A NEW METHOD OF DETECTION OF CEREAL FLOURS SEPARATELY AND IN MIXTURES BY THE "AGAR-PLATE" METHOD.

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In a previous communication (Giri, *Science*, 1935, **81**, 343), a method has been suggested for the differentiation of pure starches by their characteristic coloured zones produced when impregnated in an agar medium and flooded with iodine after hydrolysis with amylase. This method seemed so promising that in the present study the method was applied for the differentiation of cereal flours and their detection when present in mixtures. For obvious reasons, choice was made of those cereals which yield characteristic results. Those selected included rice, wheat, maize, ragi, barley, jowar and bajra.

EXPERIMENTAL.

All the cereals were powdered well and passed through a 40-mesh sieve. A known amount of the flour was taken so that the concentration of the starch in the final agar medium amounted to 0.4 per cent. The flour was then introduced into a beaker in which water was kept boiling. This procedure was necessary in order to prevent the partial hydrolysis of the starch by the enzyme which is present in the flour. The solution was kept boiling for a minute subsequent to the addition of the flour, cooled and then filtered through a fine muslin cloth. The filtered solution was directly used for the experiment.

The agar medium was prepared by autoclaving known amounts of agar in water. The concentration of agar gel was such that its concentration in the final agar medium amounted to 1 per cent. Before mixing agar with the flour extract, the pH was adjusted to 4.6 by the addition of 0.2 N acetate buffer for malt amylase and taka-diastase, and to 6.8 by N/15 phosphate buffer for salivary amylase.

The agar gel, while still warm, was mixed with an equal volume of the flour extract and immediately transferred to a number of petri-dishes. The agar sets to a jelly after cooling. A small drop of amylase [saliva, 1 : 5 dilution; taka-diastase (B.D.H.), 0.1 per cent. solution] was added on to the centre of the agar plate and allowed to diffuse at the laboratory temperature for about 24 hours. At the end of that period, iodine solution (N/200, enough to cover the surface) was

poured on the plate, and allowed to remain there for about 3–5 minutes, until the colours of all the zones have come out clearly. The iodine solution was then poured out, and the surface of the agar plate washed with water. After a further period of about 3–5 minutes, the colours of all the zones were observed.

The characteristic colours produced with various cereal flours by the three types of amylases—taka-diastase, malt amylase and salivary amylase—are described in Table I and the flours are classified accordingly.

The diffusion zones developed by different types of amylases are different in their intensity of colour and width of zones.

TABLE I.

Classification of flours	Flour	Colour zones		
		Salivary amylase	Malt amylase	Taka-diastase
Group I ..	Wheat	Central colourless zone surrounded by a deep blue zone	Central deep green coloured zone surrounded by a diffused violet zone	Central blue zone surrounded by a streak of violet
	Maize	,,	,,	,,
	Jowar	,,	,,	,,
	Bajra	,,	,,	,,
Group II ..	Rice	Central colourless zone surrounded by a deep violet zone	Central deep blue coloured zone surrounded by a diffused violet zone	Central violet zone surrounded by a streak of violet
	Ragi	,,	,,	,,
	Barley	,,	,,	,,
Group III ..	Potato	Colourless zone	Central colourless zone surrounded by a diffused violet zone	Central colourless zone surrounded by a light green zone and finally by a streak of violet

From the foregoing, it is clear that flours belonging to one group can be easily differentiated from those of the other groups by their characteristic colour zones.

DETECTION OF ADULTERATION OF FLOURS.

The method described above has been successfully applied to the detection of adulteration of flours, when hydrolysed by salivary amylase.

It was mentioned before that the colour zones given by rice and wheat flours are characteristically different from one another, the former producing a violet and the latter a blue zone when hydrolysed by salivary amylase. When present in mixtures, the coloured zones produced were found to be a combination of two colours, the violet zone being surrounded by a blue zone, the intensity of the colour tone depending on the concentration of rice present in the mixture. In Fig. 1 are given the colour patterns of the diffusion zones of (*a*) wheat, (*b*) rice, and (*c*) wheat and rice mixed in equal proportions. It has been found possible by this method to detect adulteration of wheat by rice to the extent of 20 per cent. and above. When a smaller percentage of rice flour is present, the test does not appear to be quite so sensitive. This method has also been successfully applied to indicate the presence of ragi and rice in maize, barley and ragi in wheat, and jowar in rice. In all these cases, it is usually necessary to prepare the colour standards with pure flours, simultaneously with those of the test solutions.

A rough estimate of the amount of adulterant present may also be made by examining the zones produced by mixtures of successively known dilutions of the adulterant until the difference disappears.

DISCUSSION.

The present method of differentiation of cereal flours, and their detection in mixtures is useful not only when they are present in their natural state, but also when the structure of the starch colloid is destroyed by cooking, in which case there is no method available in literature for their detection separately and in mixtures. The use of prepared foods is rapidly enhancing with the advancement of civilization. The analyst can detect this fraudulent practice in flours by applying the well-known microscopic method, but he fails to do so in the case of cooked foods. In farinaceous foods for infants and invalids, it is found that some preparations consist chiefly of unconverted starch. These foods are nearly all made up of baked dry flour of wheat or barley. In such cases, the presence of cheaper cereal flours can be easily detected.

The substitution of cheaper or inferior grades for those of higher quality—the fraudulent admixture of rice or ragi to wheat or maize preparations, is extensively practised in Southern India. In Rajputana and some other parts of Northern India, barley flour is considerably cheaper than wheat and thus in wheat preparations the presence

of barley is easily detectable. Further, in Rajputana, rice flour is more costly than wheat or maize flour, and hence the admixture of rice with wheat and maize flours is usually practised by vendors. The present method affords an easy means of detecting such adulterations.

Another point of interest which has been observed in these experiments is the reversibility of the colour zones given by pure starches (B.D.H.), as compared with those of aqueous extracts of the cereal flours themselves. This phenomenon is probably due to the fact that in the course of preparation of starch from cereals, its physical and chemical composition may change and consequently the nature of the hydrolysis of the starch by amylases is also altered. It will be of interest to further study the influence of different methods of preparation of starches from cereals on their digestibility by amylases, and compare the digestibility of starches in its natural state in cereals and when isolated in pure form. Further work in this direction is in progress.

SUMMARY.

A method has been described for the differentiation of cereal flours and their detection when present in mixtures. The application of the method for the characterisation of various flours in mixtures, and its usefulness in the detection of adulteration are discussed.

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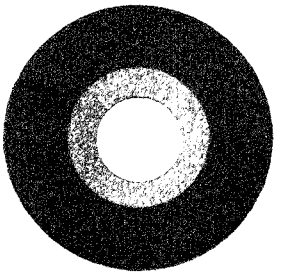
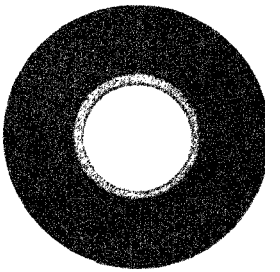


a



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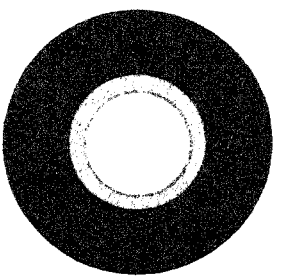

c

Fig. 1.