



## Studies on the Determination of Hydrogen Peroxide and Its Dissipation in Foods

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### ABSTRACT

An enzymatic colorimetric method for the determination of hydrogen peroxide in udon (Japanese noodles) and shark fins was developed. Hydrogen peroxide was extracted from samples with water. After filtration, the extract was treated with *o*-phenylenediamine and peroxidase in citrate buffer (pH 5.0). Color development was determined by spectrophotometry at 490 nm. Recovery studies were performed at spiked levels 0.92, 3.68 and 9.20 ppm. Average recoveries ranged 90.7~99.9 and 65.3~91.7% from udon and shark fins respectively. Coefficients of variation for average recoveries were less than 5%. The detection limit was 0.05 ppm in these commodities.

The hydrogen peroxide residues in udon do not decompose or diminish effectively during storage and boiling process. But hydrogen peroxide residues in shark fins were removed by dipping and subsequent boiling with water. Twenty samples of dried shark fins and thirteen samples of udon purchased from various markets were analyzed using the proposed method. The results showed that twelve samples of dried shark fins from grocery stores contained hydrogen peroxide residues ranged from 0.9 to 5451 ppm. Six udon samples purchased from traditional markets contained hydrogen peroxide residues ranged from 5.2 to 516 ppm. No hydrogen peroxide residue was detected in the other seven udon samples purchased from supermarkets.

**Key words:** hydrogen peroxide, enzymatic colorimetric method, *o*-phenylenediamine, shark fins, udon.

### INTRODUCTION

Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), a transparent liquid, is an effective bactericide as well as a strong bleaching agent. Its chemical reaction involves

both oxidation and reduction. It is readily decomposed by light, heat, metals, alkaline solution, and some enzymes such as catalase and peroxidase<sup>(1,2)</sup>. The adversely effect of hydrogen peroxide on animal has been reported. Rat (C57BL) of low cata-

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lase activity fed with drinking water containing 0.1 or 0.4% hydrogen peroxide could induce duodenal cancer<sup>(1)</sup>. A poisoning outbreak occurred in Tokyo (Japan) in 1971 due to the consumption of udon with hydrogen peroxide residue<sup>(2)</sup>. According to the "Application Scope and Limits of Food Additives" announced by Department of Health, hydrogen peroxide is belong to the bactericide category. It can be used in surimi-base products as well as any food products other than flour and flour related products; however, hydrogen peroxide residue in any food is not allowable<sup>(3)</sup>. The health authority in Japan regulates that hydrogen peroxide must be thoroughly removed or decomposed prior to attainment of final product<sup>(4)</sup>. According to the food regulation in America, hydrogen peroxide residue in aseptic package should be no more than 0.5 ppm, which is determined by a test method using distilled water as a solvent<sup>(5)</sup>.

The current Chinese National Standard (CNS) for testing hydrogen peroxide is a qualitative method<sup>(6)</sup>, which is low in sensitivity and readily interfered by the matrices<sup>(1,7)</sup>. To improve the current CNS method, a precise, rapid, and high sensitive analytical method was intended to develop in this study.

With respect to the analysis of hydrogen peroxide, several methods have been reported as follows: qualitative analytical method<sup>(1,6-8)</sup>, enzymatic colorimetric method<sup>(9-15)</sup>, oxygen electrode method<sup>(15-19)</sup>, iodine titration method<sup>(1,20)</sup>, high performance liquid chromatography (HPLC) method<sup>(21)</sup>, and gas chromatography (GC) method<sup>(22)</sup>. In addition, testing kits are commercially available, including spectroquant hydrogen peroxide cell test, reflectoquant peroxide test (E. Merck, Darmstadt, Germany) and quantofix peroxide 25 (Macherey-Nagel, GmbH & Co., Druen, Germany). In CNS method, titanium sulfate and vanadium sulfate are used as color-developing reagents, which can only detect as low as 10 and 20 ppm hydrogen peroxide, respectively<sup>(1)</sup>. Some special equipment is necessary to perform oxygen electrode method although it can give a higher detection sensitivity. Titration method is low in

sensitivity and the end point of titration is difficult to determine<sup>(1,10)</sup>. HPLC and GC methods are not well documented and both these two methods involve derivatization procedures, which are complicated and not suitable as a routine procedure. Enzymatic colorimetric method is used most often. The oxidizable substrates are oxidized by hydrogen peroxide while existing enzyme as catalase, generating a chromogen or fluorophore. 4-Aminoantipyrine (4-AA)<sup>(9,14)</sup>, ammonium thiocyanate<sup>(11)</sup>, sesamol dimer<sup>(12)</sup>, thiochrome<sup>(13)</sup>, and *o*-phenylenediamine (OPD) have been reported to be oxidizable substrates in literature. The reaction of this method is specific and fast; however, the oxidation-reduction reaction initiated by co-existences could interfere with the result<sup>(11,22)</sup>.

