THE EFFECT OF THE WATER ACTIVITY OF THE GROWTH MEDIUM ON PROTEIN CONTENT AND POLYSACCHARIDE COMPOSITION OF THE SUGAR TOLERANT YEAST SACCHAROMYCES MELLIS.

By

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INTRODUCTION

Yeasts occurring in substrates with high sugar content such as honey, jams, jellies, syrups, dried fruits and fruit concentrates are usually referred to as osmophilic yeasts or osmoduric yeasts. Osmophilic yeasts are those which proliferate in an environment of high osmotic concentration, while osmoduric yeasts are those which tolerate but do not multiply in environments of high osmotic concentration. Much of the interest in osmophilic yeasts has been due to their role in the spoilage of the previously mentioned high sugar content foods. The common dominator of all these foods is the low water content in relation to organic matter. This low water ratio may be achieved either by a drying process or by the addition of large amount of solute.

Scott (1957) discussed the relationship between osmotic pressure, vapor pressure, equilibrium relative humidity and water activity. The equilibrium relative humidity of a solution is the humidity at which the rates of evaporation and condensation are equal and obviously will vary with the amount and type of material in the solution, which in turn affects the water activity. Water activity is numerically equal to the corresponding relative humidity R.H. expressed as a function, i.e., R.H./100. It has been demonstrated by Shachinger and Heiss (1951) and English (1953) that it is the overall water activity of the medium, and not the nature or the amount of carbohydrates present which controls the growth of these yeasts under such conditions of high sugar content.

The purpose of the present studies however, is to show how the water activity of the growth medium — as regulaed by either glucose

or sucrose — can affect both the protein content, and the different fractions of polysacharides in the sugar osmotolerant yeast *Saccharomyces mellis*.

Materials and Methods

Test organism : The yeast used in these studies was isolated from a spolied sample of apple jelly. It can grow on up to 80% (w/v) glucose concentration. At 2% level, glucose but not sucrose, maltose or lactose was fermented. It readily assimilates glucose but poorly assimilates maltose and sucrose, while lactose was not assimilated. It does not assimilate nitrate. Cells are mostly oval, while vegetative growth by lateral budding, with no pseudomycelium formation. It forms a little ring but no pellicle. Ascospores are oval or spherical mostly one per ascus, 2-3 may be found. The yeast was identified as *Saccharomyces mellis* acording to the procedures presented by Lodder and Kreger — van Rij (1952).

Growth medium : The yeast was grown in 100 ml portions in 250 ml capacity Erlenmeyer flasks at the desired sugar concentration, using a basal medium composed of 0.5% peptone, 0.3% yeast extract, 0.2% KH_2PO_4 and 0.05% $MgSO_4.7H_20$, initial pH 6.5. Sugar solutions and basal medium were sterilized separately and then aseptic ly mixed. The water activity of the growth medium was regulated using either glucose or sucrose according to the table presented by Scott (1957). When sucrose was used to regulate the water activity of the medium glucose was added at 1% concentration. Inoculated flasks were incubated at 28°C for three days.

Washing of cells : Cells were harvested by centrifugation at 5500 r.p.m. for 15 minutes, (media with very high concentrations of sugars were first diluted with water to help easy centrifugation of cells). After three times washing with distilled water, cells were lyophilized and kept in a desiccator over calcium chloride.

Protein analysis : 100 mg portions of lyophilized cells were transferred to 15 ml capacity glass centrifuge tubes. 4 ml of 0.5N NaOH solution were added. Tubes were then put in a boiling water bath for 8 minutes. After alkali digestion, cells were centrifuged for 10 min. at 5500 r.p.m. The cell residue was washed twice with 3 ml portions of cold 0.5N NaOH solution and the combined supernatants were brought up to 10 ml. Protein analysis was run using Folin Ciocalteu method, Lowry et al. (1951). Bovine albumin was used as a standard. Absorbancy of samples was read colorimetrically at 550 mu.

