On the Fortification of Edible Fat with Vitamin A

BY U. P. BASU AND S. K. SEN GUPTA

For making up the deficiency of vitamin A edible fat is fortified with some vitamin preparation; but on storage the preparation often loses its vitamin A potency, mainly due to oxidation. The addition of a suitable anti-oxidant retards this oxidation. Baxter and Robeson[1] have further shown that vitamin A in the form of its ester (vitamin A acetate) is less susceptible to oxidation. Accordingly, it has been of interest to see how an edible fat in which vitamin A acetate had been incorporated would behave on aeration in presence of some suitable anti-oxidant.

Cow's "ghee" (butter clarified by boiling) was fortified in one case with a vitamin A concentrate made by saponification containing 0.2 million I.U. per gram and in another with a crystalline vitamin A acetate, in presence of 0.05% ethyl gallate (*cf.* Lea,[2] and Bose and Banerjee[3]) and the initial potencies of vitamin A in both the preparations were measured in terms of Carr–Price blue value. Air free from moisture and carbon dioxide was passed through both the preparations under identical conditions at 40°. The relative fall in C.-P. blue values in the two preparations was noted from time to time and the results are being recorded in the table.

TABLE I

LOSS OF VITAMIN A POTENCY ON AERATION

Period of aeration, hours	Carr-Price value in preparations With concentrate	With acetate
0	19.2	17.4
20	18.2	17.2
60	15.3	17.0
100	9.8	17.0
150	6.4	16.0
200	3.2	15.3
250	nil	12.8

The preliminary investigations indicate that vitamin A acetate may preferentially be used in fortifying an edible fat. Details of the work will be published elsewhere.

(1) J. G. Baxter and C. D. Robeson, THIS JOURNAL, **64**, 2411 (1942).

(2) C. H. Lea, *J. Soc. Chem. Ind.*, **63**, 107 (1941).

(3) S. M. Bose and B. N. Banerjee, *Ind. J. Med. Res.*, **33**, 303 (1945).

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Influence of Temperature on the Production of Glycerol during Alcoholic Fermentation

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A small amount of glycerol is always produced during alcoholic fermentation. The intermediary processes leading to this formation of glycerol are included in the current theory for the mechanism of alcoholic fermentation (see Meyerhof).[2] This scheme, however, does not provide an insight into the way in which substrate is diverted into the series of reactions leading to glycerol formation. Inasmuch as the immediate origin of glycerol involves the hydrolysis of glycerol-1-phosphate, it follows that the phosphatase activity of yeast can be assumed to play a part in its production. While certain special conditions are known to enhance glycerol formation, the influence of common environmental factors—such as temperature, oxygen tension and concentration of ammonium salts—on the production of glycerol during alcoholic fermentation remains unsettled. This paper deals with the production of glycerol in the course of fermentations maintained at 30 and 37 or 40°. As a means of throwing further light on differences in glycerol formation at these temperatures, observations were made on the phosphatase activity of yeast recovered from fermentations maintained at different temperatures.

Experimental

Yeast Strain.—All experiments were performed with a distillery strain of *Saccharomyces cerevisiae*.

Medium.—The medium contained 10 g. of glucose, 0.7 g. of dehydrated yeast extract (Difco) and 0.5 g. of potassium dihydrogen phosphate per 100 ml. This medium was autoclaved at 120° for ten minutes.

Preparation of Inoculum and Management of Fermentation.—In the development of inoculum, yeast was transferred through the following steps at twenty-four-hour intervals: (A) from the stock slant to 10 ml. of medium; (B) 1 ml. from (A) to each of two tubes containing 10 ml. of medium apiece; (C) the contents of each tube from (B) were added to 150 ml. of medium in a centrifuge bottle. All cultures were incubated at 30°. At the time of inoculation, yeast was centrifuged from the cultures prepared in step (C), washed twice with potassium dihydrogen phosphate solution (0.5 g. per 100 ml.) and transferred to 1300 ml. of medium. After a thorough mixing, 600-ml. portions of the inoculated medium were transferred to 2 sterile, 1-liter Florence flasks which were plugged with cotton and suspended in water-baths maintained at 30, 37 or 40°. Both flasks were continuously agitated.

Analytical Operations.—Immediately after inoculation and at intervals throughout the twelve–fourteen hour observation period samples were withdrawn for evaluation of glucose and glycerol concentrations. Glycerol concentration was determined by the procedure of Amerine and Dietrich.[3] Because the yeast extract present in the medium contributes to the observed glycerol concentration, the apparent glycerol concentration of the medium immediately after inoculation was subtracted from the observed concentration of subsequent samples. This difference was recorded as "glycerol formed." At the end of the indicated observation period yeast cells were centrifuged from the culture, washed with water, and dried for forty hours in a vacuum desiccator over activated alumina. A known weight of dried cells was assayed for phosphatase activity at *p*H 4.1 using disodium glycerol phosphate (Eastman Kodak Co. #644) as substrate.

Results

In Fig. 1 glycerol formation during fermentation at 30, 37 and 40° is identified as a function of

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(2) Otto Meyerhof, "A Symposium on Respiratory Enzymes," University of Wisconsin Press, Madison, Wis., 1942, pp. 3–15.

