Technical Papers

Susceptibility of the Starch in Fresh and Stale Bread to Enzymatic Digestion

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It was reported recently (*1*) that the incorporation of low levels of bacterial α-amylase[2] in bread formulation resulted in baked products normal in all respects but characterized by greatly reduced rates of staling. In this connection it was demonstrated by measurement of the susceptibility of the bread starch to hydrolysis by β-amylase (*3*) that the starch changes generally considered to be of major causative importance in bread staling (*4–6*) were absent or significently reduced in the products baked with the enzyme.

During the course of studies concerned with the nature of the starch changes accompanying the staling of bread, data were obtained which indicate that the rate of *in vitro* digestibility of stale bread by pancreatin is significantly less than that of fresh bread. Bread baked with bacterial α-amylase maintained an essentially constant rate of digestibility, equivalent to that of fresh bread, throughout the storage period.

These data, which are shown in Table 1, were obtained by a modification of the yeast fermentation method (*3*) involving substitution of pancreatin for α-amylase and buffering at pH 7.5 instead of at pH 5.2. In this method the amount of gas evolved during the digestion period, after prior removal of the carbohydrates directly fermentable by yeast, is related stoichiometrically to the amount of fermentable carbohydrate produced by the enzyme action.

TABLE 1

EFFECT OF AGE OF BREAD ON DIGESTION OF BREAD CRUMB BY PANCREATIN*

Storage at 30° C (hr)	Gas production in fermentation test (ml)		Rate of digestion†	
	Control bread	Bacterial α-amylase bread	Control bread	Bacterial α-amylase bread
20	575	587	1.00	1.02
40	518	558	0.90	0.97
60	488	562	.85	.98
80	472	560	.82	.97
100	460	565	.80	.98
120	443	560	.77	.97
140	432	570	.75	.99
160	413	563	.72	.98

* Three-hour digestions carried out at 30° C on 20 g of bread crumb suspended in 100 ml of water containing 10 ml of McIlvaine's citric-phosphate buffer, pH 7.5. Digestion by 35 mg of pancreatin.

† Rates calculated relative to that of the bread stored at 30° C for 20 hr.

As shown in Table 1, the rate of digestion of the control bread decreased progressively during the 160-hr storage period to a value approximately 75% of that when the bread was reasonably fresh (20 hr old, stored at 30° C). The breads baked with 6 SKB units of bacterial α-amylase, on the other hand, maintained a digestibility rate essentially equivalent to that of the fresh control bread throughout the entire storage period.

It would appear from these data that stale bread is less readily digested under *in vitro* conditions than fresh bread and that inclusion of bacterial α-amylase in the bread formulation prevents the decrease in rate of digestion. Evaluation of the significance of these *in vitro* findings in terms of human nutrition will not be possible until suitable *in vivo* studies are carried out. Appropriate techniques, used in studies on the digestion of bread in the human stomach, have been published (*7*).

It is necessary to reconcile these results, indicating that the starch in fresh bread is more readily digested, with other *in vitro* reports (*8–10*) that oven-fresh bread is attacked with difficulty by proteolytic and diastatic enzymes. Although the conclusions appear contrary, there is reason to believe that the difference refers, not to inherent susceptibility of the starch substrate to enzymatic action, but rather to overall availability of the bread sample to enzymic attack. Oven-fresh bread does not disperse so easily or so well as bread even a few hours older. All samples in our experiments were thoroughly dispersed by mechanical means in order to permit conclusions referring specifically to susceptibility of the starch component to enzymatic digestion. The samples in the studies reported in the literature were not well dispersed and, presumably, the data obtained reflect differences in dispersibility of the samples on aging. In any event, the particular problem of oven-fresh bread is a special one with only narrow implications since commercial bread is generally not less than 8–12 hr old when it reaches the consumer.

From studies with humans (*11*) and laboratory animals (*12*) it appears well established that the total utilization of the carbohydrates in bread is essentially quantitative regardless of the age of the bread. In view of the known retrogradation of starch during bread staling, representing a polymerization and return towards the non-gelatinized state, it appeared of value to establish whether a similar conclusion would be drawn under *in vitro* conditions. The same technique used to obtain the other data of this paper was

[1] We are indebted to F. D. Schoonover for baking the breads used in this study.

[2] The α-amylase (*B. subtilis* type) was added at the rate of 3–6 Sandstedt, Kneen- and Blish units (*2*) per pound of bread.

followed, except that the amount of pancreatin was increased to 500 mg to provide a rapid rate of hydrolysis. From 20 g of interior bread crumb, 1220 ml of gas (± 2%) were obtained regardless of the age of the bread (up to 1 week at 30° C). Thus, the *in vitro* data are in agreement with *in vivo* data (*11, 12*) indicating that the extent of digestion of the starch in bread is not influenced by the age of the bread.