Carbohydrate Analysis : Quantitative determination of the different fractions of cellular polysaccharides together with the total carbohydrate content was carried out using the procedure described by Chung and Nickerson (1954). 50 mg portions of lyophilized yeast cells were used. Anthrone reagent was used for colorimetric determination of carbohydrates. Standard mannose solution was used for mannan determination, while standard glucose solution was used for the rest of samples.

Results and Discussions

Fig. 1 presents levels of alkali extractable proteins of the yeast when grown on media the water activity of which was regulated either by glucose alone, or by sucrose keeping the glucose level at 1%. In both cases, one notes the complete similarity in the stimulatory effect of he water activity of the medium on levels of alkali extractable proteins with a peak at about 0.91 water activity. On the other hand, a reciprocal relationship is noticed between levels of alkali extractable proteins and levels of the total yeast carbohydrates as a function of the water activity of the medium. Yeast cells grown in the presence of sucrose at 1% glucose level showed lower levels of carbohydrates than yeast grown in the presence of glucose alone.

Fig. II and III show the levels of each fraction of the yeast polysaccharides when sucrose and glucose were used to regulate the water activity of the growth medium respectively. In both cases, complete similarity could be noticed for each fraction of polysaccharide. The trichloroacetic acid extractable carbohydrates which constitute mononucleotides, oligosaccharides and phosphorylated sugars (Chung and Nickerson 1954), show a very small increase in their levels as the water activity of the growth medium decreases, with a small peak at about 0.97 water activity. As the water activity of the medium decreases, an initial rise in the glycogen levels is noticed with a peak at about 0.985 water activity, followed by reduction in these levels at lower water activities. Also, a reciprocal relationship could be noticed between levels of both glycogen and mannan. However, levels of both compounds at low water activities approximate those for cells grown at high water activity. Glucan levels seem to be directly affected by the reduction in the water acivity of the growth medium.

According to Chung and Nickerson (1954), synthesis of glucan and mannan by different strains of Saccharomyces species growing

on glucose containing media, proceeds via the formation of glucose and mannose-1- phosphates respecively. Both in turn are formed from hexose-6- phosphate. Therefore, the observed reduction in levels of glucan at low water activities presumably might be due to the development of metabolic lesions at one or more steps during the synthesis of glucan from glucose-1-phosphate.

Glucan and mannan have already been shown to occur in cell walls of *Sacharomyces* species Kessler and Nickerson (1959), and any change in the ratio of these polysaccharides will presumably affect the physicochemical properties of the yeast cell wall, Dunwell et al. (1961). Therefore. possible alterations in the physicochemical properties of the yeast cell wall when grown at different levels of water activity, can be presumed in reference to the noticed difference in the ratio of both glucan and mannan obtained in the present studies. This needs further investigations. However, the stimulatory effect of lower values of water activity on levels of cellular proteins, could not be explained in relation to the yeast sugar osmotolerance.

Summary

The sugar osmotolerant yeast *Saccharomyces mellis* was grown on media the water activity of which was regulated either by glucose or sucrose. Levels of total carbohydrates were found to decrease, while levels of alkali extractable proteins increase in the yeast cells as the water activity of the growth medium decreases. Reciprocal relationship has been found between levels of glycogen and mannan under the same conditions. Glucan levels however, were found to be directly affected by the reduction of the water activity of the growth medium.

At low values of water activity, a possible development of metabolic lesion at one or more steps during glucan synthesis from glucose-1-phosphate was presumed. Also, a possible alteration in the physicochemical properties of the yeast cell wall was presumed, due to the noticed variation in ratios between glucan and mannan levels, when cells were grown at different water activities.

REFERENCES

1. Chung, C.W. and W.J. Nickerson (1954)
Polysaccharide synhesis in growing yeasts.
J. Biol. Chem. 208, 395.

2. Dunwell, J.L., F. Ahmad, and A.H. Rose (1961)
Changes in the polysaccharide composition of yeast resulting from biotin deficiency.
Biochem. Biophys. Acta 51, 604.

