THROUGH-CIRCULATION DRYING OF SEAWEED
I.—*Laminaria cloustoni* stipe

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The drying characteristics of freshly harvested seaweed stipe of the species *Laminaria cloustoni* have been investigated in a laboratory through-circulation drier. The stipes, with a water content of 83–86%, were cut on a bacon slicer into discs $\frac{1}{8}$-in. thick by 1–$1\frac{1}{4}$ in. in diameter.

Experiments on the effect of interruption of drying, initial water content, and repeatability established the experimental procedure before studying the principal factors. Variables studied were bed depth (0·5–7 in.), air temperature (120–340° F.), air wet-bulb depression (44–228° F.) and air velocity [3–9·5 lb./(sq. ft.)(min.)], slice thickness ($\frac{1}{16}$ to $\frac{5}{16}$ in.) with an experiment on agitation. Static pressure drops of air passing through beds of dried and wet stipe slices were also investigated.

As the initial drying rates of the seaweed beds were virtually constant, the value of this constant rate has been correlated with the drying factors. Drying times between water content limits of 5 to 0·15 lb. of water/lb. of bone-dry solids have been related by empirical equations to the drying conditions.

It has been demonstrated that the drying rates of seaweed beds at average water contents of 5 to 0·2 lb./lb. of bone-dry solids are directly proportional to the wet-bulb depression of the air, and a unit wet-bulb depression evaporation coefficient may be used to find approximate drying times and rates.

Tests showed that static beds of seaweed stipe were scorched at temperatures of 250° F. or higher.

Factors to be considered in the design of a continuous through-circulation drier for stipe are given.

Introduction

There are three main reasons for drying seaweed: to reduce transport costs, to enable the material to be stored without bacterial decomposition, and to allow it to be compounded easily with other products (e.g. animal feeding-stuffs). Drying and grinding of the seaweed is frequently a preliminary to the extraction of algal chemicals.

Drying must be cheap and efficient. Through-circulation of heated air appeared a promising method, and the drying characteristics of seaweed were therefore investigated in this type of drier.

Previous work on through-circulation drying

Hop kilns or oasts are natural-draught through-circulation driers. Burgess[1] has described work on hop drying from 1921 to 1938 on four small experimental kilns constructed by the Institute of Brewing. The hop cones are about 1 to 2 in. in length and $\frac{3}{4}$ to 1 in. in diameter, with initial moisture contents of approximately 80%. They are supported on a loosely-woven horsehair cloth which lies on an open slatted floor mounted several feet above an open fire, and are dried to a moisture content of about 6%. Burgess divides the drying time into two periods, the 'minimum time' required to dry a single layer of hops, and the 'extra time' necessary for the level of dryness to rise through the bed of hops. The time required to dry hops from 80 to 2% at a constant air temperature is given by the expression

$$\theta = (P - p)^{-1}(716{\cdot}5L.V^{-1{\cdot}047} + 6250V^{-0{\cdot}39})$$

where P = vapour pressure of water at temperature of drying air, in. of mercury; p = vapour pressure due to humidity of atmosphere, in. of mercury; L = loss in weight during drying, oz./sq. ft. of kiln floor. (Symbols not given will be found in a list at the end of the paper.)

Owen[2] describes a series of experiments on the desiccation of sugar-beet cossettes (2·2 in. × 0·4 in. × 0·2 in.) on the laboratory scale and on the plant scale. The factors influencing drying which were studied included the porosity and depth of the bed of sugar beet, and the volume, pressure, and temperature of the air used. These tests were also concerned with the inversion of the sugar during drying, and it was found that the critical temperature of the material in the moist state was 220° F.

Scott[3] employed through-circulation of air to study the effect of temperature on grass drying. The limiting temperature for this material was 300–350° F., above which temperature, blowholes of scorched grass rapidly developed.

Marshall & Hougen[4] studied a wide variety of materials under similar conditions of through-circulation drying, and showed that the times required were much shorter than corresponding

times for cross-circulation drying. These workers expressed the constant drying rate by the formula:

$$dW/d\theta = kG^{0.81}(H_s - H_a)$$

where k = a constant; H_s = saturation humidity at wet-bulb temperature of air and H_a = humidity of air, lb./lb. of air. The value of k was about 3–6 for clays and pigments, 25–100 for granular solids, and 110–220 for fibres.

This exploratory study was continued by Gamson, Thodos & Hougen.[5] From a study of the drying of wetted catalyst pellets, they derived an equation for the constant rate of evaporation of water from the surface of wet granular solids:

$$dW/d\theta = 0{\cdot}42aG^{0.59}(\Delta H)_m/\rho_s D_p^{0.41}$$

where a = drying area, sq. ft./cu. ft. of bed volume; ρ_s = bulk density of dry granular bed, lb./cu. ft.; D_p = average particle diameter, ft.; $(\Delta H)_m$ = log(mean of inlet and outlet humidity driving forces across the air film on the particle). A more general form of the equation was also given, covering the vaporization of liquids other than water into gases other than air.

When the modified Reynolds number for gases passing through beds of solids is greater than 350, the flow is turbulent, whereas streamline flow occurs when the value is less than 40. Wilke & Hougen[6] gave equations for the constant drying rate when the flow was in the streamline region.

Mounfield[7] investigated the drying of wheat in a batch through-circulation drier, consisting of a vertical cylinder and a conical base with provision for introducing hot air through a grating in the bottom. The wheat was continuously circulated by a vertical worm.

Brown & Van Arsdel[8] studied the through-circulation drying of white potato strips ($\frac{5}{32}$ in. square) and presented their data in nomographic form. The apparatus used was a cabinet drier baffled to direct the air downward through the vegetable bed. Perforated metal sheet was used to create a substantial pressure-drop, thus ensuring a reasonably uniform air distribution at all parts of the bed.

Ede & Hales[9] described tests on vegetable and fruit dehydration with through-draught driers. With vegetables, it was difficult to maintain steady drying conditions during a test owing to the contraction of the material, and little work was done. With fruit, however, the effect of tray loading, air speed, and specific surface on the rate of drying were investigated. Ede & Hales also investigated comprehensively the cross-circulation drying of scalded potato strips, and devised a unit wet-bulb depression evaporation-coefficient to correlate their experiments.

Allerton, Brownell & Katz[10] studied the mechanism of filter-cake drying by through-circulation of air, using glass balls (16–150-Tyler mesh) and crushed quartz (10–24-mesh) with water as the vaporizing liquid. Air flow was mainly in the streamline region. Because of the low air temperature used (85–95° F.), and the large surface exposed, the air left the bed of material virtually saturated. Evaporation was considered to take place in a narrow zone ($\frac{1}{8}$–$\frac{1}{4}$ in.) which passed through the bed. When this zone reached the end of the bed, the initial constant-rate period was succeeded by a falling-rate period. The drying rates were correlated on a vaporization-efficiency basis:

$$r/R = E = [1 - e^{-rW}] \text{ and } [Y = 2{\cdot}72(Re)^{0.215}(d)^{-0.35}W^{0.36}]$$

where r = rate of drying and R = maximum rate of drying, lb./(sq. ft.)(min.); E = vaporization efficiency; Y = drying factor, sq. ft./lb.; d = particle diameter, in.; $Re = DG'/\mu$, where D = particle diameter, ft.; μ = viscosity, lb./(ft.)(sec.); G' = mass velocity, lb./(sq. ft.)(hr.).

Hendry & Scott[11] reviewed different methods of presenting experimental data on drying and showed how these could be applied to the design of full-scale driers.

Drying tests on viscose staple fibre on the laboratory and plant scale were described by Coles.[12] The following formula was derived for the down-draught through-circulation drying of viscose fibre:

$$2{\cdot}3\log_{10} w_0/w = 0{\cdot}356G^{1.23}\theta dH$$

where w_0 and w = the initial and final free moisture content, lb./lb. of bone-dry product; dH = difference between humidity of air saturated at wet-bulb and the actual humidity of the air, lb. of water/lb. of air.

Seaweed drying

Laminaria cloustoni is the predominating sublittoral seaweed plant indigenous to the

coasts of Great Britain. It grows at depths of one to eight fathoms and consists of a rigid upright stipe or stem, surmounted by a flat palmate frond divided into downward-trailing ribbons. The plant anchors itself to rocks by means of a holdfast which resembles in appearance the roots of land plants. The water ratio of the stipe in lb. of water/lb. of B.D.S. (see Nomenclature) varies at different times of the year[13] from about 5 to 7, whereas for the frond it ranges from 3 to 7·3.

Owing to the difficulties of harvesting sublittoral seaweeds, supplies of this plant have hitherto depended almost entirely on cast weed. This was collected by crofters who cut off the fronds and holdfast and spread the stipe over walls to dry in the sun and wind. After prolonged exposure, the water content was reduced to about 50%. The air-dried plants could then be transported to a processing factory.

Mechanical seaweed-harvesting equipment is now being developed by the Institute of Seaweed Research and there is a possibility of regular supplies of freshly collected seaweed for industrial uses. This present work is part of an investigation, sponsored by the Institute of Seaweed Research, into the drying properties of freshly harvested seaweed.

Previous work

Mitchell[14] described some of the industrial uses of red and brown seaweeds and compared the methods of drying seaweed with those used for grass and vegetables.

Clark, Pratt, Coleman & Green[15] described a method of drying *Macrocystis pyrifera*, a seaweed found off the Californian coast. The chopped *M. pyrifera* (87% water) was fed into a rotary drier with an inlet air temperature of 1200–1800° F. and was discharged after 20 minutes with a water content of 40–65%. The seaweed was further dried to 5–15% moisture content on a conveyor-drier by means of air at 200–260° F. and a retention time of 30 minutes. The depth of the seaweed bed was 2–3 in.

Raw material

The seaweed used in this work was harvested from two areas (Kerrera Island, near Oban, and Inchcolm Island, Firth of Forth) by a multi-pronged grapnel towed from a motor launch. The fronds and holdfast were removed, and the stipes dried, generally within 24 hours of harvesting.

As a set of drying tests required several weeks for completion, it was impossible to ensure that all the tests were strictly comparable, because of variations in the biological nature of the material. Whenever possible, tests on the effect of one variable were performed consecutively, to reduce this variation to a minimum.

Apparatus

The laboratory drier (Fig. 1) consists of a centrifugal fan, directly coupled to a d.c. motor, which blows the air over eight 1-kw. bar-elements into a plenum chamber at the base, and thence upwards through a vertical duct (12 in. square) in which the basket of wet material rests on stout wire gauze. The lid for the drying chamber has an outlet (6 in. in diameter) fitted with a Perspex observation window surmounted by an aluminium cone, and is counterpoised for rapid opening. The outside of the drier is lagged with $\frac{1}{4}$-in. asbestos millboard. Wet-bulb and dry-bulb thermometers are fitted at the air inlet immediately below the drier bed, and at the outlet in the Perspex cylinder. Wicks on the wet-bulb thermometers dip into small tubes connected to larger external reservoirs. The inlet dry-bulb temperature is regulated by a Sunvic thermostat and relay which controls one of the heaters. A 3-kw. element fitted in a 5-in. duct permits heating of the air entering the fan.

Air velocity is varied by a nine-position starter on the fan, with fine control by a separate rheostat. An ammeter on the motor allows easy duplication of any particular fan setting. Air velocities are measured by an anemometer which was a push-fit in the top of the aluminium cone. A steam injector in the entry duct enables the humidity of the inlet air to be increased (Fig. 1 insert). The injector is controlled manually by a steam valve, and once set for a given wet-bulb temperature requires only occasional adjustment.

A static pressure-tube mounted in the inlet duct was connected by rubber tubing to a draught gauge reading to 2 in. of water gauge by 0·01 in.

The removable container for the wet material (9 in. deep, approx. 11 in. square) was constructed of sheet aluminium with a floor of $\frac{1}{16}$-in. copper gauze strengthened by a stouter $\frac{1}{4}$-in. mesh gauze. Asbestos cord prevented air leakage between the edges of the basket and the sides of the duct.

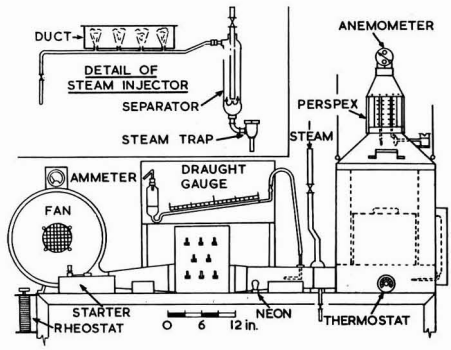

FIG. 1.—*Through-circulation drier*

Discussion of experimental procedure

Bed depth.—In through-circulation drying, one of the principal variables is the bed thickness, which is best expressed as a linear depth. It is impracticable to measure this accurately if the individual pieces are large, and the bed loading is therefore usually expressed as lb. of wet stock/sq. ft. of bed area. This is not entirely satisfactory, however, as two batches of material with the same wet loading but different water contents will have differing quantities of bone-dry material. This work has demonstrated that for seaweed within reasonably close limits of initial water content, the dry loading, L_d (lb. of B.D.S./sq. ft.), rather than the wet weight governs the drying time. This may be because the load with the higher water content will have a lower solids content, and therefore a smaller proportion of material to be dried in the diffusion period.

Although dry loading probably provides the more fundamental basis for expressing loadings, this system is inconvenient for industrial use unless frequent values of water content are available.

Weighings.—The progress of each experiment was followed by weighing the sample periodically. Ideally, this should be done continuously without removing the sample from the drier,[3, 16] but this technique requires a correction for the upthrust of the air, any error in air-flow measurement being reflected in corresponding weight errors, and air flow is often the vairable which is most difficult to measure accurately. The compensating advantages of the continuous method are that air conditions can be kept steady and there is no error due to cooling during weighing.

In the present work, the basket of seaweed was removed and weighed on a balance of 20-lb. total capacity, calibrated by 0·005 lb. Weighings were estimated to 0·001 lb. with a probable accuracy of $\pm$ 0·001 lb.

Moisture content and sampling.—In the earliest tests of this series, the water content of the seaweed was determined by taking samples immediately before the drying run. This method proved inaccurate, the calculated weight of B.D.S. sometimes exceeding the weight of dried seaweed in the basket. This may be attributed to (i) sampling error, probably due to differences in water content from plant to plant and to variations in different parts of the same stipe, and (ii) error in determining water content. This error may be considerable because of the large amount of water in the fresh seaweed. If the preparation of the material is slow, some pieces will dry out.

If, however, the water content of the dried product is determined, small differences do not affect the B.D.S. estimation seriously, and the water contents, in the second half of the test at least, can be calculated more accurately. Further, if the dried product is sampled at the end of a run, it will generally have a more uniform moisture content. The water content of the bed of seaweed is not uniform and must be expressed as an average value, which is best obtained by dividing the total weight of water by the bone-dry weight of the batch.

Particle size.—Owing to the large cross-section of the stipes, it is necessary to subdivide them to ensure reasonably short drying times. This involves an attempt to find an optimal particle size in relation to speed of drying and cost of pre-cutting. In through-circulation drying a lower limit to the piece size is reached when the particles are fluidized by the air stream.

An obvious method of reducing the stipe size is by slicing, and preliminary tests showed that $\frac{1}{8}$-in. slices dried in a reasonable time. It was necessary to consider practical means of size reduction on the industrial scale, and it was later found that pieces of approximately this size could be produced on full-size equipment.

A hinged-knife arrangement used in the earlier tests was later replaced by a bacon slicer, which gave even slicing with much greater speed, keeping the factor of slice thickness constant throughout the tests. The diameter of the stipes increases from about 1 in. at the end nearest the frond to $1\frac{1}{4}$ in. at the holdfast end, but no attempt was made to classify the pieces, as this variation would be encountered in practice. The loss of water due to slicing was probably less than 1%.

Mincing was also attempted, but it was found that the crushing action in the machine expressed cell sap, which was separated by centrifuging, giving a loss in weight of 25–30%. The advantages of mincing combined with centrifuging before drying are that the minced seaweed has a more uniform water content, the smaller pieces dry more quickly, and the evaporation load is reduced. These advantages would probably be offset, as some of the chemicals are contained in the expressed liquid and they would still have to be recovered by evaporation.

Experimental procedure

The fan was started, adjusted to the correct ammeter reading, the heaters switched on and conditions allowed to stabilize at the desired temperature (approx. 15 minutes).

Before slicing the seaweed, any barnacles or parasitic growths were scraped off the stipe, care being taken not to remove the outer skin. If not removed, these growths tended to crumble and to be blown out of the drier towards the end of the test, causing loss of dry matter.

The empty basket was counterpoised on the balance and the desired amount of stipe slices weighed into it, with random packing. A 1-in. layer of discs $\frac{1}{8}$ in. thick corresponded to 2·5 lb., and bed loadings used were multiples of this figure. As the internal area of the basket floor was 0·896 sq. ft., the tray loadings were multiplied by 1·116 to express them as lb./sq. ft.

Immediately before a run, inlet and outlet temperatures were recorded and the atmospheric humidity measured with a whirling hygrometer. The static pressure was noted when the drier was empty and closed.

The basket was then inserted in the drier, the timer was started and the lid closed. The initial static pressure was noted and the exit temperatures were taken every minute. The basket was removed, weighed, and replaced in the drier at regular intervals, five minutes for the first hour of drying, ten minutes for the second hour, and fifteen minutes thereafter. Before the lid was closed, the stopclock was reset to zero so that the measured time interval (5, 10 or 15 minutes) represented the actual time the material was in the drier, and was not affected by any variation in the time occupied by a weighing (average, about 20 seconds).

Anemometer readings were taken about two minutes before each weighing to allow the exhaust temperature to steady after the interruptions caused by the removal of the basket for weighing.

The experiment was generally concluded when the loss in weight was not greater than 0·005 lb. over 10 minutes, but this figure depended to some extent on the original weight of material used.

After drying, the seaweed was allowed to cool, and samples were taken at the centre and corners of the bed. These individual samples were combined and ground in a Christy & Norris 8-in. laboratory-mill to pass a 1-mm. screen. This powder (5 g.) was weighed into aluminium dishes ($2\frac{1}{2}$ in. in diameter × $\frac{7}{8}$ in. deep) provided with tightly fitting lids, and dried in an electric oven for five hours at 104 ± 2° C. The dishes were cooled and reweighed, the loss in weight being attributed to water. The mean of duplicate determinations was used as a basis for calculations.

Temperature and humidity measurements.—Owing to errors arising from uneven air flow at the bend beneath the bed, and to radiation from the electric heaters, the inlet dry-bulb thermometer indicated a few degrees high. As the outlet thermometer gave a much more accurate value, the air temperature was measured here at the start of a run, and the inlet thermometer was used to measure any temperature fluctuations. This indicated that the thermostat could control the temperature to ± 1° F. or better. The air-temperature variation at different parts of the empty bed was about ± 5° F., but it is probable that this scatter was reduced when the basket of seaweed was in position.

The outlet wet-bulb thermometer read a degree or two high when the drier was empty just before a test. This was attributed to the comparatively low air velocity past the bulb and to the difficulty of keeping the wick wet at higher air temperatures. A correction was made by taking the atmospheric humidity and finding the wet-bulb temperature corresponding to the dry-bulb temperature from a psychrometric chart. The difference between calculated and experimental wet-bulb temperatures agreed reasonably well with the velocity correction chart drawn up by Carrier & Lindsay.[17]

Air-velocity measurement.—The exit-air velocity was obtained by measuring the time for 1000 feet of air to be recorded on an anemometer in the outlet duct, and the value obtained corrected by the calibration factor for the instrument. The mass flow was calculated from the area of the outlet duct and the air density. The correction for the effect of density on the anemometer reading was neglected, as this is usually small for high air velocities.[18] Because of frequent opening of the lid, it was difficult to keep the drier airtight and some air leakage

took place. An experimental factor determined from mass balances in 15 tests at different air velocities was used to correct for this air leakage.

Calculation of results.—The bone-dry weight of the seaweed was found from the final weight of product in the drier and its water content. The weights of water associated with this weight of seaweed at each weighing were next calculated, and expressed as the water content (dry basis).

The curves of total water ratio versus time for each run were plotted, and the drying times between required moisture contents interpolated.

It was observed that the initial drying rates for beds of 2 in. or over were approximately constant. To enable this constant rate to be calculated accurately, the appropriate part of the graph was redrawn on a larger scale so that the slope of the line was 45°. The best straight line was drawn through the data and the two extreme experimental points which lay satisfactorily on this line were selected, and from the test data the drying rate between these two points was expressed as lb. of water/(lb. of B.D.S.)(hr.). Instantaneous drying rates at other points on the curve were measured with a tangentimeter described by Simons.[19]

The curves of water content versus time were mostly plotted as obtained, showing the variation in initial moisture contents, but for clarity, others were plotted starting from a constant water content by subtracting a constant time from each time of weighing.

