EFFECT OF THE AMOUNT OF RESIDUAL NITRITE AND VACUUM LEVEL ON COLOR STABILITY IN PREPACKAGED COOKED HAM: CHEMICAL ASPECTS.

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SUMMARY: In prepackaged cured cooked ham, the amount of residual nitrite and the vacuum level influence the color stability and then, the shelflife of the product. When residual nitrite is around 20 mg/kg, a 10-15 day color stability can be obtained only with a high vacuum (5 hPa). When residual nitrite is around 80-100 mg/kg, color is obviously stable with a high vacuum, but an acceptable shelflife is obtained with a 100-125 hPa vacuum. In this case, color turns grey-brown during the first 4 hours after packaging and then becomes satisfactory again during the next 24 hours and remains stable for 8-10 days.
The chemical reactions occuring are:
- In a first step, nitrosylmyoglobin is oxidized in metmyoglobin.
- In a second step, metmyoglobin progressively disappears and residual nitrite causes a secondary nitrosation which imparts again a suitable pink color.

INTRODUCTION: Although potassium nitrate (salpeter) has been used for meat curing for many hundred of years, its role has been understood only since the end of the 19° century. The studies of Polenski (1891), Lehman (1899) and Haldane (1901) established that nitrate must be reduced to nitrite bacteriologically before reaction occurs with myoglobin to give the characteristic color of cured meat products. Later, Kerr *et al.* (1926) established proper levels of usage for nitrite in meat curing. Then, Brooks *et al.* (1940) and Tarr (1941) offered respectively the first proof that nitrite is the agent responsible for cured flavor and has an antimicrobian function.
Further studies corroborated these findings and, simultaneously, showed that some of the added nitrite disappeared during processing storage and distribution (Fox and Thomson, 1963; Nordin, 1969; Cho and Bratzler, 1970; Greenberg, 1972; Herring, 1973).
Then, three potential problems currently associated with nitrite and cured meat had to be dealt with: firstly, the possible presence of preformed nitrosamines in the product; secondly, the presence of residual nitrite (whose consumption increases the total body burden of nitrite and can produce nitrosamines formation in the consumer's stomach) and thirdly, concern about the unknown.
If the improvement of our knowledge about the third point was (and still it is for several points) the aim of a lot of researchers (as shown by the amount of published papers), the outcome of the first two is a modification of the regulation in many countries, in order to lower the level of residual nitrite as near 0 as possible in cured meats. But, as nitrosamine formation is to be prevented and the amount of nitrite ingested

by consumers decreased, cured meat products need to have a good shelflife. Studies carried out on prepackaged cured cooked ham (Goutefongea 1980) have shown that a high vacuum (about 5 hPa) gives a good color stability for any level of residual nitrite; however, when the vacuum level reaches only 100-125 hPa, the color stability becomes largely depending on the amount of residual nitrite as follows:

- residual nitrite < 20 mg/kg: the color turns irreversibly grey-brown very rapidly;
- residual nitrite > 80 mg/kg: the color turns grey-brown during the first four hours after packaging, then becomes satisfactory again during the next 24 hours and remains stable for 8-10 days.

The aim of this work is to try to understand the chemical mechanism of these color changes.

MATERIALS AND METHODS: Paired hams from eight hogs (16 hams) were purchased from a local slaughterhouse twenty-four hours after slaughter and kept at +4°C. The next day, they were derinded, defatted, deboned and injected (stitch pumping) with brine. One ham of each pair was pumped to 115% of green weight after trimming by using a brine composed of 10% sodium chloride (NaCl) and 1000 mg/kg sodium nitrite ($NaNO_2$). This resulted in ingoing level of 150 mg/kg $NaNO_2$. The second ham of each pair was pumped to 115% of green weight after trimming with a brine composed of identical level of NaCl but lowered $NaNO_2$ concentration to 200 mg/kg. This resulted in an ingoing nitrite level of 30 mg/kg. After pumping, hams were soaked in their respective brine for 48 hours, then drained for 4 hours, put in moulds and cooked in a steam oven until the internal temperature reached 68°C. After cooking, they were pressed under 22 hPa and stored at +4°C for 2 days in a chilling room. Afterwards, they were taken out of the moulds and muscles *Biceps femoris* and *Semimembranosus* were carefully dissected with complete removal of fat and connective tissue, then finely ground.

