INFLUENCE OF THE FORM OF NITROGEN ON THE QUALITY AND OF QUANTITY "PITCH" IN DISTILLERY PRACTICE

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NITROGEN which is provided in different organic and inorganic forms, constitutes an essential nutrilite for the growth and functioning of yeasts. Although a few disputable claims have been made that yeasts are capable of fixing elementary nitrogen[1,2,3,4] it is generally accepted that the greater and the more substantial part of its nitrogen requirements, have to be met through a supply of assimilable forms of nitrogen which are covered by a wide range of the comparatively simple inorganic and the more complex organic compounds.

Lindner[5] has made a study of the various sources from which an yeast could obtain its requirements of nitrogen. He found that compounds with long hydrocarbon chains were assimilated with comparative ease as compared with the utilisation of ring compounds like histidine. More recently, Nielson[6] and Hartelius,[7] in the course of their comprehensive studies, have investigated the relative efficiency, of some 36 different amino-acids as sources of nitrogen for the growth of yeast; some of them individually and in particular combinations have been shown to exert a growth-promoting effect.

The pioneering studies of Hartelius,[6] Nielson[7] and Thorne,[8,9,10] on the relative value of amino-acids as sources of nitrogen for yeast, have revealed that :—

(1) they vary among themselves in their nutritive value,
(2) the nitrogen in amino-acids is taken up by yeast in the form of ammonia which is formed by decarboxylation,
(3) amino-acids in mixtures exhibit a growth-stimulating efficiency which is far greater than what might be expected if the effect were merely additive,
(4) some of the amino-acids when administered in minute amounts (*e.g.*, β-alanine, asparagine, aspartic acid, glutamic acid, lysine and arginine) are found to possess a stimulatory effect on yeast-growth,
(5) Yeasts differ among themselves with regard to their response to various amino-acids.

The alcohol-producing capacity or attenuative power of an yeast naturally depends upon the concentration of the zymase complex, whose content of the "pitch" would determine its overall efficiency. The elaboration of the zymase complex in yeast is intimately connected with its nitrogen metabolism. Sippel reported that the attenuative power is restored by the addition of soyabean flakes to the extent of 0·2 to 3·0 per cent.; this supplement resulted in a replenishment of the normal protein content of yeast which had decreased by about 10 per cent. Difficulties of attenuation and of harvesting of yeasts have been traced by Bishop and Whitely[11] to the low content of nitrogen in worts especially when strongly attenuating yeasts are employed.

The fall in the attenuating power of "pitches" in distillery practice, is a common occurrence in most of the Indian distilleries. This is mostly due to faulty and poor nutritional conditions under which the "pitch" is built up. One of the essential limiting nutrients is nitrogen. In view of the importance of this nutrilite in distillery practice, we have made a systematic study of the efficiency of different sources of nitrogen on the rate of growth, the extent of growth and the attenuating power of the resulting harvests of yeasts.

Part I. Rate and Extent of Growth

Experimental

In the present investigation seven sources of nitrogen have been tried, ammonium sulphate, urea, asparagine, wheat bran extract, proteolysed extracts of groundnut cake and the distillery

