TROPICAL SOILS*

By H. GREENE
Rothamsted Experimental Station

THE Universal Declaration of Human Rights (1948) includes the phrase 'The advent of a world in which human beings shall enjoy freedom of speech and belief and freedom from fear and want'. This fourth freedom, freedom from want, is a new idea. It occurs in the final but not in the first draft of the Atlantic Charter. The first draft contained the words 'freedom of speech and thought', whereas the final draft (1941) has the immortal phrase 'that all the men in all the lands may live out their lives in freedom from fear and want'. The idea of freedom from want had previously been stated by Whitehead,[1] who, with his customary insight, gave four examples of disasters that can afflict farmers: a drought, a wet summer, a bad harvest and a cattle disease; he drew the conclusion that the plain economic facts of life must be the governing force in social development.

Now although in this country our welfare depends largely on the production of coal and steel, on the chemical and engineering industries and on convenient transportation and transshipment, in many tropical areas the only obvious way of obtaining money for social development lies in the improvement of agriculture and, particularly, in producing more crops for sale in the world markets. Success in this depends on social and economic factors as well as on the physical conditions of soil and climate. But the physical conditions are of such importance that it is well worth while to study them with care. Merely to ascertain what are the relevant physical conditions makes it possible to avoid some painful errors and disappointments and we may be able to alter the physical conditions so that they conform more closely to our needs.

Three main problems

When studying soil we encounter three main problems: What kinds of soil occur? What is their distribution? What is their best use? These three problems are not entirely separate but they are sufficiently so to be tackled by different people. The first problem involves classification and has proved very difficult. It will be sufficient to notice two sharply contrasted kinds of soil: the leached red earth or red loam that is found in well drained situations of the fairly humid tropics, and the unleached grey calcareous clay, often called black cotton soil, that usually occurs in poorly drained situations of the more arid parts of the tropics. Here are two soils that look very different, that differ in their chemical and physical properties and in the crops they can best support. There are many different kinds of soil in the tropics and, although the differences between them are less extreme than in this contrast, they are nevertheless of decisive importance to the farmer; it is therefore a matter of urgency to prepare maps so that both the farmer and the administrator can know what is the distribution of these soils. This is a task in which the people of an underdeveloped tropical country can play an important part; within any area of a few thousand square miles the number of different soils is not unmanageably large, so that with tuition and practice men of ordinary ability can learn to distinguish them. Charter,[2] who is in charge of soil survey in the Gold Coast, has remarked that sharing in the making of a soil map is the best preparation for putting soils to their most appropriate use. It is indeed an admirable kind of education, combining the scientific observation of nature with economic objectives, and it makes a worthy appeal to local patriotism. The third problem—what is the best use for the different soils?—can be solved in part by the practical experience of farmers, but their work is made quicker and less painful by applying the techniques of field experimentation introduced at Rothamsted by Fisher[3] and his associates[4, 5] and now used widely and successfully.

Factors in soil formation

When working on these three major problems, soil scientists have become aware of some pervasive regularities which have convinced them that soils are not formed by chance but are the outcome of smoothly working processes operating on materials that are familiar to

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the geologist. These, the parent materials of soil, are often the loosened or slightly altered form of crystalline rocks; sometimes they are alluvial deposits or wind-borne deposits that may be new or old, loose or consolidated, coarse-grained or fine-grained, with possibly a wide range in chemical composition. One can well believe that the properties of soil will be found to depend greatly on the nature of the parent material. However, towards the end of the nineteenth century Russian scientists found that climate had what seemed to be a dominating influence on the course of soil formation: they observed that similar kinds of leached soil were formed from various parent materials in the cold moist climate of north Russia, whereas similar kinds of unleached soil, a fertile black earth, were formed from various parent materials in the warmer and drier climate some hundreds of miles further south. Thus climate came to be recognized as one of the dominating factors in soil formation. It can be readily appreciated that topography is another important factor; there are many cases where, owing to run-off, the soils near the top of a slope receive less than their share of the rainfall, whereas those at the foot of the slope receive more than their share. The effects are particularly conspicuous in some tropical areas; in Uganda and some other parts of East Africa there was found a regular pattern of red leached soils on the hills and grey soils in the valleys. The late Geoffrey Milne[6] gave the name catena to this recurring sequence, and he noted that substances leached out of the higher-lying soils assembled and accumulated in the lower-lying soils. This occurs, for example, with silica and bases. The effects of climate and topography on soil formation are well shown in the Southern Sudan.[7] One side of the Imatong Mountains receives heavier rain than the other; consequently, on the rainy side the soils are deeply leached red earths, whereas on the dry side there are relatively unleached soils, ranging from reddish grit on the slopes to grey calcareous clay on the flat land. The corresponding differences in vegetation are striking (Figs. 1 and 2).

FIGS. 1 *and* 2.—*Contrast between dry and wet sides of the Imatong Mountains, Equatoria Province, Anglo-Egyptian Sudan. The photographs were taken near the crest*

FIG. 1 (*left*). *Drought-resistant plants growing on shallow gritty soil amid bare rock*

FIG. 2 (*right*). *Giant lobelia and other luxuriant vegetation covers black surface soil overlying friable red earth which is usually deep*

It would be possible to illustrate from an adjacent area in the Southern Sudan the effect of parent material on soil formation, but instead some recent observations made in Malaya will be considered.

Weathering by sulphuric acid.—In April, 1952, Dr. Elizabeth Alexander, a geologist then resident in Singapore, showed the author deep exposures of sedimentary material ranging from stones and gravel to sand and clay. The sediments had been raised to a nearly vertical position by emergence of a granite mass now forming the central part of Singapore Island and of the Malayan peninsula. The sandy, yellow parts of the sediments showed Liesegang surfaces in close conformation, predominantly horizontal but having a wavy outline. The upper part of the exposure had red and white mottling such as is found in the humid tropics

in the deep subsoil at the foot of higher land (perhaps in this case at the foot of a higher mass of granite). The red and white mottling was evidently a later process than the Liesegang formation, which, it is suspected, resulted from the progressive oxidation of sulphide by percolating water. A newly formed gully in similar geological material a few miles from this site had exposed at depth a grey sandy sediment which, when tested with indicator, proved to be of high acidity, such as occurs when sulphur or sulphide is oxidized to sulphuric acid. It seems possible that mangroves or other plants accumulated sulphur during deposition of the sediments; when the sediments were tilted, therefore, they constituted nearly vertical sheets of porous material impregnated with residues, originally organic, that were ready to produce strong acid as soon as they were reached by drainage waters containing dissolved oxygen.

Such conditions would favour the formation, migration and hydrolysis of iron and aluminium sulphate; indeed, at this site iron concretions and bauxitic concretions are segregated in conspicuous form. It thus appears that parts of the sediments that subsequently became the agricultural and rubber-growing soils of Malaya were leached by sulphuric acid (approximately 0·1N) before they became the parent materials of the present soils. This may well have significance for present-day agriculture. It may be added that there are indications from Africa, Australia and Europe that weathering by sulphuric acid may be of wider occurrence than has hitherto been supposed.

Effect of vegetation.—Although the types of vegetation and of animal life are largely determined by climate and topography, soil scientists have found it convenient to regard plant and animal organisms as separate factors in soil formation. The effect of vegetation on soil formation is well shown in a map of the soils in Tama County, Iowa, prepared by Aandahl, Simonson & their associates.[8] In Tama County a deep deposit of glacial drift covers the limestone bedrock and is itself covered in most places by a deposit of loess—a yellowish-brown, slightly acid, silt loam that becomes calcareous at about a 10-ft. depth. This loess, the parent material of most of the soils, is unusually uniform. The map shows alluvial soils along the river system, but on the higher land there is a strong contrast between yellow areas that formerly carried trees and blue areas that formerly carried grass. In the field the soils of the yellow areas can be recognized as grey–brown podzolic soils. They are lighter in colour and are notably less fertile than the soils of the blue areas, which have been called prairie soils. In Tama County the farmer will be more prosperous if he buys land that was formerly under grass (prairie soil) than if he buys land that was formerly under trees (grey–brown podzolic soil). This is an example of the practical importance of vegetation in soil formation. Vegetation can have a marked effect of this sort because plants differ in the nutrients they absorb from soil, in the substances they give off from their roots and leaves during growth and in the nutrients they return to the soil during decay.

Bloomfield[9] has been making aqueous extracts of organic materials such as grass and pine needles, and finds that these extracts are capable in different degrees of reducing iron oxide and bringing it into solution in the form of organic complexes some of which are notably stable. His work is throwing light on the mobility of iron and aluminium in soil. This is a matter that has engaged the attention of many scientists. About twenty years ago the Russian scientist Polynov,[10] by comparing the chemical composition of the country rock with the composition of river water draining an area, came to place the common constituents in the following order of mobility:

		%
1st class	Cl	100
	S	57
2nd class	Ca	3
	Na	2·4
	Mg	1·3
	K	1·2
3rd class	SiO_2	0·2
4th class	FeO_3	0·04
	Al_2O_3	0·02

It seems likely, however, that the kind of evidence Polynov considered caused him to overlook the high local mobility of iron and perhaps of aluminium. (Dr. A. Muir notes that Polynov was discussing the residual products of weathering in well drained situations.) More recently Jackson[11] & his co-workers have constructed a weathering sequence of clay-size minerals that begins with gypsum and calcite, includes quartz, and ends with gibbsite, haematite and anatase. Nevertheless it seems likely that, in the field, conditions sometimes impose greater mobility on the sesquioxides than this list suggests. It is evident that the sesquioxides may move over relatively small distances, producing results of scientific and of agricultural interest, without necessarily contributing much to the material dissolved in river water. The products

of some of these restricted migrations of sesquioxides form conspicuous features over wide areas in the tropics. These products are lateritic materials that are found as layers within the soil or that are preserved as relics in the form of ironstone caps on hills that they have protected from erosion. Some scientists who had not seen laterite in the field were so impressed by pictures and accounts of these formations that they came to regard laterite as the final stage of soil formation. In their view there was first the fresh rock, then the juvenile soil containing many unweathered rock fragments, and then the mature soil in which the effect of climate was most typically seen; but later, as leaching continued, with progressive removal of silicon and bases, there was a further stage of degradation or senescence, until at last only the sesquioxides were left, perhaps in the form of lateritic crusts. That was the death of the soil.

This account is now suspect,[12] for different views are now held as to the origin of lateritic materials and it is known[13] that they can serve as the parent materials of new soils. It remains true, however, that the conversion of rock into soil is a slow process, which induces the washing away or segregation of some plant nutrients to leave an impoverished residue. In particular it seems reasonable to associate the formation of laterite with the leaching of some plant nutrients and the segregation of others. Prescott & Pendleton[14] mention the possible association of laterite formation with the high acidity arising from oxidation of marcasite and they refer also to the segregation[15] of molybdenum and zinc with the lateritic concretions of Western Australia and South Australia. It is probably no accident that application of molybdenum and other plant nutrients is so effective in those areas.

Possible ways of increasing agricultural production

It is essential now to present another point of view. Almost any farmer knows that he can change his soils for better or worse within a few years. We have a wonderful opportunity for increasing the productivity of the soil in those underdeveloped tropical areas where we still have an administrative responsibility, and it may be that the opportunity is no less in those countries where administrative responsibility has been given to the native people. We can consider these possibilities in relation to the soil-forming factors discussed above.

Irrigation is one of those obvious ways of increasing agricultural production. In Northern Rhodesia, Southern Rhodesia and Nyasaland there are about ten million acres where an enormous improvement in agricultural productivity may be achieved by means of irrigation and drainage. Technical studies continued over some years are needed to gauge the engineering and agricultural problems; heavy outlay of capital will be needed; the economic aspects are at present obscure. Nevertheless it seems probable that a good part of these developments will take place during the next fifty years. This seemingly practicable series of operations is in effect equivalent to altering the climate over a large area. One might expect from this a change in agricultural production, in this case an increase from almost nothing to a fairly high level. Another climatic limit on agricultural production is the seasonal incidence of some pest or disease that attacks the desired crop. Means are now at hand for removing some of these controls so that in theory it is now possible to grow crops that would not formerly survive. In theory that change in land use is likely to be associated with a change in the soil. In practice the change is more likely to be beneficial than harmful. This suggests that the use of new means of controlling pests and disease may be looked upon as equivalent in effect to a change in climate and on the whole it is likely to prove a beneficial operation.

Topography.—It is usually not practicable to alter the shape of mountains and valleys. Here and there, however, it certainly is possible to install a drainage system or to carry out works on river training that are equivalent, physically and economically, to altering the topography of an area; for example, an area that was formerly waterlogged or that suffered from a severe flood hazard may be changed into valuable agricultural land. Again, throughout human history low-lying areas have been almost closed to human occupation because of malaria or other disease. There are now obvious possibilities of occupying these lands in safety, and in many of them the alluvial soil is likely to be fertile. Here we do not alter the topography: we circumvent the effects of a topographical factor that formerly determined land use.

We noted that a parent material that had been leached with sulphuric acid might prove less fertile than other parent materials. One would be prepared to find that a soil derived from an arenaceous sedimentary rock was less well supplied with basic plant nutrients than a soil derived from a crystalline rock. Similarly a soil in a region of heavy rainfall is likely to be more leached than a soil in a region of light rainfall. In short, we expect to find that soils differ in their content of plant nutrients. During the past hundred years, however, we

have been learning to make good a deficiency in plant nutrients by use of artificial fertilizers. The work of Lawes on the major nutrients was followed by that of Brenchley[16] and Warrington[17] on boron, and it is now known that in addition to the major nutrients there are other micro-nutrients which in certain cases can play a decisive part in raising agricultural production from practically nothing to a fairly high level. Some of the most striking examples come from Australia[18] but it is most unlikely that Africa, say, or South America, will fail to benefit from similar discoveries. It may quite reasonably be suggested that the progress made in these fields has been striking and shows good promise of being extended soon. It appears that in British Colonial Territories we are not yet doing enough to explore these various possibilities of increasing agricultural production by use of fertilizers, but a start has been made in this vitally important and constructive work.

We have been considering ways in which human activities produce changes in soil. Many of the interactions are unplanned and unforeseen. Merely to stop inter-tribal warfare usually brings about a change in land use; the building of a road or a railway, the concentration of people into a village or a town, for example, all bring about changes in land use, in the values of soil and in physical and chemical properties of soil. Attention has been drawn to the waste of natural resources in the United Kingdom when valuable agricultural land is taken over for urban or industrial use. Nevertheless in underdeveloped tropical areas it may be that the general effect of improved communications is to raise and not to lower the fertility of an area; thus it is possible that an increased density of human settlement may be accompanied by more skilful and by more generous systems of land management. It may be, for example, that in a closely settled area, where formerly there was only woodland or poor grazing, people can now afford to use fertilizers and grow vegetables and fruit.

Rotation of crops.—Although soil-forming processes usually need hundreds or thousands of years in which to produce a visible effect it is possible to design an experiment in crop rotation or in systems of land use that will show striking differences within about ten years. Careful studies[19] indicate that the farmer has often a choice of a heavier or lighter investment in capital and labour, with a greater or smaller return in harvest and income. This choice is open not only to the individual farmer but to the whole group of farmers in a region. The farmers in any underdeveloped tropical area probably have a choice restricted by physical and economic conditions but also, most severely, by lack of the information that can be obtained by applying science to the problem of tropical agriculture. Their way to achieve freedom from want is to become acquainted with the relevant facts about their soils and the needs of their plants and animals. Young scientists from the United Kingdom who can help in this will be doing most useful work.

References

[1] Whitehead, A. N., 'Adventures of Ideas', 1933 (Cambridge: Cambridge University Press)
[2] Charter, C. F., *Bull. agric. Congo Belge*, 1949, **40**, 109
[3] Fisher, R. A., 'The Design of Experiments', 1947, 4th edn. (Edinburgh: Oliver and Boyd)
[4] Yates, F., *Tech. Commun. Bur. Soil Sci., Harpenden*, 1937, No. 35
[5] Stewart, A. B., & Crowther, E. M., 'Memoranda on Colonial Fertilizer Experiments', 1947, Colonial No. 214 (London: H.M.S.O.)
[6] Milne, G., *Proc. int. Cong. Soil Sci.*, (No. 3, Oxford), 1935, **1**, 345
[7] Greene, H., *Soil Res.*, 1939, **6**, 325; *Proc. Soil Sci. Amer.*, 1945, **10**, 392
[8] Aandahl, A. R., Simonson, R. W., *et al., Soil Surv. Rep. U.S. Dep. Agric.*, 1950, Series 1938, No. 22
[9] Bloomfield, C., *J. Soil Sci.*, 1953, **4**, 5, 17
[10] Polynov, B. B., 'The Cycle of Weathering' (translated by A. Muir), 1937, p. 163 (London: T. Murby and Co.)
[11] Jackson, M. L., Tyler, S. A., Willis, A. L., Bourbeau, G. A., & Pennington, R. P., *J. phys. Chem.*, 1948, **52**, 1237
[12] Greene, H., *Proc. int. Cong. Soil Sci.*, (No. 4, Amsterdam), 1950, **2**, 175
[13] Stephens, C. G., *Bull. Coun. sci. industr. Res. Aust.*, 1946, No. 206
[14] Prescott, J. A., & Pendleton, R. L., *Tech. Commun. Bur. Soil Sci., Harpenden*, 1952, No. 47
[15] Oertel, A. C., & Prescott, J. A., *Trans. roy. Soc. S. Aust.*, 1944, **68**, 173
[16] Brenchley, W. E., 'Inorganic Plant Poisons and Stimulants', 1927, 2nd edn. (Cambridge: Cambridge University Press)
[17] Warrington, K., *Ann. Bot., Lond.*, 1923, **37**, 629
[18] Davies, J. Griffiths, *Chem. & Ind.*, 1953, p. 535
[19] Black, J. D., *Proc. U.N. sci. Conf. Cons. & Util. Resources*, 1951, **6**, 97

RIGOR MORTIS IN BEEF

By B. B. MARSH

A study has been made of the onset of *rigor mortis* in the *longissimus dorsi* muscle of the ox, thus extending similar observations previously made on rabbit muscle. The changes in pH and adenosine triphosphate (ATP) content accompanying the physical manifestations of *rigor* are described. As in rabbit muscle, the development of inextensibility accompanies the dephosphorylation of ATP, and, provided the ultimate pH is low, the fall in pH runs parallel to these changes. The rate of decrease in pH is almost identical with that in rabbit muscle at physiological temperature, but differs appreciably from it at lower temperatures. The results are discussed in relation to the time required for the completion of the onset of *rigor mortis* in commercial chillers.

The importance of the changes associated with the onset of *rigor mortis* to meat quality has been discussed by Bate-Smith,[1] and the influence of various factors—glycogen reserves, degree of exhaustion and temperature—on the time course of *rigor* in rabbit muscle has been studied by Bate-Smith & Bendall.[2] The interesting view that the onset of *rigor* may be regarded as a slow and irreversible physiological contraction has been advanced by Bendall,[3] whose results emphasize the time coincidence of the physical onset of *rigor* and the partial dephosphorylation of adenosine triphosphate (ATP) in rabbit muscle. A brief study of *rigor mortis* in whale muscle[4] extended this time correlation to another species, and showed that a further physical manifestation—an appreciable decrease in fluid retention by the muscle—may accompany *rigor*.

Studies of the meat of commercially important species have been confined for the most part to the period following the completion of *rigor* changes, with the result that, although much information is available on ultimate pH,[5] ' drip ',[6] and certain storage changes,[1] very little is known of events accompanying the onset of *rigor*. Sufficient differences were detected in *rigor* studies on rabbit and whale muscle to emphasize the danger of applying results obtained on one species to another without further investigation, and information obtained in slaughterhouses does not greatly assist owing to a frequent confusion between *rigor mortis* and the ' setting ' of the fat.

The present study was considered necessary as a preliminary to projected later investigations on certain problems of the meat industry. In addition to examining certain physical and chemical changes occurring in beef during the onset of *rigor*, and the degree of variation to be expected from carcass to carcass, the project was designed to investigate the suggested relationship between shortening in *rigor* and physiological contraction.

Experimental

Materials

The animals from which samples were taken were killed according to normal slaughterhouse procedure (stunning and bleeding), and represented a wide range of breed, grading and age. Jersey, Aberdeen Angus, Hereford and Shorthorn breeds were included in the study; grading ranged from prime ox to ' boner ' (manufacturing) cow, and ages extended from two to greater than eight years.

Samples of about 1 kg. were removed from the *longissimus dorsi* muscle (the ' eye of beef ') in the vicinity of the 11th rib, and were transported to the laboratory in a plastic bag inside a steel container. At the commencement of study, about 60–80 minutes *post mortem*, the temperature had not fallen below 36–37°. Samples for analysis and for the physical detection of *rigor* were taken from the middle of the large sample to avoid complications due to oxygenation of the superficial tissue.

Methods

The onset of *rigor mortis* was recorded by an apparatus similar in principle to that described by Bate-Smith & Bendall.[2] The strip of muscle, about 5 cm. in length and 0·3–0·7 sq. cm. in cross-section, remained under a load of 37 g., except for one 15-second period during each 15 or 30 minutes when the load was manually removed. The writing arm, providing a sixfold magnification, recorded changes in length and extensibility in ink on squared paper, to give a graphic matrix facilitating accurate measurement. Shortening (s) is expressed as a percentage of the muscle length, or in some cases as a percentage of the total shortening, work performance (w) as g. cm./g. {(load × distance load is raised)/(weight of sample)}, and extensibility ($1/L$) as: (the change in length in the 15 seconds following load removal)/(loaded length of

muscle). The modulus of elasticity is also calculated on the length change in the 15-seconds period.

A similar sample of muscle from an adjacent site was suspended alongside the stretched strip for analysis. A stream of water-saturated nitrogen was passed continuously into the glass container which covered the two samples. Experiments were conducted at 7°, 17°, 27° and 37°, and a few observations were made at 33·5° and 43°.