For each ham, aliquots weighing about 50 g were packaged in transparent plastic pouches (oxygen permeability: 10 $cm^3/m^2/24h$) under residual pressure of either 5 or 125 hPa. For each vacuum condition, two kinds of samples were prepared: thick samples (> 5 mm) for color analysis by reflectance spectra and thin samples (1 mm) for chemical analysis: as a matter of fact, reflectance measurements require thick samples (theoretical thickness = ∞); on the contrary, as chemical reactions associated with color changes affect only the surface of the samples, chemical determinations have to be carried out on samples as thin as possible.

All samples were stored at + 10°C in fluorescent light.

Reflectance spectroscopy measurements were carried out on a spectrophotometer Jobin-Yvon/Hitachi model 100-80A, fitted with an integrating sphere, a 10 inch recorder and a micro-computer Apple IIe. The reflectance spectra were recorded between 700 and 400 nm and the values of the reflectance percentage, collected at 5 nm intervals, were treated on the computer to obtain color characteristics; we considered mainly the

luminance Y, and the dominant wavelenght λ_d.. Furthermore, we determined ΔE, which is the global assessment of color differences, between the initial time T_0 and the different times at which reflectance measurements were made.
Chemical determinations were carried out as following:

- Residual nitrite was determined in duplicate on samples weighing 10 grams, homogeneized in warm water (60°C) buffered with borax (5%, pH 9,0) and then heated at 100°C for half an hour. After cooling, the solutions were clarified by Carrez solution 1 (potassium ferrocyanide 15%) and 2 (Zinc acetate 30%), adjusted in volume and filtered; Nitrite was determined by diazotation with sulfanilamide-HCl and coupling with a-naphtylethylenediamine. The resulting chromophore was measured spectrophotometrically at 540 nm.
- Total pigments and nitroso-pigments were quantified (Hornsey 1956) by HCl-acetone-water extraction for total pigments and acetone-water (80/20) for nitrosopigments. Spectrophotometric measurements were made at 512 and 540 nm respectively.

Reflectance spectroscopy measurements and chemical determinations were performed at the initial step T_0 (just after packaging) then at T_0 + 4 h, T_0 + 24 h, T_0 + 48 h, T_0 + 72 h, and T_0 + 96 h.

RESULTS AND DISCUSSION: Results of chemical analysis are summarized on Fig.1. We observe that the amount of residual nitrite is about 50% of the initially added at T_0 and then, there is a depletion during storage; at T_0 + 96 h, residual nitrite is about 25% of initially added. No significant effect of the vacuum level and initial nitrite on the depletion percentage is noted. These results about disappearing of a part of the added nitrite during processing, then during storage, are well known.
Concerning total pigments, we notice, in every experimental condition, a progressive disappearance which is probably a consequence of a slow opening of the porphyrin ring under light exposure. For both nitrite levels, the disappearing rate seems to be slightly higher for higher residual pressure.
The main differences appear when considering the variation of nitrosopigments. For both levels of added nitrite, we observe on samples packaged under 5 hPa a decrease similar to the one observed on total pigments. Under a 125 hPa pressure, the phenomenon is depending on nitrite level: with a low nitrite level, there is a decrease in nitrosopigments fraction, fast between T_0 and T_0 + 4 h, then slower; with a high nitrite level, we observe a similar change between T_0 and T_0 + 4 h, but an increase from T_0 + 4 h and T_0 + 48 h, then a slow decrease.
The color characteristics calculated from reflectance spectroscopy measurements are shown Fig. 2. Their variations represent the color changes of the samples during storage: when the dominant wavelength λ_d is decreasing, color turns from pink to grey-brown, and when the luminance factor Y is increasing, color is fading; then, the variation of ΔE illustrates the global changes in color from the initial state (color change is visible by human eye when $\Delta E > 2$).
The interesting point to discuss is the chemical mechanism of color changes. As for residual pressure of 5 hPa, these changes are slow and slight, we are interested mainly in most significant changes, which occur with residual pressure of 125

