# Reduction of the Microbial Populations in Flours Incorporated into Refrigerated Foods[1]

**LAZARE WISEBLATT,**[2] American Institute of Baking, Chicago, Illinois

**ABSTRACT**

Flours were inoculated with several microorganisms representing sources of food spoilage and of food poisoning, at levels above those reported elsewhere in flours. The organisms used were *Bacillus subtilis, Staphylococcus aureus, Aspergillus flavus,* and *Escherichia coli.* Substantial reductions in the numbers of all these organisms were obtained by the use of heat and propylene oxide vapor, and by their sequential use. Effective application of these agencies does not result in loss of functional properties of the treated flours; heat or propylene oxide followed by heat leaves the organoleptic properties undamaged. A simple analytical method for propylene glycol was modified to estimate propylene oxide extracted and distilled from treated flour.

The proliferation of convenience food items in a ready-to-heat form, preserved by freezing or ordinary refrigeration, has placed severe quality-control restrictions on the ingredients. Prevention of high microbial populations is important to the preservation of organoleptic quality and reduces health hazards. Where flour forms an ingredient of such foods, the permissible numbers of microorganisms must be less than can be safely tolerated when the flour goes into adequately prebaked or precooked items. As indicated by Thatcher, Coutu, and Stevens (1), a high standard of mill sanitation will usually be the best single method of microbial control. Proper storage of the flour under conditions of low moisture will prevent growth of such organisms as are present (2).

When microbial levels in flour are unacceptably high in spite of good mill precautions and subsequent handling, products such as frozen unbaked pie doughs, refrigerated yeast doughs, refrigerated biscuit and cookie doughs, frozen canned soups, and so on, using such flour are subject to microbial growth under some conditions. Both ideally and practically, reduction of the microbial levels of the ingredients is more desirable than any treatment that might be given the prepared products before storage. In the case of flour, the applicable treatments are limited severely by the low moisture content; solid chemicals and antibiotic substances which show good antimicrobial activity in aqueous systems are generally inactive in stored flour. Volatile liquid chemicals and gases, heat, and ionizing radiations all have some good points and some drawbacks.

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[1] Research performed at the American Institute of Baking, Chicago, Illinois, under Research and Service Contract No. 12-14-100-6875 (74) with U.S. Department of Agriculture, Agricultural Research Service and supervised by the Cereals Laboratory of the Western Utilization Research and Development Division, Albany, Calif. Presented at the 51st annual meeting, New York City, April 1966.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

[2] Author's present address: The Quaker Oats Co., John Stuart Research Laboratories, Barrington, Ill. 60010.

Ionizing radiations will destroy even resistant bacteria like *Bacillus subtilis* and other spore-forming bacilli at dosage levels from 600 to 1,600 kilorep (3,4); but such levels cause excessive harm to both functional and organoleptic properties of the treated flours (5,6). Gaseous chemicals like chlorine and sulfur dioxide are not specific enough in their action on microorganisms, and cause extensive physicochemical changes in flour at all but very low levels. The epoxides, ethylene oxide and propylene oxide, show excellent microbicidal action at the low relative humidities common to flour (7,8). Heat is inexpensive compared with chemicals or ionizing radiations; this is an important factor in treating such a bulk product as flour. The possible damage to functional and organoleptic properties has to be weighed against the operating economy. The studies of Geddes (9) indicated a fairly good tolerance in flour towards heat-damage, if the moisture level for a given time and temperature of treatment did not exceed a rather sharp limit.

## MATERIALS AND METHODS

### Inoculated Flours

The organisms selected for inoculation of flour were suggested by G. M. Dack (Food Research Institute, University of Chicago) as representative of the types which are implicated in food spoilage and food poisoning (excluding, in the latter case, the anaerobic organisms which are a problem only in canned or preserved foods).

*Bacillus subtilis* (ATCC 6051) is representative of the thermoduric, mesophilic spore-forming aerobes, as a class the most resistant to destruction by thermal or chemical means. True thermophiles would be of lesser consequence in the cold-stored products here considered.