Results

In the experimental work, when a suitable particle size had been determined, the following minor factors were investigated, namely interruption of drying, repeatability, initial water content and agitation. After thus ensuring a sound experimental technique, the major factors, bed depths, slice thickness, air velocity, temperature and humidity, and pressure drops, were studied.

In all the 65 tests reported, random packing of the bed was used. The variation of the drying conditions (given on the graphs) is expressed as the standard deviation.

Particle size.—Preliminary tests were carried out to find a suitable piece size of stipe to give reasonable drying times with the available air temperatures and velocities.

Fig. 2 shows the marked effect of the reduction of size on the drying time. The size selected for later tests was a $\frac{1}{8}$-in. slice.

Interruption of drying.—Gamson *et al.*[5] found that the rate of drying of catalyst pellets was not greatly affected by the time lost when the sample was removed from the drier for weighing. This was verified for seaweed by making two comparable tests on slices cut from the same batch of stipe and dried on the same day, using a 1-in. bed and weighing at 3- and 15-minute intervals respectively. Fig. 3 shows that the points for the 15-minute test fall on

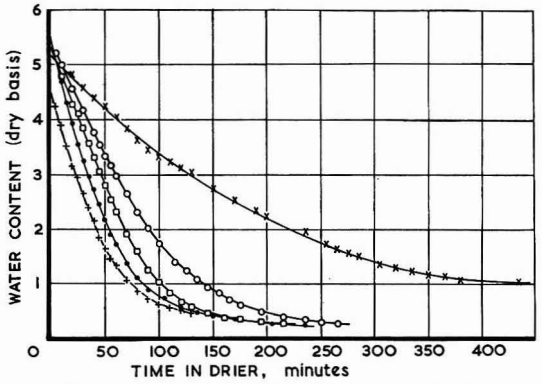

FIG. 2.—L. cloustoni *stipe*. *Effect of particle size*

Run	D.B.T., °F.	L_w	Size
×	150	12·2	1-in. slices
○	155	10·3	$\frac{1}{2}$-in. slices, $\frac{1}{2}$'s
□	160	6·3	$\frac{1}{2}$-in. slices, $\frac{1}{2}$'s
●	160	9·9	$\frac{1}{2}$-in. slices, $\frac{1}{2}$'rs
+	160	5·5	$\frac{1}{8}$-in. slices

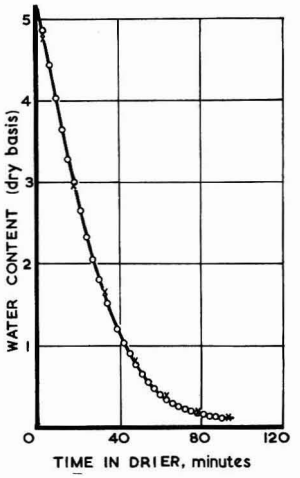

FIG. 3.—L. cloustoni *stipe*. *Interruption of drying*

$\frac{1}{8}$-in. slices
D.B.T., 156° F.; W.B.D., 73° F.

Run		L_d	G
○	3 min.	0·518	6·75
×	15 min.	0·512	6·73

the same curve as the 3-minute test, suggesting that the error caused by the weighing interval is small.

Two possible results of the interruptions were that the basket and seaweed cooled during the 20 seconds when they were outside the drier, and that the seaweed was agitated slightly when the basket was replaced in the drier. The first factor would tend to reduce the drying rate, whereas the second would increase it.

Repeatability test.—This test was designed to find how closely drying times of seaweed would agree when drying under conditions as nearly identical as possible. A batch of 5 lb. of stipe was sliced and half of the material dried. Immediately after the first half had been dried, a second test was made using the remaining seaweed, which had been stored in a large bottle. Fig. 4 shows that the curves are very nearly coincident, the slight difference being probably due to small variations in dry loading and air velocity.

It therefore seems possible, under ideal conditions, to obtain close agreement with a biological material such as seaweed, from the same batch of material.

Effect of initial water content.—A feature of the drying of biological material is the variation of initial water content which makes direct comparison of tests difficult. Brown & Kilpatrick[20] encountered this factor when drying riced potatoes, and overcame it by making small time corrections so as to base each test on a common moisture content.

Three 2·5-lb. portions of fresh stipe slices were prepared from one batch of seaweed and the first portion dried immediately. The two remaining parts were spread on trays in the laboratory and allowed to air-dry for 1 and 5 days before being dried under identical conditions.

In this way three tests were made with seaweed of different initial water content, but with the same bone-dry loading per unit of basket area. Fig. 5 shows that the three curves of water content versus time are coincident, a time correction being added to the last two to allow for their lower water content.

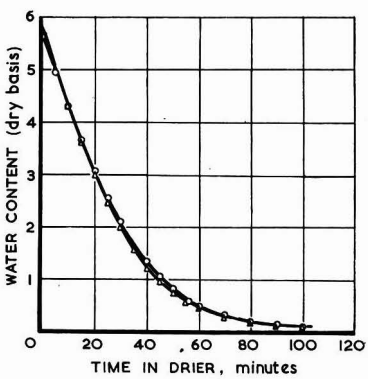

FIG. 4.—L. cloustoni *stipe. Repeatability experiment*

$\frac{1}{8}$-in. slices
D.B.T., 156° F.

Run	W.B.D., ° F.	L_d	G
△	73	0·413	6·63
○	72	0·422	6·55

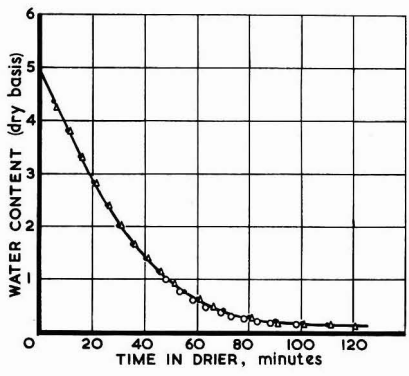

FIG. 5.—L. cloustoni *stipe. Effect of initial water content*

D.B.T., 156° F.; W.B.D., 61° F.

Run	L_w	L_d	G
●	2·79	0·473	6·75
△	2·49	0·470	6·4
○	0·96	0·479	6·65

This shows that samples of seaweed with the same bone-dry loading will require the same time to dry (through the same water-content range) under the same drying conditions, irrespective of their original water content. It follows that the amount of previous air drying does not affect the drying rates in later stages.

Agitation.—The drying rates of different layers of a bed of seaweed were studied using three baskets which fitted inside each other. Each was loaded with 2·5 lb. of seaweed, giving a composite 3-in. bed when the containers were assembled. The baskets were weighed separately so that the average water contents of each layer could be calculated (Fig. 6). This graph shows that the water contents of the three layers vary widely during drying, and maximum

deviation occurs at an average value of 3·25. At the start of the drying there is apparently no condensation of water on the upper layer, as the uppermost curve shows a small but significant loss in weight. It is also demonstrated that the constant drying rates of the composite bed are a combination of the falling drying rate of the bottom section and increasing drying rates of the upper layers. A disadvantage of static through-draught drying is disclosed, namely, that the bottom layer is subjected to the full inlet-air temperature after it has been dried until the remainder of the bed is dry. This defect is less serious in through-circulation drying than in cross-circulation drying, owing to the shorter times involved, and is often overcome in practice by inverting the bed at intervals or by reversing the air flow. Agitation has the added advantage that agglomerates are broken up and surfaces of pieces which have adhered to each other are exposed. Fig. 6 also shows that although the drying operation is nearly complete in the lower layers, the run as a whole must be prolonged until the top layer is dry. If mixing is employed, less time should be required to dry the whole bed to an average water content. Other tests on seaweed stipe with rotary driers showed that higher air temperatures than were possible with static through-circulation driers could be used before scorching resulted. This may be explained by the dual effects of increased evaporation by exposure of new surfaces and uniformity of heating and drying. If a drier is operated correctly, the material should be discharged when at an optimum water content and before overheating becomes serious.

An estimate of the expected time-reduction on agitation of the material was obtained from a test in which the stipe slices were turned over by hand every 10 minutes. A static experiment was carried out for comparison. The stirred sample dried in about 86% of the time taken by the control test, and the constant drying rate was 4·07 lb./(lb. of B.D.S.)(hr.) for agitation compared with 3·65 for a static bed.

Bed depth.—In cross-circulation drying, light loading of the wet material is generally necessary, since the air cannot penetrate readily to the lower regions of the bed and drying is thus prolonged. With through-circulation drying the air comes into contact with all parts of the bed and much heavier loadings may be practised.

Stipe slices, $\frac{1}{8}$ in. thick, were dried at an average air temperature of 157° F. with an average air flow of 5·8 lb./(sq. ft.)(min.). When these runs were made, fine speed control of the fan was not possible, so that the average air flow for a run decreased as the bed thickness was increased. The time required to dry the stipe slices from 5·0 to 0·15 water ratio was plotted against the dry loading, L_d. This curve (Fig. 7), in which time appears to be approaching a limiting value, has the empirical equation:

$$\theta = 245[\log_{10}(L_d + 0{\cdot}944) + 0{\cdot}232]$$

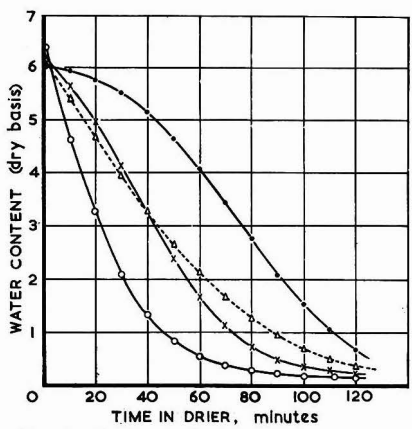

FIG. 6.—L. cloustoni *stipe. Water content of various layers in seaweed bed*

$\frac{1}{8}$-in. slices
D.B.T., 157° F.

	L_d
● Top 1-in. layer ..	0·398
× Middle	0·388
○ Bottom	0·377
△ Composite	1·163

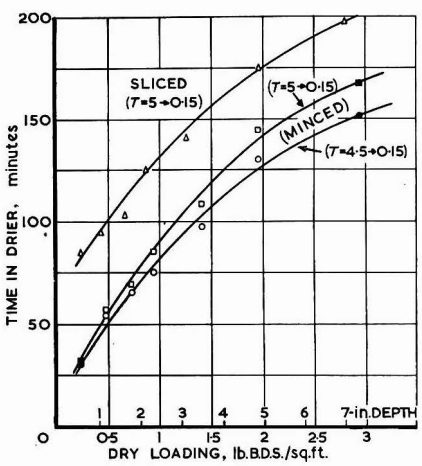

FIG. 7.—L. cloustoni *stipe. Drying time* versus *bed depth*

D.B.T., 156–160° F.; W.B.D., 74–78° F.
△ Oban: April–May
○ Inchcolm: July
● Inchcolm: February

The constant drying rate plotted against the dry loading gives a curve (Fig. 8) which may be expressed by the equation :

$$dW/d\theta = 1{\cdot}48 + 10^{1{\cdot}03 - 0{\cdot}536L_d}$$

The output of commercial dry seaweed (0·15 lb./lb. of B.D.S.), starting at an initial water ratio of 5, is given by :

$$R = (L_d \times 69)/\theta$$

When this output is plotted against the dry loading (Fig. 9), it is seen that the highest output is obtained with the deepest bed. The increase in output is most marked up to bed depths of about 3 in. ($L_d = 1{\cdot}2$), and this bed thickness was used for the remaining tests as it gave what was considered a convenient drying time. This quantity of seaweed was found just sufficient to cover the basket floor when drying was complete.

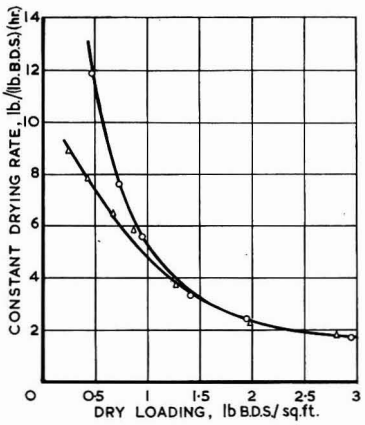

FIG. 8.—L. cloustoni *stipe*. *Constant drying rate* versus *bed depth*

D.B.T., 156–160° F.; W.B.D., 74–78° F.
○ Minced G, 5·5 ± 0·15 lb./(sq. ft.)(min.)
△ Sliced G, 5·8 ± 0·2 lb./(sq. ft.)(min.)

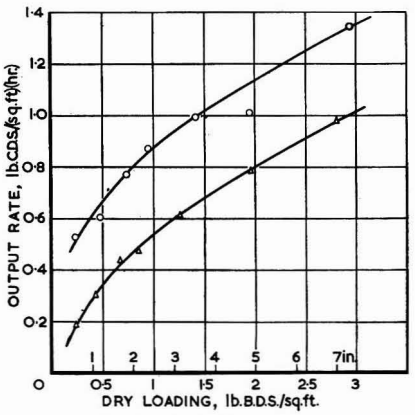

FIG. 9.—L. cloustoni *stipe*. *Output* versus *bed depth*

D.B.T., 156–160° F.
○ Minced T, 4·5 to T, 0·15
△ Sliced T, 5 to T, 0·15

A complementary series was produced on the variation of output and drying time at different bed depths using minced and centrifuged stipe in place of the sliced material. When centrifuged for two minutes (centrifugal effect = 820), the initial water content was reduced to about 4·5 lb./lb. of B.D.S. A screen analysis of this material is given in Table I.

The curve of drying time for 4·5 to 0·15 water ratio (Fig. 7) has an equation similar to that for the slices :

$$\theta = 239[\log_{10}(L_d + 0{\cdot}93) + 0{\cdot}0568]$$

Table I

Screen analysis of minced and centrifuged stipe (medium cutter)

		Differential, %	Cumulative, %
Retained on	½-in. mesh	0·00	0·00
,,	¼-in. ,,	47·10	47·10
,,	⅛-in. ,,	31·40	78·50
,,	3/32-in. ,,	4·96	83·46
,,	1/16-in. ,,	11·45	94·91
Passing	1/16-in. ,,	5·09	100·00
		100·00	

The constant drying rate decreases as the bed loading is increased (Fig. 8), and the value of the drying rate is related to the dry loading by the expression :

$$dW/d\theta = 0{\cdot}525[L_d]^{-1{\cdot}12}$$

The curve of drying time ($T = 5$ to $T = 0{\cdot}15$) versus L_d for minced stipe was obtained from the time versus water content curves by slight extrapolation, i.e. assuming the constant drying rate to have the same value at higher water contents. From this curve (Fig. 7) the drying time for $L_d = 1{\cdot}2$ lb./sq. ft. was found, and the equivalent slice-thickness corresponding to this time interpolated from Fig. 11. This gave a thickness of 0·0675 in. ($\frac{1}{16}$ in. approx.).

Slice thickness.—Fig. 10 shows the effect of slice thickness on the time of drying. The thickness has very little effect on the constant drying rate, but reduces the times in the later stages considerably. These drying tests have been correlated by the time required to dry the seaweed from a water ratio of 5 to 0·15, and Fig. 11 shows the drying time for different thicknesses. The data are represented by a smooth curve which has the equation $\theta = 411S^{0{\cdot}523}$.

When the constant drying rate is plotted against the slice thickness (Fig. 12) the points are fairly widely scattered, but lie on a smooth curve when corrected for the effect of bed depth. The specific surface (sq. ft./lb. of wet seaweed) of the slices was calculated assuming an average slice diameter of $1\frac{1}{8}$ in., and the constant-rate period when plotted against the specific surface gave a straight line. It is unlikely that the total surface of the material was exposed to the drying air, but the fractional amount exposed was possibly similar in each case.

Air velocity.—The air-flow rates used in this series ranged from about 3 to 10 lb./(sq. ft.)(min.). The fan speed was adjusted to a prearranged ammeter reading which was kept constant during a test. As drying progressed, the seaweed bed contracted, its resistance decreased, and the air flow consequently increased until approximately midway through a test, when no further shrinkage occurred (Fig. 22). The average mass air flow was taken for a run. No pronounced ' edge effect ' was apparent for the stipe slices, since the slight vibration resulting from the weighing operation caused the pieces to settle down evenly.

The plots of drying time versus air flow for 15 tests are shown in Fig. 13. These runs had to be classified into two groups, depending on the plant habitat, before correlation could be attempted. Plants from Oban require about 20% longer time for drying at higher air

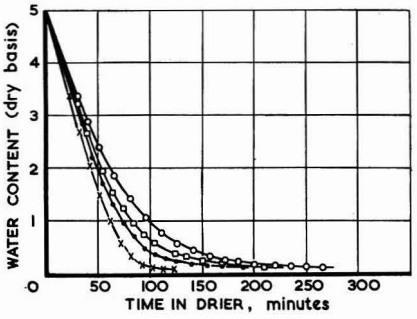

FIG. 10.—L. cloustoni *stipe*. *Effect of slice thickness. Oban : April*

D.B.T., 153–156° F.; W.B.D., 70–74° F.
G, 6·6 ± 0·15 lb./(sq. ft.)(min.)
L_d, 1·230 ± 0·43 lb./sq. ft.
× $\frac{1}{16}$ in. □ $\frac{1}{4}$ in.
● $\frac{3}{16}$ in. ○ $\frac{3}{8}$ in.

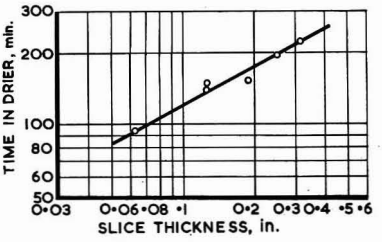

FIG. 11.—L. cloustoni *stipe*. *Drying time* versus *slice thickness*

D.B.T., 153–156° F.
T, 5 to T, 0·15

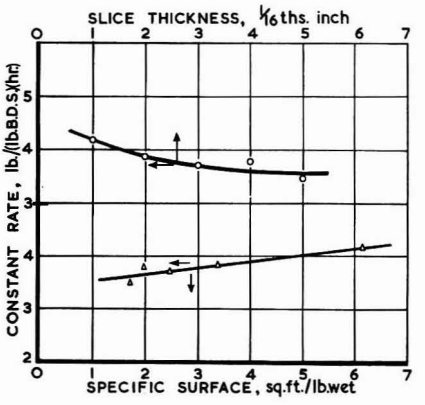

FIG. 12.—L. cloustoni *stipe*. *Effect of slice thickness on constant rate*

velocities than plants taken from the Firth of Forth. The data can be represented by two equilateral hyperbolae with equations:

$$\theta = G/(0{\cdot}0103G - 0{\cdot}02) \quad \text{. . . Oban}$$
$$\theta = G/(0{\cdot}013G - 0{\cdot}03) \quad \text{. . . Inchcolm}$$

The scattering of the data at low air velocities may be due to the Reynolds number approaching the transition value between turbulent and streamline flow. As the air flow increases the scatter is reduced, and if the same equations hold beyond $G = 10$ the drying time will approach a limit, so that any increase in air flow will have a negligible effect.

The plot of constant drying rates versus air flow (Fig. 14) is a straight line having the equation:

$$dW/d\theta = 0{\cdot}64G + 0{\cdot}34$$

By extrapolation this gives a value of 0·34 lb./(lb. of B.D.S.)(hr.) for the drying rate when $G = 0$ (i.e. static air conditions).

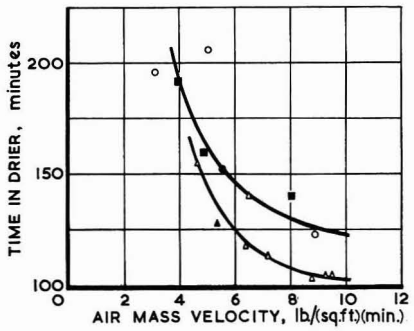

FIG. 13.—L. cloustoni *stipe*. *Drying time* (*T*, 5 *to T*, 0·15) versus *air mass velocity*
L_d, 1·20 lb./sq. ft.; W.B.D., 73° F.
D.B.T., 155–159° F.
Oban: March ○; April ■; May ●
Inchcolm: March △; May ▲

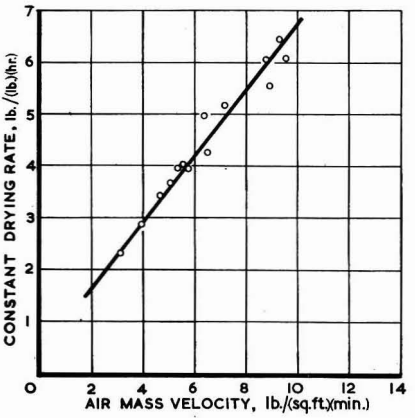

FIG. 14.—L. cloustoni *stipe*. *Air mass velocity* versus *constant rate*
D.B.T., 155–159° F.; W.B.D., 73° F.
L_w, 8·35 lb./sq. ft.; L_d, 1·20 lb./sq. ft.