Hydrogen peroxide has been used in bleaching shark fins to prolong the shelf life and to improve the appearance of final products<sup>(23)</sup>. Su *et al.*<sup>(24)</sup> investigated 18 dried shark fins and found 7 samples containing hydrogen peroxide. Hydrogen peroxide has also been reported to be illegally used in udon manufacturing for bleaching and disinfecting purposes. In this study, shark fins and udon were selected as target samples. Enzymatic colorimetric method and commercial testing kits were evaluated in order to establish a precise and rapid testing method, which could be officially used in detecting hydrogen peroxide in food. In this study, the storage stability and heat effect on hydrogen peroxide were also carried out. Results could be a reference for consumers and related authorities.

## MATERIALS AND METHODS

### I. Materials

The following samples were purchased from grocery stores, traditional markets, or supermarkets: 20 dried shark fins, 10 rehydrated shark fins, 6 shark fin cans, and 13 udon samples. Both dried shark fins and shark fin cans were stored at room temperature; while rehydrated shark fins and udon were kept in freezer before use. Ten samples of cooked shark fins and udon each were purchased

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from restaurants and tested for hydrogen peroxide on the day of purchasing.

## II. Reagents

Reagent grade hydrogen peroxide (30% of concentration) was obtained from Santoku Chemical Industries, Co., Ltd. (Tokyo, Japan). Peroxidase (Product No. P 8000) and 4-aminoantipyrine (4-AA) were the products of Sigma Chemical Co. (St. Louis, MO, USA). Regent grade *o*-phenylenediamine dihydrochloride (OPD), citric acid, sodium citrate, sulfuric acid, and phenol were purchased from Nacalai Tesque Inc. (Kyoto, Japan). OPD solution was prepared by dissolving 100 mg OPD and 10 mg peroxidase in 100 mL of citrate buffer (pH 5.0).

## III. Instrument

A spectronic 3000 Array Spectrophotometer (Milton Roy Company, New York, USA) was used in this study.

## IV. Preparation of Standard Solutions

The content of hydrogen peroxide was standardized in accordance with the "Methods of Analyzing Food Additives in Foods-with Commentary"<sup>(16)</sup>. The standardized hydrogen peroxide was diluted to a concentration of 1 mg/mL with distilled water, which was then diluted to a series concentrations ranging from 0.2 to 5.5 µg/mL ready for use as standard solutions.

## V. Qualitative Testing

A CNS 10893, N6189 testing method entitled "Method of Test for Bactericides in Food-Test of Hydrogen Peroxide"<sup>(6)</sup> was adopted.

## VI. Quantitative Analysis

### (I) Sample Preparation

Sliced udon (10 g), rehydrated shark fins (10 g), or dried shark fins (2 g) was each placed in a 50-mL tube. Thirty-five mL of distilled water or methanol was added to the tube, and mixed well. The sample was dipped and shaken gently in a shaker for 10 min or homogenized at 3000 rpm

for 3 min, then diluted to 50 mL, filtered through a filter paper (Toyo No. 5A), and more filtering through a 0.45 µm membrane if necessary. The testing solution was thus prepared.

### (II) Quantitative Procedure

#### 1. Modified OPD Colorimetric Method

A method reported by Wu and Lin<sup>(15)</sup> was modified and used in this test. Five mL of testing solution and 5 mL of OPD solution was mixed and left stand at room temperature for 10 min. One mL of sulfuric acid (10 N) was then added to fix the color. A spectrophotometry at 490 nm was used to detect the absorbance of the final solution as well as sample blank in which the testing sample was replaced by 5 mL of distilled water. Hydrogen peroxide residue in testing samples was determined by comparing to the standard curve of hydrogen peroxide.

