Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001, Pages 220-223 藥物食品分析 第九卷 第四期

Identification of Hydrogen Peroxide as a Causative Agent in Noodles Implicated in Food Poisoning

SHU-CHU SU[1,2]*, SHIN-SHOU CHOU[1], PI-CHIOU CHANG[1] AND DENG-FWU HWANG[2]

[1.] *National Laboratories of Foods and Drugs, Department of Health, Executive Yuan, Taipei, Taiwan, R.O.C.*

[2.] *Department of Food Science, National Taiwan Ocean University, Keelung, Taiwan, R.O.C.*

(Received: June 18, 2001; Accepted: September 10, 2001)

ABSTRACT

Noodles and udon noodles were involved in two food poisoning outbreaks in Taiwan in May, 2000. Samples were collected from the uneaten portions of the victims' meals. Hydrogen peroxide (H_2O_2) in samples is determined by a reflectoquant peroxide test with a detection limit of 1.0 ppm. The level of H_2O_2 in udon and noodles ranging from 597 to 1,656 ppm. The gastro-enterological symptoms of the victims along with the high content of H_2O_2 in udon and noodles consumed provided reasonable evidence that the food poisoning was directly associated with H_2O_2. Furthermore, each of the 30 samples of commercial noodles collected from markets in Taipei and Kaohsiung were determined. The ratio of detected samples was 26.7% and 3.3% in Taipei and Kaohsiung, respectively. Eight noodle samples purchased from traditional markets in Taipei contained H_2O_2 residues ranging from 50 to 1,240 ppm. One noodle sample purchased from a traditional market in Kaohsiung contained an H_2O_2 residue of 263 ppm. No H_2O_2 residue was detected in the other samples purchased from supermarkets in Taipei and Kaohsiung.

Key words: hydrogen peroxide, udon, noodle, food poisoning

INTRODUCTION

Two incidents of food poisoning due to ingesting udon and noodles, occurred in Taoyuan and Kaohsiung, Taiwan in May, 2000[(1)]. The incidents caused illness in 11 and 390 victims in Taoyuan and Kaohsiung, respectively (Table 1). Symptoms including nausea, vomiting, abdominal cramps, and prickling and burning of throat appeared soon after eating udon[(1)]. The victims recovered within 24 hours.

A poisoning outbreak occurred in Tokyo in 1971 due to the consumption of udon with H_2O_2 residue[(2)]. The gastro-enterological symptoms of these two incidents were similar to those caused by H_2O_2, and unlike those caused by *Bacillus cereus* toxin[(3)]. H_2O_2 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, however, the adverse effect of H_2O_2 has been reported, such as inducing duodenal cancer[(4)]. Although H_2O_2 residue in any food is not allowed in Taiwan, it is permitted for use in surimi-based products as well as any food products other than flour and flour related products[(5)]. The health authority in Japan regulates that H_2O_2 must be thoroughly removed or decomposed prior to attainment of final product[(6)]. According to food regulations in the US, hydrogen peroxide residue in aseptic packages should be no more than 0.5 ppm, which is determined by a test method using distilled water as a solvent[(7)].

Our objective was to investigate the association of food poisoning incidents with H_2O_2 levels of noodle samples obtained from the uneaten portions of the victims' meals. Outbreaks of H_2O_2 poisoning indicated that the residue of H_2O_2 in commercial noodles might be a serious problem in Taiwan. Hence, the noodle samples from Taipei and Kaohsiung markets were also analyzed for H_2O_2 levels.

* Author for correspondence. Tel: 02-26531263;
Fax: 02-26531256; E-mail: sushuchu@nlfd.gov.tw

MATERIALS AND METHODS

I. *Materials*

Three noodle samples were collected from the uneaten portions of the victims' meals. In addition, thirty noodle samples were collected from traditional markets or supermarkets in Taipei and Kaohsiung between June and August, 2000. All samples were stored at -20˚C until use. The content of H_2O_2 (30% of concentration, Santoku Chemical Industries, Co., Ltd., Tokyo, Japan) was standardized in accordance with the "Methods of Analyzing Food Additives in Foods-with Commentary"[(8)]. Standardized H_2O_2 was diluted to a concentration of 1 mg/mL with deionized water, which was then diluted to a series of concentrations ranging from 0.2 to 6.0 μg/mL ready for use as standard solutions.

