEK, 1979; SCOFOS, et al. 1979).

Use of sodium nitrite in cured meats as an anticlostridial agent has been well established (PERIGO and ROBERTS, 1968; CHRISTIANSEN, et al. 1973; HUSTAD, et al. 1973; ROBACH and IVEY, 1978). The inhibitory effect of sodium nitrite on food poisoning bacteria has been studied by many workers using different laboratory media as well as food products under different conditions (ASHWORTH, et al. 1974; ROBERTS and SMART, 1974; ROBERTS and DERRICK' 1978). Inhibition of several other food poisoning bacteria, such as staphylococci, by nitrite, has also been reported (FIDDLER, et al. 1972; JOHNSTON and ELLIOT, 1976). The other important function of this chemical additive is its role in formation of typical colour and flavour in cured meats (FRAZIER and WESTHOR, 1978).

In some articles (SEBRANEK and CASSENS, 1973; GRAY, 1976), the potential formation of carcinogenic compounds from nitrite in cured meats, particularly bacon, was reviewed. An increasing awareness of this possible hazard has prompted interest in the partial to complete removal of nitrites from meat products where nitrosamines has been detected. Potassium sorbate has received much attention as a potential nitrite substitute with antibotulinal activity (TOMPKIN, et al. 1974; IVEY, et al. 1978). Further, formation of nitrosamines in cured meats such as bacon is diminished by the substitution of sorbate (O'BRIEN, 1978).

Sorbic acid and its potassium salt, potassium sorbate, are both Generally Recognized as Safe (GRAS) food ingredients and have been widely used as food preservatives for over 30 years. They are antimicrobial agents that inhibits molds, yeasts, and certain bacteria including *C.botulinum* (TOMPKIN, et al. 1974).

Listeria monocytogenes can survive in, and be disseminated by different kinds of food such as milk, poultry, eggs and meat. It can survive most salting procedures used in food processing and can also survive at low temperatures in meat (KHAN, et al. 1973; SHAHAMAT, et al. 1980 a) which makes it a potential public health hazard. *Listeria monocytogenes* is listed among the psychrotrophic food spoilage microorganisms (MOSSSEL, 1971).

Relatively little research is available on the inhibition of *Listeria monocytogenes* by potassium sorbate/sodium nitrite combination in cured meats. The purpose of this study was to investigate the efficacy of potassium sorbate, sodium nitrite, alone or in combination with each other in inhibiting the growth of *Listeria monocytogenes* in sausage at refrigeration temperature.

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MATERIAL and METHODS

Test organisms:

Two strains of *Listeria monocytogenes* were obtained from R.M. Tweedt, Food and Drug Administration, Cincinnati, OH, USA. Strain Scott A was clinical isolate, serotype 4b and strain V₇ was milk isolate, serotype 1. The cultures were prepared by inoculating Tryptose Broth TB (Difco) with a loop of a slant culture of the appropriate organism.

Preparation of sausage samples:

Fresh sausage was purchased from a supermarket and divided into sterile glass blender jars, approximately 600 gram per Jar. The Jars were treated and categorized to 5 batches (I, no additives; II, 0.26% potassium sorbate; III, 156 ppm of sodium nitrite IV, 78 ppm of sodium nitrite, 0.26% potassium sorbate; V, 78 ppm of sodium nitrite, 156 ppm of sodium nitrate).

Inoculation of sausage:

For inoculation, 20 ml of Tryptose Broth culture of *Listeria monocytogenes* strains were mixed with the prepared sausage samples. The inoculation level used was 10^5 - 10^6 cells per gram sausage. The glass Jars were stored at 4°C covered with aluminium foil for survival studies at refrigeration temperature.

In the survival studies at 4°C, two 25 gram samples were taken from the sausage, before inoculation, after inoculation and daily during 6 days. The samples were homogenized with 225 ml of 0.1% peptone water in sterile blender Jar.

The pH of sausage samples was determined at the time of bacteriological analysis using a Corning research pH meter.