Drying rates are frequently expressed as a power function of air velocity. A distinction should be drawn between the index of a rate (often the constant rate) and the index for the drying time. The time index may be regarded as the inverse of an 'average rate' index between specified water contents. The velocity index often changes as drying proceeds, so that the index for the 'average rate' will depend to some extent on the limits of water content chosen.

Marshall & Hougen[4] showed that the constant drying rate of charcoal was proportional to the 0·81-power of the air flow, whereas Gamson *et al.*,[5] with wetted catalyst pellets, obtained a value of 0·59. Working with viscose rayon fibre, Coles[12] obtained a velocity index of — 1·25 for the time of drying.

Burgess[1] found that the 'minimum time' for hops was related to the — 0·39-power of the air speed, and Ede & Hales[9] found that drying rates for fruit ($T = 6$ to $T = 2$) varied as the 0·4-power of the air velocity for through-circulation drying. The plot of air velocity correction factors given by Brown & Van Arsdel for potato strips is broadly similar to Fig. 13. The index for the time of drying was — 0·4 in the range 5·7–10·3 lb./(sq. ft.)(min.).

The drying times in the region $G = 6$–9·5 lb./(sq. ft.)(min.) for Inchcolm seaweed are approximately proportional to the — 0·4-power of the air flow.

Burgess[1] pointed out that the higher velocity indices obtained by other workers on evaporation were obtained from free water surfaces or inert wetted solids and not from initially living plant material. He further suggested that the lower velocity indices for hops and plant material may be related to the time required to kill the material in the drier.

The velocity index (= 1) for the constant drying rate of sliced seaweed stipe is higher than that for inert solids, possibly because the air approaches the limit of its water-carrying capacity, owing to the use of a deeper bed.

Temperature and humidity.—For tests on temperature and humidity, a 3-in. bed of $\frac{1}{8}$-in. thick slices was used throughout. The humidity of the air is expressed as the wet-bulb depression (W.B.D.), in agreement with the reasons put forward by Ede & Hales.[9]

The tests were formed into five series (Table II).

Table II

Series	D.B.T., ° F.	W.B.D., ° F.	G, lb./(sq. ft.)(min.) average
1	120–212	44–116	7·2, Oban, Apr.
2	148–205	66–111	4·5, Inchcolm, July and Sept.
3	157	27–57	7·0, Oban, June
4	120–180	44	8·3, Inchcolm, Oct.
5	200–340	108–228	10 (smaller drier), Inchcolm, June

In Series 1, only the dry-bulb temperature (D.B.T.) was controlled so that the absolute humidity of the drying air (lb. of water/lb. of air) was that of the atmosphere. Series 2 was produced at a lower air velocity to enable higher temperatures to be attained. The humidity series (Series 3) utilized steam-injection to give higher wet-bulb temperatures (W.B.T.) at a constant dry-bulb value. The plot of constant drying rate versus W.B.D. for Series 1 and 3 (Fig. 15) and for Series 2 (Fig. 16) is a straight line passing through the origin, as follows:

$$dW/d\theta = 0{\cdot}057(t_d - t_w) \quad \ldots \text{ Series 1 and 3}$$
$$dW/d\theta = 0{\cdot}0374(t_d - t_w) \quad \ldots \text{ Series 2}$$

The corresponding points for Series 4 are in close agreement with each other, but the cluster lies above the straight line for the constant rate (Fig. 15). This is probably due to the different source of the plants used and to the higher air velocity.

The drying time gives a smooth curve when plotted against W.B.D. (Fig. 17). The curve for mass flow 7·1 was extended by results obtained at higher temperatures in a smaller similar drier which had a basket 6 in. square (Series 5).

It is often stated that when a material is nearly dry the W.B.D. of the air has little effect on the drying rate and that this rate depends mainly on the D.B.T. of the air. This effect is illustrated by Series 4, where the W.B.D. was constant but the D.B.T. was increased. With D.B.T. of 120, 140 and 180° F., the drying times were 187, 177 and 167 minutes respectively.

The plot of drying rates at different water contents versus the W.B.D. (Fig. 15) shows that although there is some scattering of the points, there is no significant deviation from a

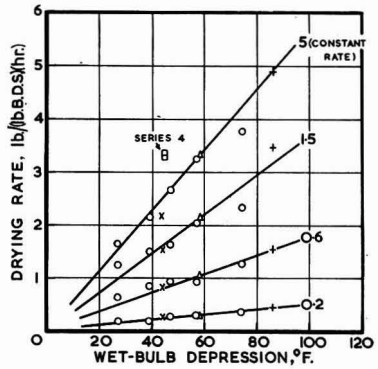

FIG. 15.—L. cloustoni *stipe. Effect of D.B.T. and W.B.D. on drying rate*

L_w, 8·35 lb./sq. ft.; L_d, 1·26 ± 0·067 lb./sq. ft.
G, 7·1 ± 0·3 lb./(sq. ft.)(min.)
D.B.T., ° F.: × 118; △ 135; ○ 156; + 173

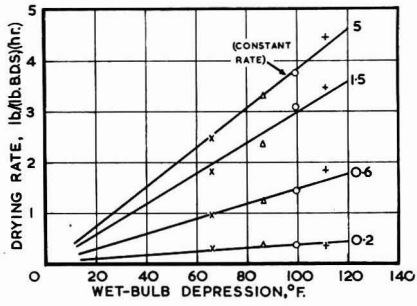

FIG. 16.—L. cloustoni *stipe. Effect of D.B.T. and W.B.D. on drying rate*

L_d, 1·334 ± 0·042 lb./sq. ft.
G, 4·5 ± 0·1 lb./(sq. ft.)(min.)
D.B.T., ° F.: × 148; △ 173; ○ 191; + 205

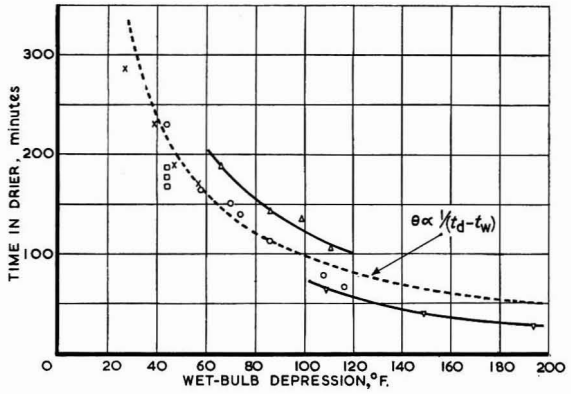

FIG. 17.—L. cloustoni *stipe*. *Effect of D.B.T. and W.B.T. on drying time*

$\frac{1}{8}$-in. slices
L_w, 8·35 lb./sq. ft ; L_d, 1·265 ± 0·067 lb./sq. ft.

Series		G, lb./(sq. ft.)(min.)
1	○	7·2 ± 0·3
2	△	4·5 ± 0·1
3	×	7·0 ± 0·3
4	□	8·3 ± 0·2
5	▽	10 (small drier)

straight-line relationship. The drying rates are therefore proportional to the W.B.D. even at water contents as low as 0·2 lb./lb. of B.D.S. These points include runs at different D.B.T. and runs at constant D.B.T. using humidified air (Series 1 and 3). It was observed that the higher W.B.D. are usually associated with higher D.B.T. and that, within limits, the W.B.D. of a sample of air with a constant absolute humidity increases in direct proportion to the D.B.T. The drying rates for Series 2 show a similar relationship to the W.B.D. (Fig. 16).

The proportionality of the drying rates to the W.B.D. at low water contents may perhaps depend on the layer drying effect (Fig. 6), which results in comparatively wet samples of seaweed being present in upper strata of the bed until near the end of a run. For a given W.B.D. these wetter pieces would dry more rapidly than the average, and would tend to increase the proportionality of the bed-drying rate as a whole.

Ede & Hales[9] dried $\frac{3}{16}$-in. × $\frac{5}{16}$-in. potato strips individually and also in a layer by cross flow of air at a D.B.T. of 158° F. They found that the drying rates, except at the beginning of a run, showed a clear departure from proportionality to the W.B.D. This departure was more pronounced in the experiments with individual strips. A further series by Ede & Hales, with $\frac{3}{16}$-in. × $\frac{1}{4}$-in. potato strips on trays with a lower bed loading and different D.B.T., indicated that the drying rates were directly proportional to the W.B.D. at water contents ranging from 5 to 0·2 lb./lb. of B.D.S. Ede & Hales attributed this difference partly to the effect of the smaller strip-dimension and the different D.B.T. The effect of the non-uniform drying on the relationship to the W.B.D. suggested for through-circulation drying may also apply to the tray-drying experiments on potatoes. Ede & Hales showed graphically the variation in water content of potato strips on a single tray during drying, and the points obtained agree fairly well with Fig. 6. The thickness of the stipe slices used in the present work ($\frac{1}{8}$ in.) is less than the smaller dimension of the potato strips, which may account for the absence of any serious departure from the proportionality to the W.B.D.

Brown & Van Arsdel[8] gave drying-time nomographs for the through-circulation drying of $\frac{5}{32}$-in. square potato strips. The first period (water contents 4 to 0·2 lb./lb. of B.D.S.) appears to be almost entirely dependent on the W.B.D. and only slightly dependent upon the D.B.T. In the range 0·2 to 0·06 the reverse is true.

It would appear that the proportionality of the drying rate to W.B.D. may be used without serious error for seaweed with average water contents down to about 0·2 lb./lb. of B.D.S., below which the D.B.T. is increasingly important.

If the drying rates at all water contents are directly proportional to the W.B.D., the time of drying should be inversely proportional to the W.B.D. The curve for this relationship (Fig. 17, dotted line) shows that the agreement with other experimental points is good except

at the extremes. This curve was based on a reference run of 164 minutes at 58° F. W.B.D., 135° F. D.B.T. and it was selected because it gave the best agreement with Fig. 15.

Ede & Hales suggested the use of the wet-bulb depression evaporation-coefficient, i.e. lb. of water/(lb. of B.D.S.)(hr.)(W.B.D. in ° F.), since the drying rates were proportional to the W.B.D. As this has been shown to hold approximately for through-circulation drying of seaweed, the basic curves for drying time and rates per unit W.B.D.(° F.) are plotted for the reference experiment at a W.B.D. of 58° F. referred to previously (Figs. 18 and 19). These curves refer to $\frac{1}{8}$-in. stipe slices at a bed loading of 1·28 lb. of B.D.S./sq. ft. and an air flow of 7·5 lb./(sq. ft.)(min.). Drying times should be divided and rates multiplied by the W.B.D. in ° F. For other loadings and air speeds, times should be divided and rates multiplied by the factors given, which are taken from Figs. 7 and 13. This basis of drier design is stated by Hendry & Scott[11] to be at least as accurate as other methods, with the additional merit of simplicity.

Static pressure drops

Static pressure drops were measured by taking the draught-gauge reading when the bed of seaweed was in position with the drier closed, and deducting the resistance of the drier and basket at the same air-flow rate. Plots of static pressure drop versus mass air flow are given in Figs. 20 and 21 for beds of fresh and dried stipe slices, using air at room temperature.

When the pressure drops are expressed as in. of water gauge/ft. of bed depth, the values for the 1-in. layers are $1\frac{1}{2}$ to $2\frac{1}{2}$ times the value for the deepest beds. This is the reverse of what would be expected if compression was taking place, and may be the result of errors being magnified when the pressure drops are very small and bed thicknesses are difficult to measure accurately. This error was negligible for depths greater than 3 in.

Using freshly sliced sugar-beet cossettes, Owen[2] found that the static pressure drop at constant air velocity was directly proportional to the bed depth, and he derived the following equation for the pressure drop:

$$X = (0{\cdot}0015V - 0{\cdot}09)Z$$

where X = static pressure drop, in. of water gauge; Z = bed thickness, in.

Spaugh[21] studied the resistance of beds of dehydrated vegetables to the through-flow of air, using bed depths up to 36 in. and air velocities of 37–170 ft./min. Spaugh found that the values of the pressure drop per unit bed depths at different heights in the bed were fairly widely scattered, and showed no definite correlation. He concluded from this that the packing effect was negligible for dehydrated vegetables up to 36-in. beds.

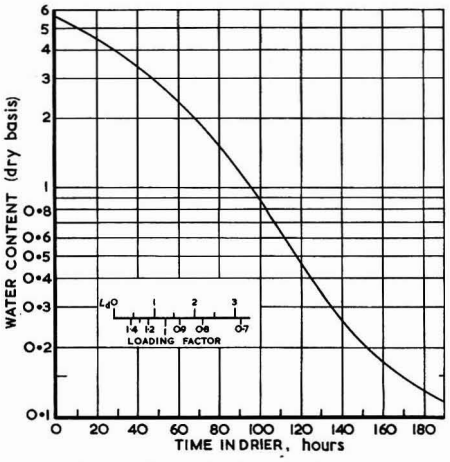

FIG. 18.—L. cloustoni *stipe. Drying times for unit W.B.D.*, ° F.
L_d, 1·28 lb./sq. ft.; G, 7·5 lb./(sq. ft.)(min.)

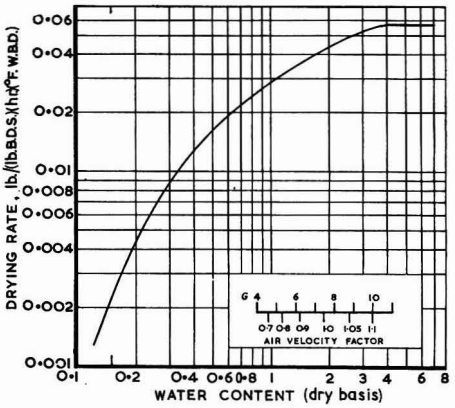

FIG. 19.—L. cloustoni *stipe. Drying rates for unit W.B.D.*, ° F. *Oban: April*, 1951
$\frac{1}{8}$-in. slices
L_d, 1·28 lb./sq. ft.; G, 7·5 lb./(sq. ft.)(min.)

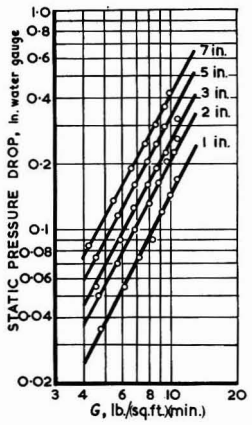

FIG. 20 (left).—L. cloustoni *stipe dried. Pressure drop* versus *air flow*

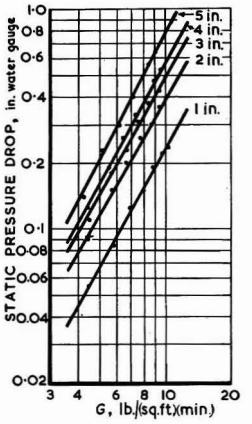

FIG. 21 (right).—L. cloustoni *stipe fresh, ⅛-in. slices. Pressure drop* versus *air flow*

A general equation for dehydrated vegetables given by Spaugh is:

$Q = (C_1V/C_2 - b)^n$, where Q = static pressure drop, in. of water gauge/ft. of bed; C_1, C_2 = experimental constants; b = ft. of perimeter/sq. ft. of bin cross-section; n = experimental exponent. Spaugh suggested that when b is greater than unity the simpler equation $Q = CwV^n$ may be used, where C is again an experimentally determined constant, and w = air density, lb./cu. ft. (For the present drier, $b = 4{\cdot}23$.) He also showed that the effect of the air viscosity could be neglected for the range of temperatures studied (room temperature to 200° F.).

The straight lines on Figs. 20 and 21 may be represented by the equation $Q = CG^n$. The exponent n varies from 1·76 to 1·88 for dried stipe and from 1·75 to 1·92 for wet stipe slices. Spaugh obtained values ranging from 1·60 for flaked onions to 1·82 for strip potatoes, and Coles gave a value of 1·5 for a bed of viscose fibre.

Equations for pressure drops of seaweed beds (random packing) have been derived from the average value of the exponents and the pressure drop from the deepest beds. These equations are:

$$Q = 0{\cdot}011G^{1{\cdot}83} \text{ for dried stipe slices, and}$$
$$Q = 0{\cdot}027G^{1{\cdot}8} \text{ for fresh stipe slices.}$$

From Fig. 22, which shows the variation of pressure drop during a drying run, shrinkage appears to be negligible after a water content of 1–1·5 lb./lb. of B.D.S. is reached.

Factors in the design of a drier

Temperature.—Tests on the smaller drier at higher temperatures revealed some scorching of the stipe at a D.B.T. of 250° F. which was serious at 300° F. It is therefore suggested that the maximum air D.B.T. should not exceed 225° F. for static through-circulation drying of *L. cloustoni* stipe. If higher temperatures are desired in the initial stages, agitation should be sufficient to ensure even drying.

Air velocity.—Fig. 13 shows that there is little to be gained by using air flows greater than 8–9 lb./(sq. ft.)(min.) (i.e. 127/143 ft./min.). The normal working limits of air velocity for through-circulation drying[22] are 100–300 ft./min.

If drying were carried out in stages, it would be advantageous to use higher air

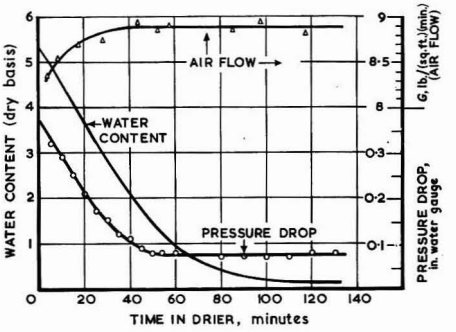

FIG. 22.—L. cloustoni *stipe. Variation of static pressure drop and air flow during a run*
⅛-in. slices; 3-in. bed; L_d, 1·33; D.B.T., 156° F.; W.B.D., 75° F.

rates initially, as the constant drying rate increases linearly with air velocity (Fig. 14). In the later stages, the air flow could profitably be reduced.

Bed depth.—The minimum bed depth should be 3 in., as the output falls rapidly below this value (Fig. 9). To obtain the maximum output a depth of at least 7 in. could be used, although this will be limited by the strength of the conveyors.

As a result of the agitation experiment, it appears to be beneficial to use a two-stage drying process. In a continuous through-circulation drier, two superimposed conveyor belts could be used with the upper one discharging on the lower. By reducing the speed of the lower belt it should be possible to increase the bed depth, thus avoiding channelling and making more effective use of the drying air.

From Fig. 22 it may be seen that shrinkage is virtually complete at a water content of 1–1·5 lb./lb. of B.D.S. and this may be a convenient point to make the change-over to the second belt.

One disadvantage of deeper beds is that the longer drying time reduces the viscosity of the alginic acid in the seaweed.[23] If quality of the alginic acid is of paramount importance, it may be advisable to operate at a lower bed loading to reduce the contact time of the seaweed with the hot air.

Nomenclature

Water contents are expressed on the dry basis as the ratio of the weight of water to the weight of bone-dry solids. This system has the advantages for drying calculations that the weight of the bone-dry material is constant throughout a run, and the water ratio is directly proportional to the evaporation load.

The term ' bone-dry solid ' (B.D.S.) is in common use in drying literature and is justified by the fact that it implies the complete absence of water, whereas the simpler term ' dry solid ' is defined by Marlow[24] as referring to material containing water in equilibrium with the atmosphere. The bone-dry seaweed is taken to be the material remaining in the dishes after oven drying under the conditions specified previously.

The phrase ' commercial dry solid ' (C.D.S.) used in this work refers to seaweed having a water ratio of 0·15 (13·04% water on the wet basis). At this water content the seaweed can be readily ground and stored safely.

Symbols

T = total water content, lb. of water/lb. of B.D.S.
G = mass air flow, lb. of dry air/(min.)(sq. ft. of cross-sectional area of bed)
V = air speed on emergence from the bed, ft./min.
L_d = dry loading, lb. of B.D.S./sq. ft.
L_w = wet loading, lb. of wet seaweed/sq. ft.
R = output rate, lb. of C.D.S./(sq. ft.)(hr.)
W = moisture content, lb./sq. ft. of cross-sectional area
θ = drying time, min. (usually for $T = 5$ to $T = 0{\cdot}15$)
$dW/d\theta$ = constant drying rate, lb. of water/(lb. of B.D.S.)(hr.)
S = slice thickness, in.
t_d = D.B.T. = dry-bulb temperature, ° F.
t_w = W.B.T. = wet-bulb temperature, ° F.
$t_d - t_w$ = W.B.D. = wet-bulb depression, ° F.
B.D.S. = bone-dry solid
C.D.S. = commercial dry solid

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VEGETABLE OILS. II.*—Further Studies of Seed Oils of Various *Strophanthus* Species

By F. D. GUNSTONE

The component acids of the seed oils of three *Strophanthus* species [*S. sarmentosus* (forest form), *S. hispidus* and *S. courmontii*] have been determined, and it is shown that 9-hydroxyoctadec-12-enoic acid is present in each seed oil.

