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The Use of the Waring Blender in Biochemical Work

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(Received 2 July 1948)

In recent years the Waring blender has found widespread application in biochemical work, owing to the ease and speed with which it disintegrates all sorts of heterogeneous materials into uniform suspensions. It is surprising that the literature contains no reference, so far as the authors are aware, to possible harmful effects of treatment in the Waring blender on the properties of biological material. Several workers seem to have noticed that the intense aeration occurring in operating the blender causes oxidation of reducing substances. This can be gathered from the fact that they use the blender *in vacuo* or in an atmosphere of inert gas. However, no definite information on this point seems to have been published. A further possibility of damage to biological material might be anticipated, viz. denaturation of proteins and hence inactivation on enzyme systems through intensive agitation.

While carrying out experiments on the reducing matter and oxidizing enzymes of wheat germ and other mill streams, the authors found that even short treatment in the Waring blender greatly affected these systems. The present paper describes these findings which were first reported at the New Zealand Science Congress held in Wellington in 1947. In the meantime Quinlan-Watson & Dewey (1948) reported inactivation of cytochrome *c* oxidase caused by treating animal tissue in the Waring blender. The main work on oxidizing enzymes of wheat will be the subject of separate papers.

EXPERIMENTAL

Quantitative evidence for the effect of the Waring blender on suspensions of various mill streams comes from the following experiments.

Effects of the Waring blender on reducing matter

Suspensions of wheat germ (e.g. 1 part germ and 11 parts of water) were made (a) by grinding with sand and water in a mortar, (b) by treating in the Waring blender, and (c) by boiling for 2 min. In the blender, the temperature of a 250 g. suspension rose by 18° within 5 min. owing to the generation of heat by friction. To avoid injurious increases of temperature on treating the suspensions for more than 5 min. they were cooled after 5 min. stirring, or ice water was used for making them.

Fig. 1. Changes of reducing matter in wheat-germ suspensions obtained A, by grinding with sand; B, by 5 min. treatment in the Waring blender; C, by boiling and allowing to stand at 40° for several hours.

The suspensions were then kept in a water bath at 40°, and samples for determination of reducing matter were withdrawn at various intervals. The results were expressed as ml. 0.01 N-I₂ solution/g. of germ. The changes of reducing matter with time are shown in Fig. 1. These graphs in the

first place demonstrate that treatment in the Waring blender caused oxidation of the bulk of the reducing matter present in wheat germ.

Fig. 1 (curve *A*) shows that in the sand-ground suspension there is an initial increase and a subsequent decrease of reducing matter. Both are due to enzyme action: they do not occur in boiled suspensions (curve *C*). The increase is probably the effect of a protease liberating thiol groups, the decrease that of an oxidizing enzyme mechanism. Curve *B* (suspension treated in Waring blender) closely resembles curve *C* (heated suspension) except that it is on a lower level. It seems to indicate that treatment in the Waring blender destroyed enzyme activities responsible for the liberation and oxidation of reducing matter (shown by curve *A*).

Sullivan, Howe & Schmalz (1936) found that wheat germ contains GSH which causes the severe damage, well known to cereal chemists, accompanying the inclusion of wheat germ in doughs. Hullett & Stern (1941) and Smith & Geddes (1942) showed that GSH could be eliminated from wheat germ by fermentation, and that such pre-fermented wheat germ had no adverse effect on the doughs. The elimination of GSH by fermentation, indicated by negative reaction with sodium nitroprusside, takes several hours; treatment in the Waring blender achieved the same result within 5 min. A wheat-germ suspension stirred in the Waring blender for 5 min. gave a negative reaction with nitroprusside and was quite harmless when included in the dough. This rapid oxidation of the thiol groups is due to an enzyme mechanism which requires the presence of O_2 . No comparable oxidation takes place in a boiled germ suspension treated in the blender.