In each experiment a total of 5–8 samples was taken at intervals for the determination of the phosphorus fractions, and the same number for pH estimations. The phosphorus fractions were determined by the same methods as employed by Bendall.[3] The pH (glass electrode) was estimated on muscle homogenates prepared in 0·005M-sodium iodoacetate in the apparatus of Marsh & Snow.[7] Trichloroacetic acid (TCA) extracts were also prepared in this homogenizer.

The mean nitrogen content (± standard deviation) of 26 muscles examined, kindly determined by Miss D. L. Johannesson, was 3·57 ± 0·15%, and the same samples had a mean water content (24 hours at 105°) of 72·3 ± 1·6%. Estimations (154) of total acid-soluble phosphorus (T.S.P.) gave a mean value of 1·72 ± 0·10 mg./g. of muscle; this compares with 2·04 ± 0·15 mg./g. in rabbit *psoas* muscle.[3] The acid-labile phosphorus of ATP (ATP-P) is expressed throughout this paper as a percentage of the total acid-soluble phosphorus.

Results

The extent of physical changes

The most obvious physical change occurring during the onset of *rigor mortis* is an increase in the modulus of elasticity, which, in rabbit *psoas* muscle,[2] rises from about 500 to values about 10,000. It was found in the present study that an even more marked change occurs in beef, where the modulus rises from a mean initial value of 1110 ± 270 to a mean final value of 34,900 ± 17,700 (28 experiments). In individual experiments the ratio of final to initial modulus ranged from 8 to 79, with a mean value of 32.

A shortening in length accompanied the change in elasticity in every experiment. Difficulty was sometimes experienced in determining the absolute shortening since an interval of ½–6 hours elapsed before the loaded muscle attained a steady pre-*rigor* length, a lengthening of up to 4% occasionally preceding the onset of *rigor*. Because of the possibility that this might have obscured the commencement of shortening, the results of several experiments have been discarded. In the remaining 21 experiments an equilibrium loaded-length was attained well before the first detectable change in extensibility. Under a load of 50–120 g./sq. cm., shortening ranged from 1·6 to 13·1% of the muscle length, representing work performances of 1·1–11·5 g. cm./g. The amount of work done was considerably greater than that performed under comparable conditions of ultimate pH and temperature by rabbit *psoas* muscle. Thus, in four beef experiments at 17°, when the mean ultimate pH of the muscles was 5·49 ± 0·09, 3·6 ± 0·5 g. cm./g. of work was performed; rabbit muscle under comparable conditions would be expected[3] to do less than 1 g. cm./g.

The variation in ultimate pH of the muscles studied in the present investigation was too small to show the dependence of shortening and work performance on pH,[3] but the effect of temperature on work was demonstrated. Table I, which includes only those muscles of ultimate pH below 5·70, summarizes the results.

Table I

The effect of temperature on work performance during the onset of rigor mortis

Temperature, ° c	7	17	27	37
No. of observations	4	4	4	8
Work done (g.cm./g.) (mean ± standard deviation)	2·0 ± 0·7	3·6 ± 0·5	5·5 ± 2·9	7·5 ± 2·8

As reported elsewhere,[8] the final extensibility ($1/L$ in full *rigor*) was found to be related to shortening, greater shortening being accompanied by a smaller decrease of extensibility during the onset of *rigor*.

Whatever the changes may be in the resolution of *rigor mortis*, they do not involve the reversal of the onset of inextensibility. A strip of muscle maintained at 7° in nitrogen for 7 days following the onset of *rigor* had the same modulus of elasticity (49,000) at the end of this period as at the beginning; a regular increase in loaded length of about 0·5%/24 hours was observed. Another sample held at 37°, after shortening by 8·1% in 6 hours *post mortem*, regained its resting length during the next 17 hours and continued to stretch further, but its modulus of elasticity remained at a value of 18,400 during the entire 6–24-hour period.

ATP and rigor mortis

If we take into account the considerable evidence available for the dependence of the onset of *rigor* on ATP dephosphorylation,[3, 9, 10] it is then necessary to quote only sufficient beef results to show their general agreement with those of the rabbit investigations. Two experiments, selected because of their similar initial, and almost identical ultimate, pH values, are represented graphically in Fig. 1 to illustrate the time coincidence between onset of *rigor* and ATP decomposition, and the time course of *rigor* at two different temperatures.

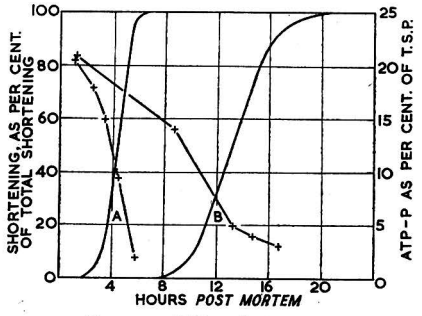

FIG. 1.—*ATP and shortening*

ATP-P shown by broken lines; shortening by continuous lines. A at 37°, B at 17°. Initial pH values 6·88 and 6·91; ultimate pH values 5·42 and 5·43; shortening 11·6% and 1·3% respectively

Throughout the investigation the same interdependence of chemical and physical events was observed. Table II, which includes analyses made in 25 experiments, summarizes this correlation. The onset of *rigor* is here expressed in terms of the decrease in extensibility ($1/L$) as a percentage of the total decrease, a measure that paralleled exactly the shortening expressed as a percentage of the total shortening. Owing to the usually low ultimate pH of the muscle, it proved impossible to investigate the dependence of the 'critical level' of ATP on ultimate pH,[3] but, for the necessarily small pH range, a close relationship between *rigor* and ATP is obvious from the Table.

In 11 experiments the estimation of creatine phosphate phosphorus (CP-P) was undertaken. In all cases CP-P had fallen to a level at or below 5% of the T.S.P. before the first physically detectable change occurred, and while the content of ATP-P was still at about the 20% level. This result is in agreement with Bendall's[3] view that, so far as *rigor* is concerned, CP acts merely as a means of re-synthesis of ATP and plays no other part in the length or extensibility changes.

Table II

The relationship between extensibility and ATP content of muscle

Extensibility decrease, %	0	1–10	11–30	31–50	51–70	71–90	91–100
No. of observations	38	53	11	12	9	15	20
ATP-P as per cent. of T.S.P. (mean ± standard deviation)	20 ± 2	19 ± 3	16 ± 3	11 ± 4	9 ± 3	7 ± 3	4 ± 2

Because of the ease of estimation of pH compared with the determination of ATP, particularly in a meat-works laboratory, it seems desirable to be able to follow *rigor* changes approximately by pH measurement. It has been clearly demonstrated[2, 10] that the onset of *rigor* in rabbit muscle is dependent, not on pH, but on the ATP content; nevertheless, at any given ultimate pH the ATP content at commencement of *rigor* is approximately constant,[3] and, in normal *rigor*, acid production and ATP decomposition run parallel.[10] Thus a knowledge of the pH at any time and of the ultimate pH allows an estimate of the progress of the onset of *rigor* in rabbit muscle. The relationship between ATP and pH in beef of ultimate pH below 5·70 is shown in Table III. A close correlation is seen to exist, which indicates that, provided the ultimate pH does not exceed 5·7, pH determination alone will supply a reasonable estimate of the decomposition of ATP, and hence (Table II) of the extent of the onset of *rigor* in beef.

Table III

The relationship between pH and ATP content in muscles of ultimate pH below 5·70

pH range	No. of observations	ATP-P as per cent. of T.S.P. (mean ± standard deviation)
Above 6·60	24	20·5 ± 1·5
6·59–6·40	16	19 ± 2·5
6·39–6·20	15	16 ± 2·5
6·19–6·00	18	13 ± 2·5
5·99–5·80	18	8 ± 3
5·79–5·60	13	5 ± 2·5
5·59–5·40	12	3 ± 1·5

Post-mortem changes of pH

Since the physical changes that occur during the onset of *rigor mortis* in beef, their dependence on the ATP content of the muscle, and the relationship of this ATP content to pH, have been determined there remain to be described the post-mortem changes of pH in regard both to the onset of *rigor* and to meat quality.

To determine the rate of decrease of pH and the influence of temperature on it, 30 experiments, each consisting of 6–10 pH estimations, were performed. The results are shown graphically in Fig. 2, which illustrates both the form of the pH/time curve and the effects of temperature change on rate.

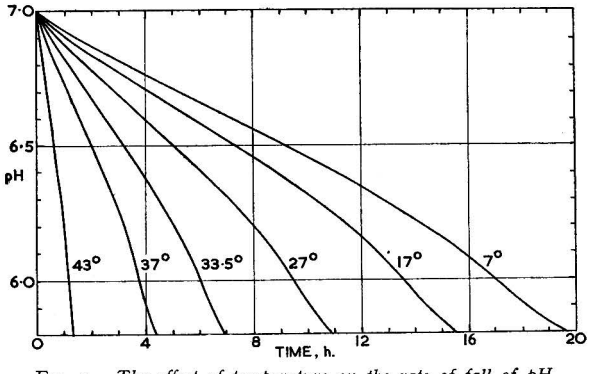

FIG. 2.—*The effect of temperature on the rate of fall of pH*

The rate of pH decrease in beef at 37° proved to be almost exactly equal to that in rabbit *psoas* muscle at the same temperature.[3] The minimum rate occurred at about pH 6·5 and the maximum at pH 6·1, values rather lower than reported for rabbit muscle (6·7 and 6·35 respectively), but the rate curves are otherwise very similar at physiological temperature. At lower temperatures, however, the rates of pH decrease in beef were found to differ appreciably from those in rabbit muscle; at 17°, for instance, the times for a pH fall from 6·8 to 6·2 were about 530 and 320 minutes in beef and rabbit respectively. Although 10° temperature coefficients (Q_{10}) calculated from these curves confirm the finding[2] that the Q_{10} is greater the higher the 10° temperature range, they also indicate that the actual Q_{10} values for particular ranges differ considerably from those in rabbit muscle. The rapid rise in Q_{10} with increasing temperature made necessary its determination over narrower temperature ranges near 37°, and experiments were therefore undertaken at 33·5 and 43°. Temperature coefficients (Q_{10}) are shown in Table IV. For direct comparison with rabbit results,[2] the mean Q_{10} for the range 17–37° in beef is about 1·9 (1·6 in rabbit), and for the range 7–17° it is about 1·25 (1·22 in rabbit, calculated on a 10° basis over the range 3·5–17°). It is thus in the range 17–37° that the difference between beef and rabbit muscle is apparent.

Table IV

The temperature coefficient (Q_{10}) of pH decrease in muscle during the onset of rigor mortis

Temperature range, °C	Q_{10}
7–17	1·25
17–27	1·43
27–33·5	2·0
33·5–37	3·7
37–43	6·8

In addition to the rate of pH decrease, the extent of the change was studied. By interpolation or linear extrapolation on the 37° pH/time curve, the approximate pH at death was determined. Considerable variation was observed, the values ranging from about 6·65 to about or above 7·4; of the 32 samples examined, 16 had estimated pH values at death of 7·20 or higher, 6 of 7·00–7·19, and 10 below 7·00.

Ultimate pH showed less variation, but occasional very high values were encountered. Although four of every five samples contained sufficient glycogen to attain pH values below 5·7, one of every six failed to reach a pH below 6·2. The extreme values observed were 5·31 and 6·84. No significant difference in ultimate pH was detected between the two main grades;

11 samples of prime ox had a mean value of 5·58 ± 0·25 and 14 of 'boner' cow averaged 5·61 ± 0·27. Increasing age appeared to elevate the ultimate pH; thus, if values above 5·7 and animals of doubtful age are excluded, samples of eight beasts of 3–5 years of age inclusive had a mean ultimate pH of 5·40 ± 0·06, and eight of age 6–8 years inclusive had a mean value of 5·55 ± 0·11.

No obvious relationship was found between initial and ultimate pH values, even those muscles of low initial pH (6·6–6·8) apparently containing sufficient glycogen to attain low ultimate pH values. When those samples of ultimate pH above 5·70 are excluded, the muscles of initial pH above 7·20 had a mean ultimate pH of 5·50, and those of initial pH below 7·20 attained a mean ultimate value of 5·49. If it is assumed that, as in rabbit, the extent of the death struggle determines the initial pH,[2] it appears that this exercise is insufficient to alter significantly the ultimate pH of the muscle.

Discussion

For the most part this study has shown that the onset of *rigor mortis* in beef closely resembles that in rabbit muscle. The coincidence in time between shortening (coupled with decreasing extensibility) and ATP decomposition provides additional evidence that the onset of *rigor* is intimately related to the dephosphorylation of this energy-rich molecule, and the correlation earlier reported[8] lends further support to the view that shortening in *rigor* may be regarded as a slow and irreversible physiological contraction. No aspect of the present investigation, therefore, is in any way incompatible with the findings of the Cambridge workers.

Nevertheless, appreciable differences exist between *rigor* in beef and rabbit muscle, the most important detected in this investigation being the effect of temperature on the rate of acid production. At physiological temperature, pH/time curves for rabbit and beef are almost identical—an indication of very similar rates of ATP turnover in surviving muscle of the two species at 37°; and in both species the temperature coefficient of acid production increases with rising temperature. The coefficient is, however, considerably greater in beef than in rabbit, the effect of a 10° decrease from physiological temperature being as great in beef as a 20° decrease in rabbit muscle. At 37° the temperature coefficient of acid production in beef has the very high value of about 4·5.

Since it has been established, for beef, that the onset of *rigor mortis* coincides with the rapid phase of ATP decomposition (Table II), and that the ATP decomposition is directly related to pH when the ultimate pH is low (Table III), the onset of *rigor* may now be followed approximately by pH determinations alone; and, from the time-course of acid production (Fig. 2), the progress of *rigor* in a muscle of low ultimate pH at a given temperature may be predicted from one determination of pH. With a knowledge of the rate of cooling of the *longissimus dorsi* muscle in the side of beef in a reasonably efficient slaughter-house chiller (unpublished data), it has been possible, with the aid of Table IV, to estimate the progress of *rigor* under practical conditions. If we assume a pH two hours *post mortem* of 7·10 and an ultimate pH of 5·30, all glycolytic changes should be complete in about 30 and 26 hours in surface (3 mm.) and deep (75 mm.) muscle respectively. This time for the completion of the onset of *rigor* will, of course, vary widely, according to chiller efficiency, initial pH, and ultimate pH; but no higher initial nor lower ultimate pH than those quoted above is likely to be encountered, and the rate of cooling could not greatly exceed that observed. Allowing for slightly more efficient cooling, it would appear that a period of 36 hours *post mortem* would allow all glycolytic changes to reach completion. With the possibility of an early introduction of more rapid freezing to hasten beef through-put in meat works, problems associated with 'thaw-*rigor*',[11] due to freezing before the completion of the onset of *rigor*, may become apparent, as indeed they have done already in the freezing of whale-meat.[12] The present work suggests that a 36-hour interval between killing and the commencement of freezing should prevent such complications in beef.

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Dominion Laboratory
Department of Scientific and Industrial Research
Wellington
New Zealand

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References

[1] Bate-Smith, E. C., *Advanc. Food Res.*, 1948, **1**, 1
[2] Bate-Smith, E. C., & Bendall, J. R., *J. Physiol.*, 1949, **110**, 47
[3] Bendall, J. R., *J. Physiol.*, 1951, **114**, 71
[4] Marsh, B. B., *Biochim. biophys. Acta*, 1952, **9**, 127
[5] Bate-Smith, E. C., *J. Soc. chem. Ind., Lond.*, 1948, **67**, 83
[6] Empey, W. A., *J. Soc. chem. Ind., Lond.*, 1933, **52**, 230T
[7] Marsh, B. B., & Snow, A., *J. Sci. Fd Agric.*, 1950, **1**, 190
[8] Marsh, B. B., *Biochim. biophys. Acta*, 1953, **12**, 478
[9] Erdos, T., *Stud. Inst. med. Chem. Univ. Szeged*, 1943, **3**, 51
[10] Bate-Smith, E. C., & Bendall, J. R., *J. Physiol.*, 1947, **106**, 177
[11] Perry, S. V., *J. gen. Physiol.*, 1950, **33**, 563
[12] Sharp, J. G., & Marsh, B. B., *Spec. Rep.*, No. 58, *Fd Invest. Bd Lond.*, 1953

THE SPECTROSCOPIC EXAMINATION OF POMEGRANATE-SEED OIL

By N. H. E. AHLERS and N. G. McTAGGART

A detailed quantitative study of the ultra-violet and the infra-red spectra of pomegranate-seed oil has been made. The ultra-violet data confirm the presence of a conjugated triene system for which the infra-red measurements suggest a *cis–cis–trans*-orientation as the most likely configuration.

Introduction

Pomegranate-seed oil (the seed oil from *Punica granatum*) has been examined by Toyama & Tsuchiya[1] and the principal component acid, punicic acid, isolated. This was later confirmed by a re-investigation of the oil by Toyama & Uozaki[2] and also by other workers.[3] From the results of ozonolysis, and the identification of the decomposition products, punicic acid has been accorded an octadeca-9 : 11 : 13-trienoic structure. However, no identity has been attached to the geometric configuration of the unsaturation other than to indicate that it is presumably a stereoisomer of elaeostearic acid, which itself is known to exist in α- and β-forms. It is therefore of interest to attempt to establish the true configuration of the double bonds present.

A number of techniques are available for the elucidation of *cis*- and *trans*-isomers. Thus the work of Alder & Vogt[4] on simple conjugated dienes, and of von Mikusch[5] on conjugated linoleates, points to the use of maleic anhydride as an aid in determining the structures of conjugated polyenes. The ability to form the semi-ring structures as indicated by atomic-model spacings is related to the ease of reaction with maleic anhydride. *X*-ray-diffraction patterns have also been used to distinguish between conjugated *cis–trans*-isomers[3, 6] but known structures are required for the assignment of structure.

More recently, absorption spectroscopy, particularly infra-red, has been successfully applied to the problem. It has been observed that the precise wavelengths of absorption bands in the 10-μ region of the infra-red spectra of conjugated-polyene fatty acids is highly characteristic of the *cis–trans*-configuration of the unsaturation. In this manner the α- and β-isomers of elaeostearic acid have been identified by the authors in a previous publication,[7] and the results confirmed independently by Wheeler,[8] who also used an infra-red spectroscopic method.

Ultra-violet absorption spectroscopy is less specific in determining the orientation of unsaturation but differences in the wavelength of the absorption maxima of *cis–trans*-isomers have been reported.[9]

The present paper is therefore concerned with a detailed quantitative study of the ultra-violet and infra-red spectra of pomegranate-seed oil, to elucidate the orientation of the unsaturation. Unfortunately a sample of punicic acid was not available but a small quantity of the oil was kindly supplied by R. T. Holman, of the Hormel Institute, for examination.

Only one reference[10] has been made in the literature to the absorption spectrum of pomegranate-seed oil. The infra-red spectrum has been recorded over the wavelength range 8·5–13·0 μ, but no attempt was made to correlate the results with the *cis–trans*-structure of the oil.

Experimental

The ultra-violet experimental results recorded here have been obtained with a Beckman ultra-violet spectrophotometer.[11] The ultra-violet spectrum of the oil has been recorded over the wavelength range 220–300 mμ with a solution of known concentration in purified *cyclo*hexane[12] in a 1-cm. quartz cell.

The infra-red experimental results have been obtained with a Perkin Elmer infra-red spectrometer.[13] In recording qualitatively the complete infra-red spectrum, the oil has been examined over the wavelength range 2·5–15·0 μ as a very thin film (25 μ) between rock-salt plates. In the more detailed quantitative observations on the intensity of the absorption bands in the 10-μ wavelength region, the oil has been examined in carbon disulphide solution of known concentration in a 1-cm. rock-salt cell. There is, of course, some slight variation in both wavelength and intensity of absorption with solvent and the measurements reported in this paper should be assumed valid only for carbon disulphide.

The extinction coefficients were measured with a fixed slit-width of 0·20 mm. because variation of absorption with slit width is quite marked.[14] In the present stage of development of infra-red spectrophotometry, the extinction coefficients determined by one worker cannot be directly employed by other workers. Variations in such factors as scattered radiation, wavelength calibration, slit-width settings and path lengths in instruments of different design make it necessary for each observer to determine the extinction coefficients on the instrument used under the exact conditions employed in the analysis.

The precise wavelengths of the absorption bands near 10 μ have been measured after calibration of the spectrometer wavelength scale with the spectrum of ammonia vapour as reference standard. The results are considered accurate to ± 0·01 μ.

Results and discussion

Ultra-violet examination

The ultra-violet spectrum, shown in Fig. 1, has three absorption maxima with the principal band at 274·5 mμ and subsidiary bands at 265 mμ and 285·5 mμ. In these respects the spectrum closely resembles that of tung oil,[15] which shows three corresponding absorption bands, displaced to shorter wavelengths, and which are known to arise from the conjugated α-elaeostearic acid (*cis–trans–trans*-octadeca-9 : 11 : 13-trienoic acid) character of the oil. Oiticica oil,[16] containing α-licanic acid (4-oxo-*cis–trans–trans*-octadeca-9 : 11 : 13-trienoic acid), also shows a comparable spectrum in this region. It would therefore appear from the spectrum that the oil contains a long-chain conjugated-triene system and the displacement in the wavelength of the absorption maxima relative to the spectra of analogous conjugated-triene systems may be related to the *cis–trans*-configuration of the unsaturation.

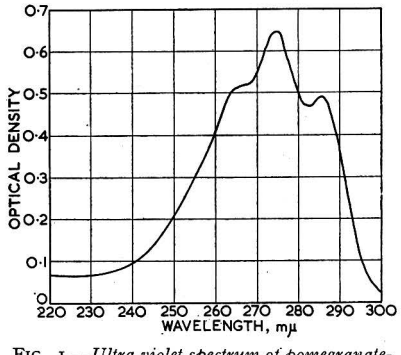

FIG. 1.—*Ultra-violet spectrum of pomegranate-seed oil (concentration, 0·0053 g./l.; path length, 1 cm.)*

Because of the lack of suitable reference compounds it is not possible to determine the content of the component acid, punicic acid, directly, although an approximate figure is gained by comparison of the intensity of the absorption maxima with the corresponding maxima of other conjugated-triene acids.