hPa. With both nitrite levels, color turning which happens squares with concomitant decrease in nitrosopigments fraction (when total pigments fraction remains unchanged); that means nitrosylmyoglobin is oxidized in metmyoglobin, as it appears on the reflectance spectra (Fig. 3 A and B).
Then, with a low nitrite level, the slow decrease in nitrosopigments after T_0 + 48 his probably associated with the decrease in total pigments under light exposure; with a high nitrite level, the recovery of good color between T_0 + 4 h and T_0 + 48 h squares with the increase in nitrosopigments fraction; the reflectance spectra (Fig. 3 B) show the progressive disappearance of metmyoglobin. So, a secondary nitrosation from residual nitrite occurs. The question is: does that secondary nitrosation affect only metmyoglobin appeared between T_0 and T_0 + 4 h or does it affect partly metmyoglobin and partly some of the originally non-nitrosated pigments? We have no categorical proof but, as nitrosated pigments seem to be more sensitive to light exposure, the second alternative could be the most likely.

CONCLUSION: These results agree mostly with those of Lin and Sebranek (1979), Amundson *et al.* (1982) and Froehlich *et al* (1983), even if the aim of these authors' works was not the same as here. Anyway, the main lesson we can draw from these results is: if we wish meat products with a low level of residual nitrite, we have to use a very high vacuum and pouches owing a low oxygen permeability to obtain good color stability and good shelflife.

REFERENCES:

- Amundson, C.M., Sebranek, J.G., Kraft, A.A., Rust, R.E., Wagner, M.K., and Gehrke, W.H. (1982) J. Food Sci. 47: 355.
- Brooks, J., Haines, R.B., Moran, T. and Pace, J. (1940) Food Invest. Bd Special Report N° 49 London.
- Cho, I.C. and Bratzler, L.J., (1970) J. Food Sci. 35:668.
- Fox, J.B.Jr and Thomson, J. (1963) J. Biochemistry, 2:465.
- Froehlich, D.A., Gullett, E.A. and Usborne, W.R. (1983) J. Food Sci.48:152.
- Goutefongea, R. (1980) 26th Europ. Meet. of Meat Res. Workers Proceedings. Colorado Springs (Aug 31-Sept.5) p.219.
- Greenberg, R.A. (1972) Meat Ind. Res. Conf. Proceedings. p.25.
- Haldane, J. (1901) J.Hygiene. 1:115.
- Herring, H.K. (1973) Meat Ind. Res. Conf. Proceedings. p.47.
- Hornsey, H.G. (1956) J. Sci. Food Agric. 7:534.
- Kerr, R.H., Marsh, T.N., Schroeder, W.F. and Boyer, E.A. (1926) J. Agr. Res. 33:541.
- Lehman, K.B. (1899) Sitzt.Physikol.Med. Ges. Wurzburg 4:57.
- Lin, H.S. and Sebranek, J.G. (1979) J. Food Sci. 44:1451.
- Nordin, H.R. (1969) Can. Inst. Food Technol. J. 2:79.
- Polenski, E. (1891) Arb. Kais. Gesundh. 7:471.
- Tarr, H.L.A. (1941) Nature 147:417.

