

**INFO #: 50691520**



**CustID: 3638**  
**Fonterra Co-operative Group Limited**  
**Interloans Librarian**  
**Library**  
**Private Bag 11029**  
**Palmerston North , New Zealand .**

Customer No: **3638 / 87054**  
Date of Order: **26/07/2010**  
Date of Shipping: **27/07/2010**  
Orderer: **Interloans Librarian**  
Department:  
Bill Ref: **Sinclair, Sue**  
Order No:  
Shipping method: **eMail**  
**interloans.pda@fonterra.com**

---

## Standard

---

Journal: JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY  
Citations: 30(\* SUPPL: \*):396-49 1982  
Author: Toyoda, M et al  
Title: Rapid procedure for the determination .....H2O2 in food.....  
ISSN: 00218561

---

**This work was copied under licence from the Copyright Agency Limited (CAL).  
A licence is required from CAL for the making of further copies by any means.**

---

**Infotrieve Australia Pty**  
344 Ferntree Gully Road  
Notting Hill VIC 3168  
AUSTRALIA

Ph: +61-3-9544 4911  
Fax: +61-3-9544 3277  
Email: [status@infotrieve.com.au](mailto:status@infotrieve.com.au)  
[www.infotrieve.com](http://www.infotrieve.com)

## Rapid Procedure for the Determination of Minute Quantities of Residual Hydrogen Peroxide in Food by Using a Sensitive Oxygen Electrode

Masatake Toyoda,\* Yoshio Ito, Masahiro Iwaida, and Masami Fujii

A new simple extraction method and a sensitive analytical apparatus (Oritector Model III) equipped with a closed cell and a sensitive oxygen electrode for determining minute quantities of residual hydrogen peroxide in food were developed. Solid food was homogenized and extracted simultaneously with 0.5%  $\text{KBrO}_3$ -0.2 M phosphate buffer (pH 7.0) previously bubbled with  $\text{N}_2$  gas to decrease the oxygen content. The filtered extract (2 mL) was put into a closed cell, and then oxygen was purged at 30 °C with  $\text{N}_2$  gas. Next,  $\text{N}_2$  gas was flowed through the headspace of the cell, until a base-line response from the oxygen electrode was obtained. After the addition of catalase, the released oxygen was measured by a recorder peak. If liquid food was used, it can be put directly into the cell. More than 80% of hydrogen peroxide spiked at the 1-ppm level was recovered from noodle, fish paste, dried fish, milk, and milk products. This method requires only 20 min for the whole procedure, and the minimum detectable level was 0.1 ppm for solid food and 0.01 ppm for liquid food.

Hydrogen peroxide has been used in many countries as a food additive for the purposes of controlling the growth of microorganisms and bleaching, but the use is limited in each country to specific foods. According to the "Code of Federal Regulations" (Office of Federal Register, 1980) hydrogen peroxide is generally recognized as safe for use as a bleaching agent in accordance with good manufacturing practice. In Japan it also had been allowed to be used as a food additive for sterilizing and bleaching purposes until Feb 1980. However, it was revealed that hydrogen peroxide caused cancer in the duodenum of mouse after it is administered in the drinking water at 0.1% and 0.4%. Accordingly, the government of Japan made a partial amendment of the Standards and Specifications (Ministry of Health and Welfare of Japan, 1959), and the amended notification says that hydrogen peroxide has to be either decomposed or removed from final products (Ministry of Health and Welfare of Japan, 1980).

This change in situation greatly enhances the need for a sensitive and accurate determination method of hydrogen peroxide in foods. Some analytical methods which had been used for the detection and determination of hydrogen peroxide residues were compared by Iwaida et al. (1981). They reported that conventional methods were not sensitive enough to detect below 1 ppm of hydrogen peroxide. More recently, Ito et al. (1981) developed and improved a 4-aminoantipyrine (4-AA) colorimetric method of determining hydrogen peroxide in foods, whereby they achieved a sensitivity of 0.05 ppm. However, this method is complicated and involves extraction of hydrogen peroxide, filtration, reaction of hydrogen peroxide with 4-AA and phenol by use of peroxidase, purification of reacted products through column chromatography, and colorimetry. Therefore, a more simple and rapid procedure with the same sensitivity was required to be applicable to many kinds of foods.