*Escherichia coli* (ATCC 11775) typifies the enteric bacteria, and is far less dangerous to use in experimental food products than other enteric types such as the *Salmonellae,* while reacting similarly to the environment.

*Staphylococcus aureus* is the species which produces the common enterotoxin associated with food-poisoning cases, usually under aerobic conditions. The strain which was used in these studies does *not* produce an enterotoxin; we are indebted for the culture to M. S. Bergdoll of the Food Research Institute, University of Chicago.

*Aspergillus flavus* (ATCC 9643) is an entirely typical mold found frequently in cereal grains and their products. The interest in development of aflatoxins in stored grains has focused attention on this and other molds (10).

The *E. coli* and *S. aureus* were introduced into flour from broth cultures in Tryptic Soy Broth (Difco 0370) as follows:

About 120 g. of flour was placed in an aluminum Waring Blendor jar; the screw cap was fitted, with the knob removed, leaving a central hole about ½ in. in diameter. The suspension of bacteria was sprayed into the flour, with the blender running at the lower of its two speeds, by means of an artist's airbrush, adjusted to give a wide conical spray. Compressed helium was used as the propellant gas, to avoid problems of filtering a compressed-air supply. The flour so treated was free of lumps and showed no tendency

to cake. It was blended in a jar mill for 10 min. to ensure uniformity, after addition of another 180 g. of untreated flour.

The *A. flavus* spores were introduced directly by growing the mold on Petri dishes of Sabouraud Dextrose Agar (Difco 0109) until they were thickly sporulated, and tapping the inverted dishes over the flour to dislodge the spores. *B. subtilis* was also introduced as spores, scraped from the surface of 3-day-old cultures on Dextrose Tryptone Agar plates (Difco 0080). The scrapings were suspended overnight in acetone (in which the spores survive very largely), filtered, and ground in a mortar with a small amount of flour to pulverize the dehydrated material. This was then blended into the bulk of the flour.

#### Counting Procedures for Organisms

Total counts of the indigenous microflora of the flours employed, or of added organisms, were done according to Method 42-11 (*Cereal Laboratory Methods,* 7th ed., 1962), with Plate Count Agar (Difco 0479) as the plating medium. *B. subtilis* was assayed by AACC Method 42-40; Medium 1 (Dextrose Tryptone Agar) was used and the plates were incubated at 35°C. instead of the specified 55°C. This lower temperature gave more consistent counts. *S. aureus* was plated on Staphylococcus Medium No. 110 (Difco 0297). *E. coli* was counted by Method 42-15, on the Desoxycholate Lactose Agar (Difco 0420) medium, and also on Violet Red Bile Agar (Difco 0012). *A. flavus* was plated on Sabouraud Dextrose Agar, which with our particular culture showed higher and more consistent counts than the media of Method 42-50. Unless otherwise indicated, reported counts are averages of three plates, taken from as many flour samples.

#### Improved Flour Dilution Technique

Because of its dough-forming properties, flour poses difficulties in preparing accurate sample dilutions for plating procedures. Such aids as sand or glass beads added to the dilution bottles so that they help to grind up lumps on vigorous shaking, or the use of a high-speed mixer, or the addition of wetting agents, do not eliminate the errors due to agglomeration. Further, the pipetting of reproducible aliquots of flour suspensions is very difficult when the flour particles are continuously settling out.

A procedure was adopted which in our hands greatly eased both the lumping and settling problems. Wide-mouthed 250-ml. Erlenmeyer flasks, each containing a 1-in. Teflon-covered magnetic stirring bar, are plugged and sterilized by autoclaving. To each flask is added aseptically 99 ml. of sterile water. Weighed flour samples (1 g.) are added to the flasks while the contents are being stirred vigorously, and stirring is continued without interruption until sampling of each flask has been completed. Pipetting of aliquots for serial dilutions or for addition to Petri dishes, done while the suspensions are kept stirred, ensures that repeated aliquots will contain identical amounts of suspended flour.