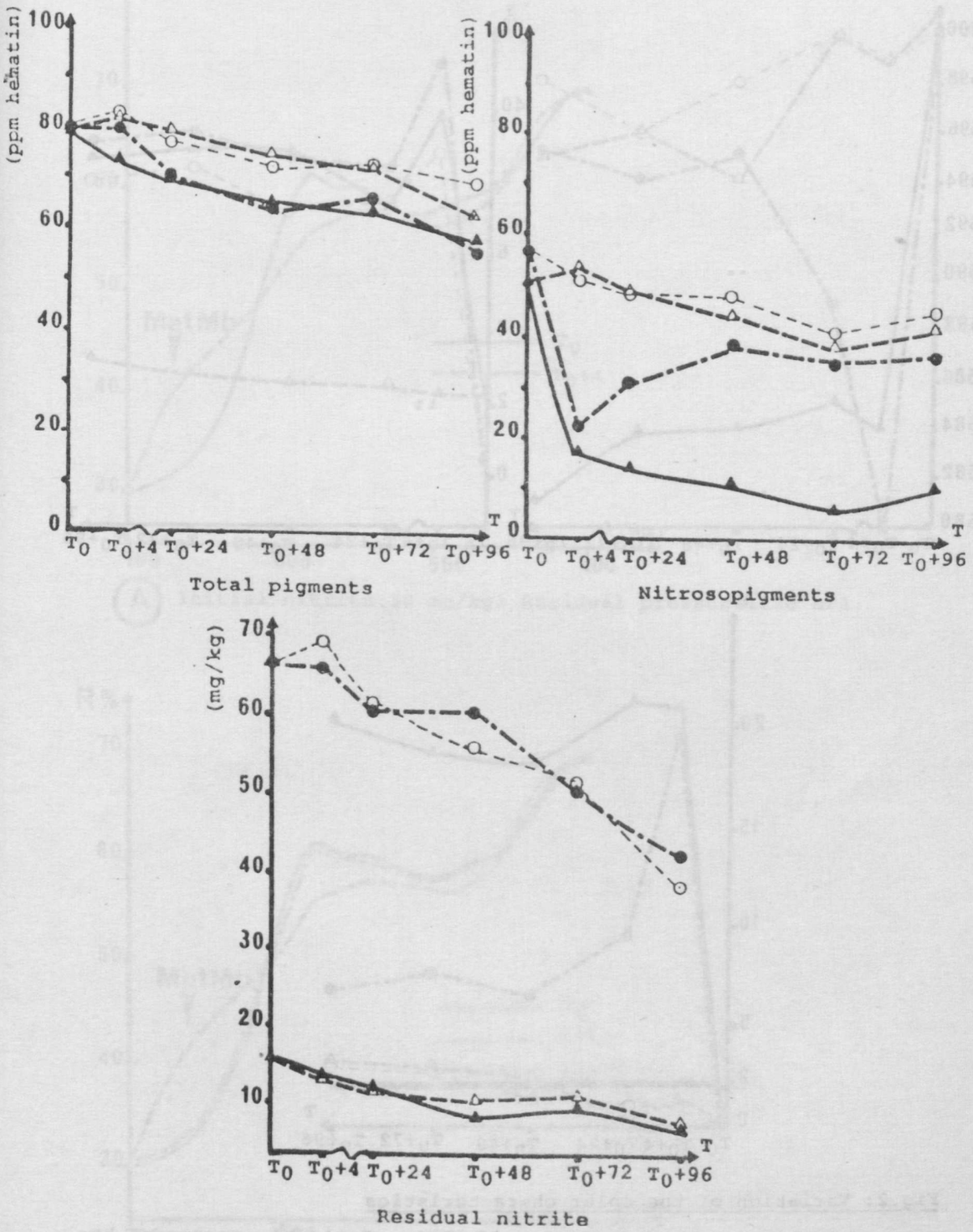


Fig.1: Variation of the chemical characteristics.