3. English, M.P. (1953)
The fermentation of malt extract by an osmophilic yeast.
J. Gen. Microbiol. 10, 15.

4. Kessler, G. and W.J. Nickerson (1959)
Glucomannan — protein complexes from cell walls of yeasts.
J. Biol. Chem. 234, 2281.

5. Lodder, J. and N.J. Kreger-van Rij (1952)
«The Yeasts» Interscience Publishers, New York, U.S.A.

6. Lowry, O., N. Rosebrough, A. Farr and R. Randall (1951)
Protein measurement with Folin phenol reagent.
J. Biol. Chem. 193, 265.

7. Scott, W.J. (1957)
Water relations of food spoilage microorganisms.
Advances in Food Research 7, 84.

8. Shachinger, L. and R. Heiss (1951)
Osmotischer Wert und Mikroorganismen wastum in Zucherlösungen.
Arch. Mikrobiol. 16, 347.

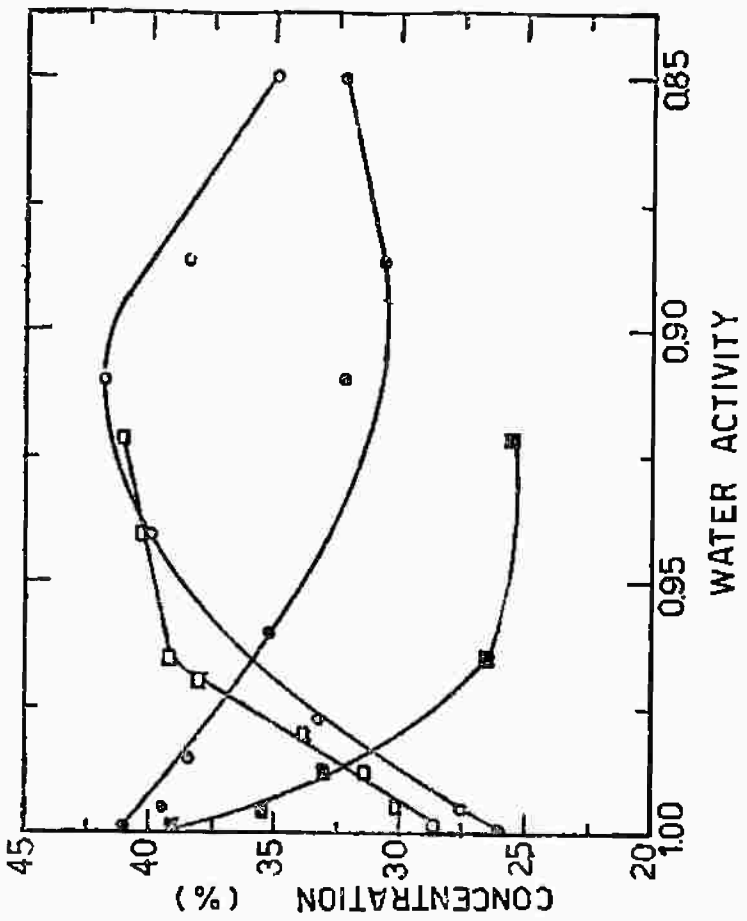
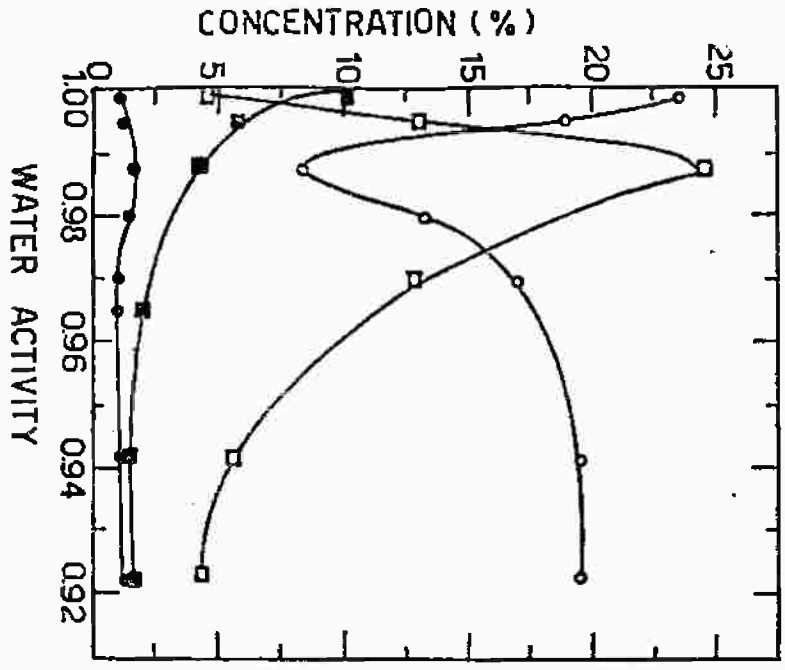


