CXLVI. THE MITTELMANN PROCESS FOR TREATING FISH-LIVERS.

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Two reports by Mittelmann on "Les graisses de la Morue et nouvelles méthodes de leur préparation" [1925] and "The manufacturing of fish conserves of Murman" [1927] are somewhat difficult of access, and this may account for the fact that certain distinctly important claims have so far neither been confirmed nor refuted in the literature. We are indebted to Bezssonoff [1929] for a summary of Mittelmann's work, the gist of which is as follows. Fresh cod-livers are sealed in evacuated tins which are then heated to 120°, the process amounting to autoclaving the livers. On opening the tins the liberated oil can readily be poured off, and it is said to be pleasant in taste and free from the typical disagreeable smell characteristic of all cod-liver oils prepared by any method used in the industry. "On testing the oil immediately after opening a tin no blue colour with antimony trichloride was obtained, but if the oil was re-examined after 10 days a positive colour test was recorded. At the same time, the characteristic unpleasant smell of commercial cod-liver oils began to manifest itself. Thus, contact with air causes the appearance of the evil-smelling substance (probably as a result of the formation of bodies with ethenoid linkages) and simultaneously brings into being the property of giving a blue colour with antimony trichloride."

The importance of these claims is considerable both from the theoretical and practical standpoints. If vitamin A is really present in the oils from freshly opened tins the antimony trichloride colour test must have serious limitations, to say the least. If, on the other hand, the colour test is definitely a valid measure of vitamin A, then since negative results are obtained with freshly opened tins, the oil from the autoclaving process must be devoid of vitamin A. As, however, the chromogenic property of the oil is acquired on standing in air, the vitamin must be an oxidation product of a precursor substance stored in the liver.

These implications made it necessary in our opinion to undertake a careful repetition of the work of Mittelmann. Accordingly arrangements were made for the livers of fish caught by Aberdeen trawlers to be canned and autoclaved at the Torry Research Station and the oils examined by the Lovibond technique applied to the antimony trichloride colour test. The tins were also to

be sent to Liverpool with the minimum delay so that a complete spectroscopic examination could be carried out. We are indebted to Mr John Bruce of Messrs Marshall and Co., Ltd., of Aberdeen for assistance in canning the livers. The tins used were lacquered on the inside to guard against contamination by the metal, the lacquer being chemically inert and oil-resisting. Tins of one pound capacity were used as these were the largest which could be sealed under vacuum. They were not large enough to contain whole livers, so that in all cases except those of small codling, the livers were cut into pieces before packing. After evacuating to 1 inch of mercury and sealing, the tins were autoclaved at 120° for 2 hours.

The first experiments were carried out on small codling caught off Aberdeen, and the livers were only about 4 hours old when canned. The oil obtained from the tins was examined the same day (19. ii. 1931) and gave a reading of 1.84 Carr-Price blue units (Lovibond). After storage under nitrogen for 6 days the value was 2.24 units, and after 13 days 2.28 units.

Ultra-violet absorption spectra for the oil from freshly opened tins, photographed less than 3 days after the fish were caught, showed that the codling-liver oils were remarkably transparent. With a 1 mm. layer of the oil itself (no solvent) 3 % of the incident light was transmitted at 299.5 $m\mu$, the curve showing a small maximum at 320–330 $m\mu$, the value for the gross uncorrected absorption at this point being $E (= \log I_0/I)$ 0.125 reduced to the basis of a 1 % solution and a 1 cm. cell. With antimony trichloride a definite blue-violet colour was produced, the solution exhibiting a well-defined maximum at 572 $m\mu$. With most cod-liver oils the blue solution is characterised by a maximum at 606 $m\mu$, the spectral absorption curve being unsymmetrical owing to the presence of greater or smaller absorption at 572 $m\mu$, which is usually less intense than that at 606 $m\mu$. With the codling-liver oil from freshly opened tins, there was definite, measurable absorption at 606 $m\mu$, but this was approximately only half as intense as the absorption at 572 $m\mu$. On standing exposed to air, or on oxidation with oxygen at 70°, the capacity to give a blue colour with antimony trichloride underwent a considerable increase. This was reflected in the spectral absorption of the blue solution by an increase in absorption at 606 $m\mu$, which was not accompanied by any appreciable increase or decrease at 572 $m\mu$.