Already an oxygen electrode has been used for the determination of as little as 5 ppm of hydrogen peroxide in boiled noodles (Ohashi et al., 1976), and in this system,

the oxygen electrode detects oxygen released from hydrogen peroxide by addition of catalase without a purge of oxygen from the test solution. We developed a rapid, simple, sensitive, and reliable method for determining hydrogen peroxide in foods by using a new hydrogen peroxide detection apparatus consisting of a special reaction cell made airtight and a sensitive oxygen electrode that may detect 0.01  $\mu\text{g/mL}$  hydrogen peroxide under a condition that most of the dissolved oxygen in the test solution has been purged with nitrogen gas. Furthermore, an excellent extraction procedure was developed for the recovery of hydrogen peroxide from foods. By combining this procedure and that apparatus, hydrogen peroxide in food can be determined within 20 min.

### EXPERIMENTAL SECTION

**Materials.** All food samples described here except herring roe were purchased from a market in Osaka from Jan to Mar 1981, and herring roe samples were sent from Hokkaido Central Fisheries Experimental Station.

**Reagents.** Special-grade hydrogen peroxide (30%) was supplied from Mitsubishi Gas Kagaku Co., Ltd., Tokyo, Japan. Antifoaming agent silicone TSA-737 was obtained from Toshiba Co., Ltd. Special-grade potassium bromate was obtained from Katayama Chemical Co., Ltd. Guaranteed-grade potassium dihydrogen phosphate and disodium hydrogen phosphate dodecahydrate were purchased from Ishizu Pharmaceutical Co., Ltd. Catalase solution (Oriental Yeast Co., Ltd.; 5000 units of catalase/mL) was used as an enzyme source. This enzyme solution is stable at least for 3 months if kept refrigerated, and it needs to be purged with  $\text{N}_2$  gas before use.

The 0.2 M phosphate buffer (pH 7.0) was prepared by dissolving 27.3 g of  $\text{KH}_2\text{PO}_4$  in 1000 mL of water and adjusting the pH to 7.0 with the solution made by dissolving 71.6 g of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  in 1000 mL of water. Extractant was prepared by dissolving 5 g of  $\text{KBrO}_3$  in 1000 mL of 0.2 M phosphate buffer and then passing  $\text{N}_2$  gas through the solution for more than 1 h during cooling by ice-water. Before use, about 90% of oxygen that dissolved in the solution should be purged from it. The stock standard solution of hydrogen peroxide was prepared by diluting 2.0 mL of hydrogen peroxide in cold water to make 100 mL and then diluting it in cold water to obtain 2.5 mg/mL hydrogen peroxide solution. The working standard solution was prepared by transferring 2.0 mL of stock standard solution into a 500-mL volumetric flask, diluting

\* National Institute of Hygienic Sciences, Osaka Branch, 1-1-43, Hoenzaka, Higashi-ku, Osaka, 540, Japan (M.T., Y.I., and M.I.), and Food Chemistry Division of the Environmental Health Bureau, Ministry of Health and Welfare, 1-2-2, Kasumigaseki, Chiyoda-ku, Tokyo, 100, Japan (M.F.).

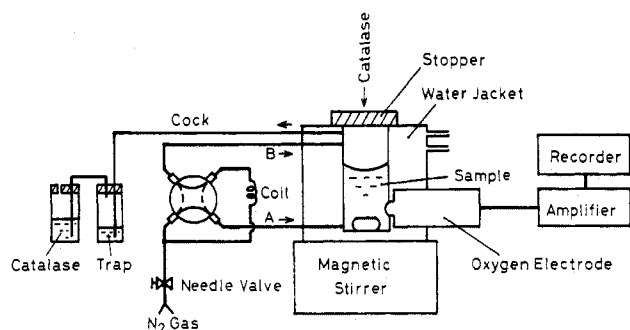


Figure 1. Scheme showing the Oritector Model III used in the determination of hydrogen peroxide.

to volume with the extractant, and then diluting 10 mL of this solution in the extractant to make 100 mL. This was prepared fresh before use.