#### 2. 4-AA Colorimetric Method

A 4-AA colorimetric method presented by Ito *et al.*<sup>(9)</sup> was used in this study. A mixture containing 5 mL of testing solution, 3 mL of 2% phenol solution, 1 mL of 0.4% 4-AA color-developing solution, and 1 mL of 0.01% peroxidase solution was incubated in a 37°C water-bath for 10 min. After cooling to room temperature, the final solution was spectrophotometrically checked for absorbancy at 505 nm. Sample blank (5 mL of distilled water instead) was also checked at the same wavelength.

#### 3. Spectroquant Hydrogen Peroxide Cell Test

A Merck brand testing kit was used. Ten mL of testing solution was placed in a spectroquant hydrogen peroxide cell. After mixing, the cell was kept standing for 3 min prior to a detection at 405 nm.

#### 4. Reflectroquant Peroxide Test

A kit for this testing was also a product of Merck Co. A test paper was immersed in testing solution. A blue oxide was formed when the test strip in test paper reacted with hydrogen peroxide.

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The concentration of hydrogen peroxide in testing solution was determined using a RQflex reflectometer to detect the blue oxides in test paper. Hydrogen peroxide content in test samples was reported on the basis of its concentration in testing solution.

### VII. Standard Curve Plotting

The standard curve of hydrogen peroxide was made by plotting a series concentrations of standard solutions, five mL of each was taken, versus their absorbances based on the modified OPD colorimetric method as mentioned above.

### VIII. Fortification Recovery Test

Fortification recovery test was carried out by spiking a concentration of 0.92, 3.68, or 9.20 ppm hydrogen peroxide into udon or rehydrated shark fin samples. Blank sample (distilled water) was also conducted. The test of each concentration was carried out in triplicate.

### IX. Detection Limit Test

Various concentrations of standard solutions were spiked into test samples and detected according to the current developed method. The limit of detection was on the basis of S/N ratio higher than 3.

### X. Stability of Hydrogen Peroxide in Boiling Water

One hundred mL of distilled water was poured into a 200-mL beaker and 1 or 2 mL of 3.68 mg/mL hydrogen peroxide was spiked. The water level was marked. The beaker was capped with a watch glass and the water was boiled for a period of 5, 10, 20, 60, 90, or 120 min. After cooling down to room temperature, the water was compensated to the mark prior to determination of hydrogen peroxide residue.

### XI. Storage Stability Test

Two portions of udon containing hydrogen peroxide were separately packed into a PE plastic bag, which was then stored at 25 or 4°C for 1 week prior to determination of hydrogen peroxide

residue.

### XII. Cooking Treatment

A cooking procedure of "Wok-boiled udon" was followed. Four portions of hydrogen peroxide-containing udon (250 g) were individually placed into a 2-L kettle which contained 500 mL of boiling water. The udon was kept boiling for 1, 3, 5, or 10 min, and then separated from water. After cooling down to room temperature, both udon and boiled water were weighed out and analyzed for the residue of hydrogen peroxide.

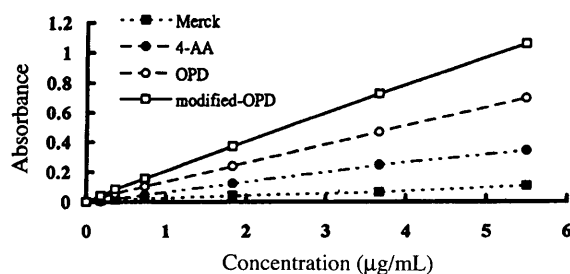
### XIII. Rehydration of Dried Shark Fins

A sample of dried shark fins (105 g), which had been determined to contain hydrogen peroxide residue, was soaked in 2 L of water overnight. The sample was heated in 2 L of boiling water (100°C) for 1 hr then transferred and kept in another 2 L of hot water (96°C) for 30 min. The procedure of transferring and keeping in another 2 L of water at 96°C was repeated in order to completely rehydrate the shark fins. The residue of hydrogen peroxide in each step of rehydration was monitored.