#### Antimicrobial Treatments

Several solid chemicals proved valueless in changing the microbial counts in flour, and are not discussed here.

*Chlorine Treatment.* Tests were conducted at two moisture levels, 12.5% ("low") and 17.0% ("high"). Flour was brought to the latter moisture content with water sprayed from the airbrush. Both flours were heavily inoculated, and portions were taken for plate counts. The flours were then spread in 6.5-mm. layers in vacuum desiccators. Each desiccator was evacuated to ½ atmosphere, and commercial cylinder chlorine was admitted to bring the desiccators back to atmospheric pressure. The sealed desiccators were held overnight at room temperature, then flushed with filtered air before the treated flours were sampled.

*Propionic Acid.* Flour at 12.8% moisture was inoculated with *B. subtilis* spores alone, to an assayed level of 3,200 per g. It was placed in a vacuum desiccator with 0.4% by weight of propionic acid contained in a shallow dish. The desiccator was evacuated to ½ atmosphere, and the sealed system was allowed to stand for 64 hr. At the end of this time, the level of *B. subtilis* was assayed again.

*Hydrogen Peroxide.* Two kilograms of flour, inoculated with *B. subtilis* to 1,000,000 per g. and at 12.8% moisture, was blended with 100 ml. of 3% hydrogen peroxide solution in a twin-shell blender. After standing for 2 hr., the damp (17.6% moisture) flour was spread thinly and held at 50°C. for 17 hr. to lower the moisture to 12.6%.

*Propylene Oxide.* In general, flour was treated by exposing 6.5-mm. layers to propylene oxide vapor in vacuum desiccators evacuated to ½ atmosphere. Under the partial vacuum the measured amounts of the reagent were completely vaporized in a short time. Liquid propylene oxide (Eastman White Label, No. 2068) was kept in a separate dish and never touched the flour.

*Heat.* Flour was spread in layers of 6.5 mm. (¼ in.) thick on metal foil trays. These were placed on the grid shelves of a reel-type, gas-fired baking oven for the required time at each temperature used. In supplemental tests, layers 6.5 mm. and 1.6 mm. thick were treated side by side, both without and with humidification of the oven atmosphere from pans of water on the grids. Also, temperature readings were made at the center of a flour layer 6.5 mm. thick, with a copper-constantan thermocouple positioned (in a metal guard tube) 3.25 mm. from the surface of a rigid metal pan. The tray remained stationary in the oven (temperature of oven, 130°C.) and readings were made with a millivolt potentiometer.

#### Analysis for Propylene Oxide in Flour

A 10-g. sample of flour is shaken with 50 ml. of absolute reagent-grade methanol for 5 min., then filtered through a medium filter paper (Whatman No. 1 or equivalent). Twenty-five milliliters of filtrate is distilled; the first 10 ml. is collected. One milliliter of the distillate is pipetted into a blood-sugar tube, and the remainder of the procedure is the same as that of Jones and Riddick (11) for propylene glycol. Methanol rather than water is, however, used for the blanks as well as the samples. As suggested by these authors, their procedure proved applicable to the epoxide as well as to the glycol. The standard curve is prepared with known concentrations of propylene oxide in methanol; 50-ml. aliquots are shaken with 10-g. portions of

flour known not to have been previously exposed to the epoxide, and the filtrates are carried through the procedure described.

## RESULTS

### Treatment of Flour

*Chlorine.* As is evident from Table I, the relatively massive dosage of chlorine used here was of little value against *B. subtilis* or *S. aureus;* while it seems to be effective against *A. flavus,* the disappearance of *E. coli* can be duplicated in a few days of storage at the lower moisture levels without any other treatment.