Fig. I

The effect of the water activity of the growth medium on levels of both alkali-extractable proteins and total cellular carbohydrates in *Saccharomyces mellis*.

Open circles and squares represent levels of alkali-extractable proteins when the water activity of the medium was regulated by glucose and sucrose respectively.

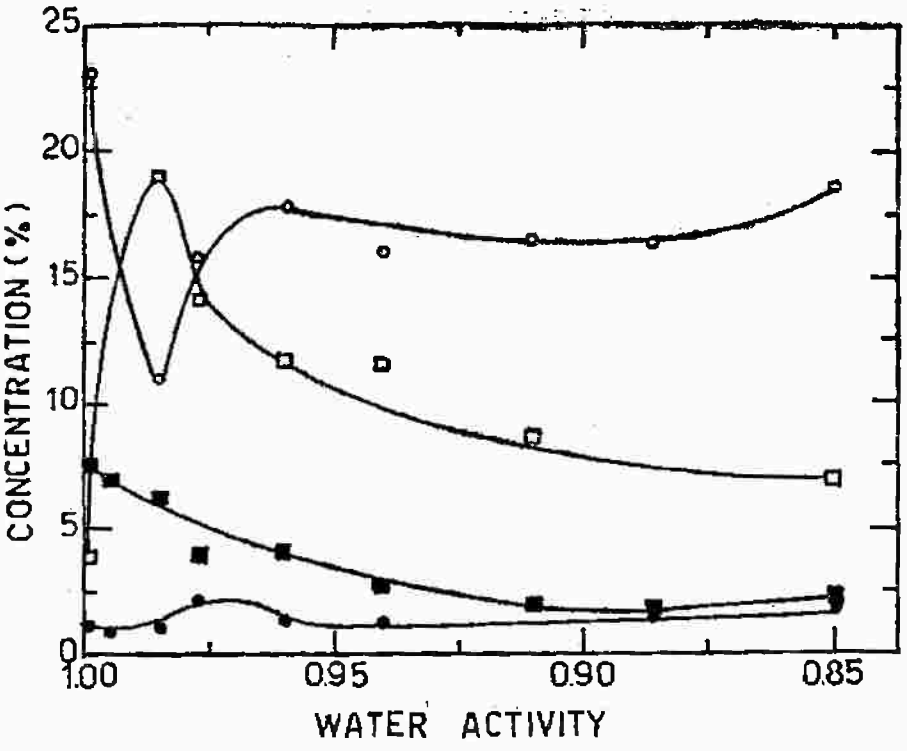
Closed circles and squares represent levels of total carbohydrates when the activity of the medium was regulated by glucose and sucrose respectively.



Legened to Fig. II

The effect of water activity of the growth medium as regulated by sucrose on levels of the different polysaccharide fractions in cells of *Saccharomyces mellis*.

Open circles and squares represent levels of mannan and glycogen respectively. Closed circles and squares represent levels of cold TCA-extractable carbohydrates and glucan respectively.



Legend to Fig. III

The effect of water activity of the growth medium as regulated by glucose on levels of the different polysaccharide fractions in cells of *Saccharomyces mellis*.

Open circles and squares represent levels of mannan and glycogen respectively. Closed circles and squares represent levels of cold TCA-extractable carbohydrates, and glucan respectively.