Effect of the Waring blender on dehydrogenase activity

The dehydrogenase activity of wheat-germ extract made from 1 part of germ and 5 parts of water, measured by the Thunberg technique at 30°, dropped from a decoloration time of 20 min. for the untreated sample to one above 4 hr. for a sample treated in the Waring blender for 2 min.

Effect of the Waring blender on ascorbic acid oxidase activity

The discovery of ascorbic acid oxidase in wheat flour by Melville & Shattock (1938) was confirmed by Sandstedt & Hites (1945) who also observed that ascorbic acid oxidase was responsible for the oxidation of the thiol groups present in wheat flours.

In experiments on the determination of ascorbic acid oxidase activity in various mill streams, the following technique was used: Mill stream (10 g.) was suspended in 170 ml. of 0.2M-phosphate buffer of pH 6.5 and digested at 30° for 0.5 hr. The suspension was shaken every 10 min. Ascorbic acid solution (20 ml., containing 20 mg. ascorbic acid and 20 mg. HPO_3) was added so as to make a total of 200 g. of suspension. Immediately after adding the ascorbic acid a sample of 20 g. was removed for determination of reducing substances. The remainder of the suspension, still kept at 30°, was aerated with a strong current of air which was stopped after exactly 20 min. Previous experiments had shown that the oxidation over the initial 20 min. was linear.

A vigorous air stream is necessary to ensure good replication of results. After 20 min. aeration a second sample of 20 g. was removed from the reaction mixture. Both samples, immediately after being taken, were deproteinized with 2 ml. of 10% (v/v) H_2SO_4 and 2 ml. of 12% sodium tungstate, made up to 200 g. and centrifuged at 2000 rev./min. Previous

experiments had shown that if mill streams were deproteinized under these conditions ascorbic acid could be recovered quantitatively, and the centrifugates were clearer than those obtained with HPO_3 .

To 50 ml. of the supernatant (corresponding to 0.25 g. of flour) were added 5 ml. of 0.002N- I_2 solution and the excess was back-titrated with 0.002N- $Na_2S_2O_3$. The difference between the readings before and after aeration, expressed in ml. 0.01N- I_2 solution/g. flour, was taken as a measure of ascorbic acid oxidase activity of the flour suspension. It was found that all readings were replicable, with a standard of ± 0.05 ml. 0.01N- I_2 solution (corresponding to roughly 2% of the initial reading), and that the initial reading corresponded to the sum of added ascorbic acid and reducing matter present in the flour.

When wheat-flour suspension was treated in the Waring blender for 2 min, and then tested for ascorbic acid oxidase activity as described above, the results differed greatly from those obtained with a control prepared by 0.5 hr. digestion at 30° (Table 1).

Table 1. *Oxidation of ascorbic acid in untreated and Waring blender-treated flour suspensions*

(Reducing substances in 50 ml. of supernatant from flour suspensions (corresponding to 0.25 g. flour + 0.5 mg. ascorbic acid) were determined before and after aeration. Results are expressed as ml. 0.01N- I_2 /g. flour. The difference between results before and after aeration is a measure of the oxidation of ascorbic acid occurring during aeration.)

Duplicate determinations were carried out on a control suspension prepared by digestion for 30 min. and on a suspension prepared by 2 min. treatment in the Waring blender.)

	Control suspension prepared by digestion (ml. 0.01N- I_2 /g.)	Suspension prepared by 2 min. treatment in Waring blender (ml. 0.01N- I_2 /g.)
Initial reading	2.41, 2.37	2.15, 2.00
Final reading	1.95, 1.93	1.24, 0.76
Difference	0.46, 0.44	0.91, 1.24

A comparison of columns 2 and 3 shows the following effects of treatment in the Waring blender: (1) duplicate determinations give widely varying results; (2) the initial readings are lower than 2.27 ml., which is the value corresponding to the added ascorbic acid; (3) differences between initial and final readings are much larger than in the control. These three effects of treatment in the Waring blender correspond exactly to what happens when ascorbic acid oxidase in a flour suspension is destroyed by high temperature or pH. Under these conditions the enzyme mechanism breaks down and is superseded by aerial oxidation. It seems very probable, therefore, that treatment in the Waring blender destroyed ascorbic acid oxidase.