In Table I are set down the ultra-violet absorption results for the samples examined, α-elaeostearic acid[15] and β-elaeostearic acid[15] (*trans–trans–trans*-octadeca-9 : 11 : 13-trienoic acid). The specific extinction coefficient, k, is derived from the relationship $D = kcl$, where D is the optical density of the sample in *cyclo*hexane solution, c is the concentration in g./l. and l is the path length in cm.

The tabulated results show that the absorption maxima for pomegranate-seed oil are shifted to longer wavelengths ($\Delta\lambda$) with respect to the *trans–trans–trans*-β-elaeostearic acid. This shift is accompanied by a decrease (Δk) in the specific extinction coefficient of the principal absorption band. (The strict comparison of results here is complicated by the fact that the unknown structure is in triglyceride form. However, only small variations in $\lambda_{max.}$ are known to be associated with the spectra of oils and the component acids and esters measured in the

Table I

Ultra-violet absorption results in cyclo*hexane solution*

Acid	Configuration	Wavelength of absorption maxima (λ) and corresponding specific extinction values (k)			$\Delta\lambda$ relative to β-elaeostearic acid at $\lambda_{max.}$	Δk relative to β-elaeostearic acid at $\lambda_{max.}$
β-Elaeostearic	*trans–trans–trans*	260 mμ	269 mμ k = 202·2	281 mμ	—	—
α-Elaeostearic	*cis–trans–trans*	261 mμ	271·5 mμ k = 168·6	282 mμ	2·5 mμ	33·6
Pomegranate-seed oil	?	265 mμ	274·5 mμ * k = 122·1	285·5 mμ	5·5 mμ	80·1

* The recorded extinction coefficient (k = 122·1 ; repeat analysis k = 122·0) suggests a conjugated-triene content of 72·4% for the oil with the reference extinction coefficient (k = 168·6) for α-elaeostearic acid. Since this analysis should be made relative to the corresponding measurement on a pure sample of punicic acid (k probably less than 168·6) which was not available, it is likely that the true conjugated-triene content of the oil is somewhat higher

same solution.) These phenomena parallel the results observed with α-elaeostearic acid, which are known to arise from the presence of a *cis*-double bond in the acid. Similar observations have been reported[6] with the conjugated diene fatty acids—thus the *trans–trans*-conjugated diene acid of von Mikusch[17] has its absorption peak at 231 mμ whereas the *cis–trans*-conjugated acids[18] absorb near 233–234 mμ.

The reference figures suggest that increase in *cis*-conjugation results in a shift of the absorption maxima to longer wavelengths. Similar observations have been reported in analogous infra-red spectroscopic studies.[7] The ultra-violet absorption spectrum of pomegranate-seed oil would therefore suggest a structure having an increase in *cis*-conjugation over that present in α-elaeostearic acid, which is known to contain one *cis*-double bond in a *cis–trans–trans*-configuration. Further evidence on the structure of the component acid of pomegranate-seed oil may be gained by a consideration of the reactivity of conjugated systems with maleic anhydride. von Mikusch[5] has recently shown that the *cis–trans*-conjugated linoleates react very slowly, if at all, with maleic anyhydride, at temperatures of about 100° or lower whereas the *trans–trans*-isomers react readily in the diene-number determinations under these conditions.

These observations may be applied to the conjugated-triene systems ; since punicic acid from pomegranate-seed oil is reported not to form a maleic anhydride adduct[3] it presumably does not have two adjacent *trans*-double bonds.

There are eight possible *cis–trans*-isomers of the conjugated-triene fatty acids that have their double bonds in the 9 : 11 : 13-positions. The structures of two are known, α-elaeostearic acid (*cis–trans–trans*) and β-elaeostearic acid (*trans–trans–trans*).

Finally, it is not unreasonable to assume that spectroscopic analysis is not capable of distinguishing between symmetrical isomers, i.e. between, say, *cis–cis–trans* and *trans–cis–cis*.

With this and other considerations noted above there are four *cis–trans*-structures as possible isomers, namely *cis–cis–cis*, *cis–cis–trans*, *cis–trans–cis* and *trans–cis–trans*. From the ultra-violet absorption results the possibility of the final structure's containing a single *cis*-double bond seems unlikely. In fact, if a shift, $\Delta\lambda$, of about 2·5 mμ in the wavelength of the ultra-violet absorption maximum, per *cis*-double bond introduced into the molecule (as noted between α- and β-elaeostearic acid and the corresponding conjugated-diene acids) is assumed, the shift observed of 5·5 mμ would suggest a structure for the acid having at least two *cis*-double bonds. It is, however, difficult to explain such a structure from consideration of the melting point of the component acid (44°) since the isomeric α-elaeostearic acid, with a single *cis*-double bond, has a somewhat similar melting point (48°) and it is generally accepted that the melting point of the unsaturated fatty acids decreases appreciably with an increase in *cis*-double-bond content.

Infra-red examination

The infra-red spectrum in Fig. 2 shows the usual absorption bands characteristic of long-chain unsaturated triglycerides.[13] Thus the strong doublet near 3·5 μ arises from the C–H valence vibrations of the methyl and methylene groups of the molecule, and the single strong absorption band near 5·7 μ is characteristic of the valence vibrations of the ester groupings.

The deformation vibrations of both the methyl and methylene groupings are evidenced by the maxima near 7·0 μ. The series of absorption bands near 8·5 μ are noted in the spectra of all the drying oils and are presumably characteristic of the glyceryl structure. The absorption band near 14·0 μ arises from the deformation vibration of the aliphatic chains.

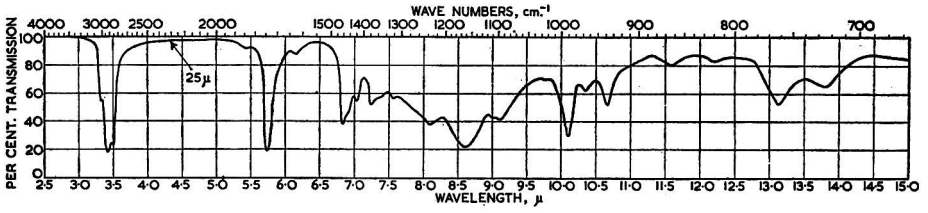

FIG. 2.—*Infra-red spectrum of pomegranate-seed oil*

There is no evidence of hydroxyl or ketone absorption in the spectrum. There are, however, unique features in the spectrum of the oil in addition to the typical absorption bands. Thus, in the 10-μ region, which is characteristic of the deformation vibrations of the C–H bonds of unsaturated groupings, three absorption bands at 10·67 μ (937 cm.$^{-1}$), 10·36 μ (965 cm.$^{-1}$) and 10·11 μ (989 cm.$^{-1}$) are noted.

Previous studies[7] in the 10-μ region of the spectra of unsaturated fatty acids have shown that the measurements of the wavelength and the intensity of the absorption bands may be used in assigning the *cis–trans*-configuration of the unsaturation present in the molecule. Detailed quantitative measurements have therefore been made on the sample in the 10-μ region. The results obtained are summarized in Table II, which includes corresponding measurements made on pure samples of α- and β-elaeostearic acids.

Table II

Infra-red absorption results in carbon disulphide solution

Acid	Configuration	Wavelength of absorption maxima (λ) and corresponding specific extinction values (k)			$\Delta\lambda$ relative to β-elaeostearic acid at $\lambda_{max.}$	Δk relative to β-elaeostearic acid at $\lambda_{max.}$
β-Elaeostearic	*trans–trans–trans*			10·06 μ k = 1·955	—	—
α-Elaeostearic	*cis–trans–trans*		10·37 μ k = 0·416	10·09 μ k = 1·425	0·03 μ	0·53
Pomegranate-seed oil	?	10·67 μ k = 0·366	10·36 μ k = 0·159	10·11 μ k = 0·611	0·05 μ	1·34

Before considering the infra-red results obtained with pomegranate-seed oil it is of value to discuss the infra-red characteristics of the isomeric elaeostearic acids that have formed the subject for previous study.[7]

Thus β-elaeostearic acid has a strong absorption band at 10·06 μ with an extinction coefficient of 1·955, which is approximately three times that given by elaidic acid at 10·33 μ and suggests a *trans–trans–trans*-configuration. The wavelength shift of the absorption band from 10·12 μ noted for the *trans–trans*-9 : 11-diene acids to 10·06 μ for the *trans–trans–trans*-conjugated triene parallels the corresponding shift in passing from the *trans*-mono-ene to *trans*-conjugated-diene fatty acids. From these data the general observation of a shift in the wavelength of the *trans*-double-bond absorption to shorter wavelengths with increase in *trans*-conjugation can be made. The truth of the converse of this statement is confirmed by the results obtained with the *cis–trans–trans*-α-elaeostearic acid with the principal absorption band at 10·09 μ, a longer wavelength arising from the increase in *cis*-conjugation.

Similarly the variation in the specific extinction coefficient may be used in evaluating the *cis–trans*-structure. Since the difference in absorption measured at 10·06 μ and 10·09 μ for the β- and α-acids respectively corresponds to approximately one *trans*-double bond these data tend to confirm the single *cis*-double bond of α-elaeostearic acid.

The principal absorption band in the 10-μ region of the infra-red spectrum of pomegranate-seed oil occurs at 10·11 μ ($k = 0{\cdot}611$). This shift in the wavelength of the principal maximum to longer wavelength as compared with the corresponding results obtained with the isomeric elaeostearic acids suggests an increase in *cis*-conjugation in the structure of the oil. The magnitude of the shift, relative to α-elaeostearic acid ($\Delta\lambda = 0{\cdot}02$ μ), is of the order of the corresponding shift ($\Delta\lambda = 0{\cdot}03$ μ) noted between the spectra of α- and β-elaeostearic acids, which is known to arise from a difference in structure of a single *cis*-double bond. In addition, the intensity of the principal absorption band ($k = 0{\cdot}611$) of the oil is of the order of the value noted for a single *trans*-double bond (methyl elaidate, $k = 0{\cdot}6$) or approximately one-third of the value noted for β-methyl elaeostearate ($k = 1{\cdot}955$).

These considerations suggest that the unknown conjugated-triene system probably contains two *cis*-double bonds. This is in agreement with the corresponding ultra-violet results.

The probable structures for the component acid are now reduced to two: *cis–cis–trans* or *cis–trans–cis*. Some observations on the more likely of these structures may be gained by a consideration of the other absorption bands in the 10-μ spectrum of the oil. A second band is noted at 10·36 μ ($k = 0{\cdot}159$) and although a band is noted at 10·37 μ in the spectrum of α-elaeostearic acid it is probable that with the oil it arises from the presence of non-conjugated *trans*-unsaturation. A third and stronger band, noted at 10·67 μ ($k = 0{\cdot}366$), forms with the 10·11-μ band a characteristic doublet which may be likened to the spectra of the conjugated *cis–trans*-linoleates that have maxima at 10·17 μ and 10·53 μ. The relative intensities of this doublet are of a similar order to those noted with the *cis–trans*-conjugated linoleates. This information suggests that only a *single cis–trans*-diene pair is present in the oil. Thus the *cis–cis–trans*-structure appears as the more likely since it is not impossible that the *cis–trans–cis*-system might function, spectroscopically, as a pair of *cis–trans*-conjugated dienes, at least in terms of intensity considerations.

Finally, the presence of an additional strong absorption band on the spectrum of the oil at 13·13 μ, not normally encountered in the spectra of vegetable oils, should be discussed. It is known that the non-conjugated *cis*-double bond in oils shows strong absorption in the 13·8-μ region. In addition the spectrum of methyl α-elaeostearate also shows a band in this region. A possible explanation of the 13·14-μ band is a correlation with a conjugated *cis–cis*-part of a *cis–cis–trans*-triene structure. The shift in wavelength from 13·8 μ to 13·14 μ may well be consistent with the shifts observed in the analogous *trans*-system.

A somewhat similar effect is observed with *cis*-double-bond absorption in the ultra-violet. Thus the mono-ene linkage absorbs near 190 mμ whereas the corresponding conjugated linoleate absorbs in the 232-mμ region.

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The Paint Research Station
Waldegrave Road
Teddington, Middlesex

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References

[1] Toyama, Y., & Tsuchiya, T., *J. Soc. chem. Ind. Japan*, 1935, **38,** 182
[2] Toyama, Y., & Uozaki, K., *J. Soc. chem. Ind. Japan*, 1937, **40,** 249
[3] Farmer, E. H., & van den Heuvel, F. A., *J. chem. Soc.*, 1936, p. 1809
[4] Alder, K., & Vogt, W., *Liebigs Ann.*, 1951, **571,** 137
[5] von Mikusch, J. D., *Angew. Chem.*, 1950, **62,** 475
[6] Nichols, P. L., Herb, S. F., & Riemenschneider, R. W., *J. Amer. chem. Soc.*, 1951, **73,** 247
[7] Ahlers, N. H. E., Brett, R. A., & McTaggart, N. G., *J. appl. Chem.*, 1953, **3,** 433
[8] Wheeler, D. H., *Off. Dig. Fed. Paint Varn. Prod. Cl.*, 1951, No. 322, p. 661
[9] Matiello, J. J., 'Protective & Decorative Coating', 1944, Vol. 4, p. 362 (London: Chapman and Hall)
[10] Paschke, R. F., Tolberg, W., & Wheeler, D. H., *J. Amer. Oil Chem. Soc.*, 1953, **30,** 97
[11] Cary, H. H., & Beckman, A. O., *J. opt. Soc. Amer.*, 1941, **31,** 682
[12] Ahlers, N. H. E., & Williamson, L., *Res. Mem. Res. Ass. Brit. Paint Mfrs*, 1950, No. 171
[13] Ahlers, N. H. E., *J. Oil Col. Chem. Ass.*, 1950, **33,** 421
[14] Hampton, R. H., *Analyt. Chem.*, 1949, **21,** 923
[15] O'Connor, R. T., Heinzelman, D. C., McKinney, R. S., & Pack, F. C., *J. Amer. Oil Chem. Soc.*, 1947, **24,** 312
[16] Bradley, T. F., & Richardson, D., *Industr. Engng Chem. (Anal.)*, 1940, **32,** 963
[17] von Mikusch, J. D., *Lack- u. Farbenchem.*, 1949, **3,** 167
[18] Holman, R. T., & Elmer, O. C., *J. Amer. Oil Chem. Soc.*, 1947, **24,** 127

FLAVOUR OF POTATOES TREATED WITH TETRACHLORONITROBENZENE AND *iso*PROPYL *N*-PHENYLCARBAMATE

By A. R. WILSON * and J. M. HARRIES †

Tests carried out by two independent taste-panels showed that autumn treatment of potatoes with 2 : 3 : 5 : 6-tetrachloro-1-nitrobenzene (TCNB) to reduce sprouting during storage resulted in a slight but significant lowering of flavour quality. This was less marked after eight months' storage than after five. A consumer-acceptance test in which potatoes stored for eight months were eaten as part of a meal failed to reveal any off-flavour. Taste-panel tests with potatoes treated with *iso*propyl *N*-phenylcarbamate (IPC, propham) gave no indication of the presence of any off-flavour attributable to treatment.

Introduction

The value of autumn treatment of ware potatoes with tetrachloronitrobenzene (TCNB) dust to reduce sprouting during storage has been demonstrated by a number of workers in Britain and elsewhere. Emilsson, Lillieroth & Nilsson[1, 2] reported that such treatment had no significant effect on flavour quality but that it led to a significant decrease in the tendency of a number of varieties to darken after boiling.[2] Mooi,[3] on the other hand, stated, without publication of supporting data, that there were indications that TCNB treatment adversely affected the taste of the tubers.

During the 1949–50 storage season samples were taken from a number of treated and untreated clamps and sent for cooking tests to the Scientific Adviser's Division, Ministry of Food. Although no significant effect of treatment on flavour was demonstrated, there was an indication of a slight down-grading of the treated samples as compared with the controls. Differences between members of the taste-panel and chance variations were considerable and it was plain that greater refinement of both sampling and tasting methods was necessary if more definite results were to be obtained. Further tests with TCNB-treated potatoes were, therefore, undertaken in the 1950–51 storage season. In addition the scope of the work was widened to include tests with tubers treated in a similar manner with a dust containing *iso*propyl *N*-phenylcarbamate (IPC, propham); this compound had been shown by Rhodes, Sexton, Spencer & Templeman[4] to be highly effective in controlling the sprouting of potatoes in storage. The results obtained are presented in this paper.

Experimental method

Rate and method of application of dusts.—Treatments were carried out in autumn, mainly during the months of September and October. The TCNB dust (nominal 3% by weight of 2 : 3 : 5 : 6-tetrachloro-1-nitrobenzene in an inert carrier) was applied at the rate of 10 lb./ton to the tubers in the carts or trailers before tipping on the clamp site. The IPC dust (nominal 2·4% by weight of *iso*propyl *N*-phenylcarbamate in an inert carrier) was applied at the rate of 2½ lb./ton, 2 lb. before tipping and the remaining ½ lb. as a surface dressing to the clamp after heaping up.

Storage.—All clamps were constructed approximately according to standard Lincolnshire practice but on bases varying from 6 to 9 ft. The tonnage clamped was from 2½ upwards.

Sampling.—Eleven clamps of TCNB-treated tubers on nine farms and two clamps of IPC-treated tubers on one farm were sampled in March, 1951. Eight clamps of TCNB-treated tubers (including seven of those sampled in March) on six farms, and seven clamps of IPC-treated tubers (including the two sampled in March) on six farms were sampled in May, 1951. Corresponding control clamps of untreated tubers were sampled at the same time in every case. The varieties treated with TCNB in clamps sampled in March were: Majestic (five clamps), Arran Peak, Doon Star, King Edward, Record and Redskin (each one clamp); in May: Majestic (five clamps), Arran Peak, Doon Star and Record (each one clamp). The varieties treated with IPC in the clamps sampled in March were Doon Star and Record (each one clamp); in May the varieties were: Majestic (two clamps), Arran Peak, Craig's Defiance, Doon Star, King Edward and Record (each one clamp).

* Agricultural Research Council Potato Storage Investigation, c/o University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leicestershire

† Ministry of Food, Statistics & Intelligence Division (attached to Scientific Adviser's Division), Great Westminster House, Horseferry Road, London, S.W.1

On each sampling date the clamps were opened at random in one place and each outer layer of tubers removed to a depth of 6–8 in.; four samples of 3–5 lb. each were then taken at random from below. These were passed over a $1\frac{3}{4}$-in. hand riddle and about 3 lb. of the ware, in each case, packed into a small open-mesh hessian sack. Two such samples from each clamp were sent to the Ministry of Food Scientific Adviser's Division in London (M.O.F.), and two to the Agricultural Research Council Potato Storage Investigation team at Sutton Bonington (A.R.C.) for independent tests.

Cooking tests.—The A.R.C. taste-panel was composed of volunteers from the staff of the University of Nottingham School of Agriculture; that of the M.O.F. was composed of members of the staff of the Scientific Adviser's Division.

Tests carried out by the A.R.C. team were based on the procedure adopted by Jameson & Tanner[5] for assessing taint in potatoes grown on land treated with crude benzene hexachloride. From each sample, 1 kg. of potatoes was washed and peeled and placed in a saucepan in 1 l. of cold water to which had been added 10 g. of common salt. The tubers were boiled until cooked, then drained and mashed in the pan. Twice as many plates as there were samples were numbered serially and allocated at random to the samples, two to each. The samples were then placed on the appropriate plates and served as rapidly as possible. One or more additional plates marked 'normal', containing untreated potatoes of the same variety, cooked in the same way, were placed in convenient positions to serve as a flavour standard. Each taste-panel consisted of twelve persons, but was not necessarily of the same composition on each occasion. The members were asked to place each test plate in one of the following categories:

A Normal
B Very slight off-flavour but palatable
C Definite off-flavour, almost disagreeable
D Disagreeable flavour
E Inedible

Apple juice, diluted 50/50 with water, was provided as a taste-buffer.

The same cooking procedure was used in the tests carried out by the M.O.F., except that no salt was added to the cooking water. The method of presentation and scoring was, however, different. The panel consisted of the same eight persons throughout and each sample was tasted by four of these, whose identity was decided beforehand by a randomized-block design. There was deliberate confounding of differences between samples from the same clamp with differences between members of the panel, but in any one session the same number of samples of treated and untreated tubers was presented, there being strict correspondence between them in every other respect. This was done in order to reduce the number of samples tasted by each member of the panel.

Each person was asked to allocate a numerical score to the flavour of each of the samples, according to the following system: a good flavour was to be given a positive score and an off-flavour a negative one. The intensity of the flavours, either positive or negative, was to be indicated by the magnitude of the score from $+2$ to -5. A score of 0 was to indicate a neutral or normal flavour. The members of the panel were asked to use scores of $+\frac{1}{2}$ or $-\frac{1}{2}$ for flavours that they could taste but not specify. A score of $+1$ or -1 or more had to be accompanied by an adjective describing the flavour. No flavour reference of 'normal' potatoes was provided nor was there any plate replication of the samples. Lime juice was used as a taste buffer instead of apple juice.

In a previous series of experiments carried out by the M.O.F., involving the tasting of samples of potatoes which had been subjected to various chemical treatments, each sample was presented to the same panel three times on the same day, at 10.30 a.m., 11.30 a.m. and 3.30 p.m. It was found that greater discrimination was possible at 11.30 a.m. than at the other times.[6] In the present tests, therefore, all tasting sessions were started at 11.30 a.m. This no doubt resulted in more adverse marking than would have occurred if the tests had been carried out at some other time of day; however, as the object of the experiment was to compare as critically as possible the effect of various treatments, not to place the palatability of the potatoes on any absolute scale related to the opinion of possible consumers, this was not considered to be a disadvantage.

Discussion of results

The results will be discussed in two parts: (1) those relating to treatment with TCNB and (2) those relating to treatment with IPC. Normally, samples of tubers treated with these two compounds were compared with corresponding samples of untreated control tubers at separate sessions of the panels, but in six cases, where the control related to both treatments,

they were compared at the same sessions. Where this was done the results for the control samples are used twice. This facilitates the comparisons without invalidating them in any way.