## RESULTS AND DISCUSSION

### I. Selection of Colorimetric Method

Colorimetric methods including 4-AA<sup>(9)</sup>, spectroquant hydrogen peroxide cell test, and OPD<sup>(15)</sup> methods were evaluated in this study. OPD method showed the highest sensitivity to hydrogen peroxide as shown in Figure 1.



**Figure 1.** Calibration curves of hydrogen peroxide determined by the OPD, modified OPD, 4-AA and Merck methods.

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4-AA method is unfavorable for hydrogen peroxide detection. Detection limit of this method is 10 ppm, which is not sensitive enough. It is readily interfered with ascorbic acid, which might exist in test samples<sup>(14)</sup>. Furthermore, the operation of this method is cumbersome. A water-bath kept at 37°C is necessary for performing this method. Although spectroquant hydrogen peroxide cell test is operation-friendly, this method is low in sensitivity (detection range between 2~20 mg/L) and high in expense. OPD method is easy to operate. In this method, a mixture of 5 mL of hydrogen peroxide and 5 mL of peroxidase OPD solution (pH 5.0) is left to stand at room temperature for 10 min. Five mL of 2 N sulfuric acid is then added to the resultant solution to fix the color, which is then monitored at 490 nm.

To understand the effects of reaction conditions on color developing, a further study was conducted to optimize OPD method.

## II. Optimization of OPD Method

### (I) Conditions for Color Developing

A concentration of 5.87 µg/mL hydrogen peroxide was used to optimize the following conditions: citrate buffer with pH range from 4 to 6, addition of OPD with concentration of 20~200 mg, addition of 0~15 mg peroxidase, a combination of sulfuric acid concentration and volume of 2 N x 5 mL, 5 N x 2 mL or 10 N x 1 mL, and a reaction time ranging from 2 to 15 min. Results showed the optimum condition for OPD method was using a pH 5.0 citrate buffer containing 100 mg OPD and 10 mg peroxidase and reaction time was 10 min. One mL of 10 N sulfuric acid was selected to be a color-fixing agent. The sensitivity using modified OPD method was higher than unmodified OPD method<sup>(15)</sup> as shown in Figure 1. The absorbance increased by 1.1 times when the amount of OPD was increased from 40 mg to 100 mg. There was no more increase in absorbance when higher than 100 mg OPD was used. Using 1 mL of 10 N sulfuric acid instead of 5 mL of 2 N sulfuric acid increased the absorbance by 1.4 times although both solutions showed the same

pH at 0.6. Modified OPD method enhanced the sensitivity in hydrogen peroxide detection by 1.5 times as compared to unmodified OPD method.

Using a modified OPD method, a standard curve with a regression line  $Y = 0.1929X + 0.0092$  (where X as concentration in µg/mL; Y as absorbance) and a regression coefficient ( $R^2$ ) of 0.9998 was obtained.

### (II) Stability of OPD-chromogen

OPD is a dye easily decomposed to a dark color substance while exposed to air. Performing this method immediately after preparation of OPD solution is strongly suggested. The light effect on the stability of OPD-chromogen was also investigated using a modified OPD colorimetric method where 5 mL of hydrogen peroxide (3.68 µg/mL) was used. Results showed that OPD-chromogen was quit stable over 3 hr either under light or excluding light (Figure 2).

### III. Effects of Extraction Methods on Recovery

The effects of different extraction methods on recovery of hydrogen peroxide were studied. Ten gram of sliced udon and rehydrated shark fins were spiked with 92.0 µg of hydrogen peroxide, immersed into 35 mL of water<sup>(1,11,12)</sup> or methanol<sup>(9,13,14)</sup>, homogenized for 3 min<sup>(9,13,14)</sup> or dipped for 10 min<sup>(18,19)</sup>, added to the volume of 50 mL with solvent, and filtered. Hydrogen peroxide in filtrates was then determined by modified OPD method. Results showed that test samples immersed in water for 10 min gave the best recovery of hydrogen peroxide (Table 1). Methanol as an extraction solvent was unfavorable because it

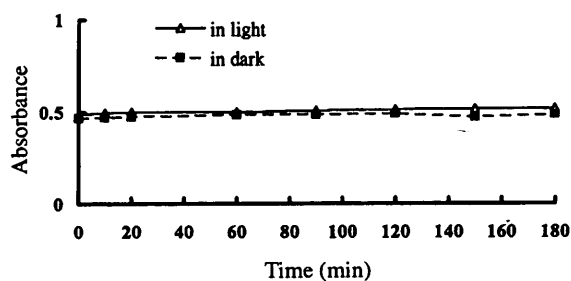


Figure 2. Stability of OPD-chromogen of hydrogen peroxide.

