MICROBIOLOGICAL ASSAY OF GROWTH FACTORS IN SOME RAW MATERIALS USED IN INDUSTRIAL FERMENTATION

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I N T R O D U C T I O N

INTRODUCTION

Different micro-organisms require more than pure sugar and salts for the development of maximum growth rates or maximum production of certain products. To attain the peak of reproduction ordinarily observed in plant extracts such as juices, molasses, wort etc., one or more of a group of substances known as bios factors must be present. These factors are inositol, pantothenic acid, biotin, thiamine, pyridoxin and nicotinic acid.

The first three vitamins have been shown to be of main importance, it is thus a matter of great practical importance to the industry that the raw materials should be assayed for these vitamins. Nutritional requirements could be specified when the purpose and conditions of certain micro-organisms have first been accurately defined. In case of lack in any vitamin, the raw materials should be supplied with other rich raw materials. It is well established now that the lack of certain bios might affect the yield in certain fermentation industries.

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butanol). Sugar cane molasses is the main raw material for the first three industries. Malt wort, rice bran extract, and corn steep liquor are also used as raw materials in certain fermentation industries. No informations concerning the vitamin content of these local raw materials are available. Also the requirements of the yeast or bacterial strains used are not known.

The present work deals with the microbiological assay of pantothenic acid and biotin in some raw materials used for several fermentation processes specially black-strap molasses.

The variation in the vitamin content according to its source, or to physical and chemical treatments was also studied. The vitamin balance in baker's yeast manufacture was also followed up.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The role of vitamins in the nutritional media of different micro-organisms had been extremely reviewed (Porter, 1947; Robinson, 1951 and White, 1954). Among these vitamins the bios factors (Inositol, Pantothenic acid and biotin)have been shown to be of main importance in fermentation industries. Consequently it is of importance to assay the vitamin content in the different raw materials used in fermentation industries.

The vitamin assay may be carried out by either chemical or biological methods. The biological methods of assay are better measures of general biological activity than are the chemical methods. For some vitamins no accurate chemical assay is yet available which is suitable for routine use with natural materials. Microbiological assays possess the advantage of speed, small requirements of space, labor, and materials as compared to other biological methods using laboratory animals or humans (Association of vitamin chemists, 1951). In some cases the chemical methods of assay are preferable to biological methods because of the inherent greater accuracy of the former. However, the difficulty lies in finding a [illegible] [illegible] methods, also the chemical methods may utilize properties unrelated to biological activities. For example D- and L- forms of a substance may be indistinguishable in the chemical assay and yet only one of the enantimorphic forms may be biologically active. Hence, chemical and biological methods of assay should not necessarily give the same answers on crude preparations because of the substances which interfere with each assay in different ways. Results of the chemical assay are usually different than the biological ones . Consequently the interpretation of a chemical assay can be certain for samples of known history, e.g. some pharmaceutical preparations, and uncertain for samples of natural origin (Kavanagh, 1960 and 1963).

As the present work concerns with the microbiological assay of pantothenic acid and biotin it is essential to review some specific informations of each of them concerning the structure, occurrence, role as coenzyme, effect of deficiency. Also informations on the role of some vitamins (with special reference to pantothenic acid and biotin) on the microbiological growth and activities, are reviewed in the following pages.

Pantothenic Acid

Pantothenic acid occurs everywhere in animal and plant tissue. The richest common source is liver but jelly of the queen bee contains six times as much as liver. Rice bran and molasses are other good sources (Stecher et al., 1960).

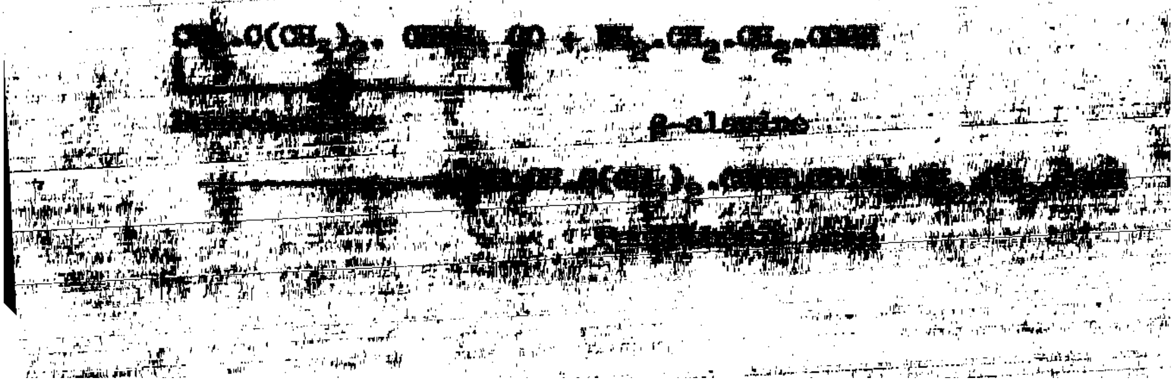
The first indication of the existance of pantothenic acid was mentioned by Williams and his associates (1933). They demonstrated the wide spread distribution of a substance that acted as a growth substance for yeast and other micro-organisms. At that time these workers applied the name pantothenic acid to the active principle. Later Williams group (1938) obtained a crude pantothenic acid concentrate from liver. Further purification yielded an amorphous product with over 11,000 times the activity of a standard rice bran extract. Afterwards the so-called antidermatitis factor from liver, also called the filtrate factor was shown to be identical with Williams pantothenic acid (Woolley et al., 1939 and Jukes, 1939). Woolley et al. (1939) showed that this factor, like pantothenic acid, is [illegible]

[illegible]

reactivated it by coupling the remaining part of the molecule with synthetic β-alanine. Stiller *et al.* (1940) were able to crystalize pantothenic acid in pure form, its structure determined, and its synthesis accomplished. They, also, demonstrated that synthetic pantothenic acid has only half the growth promoting action of the natural acid on *Lactobacillus helveticus* due to the presence of equal amounts of D and L pantothenic acid. The former was active on micro-organisms, rats and chicks, while the latter was inactive.

Chemical Structure and Physical Properties :

Pantothenic acid is α , γ -dihydroxy- β , β dimethyl butyryl- β-alanide. Thus the amino group of β-alanine is bound to the carboxyl group of a dihydroxy acid to form an acid amide group. The direct condensation of β -alanine with the lactone of the substituted butyric acid (identified as α hydroxy-β . β dimethyl-γ butyrolactone, known also as pantolactone) gives directly the pantothenic acid :



The pantothenic acid (vitamin B_5) molecular weight is 219.23, C 49.3%, H 7.82%, N 6.39% and O 36.49%. The free acid is viscous yellow oil, soluble in water and ethyl acetate but insoluble in chloroform and benzine. It is extremely hygroscopic, easily destroyed by acids, bases and heat. It is readily available as either the sodium or calcium salt. The latter salt is crystalline material fairly soluble in water (7 g. per 100 ml) and insoluble in alcohols. It is quite stable, although autoclaving destroys the activity $(\alpha)_D^{26}$ for the free acid is + 37.5 and the calcium salt + 24.3 (West et al., 1966).

Coenzyme activity :

Pantothenic acid is a part of coenzyme A of Lipmann et al. (1947) characterised by Lynen and Co-workers (1951). The CoA molecule is a nucleotide of the following composition : adenine-phosphorylated ribose (C-3)-phosphate-phosphate-pantothenic acid- β-mercaptoethylamine.

The scheme of the biosynthesis of CoA is presented by Brown (1959) as shown here in. All of the enzymes required can be found in micro-organisms and rat liver and kidney.