II. *Sample Preparation*

Each sample of noodle (10 g) was sliced and placed in a 50 mL tube, 35 mL of deionized water was added and mixed well. The sample was homogenized at 3,000 rpm (Nissei AM-3 Homogenizer, Nihonseiki Kaisha Ltd., Tokyo, Japan) for 3 min, then diluted to 50 mL, and filtered through a filter paper (Toyo No. 5A) and a 0.45 μm membrane. The test solution was thus prepared.

Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001

Table 1. Foodborne disease outbreaks caused by udon and noodles in 2000 in Taiwan

Case	Date	Place	Sample	Ratio of poisoning (%)	Time of symptom onset	Symptoms of patients
I	May 15	Taoyuan	udon	3.9 (11/280)[a]	40 min	nausea, vomiting, abdominal cramps
II	May 31	Kaohsiung	noodle	19.4 (390/2011)	20~60 min	vomiting, abdominal cramps, prick ling and burning of throat

[a] Data in parenthesis represent patients/consumed persons.

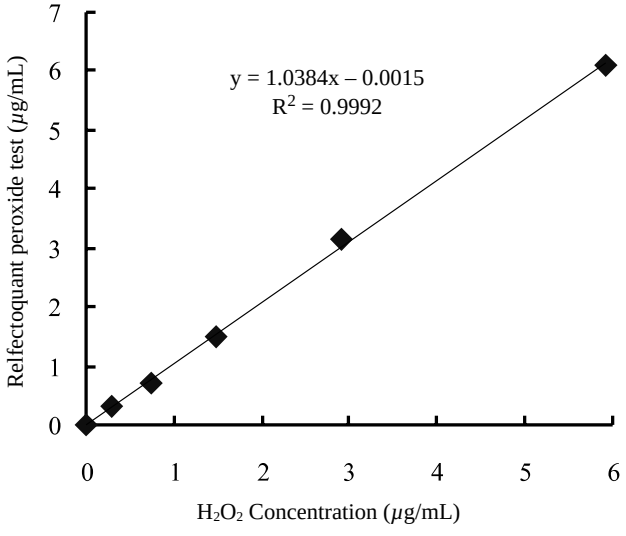

Figure 1. Relationship between hydrogen peroxide concentration and concentration detected by reflectoquant peroxide test.

The reflectoquant peroxide test applied a kit of E. Merck Co. (Darmstadt, Germany). A test paper was immersed in test solution. A blue oxide was formed when the test strip reacted with H_2O_2. The concentration of H_2O_2 in test solution was determined using a RQflex reflectometer (E. Merck Co., Darmstadt, Germany) to detect the blue oxides in test paper. H_2O_2 content in test sample was reported on the basis of its concentration in test solution.

RESULTS AND DISCUSSION

With respect to the analysis of H_2O_2, these methods have been reported as follows: qualitative analytical method[4, 9], enzymatic colorimetric method[10], oxygen electrode method[8], high performance liquid chromatography (HPLC) method[11], and gas chromatography (GC) method[12]. In addition, testing kits are commercially available, including reflectoquant peroxide test, spectroquant H_2O_2 cell test (E. Merck Co., Darmstadt, Germany) and quantofix peroxide 25 (Macherey-Nagel, GmBH & Co., Druen, Germany). Among these methods, the qualitative analytical method is low in sensitivity and readily interfered by the matrices. HPLC and GC methods are not well documented and both involve derivatization procedures, which are complicated and not suitable to routine analysis. Enzymatic colorimetric method and commercial testing kits are used most often. In a previous paper[10], we reported the reflectoquant peroxide test can generate a blue chromogen, which is less affected by the color of matrices, and is suitable for a quick in-house inspection because it is compact in size and easy to operate. The limit of detection was 1 ppm. In this study, the reflectoquant peroxide test was usable for hydrogen peroxide determination.

The standard curve of H_2O_2 was made by plotting a series of concentrations of standard solutions versus their responses based on the reflectoquant peroxide test. In the range of 0.2~6.0 μg/mL, the relationship between H_2O_2 concentration and response measured by the reflectoquant peroxide test was linear as shown in Figure 1. The correlation coefficient (R^2) was 0.999.

The recovery test was carried out by spiking a concentration of 1.48, 7.40, 14.8 or 29.59 ppm H_2O_2 into noodle samples (H_2O_2 free). A blank sample (deionized water) was also conducted. The results exhibited good recoveries (more than 95.1%) for the reflectoquant peroxide test (Table 2). It indicated that sample preparation and the determining method for H_2O_2 in this study were adequate.