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**Table 1.** Comparison of recoveries of hydrogen peroxide at a spiked level of 9.20 ppm in udon and shark fins extracted with different methods

Sample	Method	Recovery <sup>a</sup> (%)
Udon	Dipping in H <sub>2</sub> O, 10 min	99.9 (0.1) <sup>b</sup>
	Dipping in MeOH, 10 min	95.5 (2.7)
	Homogenization with H <sub>2</sub> O, at 3000 rpm, 3 min	91.6 (2.0)
	Homogenization with MeOH, at 3000 rpm, 3 min	96.0 (1.2)
Shark fins	Dipping in H <sub>2</sub> O, 10 min	91.7 (2.1)
	Dipping in MeOH, 10 min	90.4 (1.6)
	Homogenization with H <sub>2</sub> O, at 3000 rpm, 3 min	66.6 (4.8)
	Homogenization with MeOH, at 3000 rpm, 3 min	79.8 (2.8)

<sup>a</sup> average of triplicate.

<sup>b</sup> value in the parenthesis is coefficient of variation (%).

**Table 2.** Effects of food ingredient or additive in udon<sup>a</sup> on OPD color reaction

Ingredient/Additive	Test level <sup>b</sup>	Recovery <sup>c</sup> (%)
Control	—	99
Potassium bromate	10 µg	434
	5 µg	294
	1 µg	109
Soysauce	10 mg	104
	20 mg	108
L-Ascorbic acid	10 µg	81
	5 µg	93
BHT	10 µg	92
BHA	10 µg	94
Benzoyl peroxide	10 µg	97
	5 µg	98
Glucose	1 mg	100
Monosodium glutamate	100 mg	102
Potassium sorbate	200 µg	101
Sodium chloride	1 mg	98
Sodium phosphate	200 µg	99

<sup>a</sup> the udon was spiked with 9.2 ppm of hydrogen peroxide.

<sup>b</sup> in 5.0 mL sample solution.

<sup>c</sup> average of duplicate.

could result in a cloudy extract, which required to be filtered through a 0.45 µm membrane. Homogenization yielded more cloudy extracts and lower recovery of hydrogen peroxide compared to a dipping process without homogenization, implying hydrogen peroxide could be decomposed during homogenization<sup>(19)</sup>. While extraction of dried

shark fins, much less amount of sample was used in comparison of rehydrated shark fins because dried product could absorb solvent resulting in a decrease of extraction efficiency. In this study, 2 g instead of 10 g dried shark fins was used.

#### IV. Effects of Food Ingredients or Additives on Recovery

OPD method is based on oxidation of hydrogen peroxide. Any food components possessing oxidation or reduction property may inadvertently interfere with testing results. Some additives with color such as soysauce may also affect the absorbance. To understand these effects, 11 food ingredients or additives, as listed in Table 2, were individually spiked into 5 mL of testing solution, which was prepared from 10 g of udon fortified with 9.2 ppm hydrogen peroxide. Results are presented in Table 2. Potassium bromate, which was banned in food due to its carcinogenicity, greatly induced OPD color reaction. Soysauce could slightly increase the absorbance; while ascorbic acid, BHT, and BHA could reduce the color formation. The OPD color reaction was not affected by benzoyl peroxide, glucose, monosodium glutamate, potassium sorbate, sodium chloride, or sodium phosphate.

#### V. Fortification Recovery and Limit of Detection

The recoveries of hydrogen peroxide from udon and shark fins are shown in Table 3. Except for shark fins spiked with 0.92 ppm hydrogen peroxide, the recoveries ranged from 81.9 to 99.9% were obtained from both udon and shark fins. All the coefficients of variation were less than 5%. Recoveries from shark fins were less than those from udon. This could be due to the complexity of

**Table 3.** Recoveries of hydrogen peroxide spiked into udon and shark fins

Sample	Spiked level (ppm)	Recovery <sup>a</sup> (%)
Udon	0.92	90.2 (4.4) <sup>b</sup>
	3.68	96.5 (1.3)
	9.20	99.9 (0.1)
Shark fins	0.92	65.3 (3.3)
	3.68	81.9 (3.5)
	9.20	91.7 (2.1)

<sup>a</sup> average of triplicate.