It has recently been shown[1, 2] that the seed oil obtained from *Strophanthus sarmentosus* (savannah form)[3] is unique in containing a hitherto unknown hydroxy-acid, 9-hydroxyoctadec-12-enoic acid. Callow *et al.*,[3] reporting several variants of this botanical species, state that the fat content varies from 35% in savannah seeds to 20% in forest seeds. It had already been suggested[1] that a detailed comparison of these oils might be of interest. The discovery of 9-hydroxyoctadec-12-enoic acid in *S. sarmentosus* seed oil also raises the further question whether the occurrence of this acid is confined to this species or whether it is characteristic of the genus, for it is known that ' the fatty components of seeds are specific and closely related to the families in which the parent plants have been grouped by botanists'.[4] In the present paper the component acid analyses of some further *Strophanthus* oils are reported.

The seed oils

Samples of the seed oils from *S. sarmentosus* (forest form), *S. hispidus* and *S. courmontii* were kindly supplied by Dr. R. K. Callow, in addition to smaller samples of seeds similar to those from which the oils had been extracted. The extraction of the first two oils had been carried out as follows : ' The ground seed was exhaustively extracted with ethanol, the extract concentrated, diluted with water and extracted with light petroleum. The light petroleum was then evaporated ' (information supplied by Dr. Callow). *S. courmontii* seed oil had been obtained from a commercial source. These oils were used in the analyses reported below. In addition samples of the seeds (about 40–60 g.) were extracted in these Laboratories. After crushing, the oil was extracted with light petroleum in a Soxhlet extractor.

The results of these extractions and the characteristics of the oils and of the mixed acids

* Part I : *J. Sci. Fd Agric.*, 1952, **3**, 185

derived from them are listed in Table I along with the comparable figures previously reported for *S. sarmentosus* (savannah form). It will be seen that the yield of oil obtained on a small scale in the laboratory does not accord with that obtained in the large-scale extraction. This may be due to the different methods employed or to variation in the oil content of different batches of seeds, since the large-scale extractions were effected on seeds collected from different areas which had been bulked together. Two samples of *S. sarmentosus* (forest form) seeds were extracted and these are seen to differ both in average weight and in oil content, though surprisingly, both yield more oil than the savannah form. The variations in iodine value are not considered to be significant because of the difficulty of obtaining satisfactory values in the presence of 9-hydroxyoctadec-12-enoic acid.[2]

Table I

Characteristics of Strophanthus *oils*

	S.S. (S)*	S.S. (F)* (a)†	S.S. (F)* (b)†	S.H.*	S.C.*
Supplied as seeds:					
Wt. of 100 seeds, g.	1·67	3·10	2·16	1·63	2·80
Yield of oil, %	29–34	39	34	32	26
Iodine value‡	93·4	81·2	89·2	89·5	87·4
Refractive index (17°)	1·4720	1·4713	1·4707	1·4721	1·4721
Supplied as oil:					
Yield of oil, %	35	26		30	21
Iodine value‡	93·0	87·0		98·3	95·4
Saponification equiv.	292·2	300·8		293·7	294·5
Free acid (as % of oleic)	1·2	1·4		5·3	1·2
Refractive index (17°)	1·4720	1·4682		1·4655	1·4694
Mixed acids from material supplied as oil:					
Iodine value‡	97·5§	95·4		104·8	102·9
Saponification equiv.	280·5§	284·3		284·7	282·9
Absorption max. ($E^{1\%}_{1cm.}$) at 234 mμ after isomerization (180°/60° min.)	262·9§	234·2		265·6	284·3

* These abbreviations are used for *S. sarmentosus* (savannah form), *S. sarmentosus* (forest form), *S. hispidus* and *S. courmontii* respectively

† Two distinct samples of these seeds were available

‡ Iodine values of these oils are known to be unsatisfactory

§ Mixed acids from which much of the unsaponifiable material has been removed

Method of analysis

Each oil was analysed by methods which differ little from those already described.[1] The oil was first separated from a little insoluble non-fatty material. Hydrolysis gave the mixed acids which were crystallized from acetone (10 ml./g.) at − 50° to − 55° overnight, the insoluble portion being recrystallized from methanol (10 ml./g.) at − 20° overnight. Three fractions, designated A, B and C in order of increasing solubility, were thus obtained. Fraction A was methylated by refluxing with methanol and sulphuric acid, and fractions B and C were esterified with methanol and anhydrous hydrogen chloride at room temperature and subsequently acetylated by boiling with acetic anhydride. (In the analysis of both forms of *S. sarmontosus* seed oil only fraction C was acetylated.) The esters were then fractionally distilled under reduced pressure, and iodine values, saponification equivalents and ultra-violet absorption after alkali isomerization were determined as required. The results of the low-temperature crystallizations are given in Table II, but details of ester fractionation are not recorded.

Computation of the results also follows the details which have already been given, with the following exception. The acetylation of fraction B showed the presence of some hydroxy-acid which concentrated in the residue. This has, accordingly, been calculated as a mixture of unsaturated C_{18} esters having the same composition as those in the previous fraction, with methyl 9-acetoxyoctadec-12-enoate and unsaponifiable material. With *S. sarmentosus* seed oils where fraction B was not acetylated, the presence of the hydroxy-ester has been assumed to account, along with the unsaponifiable material present, for the increased saponification equivalent. These results are not so accurate as the values obtained after acetylation, but the amounts involved are not very great. This has necessitated a small correction of the results previously reported. The results obtained in the present work are given in Table III. Of the acids listed in Table III, palmitic, stearic, oleic, linoleic and 9-hydroxyoctadec-12-enoic acid (as 9 : 12 : 13-trihydroxystearic acid) have been characterized in each analysis.

Table II

Low-temperature crystallization

	S.S. (F)			S.H.			S.C.		
	A	B	C	A	B	C	A	B	C
Acids:									
Wt., g.	59·3	73·1	75·0	50·2	81·4	109·8	66·8	78·2	106·2
Percentage composition	28·6	35·2	36·2	20·8	33·7	45·5	26·6	31·1	42·3
Iodine value	20·2	93·9	154·7	5·7	98·0	150·2	25·7	100·2	158·3
$E^{1\%}_{1\,cm.}$ at 234 mμ†	—	76·9	580·9*	—	102·6	502·3*	—	142·1	539·6*
Esters:									
Iodine value	19·3	90·2	153·8	5·3	94·4	151·6	24·7	94·8	144·5
Saponification equiv.	289·3	295·2	302·6	287·4	295·6	305·9	288·5	295·9	304·9
Acetylated esters:									
Iodine value	—	—	139·7	—	91·1	129·6	—	94·7	133·6
Saponification equiv.	—	—	264·5	—	286·3	248·1	—	289·8	255·7

* In no case was there any evidence of an absorption band at 268 mμ after isomerization
† After isomerization (180° for 60 min.)

Table III

Component acids of Strophanthus *oils*

	A	B	C	Total	% (wt.)*	% (mol.)*
S. sarmentosus (forest form) seed oil:						
	(28·6)	(35·2)	(36·2)			
Myristic	0·16	—	—	0·16	0·2	0·2
Palmitic	10·96	0·92	0·24	12·12	12·2	13·4
Stearic	8·08	—	—	8·08	8·1	8·0
Arachidic†	3·06	—	—	3·06	3·1	2·8
Oleic	6·32	30·93	5·90	43·15	43·5	43·1
Linoleic	—	2·73	23·41	26·14	26·4	26·4
Hydroxyoctadecenoic	—	0·60	5·82	6·42	6·5	6·1
Unsaponifiable	0·02	0·02	0·83	0·87	—	—
S. hispidus seed oil:						
	(20·8)	(33·7)	(45·5)			
Myristic	0·13	—	—	0·13	0·1	0·2
Palmitic	10·42	1·06	0·23	11·71	11·9	13·0
Stearic	6·91	—	—	6·91	7·0	6·9
Arachidic†	1·97	—	—	1·97	2·0	1·8
Oleic	1·27	28·03	5·70	35·00	35·5	35·3
Linoleic	—	3·33	26·31	29·64	30·0	30·1
Hydroxyoctadecenoic	—	1·18	12·16	13·34	13·5	12·7
Unsaponifiable	0·10	0·10	1·10	1·30	—	—
S. courmontii seed oil:						
	(26·6)	(31·1)	(42·3)			
Myristic	0·04	—	—	0·04	0·1	0·1
Palmitic	11·74	1·23	0·27	13·24	13·4	14·6
Stearic	4·48	—	—	4·48	4·5	4·5
Arachidic†	2·80	—	—	2·80	2·8	2·5
Oleic	7·45	24·52	6·15	38·12	38·6	38·3
Linoleic	—	4·61	25·44	30·05	30·4	30·4
Hydroxyoctadecenoic	—	0·68	9·42	10·10	10·2	9·6
Unsaponifiable	0·09	0·06	1·02	1·17	—	—

* Excluding unsaponifiable material
† All acids higher than C_{18} are included as arachidic

Discussion

The results now obtained for *S. sarmentosus*, *S. hispidus* and *S. courmontii* seed oils are summarized in Table IV along with some other previously reported results.

A study of the results brings out the following points:

(i) Despite any difference there may be in the yield of oil obtained from the two varieties of *S. sarmentosus*, there is little significant difference between their component acids. Of the two samples studied the forest form gave the more saturated oil, and this is reflected in the relative amounts of oleic and linoleic acid; the more unsaturated oil actually contains more saturated acid (25·3% compared with 23·6%) than the other.

(ii) All the *Strophanthus* oils examined contain 9-hydroxyoctadec-12-enoic acid to the

Table IV

Component acids of Strophanthus *oils*, % (*wt.*)

	S.S. (S)*	S.S. (F)	S.H.	S.C.	*a*	*b*	*b'*
Myristic	0·2	0·2	0·1	0·1	—		
Palmitic	11·9	12·2	11·9	13·4	15	25·2	26·6
Stearic	9·2	8·1	7·0	4·5	7		
As arachidic	4·0	3·1	2·0	2·8	—		
Oleic	37·7	43·5	35·5	38·6	62	44·3	48·1
Linoleic	29·7	26·4	30·0	30·4	16	30·5	25·3
Hydroxyoctadecenoic	7·3	6·5	13·5	10·2	—	—	—

* Corrected (see text)

a : *S. hispidus* seed oil[6]
b and *b'* : calculated from thiocyanogen values of two samples of *Strophanthus* oil (species unstated)[7]

extent of 6·5–13·5%. It would appear that this isomer of ricinoleic acid is distributed throughout the genus rather than limited to one species. *S. hispidus* seed oil is at present the best source of this acid, though it may be that other species contain larger proportions of this acid.

(iii) Comparison of the results indicate that stearic acid (4·6–9·2%) and the hydroxy-acid (6·5–13·5%) show the greatest relative variation. It is interesting to note that, apart from the *S. sarmentosus* (forest form) seed oil, the proportions of oleic and linoleic acid are remarkably constant. The differences noted in this one case may be analogous to differences reported in sunflower-seed oils which are considered to be due to climatic conditions.[5] In the present case, however, more evidence is clearly required before there can be any certainty on this point.

(iv) Earlier studies of these oils did not reveal the presence of hydroxy-acid ; in addition, the methods of analysis used are now known to be inadequate, and it is therefore not surprising that the results differ from those now reported.

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THE FIXED OIL FROM THE SEEDS OF *CARUM COPTICUM* BENTH. (*PTYCHOTIS AJOWAN*)

By M. O. FAROOQ, S. M. OSMAN and M. S. AHMAD

The fixed oil from the seeds of *Carum copticum* Benth. of Indian origin has been analysed and the composition of its fatty acids determined by fractionation and thiocyanometric methods. Petroselinic acid has been found to be the major component of the fatty acids ; linolenic acid and any saturated acid other than palmitic are absent.

Carum copticum Benth., or *Ptychotis ajowan*, commonly known as 'Ajowan' in Hindi, is a member of the family Umbelliferae and has mainly attracted attention for its thymol content. Although its fatty oil was examined by Grimme[1] no indication is found of an attempt to analyse the fatty acids. It has been stated by Hilditch and collaborators[2, 3] that the fatty acids of the seed fats of Umbelliferae contain a considerable amount of petroselinic acid. Two samples,

(*a*) and (*b*), of the oil, obtained by extraction with light petroleum, were examined for their constants, one of which (*b*) was extracted from seeds previously exhausted by steam distillation. As expected, the oil (*a*) gave low saponification value and high unsaponifiable matter (Table I) (cf. Jamieson[4]). The composition of total acids obtained by the fractionation and thiocyanometric (T) methods agrees fairly well (Table V), except for the saturated acid, where the value given by (T) method is higher by 3·5% and may, apparently, be due to the passing over of non-fatty resinous matter into the liquid acids.[2] This is, however, a point to be further investigated. The presence of petroselinic acid was ascertained by isolation of a solid acid, m.p. 29–30° (cf. Steger & van Loon[5]), and confirmed by taking a mixed melting point of its dihydroxy-derivative with another sample of the same derivative of the acid obtained from parsley-seed oil.

Experimental

The oil (*a*) was freed from volatile essential oil as far as possible by heating *in vacuo* and then saponified with ethanolic potassium hydroxide. The ethanol-free, aqueous solution of the soap was exhausted with ether to remove unsaponifiable matter. The fatty acids liberated from the soap solution were still contaminated with non-fatty matter of resinous character. The resin acids were estimated by Twitchell's gravimetric method and found to form 2·6% of the total acids. The mixed fatty acids were then separated into solid and liquid fatty acids using Hilditch's modification of Twitchell's lead salt method[6] and found to be composed of 53·9% solid and 46·1% liquid fatty acids. (As the separation of ordinary oleic acid from petroselinic acid is not complete by this method[6] the figures may be considered to be of an approximate nature to that extent.)

The total acids therefore consist of resin acids 2·6%, solid fatty acids 52·5% and liquid fatty acids 44·9%. The constants of the three groups of fatty acids are given in Table II.

Table I

Characteristics of the oils (a) *and* (b)

	(*a*)	(*b*)
Fat content, % ..	29·5	24·0
Sp. gr. at 25° ..	0·9209	0·9201
Refractive index at 25°	1·4694	1·4635
Sap. value	145·8	174·3
I.V. (Hanus)	103·1	89·5
Acid No.	4·0	4·9
Ester No.	141·8	169·4
Unsaponifiable, % ..	14·0	2·2

Table II

	I.V. (Hanus)	Sap. value	Calc. mean mol. wt.	SCN* value
Total acids	103·7	199·8	280·8	84·0
Liquid fatty acids	124·0	198·1	283·2	85·8
Solid fatty acids	87·1	200·4	279·9	80·8

* The thiocyanogen values of the acids have been determined by following the procedure given by Jamieson.[4]

It will be observed that the mean molecular weights of all the groups of acids are fairly close to those of the acids of C_{18} series.

The liquid and solid fatty acids were separately esterified with methanol in the usual manner and the esters fractionally distilled under reduced pressure (2 mm.). The percentages of the individual acids in each fraction calculated from iodine-value and saponification equivalent (S.E.) figures, in conjunction with qualitative examination data of the fractions, are given in Tables III and IV.

Table III

Methyl esters of solid acids

Fraction	Wt., g.	I.V.	S.E.	Estimated composition	
				Palmitate	Petroselinate
S_1	10·2	71·6	279·5	1·69	8·51
S_2	11·3	74·8	285·1	1·45	9·85
S_3	12·3	79·6	290·6	0·9	11·4
S_4	8·4	85·1	292·8	—	8·4
S_5 (residue) ..	3·5	76·7	290·6	—	3·5
Totals	45·7			4·04	41·66
		% as esters		8·8	91·2
		% as acids		8·7	91·3

Table IV

Methyl esters of liquid acids

Fraction	Wt., g.	I.V.	S.E.	Estimated composition			
				Palmitate	Oleate	Linoleate	Unsapon.
L_1	6·6	119·1	280·5	0·39	3·83	2·38	—
L_2	11·8	121·2	291·3	0·5	6·96	4·34	—
L_3	9·6	126·5	292·7	—	5·11	4·49	—
L_4	13·2	129·8	294·1	—	6·52	6·68	—
L_5	9·0	128·6	293·1	—	4·57	4·43	—
L_6 (residue) ..	6·6	113·7	333·7 *	—	3·15	3·05	0·4
		* (S.E. 300·9 freed from unsaponifiable)					
Totals	56·8			0·89	30·14	25·37	0·4
		% as esters		1·6	53·0	44·7	0·7
		% as acids		1·6	52·9	44·6	0·9

Identification of fatty acids

Palmitic, petroselinic, oleic and linoleic acids were identified in the ester fractions as follows:

Fractions: S_1—Palmitic acid, m.p. 60–61°
6 : 7-Dihydroxystearic acid, m.p. 121–122°
S_4—6 : 7-Dihydroxystearic acid, m.p. 121–122°
L_1—Crude palmitic acid, m.p. 58–60°
Dihydroxystearic acid, m.p. 130–131°
Tetrahydroxystearic acid, m.p. 172–174°

No hexahydroxystearic acid could be isolated, thus indicating the absence of linolenic acid, and also no saturated acid other than palmitic was detected in any of the ester fractions.

In order to confirm the results a portion of the ester fraction (L_4) was saponified and the liberated acids were brominated by the method of Eibner & Mugganthaler.[7] No insoluble hexabromide could be isolated, and this confirmed the absence of linolenic acid. On the other hand, a white crystalline substance (tetrabromo-derivative of linoleic acid, m.p. 113–114°) was obtained on crystallization from ethanol and the presence of linoleic acid in the liquid acids was thus confirmed.

Isolation of petroselinic acid

A portion of the ester fraction (S_1) was saponified and the liberated acids were fractionally crystallized from dilute acetone. It yielded impure palmitic acid and a colourless substance which melted sharply at 29–30°, thus indicating the presence of petroselinic acid.

This solid oleic acid was also obtained as colourless shining crystals, m.p. 29–30°, by recrystallization of solid acids from 96% ethanol at 0° (cf. Steger & van Loon[5]). Further, a mixed-melting-point determination of the 6 : 7-dihydroxy-derivative obtained as described above showed no depression with a pure recrystallized sample obtained from parsley-seed oil.

Table V

Acids	Fractionation, %	Thiocyanometric (T), %
Resin acids ..	2·6	2·6
Palmitic ..	5·3	8·8
Petroselinic ..	48·1	47·2
Oleic	23·9	21·2
Linoleic ..	20·1	20·2

Table V gives the composition of total acids as found by fractionation and thiocyanometric (T) methods. In the latter case the calculation (of which we omit details) has been based on the iodine and thiocyanogen values of liquid and solid acids separately (Table V).

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A CHROMATOGRAPHIC EXAMINATION OF THE MINOR CONSTITUENTS OF VARIOUS TREE ZONES

By W. E. HILLIS

The methanol extracts of the bark, phloem, sapwood and heartwood zones of 22 species representing 11 families were examined chromatographically with three different solvents. The trees examined included some without heartwood, one with a wide sapwood, and others with heartwood of pathological and normal origin. The extracts were shown to be complex mixtures, but a ready comparison of the distribution of the minor constituents throughout one tree, and between different samples, was made possible by means of the partition chromatograms. An examination of the kinos and of extracts of the leaves and roots of certain eucalypts failed to reveal any special part played by them. Information from the chromatograms assisted the identification of rutin and ellagic acid.

Introduction

There are many trees, particularly tropical trees, which form little or no heartwood and contain a large proportion of sapwood of low durability. The factors responsible for the transformation of sapwood into heartwood are unknown, but some theories have been put forward as an explanation for this change. There has been no investigation of the chemical aspect of the transformation, although some incidental information was collected during a recent study of pine extractives.[1] In view of the increasing interest in tropical trees and the increasing demand for more durable timbers, a better knowledge of this chemical aspect should be of value.

The study of the chemical aspects of sapwood–heartwood transformation presents a complex problem. It is well known that the extractive content of heartwood is greater than that of sapwood, but the origin of the extractives has not been shown. To find the most profitable lines of attack for future work, it is necessary to obtain reliable general information of the part played by the extractives in the transformation. Consequently various zones of a wide variety of trees belonging to different families were examined. As general principles only were sought, the considerable task of isolating and identifying the minor constituents was not warranted at this stage, and paper partition chromatography appeared to be the most suitable technique for such an investigation. However, more detailed investigations are assisted by information gained from the chromatograms.

Materials

The different zones of the cross-sections of the trees listed below were used. In some cases the kinos, the zones of the roots and the leaves from the same tree were also examined.

(*a*) Trees in which no heartwood was present: one specimen each of *Alstonia scholaris* R. Br. (Apocynaceae), *Ficus stenocarpus* F. Muell. (Moraceae), *Rapanea variabilis* Mez. (Myrsinaceae), *Myrtus hillii* Benth. (Myrtaceae), *Hodgkinsonia ovatiflora* F. Muell. (Rubiaceae), *Ixora beckleri* Benth. (Rubiaceae).

(*b*) Tree with wide sapwood: one specimen of *Sloanea woollsii* F. Muell. (Elaeocarpaceae).