Treatment with TCNB.—Tables I(*a*) and I(*b*) show the frequency with which the categories and marks were allotted to the samples of treated and untreated tubers by the A.R.C. panel and the M.O.F. panel, respectively. The results are in both cases given separately for the two sampling dates, as well as in total.

Table I

TCNB Tests

(*a*) *A.R.C. panel : total frequency of allocation of flavour categories*

Flavour category*	March		May		Total	
	Control (untreated)	TCNB	Control (untreated)	TCNB	Control (untreated)	TCNB
A	389	261	311	234	700	495
B	122	185	66	123	188	308
C	14	72	7	27	21	99
D	2	6	0	0	2	6
E	1	4	0	0	1	4
Total	528	528	384	384	912	912

(*b*) *M.O.F. panel : total frequency of allocation of marks for flavour*

Flavour mark*	March		May		Total	
	Control (untreated)	TCNB	Control (untreated)	TCNB	Control (untreated)	TCNB
+ 2	4	0	0	0	4	0
+ 1	2	3	2	0	4	3
+ ½	3	4	1	0	4	4
0	49	33	18	15	67	48
− ½	15	27	10	9	25	36
− 1	11	13	9	14	20	27
− 2	2	5	3	6	5	11
− 3	0	1	1	0	1	1
− 4	0	0	0	0	0	0
Total†	86	86	44	44	130	130

* See text

† These figures are lower than would be expected from the number of clamps sampled. No duplicate samples were received from five out of eight pairs of clamps in May and one taster was prevented from tasting one sample from each of two pairs of clamps in March

There are two methods of ascertaining the significance of differences between treated and untreated tubers. First, χ^2 tests may be applied directly to the frequencies of occurrence shown in the Tables, after amalgamation of the smaller classes. The values found for χ^2 were :

	March	May	Total†
Table I(*a*)	81·02**	39·83**	118·67**
Table I(*b*)	7·86*	2·54	8·88*

* Significant ($0{\cdot}01 < P < 0{\cdot}05$)

** Very highly significant ($P < 0{\cdot}001$)

† These values were calculated from the total frequencies, not by combination of the χ^2 values for March and May

All these values are significant except that for Table I(*b*) in May ; the degree of significance is higher in Table I(*a*) than in Table I(*b*), probably owing to the greater number of estimations carried out by the A.R.C. panel.

Inspection of the Tables shows that the direction of the difference between treated and untreated samples is the same in all cases, tubers treated with TCNB being scored more adversely than untreated tubers. It is also evident that the difference was less marked in May than in March, so much so that the difference between them in the May results from the M.O.F. panel, though still apparent, was not significant.

The second method of analysis that may be applied to such data is the analysis of variance, a more powerful form of analysis than the χ^2 test, but involving more assumptions about the nature of the observations. Such an analysis may be applied directly to the results of the M.O.F. panel, and to the results of the A.R.C. panel after transforming the categories into marks. The transformation of A into 1, B into 2, C into 4, D into 6, and E into 8 was suggested by

Jameson & Tanner,[5] and Table II gives the results of an analysis of variance of the total data provided by the A.R.C. panel, adopting this procedure. The figures show not only that the treatments were a highly significant source of variance, but also that the variance between observers was the least important. Greater differences are apparent between samples from the same clamp, treated in the same way, than between members of the panel in their scoring of the samples. In the tests carried out by the M.O.F. these two sources of variance were deliberately confounded in order to reduce the number of estimations, as explained above.

Though the difference between treated and untreated tubers is significant it is slight, the average of all marks given by the M.O.F. panel being — 0·50 and — 0·24 respectively. With the A.R.C. panel, the corresponding averages, after transforming categories into marks as noted above, were 1·73 and 1·29.

Table II

TCNB tests: Analysis of variance of data provided by the A.R.C. panel

Source	Sums of squares	Degrees of freedom	Mean squares
Between treatments	85·54	1	85·54
Between clamps within treatments	218·14	36	6·06
Clamps	166·79	18	9·27
Clamps × treatments interaction	51·35	18	2·85
Samples within clamps	94·00	38	2·48
Plates within samples	116·74	76	1·59
Observers within plates	1031·38	1672	0·62
Total	1545·8	1823	

The difference between the averages for treated and untreated tubers was 0·27 in March compared with 0·23 in May (M.O.F. panel), and 0·53 in March compared with 0·30 in May (A.R.C. panel). It is thought that a comparison of the results for tubers of the same treatment in March and May is not permissible, because the differences that exist may be due just as much to a change in the panels' standard of scoring after a gap of two months as to any change in the flavour of the potatoes. Comparison of the differences between treated and untreated potatoes is legitimate, however, as it is independent of such factors. Table III shows the frequency with which descriptive adjectives were used by members of the M.O.F. panel when allotting marks of — 1 or less at tasting sessions after both dates of sampling. It is evident that the off-flavour that existed in the tubers treated with TCNB was not sufficiently strong to be described with any certainty. Both the average marks and the adjectives used suggest that there was an unspecifiable off-flavour in some of the samples of untreated tubers. The adjective most frequently used to describe the off-flavour in the treated tubers was ' metallic '.

Table III

TCNB Tests

Frequency of descriptive adjectives used by the M.O.F. panel to characterize off-flavour

Adjective	Control (untreated)	TCNB
Bitter	5	5
Metallic	3	8
Earthy	2	4
Sour	3	4
Stale	0	2
Musty, mouldy	1	4
' Chemical '	3	4
Acid	3	0
Raw	2	2
' Cardboardy '	0	3
Spongy	2	3
Others	2	0
Total	26	39

That the off-flavours were slight and that the difference attributable to treatment with TCNB was only just noticeable is also borne out by the fact that there was very little low scoring of any of the samples. In only 1% and 0·3% of readings were samples of treated and untreated tubers, respectively, accounted disagreeable in flavour by the A.R.C. panel. The lowest categories of the scale (— 4 and — 5) were never used by members of the M.O.F. panel and a mark of — 3 only twice in all.

Consumer-acceptance test with TCNB-treated potatoes.—Since it had been found that treatment with TCNB introduced significant taint when measured by analytical or laboratory tasting methods, it was decided to attempt a consumer-acceptance test in which a fairly large number of people were to be given, without any previous knowledge or training, potatoes treated with TCNB and untreated potatoes as part of a meal. Such a test was carried out at the Queen Elizabeth College, University of London, in May, 1953, the procedure being as follows:

The dining-hall in which students consumed an evening meal was divided down the centre

and on each of three evenings it was arranged for untreated potatoes to be served to those students who sat on one side of the dividing line and potatoes treated with TCNB to those on the other; the allocation of the treated and untreated potatoes to the two halves was decided at random on each occasion. Records were kept of the number of diners on each side, of the net quantity served to each side, and of the net quantity unused, whether unserved from the dishes which were placed on each table or left on their plates by the diners. The figures obtained are given in Table IV. The variation in the quantities unused are largely due to the fact that the kitchen had no previous knowledge of the exact number of diners on any occasion, so that figures of consumption per head should reflect any real difference between treated and untreated potatoes. Though there are quite large variations in consumption from evening to evening, resulting presumably from the nature of the remainder of the meal and from the previous activity of the diners, it is clear that there was no outstanding difference between the consumption of treated and untreated potatoes.

Samples of the potatoes supplied for these tests were also presented to the A.R.C. taste-panel, as, owing to the sampling variations previously noted, there might have been no taint in the treated potatoes used. The averages obtained from this test, computed as before, were 2·23 and 1·31 for treated and untreated tubers respectively, a difference that is highly significant.

Table IV

TCNB Tests
Results of consumer-acceptance test

		Quantity served (clean-weight cooked), lb.	No. of diners	Quantity unused (dish and plate), lb.	Consumption per head, oz.
First day :	Treated	23·5	64	14·875	2·15
	Untreated	25·0	70	10·563	3·30
Second day :	Treated	23·0	49	8·063	4·84
	Untreated	23·0	80	4·875	3·63
Third day :	Treated	20·0	44	5·938	5·11
	Untreated	16·25	35	2·625	6·23

Table V

IPC Tests

(*a*) *A.R.C. panel : total frequency of allocation of flavour categories*

Flavour category*	March		May		Total	
	Control (untreated)	IPC	Control (untreated)	IPC	Control (untreated)	IPC
A	91	92	240	233	331	325
B	5	3	82	93	87	96
C	0	1	14	10	14	11
D	0	0	0	0	0	0
E	0	0	0	0	0	0
Total	96	96	336	336	432	432

(*b*) *M.O.F. panel : total frequency of allocation of marks for flavour*

Flavour mark*	March		May		Total	
	Control (untreated)	IPC	Control (untreated)	IPC	Control (untreated)	IPC
+ 2	0	0	0	0	0	0
+ 1	1	1	3	2	4	3
+ ½	2	1	1	2	3	3
0	7	9	12	18	19	27
− ½	2	1	6	10	8	11
− 1	2	1	9	2	11	3
− 2	1	2	5	1	6	3
− 3	0	0	0	0	0	0
− 4	0	0	0	1	0	1
Total†	15	15	36	36	51	51

* See text

† These figures are lower than would be expected from the number of clamps sampled. No duplicate samples were received from five out of seven pairs of clamps in May and one taster was prevented from tasting one sample from one pair of clamps in March

Treatment with IPC.—Tables V(*a*) and V(*b*) are similar to Tables I(*a*) and I(*b*), in that they show the frequency with which categories and marks respectively were allotted to the samples of treated and untreated tubers.

None of the values of χ^2 computed from the results in these Tables proved to be significant. Inspection of the following average values, obtained from the A.R.C. data after transformation of the flavour categories as previously described, will confirm that there was no evidence that the tubers treated with IPC differed in flavour from those which were untreated:

	March		May		Total	
	Control (untreated)	IPC	Control (untreated)	IPC	Control (untreated)	IPC
Table V(*a*)	1·05	1·06	1·37	1·37	1·30	1·30
Table V(*b*)	− 0·20	− 0·27	− 0·51	− 0·28	− 0·42	− 0·28

These averages are, in most cases, remarkably similar; where they are not—as, for instance, in the May data from the M.O.F. panel—they show that the control samples were marked more adversely than the treated samples, but not significantly so. As for the TCNB results, it is not permissible to compare those for tubers of the same treatment in March and May.

Since no difference was found between untreated and IPC-treated tubers, no consumer-acceptance tests were carried out. It cannot, of course, be concluded that no consumer would be able to detect a taint in IPC-treated potatoes; but in view of evidence that trained analytical panels, using tested systems of scoring, usually possess considerable powers of discrimination, it may be concluded that the proportion of the public which would detect such a taint, if present, is probably small.

Conclusions

The panel tests showed that no detectable taint followed the use of IPC, whereas treatment with TCNB led to a slight but significant reduction in flavour quality, although by no means all treated samples were down-graded below corresponding untreated control-samples. Both this latter point and the stringent nature of taste-panel tests of the type employed in the experiments should be remembered when the practical implications of the results obtained are considered. The result of the consumer-acceptance test suggests that the average level of off-flavour found in the panel tests with TCNB-treated tubers would not normally be detected by most persons when potatoes are eaten as part of a meal.

It is a point of interest that, in 2·6% (A.R.C.) and 20% (M.O.F.) respectively of the assessments made by individual members of the two taste-panels, samples of 'untreated' potatoes were described as having a definite off-flavour.

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Agricultural Research Council Potato Storage Investigation
c/o University of Nottingham School of Agriculture
Sutton Bonington, Loughborough

and

Scientific Adviser's Division
Ministry of Food, Horseferry Road
London, S.W.1

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References

[1] Emilsson, B., Lillieroth, C. G., & Nilsson, R., *K. LandtbrAkad. Handl., Stockh.*, 1949, **88**, 487
[2] Emilsson, B., Lillieroth, C. G., & Nilsson, R., *K. LandtbrAkad. Handl., Stockh.*, 1951, **90**, 421
[3] Mooi, J. C., *Landbouwk. Tijdschr., 's-Grav.*, 1949, **61**, 628
[4] Rhodes, A., Sexton, W. A., Spencer, L. G., & Templeman, W. G., *Research, Lond.*, 1950, **3**, 189
[5] Jameson, H. R., & Tanner, C. C., *J. Sci. Fd Agric.*, 1951, **2**, 171
[6] Harries, J. M., *J. Sci. Fd Agric.*, 1953, **4**, 477

A COMPARATIVE STUDY OF THE PIGMENTS IN MICROBIAL FRACTIONS FROM THE SHEEP'S RUMEN AND IN THE CORRESPONDING DIET

By J. DAVIDSON

In microbial fractions separated from the rumen contents of a hay-fed sheep pigmentation was found to be caused by pigments normally associated with plant chloroplasts. The extent of this pigmentation was greater than might be expected from the concentration in the hay feed. No chlorophyll typical of the known pigmented bacteria was found. An assessment of plant-particle contamination of microbial fractions, based on the pigment concentrations found, agreed in general with an assessment by a new direct-observation technique. The lowest contamination found was calculated to be of the order of 10%.

Degradation of chlorophylls in the rumen was demonstrated and was found to be more extensive when artificially dried grass was fed than when hay was fed.

The implications of these findings are discussed.

Introduction

It is well known that the progress of nutritional studies involving the digestibility of materials passing through the alimentary tract would be greatly hastened by the use of rapid indirect marker methods instead of the long and tedious trials at present in use for evaluating digestibility.

Recently a method[1] has been published that is based on the extraction by aqueous acetone and spectrophotometric measurement, at a wavelength of 406 mμ, of a 'chromogen' present in the diet itself. The advantages of such a method, involving a marker inherent in the diet and rapidly estimated, are great. However, this 'chromogen' has not been investigated. Because of the importance of this technique in nutritional studies and before its application on an extensive scale is considered it is desirable to investigate the fundamental principles by studying the individual pigments in an acetone extract of a suitable dried herbage and fractions from the corresponding rumen contents.

It is also well known that the cellulose of the diet becomes available to the ruminant through the activity of micro-organisms inhabiting the rumen. The appreciable quantity of protein synthesized by these micro-organisms becomes available to the host animal in the abomasum and intestines. The value of this microbial protein to the ruminant has been widely studied, and assessed chemically by analysis, and biologically by growth trials with rats. When these preparations of micro-organisms have been separated directly from rumen contents, and not after culturing processes, they may be contaminated by particles of diet, and this contamination could in turn give rise to false estimates of the nutritive value of the microbial protein. The wide divergence between biological values obtained by different workers for microbial protein from the rumen is well summarized by Reed *et al.*[2] Preparations of micro-organisms from the rumen are frequently associated with a green colour, and with animals fed on fresh herbage this green colour has been noted even in preparations separated at very high centrifugal speeds.[2]

It is desirable to characterize the unknown green colour associated with microbial fractions and thus determine whether it is caused by plant-particle contamination or whether micro-organisms growing in the rumen contain pigments of their own.

To gain information of value in both these problems, the following study of organic pigments present in the rumen of the sheep was carried out.

Experimental

Animal and diet

One Cheviot wether was fitted with a permanent rumen cannula[3, 4] giving direct access to the rumen. When required, samples of rumen contents were withdrawn through this cannula by a tube and suction.

The wether was fed to appetite on 1200 g. of chopped meadow hay given in two equal portions at 8 a.m. and 8 p.m., and had access to water only between 8 p.m. and 10 a.m. All food uneaten and residual water were removed at 10 a.m. After a preliminary period the sheep was conditioned to eating all food in two hours. Water consumed and body weights were recorded so that any change in the condition of the animal during the experimental period would be noticed. The removal of residual food and water at 10 a.m. allowed samples to be taken at any desired stage in the cycle of rumen digestion without complications arising from a fresh influx of food and water.

The fractionation of rumen contents

For the study of pigments in rumen contents a procedure was developed for obtaining reproducible rumen fractions. Because of the labile nature of plant pigments, mechanical methods of separation, such as filtration and centrifuging, were used where possible. The method finally adopted was as follows:

At the chosen time after feeding, the sheep's rumen was massaged to obtain a more representative sample[5] and a 700-ml. sample was withdrawn by suction through a $\frac{1}{2}$-in.-diameter thick-walled glass tube.

The procedure then followed was that set out in Fig. 1. The sample was strained at once through four thicknesses of surgical gauze. About 50 ml. of wet food material remained on the gauze (sediment A). Liquor A was placed in sixteen 50-ml. centrifuge tubes which were centrifuged at a relative centrifugal force of 500 *g* (1500 r.p.m. with a $7\frac{1}{2}$-in. wheel radius) for ten minutes to give sediment B and liquor B.

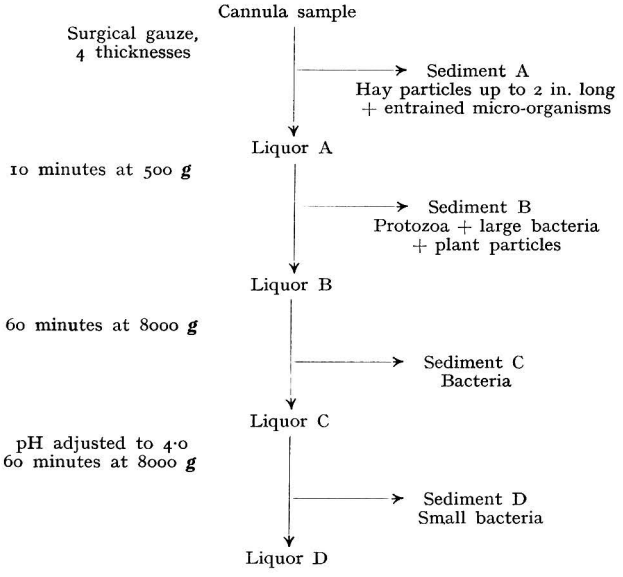

FIG. 1.—*Schematic representation of the procedure for the fractionation of rumen contents*

To reduce plant-particle contamination of sediment C as much as possible, the top 20 ml. approximately of liquor B from each tube was transferred by pipette into four 75-ml. centrifuge tubes. The remainder of liquor B was discarded and the sediments B were combined and washed three times with 0·9% sodium chloride solution. This washed sediment B was generally about 25 g. wet weight.

The 75-ml. tubes containing the upper portions of liquor B were centrifuged at 8000 *g* for 60 minutes. The supernatant liquor C obtained was removed by means of a suction bottle and tube with the tip drawn out and bent. The remaining sediments C were combined, suspended in 0·9% sodium chloride solution, and again centrifuged at 8000 *g* for 60 minutes. The washings were removed by suction and discarded. The washed sediment C weighed about 10 g. (wet weight).

Microscopical examination of liquor C showed that it still contained large numbers of the smaller bacteria. Reed *et al.*[2] have pointed out that, even after centrifuging at very high speeds of 29,000 *g*, about 20% of the smaller bacteria remain in suspension.

In view of these observations the writer decided to prepare a quantity of the smaller bacteria by adjusting the pH to 4·0, a value at which most of the proteins are insoluble. Although a proportion of the pigments in this sediment D, obtained by acidification, will probably be altered by the treatment, the amounts of degraded pigment found will still indicate the original extent of pigmentation in the smallest particles.

Therefore, liquor C, which had a pH of 7·2–7·4, was adjusted to pH 4·0 by adding 7–8 ml.

of glacial acetic acid slowly while stirring. The pH change was followed on a direct-reading meter. The suspension was transferred to centrifuge tubes and centrifuged at 8000 *g* for 60 minutes. After removal of the supernatant liquor D by suction, the slimy sediments D, comprising some 5–7 g. (wet weight), were combined in one tube but were not washed.

Sediments B, C and D were stored in the dark at — 20° until required for study.

Microscopical observations

Sediment B contained protozoa up to 200 μ long, large and small plant fragments, an oval organism (Quin's[6]), and selenomonas approximately 3 $\mu \times 6$ μ, and many organisms approximately 0·5–4 μ long.

Sediments C and D contained no protozoa, very few oval organisms, irregularly shaped particles of 5–10 μ and many organisms of 0·5–4 μ. In sediment D there appeared to be a certain amount of 'clumping', owing to the low pH.

The extraction, separation and estimation of the chief pigments

The methods used were similar to those used for hay,[7] with a modification for sediment D which was more acid than sediments B and C, namely just before rupturing the plant cells by the quick-freezing treatment, magnesium carbonate was added to sediment D in order to neutralize acids which might cause rapid pigment alteration.

An estimation of the proportion of plant material in the sediments by direct observation

Near the end of the experiment an estimate was made, by microscopy, of the proportion of plant material in each rumen sediment studied. The following method was adopted:

Freshly prepared sediments B, C and D were diluted to approximately rumen-liquor strength and mixed in an end-over-end shaker for 30 minutes. Samples (1-ml.) of each suspension were added to 5 ml. of 20% formaldehyde and shaken rapidly for 10 minutes.

Smears were made by (1) placing 0·01 ml. of the formaldehyde suspension on a slide on which a circle 4 sq. cm. in area had been inscribed by grease pencil, (2) adding 1 drop of methanol, mixing thoroughly and spreading with a platinum loop and (3) drying for one minute in the oven at 100° C. The slide was covered with carbolfuchsin stain and, after being washed, was blotted face down on lens tissue spread on filter paper.

The assumption was made that all irregularly shaped particles were plant material and all regularly shaped particles micro-organisms.

To carry out proportional counts on these slides a micrometer was placed in the left eyepiece and a graticule in the right eyepiece of the microscope. The eyepieces used throughout were × 10. Observations were made on vertical and horizontal fields across the diameters of the 4-sq.-cm. circle. The areas of these fields were measured.

It was found practical to count protozoa and large irregular particles of length 10–20 μ in fields greater than 330 μ square with × 150 magnification, and all small particles and organisms in fields about 37 μ square with × 1425 magnification.

A simple count could not be expected to indicate the volumes of each type of particle observed; the product of length times estimated cross-sectional area was therefore calculated for each large particle and for average bacteria sizes. For the assessment of particulate volume the small bacteria were estimated to be 1·5 $\mu \times 1$ μ on the average, and the larger bacteria 6 $\mu \times 3$ μ. The length and breadth of all other particle sizes were estimated during the count, and the estimated volume of each observed particle was calculated.