TABLE I
ANTIMICROBIAL EFFECTS OF CHLORINE VAPOR EXPOSURE ON FLOUR

| | Before Exposure | | After Exposure | |
|---|---|---|---|---|
| Assay | Moisture Level, 17.0% | Moisture Level, 12.5% | Moisture Level, 17.0% | Moisture Level, 12.5% |
| *per g. flour* | *thousands* | *thousands* | *thousands* | *thousands* |
| Total count | 32,000 | 35,000 | 2,000 | 120 |
| *S. aureus* | 580 | 220 | 49 | 31 |
| *B. subtilis* | 3,100 | 610 | 1,500 | 35 |
| *A. flavus* | 370 | 350 | 0.7 | 0.2 |
| *E. coli* | 2,900 | 1,000 | 0 | 0 |

The flour color was grossly affected, and the doughing properties were ruined by this treatment.

*Propionic Acid.* Far from reducing the count of *B. subtilis,* the 64 hr. of exposure to propionic acid vapor caused an increase from 3,200 to 29,000 per g.

*Hydrogen Peroxide.* The net effect of the wetting with dilute hydrogen peroxide and mild heat-drying raised the count of *B. subtilis* from $1.0 \times 10^6$ to $2.2 \times 10^6$ per g. flour.

*Propylene Oxide.* (a) *Effect of flour moisture and temperature.* One kilogram of flour, assaying $1.25 \times 10^6$ *B. subtilis* per g., was divided into two equal portions; one was moistened by airbrush spraying to 17.2%; the other remained at 12.4%. Each portion was exposed to 7 ml. (1.4%) of propylene oxide at room temperature for 16 hr. Final counts of *B. subtilis* were: at low moisture (12.4%), 9,000/g.; at high moisture (17.2%), 280,000/g.

Thus high moisture is undesirable; but the effect may be simply due to hydrolysis of the epoxide. The experiment was repeated at two temperatures, 21° and 35°C. Final counts now were:

| | *21°C.* *thousands/g.* | *35°C.* *thousands/g.* |
|---|---|---|
| At low moisture | 3 | 0 |
| At high moisture | 170 | 7.5 |

Temperature has an obvious influence, propylene oxide being more effective under warm conditions. A further test was done on flour inoculated

to 5,500,000 *B. subtilis* per g. and moistened to 17.1%. This was treated for 60 hr. at 21° and at 50°C. Final counts: at 21°C., 9,000/g.; at 50°C., 0/g. Thus, both low moisture and high temperature favor destruction of *B. subtilis* by propylene oxide.

(b) *Minimum sterilizing doses at 21° and 35°C.* Flour samples from a batch assaying 51,000 *B. subtilis* per g. were treated with 0.3, 0.6, 1.2, and 2.4% of propylene oxide, at 21° and 35°C. All were exposed for 18 hr. Final plate counts are shown in the table below.

| *Temperature* | *Propylene Oxide as Percent of Flour* | | | | |
|---|---|---|---|---|---|
| | *0* | *0.3%* | *0.6%* | *1.2%* | *2.4%* |
| °C. | | | | | |
| 21 | 53,000 | 6,300 | 3,300 | 4,100 | 600 |
| 35 | 48,000 | 1,800 | 600 | 200 | 0 |

Thus at 35°C., 0.6% of propylene oxide is as effective as four times this level at 21°C.

(c) *Effect of oven treatment after propylene oxide.* Flour assaying 4,500 *B. subtilis* per g. was treated with 0.8% of propylene oxide at 21°C. for 16 hr. A lingering odor of the reagent led to oven-heating the flour at 100°C. for 2 hr. When plated-out, the flour was sterile. Thus, if the functional properties of the flour were not injured by it, such oven-heating could reduce the minimum sterilizing dose markedly.

A more extensive experiment was done on flour containing substantial inoculated levels of the test organisms: *B. subtilis,* 450,000/g.; *E. coli,* 300,000/g. (died away rapidly); *S. aureus,* 860,000/g.; *A. flavus,* 710,000/g.; total count, 2,400,000/g.

