

XANTHINE OXIDASE-PEROXIDE INTERACTION IN
STORED DRY WHOLE MILK 2261

In storage studies of vacuum foam-dried whole milk made by the batch process, oxidative deterioration of the milk lipid was encountered. Lea and Smith (6) reported that the extent of lipid oxidation in fat-containing foods could be estimated by quantitatively determining the peroxide value of the lipid. In this study this means of estimating oxidation was employed.

Although the work on oxidized flavor development in milk has been extensive, the precise origin and mechanism is not known. One of the factors reported to promote oxidized flavor development was xanthine oxidase activity (3). However, this finding was not substantiated by others (9). Also, xanthine oxidase has been found to contribute significantly to an increase in reducing potential of dairy products (7), suggesting that it possesses potential antioxidant activity. It was this uncertainty concerning the role xanthine oxidase assumes in the oxidative deterioration of milk lipids (peroxide formation) that prompted this study.

EXPERIMENTAL PROCEDURE

Dry whole milk. Dry whole milks used in this study were produced by the batch vacuum foam drying process of Sinnamon et al. (8). They were N₂ packed in cans and stored at 23 C. At 2 wk intervals the products were removed from storage and analyzed.

A number of experimental products, to which crude xanthine oxidase or the substrate (hypoxanthine) was added, were also prepared. The crude enzyme was the buttermilk fraction recovered from churned raw cream. Also, a purified xanthine oxidase preparation (150,000 units) obtained from Nutritional Biochemical Corporation¹ was used. The substrate, hypoxanthine, was added to give a final concentration of 2.26×10^{-4} , 4.50×10^{-4} , and 5.56×10^{-4} moles per liter of milk. In all cases the additives were incorporated in the fluid milk prior to concentration. These products were also N₂-packed, stored at 23 C, and analyzed at 2 wk intervals.

Headspace O₂ content of the stored product was measured using a Beckman Model E-2 oxygen analyzer. This was generally below 0.2% by volume, but ranged from 0.1-0.7%.

Xanthine oxidase activity. The colorimetric method based on the reduction of colorless tri-

¹Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

phenyl tetrazolium chloride to the red reduced form described by Zittle et. al. (11) was employed. One unit of xanthine oxidase is defined as equivalent to an optical density of 0.036 at a wave length of 485 m μ in a Beckman Model B Spectrophotometer. Dry milks were reconstituted to fluid milk concentration (12% total solids), diluted, and analyzed immediately. Enzyme activity is expressed as units per 100 mg solids.

Peroxide value. Assuming that oxidation would probably be greatest in that portion of the milk lipid not completely protected by globular membrane, namely the free-fat phase, all analyses were performed on this lipid phase of the dry whole milk. The free fat was removed by extracting the dry milk with carbon tetrachloride. Extraction, and all subsequent steps in this analysis, were carried out in a darkened room. One hundred grams of whole milk powder were extracted for 10 min with 600 ml carbon tetrachloride, using a Wrist-Action shaker. The extract was filtered under suction and the solvent removed, using a rotary vacuum evaporator, maintaining the temperature below 30 C. Weighed portions (about 450 mg) of the free fat were placed in 10-ml volumetric flasks and dissolved in a mixture of acetic acid-chloroform (3:2). The colorimetric (iodometric) procedure of Swoboda and Lea (10) was used after modification as follows: The dissolved fat was purged for 3 min with nitrogen (solvent-saturated). Three drops of a freshly prepared saturated NaI solution were added to each sample and the reaction, under a constant flow of nitrogen, was allowed to proceed for exactly 10 min. One per cent cadmium acetate was then added to complex the excess iodide ions, and the two-phase mixture was shaken vigorously to partition the free iodine into the aqueous layer. The free iodine color was measured spectrophotometrically, using 1-cm cells in a Beckman Model B Spectrophotometer at 350 m μ . The reference curve was obtained by reacting standard solutions of KIO₃ with NaI as described above. Peroxide values are expressed as milliequivalent oxygen per kilo of fat. Four analyses were run at one time, a sample in triplicate plus a reagent blank.

RESULTS AND DISCUSSION

Figure 1 shows the xanthine oxidase and peroxide value for a dry milk stored 18 wk. These data are fairly representative of the xanthine oxidase activity and peroxide values obtained

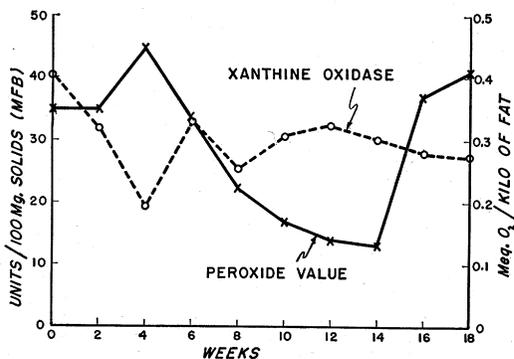


FIG. 1. Xanthine oxidase activity and peroxide value of vacuum foam-dried whole milk (batch process) stored 18 wk at 73 F.

with 17 other stored products. These data indicate that an inverse relationship exists between xanthine oxidase activity and peroxide values. Initial enzyme activity was quite high, but decreased during the early storage period. During this storage interval an increase in peroxide value was observed. Continued storage up to 12 wk resulted in a reactivation of enzyme activity, with a corresponding decrease in the peroxide value. Similar reactivation of xanthine oxidase in dry milk was observed by Greenbank and Pallansch (4).