The particulate matter observed was divided into the following groups and the percentages of each were calculated: protozoa; large irregular particles; small irregular particles; large bacteria—Quin's organism (6 $\mu \times 3$ μ) and selenomonas (6 $\mu \times 3$ μ); small bacteria (1·5 $\mu \times 1$ μ).

Sampling

During a period of one week, samples were withdrawn through the cannula at 2, 4, 6, 8, 10 and 12 hours after the 8 a.m. feed, fractionated according to the scheme already described, and stored at — 20° in the dark until required for pigment extraction. During the same week, samples of the chopped hay feed were taken daily, ground to pass through a sieve having circular holes of 0·4 mm. in diameter, and stored in darkness until required.

With the small amounts of sediments C and D available it was possible to carry out only one estimation of the pigments in each sample.

Sediment A, consisting chiefly of lengths of hay, was not examined, because it was considered that the results would simply reflect the analyses made on the hay feed.

Results

The average and range of concentration of pigments found in the hay feed and rumen sediments are shown in Table I. The time at which the rumen sample was withdrawn had no obvious effect on the type or quantity of pigments present in the various rumen sediments.

Table I

Pigment estimates, mg./100 g. of dry matter: average and range of concentrations in hay-fed sheep

Sample	Chloro-phyll-*a*	Chloro-phyll-*b*	Phaeo-phytin-*a*	Phaeo-phytin-*b*	Total tetra-pyrroles	Carotene	Xanthophyll
Hay feed	60 (55–65)	38 (34-42)	8 (6–12)	2 (1–3)	108	0·8 (0·4–1·1)	3·5 (2·6–4·2)
Sediment B	129 (104–143)	95 (76–106)	66 (53–72)	12 (8–17)	302	2·6 (1·9–3·1)	12·9 (10·6–13·8)
Sediment C	7 (5–10)	12 (8–18)	44 (34–55)	27 (19–36)	90	1·0 (0·8–1·2)	3·6 (3·0–4·3)
Sediment D	2 (2–3)	3 (2–4)	20 (19–21)	15 (9–21)	40	1·0 (0·9–1·4)	2·3 (2·0–2·6)

Table II

Summary of extraction and separation results

	Average % pigments extracted by acetone treatment			Average % pigments transferred to diethyl ether			Average % pigments lost during sucrose column separation		
Wavelength, mμ	660	450	410	660	450	410	660	450	410
Sample									
Hay	99·8	99·8	99·6	98	98	77	6	3	4
Sediment B	99·9	99·8	99·7	100	100	87	8	4	10
Sediment C	99·8	99·3	99·4	98	90	74	15	14	15
Sediment D	99·3	99·5	99·7	70	54	50	11	14	23

An assessment of losses, summarized in Table II, indicated that (1) more than 99% of the fat-soluble pigments were extracted by the acetone treatment; (2) a considerable proportion of the pigments extracted from sediment D were more soluble in dilute aqueous acetone than in diethyl ether, probably owing to pigment degradation during the acid treatment of that sediment; (3) the results shown in Table I might be low by 5–10% for the hay feed and sediment B, and 10–15% for sediments C and D.

The results in Table I show that there is a greater concentration of pigments in sediment B than in the original hay feed. The increase is twofold to threefold for the chlorophylls, sixfold to eightfold for the phaeophytins, and threefold for the carotene and xanthophyll. The increase in total tetrapyrroles is threefold. Apart from the very large phaeophytin increase, probably due to slightly acid rumen conditions, the general increase is at first surprising.

According to Schneider,[8] bacteriochlorophyll has a maximum in the red region of the spectrum at 681 mμ. The chlorophylls separated throughout this work had a maximum in the red region at 661 mμ, which confirms that these pigments have still the normal plant-chlorophyll structure.

That there is an increase in all pigments associated with plant chloroplasts may be due to the presence of plant particles in the sediment and not to a concentration of pigments within the micro-organisms. If this increase is due to particles derived from the hay feed, then these particles must be richer in chloroplast pigments than the ground whole hay. This enrichment would be possible during chewing and regurgitation if (*a*) the particles are broken off the outside leaves of the hay or (*b*) the particles are broken off clover leaves.

It is well known that the outside leaves of fresh grass, as well as clover leaves, are richer in chloroplast material than the grass stem, and this relationship will exist in the dried material.

Sediments C and D show a marked increase in the ratio of phaeophytins to chlorophylls. With sediment D the amount of chlorophylls remaining is negligible. Sediment C, which was expected to be almost free from plant material, contains, on a dry-matter basis, a tetrapyrrole concentration approaching that in the hay feed, and about the same amount of carotene and xanthophyll. Sediment D contains more than a third of the tetrapyrrole concentration of the original hay, and carotene and xanthophyll contents similar to those of hay.

Because in each sediment there is a greater amount than might be expected of all pigments present in plant chloroplasts, and because this suggests that the pigment content of the sediments might be due to contamination by plant material alone, even in sediments C and D which might normally be considered free from contamination, it was of interest to

calculate, from the pigment concentrations found, the proportion of each sediment that might be composed of plant material.

Willstätter & Stoll[9] found that fresh green leaves, on a dry-matter basis, contained on the average 0·6–1·2% of chlorophylls and 0·1–0·2% of carotenoids, of which 0·07–0·12% was xanthophyll. If it is assumed that the plant chlorophylls and phaeophytins found in the various rumen fractions are derived from similar plant-leaf material then the limits of concentration found by Willstätter & Stoll can be used to calculate the figures shown in Table III. It can be seen that if the tetrapyrrole content is derived from green leaf material then one might expect: (i) in sediment B, the proportion of leaf material to be as high as 50% or as low as 25%; (ii) in sediment C, as high as 15% or as low as 8%; (iii) in sediment D, as high as 7% or as low as 3%.

Table III

Plant-particle contamination of rumen sediments calculated from pigment contents of dry matter

	Total of tetrapyrrole pigments found, % (Table I)	Per cent. of the dry matter composed of green leaf material when the pigment content of dry leaf tissue is assumed to be:	
		0·6% of chlorophyll	1·2% of chlorophyll
Sediment B	0·30	50	25
Sediment C	0·09	15	8
Sediment D	0·04	7	3
	Total carotenoids found, %	0·1% of carotenoids	0·2% of carotenoids
Sediment B	0·016	16	8
Sediment C	0·005	5	3
Sediment D	0·003	3	2
	Total xanthophyll found, %	0·07% of xanthophyll	0·12% of xanthophyll
Sediment B	0·013	19	11
Sediment C	0·004	6	3
Sediment D	0·002	3	2

Calculations based on the concentration of carotenoid pigments suggest much lower proportions of plant material. In view of the calculated percentages mentioned above it was desirable to study each sediment microscopically to assess by direct observation the proportion of plant material to micro-organisms.

Table IV

Summary of proportional counts on rumen sediments from a hay-fed sheep

	Fields	Protozoa, %	Irregular particles, %	Bacteria, %
Sediment B	Horizontal	26	48	26
	Vertical	34	40	26
Sediment C	Horizontal	0	10	90
	Vertical	0	9	91
Sediment D	Horizontal	0	16	84
	Vertical	0	16	84

A summary of the final results given in Table IV shows that protozoa were present in sediment B only, and that irregular particles, which might be taken as plant particles, occurred to the extent of 40–50% by volume in sediment B, 10% in sediment C and 16% in sediment D. The last figure may be somewhat high owing to clumps of bacteria being counted as irregularly shaped particles.

These estimates by direct observation of plant material present in the microbial sediments agree in general with the estimates based on the assumption that all tetrapyrrole pigments extracted from sediments are derived from leaf tissue. Although neither the assumptions made in the use of Willstätter & Stoll's data to calculate the contamination of the microbial fractions, nor the approximate method employed in the direct microscopic count, can be entirely free from criticism, yet the agreement between the results supports the view that the

amounts of pigment found in sediments B, C and D arise from the presence of plant particulate matter only, and not from a concentration of any one pigment by the micro-organisms themselves.

When compared with the hay ration, microbial sediments from sheep-rumen contents show a considerable increase in the ratio of phaeophytins to chlorophylls. This indicates breakdown of chlorophylls in the rumen, probably because of slightly acid conditions.

A similar experiment in which artificially dried grass, containing more carotenoids than the hay, was fed to the same sheep gave results that led to similar conclusions, although the destruction of chlorophyll pigments in the rumen was greater than with the hay-fed sheep. The higher temperatures used in artificial drying of the grass may have affected the stability of the chlorophyll pigments, and thus favoured greater destruction of chlorophylls in the rumen.

Discussion

The proportion of plant material found in the bacterial sediments separated from rumen contents is considerably higher than might be expected, and illustrates one of the difficulties facing workers who make chemical and biological assessments on sediments taken directly from the rumen.

Recently, Reed *et al.*,[2] working with sheep fed on ' fresh green ' feeds, have pointed out that bacteria sedimented at high centrifugal speeds retain a green colour. If this is assumed to be due to plant chlorophyll material the writer considers it doubtful whether a complete separation of bacteria from plant material can be obtained by the customary methods of filtering and centrifuging. The present study shows that contamination of bacterial sediments by dietary material may be of the order of 10% in carefully prepared sediments, and this should be taken into consideration in chemical and biological investigations on these sediments.

Contamination of this nature may account for some widely divergent results that have been obtained for the feeding value of microbial protein prepared from rumen contents. Johnson *et al.*[10] found that bacteria isolated from the rumen of sheep fed on a diet containing 11% of protein contained 44·5% of protein based on the dry matter, compared with 58·8% of protein for a bacterial fraction (a yellow rod) obtained by culture on a synthetic medium. Contamination with food particles might partly account for such a difference in protein content.

In view of these observations it would be advisable, when assessing the value of chemical and biological measurements made on microbial protein derived from the rumen, to obtain an estimate of the food contamination of the rumen sediment.

As stated in the Introduction, a simple and reliable marker-method for estimating the digestibility of the diet would be of considerable value in nutritional studies. Reid *et al.*[1] described a new method which used as a marker the absorption of 406 mμ of the pigments in an acetone extract of the forages eaten and the faeces excreted. These authors obtained results on dry matter digestibility that were in close agreement with those obtained by conventional methods, and although at the beginning of their communication it was pointed out that more than one pigment might be responsible for the absorption at 406 mμ it was later argued that ' since recovery of the pigment in the faeces was so consistent for all forages studied, and since it would seem improbable that several chromogens would exhibit an absorption relationship resulting in the formation of isobestic points [frequencies at which mixtures in all proportions show the same optical density] near 406 mμ, one chromogenic substance apparently is responsible for the absorption observed '.

The present study, however, shows clearly that an acetone extract of forages contains several pigments with absorption at 406 mμ, and the total absorption at 406 mμ must be caused by a summation of the absorptions due to chlorophyll-*a* and -*b*, phaeophytin-*a* and -*b*, carotene, xanthophyll and degradation products. No fat-soluble pigment has been found that could be the ' chromogen ' responsible for constant absorption at 406 mμ.

Moreover it appears that chlorophylls are degraded in the rumen to a varying extent and it is probable that further degradation takes place in the abomasum and intestines. This may account for the results obtained by Cook & Harris,[11] who obtained by the chromogen method inaccurate digestibility coefficients for certain range forages (natural herbages) in winter.

Because one of the first essentials in a marker is stability, and because the chlorophylls at least are known to be extremely unstable, it is doubtful whether a marker method employing these plant pigments can be universally successful. Such a method should be applied extensively only after comparative trials under local conditions have shown that results are the same as those obtained by conventional methods.

One digestibility trial with hay-fed sheep, carried out at this Institute to compare the

' chromogen ' and ' dry matter consumption–excretion ' methods, has shown that the chromogen at 406 mμ ' is not completely recovered in the faeces and if used to calculate the digestibility coefficient gives low results.

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The Rowett Research Institute
Bucksburn
Aberdeenshire

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References

[1] Reid, J. T., Woolfolk, P. G., Richards, C. R., Kaufmann, R. W., Loosli, J. K., Turk, K. L., Miller, J. I., & Blaser, R. E., *J. Dairy Sci.*, 1950, **33**, 60
[2] Reed, F. M., Moir, R. J., & Underwood, E. J., *Aust. J. sci. Res.*, 1949, [B] **2**, 304
[3] Quin, J. I., Van der Wath, J. G., & Myburgh, S., *Onderstepoort J. vet. Sci.*, 1938, **11**, 341
[4] Phillipson, A. T., & Innes, J. R. M., *Quart. J. exp. Physiol.*, 1939, **29**, 333
[5] Heald, P. J., *Brit. J. Nutr.*, 1951, **5**, 84
[6] Quin, J. I., *Onderstepoort J. vet. Sci.*, 1943, **18**, 91
[7] Davidson, J., *J. Sci. Fd Agric.*, 1954, **5**, 1
[8] Schneider, E., *Ber. dtsch. bot. Ges.*, 1934, **52**, 96
[9] Willstätter, R., & Stoll, A., ' Untersuchungen über Chlorophyll ', 1913 (Berlin : Springer)
[10] Johnson, B. C., Hamilton, T. S., & Garey, J. C., *J. Anim. Sci.*, 1944, **3**, 287
[11] Cook, C. W., & Harris, L. E., *J. Anim. Sci.*, 1951, **10**, 565

THE COMPONENT FATTY ACIDS OF THE SEED OILS OF *DATURA METEL, D. STRAMONIUM* AND OF *CAPPARIS ROTHII*

By D. N. GRINDLEY

In this paper are recorded the constants of the seed oils of the species *Datura metel, D. stramonium* and *Capparis rothii* and their fatty acid compositions, which resemble those of other species of the two families that have been previously reported.

During the past two years, the component acids of a number of vegetable fats from species growing in the Sudan have been determined in these Laboratories. In order that our findings may be placed on record, a summary of some of these miscellaneous data is offered in this paper.

Datura metel L. (family Solanaceae) is a bushy herb or under-shrub which is widely distributed in the Northern Sudan, especially in Darfur, Khartoum and Kordofan Provinces. The seeds are ear-shaped and have a powerful narcotic effect; they contain about 0·2% of alkaloids, consisting of hyoscine with traces of hyoscyamine and atropine. The seeds are light-brown in colour, and larger and more flattened than those of *D. stramonium*, the well known thorn-apple, and the plant, which is more pubescent than that of *D. stramonium*, is of much more frequent occurrence in the Sudan.

D. stramonium L. is also fairly widely distributed; it is believed to have spread from the shores of the Caspian Sea through Europe and Africa in the first century A.D. It is a bushy annual growing to a height of about one metre. It occurs mainly in the Blue Nile Province and Nuba Mountains area. The seeds are dark-brown or blackish in colour, reniform in outline and are a well known poison, containing about 0·2% of mydriatic alkaloids, of which hyoscyamine is the principal. These alkaloids do not pass into the oil, which is extractable from the seeds by light petroleum. Both species yield about 16% of pale-yellow oils.

Capparis rothii Oliv. (family Capparidaceae) occurs in the Upper White Nile and Equatoria Province and is a thorny climber or small tree. The fruit is somewhat pointed, about 1½ in. across, turning orange when ripe, and contains seeds about the size of a small pea.

The seeds of these three species have been extracted with light petroleum and the oils so obtained have been analysed. Their constants are shown in Table I, together with the fatty acid compositions, as calculated from the thiocyanometric equations; the empirical value of 96 is taken for the thiocyanogen value of linoleic acid (cf. Hilditch & Murti[1]). The saturated fatty acids were also determined by Bertram's oxidation method, and the results obtained agreed closely with those derived thiocyanometrically. In each case, the aqueous solution remaining after the light-petroleum extraction of the oxidation products obtained in the course of the Bertram saturated acid determination was further extracted with ethyl ether, when large yields of azelaic acid were obtained, indicating that the first double bond occurs in the unsaturated acids in the 9 : 10-position. The absence of linolenic acid was shown in all cases by the inability of the oils to form ether-insoluble hexabromides when treated with bromine in ethereal solution at 0°, and the octadecadienoic acid present was shown to be the common *cis–cis*-$\Delta^{9:12}$ isomer (linoleic acid), common to the majority of seed oils of these types; the tetrabromostearic acid produced by bromination and recrystallization from light petroleum had in all cases a melting point of 114°.

Table I

	Datura metel	*Datura stramonium*	*Capparis rothii*
Wt. of 100 seeds, g.	1·3388	0·6393	12·92
Per cent. of kernel	—	—	30·8
Per cent. of oil in seeds	16·39	16·84	40·88 (in kernel)
Colour	Very pale yellow		Golden yellow
Free fatty acid (as oleic), %	0·230	0·226	—
Acid value, mg. of KOH/g.	0·46	0·45	—
Saponification value, mg. of KOH/g.	187·8	189·5	189·9
Mol. wt. of total fatty acids	284·5	283·4	282·9
Refractive index @ 40°	1·4681	1·4689	1·4615
Unsaponifiable matter, %	3·49	3·75	0·74
Hehner value (total fatty acids and unsaponifiable), %	96·27	95·94	95·76
Iodine value of oil	119·15	126·85	70·66
Thiocyanogen value of oil	76·08	76·68	52·94
Iodine value of fatty acids	122·55	130·47	73·84
Thiocyanogen value of fatty acids	78·74	79·44	55·32
M.p. of fatty acids	—	—	42°
M.p. of tetrabromides	114°	114°	114°
Hexabromides	Nil	Nil	Nil
Per cent. of saturated acids (Bertram)	14·41	15·24	38·1
M.p. of saturated acids	56½°	55½°	54¾°
Equiv. wt. of saturated acids	260·7	263·1	272·3
Per cent. of dibasic acids recovered	53·10	50·54	30·49
M.p. of dibasic acids	107°	107°	105°
Equiv. wt. of dibasic acids	95·0	95·3	96·0
Fatty acid percentage composition (by thiocyanogen value)			
Linoleic acid	52·16	60·75	22·05
Oleic acid	31·84	23·44	37·95
As palmitic acid	13·05	11·44	15·50
As stearic acid	2·95	4·37	24·50
Linolenic acid	Nil	Nil	Nil

The seed oil of *Capparis rothii* is thus seen to be similar to that of *Capparis tomentosa*,[2] and also to those of *Courbonia virgata*[2] and *Boscia senegalensis*,[3] which are members of the same family. The oil of *Capparis spinosa*, as reported by Zabrami *et al.*,[4] has a considerably lower percentage of saturated fatty acids. The kernel oil of *Capparis rothii* should prove to be very useful for soap-making and edible purposes, and the high oil content of the seeds may well justify the economic development of this species, especially for local requirements.

The seed oils of the two *Datura* species are typical of those of the Solanaceae, which are characterized by comparatively low contents of saturated (mainly palmitic) acid, with varying proportions of oleic and linoleic acids; linoleic acid usually predominates, although Dieterle[5] states that the seed oil of *D. alba* from East Africa only contains 28% of linoleic acid. The figures obtained in the present instance for *D. stramonium* show more linoleic acid than those obtained by Hilditch & Ichaporia,[6] but these workers were examining a sample of oil that had become partially oxidized, and they consider that their figures for linoleic acid may be lower than the true values.

It is thus found that the component acids of all the species described in this paper align themselves with those of fats previously studied from other species of the same botanical

family, affording additional evidence of the close relationship between botanical classification based on morphological considerations and the chemical composition of the seed fat.

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Wellcome Chemical Laboratories
Medical Services, Ministry of Health
Khartoum

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References

[1] Hilditch, T. P., & Murti, K. S., *Analyst*, 1940, **65**, 437
[2] Grindley, D. N., *J. Sci. Fd Agric.*, 1950, **1**, 152
[3] Grindley, D. N., *J. Soc. chem. Ind., Lond.*, 1948, **67**, 230
[4] Zabrami, D., Otschakovski, A., & Petrova, N., *Maslob.-zhirov Prom.*, 1940, **16**, (No. 5–6), 57
[5] Dieterle, H., *Arch. Pharm., Berl.*, 1926, **264**, 140
[6] Hilditch, T. P., & Ichaporia, M. B., *J. Soc. chem. Ind., Lond.*, 1936, **55**, 189T

THE PURIFICATION OF CANNERY WASTE WATERS IN BIOLOGICAL FILTERS

By DENIS DICKINSON

The waste waters from fruit and vegetable canneries are among the strongest of organic trade effluents and, moreover, occur in large volumes. By reason of the high proportion of carbohydrate they contain, their purification by conventional methods was not expected to be easy. The present paper summarizes the experience gained over a period of several years in the design and operation of a biological filtration plant for the purification of the wastes from a medium-sized fruit and vegetable cannery, operating throughout the year and manufacturing the whole range of products normally made in such factories in Great Britain.

Basic principles for design of purification plant

Experiments carried out in the laboratory and observations made under practical conditions and with pilot plant served to establish a number of principles that guide the successful purification of waste waters from fruit and vegetable canneries in biological filters.[1–3]

These may be summarized as follows:

(1) Elimination of waste wherever possible, especially as regards the indiscriminate washing of solid materials from floors to drains.

(2) Screening by mechanical means to remove gross suspended solids at the earliest possible moment.

(3) Admixture of factory domestic sewage (septic-tank effluent) with the cannery waste as a means of supplying nitrogen.

(4) Removal of grit by flow through a constant-velocity channel.

(5) Admixture with humus sludge separated from the filter effluent.

(6) Sedimentation for a period of approximately two hours in rectangular tanks of simple design.

(7) Withdrawal at a constant rate for oxidation in biological filters.

(8) Dilution before filtration with several volumes of settled filter effluent, to give a mixture having a calculated B.O.D. (biological oxygen demand) of not more than 200–250 p.p.m.

(9) Application of this mixture to the filters at a rate of 4 gal./cu. yd./hour.

(10) Dosage of the filters with a mixture containing settled factory waste for a limited period (about 12 hours) in each 24 hours.

(11) Recirculation of filter effluent at all times.