- Initial nitrite:30 mg/kg; Residual pressure:5 hPa.
- Initial nitrite:30 mg/kg; Residual pressure:125 hPa.
- Initial nitrite:150 mg/kg; Residual pressure:5 hPa.
- Initial nitrite:150 mg/kg; Residual pressure:125 hPa

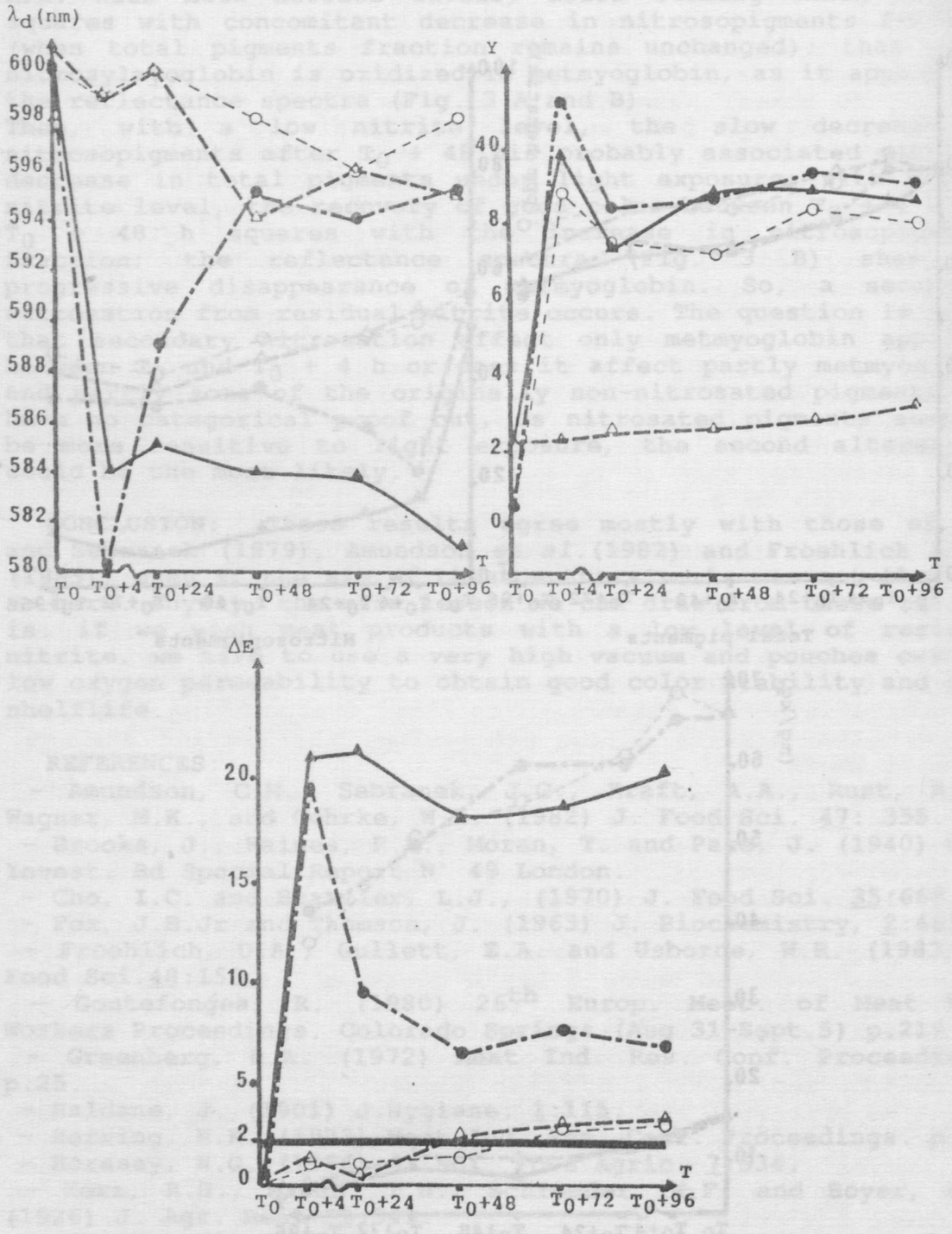
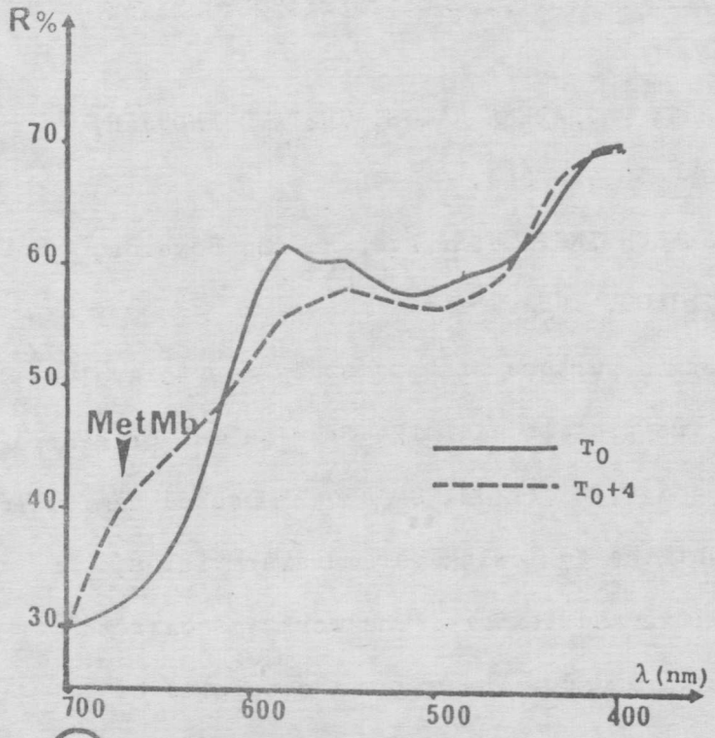
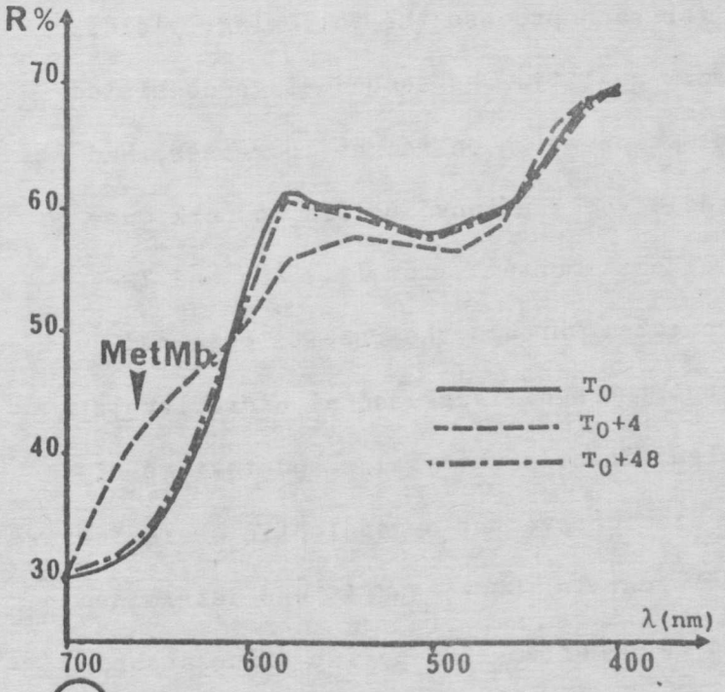


Fig.2: Variation of the color characteristics

- Initial nitrite:30 mg/kg; Residual pressure:5 hPa.
- Initial nitrite:30 mg/kg; Residual pressure:125 hPa.
- Initial nitrite:150 mg/kg; Residual pressure:5 hPa.
- Initial nitrite:150 mg/kg; Residual pressure:125 hPa



(A) Initial nitrite:30 mg/kg; Residual pressure:125 hPa.



(B) Initial nitrite:150 mg/kg; Residual pressure:125 hPa.

Fig.3: Reflectance spectra