***Listeria monocytogenes* counts:**

Serial dilutions of the sausage samples were made in 0.1% peptone water, and duplicate 0.1 ml portions of three consecutive dilutions were spread plated on McBride's *Listeria* Agar MLA (MCBRIDE and GIRARD, 1960). Plates were incubated at 35°C for 48h. Typical *Listeria monocytogenes* colonies which were smooth, bluish grey, slightly raised, translucent, watery consistency, 0.5-1.5 mm in diameter, and weakly B-haemolytic were counted and several colonies were transferred to Tryptose Agar TA slants, incubated at 35°C for 24h for confirmation. Confirmatory tests done on isolates thought to be *Listeria monocytogenes* included catalase reaction, observance of tumbling motility in TB-grown cultures incubated at 21°C for 24h (GRAY and KILLINGER, 1966) and presence of distinct blue-green colonies on TA when observed under obliquely transmitted light, as described by HENRY (1933). Serologically slide agglutination tests were done according to manufacturer's instructions on all isolates thought to be *Listeria monocytogenes* using commercially prepared antiserum (Difco) to confirm that the isolates were of serotypes 1 and 4.

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RESULTS

Results of storage studies at 4°C for the inoculated sausage are in Tables 1 and 2. *Listeria monocytogenes* strains V₇ and Scott A were not affected by sodium nitrite, potassium sorbate or sodium nitrate.

DISCUSSION

The bacteriostatic action of nitrite and potassium sorbate is pH-dependent. Sorbates are generally effective below pH 6.0 but will function up to pH 6.5 (MONSANTO, 1978). However the pH values of the stored sausage hardly changed with the time (pH 6.1 : 6.3). The bacteriostatic action of nitrite increases in an acid environment (CASTELLANI and NIVEN, 1955). Further, the pH of sausage is not optimum for the effectiveness of potassium sorbate, which only mildly inhibits lactic acid bacteria.

Potassium sorbate has been shown to be effective in inhibiting of salmonella organism in foods such as cheese (PARK, et al. 1970), milk (PARK and MARTH, 1972), poultry parts (ROBACH and IVEY, 1978), and uncured sausage (TOMPKIN, et al. 1974). Moreover, SKIJEKVALE and TJABERG (1974) observed the microflora of frankfurters, including certain pathogens, to be little affected by the level or presence of nitrite (SIMON, et al. 1973; BAYNE and MICHENER, 1975). With respect to sorbate, antibotulinal activity in uncured, cooked and then inoculated sausage has been reported by TOMPKIN, et al. (1974) while inoculated *C.perfringens* decreased rapidly with or without sorbate. In the same study, surface applied salmonellae were inhibited by sorbate while *Staphylococcus aureus* was not. Sorbate is effective against a variety of moulds, yeasts and bacteria but is said to only mildly inhibit the growth of lactic acid bacteria (MONSANTO, 1978). Yet IVEY, et al. (1978) demonstrated significant inhibition by 0.26% potassium sorbate of the lactobacillus population of bacon after 2 weeks of aerobic storage.

In practice, nitrite is said to have relatively little inhibitory on bacteria unless accompanied by other factors such as sodium chloride and low pH. SHAHAMAT, et al. (1980 a) reported that pH had a great influence on the inhibitory action of nitrite against *Listeria monocytogenes*, even concentrations as high as 2500 ppm could not prevent growth at pH 7.4.

INGRAM (1973) postulated that the optimum pH for the antimicrobial activity of nitrite is about 5.5. It thus seems that the pH is acceptable for controlling *Listeria monocytogenes*, which is amongst the psychrotrophic food spoilage flora. MOSSEL (1971) at low storage temperatures.

However, the use of potassium sorbate with or without nitrite should provide adequate protection against growth of *Listeria monocytogenes* if the pH is lowered and the product is properly refrigerated.

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Table (1)
Counts of *L.monocytogenes* V₇ on MLA for the inoculated sausage

Variable	Log ₁₀ of viable cells/g.*						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
I	7.77	8.2	8.98	10.46	10.76	11.18	12.28
II	7.77	9.98	7.84	7.18	7.28	8.72	11.65
III	7.77	9.43	10.61	7.26	8.43	9.17	11.88
IV	7.77	7.02	7.85	7	8.51	7.14	12.16
V	7.77	9.76	10.52	10.72	8.77	10.99	11.69

Table (2)
Counts of *L.monocytogenes* Scott A on MLA for the inoculated sausage

Variable	Log ₁₀ of viable cells/g.*						
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
I	8.45	9.81	10.56	10.83	11.25	12.32	13.35
II	8.45	9.76	8.12	7.39	8.77	10.94	9.83
III	8.45	6.96	7.66	8.87	10.15	9.06	14.09
IV	8.45	6.32	10.54	8.59	10.58	10.82	13.98
V	8.45	10.17	10.57	8.39	10.52	13.32	13.89

* Geometric mean of counts from 2 samples for each variable and time.