(3) Maynard A. Amerine and William C. Dietrich, *J. Assoc. Off. Agr. Chem.*, **26**, 408 (1943).

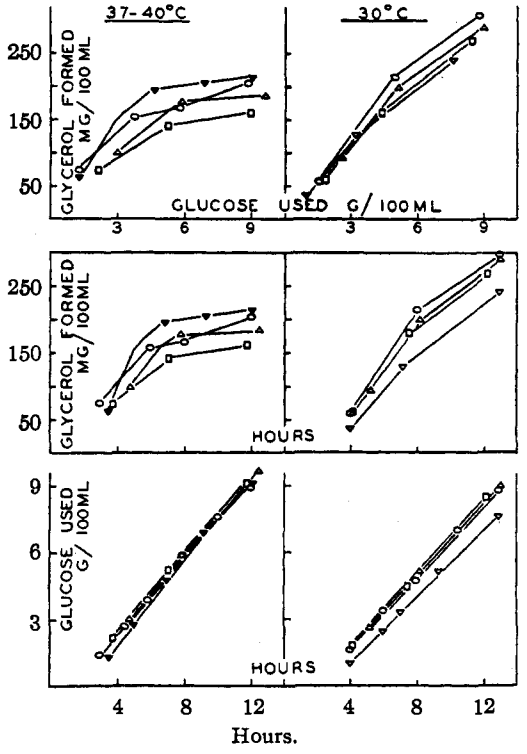

Fig. 1.—Glycerol formation and glucose utilization during four representative experiments conducted at 30° and 37 or 40° (closed triangle represents observations at 37°).

time as well as of the glucose metabolized. With fermentations maintained at 30°, the yield of glycerol after 40 to 50% of the initial sugar has been metabolized is from 3.7 to 4.2 g. per 100 g. of glucose utilized. Later, when 80 to 90% of the sugar has been metabolized, the glycerol yield is between 3.0 and 3.5 g. per 100 g. of sugar used. This represents a slight but consistent decrease in the yield of glycerol during the latter half of fermentation.

In fermentations held at 37 or 40° glycerol formation with respect to sugar utilization follows about the same pattern as at 30°, up to the point of the metabolism of 25 to 30% of the sugar initially present. When approximately half of the sugar has been metabolized, the yield of glycerol in fermentations held at 37 or 40° is 2.7 to 3.2 g per 100 g. of sugar used—a range definitely lower than that observed at a corresponding point of the fermentations maintained at 30°. By the time 85 to 90% of the sugar has been metabolized, the yield of glycerol in fermentations held at 37 to 40° amounts to only 1.8 to 2.3 g. per 100 g. of glucose utilized. Of the glycerol produced during the entire observation period, more than three-fourths was formed by the time one-half of the ultimate sugar utilization was attained.

As is evident from the glucose-used time relationships shown in Fig. 1, the rate of glucose utilization in the 30° fermentations was only slightly less than in those held at 40°. This, however, is an incomplete picture, because in fermentations held at 40°, the cell populations were only 40 to 50% of those observed at like periods in fermentations maintained at 30°. Thus, on a per cell basis, the rate of glucose utilization at 40° was always more than twice the rate at 30°.

From the observations summarized in Fig. 1, it is apparent, particularly in the fermentations conducted at 40°, that the production of glycerol was not constant throughout the observation period, although the rate of glucose utilization during the same period was nearly constant. It follows, therefore, that glycerol production must be controlled by some enzyme system not involved directly in the over-all utilization of substrate. These specifications appear to be met by the cell phosphatases capable of promoting the hydrolysis of glycerol-1-phosphate. To test this concept, yeast cells were recovered from fermentations at the end of a twelve–fourteen hour holding period and assayed for phosphatase activity. Representative results from essays are shown in Table I.

TABLE I

PHOSPHATASE ACTIVITY OF YEAST AND FERMENTATION MEDIUM AFTER TWELVE–FOURTEEN HOURS OF FERMENTATION AT DIFFERENT TEMPERATURES

Phosphatase activity[a]			
30° fermentation		37 or 40° fermentation	
For 5 mg. dry yeast	For 1 ml. medium	For 5 mg. dry yeast	For 1 ml. medium
155	205	60[b]	55
122	239	25	32
110	136	52	73
100	148	30	29

[a] Micrograms of phosphorus liberated from disodium glycerol phosphate by either 5 mg. of dry yeast or yeast present in 1 ml. of fermentation medium during ninety minutes at 30°. [b] 37°.

While the results shown in Table I indicate a considerable variation in the phosphatase activity observed in yeast cultures of comparable history, there can be little question that yeast held at 37 or 40° has far less phosphatase activity than yeast maintained at 30° over corresponding periods of time. Because the procedure employed for the assay of cell phosphatase activity is not specific for those enzymes which hydrolyze *l*-glycerol-1-phosphate to glycerol, it cannot be stated conclusively that the reduction in the amount of glycerol formed in fermentations maintained at 37 or 40° is related to a deterioration of cell phosphatase activity. However, if glycerol formation parallels the combined activities of the cell phosphatases capable of hydrolyzing the isomers of glycerol phosphate, this relationship suggests that the phosphatase activity of yeast is a significant factor in the control of glycerol formation during normal alcoholic fermentation.

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