Layers ¼ in. thick were exposed in moisture dishes to atmospheres (by means of vacuum desiccators) containing measured amounts of propylene oxide vapor; the reagent was evaporated from glass beads into the partially evacuated desiccator, with mild warming. The desiccators were held for 4 hr. at 36°C. in incubators before heat-treatment of the dishes of flour. Plate counts on flours after various combinations of propylene oxide plus heating (at 130°C.) were determined. (See Tables II, III, and IV.)

TABLE II

EFFECTS OF PROPYLENE OXIDE AND HEAT ON *B. subtilis* IN FLOUR[a]

| PROPYLENE OXIDE ON FLOUR | ASSAY OF *B. subtilis* | | | | | |
|---|---|---|---|---|---|---|
| | Heating Time at 130°C. (266°F.) | | | | | |
| | 0 Min. | 10 Min. | 20 Min. | 30 Min. | 45 Min. | 60 Min. |
| *p.p.m.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* |
| 25 | 380 | 180 | 24 | 1.6 | 0.2 | 0 |
| 50 | 310 | 51 | 3.9 | 0.6 | 0 | 0 |
| 100 | 250 | 11.0 | 1 | 0.2 | 0 | 0 |
| 200 | 110 | 4.1 | 0.7 | 0.1 | .... | 0 |
| 500 | 66 | 0.9 | 0.2 | 0 | .... | .... |
| 1,000 | 33 | 0.2 | 0 | .... | .... | .... |

[a] Propylene oxide treatment: 4 hr. at 36°C. (97°F.); *B. subtilis* spores 450,000 per g. flour.

TABLE III

EFFECTS OF PROPYLENE OXIDE AND HEAT ON *S. aureus* IN FLOUR[a]

| PROPYLENE OXIDE ON FLOUR | ASSAY OF *S. aureus* | | | | |
|---|---|---|---|---|---|
| | Heating Time at 130°C. (266°F.) | | | | |
| | 0 Min. | 10 Min. | 20 Min. | 30 Min. | 45 Min. |
| *p.p.m.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* |
| 25 | 100 | 19.0 | 2.1 | 0.1 | 0 |
| 50 | 49 | 3.8 | 0.4 | 0 | 0 |
| 100 | 17.0 | 1.7 | 0 | 0 | 0 |
| 200 | 2.7 | 0.6 | 0 | 0 | .... |
| 500 | 0.8 | 0.1 | 0 | 0 | .... |
| 1,000 | 0.3 | 0 | 0 | .... | .... |

[a] Propylene oxide treatment: 4 hr. at 36°C. (97°F.); *S. aureus* inoculum 860,000 per g. flour.

TABLE IV

EFFECTS OF PROPYLENE OXIDE AND HEAT ON *A. flavus* IN FLOUR[a]

| PROPYLENE OXIDE ON FLOUR | ASSAY OF *A. flavus* | | | | |
|---|---|---|---|---|---|
| | Heating time at 130°C. (266°F.) | | | | |
| | 0 Min. | 10 Min. | 20 Min. | 30 Min. | 45 Min. |
| *p.p.m.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* |
| 25 | 31 | 6.6 | 0.4 | 0 | 0 |
| 50 | 7.8 | 1.2 | 0 | 0 | 0 |
| 100 | 2.1 | 0.5 | 0 | 0 | 0 |
| 200 | 1.4 | 0.2 | 0 | 0 | 0 |
| 500 | 0.3 | 0 | 0 | 0 | .... |
| 1,000 | 0 | 0 | 0 | 0 | .... |

[a] Propylene oxide treatment: 4 hr. at 36°C. (97°F.); *A. flavus* inoculum 700,000 per g. flour.

The elimination of heat-treatment would require more than the 1,000 p.p.m. of propylene oxide maximum level used here, except for *A. flavus*; as noted above, heat-treatment would then become mandatory, to drive off the heavy residual odor of the reagent. Heat-treatment for, say, 20 min. after treatment with propylene oxide would be far more expensive than 45 min. of heat alone.