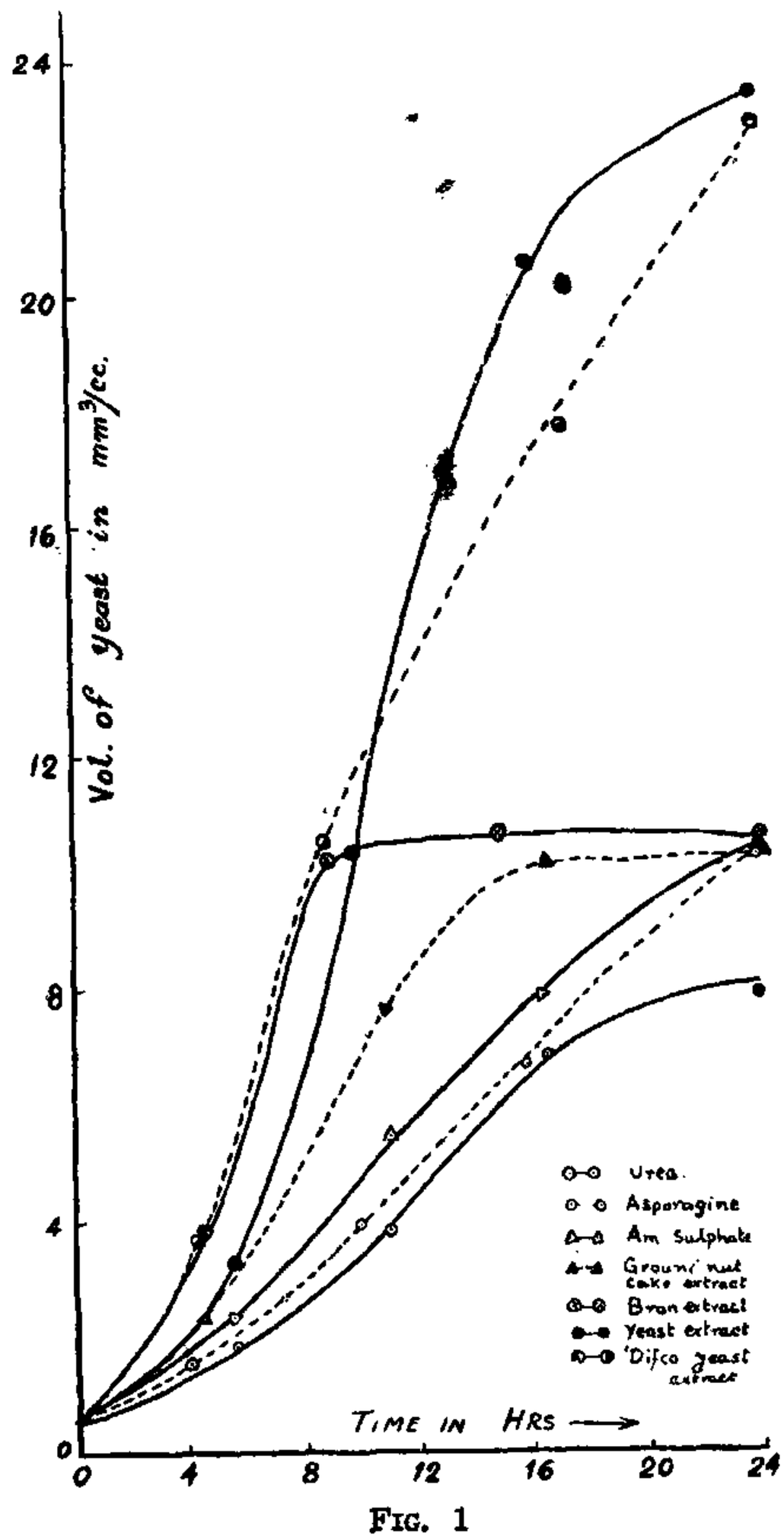

FIG. 1

yeast residues and "Difco" yeast extract. The basal medium employed in the course of these studies was similar in composition to the one used by de Souza and Sreenivasaya.[12] The organism investigated was a strain of distillery yeast developed in the section of Fermentation Technology, and the one which has proved eminently successful in yielding high concentration distillery washes. The nutrient solution was supplemented with different nitrogenous extracts to the extent of 44 milligrams per 100 ml. of the medium. The yeasts were grown in tubes bent at an angle of 45° and mounted on a shaking machine specially constructed for the purpose. The tubes were shaken 80-90 times per minute thus securing efficient aeration of the reaction medium and the cultures were allowed to grow for 24 hours. At intervals, growth of this yeast in the medium was measured by centrifuging at constant speed and for known time (10 minutes), a 2 ml. aliquot of the medium in specially made centrifuge tube provided with a calibrated capillary. The results of the rate of growth are graphically represented in Fig. 1.

A careful study of the graphs reveal that the growth-promoting efficiency of a given quantity of nitrogen as measured by the rate of growth, is the highest in the case of the "Difco" yeast extract. Next in the decreasing order, follow distillery yeast extract, bran extract, extract of groundnut cake, ammonium sulphate, asparagine and lastly, urea. The extent of the growth of yeast at the end of 16 hours with distillery yeast extract as the source of nitrogen, tends to be higher than what is apparently obtainable with "Difco" yeast extract when the overall growth at the end of the experiment is taken into consideration. During the first ten hours, the rate of yeast growth with bran extract appears to be in no way inferior to that given by the two yeast extracts; the groundnut cake extract, however, has a definitely low efficiency. The rate of growth with extracts of bran and groundnut cake is sharply arrested at the end of ten and sixteen hours respectively, indicating a practical depletion of the more easily assimilable forms of nitrogen. In striking contrast to this phenomena is the behaviour of ammonium sulphate and asparagine, both of which tend to maintain a comparatively low but a steady rate of growth.

Part II. Determination of the "Quality" of the Yeast Crops Harvested on Different Sources of Nitrogen

The organism was grown in Roux flasks containing 100 ml. of the respective media; they were placed in a flat, in a horizontal position, with a view to secure a shallow depth and a large surface for the culture solution. This facilitated aeration of the cultures essential for yeast growth. After 24 hours' growth at room temperature (27°-