**Apparatus.** An Oritector Model III (Oriental Electric Co., Ltd.) was developed and used for the determination of hydrogen peroxide. This model has a sample cell that could be sealed with a silicone stopper and  $N_2$  gas could be passed through the inside. The chart recorder was operated at 0.5 cm/min. The oxygen electrode was a type of combined oxygen electrode and used thin plastic film between the test solution and electrolyte.

**Extraction.** The sample was cut into fine pieces, and a 5-g sample was weighed into a 100-mL blending cup. Forty milliliters of extractant was added and then blended for 3 min at high speed while cooling in an ice-water bath. After addition of 1 drop of antifoaming agent, the content was poured into a 50-mL volumetric flask. An additional 5 mL of extractant was used to wash the container cup and blender blade; this was added to the flask too, made to 50 mL with extractant, and mixed well. Then the solution was filtered by using a coarse filter paper. A few milliliters of the first filtrate were discarded and another filtrate was used as fast as possible as the test solution.

**Determination of Hydrogen Peroxide.** Two milliliters of the filtrate was put into sample cell of the Oritector (Figure 1). A small amount of antifoaming agent was added and the Oritector was sealed with a silicone stopper. Nitrogen gas was passed from Figure 1A through test solution during mixing by a stirring bar. The bubbling of  $N_2$  gas and stirring was continued until most of the dissolved oxygen was removed. Then nitrogen gas infusion from A was stopped by turning a changing-over cock, and nitrogen gas was passed from Figure 1B through the headspace of the cell. After an equilibrated condition in relation to the response of oxygen electrode as indicated on the recorder chart was obtained, the base line of recorder was adjusted, and then 10  $\mu$ L of catalase solution which had been previously treated with  $N_2$  as shown in Figure 1 was injected into the cell through the stopper by using a microsyringe. The typical response pattern was shown in Figure 2. Hydrogen peroxide content was found by the response of the oxygen electrode, that is, the peak height on the recorder chart, and determined with the aid of the standard curve. If the sample is a liquid food, 2 mL of the sample could be put directly into the cell, and hydrogen peroxide content was determined in the same way as above.

## RESULTS AND DISCUSSION

So that the optimum analytical condition of hydrogen peroxide measurement could be found, analyses were carried out under varying buffer pHs, catalase concentrations, and temperatures. The effect of pH on the catalase reaction is shown in Figure 3. Within the pH range

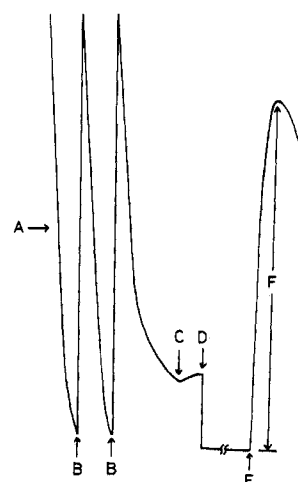


Figure 2. Typical response pattern obtained after injection of catalase solution into 0.1  $\mu$ g/mL hydrogen peroxide solution. (A)  $N_2$  gas bubbling through the working standard solution; (B) range change of amplifier; (C)  $N_2$  gas flow through the headspace; (D) base-line adjustment; (E) catalase injection; (F) peak height.

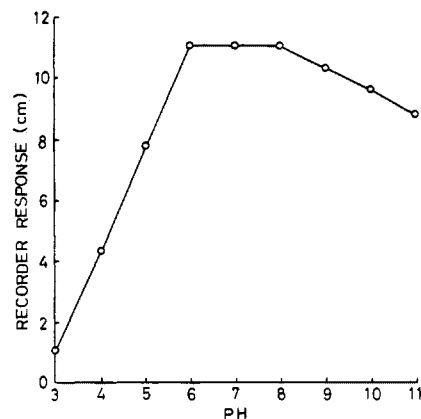


Figure 3. Effect of pH on the determination of hydrogen peroxide by catalase. 10  $\mu$ L of catalase solution was injected into 2 mL of each buffer solution containing 1  $\mu$ g/mL hydrogen peroxide. pH 3–5: 0.1 M acetic acid–sodium acetate buffer. pH 6–8: 0.1 M phosphate buffer. pH 9–11: 0.1 M borate–sodium hydroxide buffer.