To further test the validity of this apparent relationship, the data from 18 runs were combined and analyzed as follows: For a given sample the relative change in peroxide value and xanthine oxidase activity from the previously determined value was calculated; change is positive if levels increase and negative if levels decrease. Figure 2 shows a plot of the relative

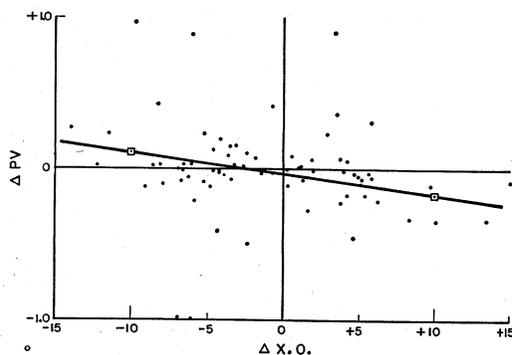


FIG. 2. Relative change in xanthine oxidase versus relative change in peroxide value.

change in xanthine oxidase activity versus the relative change in peroxide value for all samples. Analysis by means of a linear regression showed that a significant ($p = 0.05$) inverse relation exists.

Thus, the indications are that either the peroxide is inhibiting the xanthine oxidase, or the enzyme is functioning as an antioxidant. Astrup (2) recently reported that lipid oxidation in milk may be enzyme-controlled. He demonstrated that addition of copper to milk in which the xanthine oxidase had been inhibited resulted in a greater degree of oxidation than with normal milk, thus indicating the antioxidant property of the enzyme. Also, xanthine oxidase was found to play a prominent role in affecting the reducing potential of milk (7). However, it has been reported that naturally occurring substrate for xanthine oxidase is quite limited in milk and must rely on other milk enzyme systems for its necessary substrates. For this reason, an attempt to increase the activity of the enzyme was made by preparing experimental dry milks to which the substrate (hypoxanthine) was added. Also, products containing added crude enzyme were prepared.

Products prepared from milk to which 4.50 and 5.56×10^{-4} M hypoxanthine was added, unexpectedly exhibited complete inactivation of xanthine oxidase activity. This was accompanied by a sharp rise in peroxide value after 2 wk of storage and again at 5 wk (Figure 3A).

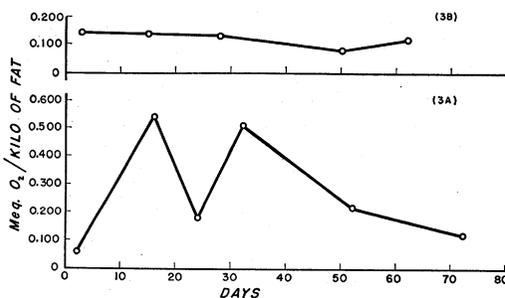


FIG. 3. Peroxide value of stored batch foam-dried whole milk. B. Crude enzyme added. A. Hypoxanthine added.

Kliman et al. (5) observed a similar double peak with peroxide value. At the 2.26×10^{-4} molar level, enzyme activity was not completely inactivated, although it was reduced. With this product, a sharp rise in peroxide value was observed only after 6 wk of storage instead of 2 wk, as with the two former products.

On addition of crude enzyme (obtained commercially or prepared from raw cream) only a slight increase in total activity was accomplished. Even so, peroxide formation throughout the storage period (9 wk) was repressed (Figure 3B).

In this series of experimental powders the possibility that the added buttermilk contains oxidation inhibitors other than xanthine oxidase was also considered. Two products containing

added buttermilk from pasteurized cream (low enzyme activity) were prepared and stored. Results obtained were inconclusive, in that one product exhibited a rise in peroxide during storage, whereas the other did not.

It is of interest to note that products of the continuous vacuum foam drying process of Aceto et al. (1) exhibited very little change in peroxide value throughout a 14 wk storage period (Figure 4). Although Figure 4 exhibits the same type of inverse behavior as the batch products, a statistical analysis, as described previously, of data from four such products showed

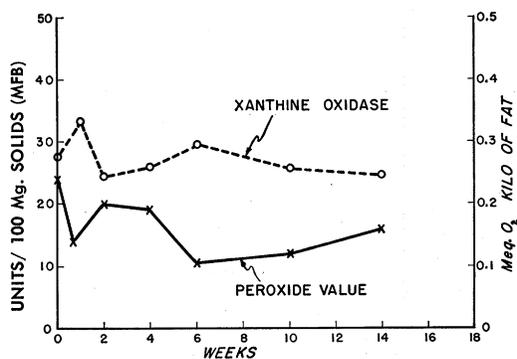


Fig. 4. Xanthine oxidase activity and peroxide value of vacuum foam-dried milk (continuous process).

no significant inverse relation between enzyme and peroxide value. The effective manner in which O_2 has been excluded during processing and packaging, as evidenced by the lack of O_2 detectable in the headspace, is responsible for the low peroxide value.

CONCLUSION

Xanthine oxidase appears to function as an antioxidant, in that it is capable of repressing peroxide formation in the free fat phase of the milk lipid. Results of this work do not demonstrate the mechanism responsible for this function. Possible mechanisms include removal of oxygen via an enzyme-catalyzed reaction with reduced substrate and the enzyme's function in the increase of the reducing potential of milk.

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