<sup>b</sup> value in the parenthesis is coefficient of variation (%).

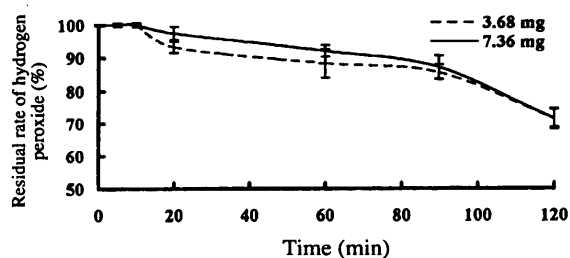
protein structures in shark fins hindering extraction of hydrogen peroxide<sup>(25)</sup>. The limit of detection was 0.5 ppm, the same as the method reported by Kikugawa *et al.*<sup>(12)</sup> and Tsukada *et al.*<sup>(13)</sup>, but still higher than the modified 4-AA method, the detection limit of which was 0.05 ppm<sup>(9)</sup>. The modified 4-AA method, however, requires a clean-up operation and a concentration processing and therefore is not a favorable choice.

#### VI. Stability of Hydrogen Peroxide in Boiling Water

The stability of hydrogen peroxide at a level of 3.68 or 7.36 mg in 100 mL of water is demonstrated in Figure 3. Hydrogen peroxide was quite stable when boiled in water for 10 min. Seventy percent of which still remained even after boiling for 120 min. Boiling process was not capable of completely removing hydrogen peroxide. This could be due to its high boiling point at 152°C<sup>(26)</sup>. Therefore, the residue of hydrogen peroxide can still exist in food matrix when food containing hydrogen peroxide is treated with regular cooking conditions.

#### VII. Storage Stability Test

The changes of hydrogen peroxide residue at the level of 90 or 480 ppm in udon during storage are shown in Figure 4. The slope of each regression curve was calculated based on hydrogen peroxide residue (Y) and storage time (X). Results showed the slopes (referring to a decline rate) for a residue of 480 ppm in udon stored at 25 and 4°C were -20.043 and -8.811, respectively. The slopes for a residue of 90 ppm at these two temperatures



**Figure 3.** Stability of hydrogen peroxide in boiling water.

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were -20.194 and -5.285, respectively. These results indicate hydrogen peroxide residue in food is relative stable after storage and its dissipation slows down at a lower temperature.

### VIII. Cooking Treatment

Udon containing 80 ppm hydrogen peroxide was cooked for 1~10 min. The residue of hydrogen peroxide in udon or soup was analyzed and

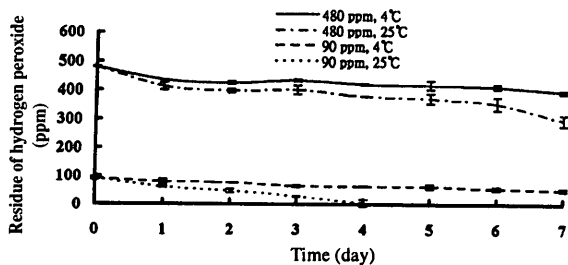


Figure 4. Change of hydrogen peroxide residue in udon during storage at 4°C and 25°C.

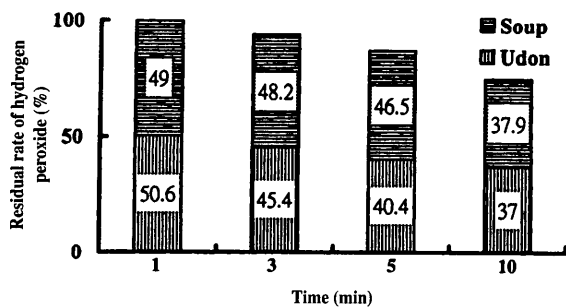


Figure 5. Effect of cooking time on hydrogen peroxide residue in udon and the soup.

results were shown in Figure 5. Almost half of hydrogen peroxide residues were transferred into soup while the total hydrogen peroxide were remained the same after cooking for 1 min. Hydrogen peroxide residues in udon and soup were 40.4 and 46.5%, respectively, 13% of which declined in total, after 5 min cooking. The residues reduced to 65% while cooking for 10 min. During cooking, udon absorbed water and swelled resulting in decrease of water. Hydrogen peroxide could transfer back to udon from soup when water kept loss after a long time cooking.