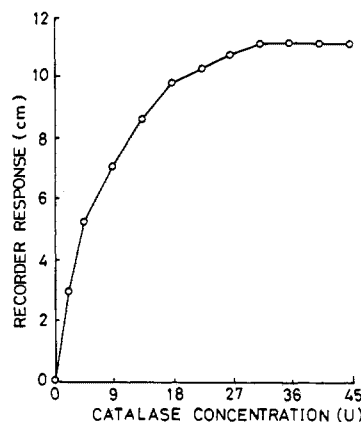


Figure 4. Effect of catalase concentration on the determination of hydrogen peroxide. 0–45 units of catalase was injected into 2 mL of 1  $\mu$ g/mL hydrogen peroxide solution.

of 6.0–8.0, relatively constant and maximal responses were obtained and indicated the optimum pH of catalase used. Accordingly, pH of the buffer solution was adjusted to 7.0 in the following experiments.

The optimum catalase amount was found by running concentrations of catalase from 4.5 to 45 units. Figure 4

Table I. Comparison of Several Extractants on the Recovery of Added Hydrogen Peroxide from Boiled and Half-Dried Larval Fish

extractants	hydrogen peroxide added		recovery, %
	0 ppm	1 ppm	
(1) water	0.32	0.36	4.0
(2) 0.5% KBrO <sub>3</sub> solution	0.31	0.97	65.9
(3) 0.5% KBrO <sub>3</sub> -phosphate buffer solution	0.36	1.07	72.9
(4) extractant 2 bubbled by nitrogen gas	0.42	1.22	80.4
(5) extractant 3 bubbled by nitrogen gas	0.36	1.26	90.3

Table II. Recoveries<sup>a</sup> of Added Hydrogen Peroxide from Various Foods

food	recovery of added 1 ppm of hydrogen peroxide, %
noodle (Namamen <sup>b</sup> )	98.3
buckwheat noodle (Namasoba <sup>b</sup> )	96.6
fish paste (Hanpen <sup>b</sup> )	76.6
rolled fish cake (Chikuwa <sup>b</sup> )	90.0
fish paste (Kamaboko <sup>b</sup> )	88.1
boiled, dried larval fish (Shirasu boshi <sup>b</sup> )	95.7
herring roe (Kazunoko <sup>b</sup> )	94.1
processed cheese	86.7
yogurt	77.2
liquid milk (packed in paper container)	98.9
long-life milk (packed in paper container)	99.0
liquid milk (bottled)	95.3
reconstituted milk (bottled)	96.7

<sup>a</sup> Average of three trials. <sup>b</sup> Japanese name.

shows that when 1  $\mu\text{g}/\text{mL}$  hydrogen peroxide solution was used, it needed approximately 30 units of catalase. Therefore, 50 units of catalase was used, considering the inhibition of the catalase reaction by food ingredients.

When the analytical temperature was considered, it was found that the response of both the electrode and catalase increased with elevation of the temperature. However, some problems occurred under the analytical condition of more than 35 °C; for example, the fixation of the membrane on the oxygen electrode became loose and this induced noise on the recorder. As a routine analysis, it is more convenient to analyze around room temperature.

But, since the enzyme reaction largely depended on a small change in temperature, it was decided to analyze at 30 °C, a little higher temperature than room temperature, by circulating controlled water into the jacket.

Under the above-mentioned detection conditions, linear standard curves of hydrogen peroxide ranging from 0.1 to 1 and 0.01 to 0.1  $\mu\text{g}/\text{mL}$  were obtained. Although the latter case was less reproducible, it gives no problem for routine analysis since the variable coefficient was within 5%.

