ORIGIN OF THE CARBON DIOXIDE PRODUCED IN THE BROWNING REACTION OF EVAPORATED MILK

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SUMMARY

To determine the relative importance of lactose caramelization in the browning of evaporated milk, uniformly labeled lactose was added to milk samples before sterilization. The CO₂ produced in the browning reaction was recovered as BaCO₃ and its specific activity was determined. It was found that about 4% of the total CO₂ produced by sterilization of milk at 242° F. for 15 min. can be traced to caramelization of lactose.

The browning of heated milk largely has been explained on the basis of the Maillard reaction (sugar-amino interaction) (1, 8, 9, 11). The contention of Kass and Palmer (4), that browning of heated milk is caused entirely by lactose caramelization, has lost considerable ground, but there are investigators (15, 17) who admit the possibility that both phenomena are involved.

Since both the Maillard reaction, through the Strecker degradation of the amino acids (2, 8, 12), and the non-amino heat degradation of lactose (2, 15), are known to generate carbon dioxide proportionately to the brown discoloration, it follows that the relative extent of the two reactions is clearly indicated by determining the amounts of gas produced by each.

EXPERIMENTAL PROCEDURE

The evaporated milk used in these experiments was obtained from a commercial condensery. The samples were removed from the conveyor before sterilization, immediately placed in ice-water, and the experiments completed on the same day.

Uniformly labeled C¹⁴ lactose was dissolved in water at 40° C. and 4 ml. of solution containing exactly 800 mg. of the radioactive compound were added to each 14 1/2-oz. can of milk. An equal volume of milk had been previously removed so that the volume of headspace would not be altered. The cans were soldered, agitated to ensure complete mixing of the added material, and sterilized in a Fort Wayne pilot batch sterilizer with the reel continuously operating in the following manner: (a) Temperature raised to 220° F. as fast as possible (6 min.); (b) temperature raised from 220 to 242° F. at the rate of 3° F. per minute; (c) temperature maintained 15 min. or longer at 242 ± 1° F., then cooled 15 min. in tap water in the sterilizer.

Recovery of CO₂. A special apparatus (Figure 1) was built, consisting of a steel clamp, a glass tube, and a hollow needle type stainless steel plunger. A

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14 ½-oz. can was put in the clamp, and the glass tube was placed on the can and tightened by means of screws. A resilient rubber gasket was inserted between the can and the glass tube, so that tightening the screws produced a gas-proof seal. The upper end of the glass tube was closed with a rubber stopper through which the hollow plunger passed, extending well into the tube with the tip touching the lid of the can. The upper end of the plunger was connected to a nitrogen cylinder. On gentle hammering at the top with a wooden mallet, the sharp end of the plunger would perforate the lid of the can and could then be pushed to the bottom. Opening the valve of the nitrogen cylinder would force the milk well into the glass tube, with vigorous bubbling. A small amount of a silicone defoamer (Dow Corning Antifoam A, Dow Corning Corporation, Midland, Michigan) applied to the walls of the glass tube prevented excessive
foaming. The gases thus liberated were led into a gas scrubbing tower. This tower contained 100 ml. of 2 M NaOH, made at the time of each trial from a saturated stock solution and CO₂-free distilled water. Repeated runs showed that 60 min. of sweeping with N₂, keeping the can in 60° C. water bath, was necessary to recover practically all the CO₂ present. The contents of the scrubbing tower were then transferred to an Erlenmeyer flask, and 10 ml. of a precipitating mixture (0.25 M BaCl₂ and 5 M NH₄Cl) added. The material was left for about 15 min. in the 60° C. bath to accelerate the precipitation of BaCO₃. The precipitate was then filtered in a standard-size Tracerlab stainless-steel filter, and the planchets thus obtained were washed with 95% ethanol and dried to constant weight.

Radioactive assay. The specific activity of the planchets was measured with a flow-gas nuclear sealer. Because of the relatively low level of activity employed, each sample was counted for the period necessary to reduce the error to 2%.

Reflectance measurements. The reflectance of the milk was measured with a Beckman DU spectrophotometer equipped with reflectance attachment; powdered magnesium oxide was used as the standard white. Nelson (7) recommended that reflectance readings be made at wavelength 520 mμ, but better results were obtained at 400 mμ (Figure 2) because the greatest reflectance drop occurs at this wave length (Figure 3).

RESULTS AND DISCUSSION

The relationship between heat treatment and total amount of carbon dioxide produced (shown in Figure 4) exhibits a sigmoid curve quite similar to the one obtained by Tarassuk and Jack (16) for the production of the same gas

![Graph](image)
during storage of air-packed whole milk powder of high moisture content. The value for amount of gas produced by heat treatment of 242 ° F. for 15 min. (66 mg. of BaCO₃) is in remarkable agreement with the value obtained by a direct absorptiometric technique (15). The correlation between carbon dioxide production and loss of reflectance is depicted (Figure 5). Summer milk of high heat stability was selected for this study, and it proved to be even more stable than was expected.

Results of the trials with radioactive lactose are given (Table 1).

![Graph showing effect of duration of heating at 242°F on reflectance spectrum of evaporated milk.](image)

**Figure 3.** Effect of duration of heating at 242°F on the reflectance spectrum of evaporated milk.

**TABLE 1**

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Heat treatment (min. at 242°F)</th>
<th>Specific activity (μc/mole of C)</th>
<th>CO₂ derived from lactose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>0.15</td>
<td>4.1</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0.21</td>
<td>5.7</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>0.27</td>
<td>7.2</td>
</tr>
</tbody>
</table>

A 14 ½-oz. can of evaporated milk contains about 40 g. of lactose (3). Therefore, addition of 800 mg. of radioactive lactose represents a 1:51 dilution of the labeled compound. The specific activity of the intact radioactive material
was 191.6 μc/mole of C, which means that, on the basis of dilution, the maximum possible activity of any planchet would be 191.6/51, or 3.7 μc/mole of C, and this reading would be obtained only if all of the CO₂ produced were coming from lactose breakdown.

The data show that caramelization is of minor importance in the browning of evaporated milk. The predominance of inert CO₂ points toward other pathways, the most important of which is undoubtedly the Maillard reaction. The contribution of this reaction could be quantitatively established by performing a set of identical trials, using carboxyl-labeled casein.

The data (Table 1) also suggest that the activation energy of caramelization is higher than that of the remaining pathways of CO₂ production, since the percentage of the gas traceable to lactose increased with increasing heat treatment of the milk.

The trial with radioactive lactose was repeated with half as much labeled compound of the same activity and the readings obtained were nearly one-half of those reported in Table 1.

It is well known that, in the case of dairy products, oxygen uptake parallels the production of carbon dioxide (14, 15, 16), but it has been amply demonstrated that browning proceeds independently of the oxygen present (5, 6, 13, 15). Browning by caramelization of lactose does require oxygen (15). However, it
is evident from the present data that only a small fraction of the oxygen used would be accounted for by caramelization. The loss of oxygen, in addition to that due to caramelization, could perhaps be explained in terms of the oxidation of sulphydryl groups which have been shown to disappear on browning (10), and to oxidative changes of ascorbic acid.

REFERENCES


BROWNING REACTION IN EVAPORATED MILK