**Heat-Treatment Alone**

Pastry flour inoculated so as to assay 540,000 *B. subtilis* per g. was treated under several combinations of temperature and time, in 6.5-mm. layers. Post-treatment counts are given in Table V.

TABLE V

ACTION OF HEAT *vs. B. subtilis* IN FLOUR[a]

| TEMPERATURE | ASSAY OF *B. subtilis* | | | | | |
|---|---|---|---|---|---|---|
| | Heating Time | | | | | |
| | 5 Min. | 15 Min. | 30 Min. | 60 Min. | 2 Hr. | 3 Hr. |
| *°C.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* | *thousands/g.* |
| 90 | .... | .... | .... | .... | 92 | 3.5 |
| 110 | .... | .... | .... | 2.4 | 1.1 | .... |
| 130 | .... | .... | 1.7 | 0.5[b] | 0.1[b] | .... |
| 150 | .... | .... | 0.7[b] | .... | .... | .... |
| 170 | 42 | 2.8[b] | .... | .... | .... | .... |

[a] *B. subtilis* spores, 540,000 per g.

[b] These treatments caused some discoloration.

Comparison of Tables II and V shows that, below 100 p.p.m., propylene oxide offers no advantages over heat alone. It is noted that an intermediate temperature range gave most pronounced destruction of *B. subtilis* spores without excessive browning of the flour; a combination of 130°C. and 45 min. was chosen as the best compromise. The results of two studies with flours carrying the test organisms are given in Table VI.

TABLE VI

ACTION OF HEAT *vs.* MICROORGANISMS IN FLOUR

Treatment: layer of flour 6.5 mm. deep, heated 45 min. in oven at 130°C. (266°F.)

| ORGANISM | INOCULUM | COUNT AFTER TREATMENT | ORGANISM | INOCULUM | COUNT AFTER TREATMENT |
|---|---|---|---|---|---|
| | *thousands/g.* | *thousands/g.* | | *thousands/g.* | *thousands/g.* |
| *B. subtilis* | 2,400 | 2.4 | *A. flavus* | 710 | 0 |
| | 14.3 | 0 | | 450 | 0 |
| *S. aureus* | 1,390 | 0.25 | *E. coli* | 300 | 0 |
| | 860 | 0 | | | |

The prescribed conditions are capable of sterilizing flour containing the test organisms at very high levels, and even spores of *B. subtilis* at levels beyond reasonable expectations.

**Heat Penetration into Flour**

To determine whether thinner layers of flour could be treated effectively in a shorter time, a layer 1.6 mm. thick was heated 25 min. at 130°C., with the 6.5-mm. layer used as a control. This was repeated with water vapor in the oven. Data for the treated layers are given below; in parentheses, figures obtained when oven was operated with water present.

| | *Control, Unheated* | *Layers 6.5 mm. Thick* | *Layers 1.6 mm. Thick* |
|---|---|---|---|
| *B. subtilis* | 320,000 | 12,000(33,000) | 4,300(5,000) |
| *A. flavus* | 1,400,000 | 1,300 | 500 |
| *S. aureus* | 400,000 | 600 | 0 |

No benefit can be expected from the use of oven humidification, unless perhaps the ovens were operated under pressure.

The reduction in microbial count in the thin layers was only slightly better

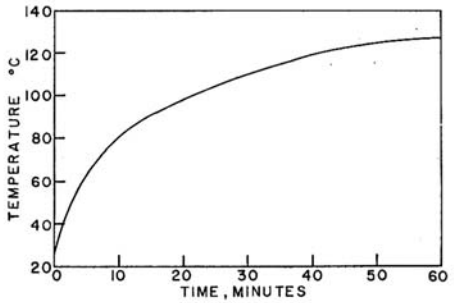


Fig. 1. Temperature at center of layer of flour 6.5 mm. deep in oven at 130°C.

than that in the 6.5-mm. layers. Therefore the slight gain in effectiveness is more than offset by the reduced capacity.

