

A Research Note

INVESTIGATIONS ON THE POSSIBLE OCCURRENCE OF NITROSAMINES IN LEBANON BOLOGNA

INTRODUCTION

RECENTLY we reported on the microbiology (Smith and Palumbo, 1973) and the processing of Lebanon bologna (Palumbo et al., 1973). This process involved the fermentation of the sugar in the bolognas by either the natural lactic acid bacteria of the meat or added lactic acid starter culture; during the 4-day fermentation, the acidity increased from a starting pH of 5.6 to a pH of 4.5. Micrococci present normally in the meat reduced the nitrate to nitrite with the concomitant formation of nitrosylmyoglobin.

Lebanon bologna is a unique system for studying the potential for nitrosamine formation because it possesses certain characteristics which are known to favor nitrosamine formation. These include: (1) low pH, 4.5–4.7 (Palumbo et al., 1973). This pH range would be conducive to nitrosamine formation since Mirvish (1970) reported that nitrosation of secondary amines is favored at low pH values. (2) Manufacture with a straight nitrate cure (1.85g KNO₃/kg meat) which may result in varying nitrite levels in the product. Since the nitrite content cannot be rigidly controlled, the possibility exists that amounts of nitrite formed by micrococcal reduction of nitrate might be in excess of that needed for optimal nitrosylmyoglobin formation, and thus provide a nitrite reservoir for nitrosamine formation. (3) A 4-day fermentation at 35°C during which certain bacterial species present in the bolognas could contribute to nitrosamine formation. Sander (1968), Klukes and Jondorf (1971) and Hawksworth and Hill (1971a, b) have shown that nitrosamines can be formed by a variety of microorganisms. These investigators observed that various nitrate reducing bacteria can form nitrosamines; early in the Lebanon bologna fermentation, there is a large population of nitrate reducing micrococci present (Smith and Palumbo, 1973). Furthermore, Hawksworth and Hill (1971a) have observed that some nonnitrate reducing lactobacilli can form nitrosamines. During the Lebanon bologna fermentation, there is a large population of lactobacilli present (Smith and Palumbo, 1973). In fact, at the end

of the fermentation period, lactobacilli are the predominant flora and remain viable for long periods of storage at 5°C. Collins-Thompson et al. (1972) observed that species of streptococci contributed to nitrosamine formation by lowering the pH. Components of the bacterial flora of Lebanon bologna could thus contribute to nitrosamine formation by: (a) lowering the pH; (b) providing nitrite through reduction of nitrate; or (c) enzymatically carrying out the nitrosation reaction. Because of these three unique characteristics, we undertook an examination of Lebanon bolognas produced in our pilot plant and some obtained commercially to determine the possible presence of six volatile nitrosamines. Examination of Lebanon bologna produced in our pilot plant was of particular interest because the exact processing and handling history of the sausages could be controlled.

EXPERIMENTAL

SAMPLES of Lebanon bologna from six different manufacturers (two prepared with starter culture) were purchased from local retail stores in the Philadelphia area and were analyzed as obtained. Natural flora and starter culture fermented Lebanon bolognas were prepared in our pilot plant as described previously (Palumbo et al., 1973), except that the starter culture Lebanon bolognas were also prepared with a straight nitrate cure. Samples of these two types of pilot plant bolognas were taken for pH, nitrite and nitrosamine analyses at 0, 1, 2, 3 and 4 days of fermentation and after 4 and 12 days storage (mellowing) at 5°C.

The bologna samples were analyzed for nitrite colorimetrically using the Griess reagent (AOAC, 1970). The pH was measured with an Orion model 801 digital pH meter after mixing 2g of bologna with 10 ml of water.

Nitrosamine analyses were performed using the method of Fazio et al. (1971) with a modification reported by Fiddler et al. (1974). An aliquot of the bologna samples with 20 ppb of six volatile nitrosamines added was carried through the entire procedure. The average recoveries were: dimethylnitrosamine, 88%, methylethyl nitrosamine, 95%; diethylnitrosamine, 95%, nitrosopiperidine, 69%; nitrosopyrrolidine, 70%; and nitrosomorpholine, 75%.

Samples of Lebanon bologna that had peaks coincident with the gas-liquid chromatographic retention times of authentic samples of nitrosamines were examined by GLC-high resolution mass spectrometry using the apparatus and procedures described by Pensabene et al. (1974).

RESULTS & DISCUSSION

EXAMINATION of commercial and pilot plant produced Lebanon bolognas failed to show detectable levels of dimethylnitrosamine, methylethyl nitrosamine, diethylnitrosamine, nitrosopiperidine, nitrosopyrrolidine, and nitrosomorpholine (the six volatile nitrosamines for which we tested). Furthermore, none of the nitrosamines were detected in pilot plant bolognas at any time during manufacture and storage. The alkali flame ionization detector employed (Howard et al., 1970) can detect as little as 0.5 ppb nitrosamine and high resolution mass spectrometry can confirm the presence of 5 ppb nitrosamine.

Pilot plant Lebanon bolognas fermented by natural flora had the highest concentration of nitrite (86 ppm) during the first 24 hr, while in the starter culture fermented bolognas, the maximum concentration of 62 ppm was produced between 24 and 48 hr. After the nitrite content peaked early in the fermentation, it rapidly decreased to a steady state value of 34 ppm in the natural flora and 24 ppm in the starter culture bolognas, where they remained during the rest of the fermentation and mellowing. This early high concentration of nitrite in natural flora fermented Lebanon bolognas corresponded with the rapid development of micrococci observed in these bolognas during the first 24 hr (Smith and Palumbo, 1973). Palumbo et al. (1973) observed that the maximum amount of nitrosylmyoglobin was also formed during the first 24 hr in bolognas fermented by natural flora. Starter culture fermented bolognas were prepared by the addition of starter culture (Lactacel MC, Merck & Co., Rahway, N.J.) to beef which had been frozen and then thawed just prior to use. The slower formation of nitrite in these bolognas probably reflects the lower concentration of micrococci found in this meat (the aged meat from which the natural flora bolognas were prepared had a high number of both micrococci and lactic acid bacteria).

As would be expected, the Lebanon bolognas prepared in our pilot plant with starter culture underwent a more rapid pH decrease and attained a lower final pH

than natural flora fermented bolognas. The pH of the starter culture bolognas was 4.5 at 1 day and 4.0 after 2 days of fermentation, while for natural flora bolognas it was 4.7 and 4.5, respectively.

Although there is a potential for their formation during the processing of Lebanon bologna, nitrosamines were not detected in either commercial or pilot plant Lebanon bolognas. Also nitrosamines were not detected at any time during the fermentation and mellowing of pilot plant bolognas. The absence of nitrosamines in finished Lebanon bologna may be explained in 2 ways. Either no nitrosamines are formed or any nitrosamines which may be formed are broken down by components of the microflora of the bolognas. We examined the pilot plant bolognas during mellowing and daily during the fermentation and found no detectable levels of the six nitrosamines at any time. Therefore, we concluded that no nitrosamines were formed. Hence, the concentration of either free secondary amine or nitrite during manufacture of Lebanon bologna may be too low to permit detectable nitrosamine formation.

Since Fong and Chan (1973) observed nitrosamine in fish broth with concentrations of nitrite and nitrate considerably lower than those found in Lebanon bologna, the probable reason for the absence of detectable nitrosamine formation is a low level of free, secondary amine.

(Precautions should be exercised in the handling of nitrosamines, since they are potential carcinogens.)

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