### IX. Rehydration Treatment of Shark Fins

A sample of dried shark fins (105 g) containing 726 ppm hydrogen peroxide was rehydrated with 20 folds of water (v/w). The amount of

Table 4. Effect of rehydration treatment on hydrogen peroxide residue in shark fins

Treatment	Time (hr)	Hydrogen peroxide residue <sup>a</sup> (ppm)
Control		726
Dipping	24	18.5
Boiling <sup>b</sup>	1	11.9
Boiling <sup>b</sup>	1.5	3.6
Boiling <sup>b</sup>	2	N.D. <sup>c</sup>

<sup>a</sup> average of duplicate.

<sup>b</sup> after previously dipped in water for 24 hr.

<sup>c</sup> not detected.

Table 5. Survey of hydrogen peroxide residues in shark fins and udon sampled from various markets

Sample	Sampling place	No. of positive samples	Hydrogen peroxide residue <sup>a</sup> (ppm)
<b>Shark fins</b>			
Dried	Grocery stores	12 (20) <sup>b</sup>	0.9, 1.1, 15.4, 26.8, 934, 1330, 1367, 1560, 2327, 3178, 4828, 5451
Rehydrated	Traditional markets	0 (10)	N.D. <sup>c</sup>
Canned	Grocery stores	0 (6)	N.D.
<b>Udon</b>			
	Traditional markets	6 (6)	5.2, 14.3, 68.8, 256, 480, 516
	Supermarkets	0 (7)	N.D.

<sup>a</sup> average of duplicate.

<sup>b</sup> total number of samples.

<sup>c</sup> not detected.



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hydrogen peroxide dropped to 18.5 ppm after a 24-hr dipping (Table 4). No residue was found when dipped shark fins was heated in 2 L of water for 2 hr, indicating a processing of dipping and changing water followed by heating could effectively decompose or wash away hydrogen peroxide. Commercial rehydration treatment involves heat treatment (about 3~5 hr) and a dipping procedure before and after heating. This processing may remove most of hydrogen peroxide from shark fins although the water used for dipping is less than 20 folds of shark fins.

#### X. Investigation of Commercially Available Shark Fins and Udon

Investigation results are listed in Table 5. Twelve out of 20 dried shark fins were determined to have hydrogen peroxide residues over the range of 0.9~5451 ppm. In most cases, the higher the hydrogen peroxide residues in products, the whiter in color on the product appearance would be. In exception, two samples with snow-white color were examined to contain only 0.9 and 1.1 ppm hydrogen peroxide. No residue was detected in rehydrated shark fins and shark fin cans.

Among 13 udon samples investigated, all 6

**Table 6.** Survey of hydrogen peroxide residues in cooked shark fins and udon from various restaurants

Sample	Hydrogen peroxide residue <sup>a</sup> (ppm)	
	Solids	Soup
Cooked shark fins		
A	2.5	4.2
B	2.1	2.4
C	1.7	2.1
D	2.2	2.7
E ~ J	N.D. <sup>b</sup>	N.D.
Cooked udon		
A	1.6	3.3
B	1.7	2.6
C	4.7	9.2
D ~ J	N.D.	N.D.

<sup>a</sup> average of duplicate.

<sup>b</sup> not detected.

samples sold in traditional markets at room temperature were found to contain hydrogen peroxide ranged from 5.2 to 516 ppm. Among them only the sample containing 480 ppm hydrogen peroxide was vacuum-packed. No residue was found in the other 7 samples (4 domestic and 3 Japanese products) sold in supermarkets at refrigerating temperature. They were all vacuum-packed and properly labeled. In this investigation, we found that the storage conditions in traditional markets were not in accordance with the instruction on labeling. The products should have been kept refrigerated, but were shelved at room temperature. Our investigation suggests it is much safer to purchase food from supermarkets than from traditional markets.