(*c*) Trees with heartwood of pathological origin: one specimen each of *Euroschinus falcatus* Hook (Anacardiaceae), *Diospyros pentamera* F. Muell. (Ebenaceae), *Bridelia exaltata* F. Muell. (Euphorbiaceae), *Croton phebalioides* F. Muell. (Euphorbiaceae), *Longetia swainii* de Beuz. and White (Euphorbiaceae); two specimens of *Nothofagus cunninghamii* Oerst. (Fagaceae).

(*d*) Trees with apparently constant and normal heartwood formation: one specimen each of *Acacia dealbata* Link (Leguminoseae), *Pithecolobium pruinosum* Benth. (Leguminoseae), *Eucalyptus baueriana* Schau. (Myrtaceae), *Eucalyptus elaeophora* F. Muell. and kino (Myrtaceae), *Eucalyptus hemiphloia* F. Muell., nine kinos, root and leaves (Myrtaceae), *Eucalyptus polyanthemos* Schau. (Myrtaceae), *Eucalyptus macrorrhyncha* F. Muell., two kinos, root and leaves (Myrtaceae), *Eucalyptus sideroxylon* A. Cunn. and two kinos (Myrtaceae), *Syncarpia laurifolia* Ten. (Myrtaceae).

Experimental

Preparation of extracts

The specimens were separated into heartwood, sapwood and other parts, and vacuum-dried (at 45°) as soon as possible, which in the case of the eucalypts was within 48 hours of collecting the sample. The zones were milled to — 50 mesh and 20 g. was extracted with methanol in a Soxhlet apparatus in semi-darkness for four days. The extract was concentrated to 15 ml. and then chromatographed.

The leaves were extracted repeatedly with water and the extract concentrated under reduced pressure. The kinos were mixed with methanol.

Chromatographic examination

Each extract was well mixed and an increasing amount of extract placed at different points on a No. 1 Whatman paper (46 cm. × 57 cm., all papers from the same ream) by means of a thin glass rod. The solvent ascent modification of Williams & Kirby[2] was used. The resolving solvents tried were: butanol : acetic acid : water (40 : 10 : 50);[3] *m*-cresol : acetic acid : water (50 : 2 : 48);[4] and phenol : 2N-hydrochloric and acetic acids (50 : 50).[5] The precautions which were deemed advisable in an earlier examination[6] were again adhered to.

The sheets were examined under ultra-violet light, the areas outlined and described and, after drying, were sprayed with ammoniacal silver nitrate at room temperature or with bis-diazotized benzidine.[7] Those areas which responded were outlined within 15 minutes.

At least two chromatograms of each extract were prepared at different times, with two different preparations of each solvent. In almost all cases the outline of the areas was within $\pm$ 0·02 R_F, but some specimens were poorly resolved with the solvents used in this work, and their outlines somewhat diffuse. Averages of the chromatograms obtained from the same quantity of extract from each zone of the sample are presented. The colours expressed have been abridged to the simplest description, and the thicknesses of the outlines of the fluorescent areas have been varied to convey an idea of the intensity.

Results and discussion

Chromatography as an aid in the identification of minor constituents

Examination of the chromatograms of the extracts of any one species (e.g. Fig. 1) shows that different areas have different R_F values when resolved with different solvents. If the R_F values and other relevant characteristics of pure components in these solvents were known, the elucidation of the composition of the extracts would be assisted. The R_F values of some compounds have been reported,[8] but there is often a lack of agreement among different workers, which is probably due to the different (and often unspecified) conditions employed. In addition, the R_F value for a component when pure may be different from that in a complex mixture, because two substances with very similar mobilities could cause altered R_F values due to a 'salting out' effect,[9] or, on the other hand, have a mutual solubilizing effect on each other.

Despite these limitations, useful deductions can be made from R_F values. Bate-Smith[8] found that rutin had the R_F values of 0·58 with butanol solvent and 0·26 with cresol solvent; these values are close to those of 0·55 and 0·15 respectively for the yellow constituent in the leaves of *Eucalyptus macrorrhyncha* (Fig. 2). The presence of rutin was confirmed by crystallizing the dried extract from water, aqueous ethanol and finally ethanol. The yellow crystals had a melting point of 187–190°, alone or mixed with authentic rutin, and a spectrum identical with pure rutin ($\lambda_{max.}$, 260 and 350 mμ; $\lambda_{min.}$, 286).

The strong mauve fluorescent component [R_F 0·55 (butanol) and 0·4 (phenol)], which is characteristic of the Myrtaceae and of *Sloanea woollsii* (Figs. 2–7), showed a yellow fluorescence when 'fumed' with ammonia, and these properties are identical with those of ellagic acid. This deduction was confirmed by evaporating the heartwood extracts, making them alkaline with sodium hydroxide, and mixing with pyridine. The liquor was poured off, the residue acidified, washed with a small amount of water, and then dissolved in a minimum amount of boiling pyridine. The long needles which formed on cooling were removed and thoroughly washed with ethanol; they were identified as ellagic acid by its tetracarbethoxy-derivative,[10, 11] m.p. 245–247°, alone or when mixed with an authentic specimen. An *X*-ray examination gave all the major reflexions of authentic ellagic acid.

A substance which reduces ammoniacal silver nitrate and has R_F 0·7 (butanol) and 0·06 (cresol) (compare Bate-Smith[8]) and 0·2 (phenol) (see Figs. 3–6) indicated the presence of gallic acid. When the chromatograms were developed with potassium cyanide solution,[12] these areas became a strong red identical with that given by gallic acid.

However, the identification of the components indicated by the chromatograms is not always easy. The four major resolvable components of the extractives of *Euroschinus falcatus*

Key to Figures.—B, bark; BR, bark root; H, heartwood; HR, heart root; I, intermediate zone; IS, inner sapwood; K, kino; L, leaves; OS, outer sapwood; P, phloem; S, sapwood; SR, sap root.
Abbreviations: b., brown; bl., black; bu., blue; d., dull; f., faint; fl., fluorescence; g., green; i., intense; in., instant; l., light; m., mauve; or., orange; p., pink; r., red; s., strong; sl., slow; t., tan; v., very; w., white; y., yellow

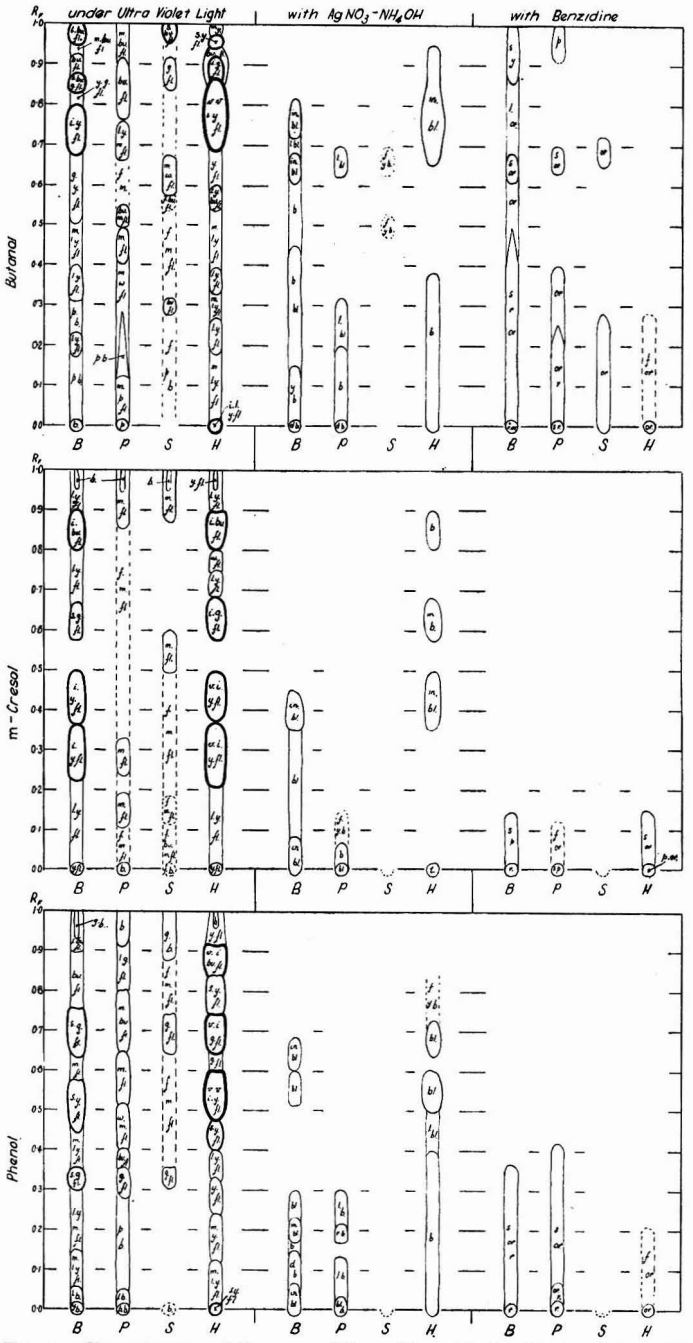

FIG. 1.—*Chromatograms of the zones of* Euroschinus falcatus *Hook. A tree with heartwood of pathological origin*

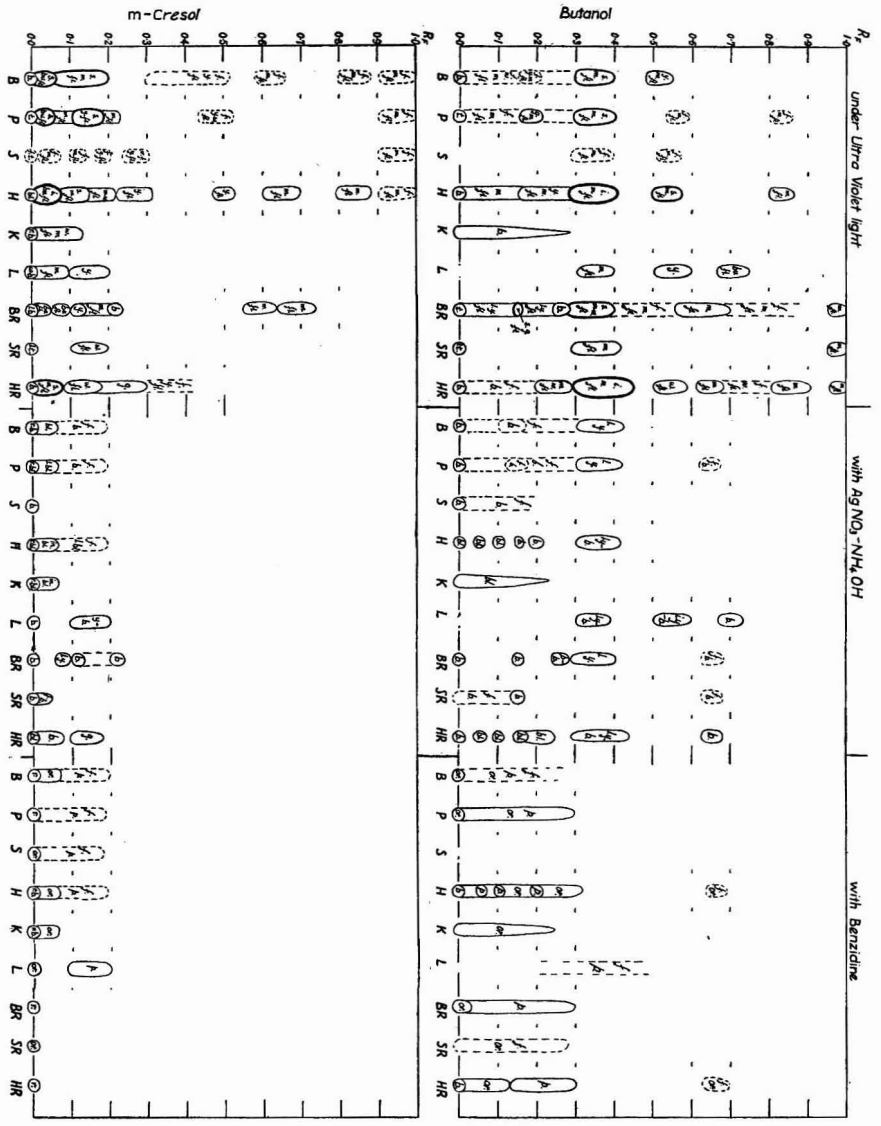

FIG. 2.—*Chromatograms of the zones of* Eucalyptus macrorrhyncha (*a 'stringybark'*). *A tree with heartwood of normal formation* (*for Key see p.* 136)

Table I

R_F of compound in butanol	R_F of compound in cresol	Compound indicated	R_F of pure compound[8] in butanol	R_F of pure compound[8] in cresol
0·95	0·71	Phloretin	0·96	0·80
0·89	0·63	Kaempferol	0·90	0·61
0·77	0·28	Quercetin	0·74	0·27
0·77	0·43	Fisetin	0·72	0·40

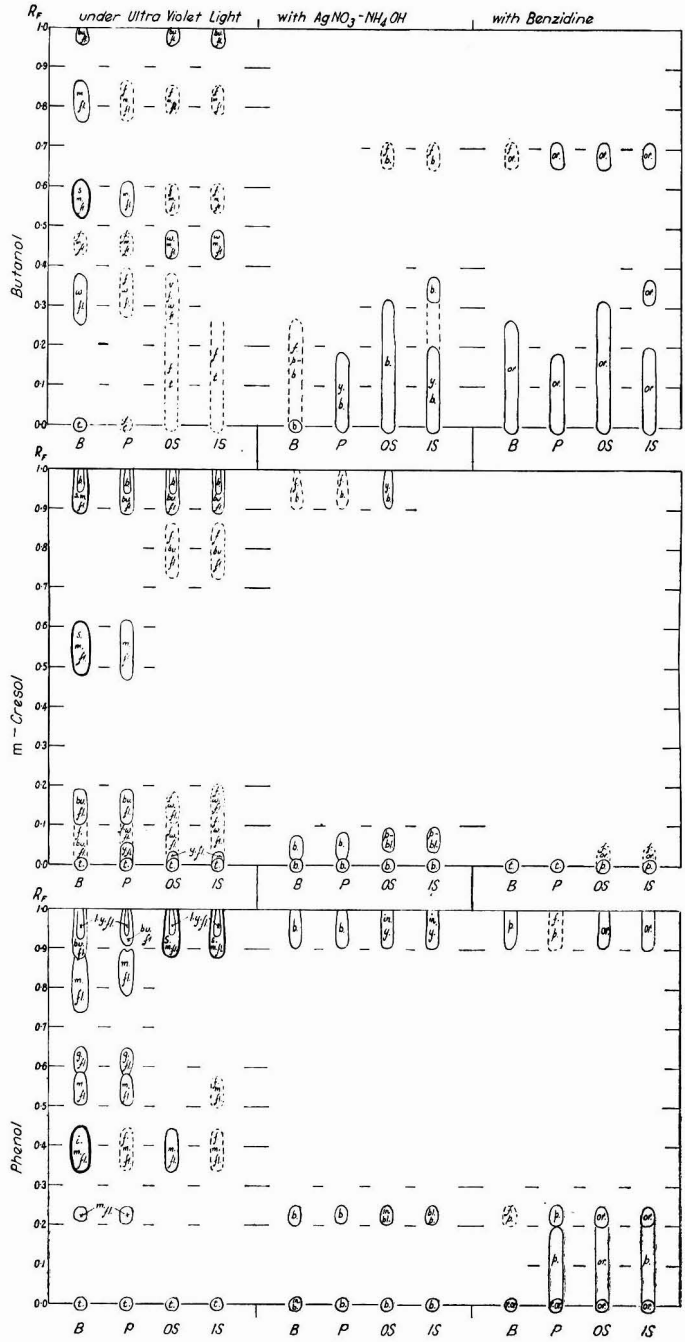

FIG. 3.—*Chromatograms of the zones of* Myrtus hillii *Benth. (about 20 cm. in diameter). A tree in which no heartwood was present (for Key see p. 136)*

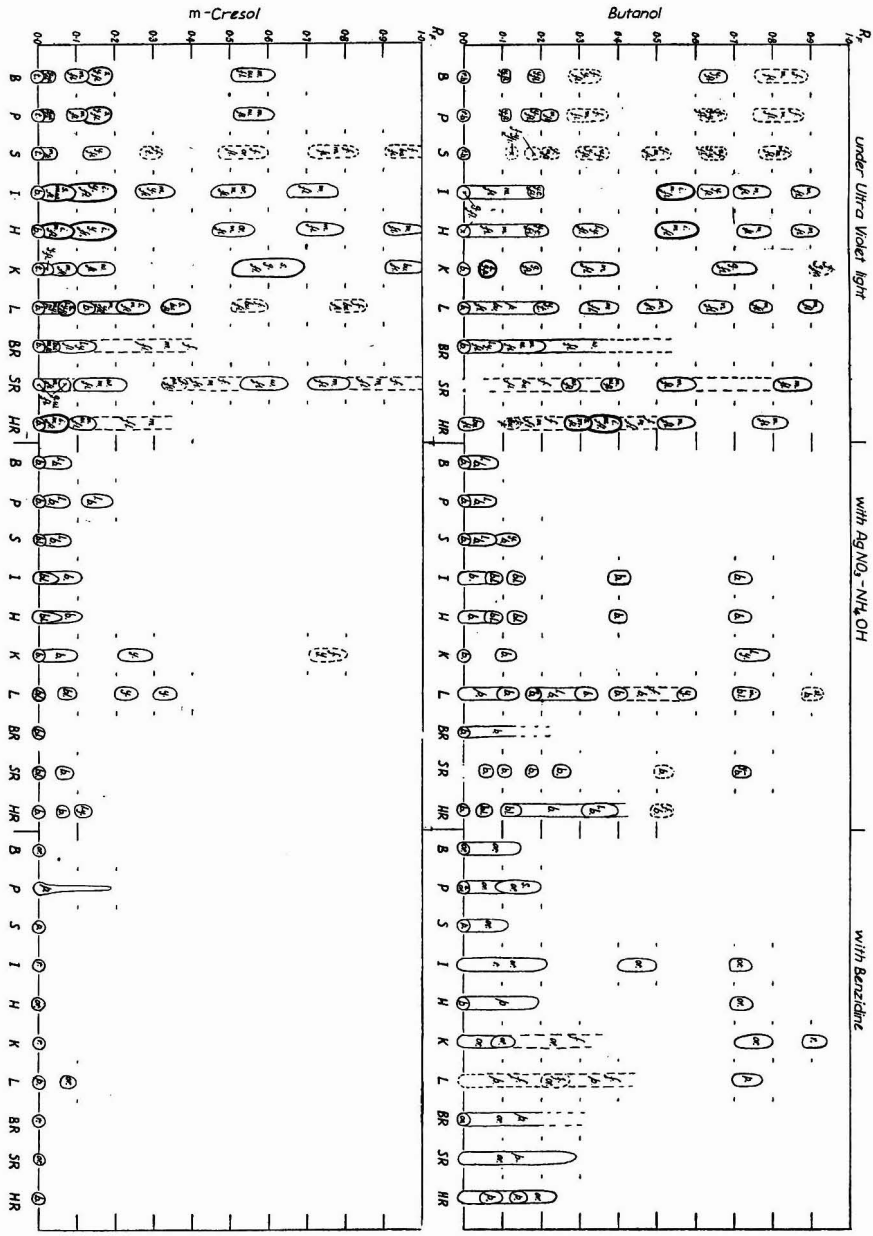

FIG. 4.—*Chromatograms of the zones of* Eucalyptus hemiphloia *F. Muell.* (*a 'box'*). *A tree with heartwood of normal formation* (*for Key see p.* 136)

