

COMBINED EFFECT OF NITRITE AND CHLORIDE ON THE ULTRASTRUCTURE OF MEAT AS SHOWN BY SCANNING ELECTRON MICROSCOPY

ABSTRACT

Scanning electron microscopy of the ultrastructure of cured beef has shown that the combination of sodium chloride and nitrite effects changes in the interfiber spaces not caused by either salt alone. The effect is attributed to catalysis of the nitrosation reaction through the formation of the more powerful nitrosating species, NOCl.

INTRODUCTION

RECENT CONCERN over the possibility of nitrite forming carcinogenic nitrosamines in meat has as one possible consequence a ban on nitrite and/or nitrate in all meat products. The effect of such a ban in terms of the specific characteristics that distinguish cured meats is not known, since we do not know precisely what the components of cure solutions do to meat components. Uncooked cured meats do not have the turgid, flexible character of fresh meat, the primary causative agent being the sodium chloride of the cure solution (Randall and Voisey, 1977). Studies have been made on the interaction of nitrite with sulfhydryl groups in meat and myosin (Kubberød et al., 1974; Mirna and Hofmann, 1969; Olsman and Krol, 1972; Walters and Casselden, 1972), but the effect of the reaction on the properties of cured meats has not been established. Nitrite has other specific actions in cured meats, producing changes in characteristics such as flavor, color, and shear values (Brooks et al., 1940; Kemp et al., 1974; Brown et al., 1974; Wasserman and Talley, 1972; Randall and Voisey, 1977), but again the studies do not provide information on the specific sites or modes of action.

Scanning electron microscopy (SEM) has been used to examine the morphology of muscle tissues before and after being cooked (Jones et al., 1977; Jones, 1977) and has shown a number of basic changes in the tissue structure. It appeared reasonable that a similar study of cured meat as compared to fresh could provide some visible evidence of the site of action of nitrite in meat. We hoped that the study would provide evidence as to the tissue components to study in terms of the mode of action.

EXPERIMENTAL

Materials

Eye of round was chilled to -2.5°C to firm the tissues, then sliced 1/4 in. thick, parallel to the fibers, from which 1 in. \times 6 in. slabs were then cut. Cure solutions duplicated a commercial dried beef cure brine and consisted of 3.08M (18%) NaCl and 31.8 mM (2300 ppm) NaNO_2 , singly or in combination.

Methods

Slices of meat prepared as described were suspended by threads in glass frames which in turn were fastened to 60 rpm clock motors and suspended in glass cylinders. The cylinders were filled with equal volumes of the different cure solutions and placed in a 7°C

Table 1—Effect of 3.0M NaCl on nitrosation reactions in mildly acidic solutions

Reaction	pH	Rate constants		Rate constant ratio
		0 NaCl	With 3M NaCl	
Nitrosation of sulfanilamide (Griess reaction)	2.81	0.46 ^a	1.56	3.40
Formation of nitric oxide (followed by formation of nitrosyl myoglobin)	5.5	0.92 ^b	4.90	5.29
Oxidation of myoglobin	5.5	1.58 ^b	3.53	2.23

^a k_1 in min^{-1}

^b k_0 in $\mu\text{M}/\text{min}^{-1}$

cold room. The frames were rotated and the solutions were stirred with magnetic stir bars during the cure process. In separate experiments with samples of 1/4 in. thickness, we found that salt penetration was complete in 2 days, so at least 3 days were allowed for cure. At the end of cure, the samples cured with sodium chloride had chloride concentrations of 10.5–11.5%, and those cured with sodium nitrite had nitrite concentrations of 1380–1520 ppm. The pH as measured on the surface ranged from 5.7–5.8. The fresh meat control was kept refrigerated at 7°C .

Samples were fixed overnight in 2% glutaraldehyde and 2% paraformaldehyde in 0.07M phosphate buffer at the pH of the particular sample. The samples were washed with water, dehydrated through increasing ethanol-water series 70%, 95% (2x), and 100% (2x). The tissue was fractured in liquid nitrogen by the cryofracture technique of Humphrey et al. (1974). The tissue was critical point dried from liquid carbon dioxide (Humphrey et al., 1974), mounted on copper stubs with silver paint, and sputter coated with gold. The samples were examined with a JEOL SEM 50-A operating at 15 kv.

We determined the effect of chloride on three nitrosation reactions by determining the rate constants with and without 3.0M chloride. We followed the nitrosation of sulfanilamide (100 μM) by 20 μM nitrite at pH 2.81 by coupling the diazo salt with 1-N-naphthalene ethylenediamine to form the diazo pigment (Griess reaction) and measuring the increase in optical absorption at 540 nm. The formation of nitric oxide from the reaction of 10 mM ascorbate and 2 mM nitrite at pH 5.5 in the presence of 0.05 mM metmyoglobin was followed by measuring the increase in absorbance at 540 nm due to formation of nitrosylmyoglobin. The oxidation of 0.05 mM myoglobin by 2.0 mM nitrite at pH 5.5 was followed by measuring the decrease in optical absorbance at 555 nm due to conversion of myoglobin to metmyoglobin.