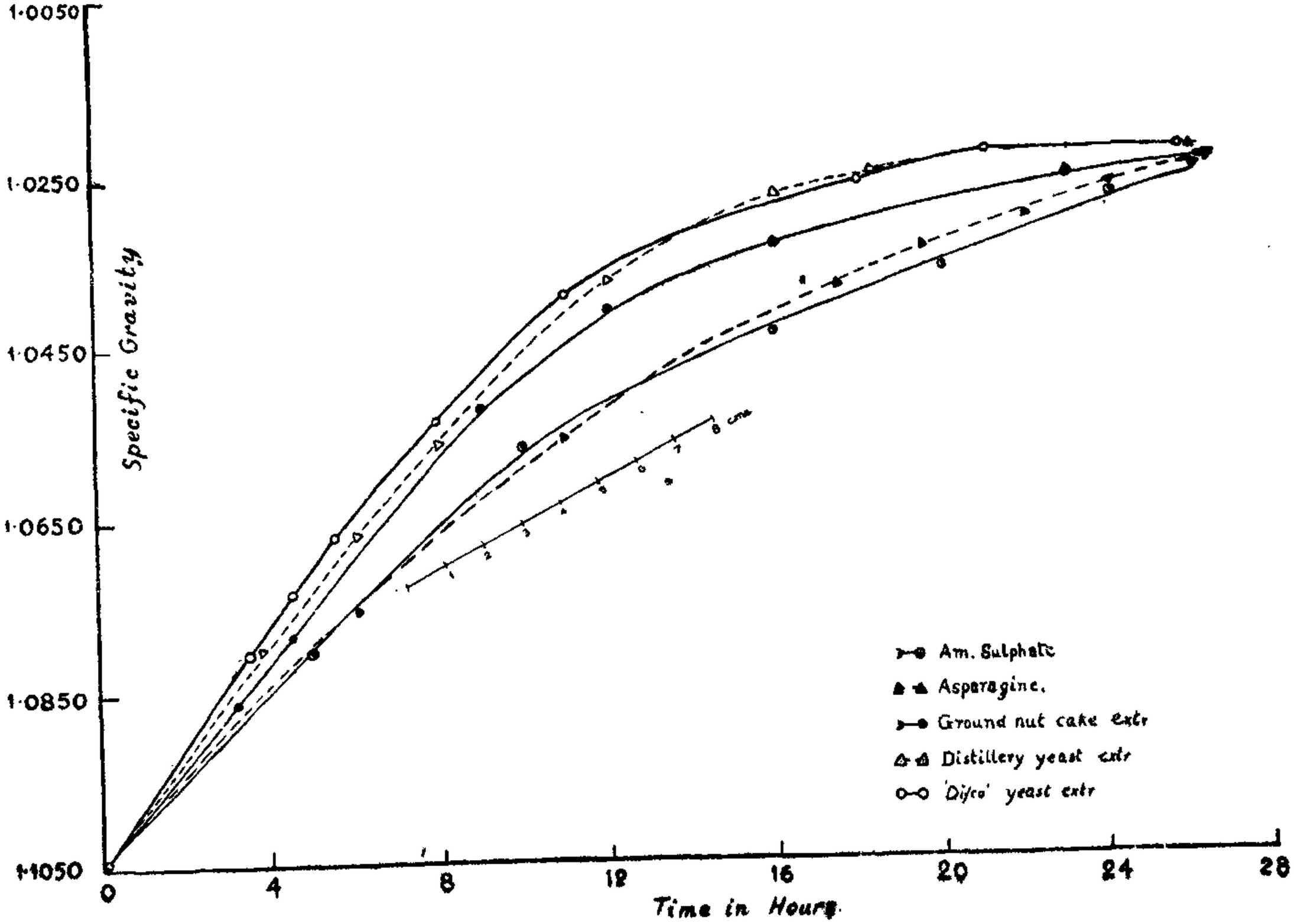

FIG. 2

28° C.) the cultures were collected and washed on the centrifuge. The yeast crop was taken up in saline, made into a thick and uniform suspension, preserved in the ice-chest and its yeast content (on the moisture-free basis) per ml. of the suspension determined by evaporating a known volume of the suspension in a platinum dish, and weighing it to constant weight. The suspension served as the inoculum; a volume of suspension equivalent to 0·4 gm. of moisture-free yeast was employed for every 30 ml. of the experimental mixture for determining the attenuating power of the yeast. The sucrose concentration of the mixture at the start was maintained at 20 per cent. Periodic determinations of attenuation were carried out by noting the specific gravity of the fermenting liquid with the aid of Westphal's specific gravity balance. The results are graphically represented in Fig. 2.

From the graphs given in Fig. 2, it will be seen that the attenuating efficiency of the yeast crops harvested on both the yeast extracts as the source of nitrogen, run very nearly parallel, that of the yeast grown with the groundnut cake extract is definitely low. The yeast crops grown on asparagine and ammonium sulphate process comparatively poor attenuating efficiencies.

In accounting for the higher "quality" of the crops grown on yeast extracts, we have to take into account the vitamins associated with the extracts. Although the strain of the distillery yeast employed in these studies have been demonstrated to be largely independent of external supplies of vitamins, their rates of synthesis by the organism do not appear to be sufficiently high to shorten the period of production of the "pitch". Through a supplementation or fortification of the nutrient media with extracts from natural sources, better harvests of yeasts with higher attenuating powers can be obtained. In this respect, yeast extracts offer the best source of supplements. These findings should find immediate practical application; steps are being taken to apply these results in large-scale distillery practice.

In conclusion we wish to tender our grateful thanks to Sir J. C. Ghosh, our Director, for the keen interest he has taken in the course of these investigations.

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