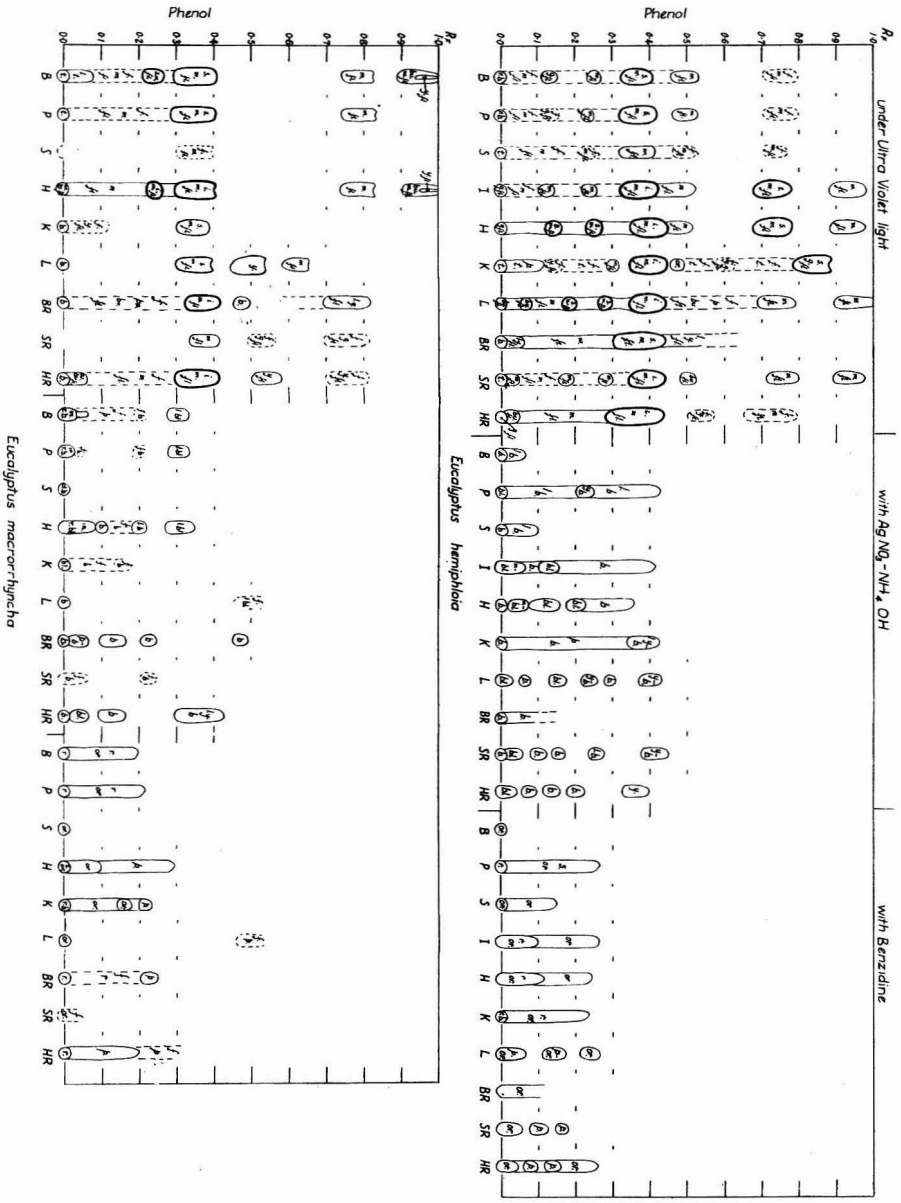

FIG. 5.—*Chromatograms of the zones of* Eucalyptus macrorrhyncha (*a 'stringybark'*). *Also of* Eucalyptus hemiphloia *F. Muell.* (*a 'box'*). *Trees with heartwood of normal formation* (*for Key see p.* 136)

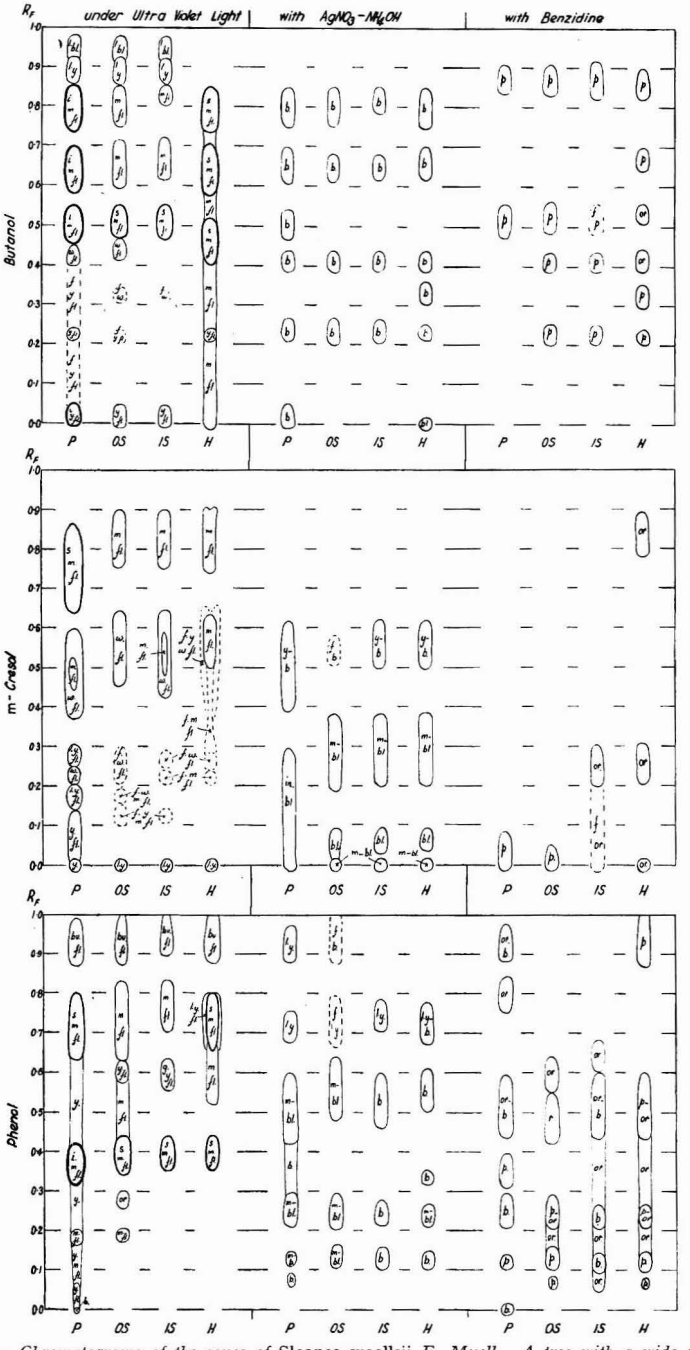

FIG. 6.—*Chromatograms of the zones of* Sloanea woollsii *F. Muell. A tree with a wide sapwood* (*for Key see p.* 136)

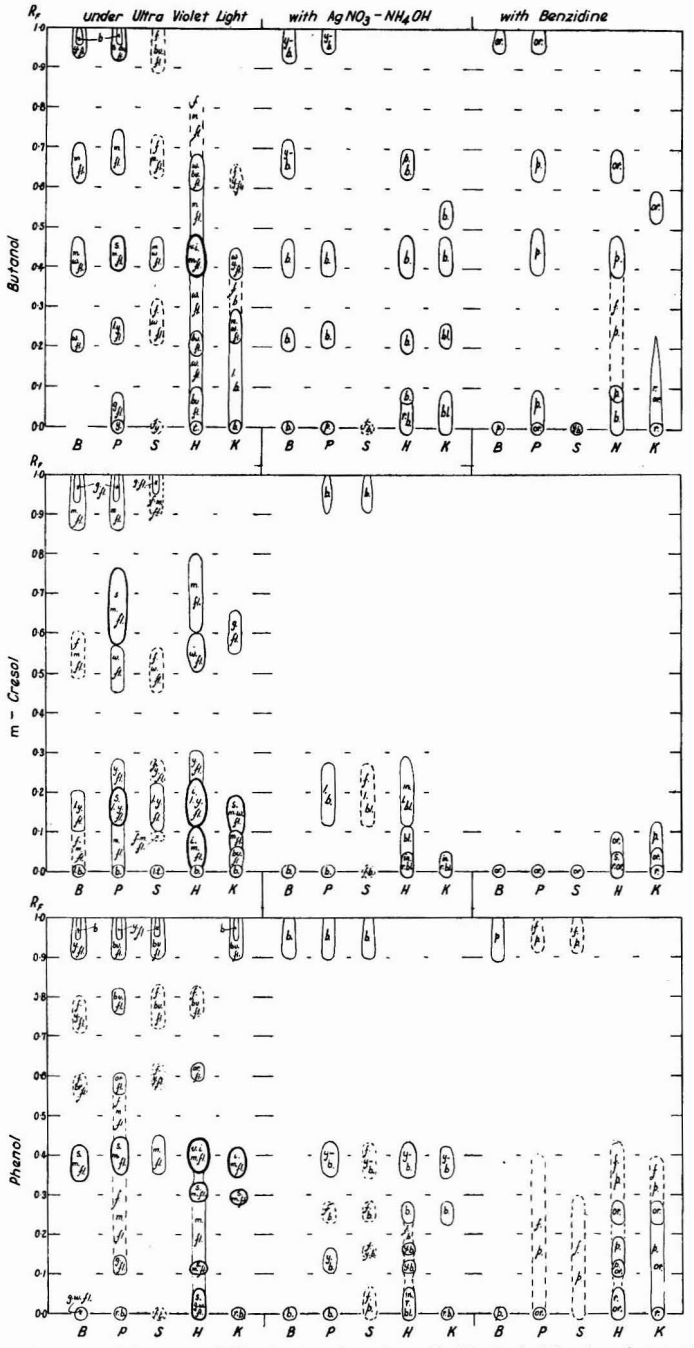

FIG. 7.—*Chromatograms of the zones of* Eucalyptus elaeophora *F. Muell.* (*a 'box'*). *A tree with heartwood of normal formation* (*for Key see p.* 136)

(Fig. 1) could be phloretin, kaempferol, quercetin and fisetin (see Table I). The last two compounds have already been isolated from other members of this family.[13]

The compound with R_F values close to that of quercetin fluoresced the most strongly and appeared to be the principal component. However, attempts to isolate the compound from the extract with solvents, by crystallization or by leaching it from the dried extract with borax solution were unsuccessful. Removal, of the appropriate area from a phenol chromatogram, and rechromatographing it in butanol, failed to produce an area from which useful information could be obtained, when a solution of it was examined spectrophotometrically.

It is obvious that partition chromatography in its present state of development can assist a detailed study of a wood extract. The phenol solvent was usually found to be the most suitable (particularly with the eucalypts) by virtue of sharpness of resolution, completeness of differentiation and stability of colours, but new solvents are needed to resolve all the materials present. The chromatograms are likely to be more complex than present tests reveal; the lignan eudesmin has been isolated from *Eucalyptus hemiphloia* kino, but a method to reveal its location has yet to be found.

Biochemistry of the formation of minor constituents

Formation of heartwood.—Chemical studies on many decay-resistant timbers have indicated that their durability may be due to the toxicity of the extractives,[14, 15] and that phenolic extractives and tannins are associated with durability. By means of chromogenic agents, phenolic and C_{15} compounds have been detected among the resolved components in the extracts examined. However, C_{15} compounds have no great proven fungicidal activity, and the insecticidal activity of both classes of compounds is unknown. The greatest amount of constituents revealed by chromatography is found in the most durable portions of the trunk, so that if these revealed components are not responsible for durability then the amount of fungicidal constituents must have increased simultaneously. There is reason to believe that deductions for one class of compounds will hold for the other.

Both normal and pathological heartwoods have a stronger fluorescence than sapwood, and sometimes the sharpness of the change and the difference in intensity are very striking. As would be expected, chromatographic examination shows a greater amount of resolvable fluorescent components in heartwood. But in most cases, the increase in fluorescence was due to the formation of a greater amount of extractives of the same general composition as found in the rest of the tree (Figs. 2 and 4–7) and not to the formation of new compounds. Those trees examined which did not show a simple increase in extractives when heartwood was formed were *Euroschinus falcatus* (Fig. 1), *Pithecolobium pruinosum* (Fig. 8) and *Acacia dealbata*; all of these gave very similar chromatograms.

Erdtman & Rennerfelt[16] observed that the formation of heartwood phenols of pines stops behind an injury to the cambium, and have suggested that heartwood is formed by the accumulation of compounds generated in the cambium and transported centripetally through the medullary rays. According to their view, one would expect to find sapwood on the pith side of large kino pockets, as these would interrupt transportation; but no sapwood is seen. Also, the major components of heartwood would be expected to occur in sapwood, but although this was true for most of the specimens examined (see also Linstedt[1]), the major heartwood extractives were not detected in the sapwood of *Euroschinus falcatus*, *Pithecolobium pruinosum* and *Acacia dealbata*. There is possibly some connexion between the sudden increase in extractives content and corresponding decrease in starch. The phenolic and C_{15} compounds could be formed from simple carbohydrates (in this case originating from starch) in a manner suggested for other plant tissues by Robinson and extended in a review by Geissman & Hinreiner;[17] Kursanov *et al.*[18] have produced some evidence for these changes. Thus the low extractive content of sapwood could be the result of normal metabolism of the cell, and the high content of heartwood might be due to a period of intense activity just before and during the death of the cell.

A thin black zone surrounds the mauve fluorescent heartwood of the eucalypts, and surrounding this again is a wider yellow fluorescent zone (both zones called the 'intermediate' zone). A similar condition was noticed with some other genera, and this difference in fluorescent colour could be due to another class of compounds representing the progenitors of the heartwood constituents. However, in those species examined, no significant difference in the chromatograms was revealed (e.g. Figs. 4 and 5).

The association of crystals in heartwood with, or in proximity to, fungal hyphae suggests that they may be directly formed by the fungus.[19] Crystals have been recovered from eucalypt heartwood and found to be ellagic acid, but chromatographic examination indicates that this

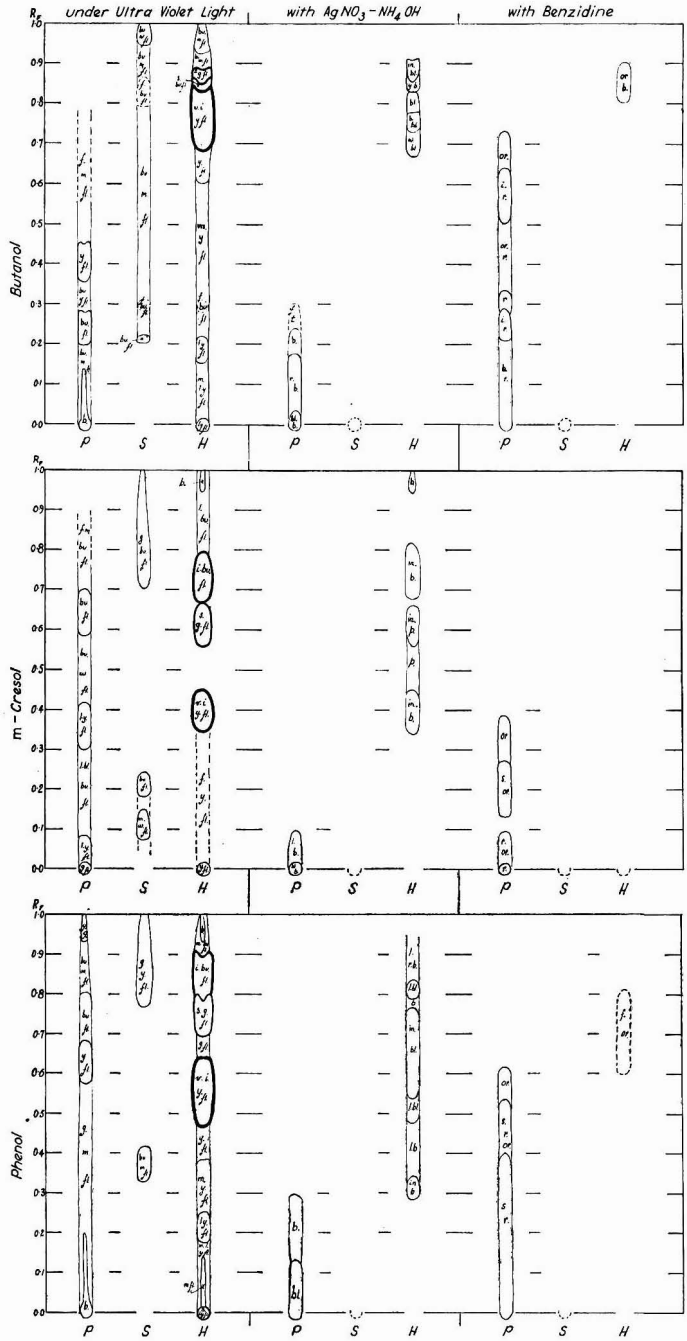

FIG. 8.—*Chromatograms of the zones of* Pithecolobium pruinosum *Benth. A tree with heartwood of normal formation* (*for Key see p.* 136).

substance is present in all eucalypt tissues, even where fungal hyphae have not been detected, suggesting that ellagic acid is a normal metabolite. Chromatograms of two samples of *N. cunninghamii* (containing heartwood of pathological origin) were identical, and the examination of a fruiting body of a fungus associated with one of the samples failed to reveal any components common to both fungus and host. In addition, extracts from several species of Myrtaceae and Leguminoseae were noticeably similar in composition within each family These observations suggest that the composition of the extractives is a characteristic of the tree, and that the fungus is not directly responsible for their formation.

Examination of trees without normal heartwood.—A feature of the trees without heartwood is the small amount of extractives present. When they are compared with the extractives of sapwood from normal heartwood trees, however, there is no great difference in the amount of resolved components, neither is there any obvious difference in their nature. *Alstonia scholaris* and *F. stenocarpus* (Fig. 9) appeared to have the smallest amount of extractives, but the other members of Group (*a*) contained about the usual amount.

The facts set out above indicate that if the sapwood cells formed more extractives in response to some stimulus, the durability would increase. This appears to have happened, for example, with *N. cunninghamii*, which often contains heartwood of pathological origin. The pathological heartwood of *Euroschinus falcatus* contains a large amount of extractives, which give a chromatogram very similar to the chromatograms of *Acacia dealbata* and *Pithecolobium pruinosum* (Fig. 8). Unfortunately there is no information available on the comparative durability of the heartwood of these trees.

Examination of a tree with a wide sapwood.—The sample of *Sloanea woollsii* examined had a wide sapwood typical of this species. The composition of the extractives did not change across the sapwood (Fig. 6), but the amount of extractives present was larger than that in the sapwood of other trees, and there was an indication that the amount was slightly greater in the inner sapwood.

Comparison of the extractives from different species.—Erdtman[20] has commented on the similarity of the products isolated from the Pinaceae, and Linstedt[1] and Linstedt & Misiorny[21] have reported the use of chromatography to characterize members of the genus *Pinus*, and to distinguish two subgenera. This present investigation has revealed a similarity within the Leguminoseae, Myrtaceae and Rubiaceae. Thus the Leguminoseae (see also Hillis[6]) yielded extracts which produced strongly fluorescent yellow chromatograms with some components common to all samples. The eight representatives of the Myrtaceae gave mauve fluorescent chromatograms with ellagic acid as a component common to all tissues. Also the R_F values and fluorescent colours of some of the components of the extracts of *Euroschinus falcatus* (Table I) suggest that they are the same as those already isolated from other members of the Anacardiaceae.[13] On the other hand the three members of the Euphorbiaceae showed no resemblance to one another.

However, chromatography of extracts cannot be used as an absolute identification of a family. There were cases where chromatograms were also similar to those of another family, e.g. *Euroschinus falcatus* (Anacardiaceae) (Fig. 1) is similar to the Leguminoseae (Fig. 8); *Sloanea woollsii* (Elaeocarpaceae) (Fig. 6) is similar to the Myrtaceae (Figs. 2–4 and 7), and *F. stenocarpus* (Moraceae) to *H. ovatiflora* (Rubiaceae).

Investigation of a few members of the *Eucalyptus* genus failed to reveal any distinctive feature which could be correlated with the popular divisions based on bark textures (i.e. boxes, gums, stringybarks). There is an indication that a yellow component (R_F 0·15 cresol) may prove to be characteristic of those trees with a box-type bark, but only single samples of the species and only a small portion, namely *Eucalyptus baueriana*, *Eucalyptus elaeophora* (Fig. 7), *Eucalyptus hemiphloia* (Figs. 4 and 5) and *Eucalyptus polyanthemos*, of the group has been examined.

Examination of tissues other than wood.—Linstedt & Misiorny[21] found that the extractives of the bark of certain pines were different from those of the sapwood and heartwood. In this work differences in the extractives between bark and wood zones were observed in only two species, *C. phebalioides* and *R. variabilis*, although some components are common throughout the trunk of these trees. The bark of some of the samples contained smaller amounts of resolvable extractives than did the phloem, which may be due to secondary changes of the bark extractives. In almost every case, however, either the bark or the phloem contained amounts much greater than those in the sapwood.