The internal temperatures of the flour layers during the treatment cycle are obviously lower than the indicated oven temperature of 130°C. These temperatures are shown in Fig. 1. This shows the internal temperature to be about 122°C. at the end of 45 min.

#### Functional Tests of Oven-Sterilized Flour

It has been shown that, functionally, this treatment does not permanently damage the flours for their intended purposes. Pastry flour with and without the heat-treatment was made into pie doughs, and the pie crusts made from these doughs were indistinguishable in appearance, color, tenderness, and flakiness. When the flour is destined as a thickening agent, this heat-treatment has an improving effect. It inactivates the amylases and improves the thickening power measurably. Pastry flour which gave an amylograph peak consistency of 650 B.U. then became constant at 530 B.U. (55 g. flour in 450 ml. distilled water) after-heat treatment gave a peak consistency of 800 B.U. and became constant at 610 B.U.

Flour to be used for yeast-leavened goods would be expected to suffer most from the heating. When the destroyed diastatic activity of heated baker's flour was restored by addition of diastatic malt (determined on the amylograph), excellent bread was made by both straight and sponge dough methods, receiving point scores (for volume, grain, texture, and color) equal to those of controls from unheated flour. The absorption was increased about 8%, owing to loss of somewhat above this percentage of moisture in the heating. It is considered that the production of satisfactory bread is as rigorous a test as can be applied to flour destined for yeast-raised goods of any sort.

In the production of baking-powder biscuits, the effect of heat-sterilization on the "all-purpose" flour was very similar to that on the bread flour. Biscuits showed reduced volume when made with the heat-treated flour; but addition of diastatic malt at the optimum level restored performance to equal that of the unheated control flour. Texture was likewise normal, and no off-flavors due to the heating were noticeable.

Organoleptically, heat-treated flour was found to impart no noticeable off-flavors to biscuits, even when held for 22 days between heat-treatment and baking of the biscuits. No rancid odor was detectable in the stored, heat-treated flours stirred with boiling water; the pH values of slurries from stored heated and unheated flours differed by not over 0.1 pH unit. Thus it seems that the abnormally dry heat-treated flour is not especially susceptible to oxidative rancidity.

#### Propylene Oxide Residues in Treated Flour

When the methanolic solutions of propylene oxide (typical of the flour extracts) were distilled, and the distillates were collected in fractions and analyzed as described, 96% of the original propylene oxide could be accounted for in the first 40% of distillate (i.e., 10 ml. distilled from a 25-ml. sample). When the methanolic distillate of propylene oxide was compared with a methanol solution of propylene glycol, computed to contain the same

molar concentration of color-reacting material, the results were:
Propylene oxide distillate, 0.029 mg./ml. in MeOH, absorbance = 0.450
Propylene glycol solution, 0.038 mg./ml. in MeOH, absorbance = 0.444

Besides the molar equivalence of the epoxide and glycol in this method, the distillates were shown to remain stable (giving constant absorbance at 595 mμ) for at least 16 hr.

The distillation step is necessary in this procedure; direct acid treatment of a flour filtrate causes severe browning of other soluble constituents, obscuring completely the ninhydrin color development. Aqueous extraction of the flours is totally unsatisfactory, as there is excessive breakdown of propylene oxide during the subsequent distillation. This is in addition to the inconvenience of clarifying aqueous flour extracts. Distillates from methanol extracts of flour not exposed to propylene oxide were put through the analytical procedure, and gave absorbances never exceeding 0.005. Thus volatile substances naturally present in flour do not interfere in this method.

With the procedure described, it was found that flours treated with propylene oxide show gradually decreasing amounts of recoverable residual epoxide. Two such flours, exposed to 1,000 p.p.m. of the reagent, showed these recoveries:

| *Days after Exposure* | *Residual Propylene Oxide* | |
|---|---|---|
| | *Pastry Flour* | *Patent Flour* |
| | *p.p.m.* | *p.p.m.* |
| 2 | 208 | 228 |
| 16 | 111 | 114 |
| 30 | 57 | 60 |

Whether these decreases are due to volatilization or to slow reaction with the flour is in question; the latter is thought to be more likely, as the flours were stored in air-tight containers.