A number of larger codling-livers were also canned and autoclaved at Aberdeen and examined spectroscopically at Liverpool. Here again, the freshly opened tins yielded oils which exhibited a small absorption band at 328 $m\mu$ and gave a colour test in which the 572 $m\mu$ band predominated over the 606 $m\mu$ maximum. It is interesting to note that the blue solution with antimony trichloride slowly faded to a clear yellow colour characterised by a sharp maximum at 460–465 $m\mu$.

The preliminary experiments on codling-livers having given indications of an increase in the chromogenic value of the oils from autoclaved livers on standing, it was decided to repeat the experiments under more defined con-

ditions and with adequate controls. Accordingly $\frac{1}{2}$ cwt. of cod-livers were obtained from fish landed at Buckie (3. iii. 1931) and gutted on shore. Nets had been out since 28. ii. 1931 so that many of the fish had been dead for a few days. The livers were conveyed to Aberdeen and each was cut into two roughly equal parts. One half of the catch was canned and autoclaved at 120° , whilst the other half was steamed with direct steam in the usual way for preparing cod-liver oil.

The Buckie cod were on spawn at the time of catching, whereas the codling already referred to were either immature or just about to spawn. It must be emphasised that the steamed liver oil was obtained from half the whole catch, whereas individual tins subjected to the Mittelmann process would be representative of two or three livers, so that the experiment could only afford a qualitative control, the well-known variations in potency of the oil from individual livers precluding strictly quantitative control.

Lovibond readings obtained at Torry Research Station on March 4th, 1931, gave readings of 4.0 and 6.4 Carr-Price units for the oils from autoclaved and steamed livers respectively. After 3 days' storage under nitrogen the oils gave values of 8.0 and 7.2 blue units. The oils and tins reached Liverpool on March 5th, and were examined on that and the following days. The spectroscopic examination of the antimony trichloride-oil solution gave results indicating that little or no change had occurred in transit. The blue solution showed a well-defined maximum at $572m\mu$ together with marked, but much less intense, absorption at $606m\mu$. The ultra-violet band with maximum near $328m\mu$ was shown very clearly, but at an intensity much greater than would be expected from the proportion of the chromogen responsible for the $606m\mu$ absorption in the colour test.

Different tins were opened at various times for several weeks after receiving the oils. It was found that in the sealed tins the oil gradually underwent a change which resulted in enhanced absorption at $606m\mu$, but within the limits of error no appreciable change occurred either in the absorption at $572m\mu$ or $328m\mu$ in the ultra-violet spectrum. It was at any rate clear that the marked predominance of the $572m\mu$ band slowly disappeared. Storage of the oil in bottles under nitrogen likewise failed to prevent a slow change resulting finally in a very marked increase in the $606m\mu$ band relatively to the $572m\mu$ band. Oxidation with oxygen at 70° or ozone at room temperatures likewise caused a marked increase in the $606m\mu$ band, which in this case was ascertainably not at the expense of a diminution either of the $572m\mu$ band or the ultra-violet band. It is noteworthy that the blue solution again faded to a bright yellow marked by a maximum at $465m\mu$.

A few comments on Mittelmann's process as a method for the manufacture of cod-liver oil are necessary. In our hands, the yield of oil is poor and variable, in some cases more water than oil being obtained. In nearly every case it was found necessary to dry the oil with anhydrous sodium sulphate before carrying out any tests. It is moreover very difficult to free the liver débris

with the steamed cod-liver oil indicated precisely the same tendency towards an increase in the blue value as the autoclaved oils, there is no evidence for regarding the Mittelmann process *per se* as having any special significance. Moreover, the ultra-violet band, which is at present perhaps the least debatable criterion of vitamin A, discloses no qualitative differences between autoclaved liver oils and oils prepared by other processes. The results agree well with the conception advanced in the following papers, that liver oils contain two distinct if not unrelated chromogens.

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REFERENCES.

- Bezssonoff (1929). *Bull. Soc. Chim. Biol.* **11**, 1146.
Mittelmann (1925). *Nouvelles Inst. Agronom. Exp.* **3**, 1.
—— (1927). *Trans. Inst. for Explor. of the North, Leningrad*, 38, 39.
Drummond and Hilditch (1930). *Empire Marketing Board, Report* 35.