An examination of the roots and leaves of *Eucalyptus hemiphloia* and *Eucalyptus macrorrhyncha* (Figs. 2, 4 and 5) did not reveal any special part played by them in the formation of extractives. No definite indication of rutin or its aglycone, quercetin, was seen in any of

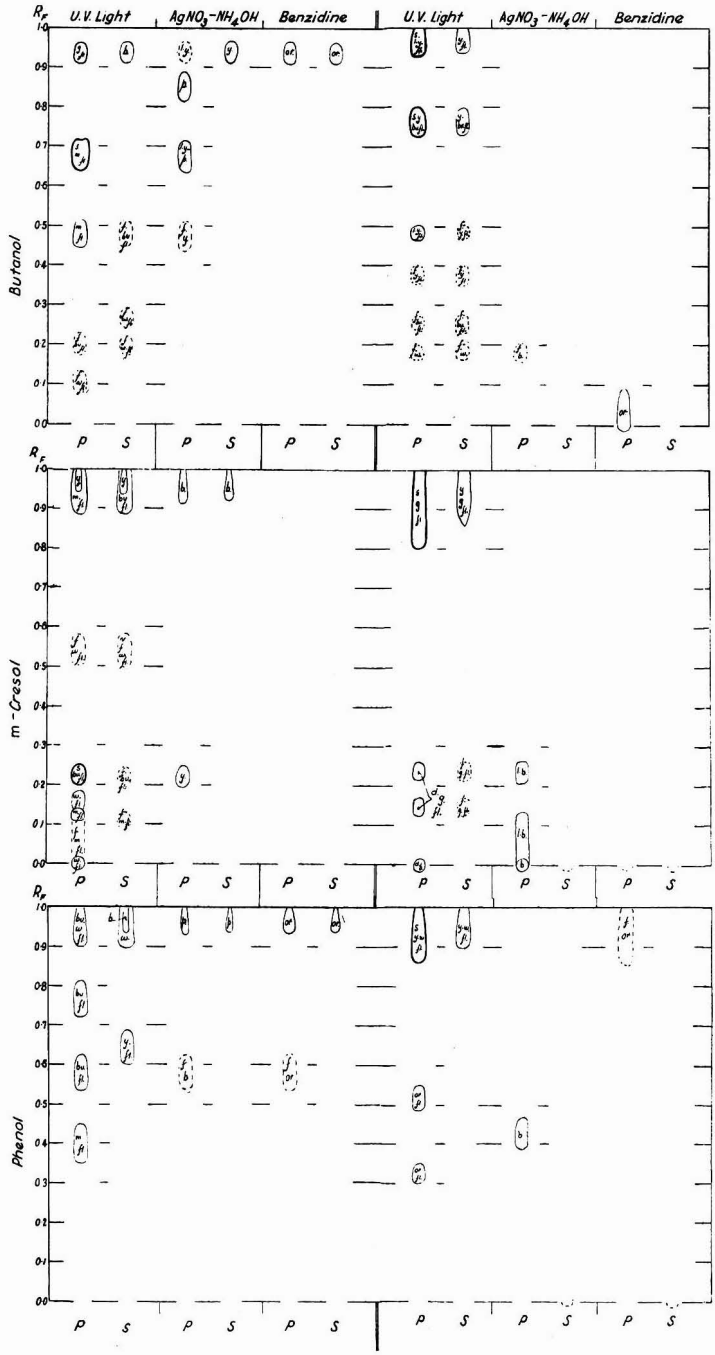

FIG. 9.—*Chromatograms of the zones of* (*a*) Ficus stenocarpus *F. Muell.* (*about* 45 *cm. in diameter*). *Also of* (*b*) Alstonia scholaris *R. Br.* (*about* 90 *cm. in diameter*). *Trees in which no heartwood was present* (*for Key see p.* 136)

the other tissues of *Eucalyptus macrorrhyncha*. Although the combined bark and phloem portions of the root of *Eucalyptus macrorrhyncha* contained two components which were not detected elsewhere, the other root tissues contained extractives similar to those found in the corresponding tissues in the trunk.

Kino.—With the exception of the kino of *Eucalyptus hemiphloia*, the eucalypt kinos examined were less resolvable than were the heartwood constituents. However, some of the kinos do contain components not found in the other tissues of the tree.

Kino veins have been associated with an earlier injury to the cambial region, and this injury may have been caused by fire, insect damage or branch shed.[22] Such injury would facilitate the entry of air and other foreign substances. Although the access of a large amount of air may be an explanation of the difference in composition of the kino from normal extractives, it should be remembered that extraneous material may form in sapwood, wound wood, or heartwood when a living cell borders on an air-filled one.[23]

Fungal hyphae have been observed in kinos,[24] and this foreign factor could gain access at an injury. Nine different samples of *Eucalyptus hemiphloia* kinos were examined, and these included samples from Queensland, New South Wales and Victoria, and from the phloem, cambium, heartwood and knotty regions. In every case the chromatogram was of the same type, although the ratio of the quantities and the amount of the initial extractives differed. A mycological investigation is now necessary to prove whether the presence of the fungi is fortuitous.

Although kino veins are of a comparatively restricted nature, kino pockets are sometimes very extensive and may hold several gallons of fluid kino. The origin of this kino is unknown; sap does not appear to play any part because, with one exception, several samples of sap from freshly felled trees failed to respond to ferric chloride or ferrous $\alpha\alpha'$-dipyridyl sulphate[25] and the only detectable phenolic component in the exceptional case was ellagic acid.

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THE ENZYMIC DEGRADATION OF PECTIN AND OTHER POLYSACCHARIDES. II*.—Application of the 'Cup-plate' Assay to the Estimation of Enzymes

By J. DINGLE, W. W. REID and G. L. SOLOMONS

The agar 'cup-plate' diffusion technique has been applied to the quantitative determination of enzyme activity, principally to amylase, polygalacturonase, cellulase, protease and pectin-esterase. With all enzymes so far examined, the relationship between diameter of zone and log(amount of enzyme) is linear over a wide range, and may be used for the quantitative estimation of the enzymes. The cup-plate assay of polygalacturonase, like viscometric methods, measures the initial destruction of the colloidal properties of pectic acid, and, owing to the complex nature of polygalacturonase, bears no simple relationship to the assay of polygalacturonase by the estimation of glycosidic hydrolysis.

Introduction

During a survey of numerous isolates of microfungi,[1] principally with regard to their elaboration of starch- and pectin-degrading enzymes, rapid methods of assay were needed, and it seemed likely that the principle of the 'cup-plate' diffusion assay for antibiotics might be applicable. It was found that various enzymes gave zones of activity when inserted into cups cut in an agar substrate gel; with all the enzymes studied, the relationship between diameter of zone of enzyme activity and log(amount of enzyme) was linear over a wide range. A quantitative cup-plate assay is now used for the routine estimation of several enzymes (amylase, polygalacturonase, protease, cellulase and pectin-esterase), and other enzymes (arabanase, xylanase and lipase) have also been determined.

Experimental

Substrates

Soluble starch. B.D.H. AnalaR.
Xylan, araban. See Reid.[2]
Sodium salt of carboxymethyl cellulose. I.C.I. Ltd.
Polyethylene glycol monolaurate. (Cithrol '10M.') Croda Ltd.
Sodium pectate and pectin. Various samples prepared on laboratory and pilot-plant scale, and commercial samples from Unipectin A.-G., Zurich.

Enzyme preparations

Enzyme preparations from microfungi were prepared both in the laboratory and on a small-scale plant by growth of selected cultures on solid media, or in submerged culture in liquid media.

Taka-diastase. Parke Davis Ltd.
Tomato pectin-esterase.[3, 4]
Orange pectin-esterase.[5]
Pectinol preparations. A sample of Pectinol '10M.' (Rohm & Haas, Philadelphia) preserved at 4° over silica gel was used as a standard preparation; Pectinol 100D was also examined.

General procedure

The technique consists in incorporating 1% of the appropriate substrate in a 2% buffered agar solution containing 0·01% salicylanilide to prevent mould growth. Gels are cast on to glass sheets in the normal manner[6] to give a 4-mm. layer. Cups, 8 mm. in diameter, are cut using a No. 4 cork-borer, and filled by means of a suitable pipette.[6] Dilutions of a standard enzyme preparation are included on every plate. With a suitable cover to prevent evaporation, the plates are incubated at 37° for 18 hours, and then sprayed or painted with the appropriate developing agent. The zone diameter is measured and the activities are calculated from the standards.

Detailed comments on the assays

α-Amylase.—The substrate is soluble starch in 0·2M-phosphate or McIlvaine buffer, pH 4·5. The plate is developed with 0·1N-iodine to give colourless zones on a blue background.

* Part I: *J. Sci. Fd Agric.*, 1950, **1**, 234

β-Amylase.—With soluble starch in 0·2M-phosphate or McIlvaine buffer, pH 4·5, samples of β-amylase gave small red zones of limit dextrin. The β-enzyme is not detectable in the presence of the α-enzyme, as the zone of activity for the α-enzyme obscures that given by the β-enzyme. The cup-plate assay may be suitable for the determination of the α-activities of materials, e.g. barley malt, containing both α- and β-enzymes.

Polygalacturonase.—The substrate is sodium pectate in 0·2M-phosphate buffer, pH 5·3. It is preferable to include 0·5% (w/v) ammonium oxalate to remove any calcium present. The plate is developed with 5N-hydrochloric acid. The zones have an opalescent halo,[7] and the inner, sharper diameter of this is measured.

Protease.—The substrate is gelatin in McIlvain buffer, pH 4·5. Usually no development is needed, as clear zones appear on an opalescent background, but the contrast may be improved by treatment with sulphosalicylic acid.

Cellulase (Cx).—The substrate is sodium carboxymethyl cellulose in Walpole's acetate buffer, pH 4·5. The plate is developed with a 10% (w/v) solution of copper acetate, when clear zones appear on an opalescent background.

Lipase.—The substrate is polyethylene glycol monolaurate in Walpole's acetate buffer, pH 4·5. The plate is developed with copper sulphate solution 10% (w/v) to give cloudy zones on a clear background.

Pectin-esterase.—The substrate is pectin in Walpole's acetate buffer, pH 4·5, containing 0·5% (w/v) calcium acetate. The plate is developed with 10% (w/v) copper acetate to yield clear zones on an opalescent background. The assay is only suitable in the absence of polygalacturonase as the latter enzyme attacks the calcium pectate formed.

Xylanase.—The substrate is xylan in phosphate buffer, pH 4·5. The plate is developed with hydrochloric acid (pure, 1 volume, plus water, 4 volumes) to give clear zones on an opalescent background.

Arabanase.—The substrate is araban in phosphate buffer, pH 4·5. The plate is developed by flooding with alcohol, to give clear zones on an opalescent background. The definition in this assay is poor.

The influence of the pH of the gel on zone size

Experiments showed that the pH of the gel had a marked effect on the size of the developed zone. The point is illustrated by the curves given for polygalacturonase in Fig. 1, where the maximum zone size was found to be given in the range pH 4·4–4·8. Similar experiments examining α-amylase activity on starch–agar plates, gave maximum zone size for Pectinol 10M. at pH 4·4, for taka-diastase at pH 5·2 and for an *Aspergillus oryzae* preparation at pH 4·4.

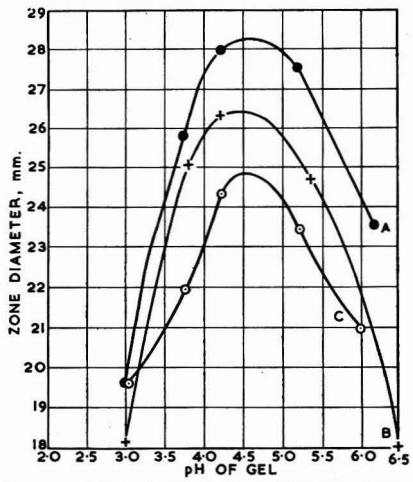

FIG. 1.—*Effect of pH of gel on diameter of zones produced in the cup-plate assay of polygalacturonase*

A. Pectinol '10M.' (Rohm & Haas Co.)
B. Preparation from *Aspergillus foetidus* Thom & Raper (surface culture)
C. Preparation from *Botrytis cinerea* (surface culture)

This effect of pH on zone size was examined by preparing agar–substrate plates in a suitable buffer. With low pH values, in order to prevent hydrolysis of the agar, the substrate and the agar were dissolved in half the total quantity of water, and the buffer salts in the other half. The two solutions at 48–50° were mixed and the plate prepared immediately. All enzyme solutions were adjusted to the pH of the plate before filling. The pH of the plate, after incubation, but before development, was checked by cutting radial sections through the plate at random, adding these to a known volume of indicator solution and comparing the agar plug with a B.D.H. capillator. With plates prepared in the manner described above, no evidence of a pH 'drift' through the zone of enzyme action was observed. If, however, the substrate–agar plates were prepared in aqueous solution and the enzyme solutions in strong buffers were inserted into the cups, it was found, on checking sections through the zone of enzyme action, that a marked pH shift had occurred. In carrying out the cup-plate assay,

it is evident that the pH of the gel should be checked, and the added enzyme solutions adjusted to the same pH before filling the plate.

It was of interest to determine the reason for the marked effect of pH on zone size. The pH of the gel might be expected to govern the rate at which the enzyme diffused from the cup, the rate at which the enzyme solutions were inactivated, and the rate of hydrolysis of the substrate in the gel. By dissection of incubated gel plates, it was found that active enzyme existed outside the edge of the developed zone, which suggested that the effect of the pH of the gel on zone size was predominantly due to its effect on the rate of substrate hydrolysis. In an attempt to examine this point, buffered agar (without added substrate) was filled into sections of glass tubing 4·5 mm. in diameter, which were mounted in a vertical position. Enzyme solution was added to the top of the gel with a micro-pipette, and diffusion allowed to take place for 18 hours at 37°. The agar column was then extruded, cut into 1-mm.-thick discs with a simple microtome device, and the discs placed on a glass sheet, which was then flooded with buffered starch–agar gel at 45° and pH 5·2. The buffer in the added starch–agar gel was sufficient to overcome the buffer in the discs.

The gel plate was then incubated and developed in the usual manner and the amylase in the discs calculated from the zone diameters, which were read off from a standard curve prepared by including in the plate similar discs of agar, to which known amounts of enzyme had been added, before they were placed on the plate. A typical result for α-amylase is given in Table I. It may be seen that the enzyme was further down the tube as the pH was raised from 4·2 to 7·2, indicating that either the rate of diffusion of the amylase was greater at pH 7·2 or that the rate of inactivation was greater at the lower pH values. A separate experiment showed that when amylase was stored in buffer solutions for 18 hours at 37°, there was marked inactivation of the enzyme in the pH range 3–5, but it was stable in the range 5–7. In the cup-plate assay, at pH 4·2 and 7·2 the zones of activity were very small, but optimal at pH 5·2. At pH 7·2 it would appear that although the enzyme had diffused into the gel and was stable at this pH, the rate of hydrolysis of the substrate was slow at a pH so far from its optimum, whereas at pH 4·2 marked inactivation of the enzyme occurred. With α-amylase, therefore, the pH of the gel to give optimum zone size was the normal pH optimum of the enzyme.

Table I

The diffusion of α-amylase (taka-diastase) and polygalacturonase (Pectinol ' IOM.*') in a column of buffered agar. Results expressed as micrograms of the original enzyme preparation per 1-mm.-thick disc*

No. of disc	Amylase pH of gel for diffusion					Polygalacturonase pH of gel for diffusion				
	4·2	4·6	7·2	3·4	3·4	4·3	5·2	5·2	6·2	7·2
1 (top disc)	5	24	1000	8	20	124	124	124	30	Trace
2	5	20	562	5	5	124	60	80	20	,,
3	4	17	251	1	3	100	50	50	12	,,
4	3	14	251	Nil	2	80	50	50	20	,,
5	2	10	178	,,	Nil	30	30	50	10	,,
6	Nil	8	150	,,	,,	30	20	30	5	,,
7	,,	5	126	,,	,,	50	20	20	8	,,
8	,,	3	126	,,	,,	30	20	20	12	,,
9	,,	2	71	,,	,,	12	20	12	5	,,
10	,,	Nil	56	,,	,,	12	12	24	4	,,
11	,,	,,	42	,,	,,	12	12	20	5	,,
12	,,	,,	16	,,	,,	12	12	12	5	,,
13	,,	,,	9							
14	,,	,,	5							
15	,,	,,	4							
16	,,	,,	3							
17	,,	,,	2							
18	,,	,,	2							

With polygalacturonase it was first shown that the enzyme was stable over the pH range 4·3–6·2 for 18 hours at 37° with accelerated inactivation outside this range. A diffusion experiment in tubes of gel was carried out, similar to that used with α-amylase (see Table I). In the pH range 4·3–5·2, active enzyme occurred further down the tube than at pH 6·2, whereas from Fig. 1, the optimum zone size in the cup-plate assay was given at pH 4·4–4·8. The results suggested that the greater zone size was given at pH 4·4–4·8 because this was the optimum pH for polygalacturonase activity. This conflicted with the figure usually quoted[8] for polygalacturonase of pH 3·5. The pH optima were therefore examined, on sodium pectate as substrate, by a viscometric assay[9] and by estimation of the glycosidic hydrolysis in the early

stages of breakdown when the colloidal properties of the substrate were still present. By both these methods the pH optimum was found to be 4·4–4·8 (see Discussion). It is probable that with all enzymes assayed by the cup-plate method, it will be found that the pH for maximum zone size will coincide with the pH optimum of the enzyme, and therefore the cup-plate method could be used for checking pH optima in certain cases.

Effect of salts on the cup-plate assay

It was found that the addition of sodium chloride to the gel media increased the zone size with amylase and pectin-esterase, presumably owing to the known salt-activation effect. Jansen, MacDonnell & Jang[10] found that Mg^{++} ions inhibited the action of polygalacturonase on pectic acid, and McColloch & Kertesz[11] stated that Cu^{++} and Ca^{++} were inhibitory. Holden[12] found that Ca^{++} inhibited the action of polygalacturonase on tobacco leaf even when the leaf was finely milled, indicating that ' it is the state of combination of pectic substances rather than their inaccessibility which influences the extent of the enzyme action '. The effect of Cu^{++}, Mg^{++} and Ca^{++} on polygalacturonase was examined by the cup-plate assay, using a purified medium ; the appropriate salt solution was added to the enzyme solution, well mixed and inserted into the cups. It was found that the presence of 5% (w/v) of calcium chloride or 8% (w/v) of magnesium chloride in the cup had no inhibitory effect on the polygalacturonase action. With Cu^{++} the presence of 4% (w/v) of copper sulphate completely inhibited enzyme action, and 0·25% reduced activity to 25% of its original figure. The effect of Cu^{++} appears to be a direct inactivating effect on the enzyme. The lack of inhibition by Mg^{++} and Ca^{++}, in contrast with the results quoted above, suggests that it is the state of combination of the pectic substances, as suggested by Holden,[12] that is important. Although large amounts of Ca^{++} and Mg^{++} were present, there was no inhibitory effect on the enzyme. The sodium pectate in the agar gel would have been completely converted to the calcium salt, and by slow precipitation in the gel the calcium pectate was probably laid down in a form readily accessible to enzyme action, whereas when precipitated as a gel from solution it is partially resistant and in plant tissue entirely resistant.[12]

Use of the cup-plate assay for screening cultures of microfungi

On screening cultures of fungi[1] it was found, with these methods, that the enzyme activity of cultures covered a wide range. As the original purpose of the assay was to select cultures having a high potentiality for enzyme production, great accuracy in the method was not essential. A method was required which was capable of assaying large numbers of cultures, and of selecting those that produced high concentrations of enzyme for more detailed study. For this purpose, the organisms were grown in both surface[7] and submerged cultures at either 25° or 37°. Liquid extracts were made under standard conditions, and these were placed in duplicate cups on the plate. A sample of Pectinol ' IOM.' preserved over silica gel at 4° was taken as the standard preparation and a series of dilutions of this were included on every plate. By plotting the zone diameter against log (enzyme in the cup), a straight-line graph was obtained, and the approximate concentration of the enzyme produced by the culture was estimated.

Use of a ' four-point ' assay

For the assay of polygalacturonase, particularly during concentration and purification, a ' four-point ' assay was used. Knudsen & Randall[13] discussed the superiority of such an assay for antibiotics and the design of such assays has been extended by Brownlee *et al.*[6] and Brownlee, Loraine & Stephens.[14] Most of the findings of Brownlee *et al.*[6, 14] are relevant to enzyme cup-plate assays, and details of design of plate may be obtained from their papers.

The replication of zone size on large plates is good with enzyme assays, but as with antibiotic assays the time taken to fill the plate is important. The last-filled cups of a given sample give smaller zones than the cups first filled, and a correction must be made by arranging the order of filling of the standard and unknown samples.

For the polygalacturonase assay, glass sheets with layers of sodium pectate–agar 30 cm. × 24 cm. were used, containing eight rows of six zones each, the centres of the cups being 4 cm. apart. Samples were analysed against the standard Pectinol sample with a ratio (high dose/low dose) of 10/1. The procedure of Brownlee *et al.*[14] was followed. The relationship between zone diameter and log(amount of enzyme) was linear over a wide range (0·6–40·0 mg./ml. of standard Pectinol) (see Fig. 2), and the zone diameter/log(amount of enzyme) curves were parallel for four commercial pectinase preparations that were examined. Assays of duplicate plates of several samples are given in Table II.