## DISCUSSION

Computation of complete cost data for flour treatment by heat, propylene oxide, or a combination of these is not possible unless the equipment to be used is definitely specified and performance figures are available. What has been done here is to estimate the cost for heat-input or the chemical agent required per unit of flour treated adequately.

Propylene oxide is quoted currently at about 65 cents per lb. in 30-lb. drums; used at 2,000 p.p.m. on flour, each 100 lb. of flour will require 2/10 lb., at a cost of about 13 cents for the reagent only. A total cost of application near ten times this figure is not unrealistic, in view of the elaborate equipment required for effective (and safe) application of propylene oxide.

To calculate heat-input requirements for heat-treatment of flour, we can assume perfect heat-transfer from the medium to the product, calculate the heat-uptake per 100 lb., and then modify this to accord with realistically acceptable thermal efficiencies. We shall here assume initial and final flour temperatures of 66° and 266°F.; specific heats for dry flour and water of

0.4 and 1.0; a latent heat of evaporation for water of 970 B.t.u./lb.; and an over-all moisture loss from 13 to 3% (slightly greater than the average noted in our experiments). Per 100 lb. of flour, the heat absorbed will be:

| | | *B.t.u.* |
|---|---|---|
| By 87 lb. dry flour | $(266\text{-}66) \times 87 \times 0.4 =$ | 6,960 |
| By 13 lb. water | $(266\text{-}66) \times 13 \times 1 =$ | 2,600 |
| By 10 lb. evaporated water | $10 \times 970 =$ | 9,700 |
| | Total | 19,260 |

If we now assume arbitrarily an over-all thermal efficiency of about 40%, the actual heat requirement will be about $(100/40 \times 19{,}260) = 50{,}000$ B.t.u. or ½ therm per 100 lb. of flour. At the current industrial rate for gas fuel of about 9 cents per therm in the Chicago metropolitan area, the fuel cost should be around 4½ cents per 100 lb. of treated flour. This is about one-third the cost of propylene oxide; the capital cost of the equipment for heating can be expected also to run substantially lower. High thermal efficiency with moderate moisture loss could be realized by the use of a screw-type feeder as the heating device.

Less sophisticated and costly heating equipment could be used, at only a modest penalty in thermal efficiency. A simple tunnel oven with radiant heating, or a through-circulation, starch-type dryer operating at low air velocity, should serve adequately. Other possibilities might be along the lines of the vertical turbodryer or the Herreshoff furnace, for maximum utilization of floor space and for intimate mixing of the flour during treatment.

So long as flours destined for use in cold-preserved, uncooked products must meet low microbial count limits, the use of antimicrobials whose efficacy is realized only in solutions, batters, or doughs is ruled out. A successful agency must operate on flour of normal moisture range, where chemical contact is limited to substances of considerable vapor pressure. Addition of moisture to the flour as a contacting aid is not only awkward and expensive but largely self-defeating; the growth of most organisms is actually facilitated thereby.

Propylene oxide, heat, and combinations of these agencies have been shown to have high effectiveness against organisms in flour, including spores of *B. subtilis,* one of the most stubborn known spoilage organisms, at levels beyond anything excusable in present-day milling and handling practices. A simple procedure was devised to estimate residual propylene oxide in flour after treatment with this substance. The recommended heating cycle was shown not to cause any damage to functional properties of flour, which could not be simply adjusted by the correct use of diastatic malt and added water.

**Acknowledgments**

Valuable consultations with Richard J. Makowski, American Institute of Baking, Department of Sanitation, on microbiological methods are gratefully acknowledged. Thanks are due to Mrs. L. Wiseblatt for performing much of the analytical work in this research.

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[Received March 7, 1966. Accepted February 24, 1967]