RESULTS & DISCUSSION

FIGURE 1 shows the scanning electron micrographs of the surfaces of the fibers fractured perpendicular to the fiber axes. No appreciable differences were found between fresh meat (Fig. 1-a) and samples treated with sodium chloride (Fig. 1-b) or sodium nitrite (Fig. 1-c), but the combination of both salts effected a gross morphological change (Fig. 1-d). With the nitrite/chloride combination the principal change occurred in the interfiber spaces which were filled with a spongy material. An examination of a large surface area was made, but nothing could be found resembling the endomysium sheaths which normally occupy these spaces in electron micrographs. The spongy appearance was more noticeable in the open spaces between three or more fibers,

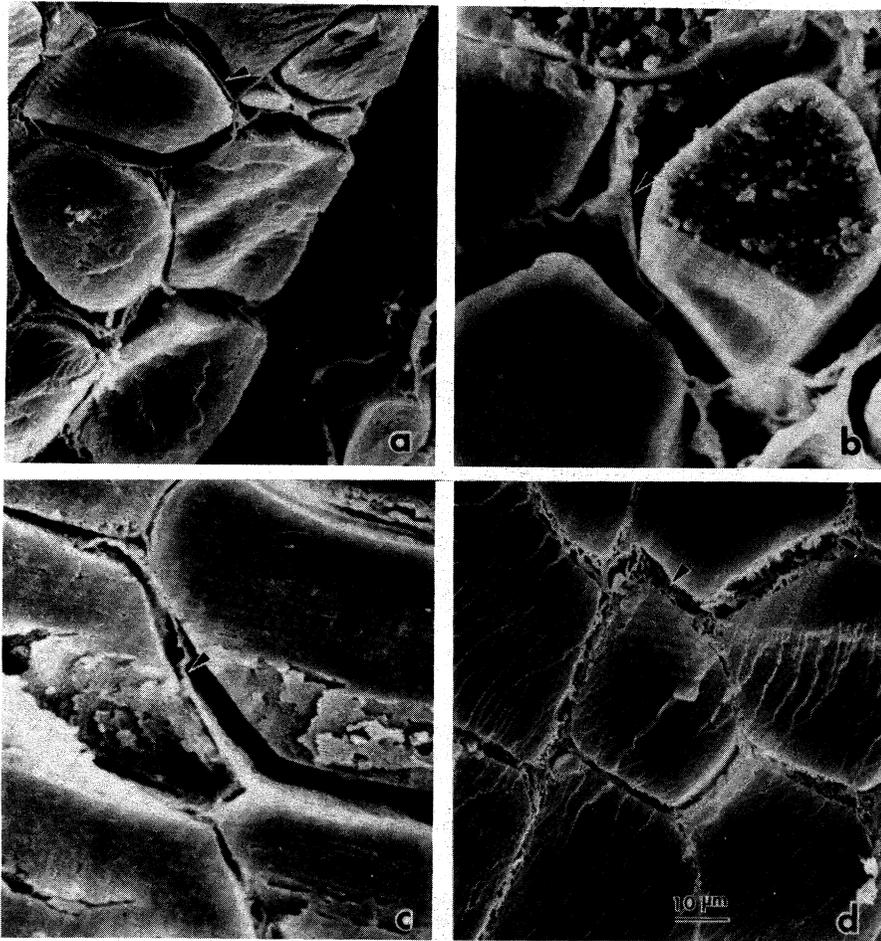


Fig. 1—Perpendicular fractures of fibers from fresh and cured meats: (a) Fresh meat; (b) cured with 3.08M NaCl; (c) cured with 32.6 mM NaNO₂; (d) cured with 3.08M NaCl and 32.6 mM NaNO₂. Arrows point to endomysium.

but a close examination of the narrow spaces between any two fibers showed the same material. In the tissue before being cured and/or prepared for SEM, the interfiber spaces contained the sarcolemma, sarcoplasmic constituents, and the endomysium. After the tissue was cured and fixed, the major remaining feature in the micrographs of all but the combined cure was the endomysium, indicated by the arrows in Figures 1a-c. In none of the micrographs of the combined cure samples was there any evidence of unaltered endomysium remaining. Whether or not other constituents of the space have also been affected is not clear, but from the total amount of material present it appears that more than just the endomysium is involved.

The increased reactivity of the combined cure components is coherent with the known reactivity of the species present. Of the two anions, nitrite is the chemically reactive species and disappears during curing through nitrosation of tissue components (Kubberød et al., 1974; Fox and Nicholas, 1974; Mirna and Hofmann, 1969). If nitrite only is present, the nitrosating species is N₂O₃, formed by a bimolecular reaction of nitrous acid (Ridd, 1961). Chloride catalyzes nitrosation reactions through the formation of NOCl, a more powerful nitrosating species than N₂O₃ (Ridd, 1961; Schmid and Pfeifer, 1953). As Cassens et al. (1979) pointed out, there is some question as to the role of chloride in nitrite reactions at higher pH values, although in most of the studies the factor studied was final product concentrations. Since catalysis refers to reaction rates, we tested the effect of chloride on the rate of some nitrosation reactions (Table 1) and found that a catalysis does take place under these conditions; in the absence of chloride, the

reaction is evidently too slow to effect any changes. We therefore attribute the observed changes in the combined cure to increased nitrosating activity. Studies are underway to determine the tissue components involved in the reaction.

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