Other effects of the Waring blender

Fresh wheat-germ suspensions have a bright yellow colour. On oxidation they assume a greyish white hue, and later, particularly on acidification, turn a deep purple on the surface and wherever else they are in contact with air as on the walls of included air bubbles. Suspensions treated in the Waring blender never proceeded beyond the greyish white stage. It was thought that tyrosinase or peroxidase might be responsible for the darkening effect. Tyrosinase determina-

tions by the chronometric method of Miller & Dawson (1941), showed that wheat germ contains no measurable amount of tyrosinase. Peroxidase, on the other hand, was present and qualitative reactions with guaiac and with benzidine, remained positive even after wheat-germ suspensions had undergone 13 min. treatment in the Waring blender. This behaviour of wheat peroxidase is in keeping with the high resistance of peroxidases from various sources to conditions which are injurious to other enzymes, e.g. high temperature. The fact that wheat peroxidase survives treatment in the Waring blender would seem to indicate that the darkening effect is not due to peroxidase action since failure to darken after treatment is not connected with destruction of peroxidase. It is to be assumed, however, that peroxidase is only one member of a more complex enzyme mechanism which may provide H_2O_2 necessary for peroxidase action. For this reason failure to darken might be due to the breakdown of some other part of this enzyme mechanism. An investigation of the darkening effect is in progress.

DISCUSSION

Potter & Elvehjem (1936) described a device for grinding tissue material, and reported that enzyme inactivation occurred on dilution of the ground material. According to their explanation, dilution causes the coenzyme concentration to drop below the level necessary to maintain enzyme activity. This explanation does not apply to the enzyme inactivations reported in the present paper. While

Potter & Elvehjem compare suspensions of different concentrations the present paper deals with suspensions of equal concentration, but obtained by different means, viz. by grinding with sand in one case and by treatment in the Waring blender in the other.

The Waring blender, with its speed of about 10,000 rev./min. is far more likely to cause protein denaturation than the grinder used by Potter & Elvehjem (1936), with its much lower speed. The present authors suggest that, while in their experiments oxidation of activating thiol groups may have been responsible for the inactivation of certain enzyme systems, the main damage caused by the Waring blender was perhaps enzyme destruction due to protein denaturation.

The experiences reported above make it justifiable to sound a note of caution with regard to the Waring blender and similar homogenizers, and to recommend restricting their use to applications which do not involve inactivation of enzymes or in which such inactivation does not matter.

SUMMARY

Treatment of mill stream suspensions in the Waring blender caused oxidation of thiol groups and inactivation of enzyme systems.

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A Mercury Gasometer for the Preparation of Gas Mixtures

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(Received 23 November 1948)

The need for a means of preparing gas mixtures containing accurately known proportions of carbon dioxide arose during manometric studies of tissue metabolism. The measurement of glycolysis in terms of CO_2 liberated from a bicarbonate buffer requires an atmosphere containing a small proportion of CO_2 , and cylinders of nitrogen and oxygen containing 5% of CO_2 are commercially available. When, however, different concentrations of CO_2 or mixtures of CO_2 with more than one other gas are required, one of the following methods must be employed:

- (1) A continuous-flow method in which flowmeters measure the rate of delivery of the individual gases.
- (2) Collection of the gases, in a graduated Mariotte bottle or other container, over a saturated salt solution in which gases are generally less soluble.
- (3) Preparation of the gas mixture in a pressure vessel fitted with a gauge.
- (4) Collection of the gases in a graduated vessel over mercury.

The flowmeter method is unreliable since the proportions in the mixture will vary widely with changes both in individual gas pressures and in resistance to