These principles have been applied successfully to the design of two full-sized treatment plants for the purification of cannery waste only (Plants A and B), and to the design of a plant for the purification of a mixture of cannery waste and town sewage.[4]

Items (10) and (11) are important, since by working in this way the load applied to the filters is varied continually, reaching a peak strength about mid-afternoon in a normal working day and a minimum strength in the early hours of the morning. Consequently this system of filtration combines to some extent the principles of recirculation and of alternating double filtration,[5] the alternating double filtration being condensed into a 12-hour cycle. It avoids storage of settled waste for more than a few hours, which effects economy in plant, and also, in conjunction with the continuous return of humus sludge to the sedimentation-tank inlet, avoids to a very large extent the development of acidity in the settled wastes by bacterial action. Further, return of the humus sludge in this manner avoids any problem of its separate disposal, all sludge being mixed together in the primary settling tanks. When this system of filtration is adopted, excessive surface growths on the filters do not occur.

Items (1) to (7) call for no further comment. Item (8), the dilution ratio, was fixed at 5 : 1 when designing Plant A in order to allow for the seasonal treatment of beetroot- and carrot-canning wastes with B.O.D. values of 1300 p.p.m. after settlement. Such wastes have occurred and in fact the maximum B.O.D. reached was 2480 p.p.m., and the maximum monthly average was 1070 p.p.m. Analysis of samples of waste water, diluted waste as fed to the filters, and of filter effluent should, in theory, allow of the calculation of the actual dilution ratio. In fact, a figure calculated on the basis of samples for any one day is frequently erroneous, largely on account of the difficulty of obtaining corresponding samples. Over a period, however, or with a sufficient number of samples, the errors cancel out, and it has been calculated from 60 sets of B.O.D. values, obtained during 1948, that the actual dilution ratio was 4·76 as against an intended 5·0.

Comparison of purification of fruit wastes and vegetable wastes

The effluents produced by Plant A have been generally very good and, indeed, they had to be, as no dilution could be relied upon on discharge. The question arises whether the actual dilution ratio, 4·76, was not excessive for wastes of comparatively low B.O.D., such as those from fruit canning, but it is shown below that the oxidative capacity of the plant was more nearly approached when treating fruit wastes than when treating vegetable wastes.

During the period of five years since Plant A was put into operation, regular samples have been collected and subjected to chemical analysis. The sampling has been done on one or two days each week, taking a fixed volume of sample each hour during the working day and analysing the mixture. The tests applied have been the B.O.D. test, the 4-hours' acid permanganate test, and the pH value, with the additional tests of stability to incubation and qualitative tests for nitrites applied to the samples of purified effluent. Several useful facts are established by the accumulated results.

From the mean monthly values of B.O.D. of the wastes before settlement, and of the purified effluent, it became apparent that, whereas the highest B.O.D. values for the wastes were found always in winter, the highest values for the purified effluent occurred in summer. This finding, which was somewhat unexpected, is well illustrated by Fig. 1 in which the mean values for the two sets of samples for each month of the year are set out. The differences between the highest, mean and lowest B.O.D. values are naturally much greater for the unpurified waste, but the inverse trend is apparent ; it recurred, moreover, each year. From these figures and from records of the flows, the actual weight of B.O.D. oxygen supplied by the plant each month has been calculated. This is plotted against the B.O.D. values for the untreated waste (Fig. 2) from which it will be seen that there is a marked positive correlation ($r = +0{\cdot}912$; $P = 0{\cdot}001$). On the other hand no correlation was found between the weight of oxygen supplied by the plant and the volume of liquid treated, so that the relationship between the oxygen supplied and the oxygen demand is not influenced by any relationship between the flow and the oxygen demand.

On the basis of pilot-plant experiments reported previously,[1] it was assumed that vegetable wastes, having the highest B.O.D. values, would prove the most difficult to purify. The present results indicate that this is doubtful. The fruit-canning season, although varying a little from year to year, normally includes the months of June, July and August ; reference to Fig. 1 shows that the B.O.D. values of the purified effluents during these months are among the highest for the year. Vegetable canning extends over the rest of the year ; hence it appears that, contrary to expectation, fruit-canning wastes are more difficult to purify than vegetable wastes, even though their B.O.D. values are in general much lower.

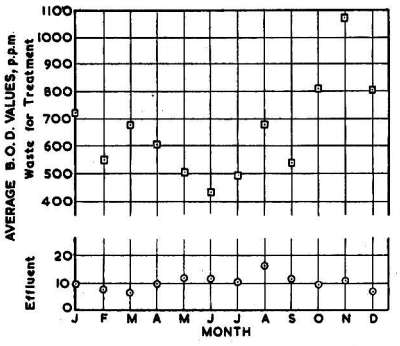

FIG. 1.—*B.O.D. values of samples from Plant A*

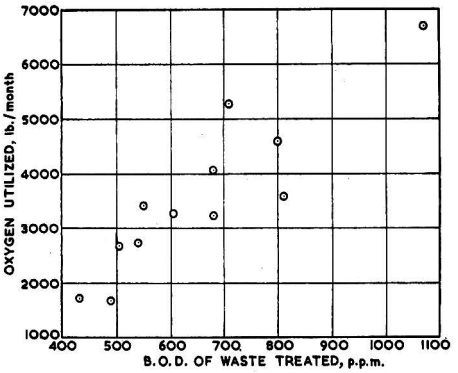

FIG. 2.—*Relationship between oxygen supplied and oxygen demand in Plant A*

It now appears that the analyses and the performance figures for the two classes of wastes, fruit and vegetable, might usefully be separated. This has been done by grouping together the figures for the months of June, July and August (fresh peas were not canned during the years under consideration) and those for December, January and February, to represent fruit- and vegetable-canning wastes respectively. The figures of most value are the analytical figures and the rate of oxidation as represented by the weight of oxygen induced by each cubic yard of filter medium.

	Fruit cannery wastes			Vegetable cannery wastes		
	Mean	Highest	Lowest	Mean	Highest	Lowest
B.O.D., p.p.m.	530	970	140	680	1100	240
Oxygen absorbed, $KMnO_4$ test, p.p.m.	195	418	18	221	424	41
pH value	5·3	6·8	4·1	5·5	6·8	4·2
Oxygen supplied, lb./cu. yd./day	0·042			0·084		
Mean flow, gal./day	13,900			21,730		

The amount of oxidation effected by each cubic yard of filter medium was exactly twice as much during the vegetable season as during the fruit season. The difference in daily volume treated may account for a proportion of this, but the tendency for the quality of the purified effluent to deteriorate during the fruit season seems to indicate that the maximum oxidative capacity of the plant was approached more nearly during such seasons. It was also found that of 12 occasions during the three years when the B.O.D. of the purified effluent sample was 20 p.p.m. or more, six were during the months of June, July and August. On the average, 23·8 cu. yd. of medium have been required to supply each pound per day of B.O.D. oxygen to fruit-canning wastes. On the other hand, vegetable wastes have required only 11·8 cu. yd. for the same amount of work, and the quality of the effluents indicates that a smaller volume would suffice for such wastes.

This method of expressing the performance figures, although helpful in comparing the behaviour of the plant when treating two classes of wastes, does not give a sound basis for the design of other installations except in circumstances that demand the same degree of purification.

It is useful to record that sudden changes in the nature of the wastes to be treated have not been found to have any appreciable effect on an established filter or its performance. Recirculation, especially with the supplementary recirculation of humus sludge, must help by evening out sudden changes in the wastes themselves.

Purification plant for effluent discharging to river

Plant A, from which these performance figures have been obtained, was designed to purify the effluent from a cannery in an isolated situation where no sizable stream was available for the disposal of the purified effluents. It was therefore necessary to ensure that the effluent would be well within the limits of the Royal Commission Standard for Sewage Effluents at all times. This necessity has also restricted experiments with the plant. The design of an installation to purify cannery waste sufficiently to permit its discharge into a fair-sized

stream without polluting the stream posed a rather different problem. In such a situation it was not essential to ensure such a high degree of purification at all times, but rather to produce an effluent of which the average B.O.D. would be 20 p.p.m. or slightly less. The principles (1)–(6) inclusive would apply, but the others might be modified. It seemed unnecessary to provide separate storage capacity to enable settled waste to be passed forward for filtration at a constant rate; the waste was therefore allowed to flow directly into a combined mixing chamber and pump-well for dilution with the purified effluent. The dilution ratio was fixed at three, based on a B.O.D. of 965 p.p.m. for beetroot waste, since this was the highest average seasonal figure found by analysis of the wastes to be purified; intermittent higher values were ignored on the principle that dilution available for disposal of the effluent during such seasons (at least 40 times) should allow for the discharge of an effluent of B.O.D. greater than 20 p.p.m. for short periods. The automatic return of three volumes of purified effluent was obtained by causing the overflow from three sides of the final settling-tank for the filter effluent (which was of the pyramidal type) to run back into the mixing well, and allowing the fourth side to discharge to the stream. As the mean rate of effluent discharge from the factory was 2500 gal./hour, the rate of application to the filters had to be 10,000 gal./hour. Provision for this was made, but the quantity can be increased or decreased by altering the level of a V-notch weir in relation to a fixed overflow pipe, both situated in the distribution chamber supplying the filters. The rate of application was decided at 6 gal./cu. yd./hour, which necessitated 1666 cu. yd. of filter medium. A somewhat larger medium was used, 3-inch washed gravel, topped with three inches of fused slag. Some doubts were expressed about the suitability of this large-gravel medium—since the stone was more smooth-surfaced and inert than limestone—but it may be stated at once that it has proved to be eminently satisfactory and is, moreover, considerably less expensive than the more usual materials. In arriving at these dimensions for Plant B, due cognizance was taken of the fact that Plant A had produced excellent effluents from vegetable wastes when they were treated at an average rate equivalent to 12·08 gal. of undiluted waste per cu. yd./day, i.e. an actual average rate of 69·5 gal. of diluted waste. In Plant B, allowing for the expected lower B.O.D. value and correspondingly lower dilution ratio, purification at approximately the same rate per cu. yd. of medium might reasonably be expected, equivalent to 17·4 gal. of undiluted waste. The

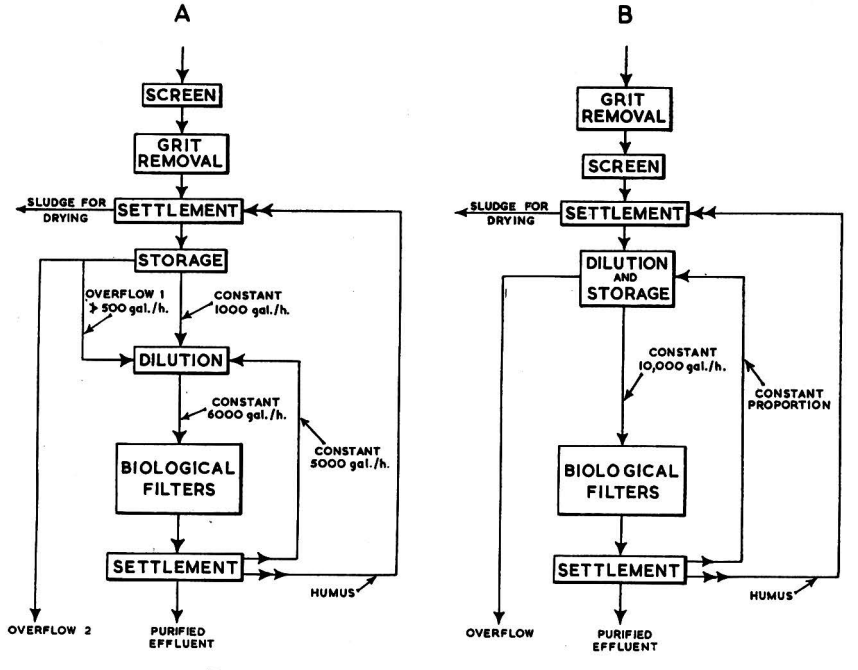

FIG. 3.—*Diagrammatic layouts of Plants A and B*

proposed elimination of storage, however, reduced the period of application from 12 hours to approximately 10 hours and therefore it was deemed advisable to reduce the load by a similar proportion, to 15·0 gal./cu. yd./day. Comparative diagrammatic representations of Plants A and B are shown in Fig. 3.

It is unfortunate that no record of flow is available at this installation (Plant B), but it is safe to say that the estimated average flow of 2500 gal./hour is fairly accurate. Emergency overflows intended to take flows greater than this have operated occasionally. Six months after first putting the plant into operation, the effluent was of excellent quality, as illustrated by the results quoted below:

	Waste before settlement	Purified effluent
Suspended solids	—	8·4 p.p.m.
Oxygen absorbed, 4-h. test	472 p.p.m.	8·6 ,,
B.O.D.	1140 ,,	3·5 ,,
Free ammonia as nitrogen	3·95 ,,	0·6 ,,
pH	7·2	7·8

It remains to be seen whether Plant B will give equally satisfactory results during subsequent fruit-canning seasons, but if the relative volumes and strengths of fruit wastes and vegetable wastes at Cannery B and at Cannery A are similar there is every reason to believe that it will.

The proportion of domestic sewage present in the effluents from the two factories may be assumed to be similar, but it is difficult to estimate just what the proportion is. It seems unlikely that it will exceed one-tenth of the total, even allowing for canteen waste.

Modification of principles

That fruit and vegetable cannery wastes can be purified successfully in biological filters with the special system of recirculation and alternation of strength, which is the subject of these studies, is adequately proved. How to calculate the size of the filters necessary to effect a sufficient but not excessive degree of purification in any set of circumstances needs to be reconsidered. When virtually complete purification is required, i.e. an effluent of B.O.D. not greater than 20 p.p.m. at any time, all the principles stated in the first paragraph must be observed, with the possible exception of item (9); the rate of application of the diluted waste to the filters may be increased above the 4 gal./cu. yd./hour there specified. The actual amount of the increase must depend to some extent on the number of hours per day during which application is necessary, but experience with Plant A indicates that it may be increased at least to 6 gal./cu. yd./hour. This effects a considerable saving in filter medium. Recent observations are that Plant A showed signs of failing owing to excessive growth when the rate of application was raised to 7 gal./cu. yd./hour with a mixture having a B.O.D. of 400 p.p.m.[6]

When there is considerable dilution available for the purified effluent on discharge, conditions need be less stringent and the suggestion is made that, instead of designing a plant large enough to purify the strongest individual waste known to be discharged by the factory, the design should be based on the highest 'seasonal average' figure, e.g. for the beetroot or carrot 'season', as applicable. Analyses carried out during one such season should provide sufficient figures; comparison of the figures obtained with published results will indicate the existence of any abnormality that requires rectification by modification of working methods. This, of course, effects considerable economy by reducing the dilution ratio. Storage of settled waste and its withdrawal at a constant rate for application to the filters appear to be unnecessary in these circumstances. The abandonment of the principle (7) means, in fact, that the dilution ratio also becomes an average figure and not a constant. The satisfactory performance of Plant B in which these modifications were incorporated confirms their effectiveness.

There is a lower limit to the degree of purification that may be effected by biological filtration. The biological filter is essentially a balanced biological system in which micro-organisms of a variety of species, and many insects, compete for supremacy. If conditions favour the development of one species of micro-organism more than another, the degree of purification will probably suffer, and mechanical choking of the filter may result from excessive growth of this species. Persistent overloading is a certain means of bringing about this very troublesome condition. If benefit is to be gained by recirculation of the filter effluent, then this effluent must be of reasonably good quality; in general it must contain nitrate and nitrite, and certainly it must contain dissolved oxygen. Consequently, it is not at present a practicable proposition to design a filtration plant that will produce a filter effluent with a B.O.D. of, say, 50 p.p.m., and that will work satisfactorily with the minimum of supervision.

If it is desired to produce a final effluent with a B.O.D. of this order, the only way to do so without continual filter trouble is to purify a portion of the effluent fairly completely in biological filters and mix this purified effluent with unfiltered effluent immediately before discharge. It is also necessary to remember that constant skilled attention at factory installations is not generally possible.

It will be noted that all these provisions relate to a cannery that processes fruit and vegetables and functions continuously throughout the year. Waste from the canning of soups, mixed meat and vegetable packs, and some milk products, are amenable to the same process. No solution is proposed for the problem of the purely seasonal fruit or pea cannery for which special methods may be required.

The Fruit and Vegetable Canning and Quick Freezing Research Association
Chipping Campden
Glos.

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References

[1] Dickinson, D., *J. Inst. Sew. Purif.*, 1946, Part II, p. 103
[2] Dickinson, D., *J. Inst. Sew. Purif.*, 1945, Part I, p. 48
[3] Jones, E. E., *J. Soc. chem. Ind., Lond.*, 1945, **64**, 80
[4] Miller, W. N., & Clark, H. W., *J. R. Sanit. Inst.*, 1950, **70**, 48
[5] *Tech. Pap. Wat. Pollut. Res., Lond.*, 1941, No. 8
[6] Dickinson, D., *Industr. agric.*, 1953, **70**, 301

RAPID DEMONSTRATION OF YEAST-CELL ACTIVITY IN PRESSURE-STORED REFRIGERATED FRUIT JUICE

By V. T. WALKLEY

A method for routine plant control is outlined whereby incipient yeast-cell spoilage in pressure-stored refrigerated fruit juice may be detected. The morphological appearance of the cells is interpreted as an average figure relative to a standard norm.

Introduction

In order to maintain adequate microbiological control covering the entire period of bulk storage of natural fruit juices, it is essential not only to obtain information regarding the total number of micro-organisms present at any given time, but also to determine the biological activity of those particular organisms. With fruit juices that contain a varied yeast-cell population, spoilage may be detected by an increase in the number of yeast cells present by employment of established plate-counting methods. However, this technique is valueless where rapid control of process is required. An increase in yeast cells, as indicated by plate-count methods, is available only after a minimum period of 48 hours' incubation. During this period of time the yeast cells may have altered considerably in numbers. Also, where fruit juice (e.g. apple juice) is stored in bulk, as in the Boehi system, in which the juice, impregnated with carbon dioxide, is stored in tanks at pressures up to 120 lb./sq. in. and at temperatures ranging from 0° to 10°, the plate-count technique is not applicable unless some method of agitating the juice is employed. As it is neither practicable nor desirable to agitate the contents of storage vessels, sampling errors arise owing to unequal distribution of the microbial population. In order to overcome these inherent variations, Marshall & Walkley[1] employed a method whereby the relative incidence of yeast cells to mould spores was treated statistically to estimate yeast-cell proliferation.

When apple juice is stored in refrigerated pressure-tanks the yeast cells present rapidly assume a spherical, ellipsoidal or typical 'resting cell' appearance and budding or vegetative cells diminish numerically. This balance is established after a few days and does not alter significantly during the entire storage period. However, we have found that, if some alteration in the physical environment of the cell takes place (such as a reduction in carbon dioxide pressure or an increase in temperature), a change in the balance of cells is evident within a very short period as conditions of storage become more favourable to fermentative spoilage.

By employing a differential yeast-cell count (described below) it is possible to detect yeast-cell activity almost immediately, and this estimation is reliable even with the extreme conditions that are encountered when the plate-count technique indicates a decreasing population owing to rapid sedimentation of the cells. Also, it has been found that in order to detect the initial onset of yeast-cell activity, the plate-count technique has the disadvantage of indicating only increased numbers of cells; this state of affairs is brought about only as a result of yeast-cell proliferation. To obtain evidence of the immediate onset of biological activity, particularly where sedimentation and unequal distribution of cells affect total counts, recourse has been directed to the morphological appearance of the yeast cell itself.

Experimental

To carry out suitable process control, the following procedure was adopted for apple juice in storage.

(i) *Total viable-cell count.*—The total viable yeast-cells were enumerated by the well known plate-count technique. Aliquot samples of the juice were diluted with sterile water to give known dilution ratios. Standard volumes of 1 ml. were pipetted into Petri dishes into which was poured the agar medium. The contents were thoroughly mixed, and when cool the plates were inverted and incubated at 25°. The medium employed had the following composition: apple juice (diluted to contain 2% of sugar) 500 ml., ammonium phosphate 1·0 g., agar-agar 10 g.

(ii) *Differential yeast-cell count.*—The technique consisted of a direct microscopical count, using a ruled counting chamber. Four separate enumerations were made on each sample and the chamber was washed, dried and refilled between each count. A total of 100 cells were counted each time, making a total of 400 cells examined on each sample. The cells were counted and separated into three arbitrary groups of (*a*) spherical cells, (*b*) ellipsoidal cells and (*c*) budding cells. The percentage figures for each group were then obtained.

Derivation of 'normal' figures.—It is essential to obtain figures for the 'normal' yeast flora of the storage vessels, as this will vary according to the conditions of temperature and pressure of storage, the type of juice stored, its chemical composition and the original complement of micro-organisms. The basic 'normal' data were derived as follows:

Weekly estimations were made of the yeast-cell population, employing both the total plate-count method and the differential (counting chamber) technique. These weekly estimations (carried out over a period of two years) were then arranged into four-week averages. In Table I are shown the figures for yeast-cell population of apple juice undergoing refrigerated pressure-storage. From this Table, the 'normal' figures were computed as follows:

(i) Total viable yeast cells:

Maximum	28,450 cells per ml. of juice
Minimum	8,000 " " " " "
Average	15,680 " " " " "

(ii) Differential count:

	Spherical	Ellipsoidal	Budding
Maximum	66	46	12
Minimum	47	29	0
% Average	56·4	38·1	5·5

In order to evaluate the 'normal' figures more closely, estimations were carried out on juices taken from a battery of ten tanks under identical conditions of temperature and pressure. It was found that the plate-count estimation was subject to a frequency distribution falling between limits of 5000 to 30,000 yeast cells per ml. of juice, despite sterilization of the sample cock and complete flushing out of the outlet tube before sampling. Chemical analysis revealed no indication of fermentative spoilage, and no evidence of fermentation was detected after a period of two years. The differential yeast-count exhibited close numerical uniformity, and over a period of two years the maximum figure for budding cells did not exceed 12%. A two-year estimation covering ten tanks gave differential figures in close conformity to those recorded in Table I, and in no case was the maximum figure of 12% exceeded.