Table II

Four-point cup-plate assay of dried-bran cultures and preparations from pectin-degrading fungi for polygalacturonase content. Results given in terms of standard Pectinol ' 10M.' assays on two plates

Prep.	Assay of prep. (× ' M.') *	
	Plate A	Plate B
Culture 187*a*	0·031	0·031
Culture 187*b*	0·112	0·117
Culture 187*c*	0·135	0·140
Extract No. 1	0·44	0·47
Concentrate 46	13·10	12·10
Extract A	0·042	0·138
Extract D	0·012	0·014

* This related to an arbitrary strength based on comparison with Pectinol ' 10M.'

Discussion

The use of the cup-plate assay for enzymes is only an extension of its use in antibiotics assays, and the use of substrate containing agar is an established technique in microbiology.[15] Sherwood[16] suggested the paper-disc modification of the assay for amylase and protease. Recently, Tamagawa[17] has used the cup-plate method for amylase and Giri & his co-workers[18] have used diffusion in an agar substrate gel for the qualitative demonstration of amylases, phosphatases, urease, tyrosinase and lipase.

The assays in the present paper were developed originally for studies on the enzyme production of microfungi. They have been used successfully for screening 120 cultures of fungi for polygalacturonase, lipase, protease, amylase and cellulase activity, both in submerged and surface culture[1] and for examining the nitrogen, carbon and growth-factor requirements for enzyme production by selected cultures from this group. The assay is of particular use in studies on microfungi, where large numbers of assays of limited accuracy are required, and the estimation of polygalacturonase, protease, amylase and cellulase has become a standard feature of the present studies. Rouse, Sui & Levinson[19] have suggested that the hydrolysis of carboxymethyl cellulose is due to an enzyme Cx which is distinct from the enzyme degrading native cellulose; if this hypothesis proves to be correct, the present assay will measure only Cx and will not give any measure of the degradation of native cellulose.

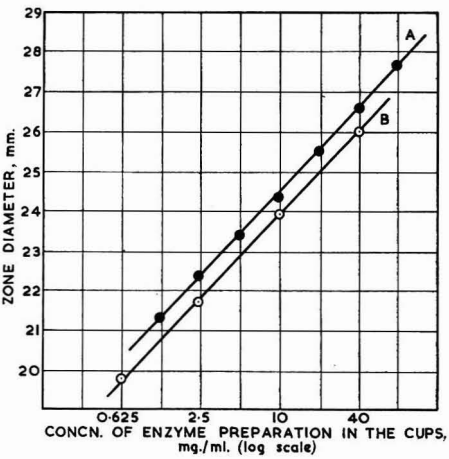

FIG. 2.—*Relationship between diameter of zone of enzyme action produced in the cup-plate assay, and concentration of polygalacturonase solution in the cup*

A, B. Standard curves from two plates on different occasions. Each point is the mean value from six zones

Pectin-esterase can only be determined in the absence of polygalacturonase; and both the original modification of this assay[2] and that given in this paper are greatly influenced by the concentration of polygalacturonase. The present assay is, however, suitable for the examination of the pectin-esterase of plants. Both orange pectin-esterase[5] and the esterase of the tomato[4] gave well defined zones by the method given in this paper, and the zone diameter was linearly related to the logarithm of the enzyme concentration in the cups. The method is of use in the technology of citrus products. It is of interest that a pectin ' depolymerase ' has been stated to occur in tomatoes,[11] and assay of tomato extracts on sodium pectate–agar gives small zones owing to the action of this enzyme. True lipase, as distinct from esterase, can be measured by its action on certain water-soluble compounds[20] and for this purpose polyethylene glycol monolaurate is suitable.

A more accurate four-point assay based on the findings of Brownlee *et al.* has been used in connexion with α-amylase and polygalacturonase.

The main emphasis of the present studies of the enzymes of microfungi has been on polygalacturonase, which is of considerable importance, both as a tool in the study of pectin structure and as an enzyme of technological importance.[8]

Polygalacturonase has usually been determined[8] either by estimation of the reducing groups liberated by its action on pectic acid or its reduction of the viscosity of the same substrate. It will be shown in a later paper that the polygalacturonase of Kertesz[8] is a complex of at least three enzymes, and that pectic acid is broken down in the following stages (Fig. 3). The initial degradation of pectic acid to the intermediate uronides results in the destruction of the colloidal properties of pectic acid and the formation of uronides which are not precipitated from solution at pH 1·0. It is this step which is measured by both the cup-plate assay and viscometric methods, when sodium pectate is the substrate. This step has an optimum pH of 4·5 for pectic-enzyme preparations from surface cultures, which degrade pectic acid to galacturonic acid; with enzymes prepared in submerged cultures, which lack the enzyme degrading the triuronide to galacturonic acid, the pH optimum of the initial step is 5·3. In each of these experiments the pH optima were determined by the cup-plate, viscometric and glycosidic-hydrolysis methods, and the results from the three methods were in agreement. The determination of the polygalacturonase content of the usual commercial pectic-enzyme preparations by the glycosidic-hydrolysis method measures the overall breakdown to galacturonic acid. Unless small amounts of enzyme are used, the initial pH optimum of 4·5 or 5·3 may be missed and a sharp peak is obtained at pH 3·5; this is in agreement with the pH optimum of polygalacturonase given by Kertesz,[8] which represents the degradation of the intermediate uronides.

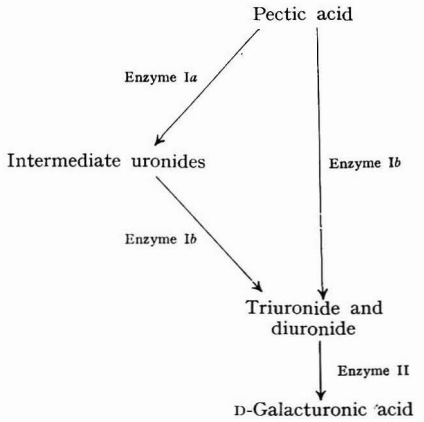

FIG. 3.—*Schematic representation of the action of pectic enzymes on pectic acid*

Enzyme I*a* (α-polygalacturonase *)	Rapidly reduces viscosity of pectic acid and pectin (without simultaneous demethylation) to uronides not precipitated at pH 1·0. Optimum pH 5·3. Enzyme inactivated at pH 5·0 and temp. 60° for 30 min. The enzyme is estimated by a cup-plate assay with sodium pectate at pH 5·3.
Enzyme I*b* (β-polygalacturonase *)	Degrades pectic acid and the intermediate uronides from the action of Enzyme I*a* with the liberation of triuronide, diuronide and galacturonic acid, at a pH optimum of 3·5. The enzyme is relatively stable at pH 5·0 and temp. 60°. The enzyme free from I*a* gives no zone on a sodium pectate plate at pH 5·3, but may be determined by a cup-plate assay with intermediate uronides from I*a* as substrate. The plate is developed with 10% calcium chloride, which precipitates the calcium salts of intermediate uronides.
Enzyme II	Hydrolyses triuronide *via* diuronide to galacturonic acid, at pH optimum 3·5. The enzyme may be determined by a cup-plate assay with triuronide as substrate. The plate is developed with 5% lead acetate, which precipitates insoluble lead salt of uronide.

* Suggested nomenclature

By using three cup-plate assays, with pectic acid, intermediate uronides and the triuronide as substrates, an approach has been made toward the estimation of the individual enzymes of the complex, during the growth of microfungi. Full details of these methods will be submitted for publication later.

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THE STORAGE OF FLOUR IN JUTE BAGS TREATED WITH INSECTICIDES

By W. G. ATKINS * and E. N. GREER †

Experiments are described in which flour stored in jute bags, the fabric of which had been impregnated with insecticides, was exposed to infestation by the species *Ephestia kühniella, Ptinus tectus* and *Tribolium confusum*. The use of 1% (w/w) of DDT in the fabric provided good protection against the first two named, and fair, but by no means absolute, protection against *T. confusum*. Transfer of insecticide to the flour occurred to an extent sufficient to be classed as contamination.

Introduction

In the past ten years, during which time contact insecticides of high efficiency and persistence, notably DDT and BHC, have become generally available, it has often been suggested that these should be used for the protection of stored products by the impregnation of containers. Jute fabrics can be treated in this way, and might thus be rendered effective in preventing the access of insects to bagged grain and flour. This subject has been studied by Parkin[1] and his collaborators at the Pest Infestation Laboratory, Slough. Parkin investigated the efficiency of various fabric treatments in protecting grain in this way, and also the extent to which the insecticides were transferred from the fabric bags to their contents.[2] The latter is of paramount importance since, no matter how effective any treatment might be in

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preventing infestation, it can scarcely be looked upon as practical if it were obtained only at the cost of contaminating the stored product.

The present experiments were carried out in 1949, when the main findings of Parkin's work had already been published, in order to obtain confirmatory evidence, to extend the work to bagged flour, and to use bags of commercial size and filling as the experimental units.

Experimental

Jute

Jute fabric impregnated at three levels of DDT, 1·0, 1·25 and 1·5% by weight, was available to us. Such levels were considered to be commercially suitable as regards cost, and a reasonable compromise between protection against infestation and contamination of flour. Only crude BHC was obtainable for purposes of impregnation and, in view of the risk of taint in the flour, it was considered inadvisable to use additions greater than 0·1%. The experiment did not therefore extend to a comparison of these two insecticides, and the data obtained for BHC treatment may be looked upon as showing no more than the effects of the presence of a low level of insecticide. The type of fabric, hessian, was deliberately chosen in preference to the more expensive twill, since it was considered that a non-returnable bag was most suitable for such treatment, as frequent cleaning and refilling gives rise to progressive loss of insecticide. The fabric construction was that known as 10 oz./40-in. hessian, 11 × 12, made up into 140-lb. flour bags. Since, in order to facilitate its processing, jute is always subjected to the preliminary softening and lubricating action of an oil-in-water emulsion, it was found convenient to include in this emulsion the appropriate amounts of insecticide. Both warp and weft yarns were treated. The success of this treatment may be judged from the results of analysis of benzene extracts of strips cut from such bags, as shown in Table I.

Table I

Insecticide	Calculated addition, %	Found, %
DDT..	1·0	1·01
DDT..	1·25	1·13
DDT..	1·5	1·57
BHC..	0·1	0·12

Bags from all the treatments listed in Table I were included in the trial, together with ' control ' bags made from fabric of the same jute batch and cloth construction, but containing no insecticide.

The activity of the insecticides was confirmed by a preliminary experiment in which adult *Calandra granaria* (grain weevils) were placed on specimens of treated fabric. It was found that after 48 hours the majority of the insects were dead, and the minority remaining died within two days of their removal from the fabric.

Flour

Five of each type of treated bag and 10 control bags were commercially filled with untreated 85%-extraction flour, which had been previously passed through an entoleter to obviate the possibility of infestation arising within the flour. The filled bags were closed by rolling the tops and sewing with untreated jute-twine.

Storage

An experimental shed, measuring 16 ft. × 9 ft. × 7 ft., was constructed from plaster board, positioned within a laboratory, and fitted with an electric heater, circulating fan and recording thermometer. The interior of the structure was painted, and all seams were sealed with Cellophane tape. It was possible to arrange thirty filled bags within the shed in six rows of five, each bag out of contact with its neighbour, but each treated bag immediately adjacent to an untreated control. During the six-month experimental period of storage, the temperature of the shed interior varied between 72 and 85° F. (average 79° F.) and the humidity was maintained above 70% R.H. (average 73%). From tests made it was noted that the flour temperature in the interior of the bags lagged considerably behind that of the surrounding air.

Insects

Three species were used, *Ptinus tectus* (brown spider beetle), *Ephestia kühniella* (Mediterranean flour moth) and *Tribolium confusum* (confused flour beetle). Two admissions of these

insects were made, one at the beginning of the experimental period of storage, when approximately 1000 of each species were liberated, and again after 10 weeks when 3000 of each were admitted. At the first admission the insects were distributed at either end of the store, but on the second occasion they were sprinkled as evenly as possible over the whole area.

Results

The experiment was carried on for 164 days, as follows:

(*a*) 12 *days' storage.*—Baking samples withdrawn from one bag of each type under test. Sampling area restricted to that portion of the flour in contact with the bag.
(*b*) 14 *days' storage.*—First batch of insects liberated.
(*c*) 77 *days' storage.*—One specimen bag of each treatment withdrawn for examination.
(*d*) 90 *days' storage.*—Second batch of insects liberated.
(*e*) 164 *days' storage.*—All remaining bags examined.

The object of withdrawing samples [see (*a*)] was to provide material for baking trials, to determine the amount of taint in the flour which was derived from the impregnated container. The test was carried out at this stage, since it was felt that in any subsequent test taint might well have been masked by secondary causes resulting from the conditions of the experiment. It was found that the flour was unaffected in its bread-making quality, all loaves being substantially equivalent. However, it was reported by the baker that the flour drawn from the bag treated with BHC had a decidedly 'mouldy' taint.

In tasting trials made upon the baked bread, it was found that this taint persisted in the loaf. Some tasters recognized a bitter taste from DDT-treated containers, but the evidence on this point was far from definite. Though it may be remarked that the crude BHC formulation used in this case was thus found to be unsuitable for the purpose, it is only fair to point out that the sample drawn represented the flour most likely to be affected, and might have passed unnoticed at the dilution which would have occurred in the 'mixing over' of the whole contents of the bag.

Infestation

At the conclusion of a total exposure period of two months, five bags, one of each type, were removed from store and examined for infestation. It was noted that large numbers of dead insects were either attached to the exterior of the treated bags or lying around their base. The flour was examined by sifting the entire contents of the bags through a sieve equivalent to No. 25 B.S. test sieve (aperture 0·575 mm.) and counting and identifying the insects remaining on it. It was found that live *Tribolium* adults were present in all bags, that these were more abundant in the control, and that although more than two-thirds of those penetrating the DDT bags were dead, the living insects behaved normally when removed and incubated on flour. *Ptinus* larvae were found in both control and BHC bags, but not in those impregnated with DDT. *Ephestia* behaved similarly. The insects were largely confined to a depth of not more than 6 in. from the top of the bags.

Since the mortality at this stage of the experiment had been heavy, it was decided to add a further batch of all three species of insects. These were put in shortly after the preliminary examination described above had been completed. Some two weeks later visual signs of breeding began to appear, the typical webbing of *Ptinus* becoming apparent on all the remaining control and BHC-treated bags. This was closely followed by moth webbing, again on the control bags, and to a lesser extent on those treated with BHC. The spread of moth webbing was rapid, ultimately obscuring most of the indications of *Ptinus*. DDT treatment seemed successful in resisting this type of infestation, and a typical example is shown in Fig. 1.

At the completion of six months' total storage, the remaining 25 bags were examined, and the work was completed in five weeks. The chief difficulty encountered was the examination of the heavily webbed flour from control and BHC-treated bags. Though such webbing was confined to a 1-in. layer of flour immediately below the fabric, its degree of concentration therein was so heavy that it was found necessary to separate and weigh it and to examine an aliquot. The population figures for these types of bags are based on this procedure.

It was found possible in this way to form an estimate of the population of *Ephestia* larvae and of the larvae and adults of *Ptinus*, but it may be noted that these estimates were far more accurate for unwebbed bags, the figures for the others being undoubtedly conservative.

FIG. 1.—*Untreated sack (left) showing* Ephestia *and* Ptinus *webbing; contrasting absence on sack (right) treated with* 1·5% *DDT*

The means of population per bag thus estimated for these two species, and the range encountered, are set out in Table II.

Table II

Type of bag	Condition of fabric	Population per bag			
		Ephestia larvae		*Ptinus* (larvae and adults)	
		Mean	Range	Mean	Range
Untreated	Heavily webbed	534	91–1720	356	6–661
0·1% BHC	,, ,,	345	63–832	139	16–237
1·0% DDT	Clear	36	16–61	5	0–10
1·25% DDT	,,	27	13–55	13	0–26
1·5% DDT	,,	21	14–28	1	0–1

It was clear from the visual condition of the bags, and from the population figures, that DDT treatment was successful in resisting infestation by these species at and above 1%. The population ranges are, however, such as to make it difficult to attach any significance to variation of the means quoted for the three levels of DDT.

In the case of *Tribolium* the same sharp division did not occur. Not only was the total population far higher, but the range encountered far wider, as shown by Table III.

Table III

Type of bag	Mean population per bag (*Tribolium*, all forms)	Range
Untreated	20,300	2,000–28,600
0·1% BHC	12,000	800–26,600
1·0% DDT	5,800	502–8,600
1·25% DDT	5,300	310–10,400
1·5% DDT	3,400	180–7,800

Despite the general trend in these figures, it will be noted that the ranges overlap in every case, and it seems probable that the explanation lies in environmental differences existing within the experimental shed, possibly deriving from inefficient circulation of the atmosphere within it, and the occurrence of steady differences in temperature. It is desirable, therefore, to apply some test of significance to the differences between the means of population found.

It was noted that the low counts occurred in bags at one end of the shed. If these were disregarded, a regular block of 15 bags, containing three of each type, could be obtained from the residue. This probably represents the most favourable case for test. The means thus obtained are shown in Table IV.

Table IV

Type of bag	Mean population per bag (*Tribolium*, all forms)	Range
Untreated	25,000	22,400–28,600
0·1% BHC	15,700	7,700–23,600
1·0% DDT	7,600	7,100–8,600
1·25% DDT	7,000	4,200–10,400
1·5% DDT	4,500	2,600–7,800

Applying an analysis of variance to these figures, the variance-ratio test indicates significant differences between means of the following magnitude:

1% level, significant difference between means, equals 7,300
5% ,, ,, ,, ,, ,, ,, 5,200

It thus appears that the evidence is sufficient to establish differences between untreated, BHC-impregnated and DDT-impregnated bags, but that the differences between various levels of DDT treatment are not sufficiently wide to have any significance.

The *Tribolium* infestation after six months' storage was found to be mainly concentrated in the top portion of the contents of the bags, as in the preliminary examination; it was obvious that the insects were able to penetrate through the fabric without much trouble in this region and where the fabric had become slack by reason of settlement of the contents of the bag. Where the fabric remained under tension no such puncturing occurred. Fig. 2 shows punctures made in the weave by this insect. The number of dead beetles found within the bags was negligible, and the abundance of larvae present left no doubt that many of the insects which reached the interior by passing through the impregnated fabric did so without impairing their fertility.

FIG. 2.—*Puncturing of treated sacking by* Tribolium *at the top of the bag*

Contamination of flour by insecticide

As has already been mentioned, whatever success impregnated jute fabric may have as a preventive of infestation, it fails if this is obtained only at the expense of contaminating the flour. The effect on taint of the insecticides under investigation has already been described,

but of course the question of the actual quantity of insecticide transferred to the flour during storage is of equal importance. At the conclusion of the storage experiments, therefore, the insects were removed from one bag of each type and the contents thoroughly mixed in a Gardner mixer and samples subsequently withdrawn for analysis by the method of Butterfield *et al.*[2] Results are shown in Table V.

Table V

Type of bag	Insecticide present in flour, p.p.m.
0·1% BHC	4·2
1·0% DDT	13·8
1·25% DDT	19·0
1·5% DDT	23·0

It is at present recommended that the tolerance permitted for DDT in human food should not exceed 7 p.p.m., or for γ-BHC, 2·5 p.p.m. There seems no doubt, therefore, that all treatments were objectionable in this sense. It is interesting to compare here the results shown in Table V which were obtained on full-size flour bags, with those of the small-scale tests conducted by Parkin, who has reported results obtained on small cotton flour-bags impregnated with insecticide after making up. Where the fabric impregnation level of such bags was 1% DDT, the insecticide content of the flour, after four months' storage, was 90 p.p.m. It was predicted from this that in a full-size 140-lb. bag it would be reasonable to assume that the final DDT concentration in the flour would be 15–30 p.p.m. The present results, though obtained in quite different circumstances, seem to afford remarkably close agreement with this estimate.

Discussion

In so far as the experiments described have any general application, it would seem that the utility of such a method of protecting stored flour is more a question of contamination of contents than insecticide efficiency. If, as is suggested, contamination is the preferable criterion, then the method outlined cannot be regarded with favour, despite the fair degree of protection it afforded against infestation. It has been pointed out by Parkin[1] that the type of weave influences the degree of protection available. Small-scale experiments carried out in the course of the present work have shown that penetration of a double layer of fabric was very difficult even for *Tribolium*. Possibly the use of such double layers, with the insecticide impregnation limited to the outer layer, would prove of use both in limiting insect penetration and flour contamination.

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ERRATUM

In the paper entitled ' The Determination of Fat Peroxide in Small Samples of the Lean and Fatty Tissues of Meat by the Ferric Thiocyanate Method ' by G. Howard Smith, *J. Sci. Fd Agric.*, 1952, **3**, 26, for ' 0·05 ml. of reagent . . .' in the 13th line from the bottom of p. 27, read ' 0·50 ml. of reagent . . .'

Journal of Applied Chemistry

The following papers are appearing in the March, 1953, issue of the *Journal of Applied Chemistry*

Acetylene studies. IV. Dehydration of dialkylethynylcarbinols
By Ernst D. Bergmann and Y. Sprinzak

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CONTENTS