Further studies are underway concerned with the nature of the changes in the starch component accompanying the staling of bread, and with the nature of the mechanism involved in the favorable action of bacterial α-amylase in preventing or retarding these changes.

References

1. SCHULTZ, A. S., *et al. Cereal Chem.*, **29**, 200 (1952).
2. SANDSTEDT, R. M., KNEEN, E., and BLISH, M. J. *Ibid.* **16**, 712 (1939).
3. JACKEL, S. S., *et al. Ibid.*, **29**, 190 (1952).
4. GEDDES, W. F., and BICE, C. W. *Q.M.C. 17–10.* War Department. Office of the Quartermaster General, Washington, D. C. (1946).
5. KERR, R. W. *Chemistry and Industry of Starch,* 2nd ed. New York: Academic Press (1950).
6. WHISTLER, R. L. *Purdue Univ. Agr. Expt. Sta. Rept.* File No. C-205 (1947).
7. ROSTORFER, H. H., KOCHAKIAN, C. D., and MURLIN, J. R. *J. Nutrition*, **26**, 123 (1943).
8. BARTELS, L. *Mehl u. Brot.*, **42**, 21, 41 (1943).
9. HABS, H., and PLAGEMANN, L. *Deut. Z. Verdauungs-u. Stoffwechselkrankh.*, **7**, 129 (1943).
10. HEUPKE, W. *Münch. med. Wochschr.*, **89**, 128 (1942).
11. HABS, H. *Deut. Z. Verdauungs-u. Stoffwechselkrankh.*, **7**, 201 (1943).
12. ABELIN, I., and BIDERBOST, A. *Biochem. Z.*, **247**, 429 (1932).

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A Simple Method for Making Transfers in Paper Chromatography

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A very efficient technique for elution of water-soluble compounds from paper chromatograms is that described by Wyatt (*1*). In this procedure the strip of paper to be extracted, cut to a point at one end, is developed as a descending chromatogram with water which is allowed to drip from the pointed end of the strip into a receiver. Compounds which travel with the front may be eluted in a few drops of water. We have been using a modification of this technique to transfer single components to another chromatographic strip for development with a different solvent. Transfer of the eluate in the ordinary way, after evaporation, is complicated by the requirement of keeping the size of the initial spot on the new chromatogram as small as possible. This necessitates performing the transfer in portions of a few microliters at a time and letting each portion dry before the next is applied. As numerous washings are required, this procedure may be very time-consuming.

It has been found possible to perform the entire

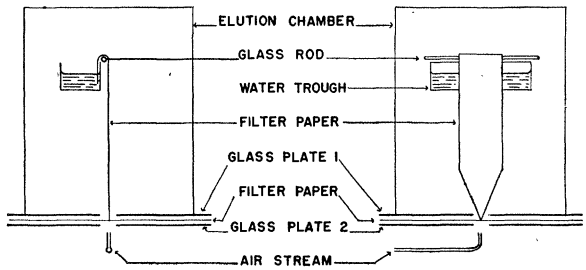

FIG. 1. Sketch of apparatus used for single stage transfer between chromatographic runs.

transfer as a continuous process by using the arrangement shown in Fig. 1. The strip of filter paper to which the transfer is to be made is sandwiched between 2 sheets of glass or plastic in which are matching round holes about 1¼ in. in diameter. The elution is performed in a small chamber resting upon these sheets so that the air in the elution chamber can be kept nearly saturated with water vapor—an essential condition if the elution is to proceed properly. The original strip is suspended from the developing trough so that its pointed tip just touches the new strip in the center of the round window. When the water front reaches this point and begins to spread on the new strip, a stream of air at room temperature, directed against the under side of the paper window, is sufficient to evaporate the eluate as rapidly as it flows. In this way, the spot size may be kept as small as is desired by regulating the air flow, and the time required for the transfer is just the time required for the elution.

Since the water flow is not observable under these conditions, the time necessary to complete the elution must be determined indirectly. The method we find most convenient is as follows. A 10-cm length of the strip (A) to be eluted is marked off in 0.5-cm divisions with lead pencil. The elution is then begun. When the water front reaches the tip of strip A and begins to spread on the new strip (B) a colored compound of known R_f value is applied at the graduation farthest from the tip of A. The subsequent travel of such a marker is linear with time during the evaporation. The R_f of the marker and the distance it has moved being known, together with the water-loading and the width of strip A, the volume of water which has been evaporated at any time may be calculated. If its R_f is sufficiently small, the indicator itself will not be eluted in the time it takes to evaporate the required 0.5 ml of water. The water-loading is determined by weighing strips of known area under similar conditions. We find about 12 mg of water/cm² in a wet strip of Whatman No. 1 paper during an elution.

As a marker an orange-colored indelible pencil (Mongol No. 962, Eberhard Faber Co.) is very convenient, since it may be inserted through a small hole in the lid of the elution chamber without altering the water saturation of the enclosed air, and the R_f of the orange dye has a suitable value—about 0.52 in our experiments.