Experimental fermentation and results

At the end of a two-year storage period, apple juice was allowed to ferment under controlled conditions. During the test, the juice was sampled at 24-hour intervals and figures obtained for total yeast-cell count (plate-count technique) and differential yeast-cell count (counting-chamber method) as previously outlined.

The first experiment was designed to allow continuous observation of apple juice in which the normal storage condition (0°; carbon dioxide pressure, 8 atm.) was suddenly altered.

Table I

Yeast-cell counts on apple juice in Boehi tank storage (0°; carbon dioxide pressure, 8 atm.)

Month	Plate count, viable yeasts per ml.	Percentage differential count		
		Spherical cells	Ellipsoidal cells	Budding cells
1	13,600	55	38	7
2	8,700	57	40	3
3	12,100	66	30	4
4	10,300	58	32	10
5	14,300	64	32	4
6	8,900	57	41	2
7	12,700	58	42	0
8	14,300	47	41	12
9	18,000	48	42	10
10	21,400	56	42	2
11	21,500	51	46	3
12	28,450	57	43	0
13	23,300	47	44	9
14	16,500	65	30	5
15	13,200	56	40	4
16	8,500	58	32	10
17	9,800	54	40	6
18	9,450	57	33	10
19	12,050	57	35	8
20	18,200	56	38	6
21	14,000	61	29	10
22	12,300	61	39	0
23	10,400	47	46	7
24	8,000	58	41	1
Maximum	28,450	66	46	12
Minimum	8,000	47	29	0
Average	15,683	56·3	38·2	5·5

The pressure was reduced to 1 atm., but the temperature was maintained at 0°. Differential counts made on this juice showed a decrease in spherical cells and an increase in ellipsoidal cells which was evident on the second day. The percentage of ellipsoidal cells continued to rise, reaching a maximum on the sixth day, with a corresponding depression in the percentage of spherical cells. Budding cells exhibited a slow continuous increase in percentage proportion, reaching a maximum in 22 days (Table II).

Table II

Differential yeast-cell counts on apple juice at 0° and atmospheric pressure

Day	Percentage differential count		
	Spherical cells	Ellipsoidal cells	Budding cells
1	60	33	7
2	54	38	8
3	43	43	14
4	39	46	15
5	40	50	10
6	30	62	8
7	32	53	15
8	37	41	22
9	39	36	25
10	47	32	21
11	44	34	22
12	43	38	19
13	45	32	23
14	46	30	24
15	46	30	24
16	40	34	26
17	32	40	28
18	21	46	33
19	15	58	37
20	8	51	41
21	10	29	61
22	9	27	64
23	12	48	40
24	16	68	16
25	19	74	7

The total plate-count figures showed no abnormality up to a period of 17 days, and during this period the plate-count figure indicated an increase from 8500 yeast cells per ml. of juice on the first day to 23,700 per ml. on the 17th day, these figures being well within the upper normal limit. The plate count indicated 31,000 yeast cells per ml. of juice on the 18th day, and afterwards the plate count increased to 1,430,000 yeast cells per ml. of juice on the 24th day and the juice exhibited signs of active fermentation.

The second experiment was arranged so that both temperature and pressure were altered, the pressure being reduced to 1 atm. and the temperature increased to 13·5°. The results were similar to those obtained in the first experiment, with the difference that yeast proliferation was extremely rapid, reaching its maximum, as evinced by the differential count, on the fourth day. Differential cell-count figures are shown in Table III, and it is readily seen that there is an indication of biological activity on the second day. The plate-count figures showed no abnormality on the second day, and even on the third day the yeast cells were estimated at only 27,000 per ml., a figure well within normal limits. The fourth, fifth and sixth days gave plate counts of 81,000, 1,800,000, and 4,200,000 yeast cells per ml. respectively.

Table III

Differential yeast-cell counts on apple juice at 13·5° and atmospheric pressure

Day	Percentage differential count		
	Spherical cells	Ellipsoidal cells	Budding cells
1	56	35	9
2	54	29	17
3	28	48	24
4	9	22	69
5	37	46	17
6	45	47	8

Discussion

It is evident that once the physical factors are permitted to alter in favour of yeast proliferation, very sudden deviations are encountered in the differential count. The percentage of spherical cells rapidly decreases and ellipsoidal and budding cells increase in numbers, and by comparison with the ' normal ' differential-count the abnormality is indicated at an early stage. Thus, under conditions where it is necessary to suppress fermentation, sufficiently early warning is obtained to ensure that corrective measures can be applied at once and in adequate time to prevent undesirable fermentative changes in the chemical composition of the juice.

The same safety margin, and degree of control, is not possible if a plate-count technique is used alone. When the pressure was reduced, and the temperature maintained at 0°, proliferation of the yeast cells was very slow. Up to a period of three days no variation in the total numbers of yeast cells was observed, and even up to the 17th day the total number of cells was well within the ' normal ' limits of variation based on direct counts on stored juice. However, the differential cell-count gave indications of abnormality on the second day, which were confirmed on the third day.

In the second experiment, where pressure was reduced to 1 atm., and the temperature increased to 13·5°, significant changes in the total plate-count figures were obtained on the fourth day, but it must be remembered that an additional two days' incubation of plates is required as a minimum, and therefore five days would elapse before the count could be assessed. However, the differential cell-count gave an indication of yeast activity on the second day; this was conclusive on the third day.

Summary

As indicated above, the plate-count technique did not show the onset of fermentative changes in stored fruit-juice until four to seventeen days had elapsed from the commencement of biological activity, whereas the differential cell-counting method gave almost immediate indication of abnormality. In the recorded experimental spoilage of juice, the conditions of pressure and refrigeration were drastically altered, and it is unlikely that such extremes would be encountered during plant operation where strict instrumentation is adopted. However, the results obtained from the differential-count are of immense value where routine control of large volumes of fruit juice in storage is necessary, particularly in conditions where agitation of the juice itself is not desirable.

Under the particular conditions of apple-juice storage described, the maximum figures of

12% of budding cells and 46% of ellipsoidal cells have been accepted as a ' normal ' differential-count. If at any time either of these figures is exceeded incipient yeast-cell spoilage is indicated.

Acknowledgment

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Cantrell & Cochrane Ltd.
Hanworth Road
Sunbury-on-Thames
Middlesex

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Reference

[1] Marshall, C. R., & Walkley, V. T., *Food Res.*, 1952, **17**, 212

THE DETERMINATION OF LIGNIN IN PLANT TISSUES: USE OF A CONTINUOUS-EXTRACTION METHOD TO REMOVE INTERFERING MATERIALS

By D. MACDOUGALL

The continuous removal of extractants during the lignin pretreatment procedure has been accomplished. In this study all the pretreatment extractions were carried out in a Soxhlet extractor. No transferring or filtering was necessary. Pretreatment involving a 6-hour continuous acid extraction gives a final lignin value similar to that obtained by refluxing with acid for 3 hours in the conventional manner. Continuous extraction with acid for 9 hours removes almost all the acid-soluble material from plant tissue. The lignin isolated by the modified procedure is lower in both nitrogen and methoxyl than that isolated by the standard A.O.A.C. pretreatment procedure. The modified method is readily applicable to routine analytical determinations of lignin.

Introduction

The necessity for removal of as large a proportion as possible of the carbohydrate and nitrogenous fractions of plant material before carrying out the final isolation of lignin with cold concentrated mineral acid has long been recognized. The pretreatment procedure most commonly employed involves exhaustive Soxhlet extraction with an ethanol–benzene mixture, refluxing with water, and refluxing with dilute mineral acid.[1] It is generally assumed that the errors avoided by use of this pretreatment procedure are much greater than any caused by solution of lignin in the solvents used.

With certain materials it is possible that the pretreatment procedures may introduce a considerable error. Cohen & Harris[2] found that treatment of wood with dilute sulphuric acid resulted in a filtrate from which a humin-like material, with properties similar to those of lignin, could be isolated. The possibility of obtaining residues of this type is much greater with tissues that contain a large amount of protein than it is with wood; the possibility of hydrolysis of reactive lignin groupings and subsequent condensation with carbohydrate or protein hydrolysis products must also be remembered. Refluxing with dilute mineral acid for three hours should be ample for the formation of humin-like materials or for condensation reactions such as those suggested above.

Norman[3] studied the effectiveness of dilute acid hydrolysis in removing nitrogenous material before the final lignin determination. He found that successive short-time extractions from straw and oak wood, with 5% sulphuric acid, gave lignin yields that were lower than those obtained when one long-time hydrolysis was used. In view of this result an extraction method that would separate the products of hydrolysis as rapidly as possible from the residue seemed desirable. For this purpose MacDougall & DeLong[4] attempted an extraction in which a continuous supply of fresh 1% hydrochloric acid was passed through the sample. Although

the lignin isolated by this procedure contained less nitrogen than that obtained by the conventional refluxing method it also contained less methoxyl. The authors suggested that there might have been some demethoxylation of the lignin.

Because of this, and since the A.O.A.C. pretreatment procedure[1] is cumbersome and not readily applicable to routine analyses, it was decided to develop a simplified pretreatment by continuous acid extraction.

Experimental

Modification of extraction procedure

In order to avoid laborious handling of material, the use of a Soxhlet extractor for all pretreatments was desirable. For acid extractions a 1-l. boiling flask was used, and the strength and volume of the hydrochloric acid were adjusted so as to give an acid concentration of approximately 1% (w/w) passing through the thimble. This was found to be the case when the boiling flask contained 500 ml. of 12·5% (w/w) hydrochloric acid. The extractors were 30 mm. in diameter and the thimbles 80 mm. × 25 mm. In order to obtain proper refluxing it was found necessary to use Friedrichs condensers, and the rate of extraction was so maintained that the acid fell from the cold finger of the condenser at the rate of 45 drops per minute. To prevent overheating of the extractor, and to allow efficient filling and siphoning, each flask was placed on a piece of Transite (asbestos-cement), 8 in. square, in which a hole 3·5 in. in diameter had been cut.

Coarse-grade alundum thimbles gave satisfactory extraction if the bottom was largely removed and a pledget of glass wool inserted, but the residues so obtained were contaminated by a large and variable amount of material from the alundum. Ordinary paper thimbles disintegrated during acid extraction unless they were centred in the extractor (by means of small glass rods) and a plug of glass wool was placed in the extractor above the thimbles. There was a small but constant loss of weight from the paper thimbles, for which a correction was made. Alundum thimbles were used in the experiments on fresh tissue and paper thimbles were used for air-dried material.

In all extractions the acid was renewed after one hour and acid extraction was always followed by a one-hour water extraction to remove the acid.

Use of the continuous-extraction method in the determination of lignin

The material used in this study was meadow fescue cut at four different stages of growth. Harvesting dates were 23 May, 6 June, 20 June and 4 July, 1949. With each sample, a portion of the fresh material was extracted according to the method of MacDougall & DeLong[4a] and the remainder was air-dried at room temperature. The air-dried material was ground in a Wiley mill to pass a 0·5-mm. screen.

Lignin determinations were carried out by the method of Manning & DeLong.[5] Lignin for nitrogen and methoxyl determinations was isolated by using naphthalene as a filter-aid; this technique was originally described by Mueller & Hermann.[6] Nitrogen was determined by the micro-Kjeldahl method, with the digestion mixture recommended by Campbell & Hanna.[7] Methoxyl determinations were carried out by the Vieboch & Schwappach modification of the Zeisel method described by Clark.[8] Samples were weighed in gelatin capsules and a suspension of red phosphorus was used in the scrubber. These modifications were recommended by Samsel & McHard.[9] The hydriodic acid was prepared in the way described by Clark.[10]

Discussion

The results of the experiments carried out on undried material are shown in Tables I and II. In this study the method previously described by the author[4a] for fresh tissue was used. In order to determine the efficiency of the continuous acid extraction, the usual 3-hour refluxing method was replaced by a 6-hour Soxhlet extraction on a portion of the sample obtained at each stage of growth.

The results in Tables I and II show that the continuous-extraction and standard refluxing methods give practically identical results in every case. Slightly less nitrogen is extracted from the young material by the continuous-extraction method than by the usual procedure. Examination of the results in Table II shows that the two procedures give almost identical lignin results. The nitrogen and methoxyl contents of the isolated lignin are also very similar.

Although the use of undried material is to be preferred[4a] it is not always convenient to carry out analyses immediately after harvesting. Routine analyses are much more commonly carried out on dry, ground plant-tissue. Therefore, the conventional and continuous-extraction

Table I

Comparison of conventional and continuous-extraction methods on undried tissue

Harvesting date	Treatment	Extractive-free residue, %	Nitrogen in extractive-free residue, %	Original nitrogen in residue, %
23 May	A	32·6	1·51	20·4
	B	35·5	1·57	23·2
6 June	A	29·9	0·83	15·9
	B	31·6	1·04	21·1
20 June	A	39·4	0·70	24·8
	B	41·6	0·68	25·4
4 July	A	37·7	0·81	25·2
	B	40·3	0·74	24·6

A: Three extractions of fresh material in Waring Blendor with ether-saturated water; 3-h. refluxing with 1% hydrochloric acid; 30-h. Soxhlet extraction with ethanol–benzene

B: Three extractions of fresh material in Waring Blendor with ether-saturated water; 6-h. Soxhlet extraction with 1% hydrochloric acid; 30-h. Soxhlet extraction with ethanol–benzene

Table II

Yields and composition of lignin fractions obtained by conventional and continuous-extraction methods on undried tissue *

Harvesting date	Treatment	Lignin,† %	Methoxyl in lignin, %	Nitrogen in lignin, %
23 May	A	4·46	5·26	5·47
	B	4·54	6·64	6·22
6 June	A	3·76	7·95	3·43
	B	3·89	7·56	4·38
20 June	A	7·21	12·11	2·26
	B	6·33	12·65	1·87
4 July	A	8·32	14·57	2·07
	B	8·06	11·36	2·40

* The pretreatment procedures to which A and B refer are shown in the footnotes to Table I

† Lignin, nitrogen, and methoxyl values are calculated on an ash-free basis

procedures were compared on samples which had been prepared in this way. For this experiment, all samples were extracted for 30 hours in the Soxhlet apparatus with ethanol–benzene (1 : 2). This was followed by Soxhlet extraction with water for three hours and with acid for 3, 6, or 9 hours. The samples prepared by the standard procedure were refluxed for 3 hours with water and for a further 3 hours with 1% hydrochloric acid. The results are shown in Tables III and IV. Each result shown is the mean of two experiments. Excellent reproducibility was obtained with the continuous-extraction procedure. The mean average deviation was approximately 2% of the weight of the residue after the pretreatments. The reproducibility was somewhat better with the 6-hour and 9-hour extractions than with the 3-hour extractions.

Examination of the results in Table III shows that as far as the amount of residue obtained and its nitrogen content are concerned, a 6-hour continuous extraction is approximately equivalent to the standard 3-hour refluxing method. Continuous extraction for 9 hours removes considerably more of the original nitrogen than is removed by the standard procedure. The results in Table IV show that, in general, a 9-hour continuous extraction results in less lignin containing less nitrogen than does the ordinary refluxing method. Comparison of the final column of Table III with the final column of Table IV shows that in cases where the removal of nitrogen during the pretreatment extractions was inefficient more nitrogen was removed during the final lignin determinations than in cases where an efficient preliminary extraction was obtained. Apparently the last portion of the nitrogen is extremely resistant to removal by hydrolysis.

The total amount of methoxyl isolated by the continuous-extraction method is less than that obtained by the standard procedure. This was true even when the time of continuous extraction was only 3 hours. Similar results were obtained by MacDougall & DeLong.[4a] These results are difficult to explain. As the residue obtained after 3 hours of continuous extraction is much greater than that remaining after 3 hours of refluxing an explanation based on more

Table III

Comparison of conventional and continuous-extraction methods on air-dried material

Harvesting date	Treatment	Extractive-free residue, %	Nitrogen in extractive-free residue, %	Original nitrogen in residue, %
23 May	C 3 h.	34·4	2·41	34·4
	C 6 h.	30·3	1·59	19·9
	C 9 h.	28·5	1·38	16·3
	Standard	31·9	1·53	20·2
6 June	C 3 h.	37·5	1·89	45·1
	C 6 h.	31·1	1·14	22·5
	C 9 h.	31·7	0·89	18·0
	Standard	33·2	1·43	30·2
20 June	C 3 h.	48·8	1·48	65·1
	C 6 h.	41·2	1·01	37·5
	C 9 h.	37·7	0·89	30·2
	Standard	39·2	1·23	43·4
4 July	C 3 h.	45·0	1·64	61·0
	C 6 h.	38·2	1·39	43·9
	C 9 h.	35·7	1·14	33·6
	Standard	40·4	1·23	41·1

C: Extraction in Soxhlet for 30 h. with ethanol-benzene; 3 h. with water; 3, 6 or 9 h. with 1% hydrochloric acid

Standard: Extraction in Soxhlet for 30 h. with ethanol–benzene; reflux 3 h. with water; reflux 3 h. with 1% hydrochloric acid

Table IV

Yields and composition of lignin fractions obtained by conventional and continuous-extraction methods on air-dried tissue *

Harvesting date	Treatment	Lignin,† %	Methoxyl in lignin, %	Nitrogen in lignin, %	Original nitrogen in lignin, %
23 May	C 3 h.	6·85	3·35	6·32	17·9
	C 6 h.	4·49	4·95	5·19	9·7
	C 9 h.	4·35	4·69	4·40	7·9
	Standard	4·98	6·07	5·12	10·6
6 June	C 3 h.	6·09	5·95	5·01	19·4
	C 6 h.	5·24	6·38	4·78	16·0
	C 9 h.	4·73	9·09	3·64	11·0
	Standard	6·06	6·79	4·20	16·2
20 June	C 3 h.	9·58	8·45	4·07	35·1
	C 6 h.	8·47	8·52	3·58	27·3
	C 9 h.	7·45	9·45	3·16	21·2
	Standard	8·28	10·26	3·31	24·7
4 July	C 3 h.	10·60	9·06	4·31	37·8
	C 6 h.	10·21	9·12	3·99	33·7
	C 9 h.	9·50	9·29	3·75	29·4
	Standard	9·99	10·91	3·13	25·8

* The pretreatment procedures to which C and Standard refer are shown in the footnotes to Table III

† Lignin values are not corrected for ash

efficient removal of methoxyl-containing carbohydrate during continuous extraction is impossible. An explanation based on solution of lignin in the continuous extraction but not with the refluxing procedure would also seem to be untenable. Demethoxylation of lignin in one case but not in the other would seem to be unlikely. It is suggested that during the refluxing procedure methoxyl-containing residues may condense with themselves or with the lignin and thus increase the total amount of methoxyl eventually isolated.

Conclusion

The continuous removal of extractants during the lignin pretreatment procedure has been accomplished in Soxhlet extractors. More time is required to remove an equivalent weight of the original material by the continuous-extraction method than by the standard refluxing procedure. The lignin isolated by this method contains less nitrogen than that isolated by the standard A.O.A.C. pretreatment procedure and would, therefore, appear to contain less contaminating material. On the other hand, the lower methoxyl content of the lignin isolated by the continuous-extraction method is surprising. It is suggested that contamination of lignin with methoxyl-containing residues may be more serious than has generally been recognized.

The method described avoids a great deal of the handling required with the methods now in use and is readily adaptable to routine analytical determinations.

Department of Chemistry
Ontario Agricultural College
Guelph, Ontario, Canada

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References

[1] 'Methods of Analysis of the A.O.A.C.', 1950, 7th edn., p. 109 (Washington, D.C.: Association of Official Agricultural Chemists)
[2] Cohen, W. E., & Harris, E. E., *Industr. Engng Chem.* (*Anal.*), 1937, **9**, 234
[3] Norman, A. G., *Biochem. J.*, 1937, **31**, 1567
[4] MacDougall, D., & DeLong, W. A., *Canad. J. Res.*, 1948, [B] **26**, 457
[4a] MacDougall, D., & DeLong, W. A., *Canad. J. Res.*, 1948, [B] **26**, 468
[5] Manning, K. R., & DeLong, W. A., *Sci. Agric.*, 1941, **22**, 69
[6] Mueller, W. J., & Hermann, W., *Papierfabrikant, Tech.-Wiss. Teil*, 1926, **24**, 185; *Chem. Abstr.*, 1926, **20**, 2746
[7] Campbell, W. R., & Hanna, M. I., *J. biol. Chem.*, 1937, **119**, 1
[8] Clark, E. P., *J. Ass. off. agric. Chem. Wash.*, 1939, **22**, 100
[9] Samsel, E. P., & McHard, J. A., *Industr. Engng Chem.* (*Anal.*), 1942, **14**, 750
[10] Clark, E. P., *Industr. Engng Chem.* (*Anal.*), 1938, **10**, 677

THE STABILITY OF VITAMIN A IN ANIMAL FEEDING-STUFFS

By ALAN W. DAVIES and ALASTAIR N. WORDEN

The stability of vitamin A in mixed rations has been studied under a variety of storage conditions. With vitamin A, added in the form of cod-liver oil (C.L.O.), stability is greater in a mash of medium particulate size than in one of coarse particulate size; this effect has been constant when light, temperature and the nature of the container, with corresponding variations in the degree of exposure to atmospheric oxidation, have been varied. The destructive action of mineral salts on this form of vitamin A has been confirmed, and found to be reversed by the addition of gelatin. The stability of vitamin A in cod-liver oil is greater in the presence of fish meal or liver meal, but less in the presence of dried brewer's yeast, than in the presence of various cereal products. 'Cubing of the diet' asserts a protective action on vitamin-A palmitate. In the particular conditions of our experiments, the stability of vitamin-A palmitate is greater than that of vitamin A in the form of C.L.O. when added to a mixed meal.