The level of H_2O_2 in udon and noodle samples implicated in both poisoning cases is shown in Table 3. All samples contained high amounts of H_2O_2 ranging from 597~1,656 ppm. The gastro-enterological symptoms of the victims along with the high concentration of H_2O_2 residues in the noodles suggested that both food poisoning incidents which occurred in Taoyuan and Kaohsiung were caused by H_2O_2.

To investigate the illegal use of H_2O_2 in commercial noodles in Taiwan markets, 30 noodle samples were collected each from Taipei and Kaohsiung and analyzed using the reflectoquant peroxide test. The level of H_2O_2 in the com-

Table 2. Recoveries of hydrogen peroxide spiked into noodles

Spiked level (ppm)	Recovery[a] (%)
1.48	95.1 (3.0)[b]
7.40	96.2 (3.1)
14.80	97.6 (2.1)
29.59	97.8 (2.5)

[a] Average of triplicate.
[b] Value in the parenthesis is coefficient of variation (%).

Table 3. The level of hydrogen peroxide in udon and noodles implicated in two food poisoning cases

Case	Sample	No. of sample	Hydrogen peroxide residue[a] (ppm)
I	Udon	1	924 (4.1)[b]
		2	933 (2.6)
		3	831 (1.0)
II	Noodle	1	597 (2.9)
		2	919 (3.1)
		3	1,656 (2.4)

[a] Average of triplicate.
[b] Value in parenthesis is the coefficient of variation (CV, %).

Table 4. Survey of hydrogen peroxide residues in noodle samples collected from Taipei and Kaohsiung, Taiwan

Sampling place	Ratio of detected samples (%)	No. of detected samples	Hydrogen peroxide[a] (ppm)
Taipei	26.7 (8/30)[b]		
Traditional market		8 (15)[c]	247±403[d] (50~1,240) [e]
Supermarket		0 (15)	N.D.[f]
Kaohsiung	3.3 (1/30)		
Traditional market		1 (15)	263
Supermarket		0 (15)	N.D.

[a] Average of triplicate.
[b] Datum in parenthesis is the ratio of detected samples to all test samples.
[c] Total number of samples.
[d] Mean ± S.D. of detected samples.
[e] Data represent the range of values from detected samples.
[f] Not detected.

mercial noodle samples is shown in Table 4. The ratio of detected samples was 26.7% and 3.3% in Taipei and Kaohsiung, respectively. Eight noodle samples purchased from traditional markets in Taipei contained H_2O_2 residues ranging from 50 to 1,240 ppm. One noodle sample purchased from a traditional market in Kaohsiung contained H_2O_2 residue of 263 ppm. No H_2O_2 residue was detected in the other samples purchased from supermarkets in Taipei and Kaohsiung. Besides noodles, dried shark fins have been reported to contain high amounts of H_2O_2[10, 13], although H_2O_2 is allowed in Taiwan in bleaching shark fins to prolong the shelf life and improve the appearance of the final products, it must be thoroughly removed or decomposed from the final products. Further investigation in other H_2O_2 abused food in Taiwan is ongoing.

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Journal of Food and Drug Analysis, Vol. 9, No. 4, 2001

引起食物中毒之麵類中過氧化氫之檢測

蘇淑珠[1,2*]　周薰修[1]　張碧秋[1]　黃登福[2]

1. 行政院衛生署藥物食品檢驗局
台北市南港區昆陽街161-1號
2. 台灣海洋大學食品科學研究所
基隆市北寧路2號

（收稿：June 18, 2001；接受：September 10, 2001）

摘　要

台灣在2000年5月發生兩起烏龍麵（udon）及麵條（noodle）所引起之食物中毒案例。採集患者之麵類食餘檢體，以reflectoquant peroxide test檢測過氧化氫，其含量範圍介於597 ~ 1,656 ppm之間，該方法之檢出限量為1 ppm。麵類檢體皆含高量之過氧化氫，配合患者之腸胃道症狀，推測過氧化氫係造成食物中毒之主因。同時，於台北市及高雄市市場抽購麵檢體各30件，過氧化氫檢出率分別為26.7及3.3%。台北市之檢體有8件檢出過氧化氫，檢出範圍為50 ~ 1,240 ppm，高雄市之檢體有1件檢出263 ppm，皆購自傳統市場，另南北兩市超級市場販賣之檢體均未檢出過氧化氫。

關鍵詞：過氧化氫，烏龍麵，麵條，食物中毒