The results of hydrogen peroxide residues in cooked shark fins and udon from restaurants are presented in Table 6. In cooked shark fins, 4 out of 10 were detected to have hydrogen peroxide (about 2 ppm) both in solid form and in soup. Three out of 10 udon samples contained hydrogen peroxide residues over the range of 1.6~9.2 ppm either in solid form or in soup. These results indicated that 30~40% shark fins and udon dishes, which prepared in restaurants contained hydrogen peroxide residues. The result that hydrogen peroxide residues existed both in cooked solids and in soup is consistent with our study that hydrogen peroxide may migrate from shark fins and udon to water phase during cooking process.

#### CONCLUSION

After comparison among CNS method, reflectoquant peroxide test, and the proposed method (Table 7), we found that CNS method was not suitable for a qualitative test because it was affected by the textures of cooked shark fins and udon as well as the color of soysauce. Modified OPD method was as effective as reflectoquant peroxide test with respect to the test for hydrogen peroxide residues, which were extracted using the developed method. Reflectoquant peroxide test is used to detect hydrogen peroxide over the range of 0.2~20.0 mg/L. The limit of detection is 1 ppm.

**Table 7.** Comparison of CNS method, reflectoquant peroxide test and the proposed method for determining hydrogen peroxide residues in samples

Sample	CNS method <sup>a</sup>	Hydrogen peroxide residue <sup>b</sup> (ppm)	
		Reflectoquant peroxide test	Proposed method
<b>Shark fins</b>			
Dried	+	2300	2327
Dried	+	18.0	15.4
Cooked	-	1.0	1.7
Cooked	-	1.8	2.2
<b>Udon</b>			
Raw	+	284	276
Raw	+	14.8	14.3
Cooked	-	5.5	4.7
Cooked	±	9.0	9.2

<sup>a</sup> qualitative method.

<sup>b</sup> average of duplicate.

This test can generate a blue chromogen, which is less affected by the color of soysauce. The device of reflectoquant peroxide test is suitable for a quick in-house inspection because it is compact in size and easy to operate. The proposed method was based on an enzymatic catalyzation that was specific, rapid, and sensitive in hydrogen peroxide detection. Test sample was extracted with water and hydrogen peroxide was catalyzed by peroxidase to form an OPD-chromogen, which can be positively identified with spectrum scanning. A satisfactory linearity over the range of 0.2~5.5 µg/mL was observed. Fortification recoveries from the samples of udon and shark fins were at the range of 65.3~99.9% and the coefficients of variation were less than 5%. The detection limit of the proposed method was as low as 0.5 ppm.

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## 食品中過氧化氫之定量及其消退情形之探討

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### 摘 要

本研究利用酵素比色法(enzymatic colorimetric method)分析食品中過氧化氫殘留量。烏龍麵及魚翅檢體以水浸泡10分鐘後過濾，濾液在室溫下，於pH 5.0之檸檬酸鹽緩衝液中與o-phenylenediamine及peroxidase反應10分鐘，再測定490 nm吸光值，由標準曲線計算過氧化氫殘留量。烏龍麵及魚翅檢體中添加過氧化氫0.92、3.68及9.20 ppm時，其回收率分別為烏龍麵90.2~99.9%，魚翅65.3~91.7%，變異係數皆小於5%。最低檢出限量皆為0.5 ppm。殘留過氧化氫之烏龍麵於室溫及冷藏儲存時，其殘留之過氧化氫皆不易消退，尤以儲藏溫度越低者為然。烹煮無法將烏龍麵中殘留之過氧化氫分解，但部分過氧化氫可從烏龍麵溶出至麵湯。魚翅復水處理時，若以多量水浸泡及加熱，且經常換水，可將過氧化氫完全去除。經依酵素比色法測定市售魚翅及烏龍麵之過氧化氫殘留量，發現20件魚翅乾品檢出過氧化氫者有12件，殘留量範圍0.9~5451 ppm。13件烏龍麵中檢出過氧化氫者有6件，皆購自傳統市場，殘留量為5.2~516 ppm，另超級市場販賣之7件包裝烏龍麵均未檢出過氧化氫。依據「食品添加物使用範圍及用量標準」之規定，過氧化氫不得殘留於食品中。本署已請地方衛生機關令其限期改善後再行檢驗。

**關鍵詞：**過氧化氫，酵素比色法，鄰-苯二胺，魚翅，烏龍麵。