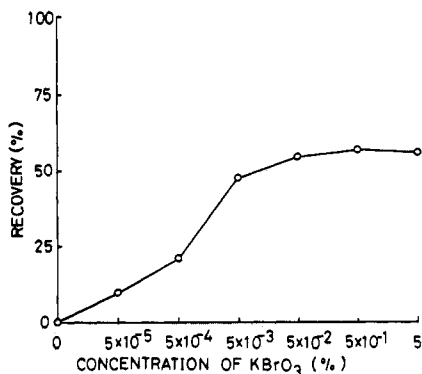
In our previous study (Ito et al., 1981), it was found that the use of KBrO<sub>3</sub> in the extraction of hydrogen peroxide with methanol was very effective for protecting the degradation of hydrogen peroxide in food. In this experiment methanol could not be used because a small amount of methanol inhibited the catalase reaction. Accordingly, at first the KBrO<sub>3</sub> aqueous solution was chosen as an extractant of hydrogen peroxide from food, and the effect of the concentration of KBrO<sub>3</sub> on the extraction of hydrogen peroxide from boiled and half-dried larval fish was studied. If boiled and dried larval fish was used, the recovery of fortified hydrogen peroxide was more than 90%. However, recoveries for the half-dried sample were as low as 67% (Figure 5). But it was still obviously effective to use KBrO<sub>3</sub> solution as an extractant and the 0.5% concentration was chosen. Furthermore, good extraction conditions were sought. Table I illustrates the superiority of the 0.5% KBrO<sub>3</sub>-phosphate buffer through which nitrogen gas had been bubbled for more than 1 h before use, as compared with the 0.5% KBrO<sub>3</sub>-phosphate buffer without treatment, and 0.5% KBrO<sub>3</sub> with treatment. It means that, under the ordinary oxygen concentration in aqueous solution, fortified or originally contained hydrogen peroxide was decomposed during homogenization. Actually, after nitrogen gas bubbling, soluble oxygen in the extractant decreased to about 10%.

Average recoveries of hydrogen peroxide from various kinds of foods at a fortification level of 1 ppm are shown in Table II. In all cases, spikes at each sample were analyzed in triplicate. Satisfactory results were obtained in all foods including fish paste, dried fish, milk, and milk products. Nowadays, in Japan, hydrogen peroxide is used only for bleaching and sterilizing purposes of herring roe, and hydrogen peroxide used is decomposed by soaking the treated roe into catalase solution. Accordingly, the detection of residual hydrogen peroxide in these herring roe is very important, and it is clear that this newly developed method is very useful for this purpose as shown in Table II.

Table III. Comparison of Hydrogen Peroxide Measurements<sup>a</sup> in Various Foods by the Electrolytic Method and Improved 4-AA Method

food	hydrogen peroxide, ppm	
	electrolytic method	improved 4-AA method
noodle (Namamen <sup>b</sup> )	0.1	0.1
buckwheat noodle (Namasoba <sup>b</sup> )	0.2	0.2
fish paste (Hanpen <sup>b</sup> )	0.1	0.2
rolled fish cake (Chikuwa <sup>b</sup> )	0.2	0.2
fish paste (Kamaboko <sup>b</sup> )	0.3	0.3
boiled, dried larval fish (Shirasuboshi <sup>b</sup> )	0.2	0.2
herring roe (Kazunoko <sup>b</sup> )	0.4	0.6
processed cheese	0.1	1.4
yogurt	1.1	1.2
liquid milk (packed in paper container)	0.04	0.1
long-life milk (packed in paper container)	0.02	0.2
liquid milk (bottled)	0.03	0.4
reconstituted milk (bottled)	0.04	0.1

<sup>a</sup> Average of three trials. <sup>b</sup> Japanese name.



**Figure 5.** Effect of  $\text{KBrO}_3$  concentration on the recovery of added hydrogen peroxide from larval fish. Hydrogen peroxide was added to boiled and half-dried larval fish at 1 ppm and was extracted with various concentrations of  $\text{KBrO}_3$  solution.

Table III gives comparative data of hydrogen peroxide residues in foods and drinks determined by this method and the improved 4-AA method. In most samples except milk and milk products, the residue levels determined by both methods were in good agreement, but in milk and milk products the values of the improved 4-AA method were higher than those obtained by this method. We are

now conducting experiments to determine the causes of their discrepancy.

This method requires only 20 min for one analysis of solid food and about 10 min for liquid food. The minimum detectable level is 0.1 ppm for the former and 0.01 ppm for the latter. Accordingly, as a routine analysis this is a time-saving method and sufficiently sensitive.