Introduction

That the vitamin-A content of a compounded ration may fall rapidly has long been recognized, and losses in carotene also are known to occur. Those whose work has a bearing upon this problem, or upon the stability of vitamin A or carotene in the preparations employed, include Fridericia,[1] Powick,[2] Mattill,[3] Holmes *et al.*,[4] Marcus,[5] Fraps & Kemmerer,[6] Lachat & Halvorson,[7] Schroeder *et al.*,[8] Whipple,[9] Lowen *et al.*,[10] Miyauchi & Sanford,[11] Record *et al.*,[12] Baird *et al.*,[13] Bethke *et al.*,[14] Heywang & Morgan,[15] Holder & Ford,[16] Almquist & Zander,[17] Brocklesby & Green,[18] Simons *et al.*,[19] Gray & Robinson,[20] Parker *et al.*,[21] Sherwood & Fraps,[22] Buxton,[23] Miller *et al.*,[24] Morgal *et al.*,[25] Williams *et al.*,[26] Williams *et al.*,[27] Temperton & Dudley,[28] Halverson & Hart,[29] Burns & Quackenbush,[30] Ringnes[31] and Tunnicliffe.[32] The problem of vitamin-D destruction in compound foodstuffs has recently been studied separately by Bauwen & van den Driessche.[33]

Ewing[34] has provided a detailed review of many of the factors affecting the stability of vitamins in feeding-stuffs, and his work should be consulted for a résumé of the role of anti-oxidants, charcoal, minerals, gelatin, fish and meat scraps and rancidity. Temperton & Dudley[28] concluded that preformed vitamin A in fish-liver oil was an unreliable source of the vitamin to be included in mash mixtures for adult fowls and growing chickens. When added at as high a concentration as 4086 i.u. of vitamin A per lb. in a starter ration for chickens during the first four weeks, it failed to prevent heavy mortality from A-avitaminosis. Similarly a concentration of 5720 i.u. of vitamin A in breeder rations was inadequate even for maintenance, and led eventually to the syndrome of advanced vitamin-A deficiency. This lack of protection was due to a rapid and extensive destruction of the vitamin after its addition in oil solution to the mashes. By chemical and biological assay it was demonstrated that losses of up to 100% of the added vitamin A took place within 24 hours of mixing. The factor responsible for the destruction was not identified, but appeared to be present in the ether extract of the fortified mashes.

Ringnes[31] reported that a premix powder prepared from synthetic-vitamin-A palmitate, and containing initially 80,000 i.u. of vitamin A/g., showed excellent stability under laboratory conditions. In an accelerated storage test, in an open container, approximately 5% of the vitamin-A activity of the powder was lost during 21 days at 45°. When mixed with a chick ration and stored at room temperature, 30% of the vitamin-A activity was lost after three months. Losses were greater when the powder came into contact with mineral salts; some 55% of the vitamin-A activity was lost after storage at 45° for 500 hours with a mineral-salt mixture. In similar conditions, 80 to 90% of the vitamin-A activity of cod-liver oil (C.L.O.) was lost. A protected form of premix powder (details not given) was found to lose only 15% of its vitamin-A activity under these conditions of storage.

Tunnicliffe[32] carried out an extensive series of observations on the effect of mixing 1% of C.L.O. with different foodstuffs. Mixing was carried out in the cold, and the storage was in very small quantities so as to permit maximum exposure to oxidation. Rapid and complete destruction of vitamin A occurred when the C.L.O. was mixed with sodium chloride, limestone flour or precipitated calcium carbonate. The destructive effect of calcium carbonate was shown to be mitigated considerably by premixing the C.L.O. with weatings. With a standard chick mash approximately 34% had been destroyed by the 14th day, 47% by the 32nd day, and 80% by the 67th day.

The problem of vitamin-A destruction has been recognized by compound-feeding-stuff manufacturers. Some commercial pig and poultry mashes contain natural stabilizing agents, and it is a common practice to put in a large surplus of vitamin A so that, even after storage for several weeks, there may be sufficient of the vitamin present to provide for the needs of the animals concerned.[35]

On oxidation, vitamin A forms free β-ionone residues and these possess a somewhat characteristic smell that may be of practical value in assessing whether a particular sample of feeding-stuff has 'gone off' in respect of its vitamin-A content.

The problem of vitamin-A destruction is not only important in itself, but may be linked, in fish-liver oils, with the possible coincident formation of products that may be toxic. This aspect will be dealt with in a subsequent publication.

The present studies were begun in the Department of Animal Health, University College of Wales, Aberystwyth, and form part of a long-term research project that is still in progress. This interim report deals with certain observations that have been made on (i) the effect of particulate size of the mash, temperature, light, air and specific ingredients on the destruction of vitamin A in added C.L.O.; (ii) the effect of 'cubing the diet' (preparing the diet as cubes) on the stability of added synthetic vitamin A; and (iii) the protective value of gelatin. A preliminary account of some of our findings has appeared elsewhere.[36]

Experimental

(1) *The effect of particulate size of the mash, temperature, light, air and specific ingredients on the destruction of vitamin A in added C.L.O.*

The mixed ration employed for these experiments was comprised of the following parts by weight: ground wheat 37·0, ground oats 30·0, ground barley 15·0, weatings 10·0, maize-gluten meal 5·5 and dried yeast 2·5. A high-grade C.L.O. (potency 700 i.u./g.) was included to give a concentration of 2%. To ensure even distribution of the oil in the meal, 40 g. of oil was added to 160 g. of meal and thoroughly mixed by hand. As the mass became uniform successive 200-g. lots were added and thoroughly mixed until a total weight of 2 kg. was obtained. This procedure was adopted for meals of both medium and coarse particulate size

and also for meals containing a commercial mineral mix. Samples were taken for immediate assay of vitamin A and the following method was used for the storage test with the coarse particulate size meal.

Sample No.	Container	Method of storage	Temperature, °c
1	Shallow trays 8 in. × 10 in. × 1 in.	Laboratory shelf	15·5
2	,, ,, ,,	Dark room	15·5
3	,, ,, ,,	Cold storage	5
4	Amber-colour screw-capped jar, 1-l. capacity	Laboratory shelf	15·5
5	,, ,,	Dark room	15·5
6	,, ,,	Cold storage	5
7	Clear-glass screw-capped jar, 1-l. capacity	Laboratory shelf	15·5
8	,, ,,	Dark room	15·5
9	,, ,,	Cold storage	5

The same procedure was adopted for 9 further samples (Nos. 10–18) comprising coarse-ground meal but containing 2·5% of a commercial mineral mix. The storage procedure was repeated with 18 samples of meal of medium particulate size, stored without (Nos. 19–27) and with (Nos. 28–36) 2·5% of commercial mineral mix.

Vitamin assay.—For the assay of vitamin A, the standard Carr–Price procedure, described by the Association of Vitamin Chemists (1947), was employed. The extracts were also subjected to chromatographic separation by methods essentially similar to those of Thompson *et al.*[37] As with the results reported by Halverson & Hart[29] the two methods gave results that were in complete agreement. All samples were analysed in triplicate and the reported results are the mean of the 3 assays. Excellent agreement between assays was obtained, the variation being $< \pm 2\%$. Results obtained during storage are shown in Tables I and II.

Stability of vitamin A in specific ingredients.—In order to determine whether any specific ingredient of the meal accelerated vitamin-A destruction, C.L.O. (potency, 700 i.u. of vitamin A/g.) was incorporated at a concentration of 2% in some ingredients commonly employed in animal feeding-stuffs. This provided an initial concentration of 13·5 i.u. of vitamin A/g. of mix. The mixes were stored in screw-capped amber-coloured glass jars of 500 ml. capacity, at room temperature. The results are shown in Table III.

(2) *Effect of cubing the diet on the stability of synthetic vitamin A and of carotene*

Experiments were carried out on a commercial diet of the following composition in parts by weight: maize meal 6, barley meal 40, bran 10, maize-germ meal 2, palm 2, pea meal 4, dried-grass meal 4, white-fish meal 6, extracted decorticated groundnut-meal 2 and Sandilands mineral mixture No. 2, 3. This diet was fortified with vitamin-A palmitate (Roche) to a concentration of 4464 i.u./lb.

Table I

Deterioration of vitamin A in meal of coarse particulate size, with and without added mineral salts

Sample No.	Storage, days														
	0	3	7	10	14	17	21	34	42	56	63	74	88	92	106
	Residual vitamin A, %														
	Without added mineral salts														
1	100	100	100	85	60	14	0								
2	100	100	100	95	82	50	29	20	12	0					
3	100	100	100	100	85	66	50	45	40	25	16	10	0		
4	100	100	100	88	73	68	61	40	35	26	22	0			
5	100	100	100	95	85	72	64	60	54	48	35	21	20	10	
6	100	100	100	100	93	85	84	78	72	67	50	36	29	20	
7	100	100	100	100	95	92	76	69	37	20	0				
8	100	100	100	100	100	93	90	85	68	50	20	20	0		
9	100	100	100	100	100	96	92	88	88	80	63	40	35	32	20
	With added mineral salts														
10	100	60	0												
11	100	100	77	37	0										
12	100	100	100	100	88	44	37	0							
13	100	100	85	70	48	25	0								
14	100	100	100	78	64	44	38	34	26	8	0				
15	100	100	100	96	84	77	51	46	40	29	20	12	0		
16	100	100	100	60	51	37	0								
17	100	100	100	87	76	40	25	20	0						
18	100	100	100	86	74	44	40	40	26	0					

Table II

Deterioration of vitamin A in meal of medium particulate size, with and without added mineral salts

Sample No.	Storage, days														
	0	3	7	10	14	17	21	34	42	56	63	74	88	92	106	120
	Residual vitamin A, %															
	Without added mineral salts															
19	100	100	100	94	77	70	28	0								
20	100	100	100	100	96	87	65	55	38	—	20	12	0			
21	100	100	100	100	90	86	80	60	54	45	39	26	20	20	0	
22	100	100	100	100	91	79	71	56	50	39	35	20	0			
23	100	100	100	100	100	83	76	69	62	53	43	40	35	20	0	
24	100	100	100	100	100	100	95	80	76	67	58	50	50	40	40	35
25	100	100	100	100	90	79	72	61	52	43	30	0				
26	100	100	100	100	100	92	86	80	67	58	43	27	25	20	20	0
27	100	100	100	100	100	100	95	90	88	80	66	50	45	40	32	15
	With added mineral salts															
28	100	100	58	40	0											
29	100	100	100	86	74	37	10	0								
30	100	100	100	100	100	88	51	30	22	0						
31	100	100	100	100	100	84	66	42	24	16	0					
32	100	100	100	100	92	78	59	42	37	20	16	0				
33	100	100	100	100	100	96	72	66	58	43	27	18	0			
34	100	100	100	100	77	44	37	20	0							
35	100	100	100	100	88	60	55	38	27	10	0					
36	100	100	100	100	100	92	59	50	32	20	10	0				

Table III

Stability of vitamin A in individual ingredients

Ingredient	Storage, days												
	7	14	21	28	42	56	60	74	88	102	116	130	144
	Residual vitamin A, %												
Weatings	80	80	76	70	70	65	62	60	—	54	50	—	30
Barley meal	90	88	—	80	—	72	—	58	—	50	39	35	20
Ground oats	78	—	70	—	56	—	45	—	33	—	25	25	20
Dried yeast	55	32	28	25	22	10	5	0					
Fish meal	98	95	—	95	—	90	84	80	—	72	—	65	60
Liver meal	95	—	90	—	86	—	72	—	60	—	50	—	55
Commercial mineral mix	20	0											

A portion of the fortified diet (A) was stored as such in paper bags at room temperature. A further batch was subjected to a commercial cubing process and a representative sample (B) of the resultant cubes was stored similarly. A part of the original diet was fortified with C.L.O. (potency 1238 i.u./g.) so as to provide a concentration of 9·64 i.u. of vitamin A/g. of diet. A representative sample (C) was subjected to the same storage procedure.

At weekly intervals aliquot portions of the 3 samples were analysed for carotene and vitamin A, carotene by a procedure essentially similar to that adopted by Halverson & Hart.[29] Results are shown in Table IV.

(3) *Protective value of gelatin*

A commercial mineral mixture (4 g.) containing salts of calcium, phosphorus, iron, copper and cobalt, and iodine, was dissolved in 50 ml. of water at 100°. Gelatin (20 g.) was added to this solution and the whole was stored until homogeneous. The product was then allowed to cool rapidly by placing it in shallow trays in a refrigerator at − 5°. After being cooled this gelatin mix was dried in a vacuum desiccator over phosphorus pentoxide for 36 hours. After a further period of 48 hours in an electric oven at 65° the gelatin became sufficiently brittle to be ground in a household coffee-grinder to a medium particulate size.

To 190 g. of a diet comprising 98 parts by weight of the mixed meal described in (1), but containing 2% of C.L.O. (potency 700 i.u. of vitamin A/g.), was added 10 g. of the gelatin–mineral mix. This gave a concentration of commercial mineral mixture of approximately 1·0%. For the control ration, 8 g. of unadulterated ground gelatin and 2 g. of commercial mineral mix were added separately (i.e. without premixing) to 190 g. of the mixed meal as employed in section (1).

The rations were then stored in paper bags at room temperature. Samples were taken

Table IV

Effect of cubing on stability of synthetic vitamin A

Storage, days	Meal + vitamin-A palmitate		Cubes (meal + vitamin-A palmitate)		Meal + C.L.O.	
	Vitamin A	Carotene	Vitamin A	Carotene	Vitamin A	Carotene
0	100	100	100	100	100	100
7	100	100	100	100	71·5	100
14	100	100	100	100	50	100
21	90	100	100	100	0	100
28	66	100	83·5	100		100
35	50	93	68	100		90
42	34	93	68	100		80
56	27	77	67	100		80
70	27	77	66·5	100		80
77	20	77	66·5	100		70
84	20	69	66·5	100		60
91	20	69	66·7	80		60
98	20	62	66·5	80		60
126	0	38	34	32		40
133		21	0	16		40

at intervals and vitamin A was assayed according to the method previously described. The results are shown in Table V.

Table V

Protective value of gelatin for meal of medium particulate size

Ration	Storage, days											
	0	7	14	21	28	35	42	49	56	63	70	84
	Residual vitamin A, %											
Gelatin–mineral mix	100	100	92	80	65	60	50	50	40	35	20	5
Control, free of mineral salts and unprotected	100	65	40	20	0							

The stability of the ration with mineral salts added in protected form is almost identical with that of the ration of medium particulate size without mineral salts, stored at room temperature in clear-glass jars

Discussion

It is appreciated that experiments of the character reported in this paper are being carried out in many different laboratories in the United Kingdom and elsewhere, and that publication of many of the results has been deferred in the hope of devising satisfactory means of stabilizing vitamin A that would be applicable on a commercial scale.

It is thought, however, that the publication of current results is justified, since the problem is of considerable significance not only commercially but to the health of poultry and other livestock generally.

Our results indicate that, under a wide variety of storage conditions, the loss of vitamin A is greater in a ration of coarse particulate size than in one of a medium particulate size. On the other hand, cubing has been found to protect vitamin A from destruction, and this protective action is even more marked with carotene. The adverse effect on vitamin-A stability of incorporating mineral salts with the ration, without taking steps to prevent their coming into close contact with the vitamin, has been amply confirmed. Some natural foodstuffs appear to assert protective action. Thus, loss of vitamin A occurs more slowly in the presence of either fish meal or liver meal than in the presence of various cereal products. Dried brewer's yeast does not confer any protection, however, and the loss of vitamin A in its presence appears to be more rapid than in the presence of cereal products. The protective value of gelatin against loss of vitamin A has been clearly demonstrated even in the presence of mineral salts that otherwise promote a rapid destruction. This is in agreement with the independent findings of Halverson & Hart,[29] whose results have been published since our relevant experiments were undertaken. They are consistent also with the well-known fact that gelatin confers a protective action on vitamin D; this is the basis of certain current commercial processes in the pharmaceutical field. With the manufacture of compound feeding-stuffs it seems doubtful whether a process employing gelatin would be economically feasible, but it is encouraging to have demonstrated that protection of vitamin A in a mixed meal containing mineral salts is possible. Burns & Quackenbush[30] have described successful stability trials in which the vitamin A was sealed into the carrier by a process that involved the use of microcrystalline wax. Coles & Thomas,[38] who cite the observations of earlier workers on the stability of

vitamin A in oil solution, have prepared aqueous dispersions of vitamin-A alcohol employing 'Lubrol W', which contains polyoxyethylene cetyl–stearyl alcohol, as the dispersing agent, and have found the resultant stability to be greater than that in arachis oil.

It is perhaps of interest to record that we have recently examined the vitamin-A content of a C.L.O. emulsion (Olyocream) that had been subjected to shelf storage for a period of exactly ten years. The vitamin-A content was still almost 60% of that present on manufacture in 1942, and no trace of the pink coloration indicative of oxidation was found during the estimation.

In the particular set of conditions operating in section (2) above, the stability of vitamin-A palmitate in a commercial premix (Roche) was considerably greater than that of vitamin A in C.L.O. From the interim results of various unpublished experiments in progress elsewhere, it is apparent that the relative stability of 'synthetic' vitamin A and of vitamin A in C.L.O. shows considerable variation in different circumstances. In the absence of precise information on the nature of the factors that facilitate or retard destruction of the vitamin, this variation is difficult to interpret, although satisfactory preparation of the premix is obviously of the greatest importance. It would appear to be advisable to determine the best source of vitamin A, and the means of protecting it against destruction, by actual tests employing the ingredients in question. Further, it must be emphasized that the value of adding C.L.O. does not lie solely in its vitamin-A content. As MacLennan[39] has stressed, the farmer is advised whenever possible to purchase C.L.O. as such, and to add it to the ration immediately before feeding; a spray-gun is suitable for this purpose.

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References

[1] Fridericia, L. S., *J. biol. Chem.*, 1924, **62**, 471
[2] Powick, W. C., *J. agric. Res.*, 1925, **31**, 1017
[3] Mattill, H. A., *J. Amer. med. Ass.*, 1927, **89**, 1505
[4] Holmes, A. D., Pigott, M. G., & Menard, D. F., *Poult. Sci.*, 1930, **10**, 37
[5] Marcus, J. K., *J. biol. Chem.*, 1931, **90**, 507
[6] Fraps, G. S., & Kemmerer, A. R., *Bull. Tex. agric. Exp. Sta.*, 1937, No. 557
[7] Lachat, L. L., & Halvorson, H. A., *Poult. Sci.*, 1937, **16**, 147
[8] Schroeder, C. H., Redding, G. K., & Huber, L. J., *Poult. Sci.*, 1936, **15**, 104
[9] Whipple, Dorothy V., *Oil & Soap*, 1936, **13**, 231
[10] Lowen, E. J., Anderson, D., & Harrison, R. W., *Industr. Engng Chem.* (*Industr.*), 1937, **29**, 151
[11] Miyauchi, D., & Sanford, F. B., *Comm. Fish. Rev.*, 1937, **9**, 7
[12] Record, R. P., Bethke, R. M., & Wilder, O. H. M., *Poult. Sci.*, 1937, **16**, 25
[13] Baird, F. D., Ringrose, A. T., & MacMillan, M. J., *Poult. Sci.*, 1939, **18**, 441
[14] Bethke, R. M., Record, P. R., & Wilder, O. H. M., *Poult. Sci.*, 1939, **18**, 179
[15] Heywang, B. W., & Morgan, R. B., *Tech. Bull. Bur. Anim. Ind., U.S. Dep. Agric.*, 1939, No. 632
[16] Holder, R. C., & Ford, S. K., *Poult. Sci.*, 1939, **18**, 345
[17] Almquist, H. J., & Zander, D., *Proc. Soc. exp. Biol., N.Y.*, 1940, **45**, 303
[18] Brocklesby, H. M., & Green, H., *Rep. Fish. Res. Bd Can.*, 1940, No. 46, p. 4
[19] Simons, E. J., Buxton, L. O., & Colman, H. B., *Industr. Engng Chem.* (*Industr.*), 1940, **32**, 706
[20] Gray, R. E., & Robinson, J. F., *Poult. Sci.*, 1941, **20**, 36
[21] Parker, W. E., Neish, A. C., & McFarlane, W. D., *Canad. J. Res.*, 1941, **19**, 941
[22] Sherwood, R. M., & Fraps, G. S., *Bull. Tex. agric. Exp. Sta.*, 1936, No. 528
[23] Buxton, L. O., *Industr. Engng Chem.* (*Industr.*), 1942, **34**, 1486
[24] Miller, M. W., Joukovsky, V., & Hokenstad, N., *Poult. Sci.*, 1942, **21**, 200
[25] Morgal, P. W., Byers, L. W., & Miller, E. J., *Industr. Engng Chem.* (*Industr.*), 1943, **35**, 794
[26] Williams, K. T., Bickoff, E., & Van Sandt, W., *Science*, 1943, **97**, 96
[27] Williams, K. T., Bickoff, E., & Lowrimore, B., *Oil & Soap*, 1944, **21**, 161
[28] Temperton, H., & Dudley, F. J., *Harper Adams Util. Poult. J.*, 1947, **32**, 83
[29] Halverson, A. W., & Hart, E. B., *J. Nutr.*, 1950, **40**, 415
[30] Burns, M. J., & Quackenbush, F. W., *Industr. Engng Chem.*, 1951, **43**, 1592
[31] Ringnes, P., *Proc. World's Poult. Congr.* (No. 9, Paris), 1951, **3**, 77
[32] Tunnicliffe, G. E., private communication
[33] Bauwen, M. A., & van den Driessche, S., *Proc. World's Poult. Congr.* (No. 9, Paris), 1951, **2**, 127
[34] Ewing, W. R. 'Poultry Nutrition', 1951, pp. 692, 793 (South Pasadena: W. Ray Ewing)
[35] Worden, A. N., *Vet. Rec.*, 1951, **63**, 89
[36] Davies, A. W., & Worden, A. N., *Vet. Rec.*, 1953, **65**, 318
[37] Thompson, S. Y., Ganguly, J., & Kon, S. K., *Brit. J. Nutr.*, 1949, **3**, 50
[38] Coles, C. L. J., & Thomas, D. F. W., *J. Pharm., Lond.*, 1952, **4**, 898
[39] MacLennan, K., private communication

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