#### LITERATURE CITED

- Ito, Y.; Tonogai, Y.; Suzuki, H.; Ogawa, S.; Yokoyama, T.; Hashizume, T.; Santo, H.; Tanaka, K.; Nishigaki, K.; Iwaida, M. *J. Assoc. Off. Anal. Chem.* 1981, 64, 1448.  
 Iwaida, M.; Ito, Y.; Tonogai, Y.; Suzuki, H.; Ogawa, S.; Hasegawa, Y.; Tanaka, K.; Kondo, M.; Fujii, M. *Nippon Nogei Kagaku Kaishi* 1981, 55, 483.  
 Ministry of Health and Welfare of Japan, Dec 28, 1959, Notification No. 370.  
 Ministry of Health and Welfare of Japan, Feb 20, 1980, Ordinance No. 24.  
 Office of the Federal Register, General Services Administration "Code of Federal Regulations"; U.S. Government Printing Office: Washington, DC, 1980; Title 21, Part 182, p 1366.  
 Ohashi, M.; Echigo, A.; Watabe, T. *New Food Ind.* 1976, 18, 17.

Received for review April 24, 1981. Revised manuscript received September 15, 1981. Accepted October 27, 1981.

## Heterogeneity and Properties of Heat-Stable Ovalbumin from Stored Egg

Jun-ichi Kurisaki,\* Yasuyo Murata,<sup>1</sup> Shuichi Kaminogawa, and Kunio Yamauchi

The changes in the properties of ovalbumin during storage were examined, especially with regard to the heat stability. Two fractions of heat-stable ovalbumin were separated from the stored egg. One was a minor new protein which was slightly acidic compared with the fresh ovalbumin. Another major ovalbumin could not be distinguished in properties, except for the stability to heat denaturation, from native ovalbumin, since the major ovalbumin from the stored egg showed the same behaviors in electrophoresis and chromatography as did the fresh ovalbumin. These results show that the transformation of ovalbumin to the heat-stable form cannot be explained by the change in the net surface charge.

The occurrence of the more stable form of ovalbumin (S-ovalbumin), first reported by Smith (1964), is one of the most interesting changes in the white, as well as the thinning, during the storage of eggs. No distinct difference in physical and chemical properties, however, was found by Smith and Back (1965, 1968b) between native ovalbumin and S-ovalbumin, except for the difference in the stability to heat or denaturing agents (Smith and Back, 1968a).

Recently, during the transformation of ovalbumin to S-ovalbumin, slight conformational changes were detected by a Raman difference spectroscopy (Kint and Tomimatsu, 1979) and changes in the surface charge were reported (Nakamura et al., 1980). However, the mechanism of the conversion is still a matter of speculation.

In the present study, we examined the correlation between the heat stability and other chemical or physical properties of ovalbumin from the fresh or stored eggs and found that two types of heat-stable ovalbumin, more acidic

ovalbumin and ovalbumin with the same surface charge as the fresh ovalbumin, were formed during storage.

#### MATERIALS AND METHODS

**Source and Storage of Eggs.** Eggs from White Leghorn (Babcock) were supplied from the National Institute of Animal Industry (Chiba, Japan). Only the eggs with type AA ovalbumin of genetic variants (Lush, 1964) were used to avoid confusion in the interpretation of the results. Shell eggs were stored on a plastic tray at  $30 \pm 2^\circ\text{C}$  for 1, 2, 3, and 4 weeks. This temperature was chosen to accelerate the formation of heat-stable ovalbumin. The pHs of the white from fresh eggs and stored eggs were 8.52-8.80 and 9.35-9.62, respectively.

**Fractionation of Ovalbumin.** Eggs were broken carefully and egg white was separated. Egg white was homogenized with a Polytron (Kinematica, Switzerland) at scale 5 for 15 s at  $5^\circ\text{C}$ , dialyzed against deionized water, and lyophilized. The dried sample (1500 mg) was dissolved in 0.05 M imidazole hydrochloride buffer (pH 6.20) containing sodium azide (200 mg/L) and centrifuged at 15000 rpm for 60 min at  $15^\circ\text{C}$ . The supernatant was equilibrated with the same buffer for at least 24 h and fractionated by ion-exchange chromatography on DEAE-Sepacel (Pharmacia Fine Chemicals). The column was 21

Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan.

<sup>1</sup>Present address: Department of Nutrition, Kagawa Nutrition College, Sakado-shi, Saitama-ken 350-02, Japan.